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SIMILAR, YET DISTINCT, PATHOGENIC PATHWAYS OF PLASMACYTOID DENDRITIC CELL-DERIVED TYPE-IINTERFERON-DRIVEN CUTANEOUS INFLAMMATION IN ROSACEA AND PARADOXICAL PSORIASIS

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Lausanne, le 24 novembre 2017

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Daniel Speiser

Dedicated to my dear parents

Also, to everyone who wonders if I’m dedicating this to them. Yes. I am.

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ABSTRACT

Cutaneous immunity coordinates necessary protection of the host against exogenous insults, yet its deregulation can have profound pathogenic consequences which can lead to development of disease.

Type-I interferons are a class of pro-inflammatory cytokines with fundamental roles in innate and adaptive immune responses. In the skin, they not only mount responses against pathogens and tumours, but also sustain re-epithelialisation following injury, and provide tonic signals for maintaining homeostatic balance. Importantly, they are also involved in the pathogenesis of a number of organ-specific and systemic auto-immune diseases such as systemic lupus, type-I diabetes, and thyroid disease, and in the skin they are important drivers of psoriasis and discoid lupus. Exactly how perturbations of type-I interferons lead to cutaneous disease is still a hotly debated subject of research.

Production of type-I interferons, in particular IFNβ, is achieved by all nucleated cells, yet small relative numbers of professional producers exist in the circulation and lymphoid organs, which produce many fold higher amounts of these inflammatory cytokines. Plasmacytoid dendritic cells (pDCs), though generally dispensable for many immune responses, are implicated in several type-I interferon-driven auto-immune diseases. Psoriasis, a T_H1/T_H17 disease with an important role for Tumour Necrosis Factor (TNF), is just such a disease. Intriguingly, treatment of psoriasis (or other diseases) with a class of biologics called anti-TNFs is effective in most patients, but can also result in the development of novel psoriasiform lesions in about 2-5% of all treated individuals. This side-effect of anti-TNFs, called paradoxical psoriasis, has many similarities to classical psoriasis yet we know little of its pathogenesis. We find that paradoxical psoriasis lesions are characterised by uniform overexpression of type-I interferons with concurrent pDC accumulation. Intriguingly, TNF directly regulates production of type-I interferons through maturation of pDCs *in vitro*, and in pDCs recruited to the site of inflammation in a novel mouse model recapitulating paradoxical psoriasis. The resulting inflammation is type-I interferon-driven yet, unlike classical psoriasis, independent of T-cells and adaptive immunity.

Rosacea is a common cutaneous disorder affecting the facial convexities, characterised by recurrent flares of disease, with apparent localised microbial infestation and aberrant expression of cathelicidin antimicrobial peptides. We find that specifically during acute flare-ups, type-I interferons are uniformly and selectively overexpressed. In addition, stabilised lesions display T_H1/T_H17 signatures and upregulated interferon-response gene expression suggesting previous interferon-bursts. Using a pre-clinical mouse model of rosacea, we find that type-I interferons are produced by pDCs and that they are responsible for the T_H17-related cytokine expression. Interestingly, killing of rosacea-associated bacteria by cathelicidin antimicrobial peptides is sufficient to drive this pathogenic signature.

Taken together, our observations indicate that pDCs drive a type-I interferon-dependent innate skin inflammation in distinct cutaneous manifestations. These can be triggered by cytokine imbalances, such as for paradoxical psoriasis, or bacterial infestation and overexpression of antimicrobial peptides, such as for rosacea, and prime downstream pathogenic innate immune responses. These findings re-centre attention towards a pathogenic role for pDC-derived type-I interferon, and provide a rationale for targeting this particular axis whilst leaving intact interferon production by other cells and protective immune responses.

RÉSUMÉ

L'immunité cutanée est responsable de coordonner la protection de l'hôte contre des dommages exogènes, néanmoins sa dérégulation peut avoir des conséquences pathogéniques qui peuvent engendrer le développent de maladies.

Les interférons de type 1 sont une classe de cytokines pro-inflammatoires avec plusieurs rôles dans l'immunité. Dans la peau, non seulement ils génèrent des réponses immunitaires contre pathogènes envahissants et tumeurs, mais ils garantissent la réépithélialisation suite aux lésions, et fournissent des signaux toniques pour maintenir la balance homéostatique. Surtout, ils jouent un rôle dans la pathogénèse de maladies auto-immunes ciblées à des organes et systémiques telles que le lupus systémique, le diabète de type 1, et la maladie thyroïdienne. Dans la peau, ce sont des facteurs importants dans le développement du psoriasis et du lupus discoïde. Le mécanisme par lequel ces perturbations des interférons de type 1 mènent à des maladies cutanées reste un sujet d'intense recherche.

Toutes les cellules nucléés sont capables de produire des interférons de type 1, en particulier l'IFNβ, néanmoins une petite population professionnelle de cellules productrices existe dans la circulation et les organes lymphatiques, et peux produire une quantité plus importante de ces cytokines inflammatoires. Les cellules dendritiques plasmacytoïdes (pDCs), tandis qu'elles soient en général dispensables dans la génération de plusieurs réponses immunitaires, sont impliquées dans de nombreuses maladies auto-immunes menées par les interférons de type 1. Le psoriasis, une maladie T_H1/T_H17 avec un rôle important de la cytokine pro-inflammatoire Tumour Necrosis Factor (TNF), est justement une maladie de ce type. De façon intrigante, le traitement du psoriasis (et d'autres maladies) avec une classe de biologiques appelés les anti-TNFs résulte dans un traitement efficace dans la majorité des patients, mais aussi dans le développement de nouvelles lésions psoriasiformes dans 2-5% d'individus traités. Cet effet secondaire des anti-TNFs, appelé psoriasis paradoxal, a plusieurs similarités avec le psoriasis classique mais peu est connu sur sa pathogénèse. Nous trouvons que les lésions de psoriasis paradoxal sont caractérisées par la surexpression uniforme des interférons de type 1 avec concomitante accumulation de pDCs. Nous décrivons que le TNF régule la production d'interférons via la maturation des pDCs tout aussi bien *in vitro* que des pDCs recrutées au site d'inflammation psoriasiforme dans un modèle murin. L'inflammation qui en découle est dirigée par les interférons de type 1 mais, en contraste avec le psoriasis classique, est indépendant des lymphocytes T et du système immunitaire adaptatif.

La rosacée est une maladie commune des convexités du visage, et caractérisée par des poussées récurrentes de la maladie. Nous découvrons que spécifiquement pendant des poussées aigües de la maladie, les interférons de type 1 sont surexprimés de façon sélective et uniforme. Les lésions stabilisées, tout comme les lésions de poussée aigüe, sont caractérisées par des signatures T_H1/T_H17 ainsi que de surexpression de gènes de réponse à l'interféron, suggérant des précédentes flambées d'interféron. En utilisant un modèle préclinique murin de rosacée, nous décrivons que les interférons de type 1 sont produits par les pDCs et qu'elles sont responsables pour l'expression associée aux cytoquines T_H17. De façon intrigante, le tuage de bactéries associées à la rosacée par les peptides antimicrobiens cathelicidin est suffisant pour engendrer cette signature.

Mis ensembles, nos observations indiquent que les pDCs mènent une inflammation dépendant des interférons de type 1 dans distinctes manifestations dans la peau. Celles-ci peuvent être déclenchées par des déséquilibres cytokiniques, comme pour le psoriasis paradoxal, ou l'infestation microbienne et la surexpression de peptides antimicrobiens, comme pour la rosacée, et initier des réponses immunitaires innées pathogéniques. Ces observations recentrent l'attention vers un rôle pathogénique de l'interféron de type 1 produit par la pDC, et justifient le ciblage de cet axe en particulier qui laisserait ainsi intacte la production d'interférons provenant d'autres cellules, et l'induction de réponses immunitaires protectrices.

1. INTRODUCTION

The immune system of the skin is a complex network of cells mounting important protection of the host against exogenous insults, but it can also be at the root of a great number of human disorders. The following introduction aims at providing a broad overview of the cutaneous immune system and the functions it serves, as well as the aberrant responses which can be detrimental to the host and lead to the development of diseases of the skin and often beyond.

1.1. THE INNATE AND ADAPTIVE IMMUNE SYSTEM IN THE SKIN

The central dogma of the immune system describes two principal components. An innate immunity arm, which provides immediate, first-line of defence, but lacking pathogen specificity.[1] An adaptive immunity arm which in contrast to innate immunity, provides high specificity for antigens found in pathogens, maintains long-lived "memory", but requires time to be primed, typically five to six days.[1] Making up the very first-line of defence against foreign insult are barrier sites such as the skin, and they provide separation from exogenous compounds and microorganisms, but also active protection.[2]

1.1.1. THE SKIN, MORE THAN JUST BARRIER

Physical barriers such as epithelial linings of respiratory, gastrointestinal, and genitourinary tracts, and the skin provide a protective separation between the host and exogenous insults. The skin plays a fundamental role in keeping microorganisms, chemicals, toxins, irritants and radiation at bay, yet it is becoming increasingly clear that this is but one facet of the largest organ of the human body.

The skin is composed of an outer layer called epidermis, an inner compartment called dermis, and the innermost layer called hypodermis. Each has a diverse set of cell types and responsibilities that make for unique environments with distinct functions for the host. The epidermis is composed of keratinocytes, a cell type whose main function is to work in unison with neighbouring keratinocytes for a timely and continuous differentiation. Basal keratinocytes proliferate giving rise to daughter cells which form a stratified layer several cells thick, and which undergo continuous differentiation, progressively losing their status as living cells, and becoming an outer shell that makes up the corneal layer. During homeostasis, this process has well defined timeliness averaging 4 weeks form initial proliferation from the basal membrane, up to final cornification. Acceleration of this process results in desquamation, whereas a delay can result in delayed and/or impaired wound healing. Other cell types making up the epidermis are melanocytes and Langerhans cells, which are both dendritic and phagocytic in nature, and the latter is a proficient antigen-presenting cell.

The dermis is found beneath the epidermis and is composed of fibroblasts, which form the connective layers; vascular epithelial cells, forming blood vessels which irrigate the surrounding tissues; macrophages, which act as sentinels for maintaining homeostasis; and mast cells which act as sensors for mechanical and chemical stresses. The papillary dermis makes up the intrafollicular spaces, intertwining with the rete ridges (papillae), and contains a web of blood capillaries that reach the lower layers of the epidermis. The reticular dermis is the

most ample skin segment, and forms the lower layer of the dermis, and is formed of dense collagenous, reticular fibres that give it its flexible, elastic and sturdy characteristics.

The hypodermis, or superficial fascia, is mostly composed of adipocytes which store fat; epithelial cells forming the lymphatic and vascular vessels; and muscle cells forming a flat sheet of muscle in certain anatomical locations. Immune cells reside within the hypodermis, such as mast cells, macrophages, and T-cells, and contribute to skin homeostasis.

The skin is a large organ. Widely accepted surface area values, based on anecdotal calculations of the surface area of skin, are of *ca*. $2m^2$. New interest on the microbial environment of the skin[3] has led to a novel appreciation of the true size of skin. By taking into account skin appendages, which average to about 3mm deep and with 0.5mm diameter, with over $5x10^6$ follicles, it is now estimated that the skin composes at least $25m^2$ of surface area,[4] well over the previous estimates. The microbial flora is also known to reside in large numbers within skin appendages, as these naturally funnel microorganisms, making it an important area for host-microorganism interactions. In fact, it is now well-recognised that because skin is one large reservoir of microorganisms such as bacteria, fungi, and viruses, it forms an important interface between the host and microbial flora. Interestingly, it is now argued that bacteria penetrate actively into the deeper layers of skin, through use of proteases,[5] and therefore continuous innate antibacterial activity is required to impede skin infections.

The epidermis forms a barrier against exogenous compounds and microorganisms, through a thick corneal layer and active renewal of keratinocyte layers. Among important innate host defence mechanisms produced by keratinocytes, are the antimicrobial peptides. These, usually low molecular weight, bactericidal, fungicidal and viricidal compounds can be produced by keratinocytes throughout differentiation and form a concentration gradient keeping exogenous microorganisms away from the deeper epidermal layers, and the dermis. Human Beta Defensins (HBD)-1, -2 and -3 are cysteine-rich cationic antimicrobial peptides. HBD1 is found constitutively expressed in keratinocytes with potent antimicrobial activity against Gram-negative bacteria. HBD2 and HBD3 are induced in keratinocytes upon bacterial challenge, but also by pro-inflammatory cytokines such as Interleukin-1 (IL-1) and Tumour Necrosis Factor (TNF). Cathelicidin antimicrobial peptides (CAMPs) such as LL-37 are also produced upon bacterial triggers and pro-inflammatory cytokines, including during skin injury.[6] Human cathelicidin antimicrobial protein 18 (hCAP18) is the precursor protein, which requires proteolitic processing by proteases for activation, and in skin by the serine protease kallikrein 5 (KLK5)[7,8]. Further proteolitic processing of LL-37 can yield shorter peptides such as RK-31 and KS-30, which display increased antimicrobial activity, and interestingly these have been identified in human sweat and in sweat glands, in close proximity to skin appendages. Furthermore, antimicrobial peptides such as LL-37 are also potent chemoattractants for neutrophils, which themselves also produce β-defensins and cathelicidins, and are proficient antibacterial cells,[9] thus further reinforcing antimicrobial activity.

Skin cells need to initiate recruitment of more specialised cell types, both for mounting immunological responses against microorganisms, but also for proper wound healing. Keratinocytes are found to be able to release active IL-1α, but also to be able to produce pro-IL-1β, in response to epidermal injury. These inflammatory mediators are thought to act in a

paracrine manner to initiate inflammatory and repair processes. They can also directly produce chemokines such as CCL17, which binds to CCR4 expressed by T-cells and DCs; CCL27, which binds to CCR10 expressed by CD8 T-cells; and CCL20, which binds CCR6 expressed by T-cells and in particular T_H17 cells. They can secrete IL-7, an important growth factor for lymphocytes, suggesting a possible role in the survival and maintenance of recruited T- and B-lymphocytes.

Fundamentally, the skin provides a protective barrier against insults from the outside world, but is also responsible for a complex cross-talk with the environment, and of orchestrating innate immunity for more specialised and rapid responses.

1.1.2. Innate Immunity Against Exogenous Insults

The innate immune system is at the first interface between host and invading pathogen upon barrier disruption. In the skin, this is initiated at keratinocytes which signal, repair-focused, inflammatory cascades which rely on rapid, innate immune responses (Figure 1).

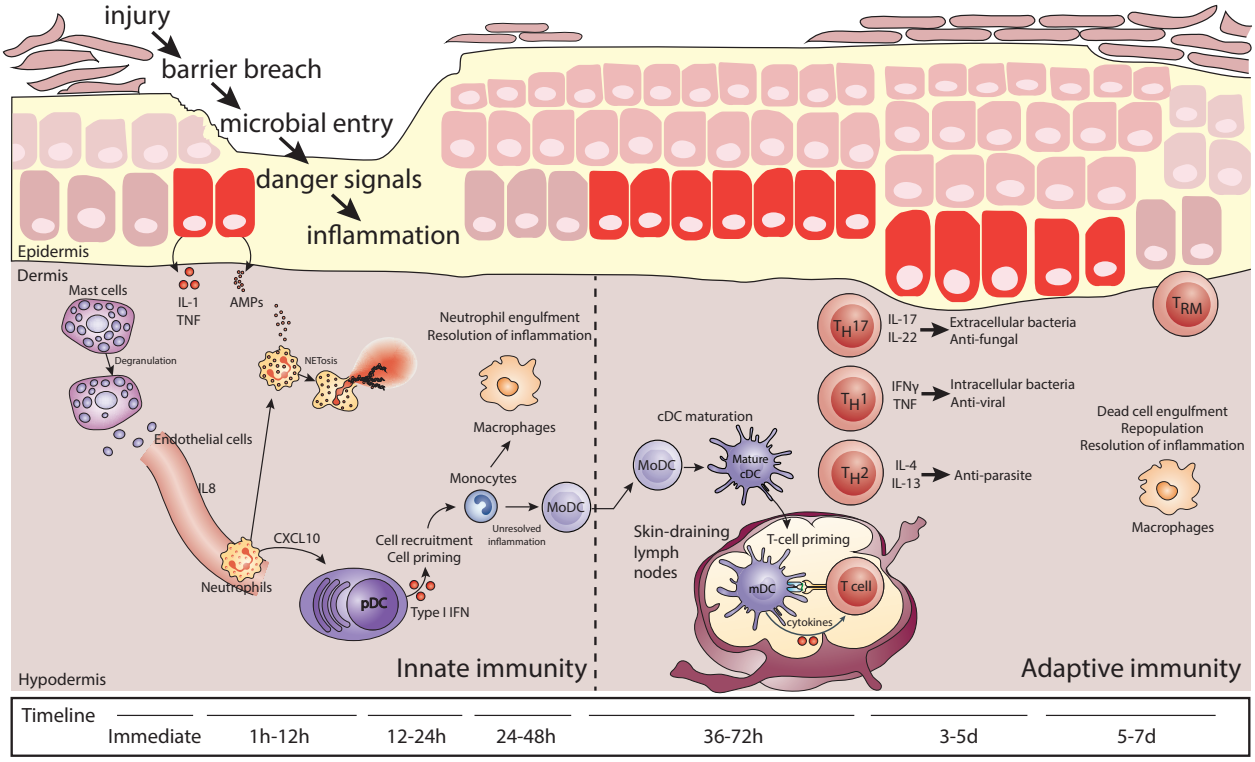

Figure 1: Schematic representation of innate and adaptive immunity in skin. Upon skin barrier disruption, microbial patterns which provide danger signals trigger immediate mast cell degranulation and secretion of pro-inflammatory cytokines. Antimicrobial peptides are produced by resident cells and recruited neutrophils to control invading microorganisms and NETosis slows the microbial spread. Type-I interferons produced by recruited pDCs leads to induction of chemokines and priming of inflammatory cells. Recruited monocytes differentiate in either macrophages which resolve inflammation by engulfing dying and dead cells, or into Mo-DCs which capture antigens and prime adaptive immune responses in skin-draining lymph nodes. Antigen-experienced T-cells can be polarised into TH1, TH2, or TH17 cells which are recruited to the site of inflammation for targeted immune responses. Macrophages promote a return to homeostasis and TRM cells populate the site of injury for future reactivation. IL: interleukin; AMP: antimicrobial peptide; pDC: plasmacytoid dendritic cell; IFN: interferon; MoDC: monocyte-derived dendritic cell; TH: helper T-cell; TNF: tumour necrosis factor; TRM: resident-memory T-cell. Schematic drawings modified and re-used with permission from Nature Publishing Group.

Arguably, skin repair cascades are centred around the need to keep barrier breaches under control so that exogenous microorganisms remain on the outside. Mammals have evolved 13 known Toll-like receptors (TLRs) to sense a myriad of ligands found on exogenous organisms. Danger signals such as cell wall components of bacteria including Lipoteichoic acid (LTA; gram-positive bacteria) or Lipopolysaccharide (LPS; gram-negative bacteria), or unmethylated nucleic acids (NA) such as those contained in viruses and bacteria (including CpG motifs) to name a few, are recognised by TLRs and other sensors expressed on skin resident cell-types including keratinocytes and macrophages. TLR engagement (LTA through TLR2; LPS through TLR4; NA through TLR3, TLR7, TLR8, TLR9, STING-converging sensors,[10] etc..) leads to production of pro-inflammatory mediators such as IL-1, TNF, chemokines including IL-8 (CXCL1/15 in mouse), and antimicrobial peptides, which in unison work to prime and recruit neutrophils.

Among first-responders are neutrophils, which are found in abundant numbers in the circulation, patrolling the periphery. Factors produced during danger sensing are released within the circulation, priming neutrophils, and chemotactic factors control neutrophil transmigration through endothelial walls. Primed neutrophils recognise and phagocytose microorganisms, which are then killed through reactive oxygen species and antimicrobial peptides from the fusion of granules with the phagosome. Of notable mention, neutrophils can also employ another method for rapidly hampering progression of bacteria within the skin compartments, called neutrophil extracellular trap (NET).[11,12] NETosis (the act of making NETs) culminates in the extrusion of nuclear and/or mitochondrial DNA through rapid decondensation of chromatin, and resulting in a landslide release of granule components. All in all, neutrophils are rapid responders at the site of barrier breaches and can both phagocytose target organisms, as well as create temporary barriers to slow down invasion from microorganisms. Correspondingly, neutropenic patients, either from inherited disorders or medically induced neutropenia (e.g. chemotherapy), have increased risk of infections from bacteria and fungi. Depending on the bacterial burden, this process can be lengthier thus leading to longer lasting recruitment of neutrophils, and the apparition of scars.[13] Once microbial control is achieved, dying neutrophils are cleared by macrophages. Historically, neutrophils have been considered as end-stage cells with limited metabolic and transcriptional capacity for *de novo* production of factors. This aspect of neutrophil biology has been revisited and compelling evidence has now accumulated as to the ability of neutrophils to produce cytokines and chemokines.[14,15] There is increasing evidence that neutrophils are able to produce key chemokines for amplifying their own recruitment, but also to promote monocyte, DC, NK and T-cell recruitment. In recent, as yet unpublished observations, it is found that neutrophils are essential in the production of CXCL10 during skin inflammation, a chemokine known to bind CXCR3, and found to recruit plasmacytoid dendritic cells (pDCs) into inflamed skin.[16,17]

Isaacs and Lindenmann (1957) first discovered that “viral interference”, the act of one virus interfering with the infectious capacity of another, was due to a soluble factor released by host cells, and not a component of the virus as previously hypothesised.[18] Years later, it was determined that one small proportion of cells in peripheral blood were the main producers of type-I interferon[19,20] and the hunt for the principal interferon producing cell type led to the identification of the Natural IFN-producing cell (IPC),[21,22] later called plasmacytoid dendritic

cell. pDCs are a rare cell type, about 0.3% among leukocytes in peripheral blood, expressing $CD123^+$ $BDCA2^+$ $BDCA4^+$ $CD4^+$ $CD11c^-$ $lineage^-$ in humans, and $PDCA1^+$ $B220^+$ $CD11c^{int}$ $Siglec\text{-}H^+$ in mice.[23,24] They are virtually absent from human skin during homeostasis, but play an important, though seldom indispensable, role in protection from infection. pDCs are found to produce 10- to 100-fold more type-I interferon than other cell types, but also cytokines such as TNF and IL-6, and chemokines such as CXCL10, IL-8 and CCL5. Specific bacterial and viral nucleic acid ligands which reach the endosomal TLRs -7 and -9, converge signalling onto MyD88, and trigger activation of IRF-7, inducing strong IFNα gene expression, and IRF-3, inducing mostly IFNβ (Figure 2). Importantly, pDCs express high constitutive levels of TLR7/9[25] as well as of IRF-7,[26] which makes them potent producers of type-I interferon. CpG-rich regions of DNA viral genomes, such as from herpes simplex virus (HSV) 1/2 and cytomegalovirus, activate TLR9;[27–29] whereas single-stranded RNA (ssRNA) virus motifs, such as from influenza, arenavirus, or vesiculostomatitis virus activate TLR7.[30–33] Type-I interferons can inhibit viral replication by induction of antiviral molecules,[34,35] apoptosis of infected cells,[36,37] and mounting antiviral innate and adaptive immune responses. They are activators of antiviral cytotoxicity from natural killer (NK) cells and CD8 T-cells,[38,39] and B-cell humoural responses.[40–44] Yet, pDCs are not crucially required in mounting immune responses, as other cell types can compensate the production of type-I interferon leading to, albeit delayed, viral clearance.[45] pDCs seem to play a role in skin injury, however, during early inflammatory responses,[6,46] though the exact mechanism is unknown.

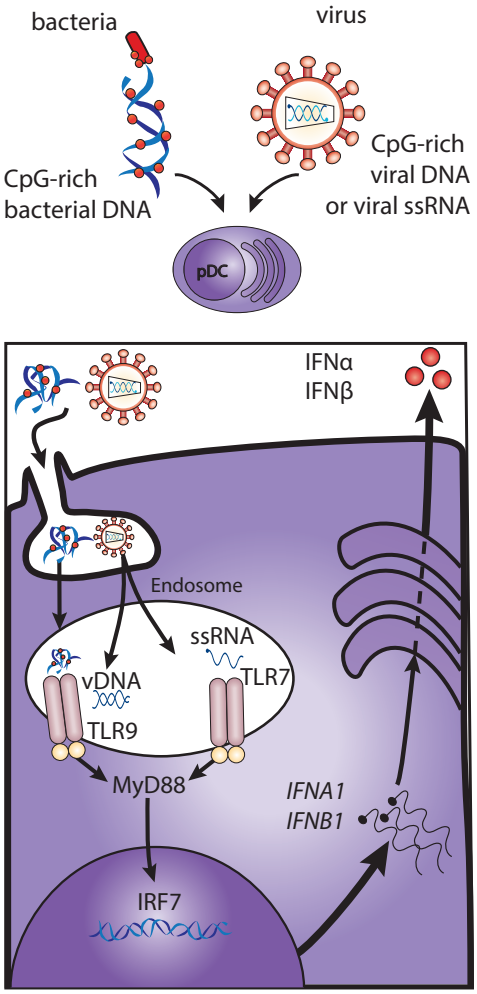

Figure 2: Microbial pattern recognition by plasmacytoid dendritic cells. Endocytosed CpG-rich motifs from extruded bacterial DNA or viral DNA, or single-stranded RNA trigger endosmal TLR9 or TLR7 respectively. These signals converge onto adaptor molecule MyD88, which activates and causes translocation of IRF7 into the nucleus. IRF7 binds to the promoter sequences driving transcription of IFN alpha and beta genes resulting in production of type-I interferons. pDC: plasmacytoid dendritic cell; ssRNA: single-stranded RNA; vDNA: viral DNA; TLR: Toll-like receptor; IFN: interferon. Schematic drawings modified and re-used with permission from Nature Publishing Group.

Monocytes are large mononuclear cells with multiple known subpopulations. In mice there are two described subsets in mice, one Ly6C^{hi} CCR2^{+}, and one Ly6C^{low} CCR2^{-} CX3CR1^{+},[47] whereas in humans three have been described, one CD14^{hi} CD16^{-} classical monocyte, one CD14^{+} CD16^{hi} non-classical monocyte, and one CD14^{hi} CD16^{+} intermediate subsets. Classical monocytes express preferentially CCR1 and CCR2, and are therefore recruited by CCL2 and 5. Non-classical monocytes express CX3C chemokine receptor 1 (CX3CR1), which is bound by fractalkine (CX3CL1). Intermediate monocytes express a combination of CCR1, 2, 5, 7, 9 and CX3CR1.[48] Once recruited to the site of inflammation, monocytes differentiate into macrophages and dendritic cells, depending on the requirements of the inflammatory environment. It is known that macrophage colony stimulating factor (M-CSF) and IL-6 produced by fibroblasts during steady-state leads to preferential macrophage differentiation, which results in engulfment of dead and dying cells, and a return to homeostasis. However, in the inflamed tissue, pro-inflammatory cytokine TNF promotes differentiation into cells with dendritic morphology, expressing HLA-DR^{hi} CD83^{+}.[49] Type-I interferons, in combination with granulocyte M-CSF (GM-CSF), can also lead to generation of CD25, CD40, CD80, CD86 co-stimulatory molecule-bearing Mo-DCs which express CCR5 and 7, and can produce T-cell polarising cytokines such as IL-1β, IL-6, IL-23, TNF and IL-12 among others.[50] Monocytes are recruited through chemokines CCL2, CCL5, CCL7, CCL12 and CX3CL1, the four former of which are type-I interferon inducible.[51,52]

More recently, a novel cell type has been described called innate lymphoid cells.[53–57] These cells have been subcategorised into group 1 (ILC1), group 2 (ILC2), and group 3 (ILC3) cells based on their cytokine and functional characteristics. Though lineage classification contradicts this, often oversimplified scheme,[58] they are the innate immune's system parallel of the adaptive's immune system T_H1, T_H2, and T_H17 T-cells. Although difficult to study due to their relatively low numbers, and them being negative for common lineage markers, they are found mostly in barrier surfaces including lung, gut and skin. Much like their adaptive counterparts and Natural Killer (NK) cells, ILC1s express transcription factors T-bet and Eomes,[59] and depend on IL-12 for their function and production of IFNγ and TNF. ILC2 mediate type-2 inflammation for expulsion of helminths, express RORα and GATA-3, and produce IL-4, IL-5, and IL-13 in response to IL-17E and IL-33. ILC3s, which include lymphoid tissue inducer (LTi) cells, express RORγt and/or the aryl hydrocarbon receptor (AhR), and produce IL-17 and IL-22 following stimulation.[60] Typically, healthy human skin maintains mostly ILC2 populations[61,62] and are thought to switch to ILC3 or ILC1 depending on the environmental needs.[63]

In summary, skin barrier disruption leads to microorganism entry into normally protected areas. This triggers sensing of danger signals by specialised receptors which leads to rapid secretion of pro-inflammatory factors by specific skin resident cells such as mast cells and keratinocytes. Paracrine signalling promotes production of antimicrobial peptides and rapid recruitment of neutrophils which provide specialised microbicidal activity and a temporary barrier function to slow microorganism spread. Neutrophils can also produce chemokines promoting recruitment of more specialised cell types such as pDCs, depending on the inflammatory needs. Finally, monocytes are recruited, in part in a type-I interferon-dependent manner, and differentiate according to the micro-environment resulting in a return to homeostasis. If the innate inflammatory response is not sufficient for controlling the invading

pathogens, neutrophil infiltration is sustained, and more specialised cell types are called into action eliciting adaptive immune responses.

1.1.3. Skin Adaptive Immunity in Response to Foreign Invasion

Adaptive immunity, though less flexible than innate immunity, provides targeted responses to invading pathogens when innate immunity is not sufficient, as well as swifter and more ordered responses and upon re-challenge.

A lot is known regarding T-cell responses in skin, thanks to extensive work performed using mouse models with primarily viral,[64–68] and to a lesser extent parasitic,[69] and bacterial,[70] infections. The current model supports that, upon first encounter with pathogens in the skin, danger signals recognised by keratinocytes and resident immune cells lead to activation of DCs. Local DCs migrate to draining lymph nodes where they present processed antigens from the pathogen to naïve T-cells. Through upregulation of co-stimulatory molecules CD80, CD83 and CD86, as well as HLA, they are able to activate T-cells by interacting with the T-cell receptor (TCR). Though not fully elucidated, type-I interferons and TNF play a role in maintenance of T-cells within secondary lymphoid organs, thus increasing the chance of locating their cognate antigen.[71] Thereafter, antigen-activated T-cells then express skin-homing molecules such as CCR6, CCR4, CCR10, and/or $\alpha_1\beta_1$ integrin, cutaneous lymphocyte antigen (CLA) and other chemoattractant receptors which guide them to inflamed skin for entry into tissues. Depending on the polarising environment during T-cell receptor (TCR) engagement, as well as the local cytokine environment at the site of inflammation, T-cells can be driven towards T-helper 1 (T_H1), T_H2, and/or T_H17 responses which are appropriate for the encountered pathogen. Exposure to IL-12 induces predominant T_H1 polarisation, with strong IFNγ responses which are particularly effective in response to viral and intracellular bacteria challenge. Cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 lead to generation of T_H2 cells which in turn produce cytokines such as IL-4, IL-5, and IL-13 which are effective against parasites. IL-23, IL-6, and IL-1β promote development of T_H17 cells, whose signature cytokines include IL-17A, IL-17F, and the IL-10 family of cytokines IL-22 and IL-26. These are particularly suited for responses to extracellular bacteria. T-helper responses can then assist effector CD8 responses in the killing of pathogens, or of pathogen-infected cells. Type-I interferons contribute to T-cell mediated immunity at the interface between innate and adaptive immunity, both by priming and promoting DC antigen presentation, enhancing CD4 and CD8 T-cell responses,[72,73] and contributing to their recruitment to sites of inflammation.[74] Importantly, antigen presentation in the absence of danger signals results in the formation of immunosuppressive regulatory T-cells (T_{reg}) which produce IL-10 and may limit clonal expansion of antigen-specific T-cells during secondary immune reactions in the periphery. A similar mechanism is thought to take place in low immunogenic tumours of the skin, which mask pro-inflammatory cytokines, danger signals, and metabolic processes, thus priming dysfunctional, hyporesponsive, or immuno-regulatory T-cell responses.[75]

B-cells are producers of antibodies, but can also produce cytokines. Though a lot is known about the role of adaptive B-cell immunity against systemic viral infections,[76] less is known about the role of humoural adaptive immunity in skin. The most comprehensive evidence for a role B-cells in skin immunity comes from the skin manifestations in patients with

clinical hypogammaglobulinemia and, more rarely, patients with hypogammaglobulinemia following therapies, such as with rituximab (anti-CD20).[77,78] Most commonly, B-cell deficiencies resulting in poor antibody production lead to loss of control of commensal or dormant infections of the skin,[79] including by bacteria *Flexispira*, *Helicobacter*, and *Campulobacter*,[80–82] and viruses such as herpes zoster.[83] Like pDCs, B-cells express TLR-7 and TLR-9 making them direct targets of exogenous nucleic acids from microorganisms, especially viruses. Additionally, they also express TLR-4, allowing responses against bacterial patterns. TLRs -7 and -9 converge on MyD88, whereas TLR-4 signals through both MyD88 and TIR domain-containing adaptor-inducing interferon-β (TRIF). Antigen recognition through the B-cell receptor (BCR) coupled with additional signals from helper T-cells, leads to activation of B-cells. It is well-described that B-cells rely on T-cell help for proper immunoglobulin production, particularly T follicular helper (T_{FH}) cells. Activated T-cells can stimulate B-cells in a number of ways. They can stimulate contact-dependent mechanisms through CD40L-CD40 interactions,[84,85] and inducible T-cell costimulator (ICOS)-ICOSL interactions[86–88] which are essential for B-cell survival and proliferation. They stimulate immunoglobulin (Ig) class-switching, cell survival and plasma cell differentiation via soluble factors such as IL-4, IL-21, B-cell activating factor (BAFF), IL-17, and TGFβ[89] among others, and chemoattraction via production of CXCL13 and binding to B-cell CXCR5 for recruitment to germinal centres.[90] Other factors are important in antibody production, including type-I interferons and IL-6 which are produced by pDCs and lead to proliferation and more potent antibody production.[44,91,92] Skin associated B-cell responses are thought to be achieved by CLA expression which guides them to cutaneous sites, though less is known about subsequent steps of B-cell homing. CXCR3 and CCR6 are highly expressed on B-cell subsets, and its ligands are strongly expressed in skin upon inflammation. Whether they play a role in B-cell migration to the skin is unclear.

Both T-cell[93,94] and B-cell[76] mediated adaptive immunity depends on the generation of long-lived, central memory and sometimes tissue-resident, cells. Importantly, they are able to rapidly proliferate in response to secondary challenge. After resolution of an infection at a barrier tissue, circulating memory T-cells (also called central memory T-cells [T_{CM}]) and resident memory T-cells (T_{RM}) are generated. T_{CM} have the ability to travel between lymphoid organs, circulation and tissues, whereas T_{RM} retain tissue homing molecule expression leaving them anchored to tissues. Skin T_{RM} also have the remarkable ability to colonize the rest of the skin surface. Effector memory T-cells can be heterogeneous in their polarisation, and produce all the prototypic cytokines associated with T_H1, T_H2 and T_H17 cells. Memory B-cells are found adjacent to contracted germinal centres in lymph nodes and spleen and close to effector T_{FH} cells, but also in the circulation. Because plasma cells are more stringently selected, memory B-cells constitute the main pool for reactivation of humoural responses. Little is known about tissue-resident B-cells. As opposed to T-cells, B-cells are found in very low numbers in homeostatic skin, yet display clonally restricted patterns, alluding to the possibility of these being memory B-cells resident within skin.[95]

The adaptive immune system is engaged when innate immunity is insufficient for control of pathogens, and results in the formation of long-term memory for rapid and powerful responses upon re-challenge. Though little is known about skin associated B-cells, T_{RM} cells are found in great numbers in human skin, patrolling the barrier tissue for antigens which may reactivate

them. Tight regulation of T_{RM} re-activation is important as inappropriate triggering of these cells can have pathogenic consequences.

1.2. Pathogenesis and Treatment of Psoriasis

Inflammation in the skin is a finely choreographed set of immunological events that strive to maintain homeostatic balance. Yet, unbalanced responses can lead to a pathological outcome in predisposed individuals. Psoriasis as a disease, is just as perfect an example of an environmental trigger acting on a background of genetic susceptibility.

Psoriasis is a chronic autoinflammatory disease of the skin and joints that has wide-reaching systemic involvement. It is estimated to affect 2–3% of individuals worldwide, and can manifest as a broad spectrum of phenotypes including plaque-type, guttate, inverse, pustular, palmoplantar, and erythrodermic psoriases, which can sometimes occur, in combination, simultaneously in the same individual.[96] Symptoms are shared between subtypes, and can include scaling and redness, itching and burning sensations, and soreness. Psoriasis follows a bimodal distribution with incidence peaking at [15-30] and [50-60] years of age. Overall, there is a cyclic evolution to the disease, with flaring for a variable period of time that can be up to months-long, followed by a phase of relative quiescence or even remission. It is well established that both the innate and adaptive immune systems play fundamental roles in disease, and complex interactions between keratinocytes, dendritic cells and T-cells lead to the pathogenesis of psoriasis. Importantly, psoriasis has one of the strongest genetic associations, with polygenic contributions.

1.2.1. The Role of T-cells and T_H17/22 Cytokines in Driving Disease

T-cells are known to be key effectors in the pathogenesis of psoriasis. The first anecdotal evidence indicating T-cell involvement comes from unexpected observations that treatment with cyclosporine in patients under organ transplantation, which co-incidentally had active psoriasis, led to amelioration of disease. It is now well established that cyclosporine, a generalised immunomodulator of T-cells, is an effective treatment for psoriasis[97–99]. Over time, evidence stemming from genetic associations, pre-clinical studies, and successful therapy has accumulated, thus defining an instrumental role of T-cells in mediating disease.

1.2.1.1. Evidence for Recognition of Skin-Associated T-Cell Antigens

Genetically, psoriasis is mostly dominated by variations spanning the psoriasis susceptibility locus 1 (*PSORS1*) of the human leukocyte antigen-C (HLA-C) accounting for over 40% of detectable heritability[100,101]. This suggests a likely class-I to T-cell Receptor (TCR) interaction as a potential culprit to the disease with potential CD8 T-cell involvement. Furthermore, almost 65% of patients with psoriasis are *HLA-Cw*0602* positive, with younger age of onset and more severe clinical presentation than those that are negative.[102]

Histologically, psoriasis is characterised by acanthosis, which is a thickening of the epidermis due to hyperproliferation of keratinocytes; parakeratosis, which is a retention and accumulation of nuclei in the stratum corneum due to incomplete cornification and premature keratinocyte maturation; and increased vascularisation with dermal and epidermal immune cell infiltrates. The parakeratosis, acanthosis and increased vascularisation are the histological

hallmarks that associate with typical psoriasis lesion signs of silvery, well-demarcated and raised, erythematous plaques. Importantly, CD4 T-cells are found infiltrating the upper dermis accompanied by dendritic cells, whereas CD8 T-cells mostly reside within the epidermis alongside keratinocytes. This spatial proximity of T-cells to the main affected cell types, including keratinocytes and vascular endothelial cells, supports a role for T-cells in the appearance of the psoriatic phenotype. In fact, T-cells are required for the development of psoriatic lesions and are already present in the skin,[103] suggesting that these may be resident-memory T-cells. Furthermore, expression of select integrins allows for CD8 T-cell entry within the epidermis and are required for full-blown development of psoriatic lesions.[104] Moreover, T-cells found in psoriasis lesions are largely monoclonal for CD8 and oligoclonal for CD4 cells,[105,106] suggesting that following recruitment, these cells expand *in situ*, potentially in response to a specific antigen.

Antigens proposed to elicit T-cell responses in psoriasis are argued to be self-antigens given the perpetuity and relapse of the disease. One theory suggests that immune responses against bacteria, such as streptococci, go awry and due to significant overlap between bacterial proteins and self-proteins there is an inappropriate response against self. This hypothesis of molecular mimicry is supported by several lines of evidence. One, streptococcal throat infections are known to re-activate or exacerbate ongoing psoriasis, guttate psoriasis in particular,[107] suggesting the possibility of cross-reactive T-cells. Two, streptococcal M protein shares considerable structural homology with type-I keratins such as K17. Three, T-cells respond to M protein among other cell wall membrane components,[108] as well as K17 and shared peptide sequences, including sequences predicted for HLA-Cw6 binding.[109] These results remain controversial as others have failed to reproduce reactivity to M proteins, and rather find that other cell wall components are responsible for the observed result.[110,111] More recent reports identify auto-reactivity to different keratinocyte-associated proteins ezrin, maspin, hsp27, PRDX2, and K6,[112] thus further lengthening the list of proteins liable to self-reactivity via molecular mimicry. Other antigens have also been proposed, in different cell types, including melanocyte-associated protein ADAMTSL5,[113] and the antimicrobial peptide LL-37.[114] The exact mechanism involved in the generation of T-cells that are not negatively selected for displaying intolerance to self, remain to be elucidated.

Much work is needed to understand why only certain individuals develop auto-reactivity to self-antigens during what are likely to be canonical immunological responses to environmental cues. Other outstanding questions include why this auto-reactivity remains localised to specific areas, and what triggers dictate resolution and re-occurrence of psoriatic lesions.

1.2.1.2. TNF Governs the Inflammatory Milieu in Psoriasis

Tumour Necrosis Factor (TNF) is a pro-inflammatory cytokine with established roles in homeostatic functions as well as pathology. In the context of skin inflammation, it is well established that TNF plays a non-redundant role in psoriasis (Figure 3).

In established chronic plaque psoriasis there are multiple sources of TNF, and the vast majority of cells can produce it. In the skin, at the site of inflammation, keratinocytes,[115] dendritic cells,[116,117] macrophages,[118] T-cells,[119] and adipocytes[120] are thought to be major contributors of TNF production, but by no means the only contributors. Keratinocytes,

especially basal keratinocytes, stain the most positive for TNF in chronic disease, likely due to persistent priming from the pro-inflammatory environment. Dendritic cells are a well-known source of TNF during immune responses, and in psoriasis a subpopulation of DC-LAMP expressing, non-Langerhans, non-monocytic, Tip-DC (TNF and inducible nitric oxide synthase producing)-resembling dendritic cells provide continuous TNF production. Macrophages are widely regarded as *bona fide* TNF producers upon toll-like receptor ligation, and in psoriasis they contribute to clinically relevant amounts of TNF.[118] T-cells have also been found to be important producers of TNF, in particular cytotoxic T-cells infiltrating the epidermis.[119] Adipocytes produce TNF, and increasing evidence suggests that obesity, a frequent co-morbidity of psoriasis, influences severity of disease[121] and this could be through contribution to TNF production. Likewise, the TNF receptors TNFR1 and TNFR2 are widely expressed across cell types, including both haematopoietic and stromal cells, thus making responses to TNF pleiotropic.

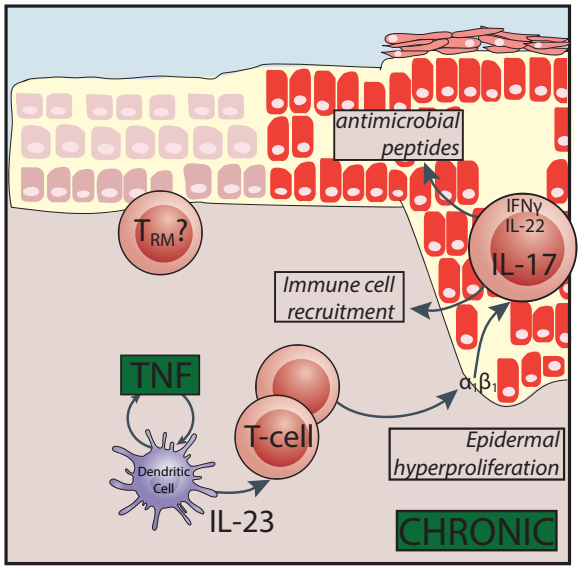

Figure 3: Chronic plaque-type psoriasis is mediated by T-cells. Following T_H17 polarisation, T-cells are recruited to the site of inflammation. The TNF-driven inflammatory environment leads to IL-23 production by dendritic cells, which maintain T_H17 polarising environment for T-cells. Expression of $\alpha_1\beta_1$ integrin allows for T-cell entry within the epidermis where they produce IL-17, IL-22 and IFNγ. Pathogenic cytokines expressed at lesion sites cause epidermal hyperproliferation with loss of granular layer and parakeratosis, immune cell recruitment and in particular neutrophils leading to microabscess formation, and expression of antimicrobial peptides which aggravate overall inflammation. Biologic targeting of IL-17, IL-23 or TNF leads to resolution of psoriasis. Even following successful treatment of psoriasis, specific T-cell associated genes are found expressed at sites of resolved lesions, raising the possibility that T_{RM} are present and responsible for re-appearance of psoriatic lesions. TNF: tumour necrosis factor, T_{RM}: resident-memory T-cell. Schematic drawings modified and re-used with permission from Nature Publishing Group.

Blockade of TNF is used as therapy for several inflammatory diseases of joints, bowel, and skin, as well as for the off-label treatment of more disparate, TNF-mediated, disorders. It has now become a gold-standard treatment for chronic plaque psoriasis. Treatment results in progressive regression of psoriasis lesions though time to response varies between patients and depending on starting psoriasis area and severity index (PASI). Histological amelioration precedes clinical response, with the former displaying visible differences as early as week 2, and the latter may take a mean time of about 12 weeks. In order to elucidate how TNF mediates cutaneous inflammation, histological and gene expression studies have been undertaken of regressing lesions over time.[122–124] Among the first signs of amelioration, is the apparent diminution of the number of infiltrating dermal and epidermal T-cells, already by week 2 of treatment. This coincides with a reduction of proliferating Ki67 positive basal

keratinocytes. Select chemokines such as CCL20 involved in T_H17 recruitment are drastically reduced by the first week of treatment. Concurrently, IL-17 levels are drastically reduced, whereas IFNγ and IL-22 drag behind and only return to normal by week 12. By week 4, DC numbers also start to diminish, and their maturation markers CD83, CD86, CD40, and activation markers HLA-DR and CD11c consistently drop. Interestingly, these follow similar kinetics as the reduction of IL-23 and IL-12 which start to return to basal levels by week 4. By week 12, epidermal hyperplasia is resolved, and concurrently keratinocyte differentiation returns to normal. All in all, TNF seems to control chemotaxis of T_H17 cells, as well as activation of DCs which produce IL-23 and other cytokines involved in maintaining IL-17 production from T-cells. As T-cells start to exfiltrate, keratinocyte hyperproliferation comes to halt, followed by reduction of DC numbers. Finally, other pathogenic markers return to baseline levels as lesions are visibly resolved. These lines of evidence suggest that TNF controls IL-17 production *in vivo*.

In summary, most cell types produce TNF in the inflammatory milieu of psoriasis lesions. Similarly, many others express the TNF receptor, suggesting that once established, disease can become self-driven by unrelenting TNF signalling, and proper regulation of TNF production is essential for a timely return to homeostasis. TNF has long been established to be a viable target for the treatment of several inflammatory and autoimmune diseases, including psoriasis. Currently, five anti-TNF agents exist, and have been both scientific and commercial successes, allowing treatment of millions of patients with TNF-mediated diseases. Yet, as mechanistic knowledge of the pathogenic pathways continues to expand, new targets are being unravelled and more success stories described.

1.2.1.3. PSORIASIS IS MEDIATED BY T-CELL DERIVED T_H17 CYTOKINES

The role of T-cells and TNF is well established in the pathogenesis and maintenance of psoriasis, with abundant evidence from clinical observations, as well as proof-of-concept pre-clinical studies. Yet, TNF is but one cytokine, and treatment efficacy is never of full-penetrance. TNF is a pervasive target in inflammation, but it is thought to be a chronic inflammation "umbrella" upstream signal. As such, downstream signals more specific to pathology must exist.

Psoriasis has long been thought of as a type-1 (T_H1) disease, due to the important levels of IFNγ found in lesions. T_H1 differentiation, as discussed in more detail in earlier sections, does not critically depend on TNF *in vitro*. The addition of TNF does however further reinforce T_H1 polarisation, and stimulate increased production of IFNγ. Though IFNγ can play a fundamental role in skin inflammation,[125] it has proven to be a less-than-optimal therapeutic target for the treatment of chronic plaque-type psoriasis in phase II dose-escalation trials, especially when compared to the gold-standard anti-TNFs. It is still unclear as to exactly why, despite such high levels of IFNγ, there is no fundamental role for the cytokine in the disease. One prevailing hypothesis suggests that IFNγ may play a role during the early pathogenesis of psoriasis, and that the high levels detected throughout chronicity are due to high numbers of infiltrating T-cells which will strongly produce the cytokine irrespectively of their polarising environment.

More recently, focus has been shifted to T_H17-related cytokines. Initially thought to specifically target IL-12, and thus inhibit T_H1 polarisation, Ustekinumab is now an effective treatment for psoriasis.[126,127] The antibody targets the shared p40 subunit of both IL-12 and IL-

23, effectively blocking downstream signalling of both cytokines. This serendipitous finding led to further work which pinpointed IL-17 and T_H17 polarisation as a key inflammatory component of chronic psoriasis. Targeting IL-17 has recently become a major success as, within 5 years of phase trials, drugs targeting it have demonstrated tolerability and high efficacy, including for severe psoriasis with >75 PASI.[128] Interestingly, another T_H17 cytokine – IL-22 – with similar function on epithelial cells, and with promising pre-clinical data, has never passed a phase I trial (NCT00563524). It is not clear exactly why targeting IL-22 does not provide clinical efficacy, despite clear dependency for many murine models. Though more work is needed to elucidate this, it may be that IL-22 is regulated differently to IL-17, thus potentially allowing for compensation through other pathogenic pathways. In light of the discovery of IL-23 as an upstream cytokine of IL-17 and T_H17-related cytokines, effort has been put into generating IL-23–specific biologics which are now in phase trials and show greater efficacy as compared to anti-IL12/23.[129,130]

Whereas TNF is not required for the *in vitro* differentiation of T-cells into T_H17, several lines of evidence have pinpointed that TNF affects IL-17 signalling. For one, TNF has been demonstrated to work in synergy with IL-17 leading to more potent inflammatory outcomes.[131,132] This has been found to be by both initiating transcription of gene transcripts stabilised by IL-17,[133] but also through upregulation of IL-17R on keratinocytes.[134] Additionally, in clinical responders to anti-TNF therapy, IL-17 expression in the skin is found to be dependent on TNF.[124] This, coupled to compelling evidence from the successful IL-17 and IL-23 trials, really highlights the importance of T_H17-mediated pathology in psoriasis.

1.2.1.4. MOUNTING A T_H17 RESPONSE IN THE SKIN

Several types of T helper responses exist, and they are thought to play important roles in host defence at barrier sites. Whereas T_H1 cells are important in antiviral[135] and intracellular bacterial defence,[136] T_H17 are particularly proficient in fighting extracellular bacterial and fungal pathogens.[137] Exactly why there is a T_H17 signature in the skin lesions of psoriasis, and whether there is a physiological reason for it, remains a subject of controversy.

The CD4 T-cell lineage 17 – termed T_H17 – was discovered over 10 years ago,[138–140] and defines a third subset of T-cells based on cytokine requirement, pathway engagement, and transcription factor expression. Its main distinguishing factor is the production of IL-17, which has strong association with inflammatory diseases of the joints (rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis) and an established role in psoriasis. With six members discovered to date (A through F), T-cells are the principal producers of IL-17A and IL-17F. Differentiation requires principally cytokine stimulation from IL-23, and can be suppressed by type-I and type-II interferons, as well as IL-4,[140] suggesting that T_H17 cells live in competition with T_H1 and T_H2 cells. Additional cytokines have been identified to enhance T_H17 function, including IL-6 and IL-1β,[141,142] which are abundant and indiscriminately expressed at inflammation sites. Importantly, polarisation of naïve CD4 T-cells depends on IL-23 and inflammatory dendritic cells[143] which provide co-stimulatory signals and TCR engagement, as well as IL-23 itself. Activating signals for DCs to induce T_H17 polarisation include microbial stimuli (TLR2/1-ligands) as well as contact co-stimulation (CD40L).[143] As T_H17 are required for immunological responses to invading pathogens such as *Candida albicans* and *Staphylococcus*

aureus,[144] it is likely that more clues will be unveiled regarding the processes and stimuli for T_H17 polarisation, and the events that lead to pathological T_H17 polarisation. Transforming Growth Factor β (TGFβ) is also described to play an important role in differentiation of mouse naïve CD4s,[142,145] though this remains controversial as in humans it inhibits differentiation *in vitro*,[141,142,146] yet "natural" polarisation using inflammatory DCs required TGFβ.[143] It is likely that several different pathways exist for generation of T_H17, and our current paradigm of single cytokine and transcription factor characterisation may not explain heterogeneity within effector T-cells. Key surface markers distinguishing T_H17 polarised cells include CCR6 and CCR4, which are required for homing to lymphoid tissues as well as certain tissue microenvironments with pro-inflammatory chemokines, particularly at barrier sites including skin and gut. As such, a paradigm for skin T_H17 responses is starting to take shape, whereby barrier disruption by certain triggers, likely microbial but potentially also environmental stresses, induces activation and migration of dendritic cells to lymphoid organs, where they present antigens and provide activating polarising signals to naïve T-cells. DC-mediated T_H17-polarisation through IL-23, leads to CCR6 and CCR4 expression on T-cells, allowing them to be recruited to appropriate sites of inflammation where they are needed. The inflammatory milieu then maintains and enhances T_H17 function through IL-1β, IL-6 and TGFβ production, supplied for by DCs and other inflammatory cells present. Production of IFNα, β, γ and/or IL-4, in response to the evolving inflammatory requirements dictated by the environment, inhibit IL-17 production, thus providing negative regulatory signals for T_H17 responses. Depending on the magnitude of inflammation, T-cells can then reside in tissues becoming resident-memory T-cells, or recirculate in blood and lymphoid tissues, becoming long-lived central memory T-cells, with their phenotypic polarisation, and awaiting activating signals.

The role of T-cells, and in particular T_H17 cells which by definition are CD4 helper cells, and of the cytokine IL-17, is well established in psoriasis. Interestingly, there is evidence that CD8 T-cells can also produce IL-17 (T_C17), including in a pathogenic context.[147,148] It is still unclear whether CD8 T-cells, which in psoriasis are predominantly intraepidermal, can produce IL-17 and whether they play a role in pathological outcome.

1.2.2. Type-I Interferons and Plasmacytoid Dendritic Cells Initiate Psoriasis

T-cells, TNF, and the IL-23/IL-17 axis are front and centre in established chronic plaque-type psoriasis, and they provide valuable targets for its treatment. Yet, for obvious reasons, very little is known about the initiating factors in the pathogenesis of the disease.

Clinical signs of psoriasis appear, inevitably, once disease is well established. As such, studies on the mechanisms initiating disease are few, and rely on the use of *in vivo* models. The use of murine models over the years has shed light on several aspects of the disease, but this is confounded by wildly different genetic alterations and/or triggers, and relatively different fidelity to the human condition. Though several spontaneous models exist, few are bona-fide models of human psoriasis. Xenograft models, which involve the transplantation of human pre- or fully developed psoriatic plaques onto immunodeficient mice, are currently the closest models incorporating phenotypic, genetic, and immunopathogenic processes of psoriasis.[103,149]

Several landmark observations were made using this model. First, and foremost, engraftment of pre-psoriatic skin biopsies from patients with established chronic plaque-type

psoriasis onto mice leads to spontaneous conversion of the graft into fully-fledged psoriasis. Through the mechanical stresses of transplantation, a series of events is set in motion that lead to localised expansion of T-cells already present in the skin, and characteristic histological signs start to become apparent culminating by 6 to 8 weeks post-transplantation. Resolved lesions of psoriasis maintain several T-cell signature genes,[150] suggesting that even though their activity may be targeted with biologic therapy, they remain localised at continuously recurring developing lesion sites. These findings indicate that dormant, resident effector-memory T-cells (T_{REM}) are found residing in the skin of patients of psoriasis, and await a trigger for reactivation and pathologic outcome.[93,94] Second, blockade of T-cells, like for TNF, is sufficient to impede progression of the pathology, thus confirming the pathogenic role of T-cells. Third, dermal to epidermal transition of T-cells is responsible for the conversion from uninvolved to psoriatic skin.[104] This T-cell migration takes place during phenotypic conversion of the lesion, but the initiating triggers of psoriasis and of T-cell re-activation were only starting to be elucidated.

Plasmacytoid dendritic cells (pDCs) are the immune system's professional producers of type-I interferon. It was identified that early after transplantation, type-I interferon signatures were upregulated, preceding apparition of the phenotypic conversion.[151] Concurrently, psoriatic patients have detectable upregulation of downstream interferon-response genes along with prominent pDC infiltrates. Importantly, inhibition of either type-I interferon signalling or of pDCs leads to blockade of the psoriatic conversion in the xenotransplantation model, demonstrating a key role for early pDC-derived type-I interferon signalling in driving psoriasis.[151] Furthermore, it was later found that antimicrobial peptides such as beta-defensins and cathelicidins are abnormally overexpressed in psoriasis and, through highly cationic charges, can bind to nucleic acids and mediate internalisation into pDCs. This allows nucleic acids to be able to reach and activate endosomal toll-like receptors 7 and 9,[152,153] thus culminating in the production of high levels of type-I interferons. As for the trigger that elicits antimicrobial peptide expression in skin, mechanical injury is sufficient to induce antimicrobial peptides and this is followed by pDC recruitment and activation,[6,46] thus providing a rationale for the Koebner phenomenon observed in psoriasis and a role for pDCs and type-I interferon in the initiation of psoriasis, but during skin homeostasis following injury (Figure 4).

In summary, the xenotransplantation model has elucidated that an external, Koebner-like, trigger induces strong expression of antimicrobial peptides which potently activate pDCs to produce type-I interferon. Resident T-cells are activated and expand *in situ*, leading to transition into the epidermis via integrin expression. Although it remains to be elucidated what the exact role of type-I interferons is on T-cells in psoriasis, there is evidence that type-I interferons can upregulate specific integrins,[154] and circumstantial evidence that viral infection leads to integrin upregulation in CD8 T-cells.[155] Therefore, one may envisage that innate interferon production may stimulate the adaptive T-cell arm in the cascade of events that precede phenotypic conversion. It also remains to be determined whether this epidermal transition is mostly mediated by CD8 T-cells, and what their functional contribution is to psoriasis.

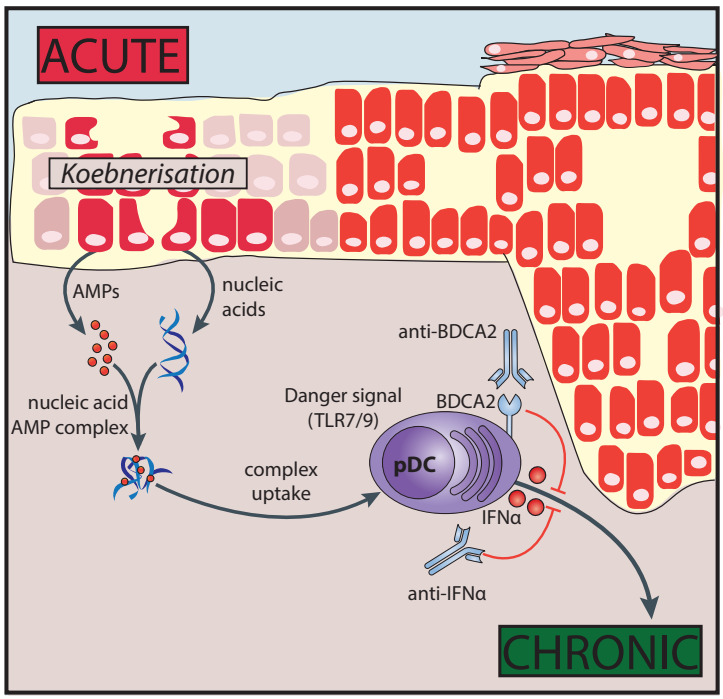

Figure 4: Plasmacytoid dendritic cells initiate psoriasis. During koebnerisation, nucleic acids released by dying cells are bound by positively charged antimicrobial peptides produced during skin injury. Nucleic acid condensation due to complexing with AMPs results in uptake into pDCs and activation of danger signalling via TLR7 and/or TLR9. This results in the production of IFNα and induction of pro-inflammatory cascades culminating in the development of chronic inflammation. In the xenotransplantation model of spontaneous psoriasis development, treatment with anti-BDCA2 antibodies, which inhibit IFNα production by pDCs, or with IFNα neutralising antibodies impedes development of the psoriatic phenotype. The precise mechanism by which pDC-derived IFN drives psoriasis remains to be elucidated. AMP: antimicrobial peptide; TLR: Toll-like receptor; pDC: plasmacytoid dendritic cell; BDCA2: blood dendritic cell antigen 2; IFN: interferon. Schematic drawings modified and re-used with permission from Nature Publishing Group.

1.2.3. Anti-TNFs and Recent Advances in Biologic Therapy

Though TNF is well-described to be required for homeostasis, including defence against pathogens, lymphoid-organ architecture, tissue repair, tumour control, and resolution of inflammation,[156–158] it is also involved in many pathogenic processes. Psoriasis, and many other joint- and gut-related inflammatory conditions, have benefited immensely from targeting of TNF. Yet, given the complexity of TNF signalling, there have been counterintuitive, and sometimes contradictory, results from TNF blockade in the clinic (Figure 5).

Currently, there are five TNF inhibitors – infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol – which have successfully been used in the treatment of TNF-mediated diseases over the last two decades. Infliximab is a human-murine chimeric monoclonal IgG1 antibody, with a murine variable region and human constant domain, developed for pre-clinical testing as far back as 1993, and commercialised by Janssen Inc. (formerly Centocor Ortho Biotech) under the name Remicade. Etanercept is an engineered compound composed of the TNFR2 fused to a human IgG1 Fc domain, produced by Amgen and Pfizer. Adalimumab and Golimumab are fully human monoclonal antibodies, respectively Humira (AbbVie, Inc.) and Simponi (Janssen, Inc.). Certolizumab pegol is an Fab fragment of a humanised monoclonal antibody, and PEGylated for increased stability, produced by UCB Pharma SA under the name Cimzia. Indications are similar between the different compounds, and diseases treated include Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, hidradenitis suppurativa, and chronic plaque-type psoriasis. Off-label use include the treatment of vasculitis and Behçet's disease.

Generally, anti-TNFs are considered more efficacious and safer than non-specific immunomodulators. Nevertheless, like most drugs, they too come with an array of possible adverse events, which can be quite disparate in nature. Among the most prevalent adverse

effects of systemic TNF inhibition are opportunistic infections, lupus-like disease, sarcoidosis, demyelination, alopecia, vasculitis, vitiligo, and psoriasis or psoriasiform inflammation. The latter is also termed "paradoxical psoriasis" due to the apparent paradox of anti-TNF being an effective treatment for psoriasis, and able to induce it *de novo* in a small proportion of patients treated with the drugs. Interestingly, anti-TNFs are abnormally immunogenic as compared to other biologics, which is believed to contribute to progressive loss of efficacy of treatment.[159,160] Moreover, the formation of self-reactive antibodies, mostly against DNA and nuclear components, is increased in patients under anti-TNF, which is thought to contribute to the increased incidence of lupus-like syndrome.[161,162] Whether the two are linked remains to be elucidated.

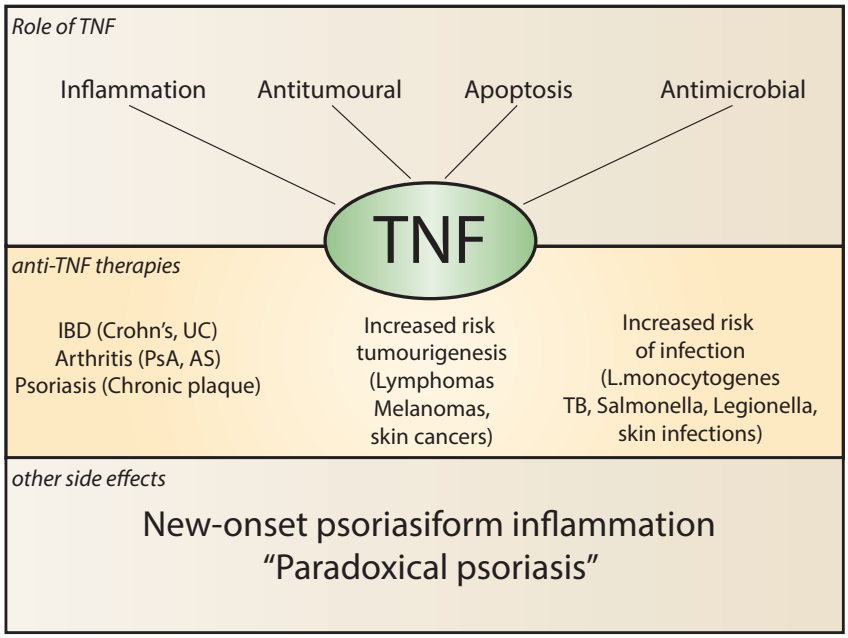

Figure 5: Homeostatic requirement of Tumour Necrosis Factor, treatment with anti-TNFs, and adverse events due to anti-TNF therapy. TNF is involved in many homeostatic processes that include inflammation, tumouricidal immunity, apoptosis-related processes, and microbial control. Anti-TNF therapies are used for the treatment of inflammatory disease bowel diseases such as Crohn's disease and ulcerative colitis; arthritides such as psoriatic arthritis and ankylosing spondylitis; and skin diseases such as chronic plaque psoriasis. Currently, for many of these diseases, anti-TNFs are the gold-standard reference treatments. Biologically, several side-effects have been proposed to be likely to arise due to TNF blockade, such as increased risk of cancers and infections. With over 20 years of experience with anti-TNFs in the clinics, a few have been proven whilst others have been disproven. Yet, other unexpected adverse events are now recognised which, paradoxically, resemble psoriasis in certain ways.

Anti-TNFs are highly efficacious in the treatment of many inflammatory diseases, and as such new strategies are being devised to reduce adverse effects and maintain long-term treatment efficacy. New compounds targeting TNFR1 now exist (ATROSAB,[163] MDS5541,[164] TROS) and are being tested for their effectiveness. More recently, as patent restrictions on anti-TNFs are being lifted, anti-TNF biosimilars have started to be produced which carry much reduced costs. Infliximab biosimilar CT-P13 (Remsima, by Celltrion) has been tested in chronic plaque psoriasis with success, and is now approved.[165] Interestingly, patients which develop anti-drug antibodies to anti-TNFs, and display reduced drug efficacy, when switched to CT-P13 they also display reduced responsiveness to treatment. Concurrently, rates of induction of anti-drug antibodies is similar between the two compounds.[166,167] Together, these observations validate the almost indistinguishable nature between biosimilars and the reference products.

Riskier approaches are being undertaken in patients that exhibit secondary failure of response to TNF inhibitors, such as vaccination to TNF. Although there is variable efficacy using this approach, patients produce polyclonal antibodies specific for TNF and show a modest but correlated clinical response.[168] Other approaches have been to use dual cytokine inhibition, or even dual-specificity (or bispecific) biologics. Though concurrent TNF and IL-1 inhibition have not yielded increased efficacy for the treatment of rheumatoid arthritis,[169] novel approaches are being undertaken such as the use of IL-17 and TNF bispecific antibodies.[170] Due to the synergistic effect of TNF and IL-17 in inflammation, this may provide increased efficacy for patients with limited therapeutic responses to single cytokine inhibition. Other dual-inhibition methods, informed by experimental observations, may prove to be useful in the treatment of psoriasis.[171]

Anti-TNF therapy is a powerful treatment option for a growing number of inflammatory conditions, several of which do not have alternative treatment options. As for most drugs, undesired effects due to anti-TNF therapy exist, but with proper clinical evaluation and regular patient follow-ups these can be diminished. A few other side-effects of anti-TNFs do not display pre-treatment predicting power, and can lead to discontinuation of treatment.

1.3. Anti-TNF Induced Paradoxical Psoriasis

With more than 20 years of experience of the anti-TNF class of drug in the clinic,[172,173] several side-effects have become apparent over time. Chronic TNF neutralisation, as expected, increases susceptibility to infections[174,175] and there is evidence of increased chance of developing cancer,[176] though this latter finding is disputed by more recent studies.[177–179] Yet, there is no doubt about another significant but intriguing side-effect of anti-TNF therapy called paradoxical psoriasis, which has countless case-reports and is confirmed by several large-scale studies.[180–188]

1.3.1. Clinical, Epidemiological and Histological Considerations

Adverse events due to anti-TNF therapy occur most commonly on the skin, with 20 to 25% of patients developing one form or another of skin complication. Inflammatory, psoriasiform skin lesions are reported in about 0.6-5.3% of patients under anti-TNF therapy from a period of weeks to months after treatment start.[181] Because histologically these lesions resemble psoriasis, these adverse manifestations are termed “paradoxical psoriasis” as anti-TNFs are widely used to successfully treat psoriasis.

Paradoxical psoriasis can manifest irrespectively of the underlying disease being treated, and without association to personal or familial history of psoriasis. Eruptions occur frequently, but not exclusively, at sites of frequent koebnerisation such as the scalp (30%), skin folds (20%), palms and soles (20%), pubic region (15%), knees and elbows (5%), but also trunk (30%), arms and legs (40%), and face (40%).[181,182] Clinical presentation can vary between patients, with plaque-type, guttate, and/or pustular features being observed like in classical psoriasis. Unlike classical psoriasis however, palmoplantar pustular psoriasis presentation rates (40%) are more frequent in paradoxical psoriasis.[189] Mean time to incidence ranges between 30 and 40 months, and cumulative incidence increases over time from 1% after the first year of treatment, up to 30% following 10 years. Psoriasiform lesions under anti-TNF can often lead to

discontinuation of treatment, depending on the severity and manageability of the adverse event. Reports differ, with overall estimates at between 20 and 40% of patients that develop psoriasiform lesions.[190–192] New recommendations, however, suggest specific topical and eventually systemic treatments should be attempted before discontinuation, and that the percentage of patients discontinued from anti-TNF therapy may drop down to 5%-10%.[181] Strikingly, switching to a different anti-TNF leads to re-appearance of the skin lesions in 85% of individuals,[189] really pointing to a class-effect of anti-TNF drugs. Incidence is a marginally higher in female patients (58%), and higher for Adalimumab (65%) as compared Infliximab and Etanercept, but this is argued to be inflated for Adalimumab as it is not a first-line anti-TNF. Often, patients are switched to Adalimumab following an adverse reaction to other anti-TNFs in the hope of reducing symptoms, and because of the high rate of reappearance or maintenance of paradoxical psoriasis this is overrepresented in second- and third-line anti-TNFs. Overall, it is recognised that the true prevalence and incidence is unknown and likely underreported, as case definition may vary between studies and diagnosis may not systematically be confirmed by a dermatologist.

Histologically, paradoxical psoriasis is partly reminiscent of classical psoriasis on a few aspects. Typical features of psoriasis have been reported in psoriasis developing under anti-TNF treatment, including hyperplasia of the epidermis with elongation of rete ridges, diffuse parakeratosis, dermal perivascular infiltration, and where pustular lesions are observed, intraepidermal and subcorneal collection of neutrophils termed Munroe's microabscesses. Many manifestations reveal a lichenoid and spongiotic inflammation, accompanied by focal necrotic keratinocytes.[193,194] Treatment of paradoxical psoriasis differs depending on the severity of symptoms, with most cases treated with psoralen coupled with UVA, or UVB phototherapy, or steroids, or methotrexate, whilst maintaining current anti-TNF treatment. More severe cases are discontinued of anti-TNF therapy, but symptoms may persist beyond treatment cessation. In these cases, use of other biologics is sometimes warranted, usually with the goal of concurrently treating the underlying condition. Whereas antibiotics are normally used to treat cutaneous infections which arise from anti-TNF therapy in about 5% of patients,[195] only few case reports exist of successful treatment of psoriasiform eruptions using doxycycline.[196] Though the patient was treated with antibiotics because he had signs resembling reactive arthritis with skin involvement (Reiter's), it is intriguing that in patients with paradoxical psoriasis without apparent signs of infection, 25% have in fact bacterial infections superimposed on their lesions.[182] Whether there is a link between microbial overgrowth and paradoxical psoriasis remains unclear, though often dry skin preceded psoriasiform eruptions, possibly suggesting that an impaired barrier might facilitate microbial entry.

It is well described that a proportion of patients that undergo paradoxical psoriasis will experience exacerbation of their existing lesions.[183] Remarkably, many patients with psoriasis treated with anti-TNF, but which subsequently develop *de novo* paradoxical psoriasis, usually see their underlying psoriasis improve.[189] As to why certain patients develop *de novo* psoriasis-like inflammation and simultaneously see an amelioration of their pre-existing psoriasis, is very much still unclear. This is all the more puzzling as paradoxical lesions present histologically identical to classical psoriasis. Many hypotheses have been postulated as to the pathological mechanisms of paradoxical psoriasis, several of which are reviewed hereafter.

1.3.2. PUTATIVE GENETIC PREDISPOSITION

Ground-breaking work has revealed strong genetic association for diseases such as psoriasis,[197] arthritis,[198] and inflammatory bowel disease (IBD).[199] To date, no genome-wide association studies have been published demonstrating a genetic association between risk alleles and development of paradoxical psoriasis. Yet a few studies have aimed at identifying genetic association for paradoxical psoriasis with risk alleles from classical psoriasis and IBD.

There is considerable genetic linkage between diseases where anti-TNF is used as a treatment, including Crohn's disease and psoriasis. In one study, loci linked to both Crohn's and psoriasis were screened in patients under anti-TNF which develop or not skin adverse events. Single Nucleotide Polymorphisms (SNPs) with association to development of skin lesions were linked to *IL12B*, *COG6* and to a lesser extent *IL23R* and *IL12RB2*, among the 7 tested gene loci.[182] In a different study, five SNPs linked with genes *IL23R*, *FBXL19*, *CTLA4*, *SLC12A8*, and *TAP1* were found to be associated with development of paradoxical psoriasis.[200] *IL23R* is by far the gene most confirmed by this type of study, with a third report showing association.[201] The fact that strong association exists with psoriasis may have been explanatory for a histologically similar type of skin pathology, yet it seems that paradoxical psoriasis associates with the protective allele of *IL23R*,[200] as this variant reduces IL23-mediated T_H17 signalling.[202] As to how this contributes to the development of paradoxical psoriasis is still unclear, but it seems to be related to a reduction of IL-23 signalling. Other genes are associated with T-cell function (CTLA4 and TAP1), keratinocyte proliferation (SLC12A8), among other unknown functions.

Much work needs to be done to determine genetic associations in patients that develop paradoxical psoriasis, as well as other cutaneous manifestations during anti-TNF therapy. This will shed light into the pathological mechanisms that may lead to better management of symptoms, as well as potential predictors of therapeutic outcome.

1.3.3. MOLECULAR PATHWAYS INDUCED BY TNF BLOCKADE

Several hypotheses have been put forth regarding the aetiology of paradoxical psoriasis. Chief among those is the hypothesis of cytokine imbalance, where loss of TNF leads to deregulation of other cytokines, with consequent pathogenic outcome.

Early observations from patients treated with anti-TNF led to the identification of detectable increases of anti-dsDNA antibody titers.[203] Further studies confirmed this finding, and identified anti-nuclear antibodies (ANA) as well as anti-dsDNA antibodies with incidences of up to 76.7% and nearly 50% respectively.[162,204,205] The role of type-I interferon in the establishment of autoimmunity, and of induction of autoantibodies was well-known and particularly apparent in patients receiving IFNα therapy for the treatment of chronic viral infections.[206] As such, it was reasoned that anti-TNF could lead to deregulation of type-I interferons. Indeed, patients under anti-TNF therapy display increased interferon-response signatures in the circulation, which were absent before initiation of treatment.[207] In a proof-of-concept approach, it was demonstrated that TNF governs plasmacytoid dendritic cell (pDC) maturation and cessation of IFNα production, as well as the generation of pDCs from progenitor cells. As described in a previous section, type-I interferon production by pDCs is an initiating early trigger for acute development of classical psoriasis.[46,151,208] As such, it was found

that type-I interferon signalling is in fact pronounced in lesions from paradoxical psoriasis.[209] Though the exact pathological mechanism remains to be determined, this finding, coupled with increased infiltration and correlation with CXCR3+ cells, suggests a pathogenic role for type-I interferon signalling and activated T-cells in paradoxical psoriasis.

Other cytokines have been postulated to play a role in the development of paradoxical psoriasis, particularly in light of putative genetic predisposition(s). IL-12 and IL-23, two pro-inflammatory cytokines related to T_H1 and $T_H17/22$ polarisation respectively, may be involved, but it is unclear how loss of TNF affects their regulation within the inflammatory milieu. Experiments performed using the CD4+ $CD45RB^{high}$ cell adoptive transfer set-up, indicate that TNF blockade leads to enhanced T_H17 with decreased T_{reg} responses, and a mildly exacerbated phenotype.[210] Interestingly, in this skin inflammation model, there is little detectable TNF in skin lesions, suggesting that the effect of TNF is elsewhere. Seen as it is a T-cell mediated inflammatory model, it may be that TNF inhibition affects a finely-tuned balance of T_{reg}-T_H1-T_H2-$T_H17/22$ pro-inflammatory cytokines, at distal sites, potentially lymphoid tissues. Another unexpected finding from this model, is that TNF is highly induced in the serum of mice treated with the anti-TNF, and that there is a sharp decrease of IL-12 and IL-23. This is hard to reconcile with the functional data demonstrating an amelioration of the phenotype upon IL12/23p40 blockade. It is also unclear whether the exacerbation induced by TNF blockade goes through the IL12/23 axis. As such it may be that two independent pathways are at play, and the authors suggest a decrease in Foxp3+ T-cells and their capacity to produce IL-10 may be responsible for the aggravation upon TNF blockade.

All in all, little is known about the molecular mechanism behind anti-TNF induced skin inflammation. Current knowledge points to type-I interferon signalling and IL12/23-mediated inflammation as a possible culprit, with concurrent loss of the anti-inflammatory cytokine IL-10, but there is little experimental evidence to support this. Furthermore, there is even fewer indication as to how exactly the crosstalk within these networks takes place.

1.4. Rosacea and Innate Inflammation

Rosacea is a chronic inflammatory disorder that selectively affects the facial convexities, including nose, cheeks, forehead and chin. Clinical and histological presentation can be different between patients, with the hallmark defining feature being non-transient centro-facial redness. Differential diagnoses include Cutaneous Lupus Erythematosous, seborrheic dermatitis which can be concomitant and more rarely cutaneous sarcoidosis, all of which require careful clinical consideration. This poorly understood disease is highly debilitating causing profound emotional distress in affected patients. Currently there is only limited and transient efficacy of available treatments, and therefore there is a need for a deeper understanding of the pathological mechanisms in order to define treatment strategies with biological rationale.

1.4.1. Clinical, Epidemiological and Histological Considerations

Rosacea presents in the clinic as four distinct subtypes, which include erythematotelangiectatic (stage I), papulopustular (stage II), phymatous (stage III), and ocular (stage IV) subtypes. Reported incidence lies between 0.1% and 22%, and can vary between geographical locations, with highest frequency in persons of northern and western European descent, and lowest among African, Central and South American, and Asian individuals. Mean prevalence is 10%, with 14% of women and 5% of men affected. Frequencies from Swedish and Greek large series studies are in agreement, with stage I (70%) being more common than stage II (26%) and stage III (4%).[211–213] Stage IV rosacea is concomitant with other subtypes in upwards of 4 of 5 cases.

Stage I erythematotelangiectatic rosacea presents as nontransient episodes of flushing with mostly central facial erythema that may involve also the neck, ears and upper chest, and may be accompanied by telangiectasias. Stage II papulopustular rosacea often encompasses the characteristics from stage I, with the addition of transient papules or pustules across the face. Both stage I and II rosacea are more commonly linked to female patients. Stage III phymatous rosacea is characterised by skin induration with irregular nodules and can be distributed along any facial sebaceous regions, with the nose as the predominantly affected site. Stage III rosacea affects more commonly male patients. Stage IV ocular rosacea is associated with bloodshot appearance with telangiectasias of eyelids, or with periocular erythema.

Despite the observations that stage II rosacea encompasses stage I attributes, that stage III rosacea occurs mostly in elderly following chronic inflammation from rosacea, and that sometimes all four stages may be present in patients, few scientific investigations have been conducted to elucidate progression between subtypes. Though this remains a controversial subject, there is new evidence suggesting that stage I precedes stages II and III, that stage II precedes stage III, and that the majority of patients developed cutaneous features before ocular stage IV symptoms.[214]

Histopathological examinations of the different subtypes are disparate and usually nonspecific. Stage I classical features include dilated superficial blood vessels with perivascular inflammatory lymphohistiocytic and plasma cells. Papules and pustules from stage II also include neutrophil accumulation at hair follicles and beyond through to the mid-dermis. Stage III rosacea is characterised by sebaceous gland hyperplasia, accompanied by widespread fibrosis and dermal thickening. Histolopathological examinations are generally not required for a diagnosis of rosacea. In some cases, there is need to rule-out lupus, and generally only then are biopsies taken to determine whether there is Ig-deposition along the epidermal-dermal interface. Frequent histological observations include the presence of *Demodex folliculorum* mites in 50-90% of patients,[215] with marked increased in the density of the mites ranging from 5 to 30 mites per follicle, depending on sites investigated.[216] Though their exact contribution to the disease is unclear, they are carriers of microorganisms such as *Bacillus oleronius* and *Staphylococcus epidermidis* in their hindgut, thus significantly increasing the bacterial load in the skin of patients. Additionally, they are a relatively efficacious treatment target, indicating a possible role for microorganisms in the disease.

1.4.2. Rosacea Management and Available Treatments

Rosacea management consists in reducing the exposure to environmental factors known to trigger flushes. Though no cure exists for rosacea, drugs are used to manage symptoms. Among approved drugs for the treatment of rosacea, topical medications exist, which typically aim to reduce visible signs of the disease, and few systemic treatments are available, generally aiming at reducing the microbial burden.

Known triggers of rosacea exacerbation include ultraviolet (UV) light radiation, alcohol consumption, heat, ingestion of certain spices, and emotional stresses.[217,218] It is not entirely clear how these contribute to the disease, though it is hypothesised that all of these factors induce vasodilation, through a direct effect or neuromediators, rendering the erythema and telangiectasias apparent. The ensuing inflammatory exacerbation may lead to the worsening of existing papules and pustules.

Approved topical treatments for rosacea include formulations whose main component is sodium sulfacetamide, metronidazole, azelaic acid, or alpha-adrenergic agonists. Sodium sulfacetamide has considerable efficacy in reducing symptoms including facial erythema and inflammatory lesions,[219,220] though the mechanism remains unknown and is generally regarded to be an unspecific anti-inflammatory effect. Other topical drugs with anti-inflammatory action are metronidazole and azelaic acid, which have reported efficacies in placebo-controlled trials,[221,222] and are thought to reduce production of and inactivate reactive oxygen species (ROS).[223,224] Additionally, topical alpha-adrenergic receptor agonists which cause blood vessel constriction by targeting superficial smooth muscles, have been found to reduce erythema[225] and can be used in combination with anti-inflammatory agents. Recently, Ivermectin, an anti-parasitic agent used in the treatment of demodicosis, has received approval for treatment of rosacea.[226] Its main mode of action is thought to be through the reduction of the microbial burden.

The most efficacious systemic therapeutic options currently approved for rosacea are tetracycline drugs. These are potent antibiotics highly efficacious in the treatment of rosacea[227] and of other skin conditions such as acne.[228] Because they carry undesirable side-effects such as selection of drug-resistant bacteria at high doses (100-200 mg doxycycline daily), they have been trialled at lower doses (40-50mg doxycycline daily) which avoid development of drug-resistant microbes. They are argued to provide beneficial outcomes in patients through an anti-inflammatory action that is independent of antibiotic effect, but it is unclear whether smaller doses affect the increased bacterial burden observed in rosacea patients.

Currently, no approved treatments exist with a well-defined pathomechanistic rationale. These rather aim at reducing the inflammatory and microbial burden. Based on recent findings of the implication of the innate immune system, new emerging therapies are being tried in small scale pilot studies.

1.4.3. Anti-microbial Peptides and Emerging Therapies

Given the apparent role for antibiotics in the management of rosacea symptoms, it is likely that microorganisms, and thus by extension the innate immune system, are implicated in the

pathogenesis of rosacea. Cathelicidin, along with its predominant serine protease responsible for its cleavage and activation, kallikrein 5 (KLK5), are found abundantly overexpressed in rosacea skin.[7] In addition, cathelicidin fragments of various sizes have been described from patient skin, and several of these peptides can elicit inflammation macroscopically reminiscent of human rosacea when injected into mouse skin. But little is known as to the purpose of the different sized fragments, and how they may contribute to the inflammatory environment.

It is not entirely clear whether aberrant KLK5/cathelicidin expression comes as a final stage in the pathogenesis of rosacea, or whether it is an early trigger of the inflammatory cascade. From *in vitro* studies, cathelicidins have been shown to be inducible in keratinocytes by vitamin D,[229] a factor borne from UV light exposure, linking a known trigger of rosacea such as UV light to cathelicidin-mediated inflammation. Upregulation of KLK5 in rosacea has been attributed to increased levels of TLR2, found overexpressed in rosacea, whose triggering via canonical TLR2 stimulation leads to KLK5 production and release from keratinocytes.[7,230] There are multiple potential triggers for TLR2 that are known to be present in rosacea, such as the gram-negative bacterium *B.oleronius* which produces gram-positive cell wall components, and Demodex mites carrying chitin which is a ligand for TLR2.[215,231]

Mast cells and Matrix Metalloproteinases (MMPs) have also been suggested to play a role in rosacea. KLK5 is first released as a pre-proenzyme and requires enzymatic processing for its activation in what is an intricate regulatory framework.[232] One such enzyme identified to carry out proteolytic activation of KLK5 and that is found overexpressed in rosacea is MMP-9.[233] In fact, it is suggested that mast cells are important sources of MMP-9, and that they are needed for proteolytically activating KLK5 which in turn results in processed cathelicidins such as LL-37 being produced.[234] The study goes further in suggesting that mast cells are responsible for the redness seen in rosacea as mast cell deficient KitW-sh mice do not develop the characteristic erythema seen upon intradermal injection of LL-37. In line with this finding, blockade of mast cell degranulation using systemic administration of cromolyn sodium also prevents development of redness. It is unclear what the contribution of neutrophils is, as they are also known producers of many MMPs such as MMP-9 as well as cathelicidin,[235] but it is suggested that neutrophil chemoattraction to the site of LL-37 injection is impaired in mast cell deficient mice.

KLK5 activity is positively regulated by MMP-mediated proteolitic activation, but its regulation goes beyond just positive signals. Protease inhibitors can also negatively modulate KLK5 activity, providing balancing signals for the timely termination of protease activity. Unabated KLK5 activity has been attributed as the defining cause of diseases involving skin barrier abnormalities. One such example is Netherton syndrome, a severe type of ichthyosis that affects skin, hair, and immune system, and presents as red scaly skin in newborns. Mutations in *SPINK5* (serine protease inhibitor, Kazal-type 5) have been identified in families with Netherton's, and these lead to null expression of the protein in patients through premature stop codons.[236] Incidentally, it was later discovered that the protein encoded by *SPINK5*, called Lympho-epithelial Kazal type inhibitor (Lekti), directly inhibits KLK5 and other serine proteases such as KLK7, trypsin, subtilisin A, plasmin, cathepsin G, and neutrophil elastase,[237] and that this regulates physiological desquamation processes. *Spink5*$^{-/-}$ animals develop cutaneous inflammation with detachment of the stratum corneum from the granular

layer via premature and unabated degradation of desmosomal cadherins, and importantly succumb to lethal dehydration few hours post-partum.[238] KLK5 overexpression under the involucrin promoter leads to exfoliative erythroderma and scaling throughout the body only a few days after birth, reminiscent of Netherton's hallmark features.[239] Importantly, loss of KLK5 rescues the clinical and most histological presentation in *Spink5*-deficient animals,[240] suggesting a key role for KLK5 in skin inflammation, and highlighting the importance of tightly regulated networks of enzyme processing, and how imbalances inevitably lead to pathologic outcome.

Cathelicidins, and by association upstream regulators KLK5 and Lekti, are involved in skin inflammation and the pathogenesis of rosacea. Many players have been identified with a potential involvement in the pathogenesis of rosacea, but given the lack of comprehensive *in vivo* investigations it is still unclear how they all fit together. It is not known whether KLK5/cathelicidin is an upstream trigger or downstream consequence of inflammation. Furthermore, though it is widely described that cathelicidins are potent pro-inflammatory alarmins,[10,241,242] how they mediate pathogenic outcome in rosacea is still poorly understood. The most prominent hypothesis pertains to the induction of IL-8, CXCL1 and CXCL2 production from keratinocytes,[234,243,244] or that LL-37 itself is able to bind CXCR2[245] leading to strong neutrophil chemoattraction. This in turn leads to inflammation and to the characteristic papules and pustules found in stage II rosacea. Continuous recruitment of neutrophils and their activation in the inflammatory environment are thought to lead to collagen breakdown and loss of elasticity resulting in skin induration, a principal feature of stage III phymatous rosacea. As for the erythema and telangiectasias, prominent in stage I rosacea but present throughout, these are thought to be a direct effect of aberrantly processed forms of cathelicidins[7] as LL-37 is shown to lead to angiogenesis.[246] This is controversial as it is disputed by recent findings suggesting that angiogenesis is a late, stage III feature of rosacea.[217]

Based on the recently proposed pathomechanism of cathelicidin-mediated inflammation,[7,229,230,247,248] emerging therapies have been proposed to inhibit aberrant processing of the antimicrobial peptides. Serine protease inhibitors exist in topical formulation, using ε-aminocaproic acid as the main active component, and are approved for the treatment of bleeding disorders.[249] In a small 12-week long case series, there was significant improvement in the group receiving treatment as compared to placebo controls particularly in erythema.[250] Concurrently, protease activity as measured from superficial skin sampling was significantly reduced. Mast cells have also been targeted in a different small-scale study using cromolyn,[234] a well-described mast cell stabiliser with unknown mode of action.[251] The authors demonstrate a decrease in protease activity in a pre-clinical model and in patients treated with the compound, and mention efficacy in reduction of erythema.

Despite a clearer understanding of the implication of the innate immune system in the pathogenesis of rosacea, we are only starting to scratch the surface when it comes to targeted therapies with an established pathomechanistic rationale.

2. AIMS OF THE STUDY

The following study aims at a elucidating the pathological mechanisms of paradoxical psoriasis induced by anti-TNF, based on observations that 1) paradoxical psoriasis has an increased interferon signature,[209] 2) TNF regulates pDC maturation and production of type-I interferons,[207] and that 3) psoriasis is initiated by pDC-derived type-I interferon.[252]

It aims to propose novel therapeutic approaches for rosacea based on a pathomechanistic rationale, and based on observations that 1) cathelicidin antimicrobial peptides are aberrantly expressed in rosacea lesions,[7] 2) cathelicidin LL-37 can potently activate pDCs to produce type-I interferons,[152] and 3) that rosacea has a predominant innate inflammatory profile with a strong microbial contribution.[247,253]

It aims at an understanding of the importance of the IL-23/T_H17 axis in non-psoriasis skin diseases with a psoriasiform pattern of inflammation, such as for pityriasis rubra pilaris, based on observations that 1) PRP has considerable overlap with psoriasis,[254] 2) can be treated with the IL-12/23 inhibitor ustekinumab.[255]

Finally, it aims at elucidating the importance of epidermal CD8 T-cells in the development of psoriasis, based on observations that 1) psoriasis is a T-cell mediated disease that can be modelled using a xenotransplantation approach,[103] 2) conversion of non-lesional to lesional psoriasis involves expression of integrins that allow dermal to epidermal transition,[104] 3) psoriasis has a strong genetic association to class I HLA-C molecules.[100]

3. RESULTS

The following section is composed of two manuscripts which are recently submitted (Appendix 1) or in preparation (Appendix 2), and two peer-reviewed manuscripts (Appendices 3 and 4).

They are integrated in the thesis manuscript with for each a summary, an introduction, a description of thesis work involved and step-by-step approaches, and a discussion and outlook. Contributions for each manuscript are outlined at the end.

Appendix 1 – TNF blockade induces a dysregulated type I IFN response without autoimmunity in paradoxical psoriasis

Summary. TNF is an important target in the treatment of psoriasis yet, in a subset of patients, there is development of psoriasiform lesions resembling psoriasis due to anti-TNFs. Histopathologically, lesions have many similarities to classical psoriasis and expression of many innate and effector cytokines follow similar expression, with the exception of type-I interferons which are overexpressed in comparison to classical psoriasis, with striking infiltrates of plasmacytoid dendritic cells (pDCs). In this work, we find that anti-TNF greatly potentiates and sustains IFNα production by pDCs *in vitro* with concurrent loss of maturation, and that pDCs are maintained in skin upon injury *in vivo*. This is reflected by marked overexpression of type-I interferons through loss of TNF-mediated maturation of pDCs. Importantly, mice treated with anti-TNF exhibit psoriasiform inflammation reminiscent of paradoxical psoriasis. Blockade of type-I interferons prior to induction of anti-TNF-driven inflammation leads to almost complete amelioration of the psoriasiform phenotype, pinpointing that the induction of type-I interferons is in fact pathogenic in this inflammatory context. Unlike classical psoriasis however, this inflammatory outcome is independent of T-cells and of adaptive immunity, indicating that anti-TNF triggers paradoxical psoriasis through the removal of an important brake on type-I interferon production by pDCs, and that this results in an unabated innate skin inflammation.

Introduction. Biologic therapeutics targeting TNF – called anti-TNFs – have been an immense scientific and clinical advancement and revolutionized the treatment of many debilitating chronic inflammatory disorders, such as ankylosing spondylitis, rheumatoid arthritis, psoriatic arthritis, inflammatory bowel disease, psoriasis, hidradenitis suppurativa, or uveitis. To date, more than 2.5 million patients have been treated with anti-TNFs and three different anti-TNF agents are currently among the top 10 best-selling drugs worldwide. With broad usage and increasing clinical experience, several side effects have become apparent. Surprisingly, anti-TNF treatment can induce new autoimmune diseases, including systemic lupus erythematosus and paradoxical psoriasis. These adverse events represent extremely important side effects in the treatment of major chronic autoimmune diseases as they potentially necessitate treatment cessation. The aims of the thesis work was to determine the pathogenic pathways involved in development of paradoxical psoriasis in order to further elucidate therapeutic options, and patient management.

Description of thesis work and step-by-step approaches. The initial approach undertaken was to perform comparative gene expression analyses of genes involved in known skin inflammatory processes,[256] using biopsies from paradoxical psoriasis lesions compared to chronic plaque psoriasis and healthy skin. By doing so, we identify type-I interferons significantly overexpressed as compared classical psoriasis, which indicated an interesting specificity of these cytokines for paradoxical psoriasis. To understand whether plasmacytoid dendritic cells (pDCs) may be involved in their production, we stained for specific markers in paradoxical psoriasis histology and found that they heavily infiltrate lesions, correlating with interferon expression. To determine whether pDCs are responsible for type-I interferon production in skin, we used an established tape-stripping model[6] in mice genetically modified to express the diphtheria toxin-receptor specifically under the promoter of the *Blood Dendritic Cell Antigen-2* (*BDCA2*) gene, essentially allowing the specific deletion of pDCs upon DT injection.[38] Upon DT injection, type-I interferon expression in skin lesions was abrogated, indicating that in skin pDCs are responsible for the bulk of type-I interferon expression. Because it is known that TNF can be produced by a vast number of cells (both immune and epithelial cells) including pDCs, and because TNF-receptor 1 (TNFR1), which binds soluble TNF, is expressed in most cell types including pDCs, we wondered whether TNF blockade intrinsically could affect interferon-production by pDCs. Importantly, previous observations by others indicate that TNF negatively regulates interferon-production by pDCs, likely through induction of maturation.[207] As postulated, stimulation with DNA/LL-37 complexes (10ug/mL and 50ug/mL pre-incubated for 15 minutes as determined previously[152]) or CpG-B (1uMm, as determined previously[152]), leads to rapid production of IFNα with subsequent maturation. The addition of TNF (100ng/mL, as defined previously[207]), leads to premature loss of IFNα producing capacity, paralleled by increased maturation. Conversely the addition of anti-TNF (1ug/mL of infliximab, adalimumab or certolizumab pegol as determined by log-dilutions on pDC cultures using infliximab) leads to sustained production of IFNα and reduction of maturation marker expression, as expected. This defines a model whereby TNF negatively regulates IFNα production, an effect that is intrinsic in pDCs, as gene expression profiles of stimulated pDCs reveal rapid expression of *IFNA2*, followed by replacement by *TNF*. To determine whether these observations hold *in vivo*, we took advantage of a skin injury model which is known to induce pDC infiltration peaking at around 24h post injury.[6] For *in vivo* use, we calculated the murine equivalent of the anti-TNF to be used, with the following formula (used for pre-clinical to clinical translation)

$$\boldsymbol{Human\ equivalent\ dose} = \boldsymbol{Animal}\ \mathbf{dose}\ \mathbf{x}\ \frac{\mathbf{Animal\ Km}}{\mathbf{Human\ Km}}$$

where the Animal (murine) Km = 3, the Human Km = 37, and this equation can be re-interpreted as

$$\boldsymbol{Animal}\ \mathbf{dose} = \boldsymbol{Human\ equivalent\ dose}\ \mathbf{x}\ \frac{\mathbf{Human\ Km}}{\mathbf{Animal\ Km}}$$

or

$$\boldsymbol{Animal}\ \mathbf{dose\ (mg/kg)} = \mathbf{5}\boldsymbol{mg/kg}\ \mathbf{x}\ \frac{\mathbf{37}}{\mathbf{3}}$$

thus

$$\boldsymbol{Animal}\ \mathbf{dose\ (mg/kg)} = \frac{\mathbf{5}\ \boldsymbol{mg}}{\boldsymbol{kg}}\mathbf{x}\ \frac{\mathbf{37}}{\mathbf{3}} \approx \mathbf{1500ug/25g}$$

We find that mice treated with anti-TNF have pDCs in lesions displaying lower expression of maturation markers, and their numbers are maintained for much longer as compared to control mice reflecting accumulation of pDCs in paradoxical psoriasis lesions. Blockade of IFNAR signalling leads to much reduced pDC infiltration suggesting that sustained production of type-I interferons by pDCs maintain pDC infiltration in skin lesions. This is reflected by the increase in expression of *Cxcl9*, *Cxcl10*, and *Cxcl11* during anti-TNF treatment, which we believe participate in the recruitment of pDCs in skin, and their abrogation during IFNAR-blockade, suggesting that interferon-induced chemokine expression causes pDC maintenance in skin lesions. Importantly, histological analyses reveal that there is clear epidermal thickening, with focal sites of parakeratosis, as in histology from paradoxical psoriasis lesions, accompanied by hallmark signs of psoriasiform inflammation including basal keratinocyte hyperproliferation and loss of terminal differentiation of keratinocytes of the granular layer demonstrated by confocal microscopy of Ki67 and involucrin (expressed on undifferentiated keratinocytes) and loricrin (expressed in fully differentiated keratinocytes). Remarkably, IFNAR-blockade leads to an almost complete rescue of the observed epidermal thickening highlighting that the abnormal increase in type-I interferons locally leads to the paradoxical psoriasis outcome. Because a similar pathway involving pDC activation and type-I interferon production is involved in driving T-cell dependent inflammation in classical psoriasis, we wondered whether a similar mechanism may take place. Yet depletion of conventional T-cells expressing TCRαβ (using anti-TCRβ depletion prior to induction of the model, amount determined for complete depletion in injured skin) does not affect the phenotype in any apparent way. In mice, several pre-clinical models pinpoint unconventional γδ-T-cells as important effectors in driving psoriasiform inflammation.[257–261] Because there is no clear consensus on whether γδ-T-cells can be successfully targeted through antibody-mediated depletion,[262] we opted for the use of mice lacking recombination-activating gene 2 (*Rag2*$^{-/-}$), thus lacking all T-cells (and B-cells). We reasoned that, in light of previous results targeting conventional T-cells, should there be an amelioration of the phenotype that this may be due to either lack of unconventional γδ-T-cells, or the lack of humoural immunity. This did not warrant further investigation, as the phenotype was recapitulated entirely in mice lacking an adaptive immune system, suggesting that neither conventional nor unconventional (nor humoural immunity) is required for the development of anti-TNF-induced paradoxical psoriasis inflammation. Classical psoriasis is well-known to be mediated by T-cells, in particular intraepidermal CD8 T-cells. Indeed, CD8 T-cells are heavily infiltrating the dermis and epidermis of classical psoriasis, in sharp contrast to paradoxical psoriasis where only few can be found in the epidermis, further supporting the lack of a role for T-cells in paradoxical psoriasis.

Discussion and outlook. In summary, this work pinpoints an innate skin inflammation pathway, linked to cytokine deregulation and loss of the TNF-mediated break on type-I interferon production by pDCs. It highlights a role for the pDC–interferon axis in the pathogenesis of

paradoxical psoriasis, and pinpoints a major difference with classical psoriasis despite similar histopathological presentation. The pre-clinical determination of the pathogenic role of the pDC–interferon axis raises the possibility for targeting this pathway in patients that develop paradoxical psoriasis. This may be of further interest in patients which have an underlying moderate/severe psoriasis that is ameliorated with anti-TNF (but that subsequently develop paradoxical psoriasis) and thus cannot be treated with anti-TNFs. Thus switching them to biologics targeting the pDC–interferon axis would treat paradoxical psoriasis and prevent new flares of classical psoriasis, as these are thought to be initiated by the same pathogenic axis. Currently, there are several anti-IFNα and anti-IFNAR biologics which have been found to be effective in other interferon-driven diseases,[263,264] have passed safety and tolerability trials, and are currently in Phase III trials (anifrolumab, AstraZeneca [MedImmune]; sifalimumab, AstraZeneca [MedImmune] discontinued due to superior efficacy of anifrolumab). Importantly, there are several biologics targeting pDCs and their capacity to produce type-I interferons upon TLR-ligation,[265] which again demonstrated efficacy in small case studies and are currently in either Phase I (MEDI7734 anti-ILT7, AstraZeneca [MedImmune]) or Phase II trials (BIIB059 anti-BDCA2, Biogen). The latter group of biologics may be more versatile in the treatment of diseases driven by a specific pDC–interferon axis, whilst leaving immunity by stromal and other non-pDC cells unscathed.

Contributions to Appendix 1 – TNF blockade induces a dysregulated type I IFN response without autoimmunity in paradoxical psoriasis

Figure 3f – figure, plus repetitions
Figure 3g – figure
Figure 3h – figure

Figure 4a – repetitions (x4)
Figure 4b – repetitions (x4)
Figure 4c – repetitions (x4)
Figure 4d – figure, plus repetitions
Figure 4e – figure, plus repetitions

Figure 5 – figure, plus repetitions

Figure 6a – figure, plus repetitions
Figure 6b – figure, plus repetitions
Figure 6c – figure, plus repetitions

Supplementary Figure 1a – figure, plus repetitions
Supplementary Figure 1b – figure, plus repetitions

Supplementary Figure 2 – repetitions (x6)

Supplementary Figure 3 – figure, plus repetitions

Supplementary Figure 5 – figure, plus repetitions

Supplementary Figure 6 – figure

Supplementary Figure 7 – figure, plus repetitions

Critical appraisal of the manuscript, and redaction of parts of the Methods section.

Appendix 2 – Rosacea-associated bacteria activate plasmacytoid dendritic cell-derived type-I interferon driving flare-ups of disease

Summary. Rosacea is a debilitating skin inflammatory disorder affecting the facial convexities, characterised by repeated flare-ups of disease. There is clinical and molecular evidence for a progression in rosacea, and this may be linked to the repeated inflammation cycles during flare-ups. Cathelicidin antimicrobial peptides have been known to be implicated in the pathogenesis of rosacea, particularly through differential processing of active peptides by its serine protease kallikrein 5. Yet little is known about the pathological mechanisms behind cathelicidin-mediated inflammation. Recently, there has been renewed interest on the role of the innate immune system in driving inflammatory cascades in rosacea. In this work, we find that rosacea lesions present T_H1/T_H17 genes and related signatures, with no T_H2 expression in comparison to healthy skin. Importantly, we report that, specifically during flare-ups of rosacea, type-I interferons are uniformly and selectively overexpressed as compared to stabilised lesions. We determine that plasmacytoid dendritic cells (pDCs) are required for this production using a pre-clinical model, and that targeting them or the type-I interferon signalling pathway leads to loss of several T_H17-polarising pro-inflammatory genes and IL22, but not T_H1/T_H2-related cytokines. This induction of interferons is dependent on the surface commensal bacteria, as topical antibiotic treatment leads to loss of the type-I interferon signature, and commensal bacteria such as *Bacillus oleronius*, which are found infesting rosacea lesions, are sufficient to drive this pathogenic signature. Importantly, *B.oleronius* is exquisitely sensitive to killing by cathelicidin antimicrobial peptides, much more than other commensal and non-commensal bacteria tested, providing evidence that their killing may be the trigger for pDC-derived type-I interferon-driven flare-ups of rosacea.

Introduction. Rosacea is an inflammatory condition affecting the facial convexities and characterised by recurring flare-ups of disease. Microbial dysbiosis is a defining feature of rosacea, with apparent infestation with *Demodex folliculorum* as a diagnostic criterion in the vast majority of cases. These commensal mites flourish in hair follicles deep in the dermis of rosacea and are the base of deep granulomatous formations. *Bacillus oleronius* has been identified as the principal bacterium harboured by *D.folliculorum*, and is thought to be continuously released. This reservoir for bacteria has been suggested to be the reason for the relative inefficacy of long-term antibiotics in the treatment of rosacea, and as such anti-mite insecticides are also used in the clinic. Though several disease management strategies are available, little is known about the pathological mechanisms of flare-ups of disease. Microbe-associated molecular patterns are well described to induce antimicrobial peptides for controlling foreign agents. Cathelicidin antimicrobial peptide has been identified as one such antimicrobial agent induced in keratinocytes downstream of TLR2, and needing proteolitic processing for activation. Cathelicidin has been found overexpressed in rosacea and its enzyme processing by kallikrein 5 (KLK5) has been pinpointed as an important component of the disease. LL-37 has been shown to be able to bind bacterial as well as human DNA, to allow its condensation and thus to be able to activate endosomal toll-like receptors- (TLR-) 7 and 9 in plasmacytoid dendritic cells (pDCs). Notably, stimulation of pDCs with these indissociable complexes leads to production of vast amounts of type-I interferons which have been implicated in the pathogenesis of other inflammatory skin diseases such as psoriasis.

Description of thesis work and step-by-step approaches. Previously, we had observed that type-I interferon-response genes such as *MX1* were upregulated in established stabilised lesions of rosacea, yet intriguingly could not find type-I interferons expressed. We reasoned that this may be indicative of a previous interferon burst long after type-I interferon genes may have died down. We collected samples taken from patients with rosacea during active flare-ups of disease, and to our astonishment found that they all had massive upregulation of the type-I interferon genes, compared to both healthy skin and stabilised lesions, along with concurrent overexpression of response genes. Importantly, all other genes showed similar expression between flare-ups and stabilised lesions, suggesting that the abnormality in the "life-cycle" of flare-up/stabilisation are selectively type-I interferons. This was with the remarkable exception of *TNF* which was marginally but significantly upregulated in stabilised lesions compared to flare-ups, and could be a parallel of TNF regulation of type-I interferons. Staining of pDCs in lesions revealed large infiltrates of cells, which were quantified with the aid of image analysis tools for unbiased counting of total infiltrating cells (defined by their round nuclei), and the manual counting of plasma-like cells with round nuclei and positive staining. The percentage of cells correlated closely with type-I interferon expression, which may indicate that 1) pDCs produce type-I interferons and thus transcript levels reflect pDC infiltration, or 2) that pDC infiltration results from type-I interferon expression, as type-I interferons are known strong inducers of chemokines. To determine whether either (or both) of these hypotheses is true, we turned to an *in vivo* system. Previously, work by others indicated that cathelicidin antimicrobial peptide injection in skin leads to inflammation and erythema with many features resembling rosacea (more important of which, the aberrant presence of cathelicidins),[7] we wondered whether cathelicidins could be directly responsible for induction of type-I interferons, and whether they could be produced by pDCs. We consistently found that induction of type-I interferons increased over time as compared to injection of the vehicle (saline) and these were closely paralleled by pDC infiltration, reproducing the human observations. Remarkably, prior depletion of pDCs using antibodies specifically directed to them, or performing these experiments in mice genetically modified for selective depletion of pDCs using diphtheria toxin (hBDCA2-DTR), lead to complete abolishing of IFNα genes, and marked significant reduction of IFNβ. Similarly, interferon-response genes were severely reduced, though not completely abolished as compared to type-I interferon signalling blockade. This indicated clearly that pDCs are the major contributors to type-I interferon production in skin in the context of rosacea. Strikingly, pDC infiltration was also abolished upon type-I interferon blockade, indicating that type-I interferons produced by pDCs lead to further pDC recruitment. Thus, both hypotheses are true, pDCs are the producers of type-I interferons and type-I interferon leads to pDC recruitment. This latter finding was further confirmed in the paradoxical psoriasis mouse model whereby IFNAR-blockade leads to almost complete abolishing of pDC infiltration, in the tape-stripping skin injury model where baseline infiltration of pDCs is abolished, and in intradermal injections of type-I interferons which lead to marked pDC infiltration. This is suggestive that pDCs produce type-I interferons but continuous stimulation is required for sustained interferon expression and pDC accumulation. This observation led us to hypothesise that type-I interferons are responsible for chemokines to be expressed at the site of skin inflammation. As rosacea is well-described to be highly expressing in several families of chemokines,[266] we tested the most highly expressed chemokines in this pre-clinical model of rosacea, and found that select chemokines of the CXCR3 and CCR2/5 families were strongly dependent on type-I

interferons and pDCs. Intriguingly, T_H17-related and polarising pro-inflammatory cytokines, as well as *IL22*, were significantly dependent on type-I interferons, but not T_H1 nor T_H2 genes. Given the clear microbial dysbiosis in rosacea, and because pDCs are strongly activated by microbial CpG-rich DNA, we wondered whether the commensal bacteria might play a role in the induction of the pathogenic pDC-derived type-I interferon-driven pathway. Topical antibiotic treatment reduces growth of skin surface bacteria from 50-150 CFU/cm^2 of swab area down to nil. Importantly, the induction of type-I interferons upon cathelicidin injection was clearly dependent on this microbial load at concentrations below 200uM of LL-37. Intriguingly, concentrations in excess of 250uM could induce type-I interferons independently of microbial presence, suggesting that potential massive host cell killing could also be a driver of cathelicidin-mediated inflammation. *In vitro*, skin commensal bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Bacillus oleronius*, were more susceptible to cathelicidin-mediated killing compared to non-skin associated, and generally non-commensal bacteria such *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Remarkably, *B.oleronius* is far more sensitive to killing by cathelicidins. Pre-incubation of heat-killed *B.oleronius* with cathelicidin peptides and stimulation of pDCs *in vitro* leads to potent production of IFNα. Similarly, *in vivo* injection of B.oleronius pre-incubated with LL-37, for the same concentration of LL-37, leads to substantially increased type-I interferon expression. It is important to note that *B.oleronius* at CFUs in excess of 100 (determined after overnight culture in blood agar, 37°C) per injection can, by itself, be stimulatory of type-I interferons over time. Finally, pre-treatment of skin with antibiotics (which abolishes the LL-37 [100uM] mediated induction of type-I interferons) and injection of heat-killed *B.oleronius* pre-incubated with LL-37 restores the type-I interferon induction, indicating that cathelicidin-mediated killing of commensal bacteria is sufficient for the induction of the type-I interferon signature.

Discussion and outlook. Our findings point to the importance of pDC-derived type-I interferon production during flare-ups of rosacea, and raise the possibility of using it as a therapeutic target to prevent one of the hallmark signs of the disease. Like for paradoxical psoriasis, targeting of the type-I interferon pathway, or pDCs directly, are a feasible and foreseeable option for the treatment of rosacea flares, and could essentially hamper the progression of the disease (see Discussion and Outlook for Appendix 1). Our data indicate that an understanding of the factors that govern microbial infestation in rosacea might lead to better prevention and management of the disease. Importantly, we don't know how the demodex mite may contribute to the disease flare-ups. Though we think that it acts as a reservoir for the bacterium, it's still not clear why it is not controlled in patients with the disease. Unfortunately, culturing is difficult and it does not take well in wild-type mice. Interestingly, strains deficient in adaptive immunity, in particular T_H2 responses, were more susceptible to spontaneous demodicosis with mouse-specific commensal mites. It's unclear whether this is reflected by heightened T_H1/T_H17 responses without T_H2 signatures in lesions of rosacea, and whether type-I interferon in response to bacterial triggers might play a direct role in this. For now there is a controversial link between the two, with reports suggesting that type-I interferons are required for T_H2 responses via priming of DCs,[267,268] whereas other reports suggest that they restrict T_H2 immunity through regulation of ILC2.[269] Though currently unclear whether IL17 plays a pathogenic role in rosacea, this is currently being investigated in an open-labelled phase 1b trial using secukinumab in an estimated 24 patients (trial NCT03079531). It would be interesting to see whether inhibition of IL17 may lead to more directed anti-parasitic T_H2

responses which could lead to control of mite infestation. Additionally, more work is needed to elucidate the link between environmental and intrinsic triggers of flare-ups and type-I interferons. We show that commensal bacteria can trigger type-I interferon-driven flare-ups of rosacea, but we don't know how these may be related to environmental triggers such as UV light exposure and alcohol consumption, or emotional stress and the autonomic nervous system. Whether these influence stress or killing of the mites, thus leading to massive release of bacteria and initiation of the cascade, is unclear.

Contributions to Appendix 2 – Rosacea-associated bacteria activate plasmacytoid dendritic cell-derived type-I interferon driving flare-ups of disease

Writing of the manuscript.

Appendix 3 – Interleukin 23-helper T cell 17 axis as a treatment target for pityriasis rubra pilaris

Summary. Pityriasis rubra pilaris (PRP) is a sporadic chronic inflammatory skin disease whose major differential diagnosis is psoriasis. Therefore, many treatments for psoriasis are used for the treatment of PRP solely on their resemblance, and not on empirical evidence. In this work, we examined the expression of inflammatory cytokines in three patients that develop PRP and identify a notable T_H1/T_H17 signature, with upregulation of both *IL12B* and *IL23A*. Given the effectiveness of ustekinumab, an anti-IL12/23 monoclonal antibody, for the treatment of moderate-to-severe psoriasis, we treated one severe PRP case that did not show signs of spontaneous remission. Like for psoriasis, there was a clear amelioration four weeks after a single infusion and a return to homeostasis after 16 weeks. Intriguingly, T_H17 cytokines *IL17A* and *IL17F* were quickly diminished due to the treatment, but not the T_H1 cytokine *IFNG*, thus providing evidence that blockade of IL23 may be of interest in PRP rather than IL12.

Introduction. Pityriasis rubra pilaris is a rare inflammatory skin disorder which often appears sporadically. Histopathologically, there is remarkable similarity with psoriasis, with apparent branny scale, orange-red erythema, palmoplantar keratoderma, and histological features such as epidermal thickening, immune cell infiltration, and parakeratosis. There's a particular urgency, in particular for erythrodermic PRP, for efficacious and fast-acting treatments. Similarities between psoriasis and PRP warrant a better understanding of the pathomechanistic pathways involved in PRP. One particularly effective treatment option for moderate-to-severe psoriasis are biologics targeting the TNF-IL23-IL17 axis. There are several case reports of successful treatment of PRP with anti-TNFs,[270,271] and sporadic cases with anti-IL12/23 but with no pathomechanistic rationale.[255] As such, we wondered whether patients with PRP had a particular cytokine signature that may warrant anti-IL12/23 therapy, and which pathway (be it IL12-T_H1, or IL23-T_H17) is likely to play a role.

Description of thesis work and step-by-step approaches. Samples were collected from lesions of three patients with PRP, and gene expression analyses performed of notable innate and adaptive T_H1, T_H2, and T_H17 inflammatory cytokines. One patient displayed particularly recalcitrant disease, and based on inflammatory T_H1/T_H17 cytokine profiles was given subcutaneous anti-IL12/23 along same dosages given to patients with severe psoriasis. Lesional skin samples were taken for gene expression analyses, at weeks 4 and 28 post treatment initiation. Concurrent with marked and rapid amelioration, T_H17 cytokines *IL17A* and *IL17F* were abolished first by week 4, and to some extent *IL22*, with *IFNG* and *TNF* lagging behind the phenotypic and histological changes due to therapy.

Discussion and outlook. This study gives evidence that a similar IL23-T_H17 axis exists between psoriasis and PRP, and points to an immunopathological rationale for targeting IL23 in the treatment of refractory PRP. However, there is need for further validation of the pathway with large scale studies. Importantly, there are striking differences between PRP and psoriasis, including follicular hyperkeratosis, orange-red waxy keratoderma, and absence of neutrophils. Thus, further work is needed to clarify pathologic mechanisms and effector cell types that mediate this effect, and whether T-cells play a role in PRP.

Contributions to Appendix 3 – Interleukin 23-helper T cell 17 axis as a treatment target for pityriasis rubra pilaris.

Figure 1c – figure

Figure 2 – figure

Figure 3b – data from figure

Critical appraisal of the manuscript, and redaction of parts of the Methods section.

Appendix 4 – Targeting $CD8^+$ T cells prevents psoriasis development

Summary. Psoriasis is a T-cell mediated disease,[103] and it is well-described that effector cytokines IL17A, IL22 and IFNγ produced by $CD4^+$ T-helper cells actively contribute to disease. We know that T-cells require expression of integrin $\alpha_1\beta_1$ for dermal to epidermal transition and that it can be targeted to prevent development of psoriasis.[104] Yet, epidermal T-cells are mostly $CD8^+$ T-cells, and their role in the pathogenesis of psoriasis has not been fully elucidated. Here, we find that $CD8^+$ T-cells can be targeted for the treatment of psoriasis, as epidermal expansion of $CD8^+$ T-cells closely mirrors psoriasiform inflammation and epidermal hyperproliferation, along with pronounced production of IL17A as compared to dermal T-cells.

Introduction. T-cells have long been known to be involved in the pathogenesis of psoriasis, with large dermal and epidermal infiltrates. Importantly, targeting IL23, and downstream T-cell derived cytokine IL17A, have proven to be effective treatment options for even the most difficult cases. As these cytokines are historically known to be produced efficiently by $CD4^+$ T-helper cells, they have been regarded as the key effector cell type mediating pathology. There is substantial evidence, however, that $CD8^+$ T-cells can produce pro-inflammatory cytokines including IL17A.[272] Being present mostly in the epidermis, $CD8^+$ T-cells may recognise antigens via MHC class I, which in psoriasis carry one of the strongest susceptibility allele – *HLACw6*.[273] Therefore, we wondered whether $CD8^+$ T-cells might participate, or even be required for the development of psoriasis.

Description of thesis work and step-by-step approaches. Samples were taken from engrafted mice treated with anti-TNF (infliximab) as a positive control, anti-CD8 depleting antibody (M-T807), or isotype treated mice, and histology assessed. $CD8^+$ T-cell depletion stopped the spontaneous development of the psoriasiform phenotype, similarly to anti-TNF treatment.

Discussion and outlook. The advancement of biologic therapies targeting cytokines have brought conclusive and definitive evidence that T-cells, and the IL23-IL17A axis are key drivers for chronic plaque psoriasis. Historically, $CD4^+$ T-helper cells are known to produce large amounts of cytokines, and provide "help" for immune responses, whereas $CD8^+$ T-cells are known to perform a cytotoxic function. Therefore, one may indeed postulate that CD4+ T-helper cells are key effector cells in the pathogenesis of psoriasis, as they respond to IL23 and produce large amounts of IL17A. Our data demonstrate that $CD8^+$ T-cells, are key drivers of the development of psoriasis. Moreover, the high association between psoriasis and mutations in the class I molecule *HLACw6* may be suggestive that their epidermal localization is important in the recognition of antigens involved in the disease. Ideally, the specific depletion of these cells or their cognate antigen or trigger may lead to a permanent cure for the disease.

Contributions to Appendix 4 – Targeting $CD8^+$ T cells prevents psoriasis development

Figure 2g – figure (histology)

Critical appraisal of the manuscript.

4. Discussion and Perspectives

Type-I interferon is well-known to be essentially necessary for the cell-intrinsic antiviral and antibacterial effects,[274] though newer evidence points to a balancing act of interferons and highlights another cell-intrinsic effect that has instead detrimental potential to the host.[275–279] Type-I interferon is also important the proper priming of immune antiviral[72,74] and anti-tumoural[280–283] responses, yet this has been also found to have profound detrimental effects in autoimmune, adaptive immunity-driven diseases such as psoriasis[46,151–153,208] and systemic lupus.[284–287] In work outlined in Appendices 1 and 2, we describe a detrimental effect of type-I interferons in driving cutaneous inflammation via pDCs, one of which is through a mechanism related to cytokine imbalance, and the other through commensal bacteria initiated pathogenic responses.

In this section, major differences between adaptive immunity-driven *versus* innate immunity-driven pathology initiated by type-I interferons are outlined. Furthermore, therapeutic intervention on the type-I interferon pathway may not be a viable target, as immune-mediated pathology due to cytokine imbalances may be more commonplace than previously appreciated, as taught by lessons from anti-TNF biologics. Importantly, selective inhibition of cell-specific production of cytokines may be the future approach for the treatment of diseases. Finally, the possible upstream triggers and downstream targets of type-I interferon will be discussed in light of these findings.

4.1. Type-I Interferon at the Crossroads of Pathogenic Innate and Adaptive Immunity

The inter-regulation between TNF and type-I interferon has long been appreciated[207] and compelling evidence indicates that TNF blockade is associated to type-I interferon expression in paradoxical psoriasis.[209] In work performed herein, we find that pDCs are responsible for *in situ* uniform overexpression of type-I interferons upon anti-TNF induced paradoxical psoriasis, and also for a cutaneous disease, rosacea. Using a novel mouse model, we describe an inflammatory mechanism for paradoxical psoriasis which, unlike classical psoriasis, is independent of T-cells and adaptive immunity.

It is unclear exactly how type-I interferon initiates chronic plaque-type psoriasis. It is thought that type-I and type-II interferons play a priming role during initial phases, before establishment of chronic disease, as neither anti-IFNα nor anti-IFNγ treatments demonstrated increased effectiveness for therapy of plaque-type psoriasis.[288,289] The role of early priming may be in the generation of pathogenic T-cells in secondary lymphoid organs. This is evidenced by the role of interferons in driving adaptive T-cell responses, both against viruses and tumours, and that mechanistically they can both upregulate co-stimulatory molecules and HLA expression on APCs, and maintain T-cells within DC compartments for increased interaction and potential recognition of cognate antigen. Once pathogenic T-cells are established and become skin resident at lesion sites, type-I interferon may act to maintain them in an effector state and expanding. Dendritic cell activation by type-I interferons *in situ* is also likely to influence T-cell activity, as it is known to drive maturation of DCs. Additionally, evidence from viral infection models points to a direct effect on T-cells as well. Antigen-specific CD8 and CD4 T cells use IFNAR signalling for clonal expansion.[72,73] LCMV-specific, antigen-experienced T-cells from *ifnar*$^{-/-}$ mice adoptively transferred into recipient, LCMV infected mice either fail to expand

(CD8 T-cells <1%), or their expansion is severely impaired (CD4 T-cells <10%). This is proposed to be mediated by poor survival of daughter cells upon antigen recognition. Thus one may envisage that in psoriasis, at the site of inflammation, T_{RM} may require type-I interferons for clonal expansion during the initiation of response, but once plaques are established, pre-existing and expanded (self)-antigen experienced T-cells can mediate pathology without the need for type-I interferon.

This role of type-I interferon on adaptive immunity which drives skin inflammation is in stark contrast with our observations on paradoxical psoriasis. Whereas in classical psoriasis the pDC-IFN axis is acting on T-cells, in paradoxical psoriasis inflammation is entirely independent of T-cells and the adaptive immunity branch, as evidenced by sustained inflammation in *rag2*$^{-/-}$ and mice depleted of αβ-T-cells and CD3 cells (not shown). As such, a new model is starting to emerge whereby the pDC-IFN axis is no longer inhibited by TNF and can stimulate the innate immune system generating pathological outcome in the skin (Figure 6).

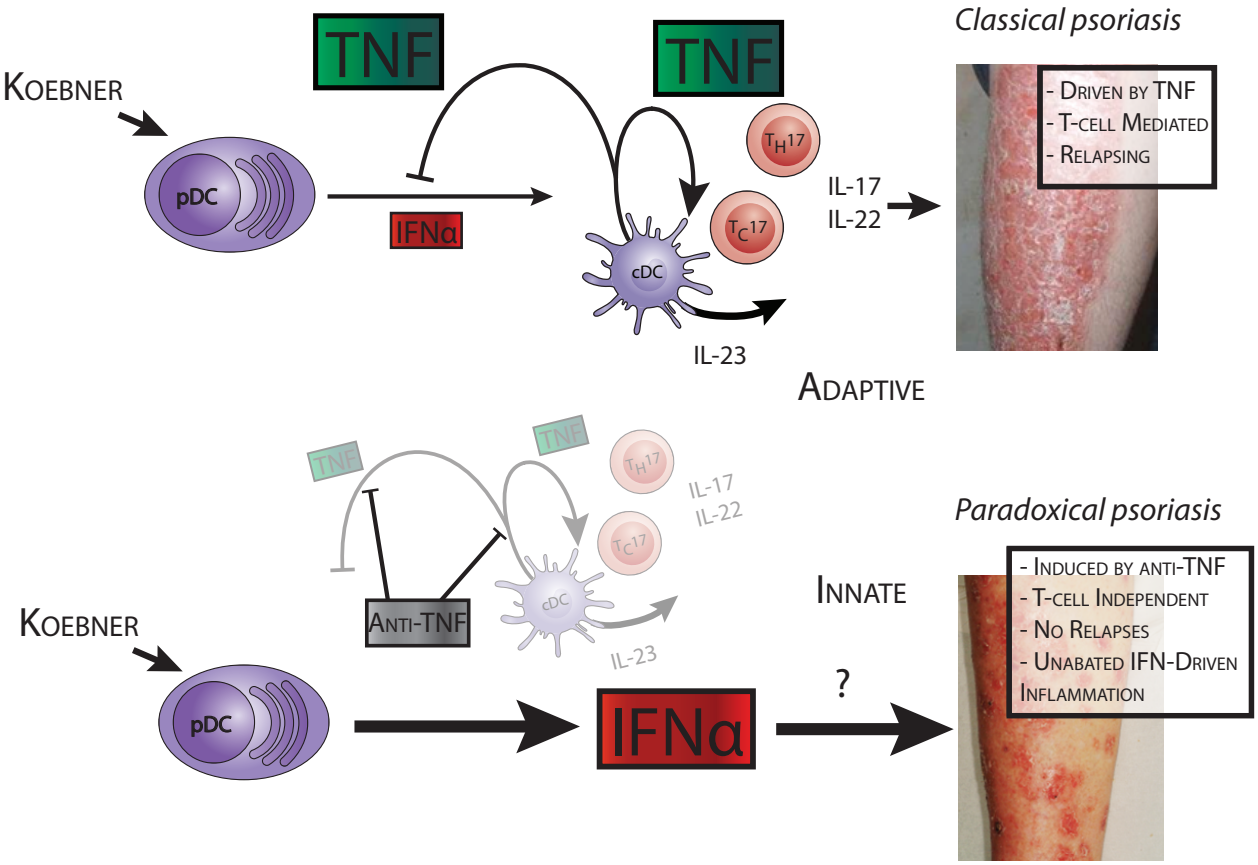

Figure 6: Anti-TNF induced paradoxical psoriasis model. In classical psoriasis, skin injury (Koebner phenomenon) ellicits pDC-derived type-I interferon production, which drives activation of skin T-cells. Dendritic cells produce copious amounts of TNF, thus inhibiting pDCs and type-I interferon production, and sustain a TNF-driven inflammatory environment. This maintains IL-23 production and T_H17 cytokines from T-cells, giving rise to pathological outcome. In contrast, in paradoxical psoriasis, anti-TNF therapy blocks TNF, thus raising inhibition from pDCs and leading to unabated production of type-I interferon. This in turn drives a T-cell independent innate inflammation resembling classical psoriasis but without relapses. Schematic drawings modified and re-used with permission from Nature Publishing Group.

Though the precise mechanisms of type-I interferon-driven innate skin inflammation remain to be determined, one might envisage anti-IFNα therapy in difficult-to-treat paradoxical psoriasis where anti-TNF discontinuation is either not an option or not sufficient to reverse the cutaneous manifestation. Yet, as from lessons learned from anti-TNF therapy, type-I interferon inhibition may lead to a domino effect-type of imbalances of other cytokines.

4.2. Undesired Effects of Type-I Interferon Blockade and of Cytokine Imbalance

Targeting of the type-I interferon pathway in the clinic is now coming to fruition after many years of research in pathogenic mechanisms of interferon-driven diseases. Systemic Lupus Erythematosous (SLE) is just one such disease which has the potential to benefit greatly from targeting of the type-I interferon pathway.[290]

SLE is a disease which for almost a century was thought to be restricted to skin, hence the clinical designation "lupus erythematosous". Later the systemic aspect of the disease was discovered, and the disease called SLE. Though all organs are usually affected in SLE (skin, heart, lungs, kidneys, joints, and nervous system), discoid lupus affects skin specifically. Skin manifestations in lupus, both systemic and discoid, frequently (but not exclusively) involve the famous butterfly (malar) pattern rash reminiscent of rosacea. Indeed, one differential diagnosis of rosacea is often lupus, as systemic inflammation needs to be ruled out. Similarities between lupus and rosacea are many (skin location, female incidence bias, and now type-I interferon[291,292]) though it is not known whether they share common pathogenic pathways, and one major difference is the presence of autoimmunity driven by T- and B-cells in SLE.

New compounds which target specifically IFNα cytokines[263] or the type-I interferon receptor[264] have been developed and recently trialled in lupus. Medium-scale phase IIb trials have recently been published revealing significant efficacy at up to 52-weeks of treatment, and interestingly stratification by interferon-response expression levels reveals even greater efficacy. All patients that displayed amelioration had marked resolution of skin rashes. For lupus trials this is remarkable, particularly in patients without severe nephritis where other biologics to date have either failed to achieve efficacy endpoints or have been aborted because of an unfavourable balance between benefits *versus* adverse events.[293,294]

Both interferon-targeting trials reported statistically significant incidence of infections with Herpes zoster (5.9% *vs* 0.9% and 6.9% *vs* 2%) or influenza, in a dose-dependent manner. Antiviral therapy resolved the infections in all patients. Few other adverse effects have been reported over the 52-week trials, without difference with placebo-controlled groups. It remains to be established whether longer duration treatment increases viral susceptibility further, and whether this treatment will have higher rates of malignancies.

Total blockade of type-I interferon signalling has been proven successful in the treatment of lupus and a role for this pathway in the pathogenesis of the disease is proven clinically. Longer duration studies may reveal more serious complications which may take years to fully develop. As such, another strategy is likely to be sought, one that targets the pathway straight at its pathogenic source.

4.3. TYPE-I INTERFERON PRODUCTION BY PDCS AS A DRUGGABLE TARGET

Type-I interferon is an important pro-inflammatory cytokine that plays a fundamental role in viral and tumour defence as demonstrated by mouse studies. As such, strategies seeking to target only the pathogenic type-I interferon component are likely to be sought. One such approach may be to target pDCs specifically, as they are rarely critically required for mounting of immune responses. In contrast they have often been implicated as directly necessary in the pathogenesis of several disease models.

The search for pDC-specific cell surface markers to study the, at the time, recently discovered cell type led to the identification of the blood dendritic cell antigen -2 (BDCA2),[295] a C-type lectin receptor (CLR) which coincidentally negatively regulates pDCs when engaged. CLRs are part of the family of pattern recognition receptors (PPRs) such as TLRs, Nod-like receptors (NLRs) and retinoic acid-inducible gene 1 (RIG-1), and interact with pathogen-associated molecular patterns (PAMPs) for their recognition. CLRs are mostly expressed by DCs, and function by interacting with mannose, fucose and glucan carbohydrates found on pathogens (mannose on viruses, fungi and mycobacteria; fucose on certain bacteria and helminths; and glucans on mycobacteria and fungi).[296,297] Triggering of CLRs results in the internalisation and processing of pathogens for degradation and antigen presentation. It also results in modulation of signalling pathways and of other PPRs such as TLRs.

BDCA2 is expressed specifically on pDCs, is found to interact with immunoreceptor tyrosine-based activation motif (ITAM)-containing Fc receptor γ-chain (FcRγ). Other receptors that pair with FcRγ are found to activate Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling and transcription of downstream targets. Ligation of BDCA2 on pDCs does not induce NF-κB activation or cytokine production, instead it abolishes TLR-mediated activation and production of type-I interferons.[298,299] It is still not clear whether other pathways may be triggered by BDCA2 ligation, as some propose engagement of BCR-like signalling in pDCs, or what exactly the natural ligands of BDCA2 do on pDCs and how *in vivo* triggering of BDCA2 may modulate lack thereof natural ligation. Nonetheless, there is recent interest on targeting BDCA2 for the inhibition type-I interferon pathway, as new antibodies are being produced and tested,[300] and others tested in small scale human proof-of-concept studies.[265] As these studies show good drug tolerability, more are bound to follow in the near future with potential for phase trials.

In our recent study, we find that directly targeting pDCs yields a similar beneficial effect as targeting the type-I interferon pathway. Other recent work highlights the pathogenic impact of pDC-derived type-I interferon in response to viral infection.[301] Therefore, this strategy has potential for clinical relevance. Another approach may be to target the pathogenic trigger upstream of type-I interferon and pDC activation.

4.4. Upstream Pathogenic Triggers of Type-I Interferons in Skin Disease

Upstream activation of type-I interferon production is induced by PAMP-recognition. Viruses, bacteria, fungi, and parasites are all activators of the pathway, but self-nucleic acids are also known to be triggers.[152,153]

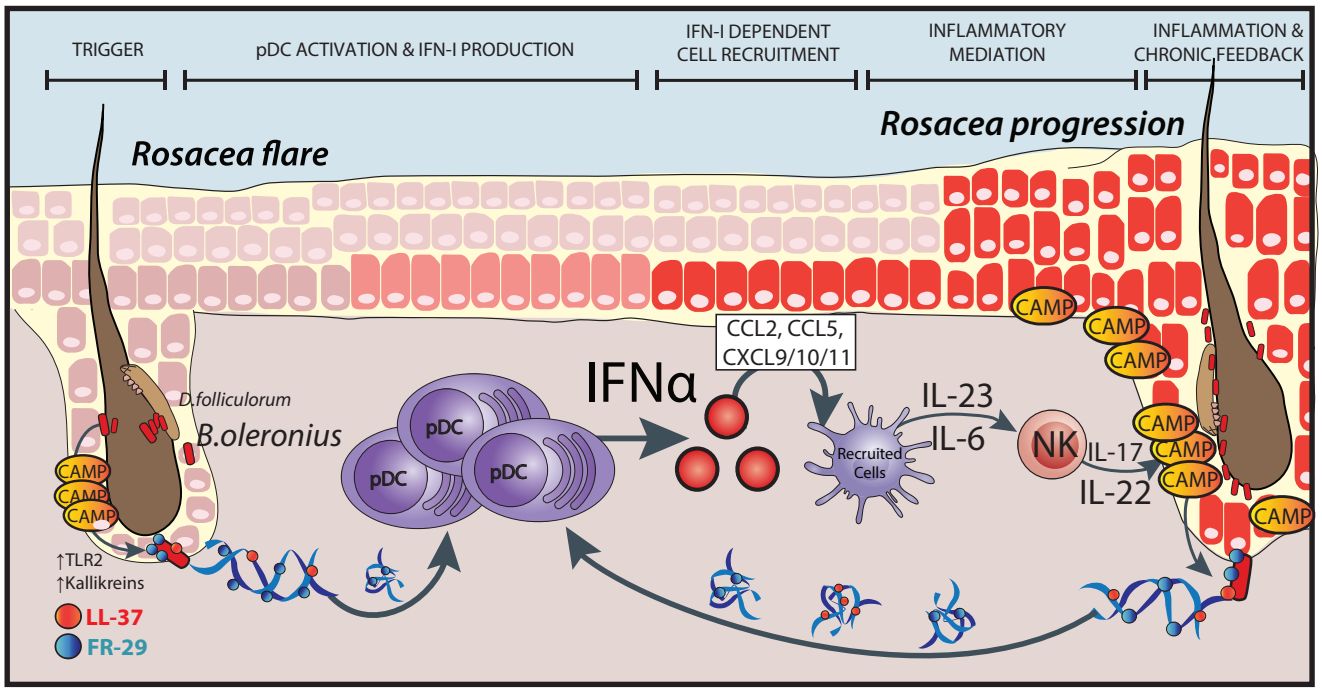

Figure 7: Plasmacytoid dendritic cell-derived type-I interferon drives flares of rosacea. In our proposed model, we identify type-I interferons selectively produced during flare-ups of rosacea. We find that cathelicidins induce pDC-derived type-I interferons which drive a T_H17-polarising cytokine environment and IL-22 at the site of inflammation, along with select CXCR3 and CCR1/5-binding chemokine expression. The resulting effector responses are known to induce further cathelicidins and exacerbate inflammation resulting in further activation of pDCs and leading to a recurring feedback loop of interferon production. Intriguingly, we find that *Bacillus oleronius*, a commensal bacterium that resides deep within the hair follicles, is exquisitely sensitive to cathelicidin peptides specifically identi-fied in rosacea. Furthermore cathelicidin peptide-mediated killing of the bacterium lead to potent activation of pDCs for production of type-I interferons. Protection of B.oleronius in the gut of *Demodex folliculorum* may provide it with a perfect breeding environment away from immune responses, thus remaining a continuous trigger in rosacea. *D.folliculorum*: *Demodex folliculorum*; *B.oleronius*: *Bacillus oleronius*; CAMP: Cathelicidin-antimicrobial peptide; TLR2: Toll-like receptor 2; pDC: plasmacytoid dendritic cell; IFNα: interferon alpha; NK: Natural killer cell; IL: interleukin. Schematic drawings modified and re-used with permission from Nature Publishing Group.

In the context of rosacea (Figure 7), we find that commensal bacteria are critically required and sufficient for cathelicidin-initiated, pDC-driven inflammation. This is in line with the use of topical and systemic antibiotics and anti-parasitic drugs. This strategy poses several issues, as indiscriminately targeting of the microflora, particularly in the gut, can be detrimental to the host in the long run. Interestingly, low-dose non-antimicrobial

administration of tetracycline drugs (40mg/day) also show efficacy.[302] These dosages have been proven to be non-antimicrobial *in vitro* and to act directly on the immune system via inhibition of mitochondria, though the effect on bacteria *in vivo*, over the course of treatment, has never been assessed. The metabolic state of microbiota and the metabolites produced have recently been the subject of keen interest by the community. In studies involving drug-resistant bacteria, it was found that inducing metabolic activity in bacteria led to loss of resistance to antibiotics.[303,304] As such, it is unclear whether low-dose tetracyclines may have an effect on bacteria specifically in inflammatory lesions which are metabolically active. Unsurprisingly, the total metabolome in the gut of the host is affected by antibiotic treatments,[305,306] leading to increased susceptibility to infection.[307] It remains to be determined whether metabolites produced by bacteria may be affected by low-dose antibiotic treatments, and whether this may have a downstream inflammation-modulating consequence.

For paradoxical psoriasis (Figure 6), we know that there is a cell-intrinsic effect on pDCs which causes increased pathogenic type-I interferon production. It is not known whether in paradoxical psoriasis TNF blockade may also play in concert with type-I interferon[275] in maintaining a pro-bacterial environment which drives an even further enhanced type-I interferon production. Moreover, this may influence downstream inflammation and contribute directly to type-I interferon-driven pathology in paradoxical psoriasis. Increased rates of superinfection in lesions of paradoxical psoriasis[182] may reflect this, though it is unclear whether it affects the inflammatory environment and how. Furthermore, it is unclear whether the virome, fungiome, and parasitome may also play a role.

Understanding of upstream activators of the pathogenic pathway in rosacea and paradoxical psoriasis may elucidate targets for therapeutic intervention. Another approach, which has been successful for the treatment of psoriasis, is to target downstream effectors.

4.5. Downstream Innate Mediators of Type-I Interferon-Driven Skin Inflammation

Currently, the treatment of psoriasis relies on targeting of the TNF-IL23-T_H17 axis. Targeting of the T_H1 or T_H22 component of psoriasis has not yielded particular efficacy. Other diseases, which have considerable clinical and molecular similarities, may also benefit from similar treatment strategies.

Pityriasis rubra pilaris (PRP) is a rare skin disorder of unknown aetiology that causes orange scaly patches with well-defined borders and may affect the entire body. Each case is unique, and no specific or consistently effective therapy exists. We find that targeting of the IL-12/23 cytokines leads to pronounced amelioration of the condition, and loss of IL-17 expression. Intriguingly, IFNγ and TNF stabilisation lags behind the amelioration, suggesting that IL-23/T_H17 (and not IL-12/T_H1) is specifically targeted (Appendix 3).[308] We find that, like psoriasis and PRP, rosacea and paradoxical psoriasis have an important T_H1 and T_H17 component, with clear upregulation of IL-17, IL-22, IL-23, IL-12, and IFNγ. Targeting IL-23/T_H17 is effective for a surprising number of skin, but also joint and gut-associated, diseases and thus may be worth exploring for rosacea and paradoxical psoriasis. This is especially interesting in light of a conserved pathogenic role for the type-I interferon pathway, but also given overexpression of T_H1/T_H17- (but not T_H2)-associated cytokines.[291,309,310] Using pre-clinical models of disease, such as the paradoxical psoriasis model, or the rosacea-reminiscent KLK5-

mediated spontaneous inflammation genetic model, could provide important clues as to the potential efficacy of treatment via targeting of downstream cytokines which are pathogenic in other skin disorders.

Ongoing work will elucidate whether, during innate inflammation such as for paradoxical psoriasis, type-I interferon acts upstream of an IL-23/T_H17-related axis that may be targeted as in psoriasis.

5. APPENDICES

Five appendices are attached to this thesis.

Appendix 1 – TNF blockade induces a dysregulated type I IFN response without autoimmunity in paradoxical psoriasis

Appendix 2 – Rosacea-associated bacteria activate plasmacytoid dendritic cell-derived type-I interferon driving flare-ups of disease

Appendix 3 – Interleukin 23-helper T cell 17 axis as a treatment target for pityriasis rubra pilaris

Appendix 4 – Targeting $CD8^+$ T cells prevents psoriasis development

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TNF blockade induces a dysregulated type I IFN response without autoimmunity in paradoxical psoriasis

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ABSTRACT

Although anti-Tumour necrosis factor (TNF) agents are highly effective in the treatment of psoriasis, 2-5% of treated patients develop psoriasis-like skin lesions called paradoxical psoriasis. The pathogenesis of this side effect and its distinction from classical psoriasis remain unknown. Here, we show that skin lesions from patients with paradoxical psoriasis are characterized by a selective overexpression of type I interferons, dermal accumulation of plasmacytoid dendritic cells (pDC), and reduced T cell numbers, when compared to classical psoriasis. Anti-TNF treatment prolongs type I interferon production by pDCs through inhibition of their maturation. The resulting type I interferon overexpression is responsible for the skin phenotype of paradoxical psoriasis, which, unlike classical psoriasis, is independent of T cells. These findings indicate that paradoxical psoriasis represents an ongoing overactive innate inflammatory process, driven by pDC-derived type I interferon that does not lead to T cell autoimmunity.

INTRODUCTION

Tumour necrosis factor (TNF) is a homotrimeric cytokine produced by immune and epithelial cells in response to infection or tissue injury[1,2]. TNF exerts potent pro-inflammatory functions via activation of immune cells and vascular endothelial cells[2-4]. Increased TNF expression levels can be found at sites of inflammation in many autoimmune diseases, such as rheumatoid arthritis, Crohn's disease, or psoriasis[5-7]. TNF blockade is highly efficacious and has become the benchmark in management of these diseases[8-11]. As such, more than two million patients have been treated with TNF blockers.

Nevertheless, TNF blockade as a therapeutic option has its limitations. Long-term TNF neutralization increases susceptibility to infections and skin cancer[12,13]. Another common side effect of TNF blockade is the development of inflammatory skin lesions, which resemble psoriasis and are observed in 2-5% of patients receiving anti-TNF therapy[14-18]. These skin manifestations are called “paradoxical psoriasis”, as TNF blockade is usually highly efficacious in psoriasis treatment. Notably, this side effect even occurs in patients undergoing successful psoriasis treatment with anti-TNFs. More severe cases necessitate interruption or complete cessation of anti-TNF therapy and, for several diseases, no equivalent alternative treatments exist. Therefore, understanding the pathogenic mechanism underlying paradoxical psoriasis, and its distinctions from classical psoriasis, remains a critical issue for the future design of successful therapeutic and preventive measures.

Classical psoriasis is a chronic, autoimmune skin disease mediated by T cells[19-21]. Evidence for a pathogenic role of T cells stems from the following observations: first, T cell targeted therapies including cyclosporine (inhibition of calcineurin in activated T cells), DAB-IL-2 (interleukin-2 receptor-specific fusion toxin)[22], and inhibitors of T cell costimulation, including alefacept[23],

efalizumab[24], and CTLA-4-Ig[25], are efficacious in psoriasis treatment; second, *HLA-Cw6* represents the strongest genetic risk variant associated with psoriasis[26]; third, clinically-relevant xenotransplant models of psoriasis are dependent on T cells[27-29]; and, finally, lesional T cells are oligoclonal and recognize epidermal autoantigens[30-34]. These pathogenic T cells mediate the chronic and relapsing course of psoriasis and define it as an autoimmune disease.

Autoimmune T cell responses in psoriasis are initiated by a subset of dendritic cells called plasmacytoid dendritic cells (pDCs), which infiltrate pre-psoriatic skin and are activated to produce type I interferons (IFN)[35]. pDC-derived type I IFNs unleash the autoimmune response by promoting activation and maturation of conventional DCs (cDCs) that stimulate expansion of autoreactive T cells. These autoreactive T cells – in particular $CD8^+$ T cells – migrate into the epidermis, where they recognize keratinocyte autoantigens and induce keratinocyte hyperproliferation[28,36]. Whether paradoxical psoriasis follows a similar pathomechanism remains unknown.

Here, we show that paradoxical psoriasis induced by anti-TNF is characterized by an exaggerated type I IFN response, which does not lead to T cell autoimmunity. Anti-TNF antibodies directly increase the capacity of pDCs to produce type I IFNs, by inhibiting their maturation. The exaggerated type I IFN response induced by anti-TNF treatments is sufficient to trigger a psoriatic skin phenotype. However, in contrast to classical psoriasis, type I IFN fails to induce cDC maturation and the subsequent activation of autoimmune T cells that is required for a chronic-relapsing disease course. Thus, paradoxical psoriasis is a side effect of an anti-TNF treatment stemming from an overactive, but self-limiting innate inflammation driven by pDC-derived type I IFN.

RESULTS

Clinical characterisation of paradoxical psoriasis

We analyzed 25 paradoxical psoriasis patients as summarized in Supplementary Table 1. Mean age of the patients was 44.8 years (range 15 to 73 years). Mean duration of anti-TNF treatment until onset of paradoxical psoriasis was 9.5 months (range 3 weeks to 5 years). Anti-TNF therapy indications include Crohn's disease (n=6), psoriasis and/or psoriatic arthritis (n=8), ankylosing spondylitis (n=8), rheumatoid arthritis (n=1), as well as SAPHO (n=1) and juvenile rheumatoid arthritis (n=1). Patients were treated with the anti-TNF antibodies infliximab (n=10), adalimumab (n=10), certolizumab (n=1), and golimumab (n=2), and the TNF-receptor fusion protein etanercept (n=2). Anti-TNF-induced paradoxical psoriasis appeared independent of the underlying diseases or the type of anti-TNF agent used (SupplementarySupplementary Table 1). Paradoxical psoriasis regressed in all patients when anti-TNF therapy was discontinued, but relapsed or persisted in 7 of 11 cases (64%) when anti-TNF treatment resumed. These relapses occurred despite switching to another anti-TNF agent. Importantly, no relapses were seen upon discontinuation of anti-TNF treatment, which suggests that paradoxical psoriasis does not represent de-novo psoriasis. The clinical presentation showed great variations reminiscent of classical psoriasis in its clinical forms (plaque-type, guttate, pustular) or particular sites of involvement (palmoplantar, scalp, skin folds) (Figure 1 and Table 1). However, we also observed some clinical particularities of paradoxical psoriasis, including a higher frequency of palmoplantar involvement as compared to classical psoriasis (80% versus 2-19% [37,38]) and severe noncicatricial alopecia, in numerous cases with scalp involvement (Figure 1). Histopathology of paradoxical psoriasis showed a large spectrum with three identifiable patterns: an eczematiform spongiotic pattern, a psoriasis-like pattern (with different amounts of intraepidermal or

subcorneal neutrophilic infiltration), and a lichenoid pattern with focal interface dermatitis (Figure 1). However, these patterns were usually overlapping, presented at variable degrees in most cases, and did not correlate to the clinical presentations. These findings suggest that paradoxical psoriasis is a transient side effect induced by TNF blockade independent of treatment type (class effect) with diverse clinical and histological presentations resembling psoriasis.

High IFN expression and PDC numbers in paradoxical psoriasis

We analyzed mRNA expression levels of selected innate cytokines involved in the pathogenesis of psoriasis to identify expression patterns unique to paradoxical psoriasis. We observed no significant difference in the expression levels of *TNF*, *IL23A*, *IL12A*, *IL36G*, *IL8* (*CXCL8)*, *IL6*, and *IL1B* when comparing skin lesions from paradoxical psoriasis with classical psoriasis (Fig. 2a). In contrast, type I IFNs *IFNA2* and *IFNB1* expression was greatly increased in paradoxical psoriasis relative to chronic plaque psoriasis (Fig. 2a). Importantly, high levels of type I IFN expression were observed in all samples, despite the variability in clinical and histological presentation. Thus, uniform high levels of type I IFN expression in lesional skin characterize anti-TNF-induced paradoxical psoriasis. Interestingly, adaptive T cell derived cytokines *IL17A*, *IL17F*, *IL17C*, *IL26*, *IFNG*, *IL4*, and *IL10* show comparable levels in skin biopsies from paradoxical and classical psoriasis (Fig. 2b). However, we found significantly increased *IL22* expression in paradoxical psoriasis, which correlated significantly with the increased type I IFN expression (*IFNA2* $r=0.567$, $p<0.005$; *IFNB1* $r=0.474$, $p=0.017$; *calculated by Spearman's rank-correlation*).

IFNs are preferentially expressed by pDCs, or natural type I IFN-producing cells. They can produce 50-100-fold more type I IFNs than any other cell type. We therefore investigated

whether pDCs are present in paradoxical psoriasis skin lesions by staining paraffin-embedded sections with CD123 (IL3RA). $CD123^+$ lymphoid cells in skin represent bona-fide pDCs[35], as demonstrated by co-staining with BDCA2 (CLEC4C) in selected cryo-samples of paradoxical psoriasis (Fig. 3b). pDCs were absent in normal skin from healthy volunteers. However, confirming previous studies [39], we found large numbers of pDCs in paradoxical psoriasis skin lesions (Fig. 3a). This increase was significantly greater than the number of pDCs found in classical plaque psoriasis (Fig. 3c). Expression of both *IFNA2* (Fig. 3d) and *IFNB1* (Supplementary Figure 1) significantly correlated with pDCs quantity, suggesting that they represent the principal source of type I IFN. Notably, pDC accumulation coincides with elevated type I IFN expression at a uniform rate regardless of the clinical or histological phenotype in paradoxical psoriasis.

Anti-TNF enhances IFN by inhibiting PDC maturation

Given the increased *IFNA2* expression in anti-TNF-induced paradoxical psoriasis, we investigated whether TNF blockade would enhance IFN-α production by pDCs directly. As LL37 complexed with DNA has been shown to activate pDCs in psoriasis[40,41], and because *CAMP* mRNA expression (*corresponding to LL37*) in paradoxical psoriasis was comparable to psoriasis (Supplementary Figure 2), we used LL37/DNA complexes as stimulus to activate enriched human peripheral blood pDCs in the presence or absence of anti-TNF antibodies. TNF blockade significantly enhanced IFN-α production by stimulated pDCs measured 48 hours after stimulation (Fig. 3e,f). This was direct effect of TNF blockade and not mediated by Fc-receptors as shown by a similar IFN-α increase when using certolizumab, a Fc-free Fab-fragment of a monoclonal antibody, but not an irrelevant human IgG antibody (Supplementary. Figure 3a,b).

Furthermore, addition of recombinant TNF to the culture strongly suppressed IFN-α production by activated pDCs (Fig. 3f) indicating that TNF controls IFN-α production by pDCs. To gain further insights into the mechanisms by which TNF controls IFN-α production, we performed time course analyses of cytokine expression in activated pDCs. *IFNA2* expression occurred early, peaking at 24 hours, whereas *TNF* expression increased at later time points (48 hours and 72 hours after stimulation) and coincided with the decrease of *IFNA2* expression (Fig. 3g). Anti-TNF antibodies did not affect early *IFNA2* expression but markedly increased its levels at 48 hours and 72 hours, indicating that TNF blockade prolongs the ability of pDC to produce type I IFNs (Fig. 3h and SupplementarySupplementary Figure 4). Moreover, addition of recombinant TNF to the culture shortened *IFNA2* expression by pDCs (Fig 3h). Together these data show that IFN-α precedes TNF expression and suggest that TNF replaces IFN-α by inhibiting its expression. Because TNF drives pDC differentiation into mature DCs which lose their ability to produce IFN-α[42], we hypothesized that anti-TNF would prolong type I IFN production of activated pDC by inhibiting their maturation. Indeed, anti-TNF significantly decreased maturation of pDCs as shown by reduced surface expression of HLA-DR (CD74) 48 hours after activation (Suppl Figure 5a,b). Anti-TNFs also reduced expression of costimulatory molecules CD80 and CD86, as well as maturation marker CD83, on activated pDCs (SupplementarySupplementary Figure 5c-g). Addition of recombinant TNF, which suppressed IFN-α production by pDCs, strongly upregulated expression of CD80, CD86, and CD83 (SupplementarySupplementary Figure 5c-g). These data suggest that TNF controls the duration of IFN-α production by promoting differentiation of pDCs into mature DCs. Consequently, TNF blockade inhibits pDC maturation and prolongs their ability to produce IFN-α, providing an explanation for high levels of type I IFN in anti-TNF induced paradoxical psoriasis.

ANTI-TNF INCREASES IFN AND PDC NUMBERS IN THE SKIN

Next, we studied whether anti-TNFs are sufficient to increase type I IFN production in-vivo utilizing a skin injury mouse model. In this mouse model, repetitive tape stripping leads to a short-lived pDC infiltration into injured skin, peaking at 24 hours and declining at 48 hours (Fig. 4a)[43]. Anti-TNF treatment promoted significant increased and sustained pDC infiltration (Fig. 4a, b), which paralleled prolonged type I IFN expression (Fig. 4c). Importantly, pDC depletion largely abrogated this type I IFN expression, which confirmed in-vivo that pDCs are the principal source of type I IFNs following TNF blockade (Supplementary Figure 6). Similar to the human in-vitro data, TNF blockade in-vivo significantly inhibited pDC maturation as shown by a decreased expression of Cd80 and Cd86 (Fig. 4d). Interestingly, blocking type I IFN-signaling by an anti-type I IFN-receptor (anti-IFNAR) antibody significantly reduced the numbers of pDCs infiltrating injured skin (Fig. 4e). As CXCR3-ligands CXCL9, CXCL10, and CXCL11 are induced by type I IFNs and mediate pDC migration into the skin[44,45], we analyzed their expression in our mouse model. Indeed, we found a significant, type I IFN-dependent overexpression of *Cxcl10* and *Cxcl11* in the skin of anti-TNF treated mice at 24, 48, and 72 hours, as anti-IFNAR treatment completely abrogated their expression (Supplementary Figure 7). These data show that type I IFN production sustains skin infiltration of pDCs and suggest an amplification loop in which type I IFN produced by pDC promotes additional pDC infiltration into the skin. These data demonstrate that blocking TNF decreases pDC maturation and enhances type I IFN production by pDCs to amplify their skin infiltration.

Anti-TNF promotes paradoxical psoriasis via IFN

Transient type I IFN production by pDCs promotes wound healing[43], whereas sustained expression may initiate classical psoriasis development[35,46]. Because anti-TNF treatment in wild-type mice increases pDC accumulation and type I IFN production in the skin, we determined whether it also induced a psoriasis-like phenotype. Indeed, 6-7 days after tape stripping, the epidermis of anti-TNF treated mice showed typical hallmarks of psoriasis including acanthosis, parakeratosis, and a focal loss of the granular layer. In addition, we observed basal and suprabasal Ki67 expression indicative of keratinocyte hyperproliferation and involucrin expression throughout the entire epidermis suggesting abnormal keratinocyte differentiation (Fig. 5a-d). In contrast, the epidermis of control mice was similar to untreated skin showing minimal Ki67-positive keratinocytes and involucrin expression within the upper epidermal layers (Fig. 5a-d). We then treated mice with anti-IFNAR antibodies to determine if enhanced type I IFN induced the psoriatic phenotype. Inhibition of type I IFN-signaling decreased the anti-TNF-induced psoriatic phenotype to levels indistinguishable from control mice (Fig. 5e,f). Together, these data indicate that anti-TNF induces a psoriatic phenotype through enhanced and sustained type I IFN production by pDCs. These data provide a mechanism that underlies paradoxical psoriasis.

Development of paradoxical psoriasis is T cell-independent

Type I IFN production by pDCs triggers classical psoriasis[35] through activation of conventional DCs (cDC) and expansion of autoimmune T cells. These pathogenic T cells are direct triggers of epidermal hyperproliferation and their persistence in the skin and circulation of psoriasis patients are responsible for chronicity and the recurrent disease course[19-21]. Because paradoxical psoriasis does not represent true psoriasis as it never relapses upon cessation of anti-TNF (Supplementary

Table 1), we next asked whether T cells play a role in paradoxical psoriasis. We depleted conventional T cells in our paradoxical psoriasis mouse model using anti-TCR-beta antibody administration. T-cell depleted mice treated with anti-TNF developed a psoriasis-like phenotype with increased acanthosis that was similar to non-depleted control mice treated with anti-TNF (Fig. 6a,b). Because unconventional T cells such as γ/δ-T cells have been implicated in the development of psoriasiform skin inflammation in mouse models[47], we treated *Rag2*$^{-/-}$ mice, which are deficient of both conventional α/β-T cells and γ/δ-T cells, with anti-TNF. Similar to wild type mice, anti-TNF treated *Rag2*$^{-/-}$ mice developed a psoriatic phenotype with significantly increased epidermal thickness (Fig. 6a,c). These data indicate that neither conventional T cells, nor γ/δ-T cells are required for the type I IFN-driven keratinocyte hyperproliferation. To investigate the role of T cells in human paradoxical psoriasis, we quantified CD8$^+$ T cells infiltrating the epidermis, which represent the pathogenic T cell subpopulation in psoriasis[28,36]. Compared to the large numbers of CD8 T cells present in the epidermis of classical psoriasis (n=11), a significantly lower number of CD8$^+$ T cells was present in the epidermis of paradoxical psoriasis (n=16) (Fig. 6d-f). CD8$^+$ T cells were completely absent in normal skin of healthy donors (n=5). Because mature cDCs in psoriatic skin represent the key stimulators of pathogenic CD8$^+$ T cells to migrate into the epidermis, we next quantified mature cDCs in skin samples using the maturation marker LAMP3. We found a significantly increased number of LAMP3$^+$ cDCs in the skin of classical psoriasis as compared to skin from healthy donors (Fig. 6g). In contrast, there were significantly fewer LAMP3$^+$ cDCs in paradoxical psoriasis suggesting a lack of cDC maturation despite the increase type I IFN expression (Fig. 6g-i). Taken together, these data suggest that paradoxical psoriasis represents an overactive type I IFN-driven innate

inflammation that does not lead to cDC maturation with consequent T cell-mediated autoimmune response as in classical psoriasis.

DISCUSSION

This study identifies the pathophysiological mechanism underlying anti-TNF-induced paradoxical psoriasis. By comparing skin lesions of paradoxical psoriasis with classical psoriasis, we found a selective and uniform increase of type I IFN expression along with a marked dermal accumulation of pDCs. Using in-vitro and in-vivo models, we then demonstrated that anti-TNFs directly prolong the ability of pDCs to produce type I IFN. The resulting overexpression of type I IFNs is sufficient to drive the development of the psoriatic skin phenotype observed in paradoxical psoriasis, which, in contrast to classical psoriasis, is independent of T cells.

A link between anti-TNFs and increased type I IFN expression has been suggested by previous findings that anti-TNF therapy induces a type I IFN signature in blood of juvenile arthritis patients[48] and can exacerbate lupus, a well-known type I IFN-driven autoimmune disease[49,50]. Using a combination of in-vitro and in-vivo studies, we now unravel the mechanism by which this occurs: TNF temporally controls and limits type I IFN expression by pDCs, and this effect can be reversed by anti-TNFs. Upon stimulation of pDCs, type I IFN production occurs first and is subsequently relayed by TNF production, which drives pDC maturation into DCs that lose the ability to produce type I IFNs[51]. Therefore, by promoting pDC maturation, TNF directly controls and limits the duration of type I IFN production by pDCs. On the other hand, blocking of TNF activity by anti-TNFs decreases pDC maturation and thereby prolongs the ability of pDC to produce type I IFN. Together our findings suggest a yin-yang model in which there is a temporal equilibrium between early type I IFN and late TNF expression[48] that is shifted by TNF blockade towards a prolonged and excessive type I IFN response.

Our study shows, that the type I IFN overproduction in paradoxical psoriasis is required for the development of a psoriatic skin phenotype. This finding raises questions about the differences

between paradoxical psoriasis and classical psoriasis, which is also driven by an early type I IFN production by pDCs[35]. Our data show that, unlike classical psoriasis, which is a T cell-mediated autoimmune disease, development of paradoxical psoriasis is independent of T cells. Therefore, both paradoxical psoriasis and classical psoriasis are triggered by pDCs and type I IFN, but only classical psoriasis develops into a T cell-mediated relapsing autoimmune disease. In contrast, paradoxical psoriasis fails to elicit an adaptive immune response and remains fixed in an ongoing pDC-driven innate immune response. These findings explain why there is no disease memory in paradoxical psoriasis while classical psoriasis is characterized by T cell-mediated recurrent flare-ups. There are two possible explanations for the lack of T-cell autoimmunity in paradoxical psoriasis. In classical psoriasis, the type I IFN response is rapidly replaced by increasing levels of TNF, which is critical for the maturation of cDCs that stimulate T cells[46]. In the context of paradoxical psoriasis, TNF blockade inhibits the induction of mature cDC and subsequent T cell activation, while magnifying type I IFN-driven innate inflammation. Another possibility is that paradoxical psoriasis patients lack genetic risk variants that drive and regulate T cell autoimmunity. In fact, variants involving T cell activation and Th17 differentiation including *IL23A*, *IL23R*, *IL12B*, *HLACw6*, *RUNX3*, *STAT3*, and *TRAF3IP2* genes have been identified in classical psoriasis[52,53].

The mechanisms by which type I IFNs promote the psoriatic skin phenotype are currently unclear. Type I IFN itself does not induce keratinocyte proliferation nor is it responsible for the altered differentiation[54]. Most likely, type I IFN activates immune cells releasing cytokines that drive the development of a psoriatic phenotype. One possible link between type I IFN and keratinocyte hyperproliferation is IL22, which induces epidermal remodeling by promoting proliferation of keratinocytes[55,56]. Indeed, type I IFN drives *IL22* expression, as absence of type I

IFN-signaling completely abrogates induction of *IL22* expression in skin[43]. Accordingly, *IL22* is selectively upregulated in paradoxical psoriasis and significantly correlates with type I IFN expression. The cellular source of IL22 remains unclear. Because T cells do not play a role in paradoxical psoriasis, potential candidates include innate lymphoid cells (ILC3), NK cells[57], mast cells[58], and neutrophils[59], which have all been reported to express IL22.

In addition to increased type I IFN expression, higher numbers of skin pDCs are observed in paradoxical psoriasis as compared to classical psoriasis. The increased pDC numbers is not a direct anti-TNF effect, but rather dependent on the type I IFN overexpression induced by TNF blockade. Although the exact mechanisms by which type I IFN drives pDC infiltration in paradoxical psoriasis remains to be elucidated, CXCR3-ligands induced by type I IFNs may prolong the recruitment of pDCs into the skin in a self-amplifying loop[44].

Our study also identifies a new mouse model for the induction of a psoriasiform skin phenotype with acanthosis, basal keratinocyte hyperproliferation, and altered epidermal differentiation. This model displays the following three key features of human paradoxical psoriasis, which are clearly distinct from classical psoriasis. First, the psoriatic phenotype in this model is induced and not blocked by anti-TNFs; second, like in paradoxical psoriasis, the phenotype in this model is T cell independent, whereas classical psoriasis is a T cell mediated disease; and finally, the model shows sustained type I IFN expression, which is in line with the persistent type I IFN expression in the skin of paradoxical psoriasis but not classical psoriasis. An intriguing question is why anti-TNF is able to induce a psoriatic phenotype in wild-type mice and enhance type I IFN production by pDCs from blood of healthy donors, but only 2-5% of anti-TNF treated patients develop paradoxical psoriasis. The future identification of specific genetic variants potentially involving pDC activation and/or type I IFN-signaling may provide an explanation for an increased

susceptibility of these individuals to develop paradoxical psoriasis in the context of anti-TNF treatment.

In conclusion, this study identifies the relevance of the temporal equilibrium of TNF and type I IFN (TNF-IFN yin-yang) in the pathogenesis of paradoxical psoriasis. While TNF controls type I IFN under steady-state conditions, anti-TNF treatment may tip the balance towards type I IFN ultimately driving the psoriatic phenotype in paradoxical psoriasis. In contrast to classical psoriasis, paradoxical psoriasis fails to turn into a T cell-mediated autoimmune disease with a relapsing course but remains a drug-related side effect in which inflammation perpetuates in self-amplifying innate immune response. These findings provide the basis for the design of new strategies targeting pDCs and type I IFN for the treatment and prevention of paradoxical psoriasis.

METHODS

CLINICAL DATA

This study was performed in accordance with the guidelines of the Declaration of Helsinki and was approved by the local ethics committee (Ethics Committee Vaud, swissethics). Clinical data of 25 patients with paradoxical psoriasis were collected at the Department of Dermatology, University Hospital CHUV, Lausanne (n=16) and the Department of Dermatology, University Hospital of Zurich (n=9) between 2011 and 2013. Paradoxical psoriasis was defined as newly appearing psoriasiform skin lesions under anti-TNF therapy despite response to treatment.

Skin biopsies

Skin biopsies were taken from patients with paradoxical psoriasis or untreated classical plaque psoriasis after written informed consent was obtained. Human samples were fixed using 4% paraformaldehyde for immunohistochemistry (samples available from all 25 patients) or snap-frozen and stored at -80ºC for RT-PCR (cryomaterial available from 14 out of the 25 patients). Paraffin-embedded skin sections were deparaffinized, stained with anti-CD123 (BD Pharmingen), anti-LAMP3 (Sino Biological, 10527-RP02-50), or anti-CD8 (DAKO, C8/144B), and visualized using standard horseradish peroxidase-technique. For quantitative RT-PCR, cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen) and relative Gene expression was quantified using specific Taqman probes (Life technologies, Supplementary Table 2) and expressed as $2^{-\Delta\Delta CT}$ using *GAPDH* as endogenous control.

For immunofluorescence analyses of mouse tissue, cryopreserved skin samples were stained with anti-involucrin (Covance, PRB-140C-200, 1/400) or anti-Ki-67 (eBiosciences, SolA15, 1/1000) followed by labeled secondary antibody. For flow-cytometry analysis, mouse skin was digested

with Dispase (Sigma-Aldrich) and collagenase (Invitrogen) and stained with anti-B220 FITC (BD Pharmingen, RA3-6B2, 1/400), anti-CD45 PerCp-Cy5.5, (BD Pharmingen, 30-F11, 1/400), anti-CD11c PE, (eBioscience, N418, 1/800), and anti-PDCA1 APC (Biolegend, 927, 1/400), anti-CD80 PE (BD Pharmingen, 16-10A1, 1/800) or anti-CD86 PE (BD Pharmingen, GL1, 1/800). For flow-cytometry analyses of human pDCs, antibodies used include anti-CD123 APC (Biolegend, 6H6, 1/400), anti-BDCA2 PE (Miltenyi, AC144, 1/400), anti-BDCA4 APC (Miltenyi, REA-380, 1/400) anti-CD80 PE (BD Pharmingen, 16-10A1, 1/800), anti-CD83 FITC (eBioscience, HB15e, 1/400), anti-HLADR (BD Pharmingen, G46-6, 1/400). Assessors were blinded for all histological quantifications.

CELL CULTURE EXPERIMENTS

Plasmacytoid DCs (pDCs) were purified from peripheral blood mononuclear cells obtained from blood buffy coats of healthy donors by Ficoll separation followed by enrichment using a CD304 Microbeads kit (Miltenyi Biotech). pDCs were cultured in RPMI 1640 + GlutaMAX (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin and stimulated with 10µg/ml human DNA (Biochain) complexed with 50µg/ml LL-37 (Proteogenix) with or without 1µg/ml anti-TNF antibodies (Adalimumab, Humira®), or 100ng/ml recombinant human TNF (RnD). After 48 hours of culture, interferon (IFN)-α was measured in cell-free supernatants by ELISA (Mabtech).

MOUSE MODELS

All animal experiments were performed according to institutional guidelines and Swiss federal and cantonal laws on animal protection. Ethical approval was obtained for all described experimentation according to regulations by the Federal Food Safety and Veterinary Office

(FSVO). Animals were maintained and bred in pathogen-free facilities. Age- (8-10 weeks old) and sex-matched mice were used for all experiments. Female wild type Balb/c mice were purchased from Jackson Laboratory, *hBDCA2-DTR* (*hCLEC4C-DTR*) mice were bred at our facility. Skin injury was performed as previously described[43]. Briefly, mice were anaesthetized and their lower backs shaved using clippers, and then depilated using the commercially available Veet® cream. After cream removal with a paper tissue, 10 gentle strokes of commercially available tape (Scotch™, 3M) were applied to the lower back. Dosage of antibodies applied was deduced from therapeutic use in humans and injected intraperitoneally as follows: 1500µg of anti-TNF (Infliximab, Remicade®) at days -1 and 0; 200µg of anti-TCRβ (BioXCell, h57-597) at days -2, 0, 2, and 4; 250µg of anti-IFNAR (BioXCell, MAR1-5A3) at days -1, 0, 1, and 3. We used Remicade because it was previously shown to efficiently block both human and mouse TNF[60]. However, as a positive control, we used a mouse-specific anti-TNF antibody (Supplementary Figure 8). As a negative control, we used an irrelevant human IgG antibody (Supplementary Figure 8). Effective blockade of type I IFN-signalling by the anti-IFNAR antibody is demonstrated by the absence of type I IFN-response genes at day 7 after mechanical injury (Supplementary Figure 9). For pDC-depletion experiments, 120ng of diphtheria toxin was injected intraperitoneally into *hBDCA2-DTR* mice at day -1. At indicated time points, injured skin was excised for histology, flow cytometry, and gene expression analysis.

STATISTICS

Unpaired non parametric Mann-Whitney U test was used for analysis of human gene expression and histological analysis. To investigate an association between pDCs and type I IFN gene expression, the Spearman's rank-correlation coefficient was calculated. For preclinical mouse data, Student's t-test was used to perform statistical analyses. All testing was two-sided, and a p

value of less than 0.05 was considered to indicate statistical significance. All analyses were performed with GraphPad Prism 6.0.

DATA AVAILABILITY

All relevant data are available from the corresponding authors upon reasonable request.

REFERENCES

1. Beutler, B., Milsark, I.W. & Cerami, A.C. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229**, 869-871 (1985).
2. Beutler, B. & Cerami, A. The biology of cachectin/TNF--a primary mediator of the host response. *Annual review of immunology* **7**, 625-655 (1989).
3. Kneilling, M.*, et al.* Direct crosstalk between mast cell-TNF and TNFR1-expressing endothelia mediates local tissue inflammation. *Blood* **114**, 1696-1706 (2009).
4. Vassalli, P. The pathophysiology of tumor necrosis factors. *Annual review of immunology* **10**, 411-452 (1992).
5. Di Giovine, F.S., Nuki, G. & Duff, G.W. Tumour necrosis factor in synovial exudates. *Ann Rheum Dis* **47**, 768-772 (1988).
6. Breese, E.J.*, et al.* Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. *Gastroenterology* **106**, 1455-1466 (1994).
7. Kristensen, M.*, et al.* Localization of tumour necrosis factor-alpha (TNF-alpha) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. *Clinical and experimental immunology* **94**, 354-362 (1993).
8. Moreland, L.W.*, et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *The New England journal of medicine* **337**, 141-147 (1997).
9. Leonardi, C.L.*, et al.* Etanercept as monotherapy in patients with psoriasis. *The New England journal of medicine* **349**, 2014-2022 (2003).
10. Rutgeerts, P.*, et al.* Infliximab for induction and maintenance therapy for ulcerative colitis. *The New England journal of medicine* **353**, 2462-2476 (2005).
11. Taylor, P.C. & Feldmann, M. Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis. *Nat Rev Rheumatol* **5**, 578-582 (2009).
12. Bongartz, T.*, et al.* Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* **295**, 2275-2285 (2006).
13. Burmester, G.R., Panaccione, R., Gordon, K.B., McIlraith, M.J. & Lacerda, A.P. Adalimumab: long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and Crohn's disease. *Ann Rheum Dis* **72**, 517-524 (2013).
14. Baeten, D.*, et al.* Systematic safety follow up in a cohort of 107 patients with spondyloarthropathy treated with infliximab: a new perspective on the role of host defence in the pathogenesis of the disease? *Ann Rheum Dis* **62**, 829-834 (2003).

15. Sfikakis, P.P., Iliopoulos, A., Elezoglou, A., Kittas, C. & Stratigos, A. Psoriasis induced by anti-tumor necrosis factor therapy: a paradoxical adverse reaction. *Arthritis and rheumatism* **52**, 2513-2518 (2005).
16. Cohen, J.D.*, et al.* Psoriasis induced by tumor necrosis factor-alpha antagonist therapy: a case series. *The Journal of rheumatology* **34**, 380-385 (2007).
17. de Gannes, G.C.*, et al.* Psoriasis and pustular dermatitis triggered by TNF-{alpha} inhibitors in patients with rheumatologic conditions. *Arch Dermatol* **143**, 223-231 (2007).
18. Brown, G.*, et al.* Tumor necrosis factor-alpha inhibitor-induced psoriasis: Systematic review of clinical features, histopathological findings, and management experience. *Journal of the American Academy of Dermatology* **76**, 334-341 (2017).
19. Nestle, F.O., Kaplan, D.H. & Barker, J. Psoriasis. *The New England journal of medicine* **361**, 496-509 (2009).
20. Griffiths, C.E. & Barker, J.N. Pathogenesis and clinical features of psoriasis. *Lancet* **370**, 263-271 (2007).
21. Lowes, M.A., Suarez-Farinas, M. & Krueger, J.G. Immunology of psoriasis. *Annual review of immunology* **32**, 227-255 (2014).
22. Gottlieb, S.L.*, et al.* Response of psoriasis to a lymphocyte-selective toxin (DAB389IL-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nature medicine* **1**, 442-447 (1995).
23. Ellis, C.N., Krueger, G.G. & Alefacept Clinical Study, G. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *The New England journal of medicine* **345**, 248-255 (2001).
24. Lebwohl, M.*, et al.* A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *The New England journal of medicine* **349**, 2004-2013 (2003).
25. Abrams, J.R.*, et al.* CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *The Journal of clinical investigation* **103**, 1243-1252 (1999).
26. Nair, R.P.*, et al.* Sequence and haplotype analysis supports HLA-C as the psoriasis susceptibility 1 gene. *American journal of human genetics* **78**, 827-851 (2006).
27. Boyman, O.*, et al.* Spontaneous development of psoriasis in a new animal model shows an essential role for resident T cells and tumor necrosis factor-alpha. *The Journal of experimental medicine* **199**, 731-736 (2004).
28. Conrad, C.*, et al.* Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nature medicine* **13**, 836-842 (2007).
29. Wrone-Smith, T. & Nickoloff, B.J. Dermal injection of immunocytes induces psoriasis. *The Journal of clinical investigation* **98**, 1878-1887 (1996).
30. Prinz, J.C.*, et al.* Selection of conserved TCR VDJ rearrangements in chronic psoriatic plaques indicates a common antigen in psoriasis vulgaris. *European journal of immunology* **29**, 3360-3368 (1999).

31. Vollmer, S., Menssen, A. & Prinz, J.C. Dominant lesional T cell receptor rearrangements persist in relapsing psoriasis but are absent from nonlesional skin: evidence for a stable antigen-specific pathogenic T cell response in psoriasis vulgaris. *The Journal of investigative dermatology* **117**, 1296-1301 (2001).
32. Sigmundsdottir, H.*, et al.* Circulating T cells of patients with active psoriasis respond to streptococcal M-peptides sharing sequences with human epidermal keratins. *Scandinavian journal of immunology* **45**, 688-697 (1997).
33. Lande, R.*, et al.* The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nature communications* **5**, 5621 (2014).
34. Arakawa, A.*, et al.* Melanocyte antigen triggers autoimmunity in human psoriasis. *The Journal of experimental medicine* **212**, 2203-2212 (2015).
35. Nestle, F.O.*, et al.* Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *The Journal of experimental medicine* **202**, 135-143 (2005).
36. Di Meglio, P.*, et al.* Targeting CD8(+) T cells prevents psoriasis development. *J Allergy Clin Immunol* **138**, 274-276 e276 (2016).
37. Ashurst, P.J. Relapsing Pustular Eruptions of the Hands and Feet. *The British journal of dermatology* **76**, 169-180 (1964).
38. Enfors, W. & Molin, L. Pustulosis palmaris et plantaris. A follow-up study of a ten-year material. *Acta Derm Venereol* **51**, 289-294 (1971).
39. Seneschal, J.*, et al.* Cytokine imbalance with increased production of interferon-alpha in psoriasiform eruptions associated with antitumour necrosis factor-alpha treatments. *The British journal of dermatology* **161**, 1081-1088 (2009).
40. Lande, R.*, et al.* Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med* **3**, 73ra19 (2011).
41. Lande, R.*, et al.* Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564-569 (2007).
42. Kadowaki, N., Antonenko, S., Lau, J.Y. & Liu, Y.J. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *The Journal of experimental medicine* **192**, 219-226 (2000).
43. Gregorio, J.*, et al.* Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. *The Journal of experimental medicine* **207**, 2921-2930 (2010).
44. Vanbervliet, B.*, et al.* The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. *The Journal of experimental medicine* **198**, 823-830 (2003).
45. Meller, S.*, et al.* Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: An amplification cycle triggering cutaneous lupus erythematosus. *Arthritis and rheumatism* **52**, 1504-1516 (2005).

46. Conrad, C., Meller, S. & Gilliet, M. Plasmacytoid dendritic cells in the skin: to sense or not to sense nucleic acids. *Semin Immunol* **21**, 101-109 (2009).
47. Cai, Y.*, et al.* Pivotal role of dermal IL-17-producing gammadelta T cells in skin inflammation. *Immunity* **35**, 596-610 (2011).
48. Palucka, A.K., Blanck, J.P., Bennett, L., Pascual, V. & Banchereau, J. Cross-regulation of TNF and IFN-alpha in autoimmune diseases. *Proc Natl Acad Sci U S A* **102**, 3372-3377 (2005).
49. Bengtsson, A.A.*, et al.* Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. *Lupus* **9**, 664-671 (2000).
50. Blanco, P., Palucka, A.K., Gill, M., Pascual, V. & Banchereau, J. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* **294**, 1540-1543 (2001).
51. Soumelis, V. & Liu, Y.J. From plasmacytoid to dendritic cell: morphological and functional switches during plasmacytoid pre-dendritic cell differentiation. *European journal of immunology* **36**, 2286-2292 (2006).
52. Tsoi, L.C.*, et al.* Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nature genetics* **44**, 1341-1348 (2012).
53. Tsoi, L.C.*, et al.* Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. *Nature communications* **6**, 7001 (2015).
54. van der Fits, L., van der Wel, L.I., Laman, J.D., Prens, E.P. & Verschuren, M.C. In psoriasis lesional skin the type I interferon signaling pathway is activated, whereas interferon-alpha sensitivity is unaltered. *The Journal of investigative dermatology* **122**, 51-60 (2004).
55. Boniface, K.*, et al.* IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *Journal of immunology* **174**, 3695-3702 (2005).
56. Wolk, K.*, et al.* IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. *J Mol Med (Berl)* **87**, 523-536 (2009).
57. Colonna, M. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* **31**, 15-23 (2009).
58. Mashiko, S.*, et al.* Human mast cells are major IL-22 producers in patients with psoriasis and atopic dermatitis. *J Allergy Clin Immunol* **136**, 351-359 e351 (2015).
59. Chen, F.*, et al.* mTOR Mediates IL-23 Induction of Neutrophil IL-17 and IL-22 Production. *Journal of immunology* **196**, 4390-4399 (2016).
60. Lang, K.S.*, et al.* Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nature medicine* **11**, 138-145 (2005).

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Author contributions

C.C. and M.G. formulated the hypothesis, designed and supervised study and experiments, interpreted data, and wrote the manuscript. C.C. additionally provided human samples and performed histological quantifications. J.D. and A.M. planned and performed most of the experiments, including all in-vivo experiments, and gave input for drafting the manuscript. C.D. performed several in-vitro culture experiments with pDCs. O.M. performed PCR experiments on human samples and critically revised the manuscript for content. A.A.N, A-K.L., and L.E.F. provided human samples. M.V. performed histological analyses of human paradoxical psoriasis samples. All authors gave final approval of the manuscript to be published.

Competing financial interests

The authors declare no competing financial interests.

Figure 1

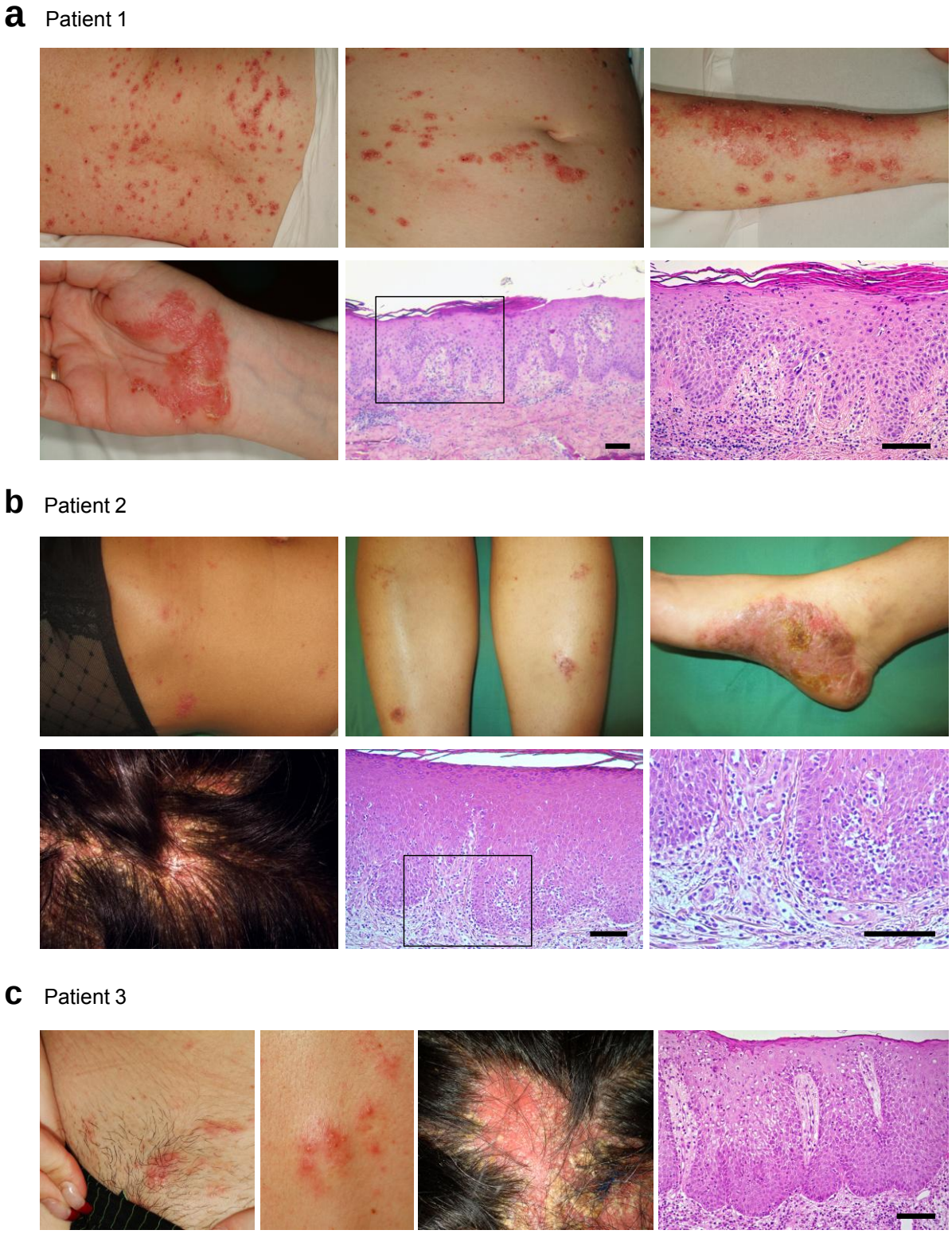


Fig. 1. Clinical and histological presentation of paradoxical psoriasis induced by anti-TNF. (**a-c**) Photographs of cutaneous lesions and corresponding histopathology of three individual patients presenting paradoxical psoriasis. (**a**) Patient 1 with small erythemato-squamous plaques disseminated over the entire body resembling guttate psoriasis and palmoplantar psoriasis-like lesions. Histology with a classical psoriasis pattern with acanthosis, papillomatosis, parakeratosis, and loss of the granular layer, but with spongiosis. (**b**) Patient 2 with partially crusted, eczematiform lesions on her legs and trunk, palmoplantar psoriasis-like lesions, and severe scalp involvement. Histology with acanthosis, papillomatosis, also in addition to spongiosis and minimal interface dermatitis. (**c**) Patient 3 with small erythematous plaques and pustules. Non-cicatricial alopecia on the site of scalp involvement. Histology with acanthosis, papillomatosis, and spongiosis. *Scale bars represent 100µm. Clinical signs and histopathology of the patients shown are representative of the patient population in this study.*

Figure 2

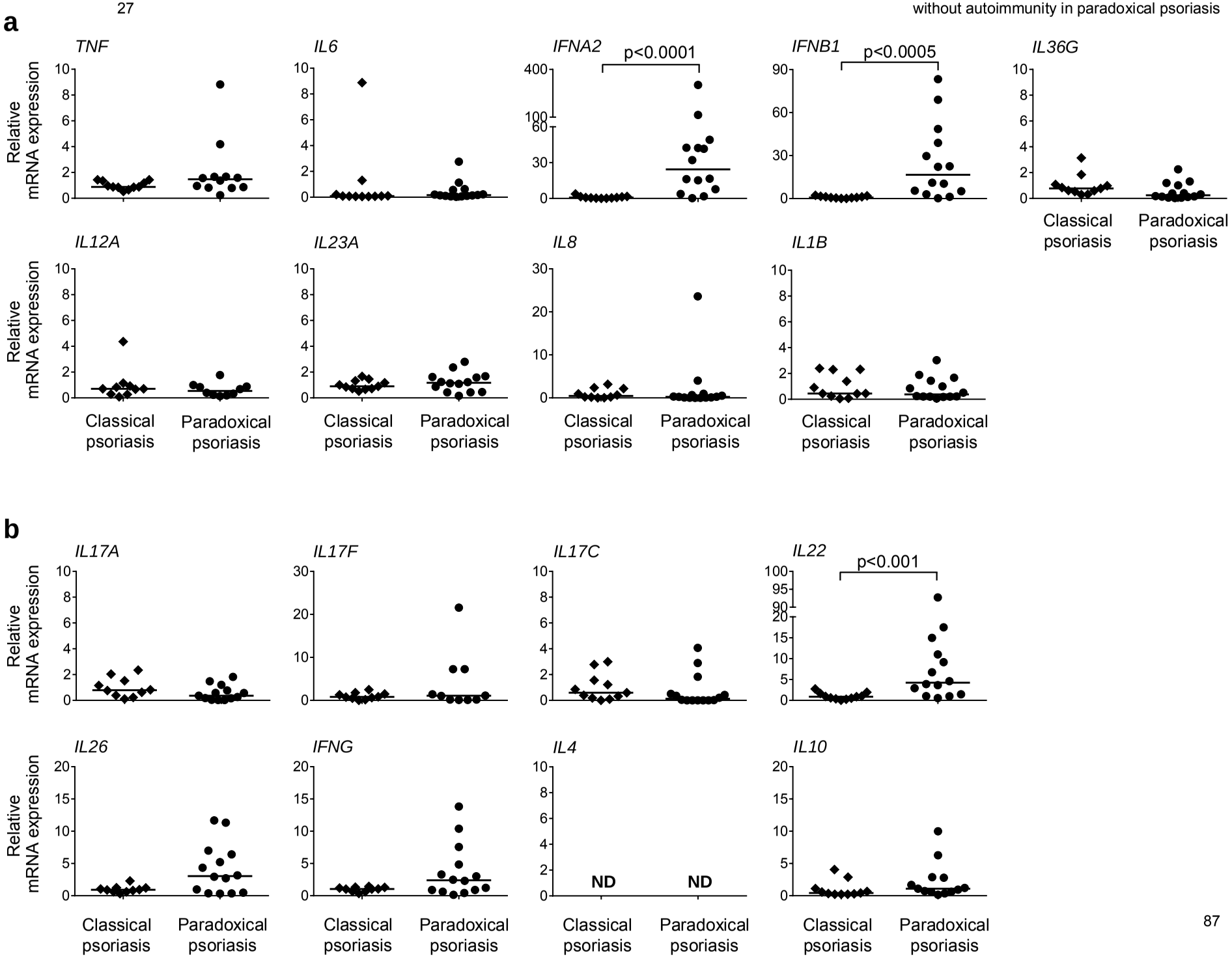

a
TNF
IL6
IFNA2
p<0.0001
IFNB1
p<0.0005
IL36G
IL12A
IL23A
IL8
IL1B
Relative mRNA expression
Classical psoriasis
Paradoxical psoriasis
b
IL17A
IL17F
IL17C
IL22
p<0.001
IL26
IFNG
IL4
IL10
ND
ND

Fig. 2. Anti-TNF leads to increased type I interferon production in skin lesions of paradoxical psoriasis. (**a**) mRNA expression analysis of proinflammatory cytokines *TNF*, *IL6*, *IFNA2*, *IFNB1*, *IL36G*, *IL12A*, *IL23A*, *IL8 (CXCL8)*, and *IL1B* relative to *GAPDH* in skin lesions of paradoxical psoriasis compared to classical plaque psoriasis. (**b**) mRNA expression analysis of adaptive T cell-derived cytokines *IL17A*, *IL17F*, *IL17C*, *IL22*, *IL26*, *IFNG*, *IL4*, and *IL10* relative to *GAPDH* in skin lesions of paradoxical psoriasis as compared to classical plaque psoriasis. *Dots represent individual patient and horizontal bar denotes the median value. Data shown as mRNA expression level relative to mean expression in classical psoriasis (mean value for classical psoriasis was set at 1). Statistical analysis was performed with unpaired non parametric Mann-Whitney U test. ND = not detected.*

Figure 3

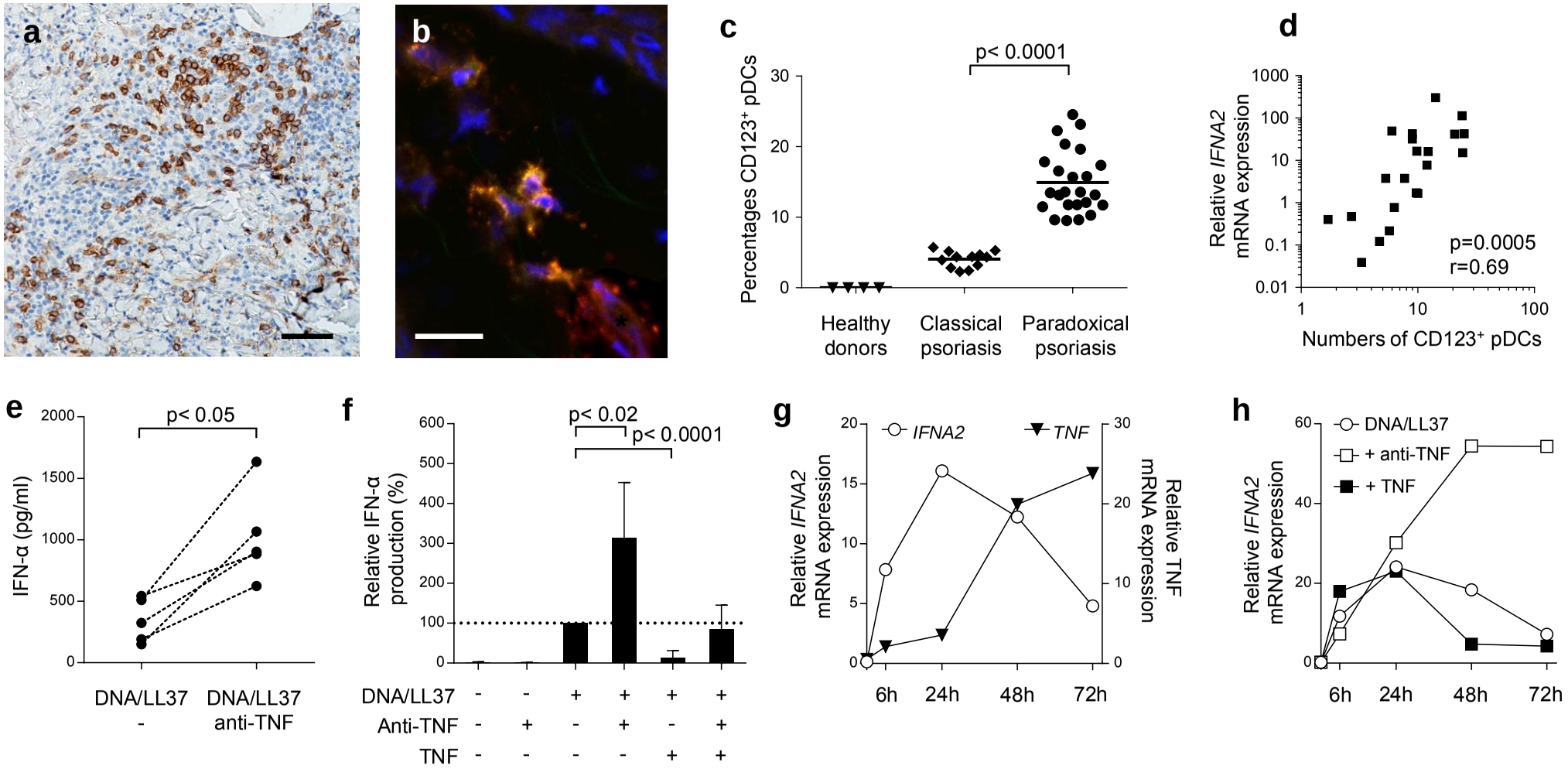


Fig. 3. Anti-TNF leads to dermal accumulation of plasmacytoid dendritic cells and increases and extends their type I interferon production. (**a**) Representative immunohistochemical CD123 (IL3RA)-staining of skin from a patient with paradoxical psoriasis. (**b**) Representative confocal laser scanning microscopy of paradoxical psoriasis stained for BDCA2 (CD303, green), CD123 (red), and DAPI (blue) shows plasmacytoid dendritic cells (pDCs) costaining for BDCA2 and CD123 (yellow) and CD123 single-positive endothelial cells (*, red) (**c**) Histological quantification of CD123-positive pDCs per total dermal infiltrate in skin from healthy donors, psoriasis, and paradoxical psoriasis. (**d**) Correlation of numbers of CD123-positive pDCs with gene expression of *IFNA2*. (**e**) IFN-α produced by pDCs enriched from peripheral blood mononuclear cells of healthy volunteers 48 hours after stimulation with DNA/LL37 complexes with or without addition of anti-TNF antibodies. (**f**) Relative amount of IFN-α produced by pDCs from healthy volunteers at 48 hours, unstimulated or upon stimulation with DNA-LL37 complexes with or without anti-TNF antibodies, with or without addition of TNF. (**g**) Relative *IFNA2* and *TNF* mRNA expression by pDCs isolated from healthy volunteers, stimulated with DNA/LL37, and kept in culture for 6, 24, 48, or 72 hours respectively. (**f**) Relative *IFNA2* mRNA expression by pDCs from healthy volunteers 6, 24, 48, and 72 hours upon stimulation with DNA/LL37 complexes either with anti-TNF antibodies or addition of TNF. *Dots represent individual patient/healthy donor (**c**,**d**) and horizontal bar denotes the mean value (**c**). Data in (**f**) depicted as relative expression (percentage) over amount of IFN-α produced upon stimulation with LL37/DNA (set at 100%); data shown as mean +/- S.D. of six independent experiments with blood from six healthy volunteers (for DNA/LL37 + anti-TNF + TNF; n=3). Data in (**g, h**) depicts one representative of four independent experiments with cells from four different healthy individuals. Scale bars represent 40μm in (**a**), 20μm in (**b**). Statistical analysis was performed in (**c**) with unpaired Student's t-test and in (**e, f**) with paired Student's t-test, in (**d**) the Spearman's rank-correlation coefficient was calculated.*

Figure 4

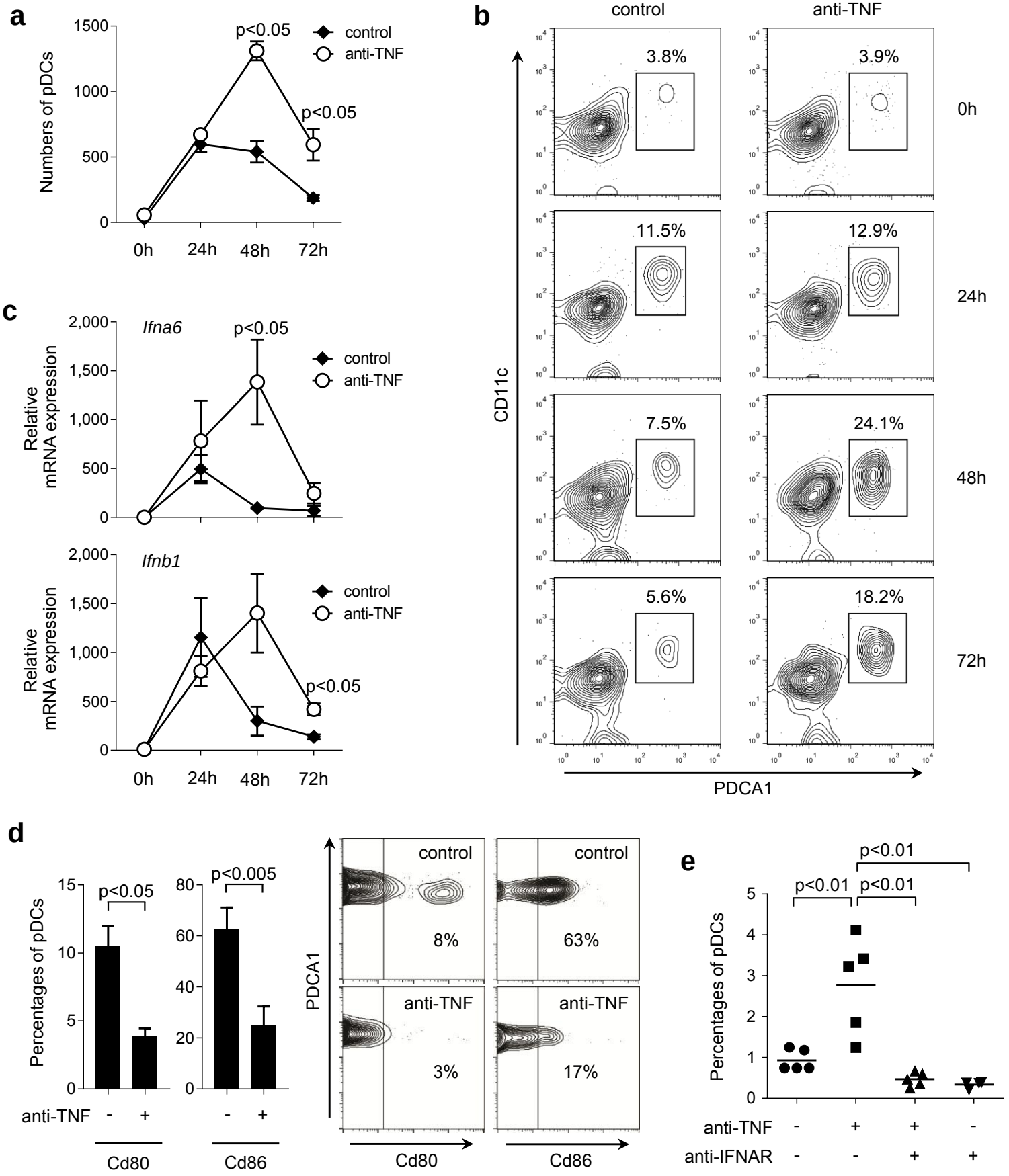

a
Numbers of pDCs
1500
1000
500
0
p<0.05
p<0.05
control
anti-TNF
0h
24h
48h
72h
b
control
anti-TNF
3.8%
3.9%
11.5%
12.9%
7.5%
24.1%
5.6%
18.2%
0h
24h
48h
72h
CD11c
PDCA1
c
Ifna6
Ifnb1
Relative mRNA expression
2,000
1,500
1,000
500
0
p<0.05
p<0.05
control
anti-TNF
0h
24h
48h
72h
d
Percentages of pDCs
15
10
5
0
p<0.05
80
60
40
20
0
p<0.005
anti-TNF - + - +
Cd80
Cd86
PDCA1
control
8%
63%
anti-TNF
3%
17%
Cd80
Cd86
e
Percentages of pDCs
p<0.01
p<0.01
p<0.01
5
4
3
2
1
0
anti-TNF - + + -
anti-IFNAR - - + +

Fig. 4. Anti-TNF increases plasmacytoid dendritic cell infiltration and type I interferon production in vivo. (**a**) Plasmacytoid dendritic cell (pDC) numbers infiltrating the skin upon mechanical injury of the back of mice treated with or without anti-TNF. pDCs quantified by flow cytometry at indicated time points. (**b**) One representative contour plot for each group at indicated time points. (**c**) Total skin mRNA expression of the type I interferons *Ifna6* and *Ifnb1* upon mechanical injury of mice treated with or without anti-TNF at indicated time points. (**d**) Expression of co-stimulatory molecules Cd80 and Cd86 on skin infiltrating pDCs 48 hours after mechanical skin injury of mice treated with or without anti-TNF. (**e**) Percentage of pDCs infiltrating the skin of mice upon mechanical injury in the presence or absence of anti-TNF and/or anti-IFNAR antibodies. *Experiment depicted in (**a, c**) is representative for at least 3 independent experiments using at least 3 mice per group. Bar charts in (**d**) show mean values plus S.E.M. of 6 mice, with pDCs from skin of 2 mice pooled for each data point; one representative contour plot for each group (2 mice pooled) is depicted in the right panel (**d**). All statistical analyses were performed with unpaired Student's t-test.*

Figure 5

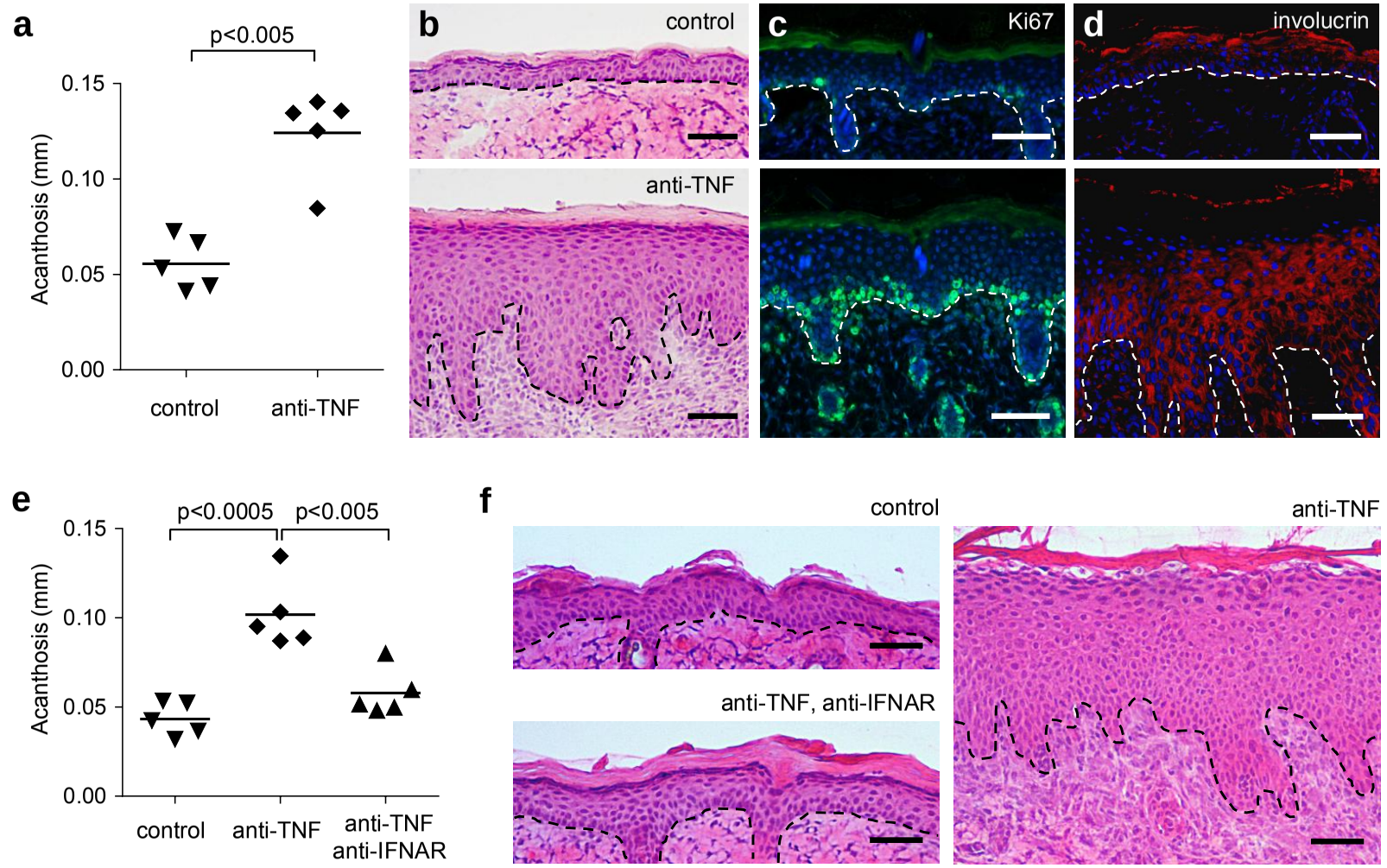


Fig. 5. In a skin injury mouse model, anti-TNF induces a psoriasis-like skin phenotype via type I interferon. (**a**) Quantification of epidermal thickening (acanthosis) in mice treated with or without anti-TNF antibody 7 days after mechanical injury by tape stripping. Representative HE-staining (**b**), immunofluorescence staining for Ki-67 (proliferation, **c**), and involucrin (differentiation, **d**) for both untreated and anti-TNF-treated mice 7 days after mechanical injury. (**e**) Quantification of acanthosis 7 days after mechanical injury of mice treated with or without anti-TNF, with or without anti-IFNAR antibodies. (**f**) Representative HE-staining of skin 7 days after mechanical injury of untreated mice and mice treated with anti-TNF antibody alone or anti-TNF and anti-IFNAR antibodies combined. *Experiment depicted is representative for at least 3 independent experiments. Scale bars represent 50μm. Dashed line (**b-d,f**) represents border between epidermis above and dermis below. All statistical analyses were performed with unpaired Student's t-test. anti-IFNAR = anti-type I interferon receptor-antibody.*

Figure 6

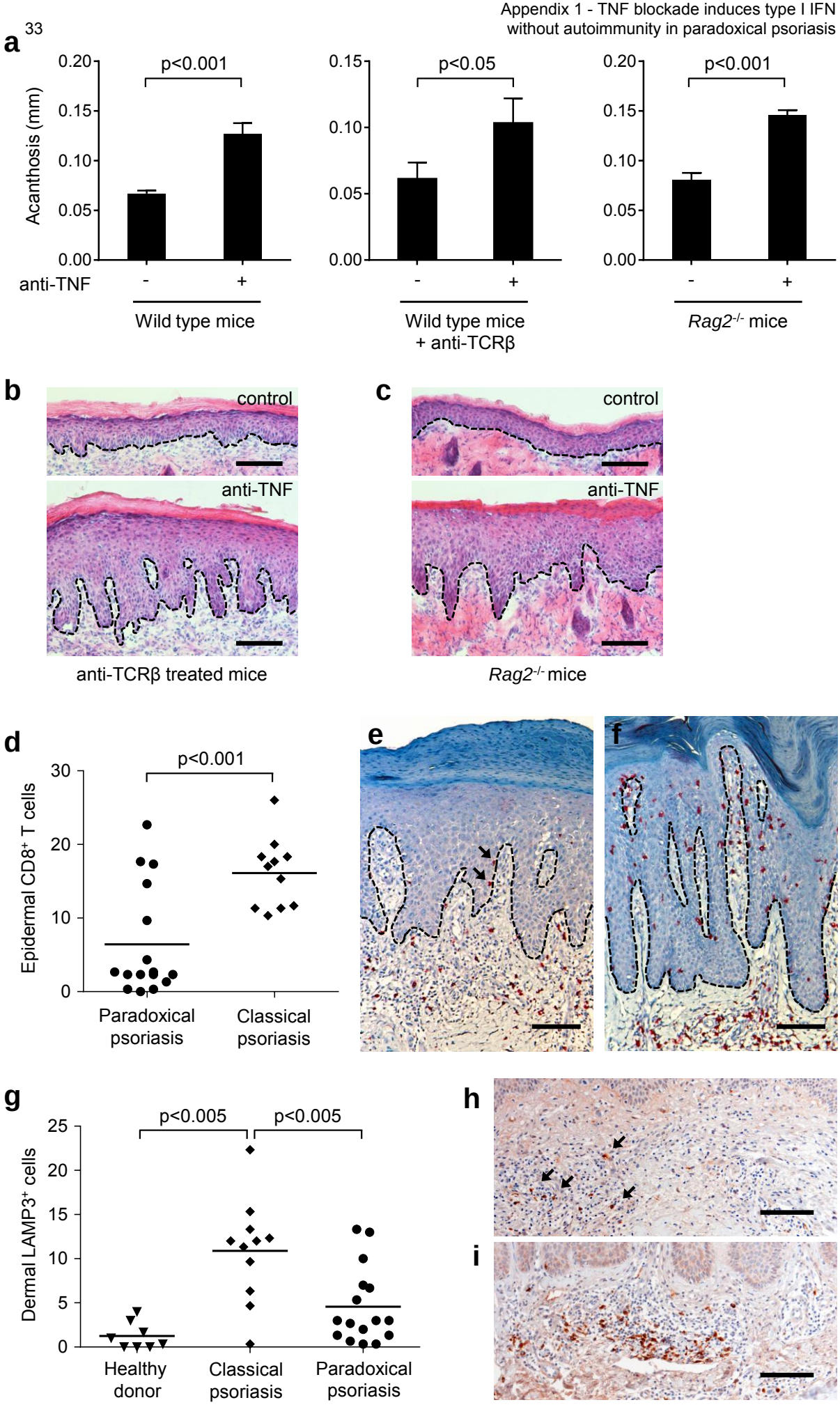
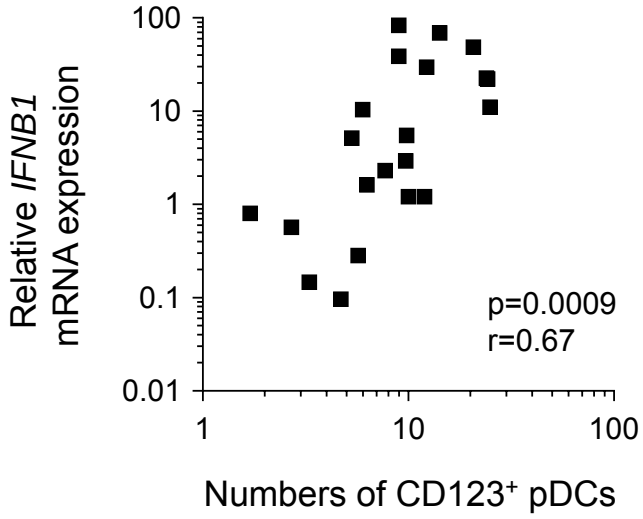
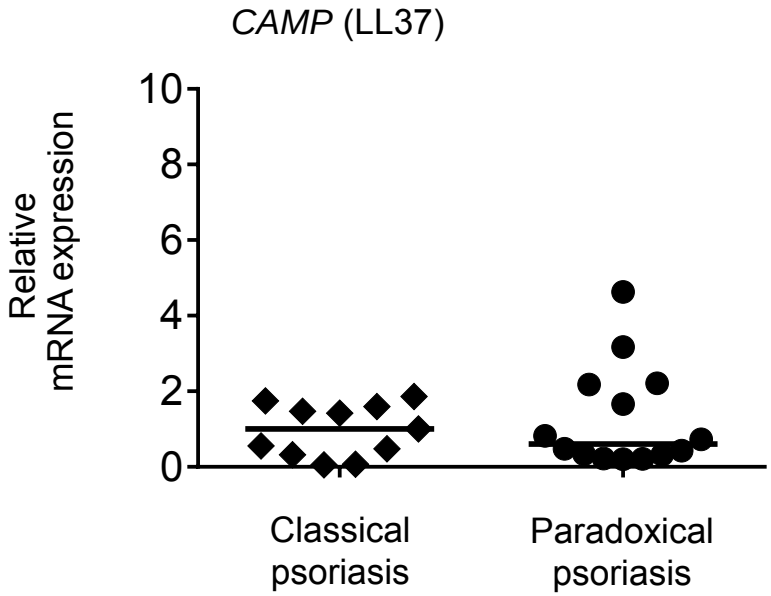

a
33
Acanthosis (mm)
p<0.001
p<0.05
p<0.001
anti-TNF
Wild type mice
Wild type mice
+ anti-TCRβ
Rag2-/- mice
b
control
anti-TNF
anti-TCRβ treated mice
c
control
anti-TNF
Rag2-/- mice
d
Epidermal CD8+ T cells
p<0.001
Paradoxical psoriasis
Classical psoriasis
e
f
g
Dermal LAMP3+ cells
p<0.005
p<0.005
Healthy donor
Classical psoriasis
Paradoxical psoriasis
h
i

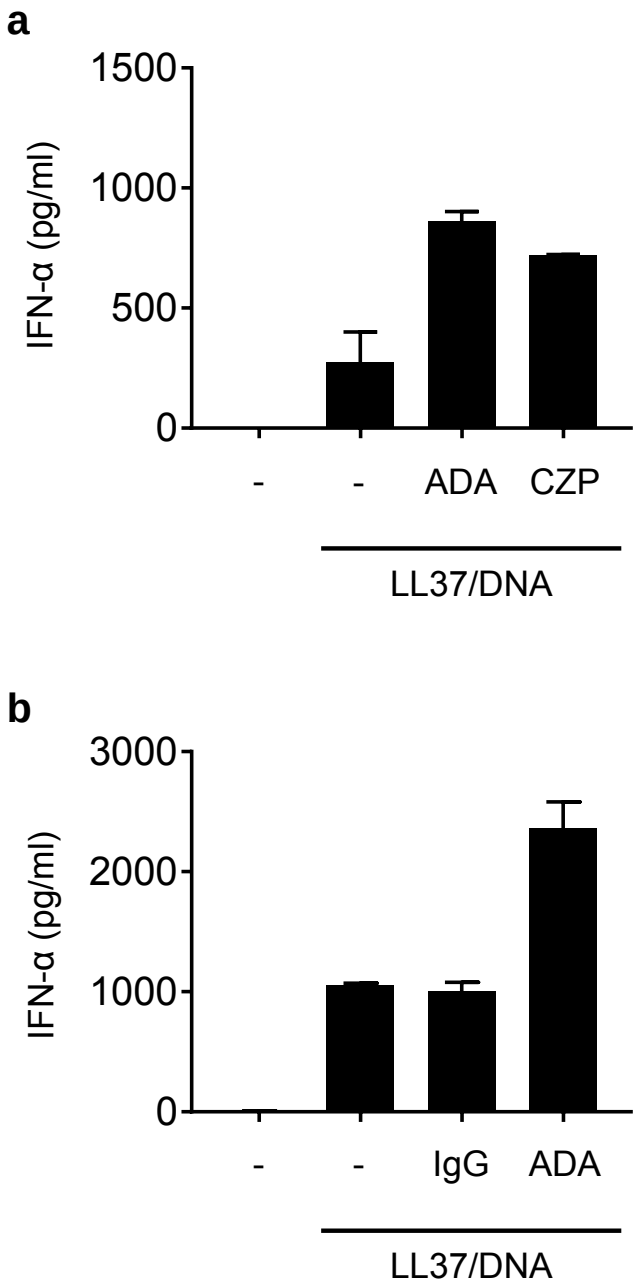
Fig. 6. Induction of paradoxical psoriasis is independent of conventional T cells. (**a**) Quantification of acanthosis 7 days after mechanical injury of wild type mice, wild type mice treated with anti-TCRβ antibody, and *Rag2*$^{-/-}$ mice, all of which were treated with or without anti-TNF antibody. (**b**) Representative HE-staining of skin 7 days after mechanical injury of mice treated with anti-TCRβ antibody alone or anti-TCRβ and anti-TNF antibodies combined. (**c**) Representative HE-staining of skin 7 days after mechanical injury of *Rag2*$^{-/-}$ mice treated with or without anti-TNF antibody. (**d**) Number of epidermal CD8^{+} T cells per high-power field in skin lesions of patients with classical psoriasis and paradoxical psoriasis. (**e**,**f**) Representative CD8-staining of paradoxical psoriasis (**e**) and classical psoriasis (**f**). (**g**) Number of dermal LAMP3^{+} cells per high-power field in skin of healthy donors as well as in skin lesions of patients with classical psoriasis and paradoxical psoriasis. (**h**,**i**) Representative LAMP3-staining of paradoxical psoriasis (**h**) and classical psoriasis (**i**). *Experiment depicted (in **a**-**c**) is representative for 2 independent experiments. Bar charts in (**a**) show mean values plus S.E.M. of 5 mice each group. Dashed line in (**b**,**c**,**e**,**f**,**h**,**i**) represents border between epidermis above and dermis below. Arrows point at intraepidermal CD8^{+} T cells in paradoxical psoriasis (**e**) or dermal LAMP3^{+} cells (**h**) respectively. Scale bars represent 100μm in (**b**,**c**,**e**,**f**,**h**,**i**). All statistical analyses were performed with unpaired Student's t-test. anti-TCRβ = anti-T cell receptor beta chain-antibody.*



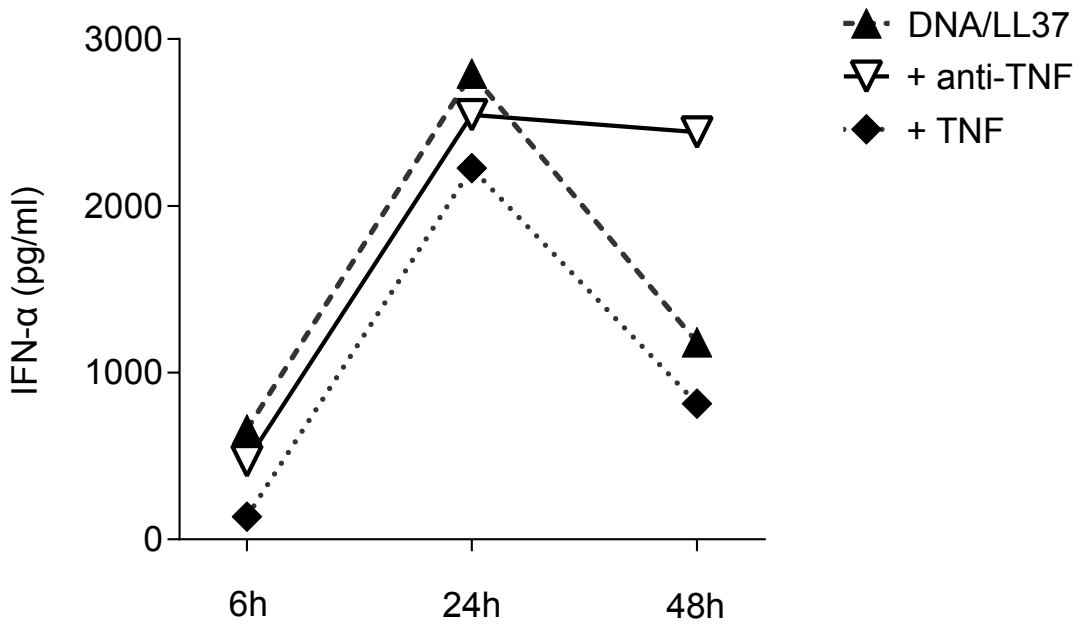
Supplementary Figure 1: Correlation of plasmacytoid dendritic cells and IFNβ. Correlation of numbers of CD123-positive plasmacytoid dendritic cells with gene expression of *IFNB1* in skin lesions of paradoxical psoriasis. Dots represent individual patient. For statistical analysis, the Spearman's rank-correlation coefficient was calculated. pDCs = plasmacytoid dendritic cells.



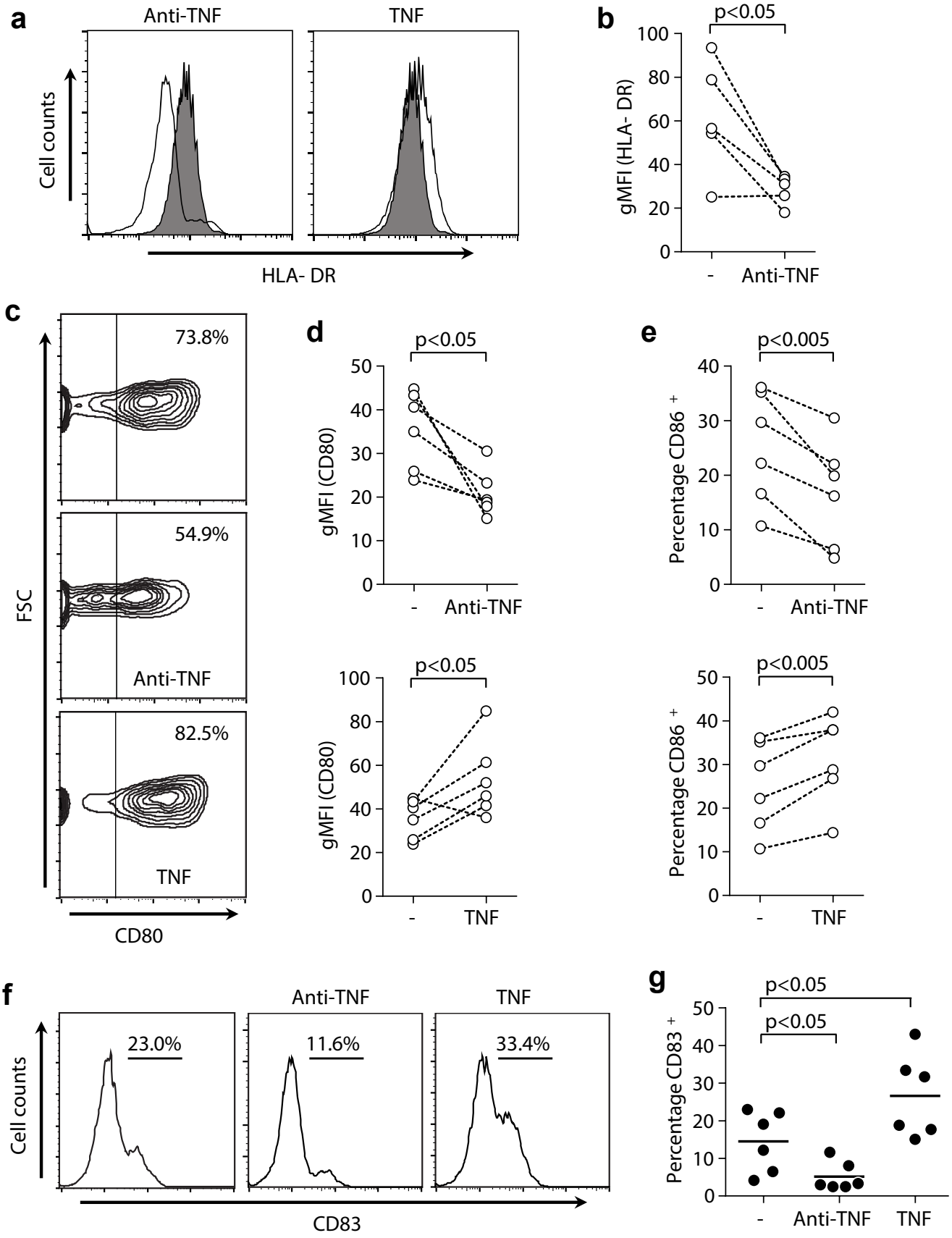
Supplementary Figure 2: CAMP expression in classical and paradoxical psoriasis. mRNA expression analysis of *CAMP* (LL37) relative to GAPDH in skin lesions of paradoxical psoriasis compared to classical plaque psoriasis. Dots represent individual patient and horizontal bar denotes the median value. Data shown as mRNA expression level relative to mean expression in classical psoriasis (mean value for classical psoriasis was set at 1). Statistical analysis was performed with unpaired non parametric Mann-Whitney U test. CAMP = cathelicidin antimicrobial peptide.



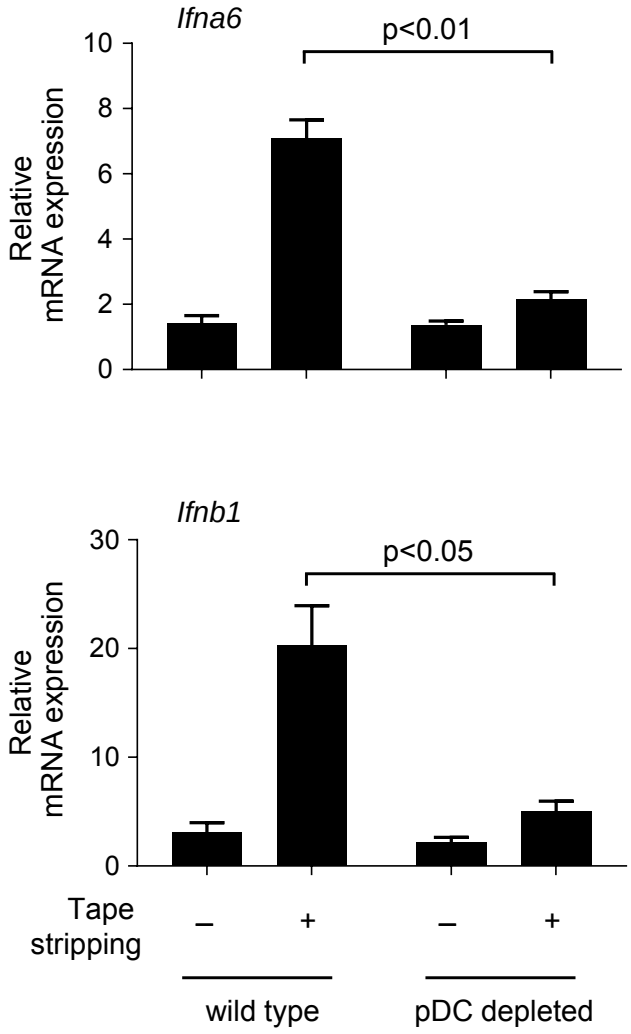
Supplementary Figure 3: Promotion of IFN-α by different anti-TNF antibodies. IFN-α produced by plasmacytoid dendritic cells isolated from healthy volunteers, 48 hours upon stimulation with DNA-LL37 complexes with or without **(a)** anti-TNF agents – either a monoclonal antibody (adalimumab) or a FC-free antigen-binding fragment (Fab') of a monoclonal antibody (certolizumab pegol) or **(b)** an irrelevant human IgG antibody or a monoclonal anti-TNF antibody (adalimumab). Data depicts one representative experiment of five (a) or three (b) independent experiments with cells from different healthy volunteers for each experiment. ADA = adalimumab (Humira®), CZP = certolizumab pegol (Cimzia®). Error bars in represent S.D. of duplicate wells.



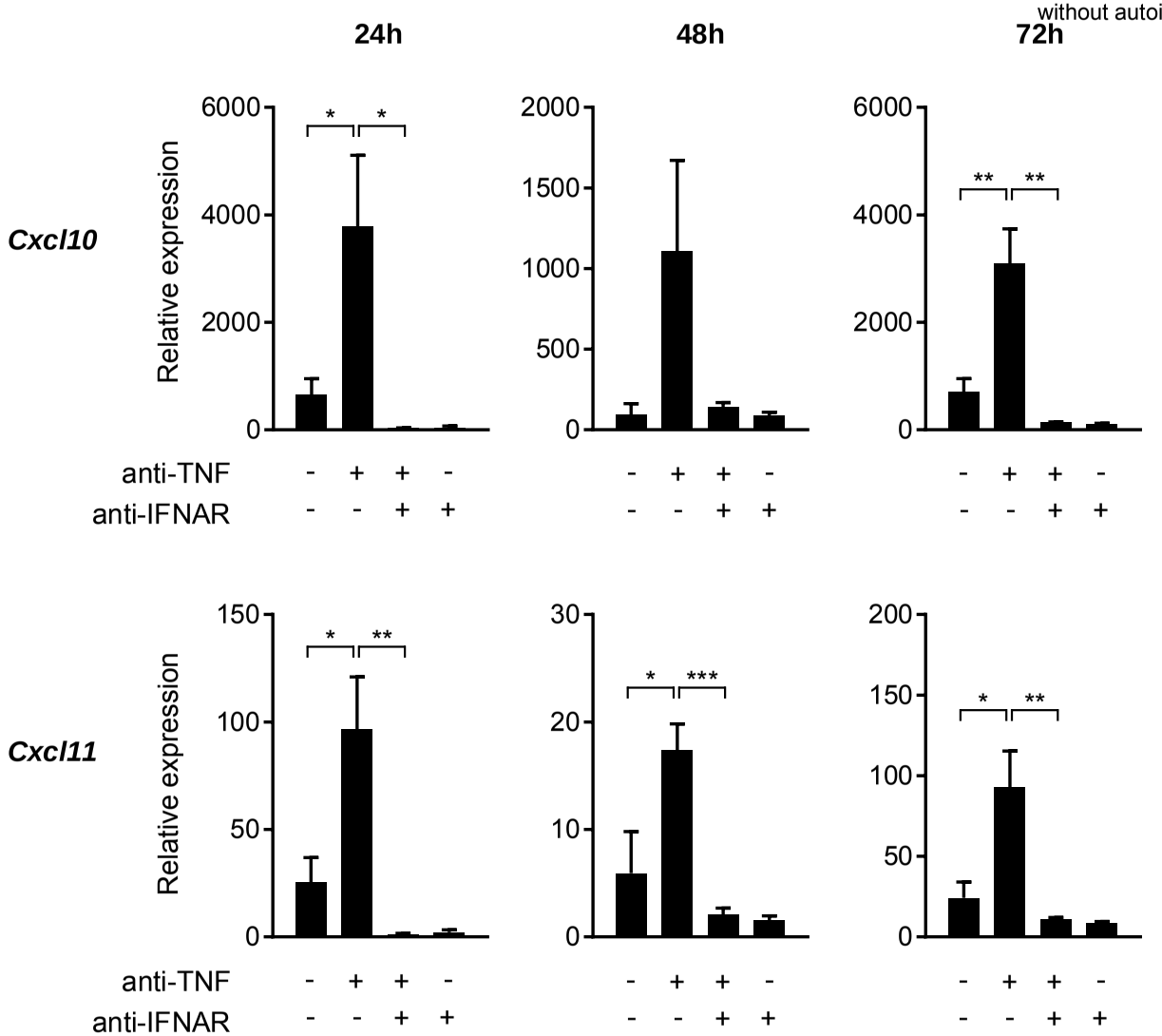
Supplementary Figure 4: Extended IFN-α production promoted by anti-TNF. IFN-α produced by plasmacytoid dendritic cells isolated from healthy volunteers and kept in culture for 6 hours, 24 hours or 48 hours respectively, upon stimulation with DNA-LL37 complexes either with anti-TNF antibodies or addition of TNF. Data depicts one representative of three independent experiments with cells from three different healthy individuals.



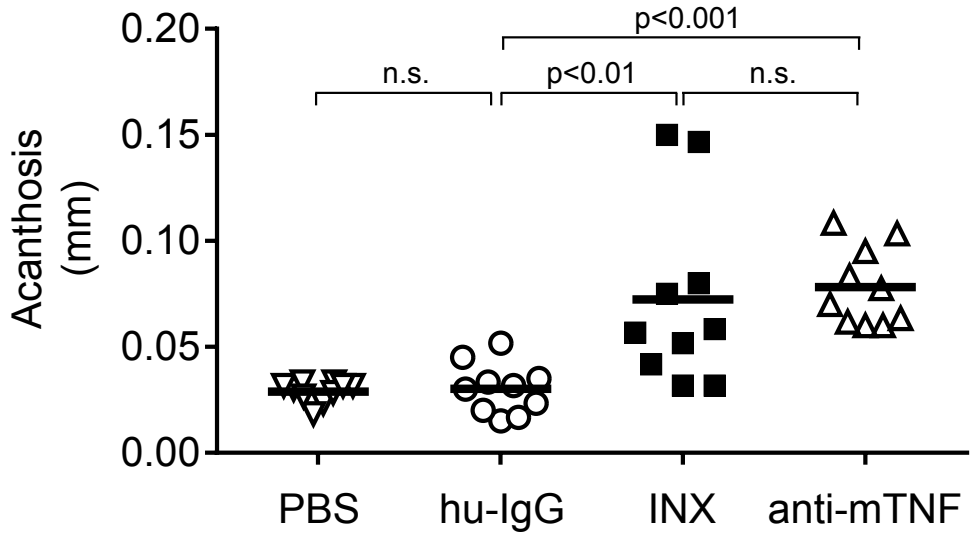
Supplementary Figure 5: Inhibition of plasmacytoid dendritic cell-maturation by anti-TNF. HLA-DR (CD 74) expression on plasmacytoid dendritic cells isolated from peripheral blood of healthy volunteers 48 hours upon activation with DNA-LL 37 complexes with (black line) or without anti-TNF antibodies (gray shaded area), with (black line) or without TNF (**a**, **b**). Expression of co-stimulatory molecules CD 80 (**c**, **d**) and CD 86 (**e**) as well as maturation marker CD 83 (**f**, **g**) on plasmacytoid dendritic cells isolated from healthy volunteers 48 hours after stimulation with 1µM CpGB in the presence or absence of anti-TNF or TNF. Experiments depicted in (**a**, **c**, and **f**) are representative for at least 5 independent experiments, each with blood from different healthy volunteers. Statistical analyses were performed with paired Student's t-test. gMFI = geometric mean fluorescence intensity, FSC = forward scatter.



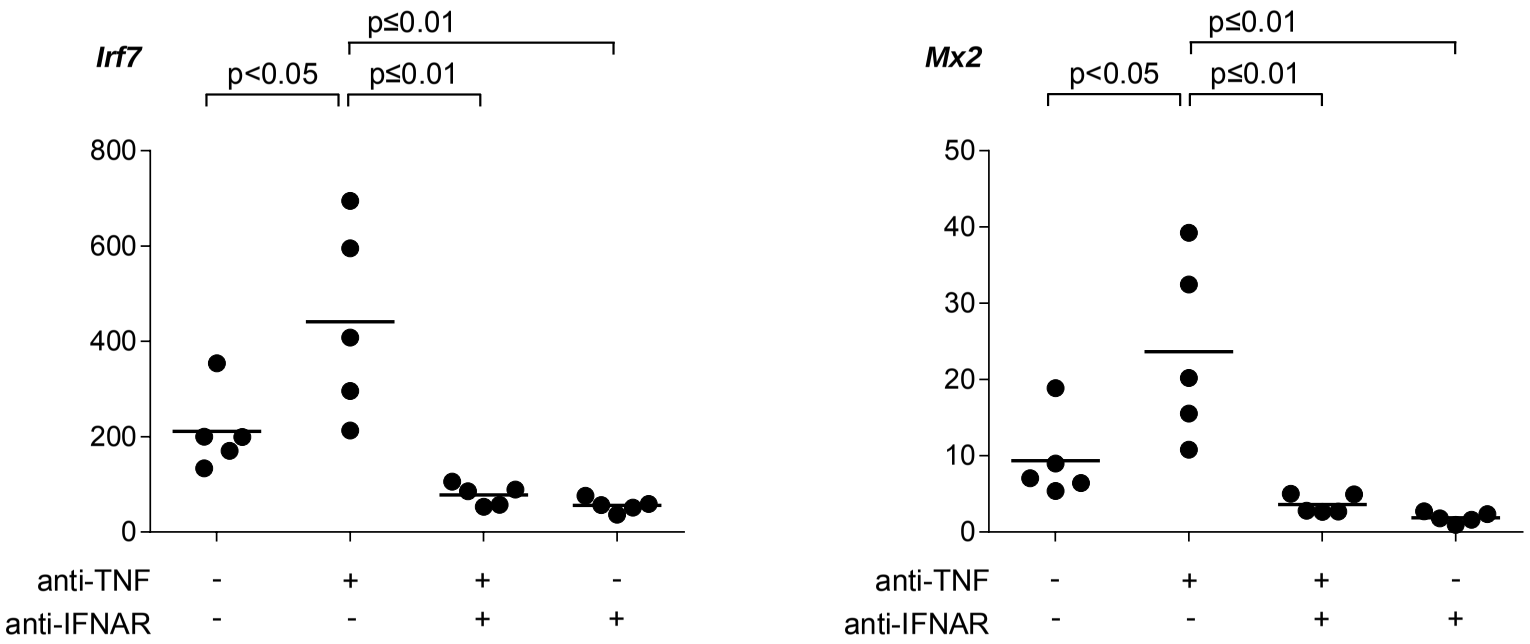
Supplementary Figure 6: Plasmacytoid dendritic cell-dependent type I interferon overexpression. Gene expression of the type I interferons *Ifna6* and *Ifnb1* in anti-TNF-treated mice in uninjured skin and upon mechanical injury in the presence or absence of plasmacytoid dendritic cells. The mean ± SEM is given and represent data from three mice. Experiment depicted is representative for at least 2 independent experiments. Statistical analyses were performed with unpaired Student's t-test. pDC = plasmacytoid dendritic cells.



Supplementary Figure 7: Expression of CXCR3-ligands dependent on type I interferons. Gene expression of chemokines *Cxcl10* and *Cxcl11* in the skin of mice treated with or without anti-TNF and/or anti-IFNAR antibodies 24 hours, 48 hours, and 72 hours after mechanical injury. The mean ± SEM is given and represent data from five mice. Experiment depicted is representative for at least 2 independent experiments. Statistical analyses were performed with unpaired Student's t-test. anti-IFNAR = anti-type I interferon receptor antibody. * $p<0.05$, ** $p\leq0.01$; ** $p\leq0.001$.



Supplementary Figure 8: Similar skin phenotype induced by anti-murine anti-TNF. Quantification of epidermal thickening (acanthosis) in mice treated with PBS, irrelevant human IgG antibody, the anti-TNF antibody infliximab (Remicade®), or an anti-mouse anti-TNF antibody 7 days after mechanical injury by tape stripping. Statistical analyses were performed with unpaired Student's t-test. PBS = phosphate buffered saline, hu-IgG = irrelevant human IgG antibody, INX = infliximab, anti-mTNF = anti-murine anti-TNF antibody, n.s. = not significant



Supplementary Figure 9: Blockade of type I interferon-induced gene expression by anti-IFNAR. Gene expression of the type I interferon response genes *Irf7* and *Mx2* in the skin of mice treated with or without anti-TNF and/or anti-IFNAR antibodies 7 days after mechanical injury. Statistical analyses were performed with unpaired Student's t-test. anti-IFNAR = anti-type I interferon receptor antibody.

Supplementary Table 1: Characteristics of the Patients with Paradoxical Psoriasis Induced by Anti-TNF Therapy.

Characetristic	Age (years)	Sex	Diagnosis/Indication for anti-TNF	Anti-TNF agent	Duration of anti-TNF therapy until onset of paradoxical psoriasis	Localization**	Anti-TNF stopped or interrupted	Re-introduction of ant-TNF	Anti-TNF agent	Relapse	Therapy of paradoxical psoriasis***
Patient 1	22	M	Crohn's disease	Infliximab	18 months	B	Yes	Yes	Certolizumab	No	TS, TCI, SS
Patient 2	66	F	Plaque psoriasis and psoriatic artrithis	Adalimumab	2 months	P, B	Yes	Yes	Adalimumab	**Yes**	TS, SR, ustekinumab
Patient 3	36	M	Ankylosing spondylitis	Infliximab	5 months	P	No	Yes (anti-TNF continued)	Infliximab, Adalimumab	**Yes**	TS
Patient 4	48	F	Ankylosing spondylitis	Golimumab	2 months	P	Yes	Yes	Etanercept	No	TS, PUVA
Patient 5	68	F	Ankylosing spondylitis	Adalimumab	18 months	S, P, B	Yes	Yes	Etanercept	**Yes**	TS, PUVA, CsA
Patient 6	20	F	Crohn's disease	Infliximab	11 months	S, I	No	Yes (anti-TNF continued)	Infliximab	**Yes**	TS
Patient 7	60	M	Ankylosing spondylitis	Infliximab	8 months	B	No	Yes (anti-TNF continued)	Infliximab, Golimumab	No	TS
Patient 8	27	M	Crohn's disease	Infliximab	5 months	P	No	Yes (anti-TNF continued)	Infliximab, Adalimumab	No	TS, SR
Patient 9	70	M	Rheumatoid arhritis	Adalimumab	5 months	B	Yes	Yes	Etanercept	**Yes**	TS, SS
Patient 10	65	F	Plaque psoriasis and psoriatic arthritis	Etanercept	6 months	S, B	Yes	Yes	Etanercept	**Yes**	TS, SS, HXC, CsA
Patient 11	45	M	Ankylosing spondylitis	Adalimumab	3 years	S, I, B	Yes	Yes	Adalimumab	**Yes**	TS
Patient 12	39	F	Ankylosing spondylitis	Infliximab	2 months	S, P	Yes	No	None	No	TS
Patient 13*	64	F	Plaque psoriasis and psoriatic arthritis	Adalimumab	5 years	S, I, B	Yes	No	None	No	TS
Patient 14*	33	F	Crohn's disease	Adalimumab	24 months	I, B	Yes	No	None	No	TS
Patient 15	73	M	Plaque psoriasis	Etanercept	3 months	S, I, B	Yes	No (switch of class to ustekinumab)	None	No	PUVA
Patient 16	36	F	Palmoplantar psoriasis and psoriatic arthritis	Golimumab	3 months	S, P, B	Yes	No (switch of class to ustekinumab)	None	No	TS, PUVA, HXC
Patient 17	57	F	Plaque and palmoplantar psoriasis	Adalimumab	2 months	P, B	Yes	No	None	No	TS
Patient 18	42	F	Crohn's disease	Certolizumab	2 months	P, B	Yes	No	None	No	TS, TCI, SS, CsA
Patient 19	33	M	Plaque psoriasis and psoriatic arthritis	Infliximab	7 months	P, B	Yes	No	None	No	TS, TCI, MTX, CsA
Patient 20	55	F	Ankylosing spondylitis	Infliximab	2 months	S, P, I, B	Yes	No	None	No	TS, TCI, MTX
Patient 21	57	F	SAPHO/psoriatic arthritis	Adalimumab	3 weeks	S, P, B	Yes	No	None	No	TS, MTX
Patient 22	23	F	Crohn's disease	Infliximab	6 months	S, P, B	Yes	No	None	No	TS, SS, MTX
Patient 23	41	F	Ankylosing spondylitis	Infliximab	3 months	S, P, B	Yes	No	None	No	TS, SS, PUVA, UVB, CsA
Patient 24	15	F	Juvenile rheumatoid arhritis	Adalimumab	5 months	S, P, B	Yes	No	None	No	TS
Patient 25	25	F	Plaque psoriasis	Adalimumab	4 months	S, B	Yes	No	None	No	TS, CsA

* History of possible previous paradoxical psoriasis (infliximab and adalimumab respectively)

** S denotes scalp, P palmoplantar, I inverse, B rest of the body

*** TS denotes topical steroids, TCI topical calcineurin inhibitors, SS systemic steroids, SR systemic retinoids, PUVA psoralen + UVA therapy, CsA cyclosporine A, HXC hydroxychloroquine, MTX methotrexate

Supplementary Table 2: quantitative polymerase chain reaction (qPCR) probes.	
Human probes	**Mouse probes**
TNF: Hs00174128_m1	*Ifna6*: Mm01703458_s1
IL6: Hs00174131_m1	*Ifnb1*: Mm00439552_s1
IFNA2: Hs00265051_s1	*Cxcl10*: Mm00445235_m1
IFNB1: Hs01077958_s1	*Cxcl11*: Mm00444662_m1
IL36G: Hs00219742_m1	*Irf7*: Mm00516793_g1
IL12A: Hs01073447_m1	*Mx2*: Mm00488995_m1
I*L23A*: Hs00372324_m1	
IL8: Hs00174103_m1	
IL1B: Hs01555410_m1	
IL17A: Hs00174383_m1	
IL17F: Hs01028648_m1	
IL17C: Hs00171163_m1	
IL22: Hs01574154_m1	
IL26: Hs00218189_m1	
IFNG: Hs00989291_m1	
IL4: Hs00174122_m1	
IL10: Hs00961622_m1	

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Rosacea-associated bacteria activate plasmacytoid dendritic cell-derived type-I interferon driving flare-ups of disease

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One Sentence Summary: Flare-ups of rosacea are driven by type-I interferon production by pDCs which can be triggered by commensal bacteria, thus providing novel therapeutic targets.

Abstract: Rosacea is a chronic skin inflammatory disease characterised by recurrent flare-ups, but it is unclear how they are induced. Microbial pattern recognition is thought to trigger flare-ups, and is known to induce potent innate antimicrobial responses. Cathelicidin antimicrobial peptides have been identified in rosacea, and are aberrantly processed by Kallikrein 5 proteases leading to potent pro-inflammatory responses. Mature cathelicidin LL-37 is known to activate plasmacytoid dendritic cells (pDCs) to produce large amounts of type-I interferons (IFN-I) via internalisation of exogenous nucleic acids. We find overexpression of IFN-I specifically during flare-ups of rosacea, with concomitant pDC infiltration. T_H1, T_H17 and T_H22 signature genes are elevated across the entire cohort, irrespective of active flare or stabilised lesions. Using an intradermal injection model of rosacea, we find that pDCs are the major producers of IFN-I and that, as with blockade of IFN-I, there is a substantial reduction of T_H17-related cytokines and a loss of IL22. Moreover, we find that *Bacillus oleronius*, a bacterium-associated and infesting lesions of rosacea, is most sensitive to cathelicidin-mediated killing and a potent activator of pDCs and IFN-I production both *in vitro* and *in vivo*, suggesting that it may be a trigger of IFN-I driven flare-ups of rosacea. Furthermore, we find that commensal skin bacteria are required for induction of type-I interferons *in situ*, and that cathelicidin-killed *B.oleronius* is sufficient to drive type-I interferon expression. Among rosacea-associated cathelicidins, we find that FR-29 is more potent than LL-37 for nucleic acid binding, internalisation into pDCs and bacterial killing, leading to more potent IFN-I production both *in vitro* and *in vivo*. Our observations indicate that aberrant processing of cathelicidins can result in microbial-dependent, pDC-driven flare-ups of rosacea.

Introduction

Rosacea is an inflammatory condition affecting the facial convexities and characterised by recurring flare-ups. Microbial dysbiosis is a defining feature of rosacea, with apparent infestation with *Demodex folliculorum* as one hallmark diagnostic criterion in the vast majority of cases. These commensal mites flourish in hair follicles deep in the dermis of rosacea and are the base of deep inflammatory infiltrates. *Bacillus oleronius* has been identified as the principal bacterium harboured by *D.folliculorum*, and is thought to be shed by the mite following death of the obligate ecto-parasite. This reservoir for bacteria has been suggested to be a trigger for disease and antibiotics are routinely used in the treatment of rosacea.

Microbe-associated molecular patterns are well described to induce antimicrobial peptides for controlling foreign agents. Cathelicidin antimicrobial peptide (CAMP) has been identified as one such antimicrobial agent induced in keratinocytes downstream of bacterial pattern recognition receptors such as TLR2, and undergoing proteolitic processing for activation. Enzyme processed forms of CAMP such as LL-37, FR-29, FA-29 and DI-27 have been identified in lesions of rosacea, but only LL-37 and FA-29 have been described to cause erythema in mice. LL-37 has been shown to be able to bind bacterial as well as human nucleic acids, to allow its condensation, and thus to be able to activate endosomal toll-like receptors-(TLR-) 7 and 9 in plasmacytoid dendritic cells (pDCs). Notably, pDCs produce vast amounts of type-I interferons and have been described to be implicated in the pathogenesis of other inflammatory skin diseases such as psoriasis.

Recently, it has been reported that rosacea has a predominant T_H1, $T_H17/22$ cytokine signature with several chemokines expressed across different stages of rosacea. Flare-ups are thought to be triggered by external stimuli such as from bacteria, but little is known about the pathogenic mechanisms that subsequently drive inflammation during flare-ups. We report that type-I interferons are overexpressed selectively in acute flare-ups of rosacea, and that pDCs are required for this signature. *In vivo* blockade of either IFNAR signalling or pDC depletion result in a loss of the IL22 and T_H17-related cytokine response induced by cathelicidin driven inflammation, as well as of select chemokine families. Among cathelicidin antimicrobial peptides identified in rosacea, we identify FR-29 as being able to activate pDCs to produce type-I interferon more potently than LL-37, through increased nucleic acid binding and pDC internalisation capacity. Surface commensals are necessary for induction of type-I interferons, as topical antibiotics abolish the interferon signature. Finally, we find that killing of the rosacea-associated bacterium *B.oleronius* by cathelicidins is sufficient to drive type-I interferon *in vivo* at the site of inflammation, providing a possible mechanism for initiation of the inflammatory events during acute flare-ups of rosacea.

Results

Type-I interferon overexpression correlates with pDC infiltration in flares of rosacea

Cathelicidin peptides such as LL-37 were identified in lesional rosacea skin.*(1, 2)* As the peptide LL-37 has been described to be an inducer of type-I interferons during the pathogenesis of psoriasis,*(3–7)* we wondered whether there may also be a detectable interferon signature in rosacea. We obtained biopsies from lesions of 8 patients with rosacea during stabilised chronic

inflammation and from 16 others during acute flare-ups. The cathelicidin transcript *hCAP18* was upregulated in all patients with rosacea as compared to healthy donours (Fig.1a), corroborating previous observations.*(1)* Type-I interferon genes are significantly upregulated selectively in patients with active flare-ups of rosacea as compared to healthy donours (*IFNA2*: $p<0.0001$, *IFNB1*: $p<0.0001$). Chronic lesions display low type-I interferon expression, similar to healthy skin, indicating a selective overexpression early during rosacea flare-ups. Interferon-response gene *MX1* was significantly overexpressed as compared to healthy donours ($p<0.0001$), with no significant difference between sub-acute and acute flare-ups ($p=0.6529$), suggesting a previous interferon burst in stabilised lesions.

Given the magnitude of expression of type-I interferons, we investigated the presence of pDCs in lesional skin of rosacea. Paraffin-embedded sections of the same biopsies from the 16 patients taken during acute flare-ups, reveal pDC stainings in the dermis across all patients (Fig.1b) and samples were overlade with positive cells in relation to other infiltrates (Fig.1c). When plotting the percentages of CD123+ cells along with IFNA2 (Fig.1d) and IFNB1 (not shown) we found statistically significant (*IFNA2*: $p=0.0011$, *IFNB1*: $p=0.0002$) monotonic positive correlation between the two (*IFNA2*: $r^2=0.5422$, *IFNB1*: $r^2=0.6322$), indicating that pDCs may be the principal source of type-I interferons during flare-ups of rosacea.

Innate and adaptive cytokines associated with $T_H1/17/22$, but not T_H2, are overexpressed in rosacea

Because type-I interferon genes are strongly overexpressed exclusively and only during acute flare-ups of rosacea, we wondered whether other pro-inflammatory cytokines might be differentially expressed. We assessed conventional pathogenic innate and adaptive cytokine expression. We find a strong T_H1 signature with significant overexpression of *TNF*, *IFNG, IL12B* (Fig.2a and b). There is no detectable expression of the T_H2 cytokine *IL4* and no overexpression of *IL13*. In contrast, T_H17 and T_H22 cytokines *IL17A*, *IL17F* and *IL22* were significantly overexpressed as were cytokines known to be required for their induction including *IL6*, *IL23A*, *IL1B* but not the IL1-family *IL36B*. Among cytokines tested, only *TNF* is significantly underexpressed in acute rosacea flare-ups as compared to stable rosacea (Fig. 2c), in stark distinction to IFNα and β. Other than type-I interferons, only *IL10* displays significant overexpression in acute rosacea flare-ups compared to stabilised lesions (Fig. 2c).

Type-I interferon expression is critically dependent on pDCs in a mouse model of rosacea

The positive correlation between pDCs and type-I interferon expression suggests that pDCs may be responsible for the observed overexpression. To determine whether pDCs might be directly responsible for interferon production during flare-ups of rosacea, we sought to verify if the pDC-IFN axis is relevant in a previously described *in vivo* model of rosacea.*(1)* We find *in situ* pDC recruitment (Fig.3a) and accumulation over time (Fig.3b), which correlate ($r^2=0.992$) significantly ($p=0.004$) for both IFNα and β genes (Pearson correlation). Type-I interferon genes are induced and increase over time, concurrently with pDC infiltration (Fig.3c). Antibody-mediated cell depletion of pDCs negates most induction of interferon α and severely diminishes interferon β gene expression (Fig.3d). Downstream interferon stimulated genes *ISG15* and *IRF7* are largely abolished, with a significant reduction of *MX2* and *IFI202b*. Similar results were achieved using a different pDC depletion system, the BDCA2-DTR transgenic mouse (Suppl. Fig.1). Type-I interferon signalling blockade leads to complete loss of all interferon-response genes, as expected.

Type-I interferon blockade and pDC depletion abolishes select Th1/22 signatures, and CXCR3 and CCR1/5-family chemokines

To understand the contribution of type-I interferon genes and pDCs to inflammatory cytokines, we investigated *in situ* expression of cytokines found overexpressed in rosacea. Inflammation brought on by LL-37 induces strong innate inflammatory cytokines including Th1-polarising cytokines *IL12A* and *TNF*, Th17/22-polarising cytokines *IL1B*, *IL23A*, *IL36B*, and *IL6*, and *IL10* (Fig.4a). Of these, *TNF*, *IL1B*, *IL23A*, and *IL6* were significantly dependent on pDCs and type-I interferon signalling, whereas *IL12A* was selectively dependent on pDCs. *IL36B* and *IL10* were induced independently of type-I interferons and pDCs. Adaptive cytokines of the $T_H17/22$ family *IL17A*, *IL17F* and *IL22* were induced by the cathelicidin LL-37 *in situ*, as were T_H2 cytokines *IL4* and *IL13*, and Th1 cytokine *IFNG* (Fig. 4b). Among these, only IL22, and to a lesser extent IL17F, were highly dependent on pDCs and type-I interferon signalling. Furthermore, several chemokines shown to be overexpressed in rosacea are strictly dependent on IFNAR signalling and pDCs, such as the CXCR3-family chemokines CXCL9, 10 and 11 and to some degree chemokines of the CCR-family CCL2 and CCL5 (Fig.4c). Unexpectedly, CXCL1 is also significantly reduced upon loss of type-I interferon signalling, but not the IL-8 functional homologs in the mouse CXCL2 and CXCL15. Other chemokines described to be upregulated in rosacea do not appear to be dependent on type-I interferon such as CXCL12, CXCL13, CCL19 and CCL20. Strikingly, interferon α/β genes were also abrogated upon IFNAR blockade (Fig.3d), suggesting that interferon requires a self-propagating loop to achieve its full breadth of expression. Moreover, IFNAR blockade led to a significant reduction of pDC infiltration (Suppl. Fig. 2) in line with loss of type-I interferon gene expression. Put together, these results indicate that, during skin innate inflammation induced by cathelicidin LL-37, pDCs are required for type-I interferon expression, and that the latter is necessary for interferon feed-forward mechanisms. Blockade of the IFNAR pathway, both by depletion of pDCs or direct blockade, did not influence by a noticeable degree the observable erythema (Suppl. Fig. 3). Put together, these data suggest that type-I interferon derived from pDCs is an important inducer of IL22 and of known T_H17-polarising cytokines in the skin, potentially via targeted recruitment of specialised cell types, yet this does not considerably affect erythema using concentrations found in chronic disease.

FR-29 induces type-I interferons by strongly binding and complexing DNA more potently than other cathelicidins found in rosacea

It is widely established that LL-37 is able to activate pDCs to produce large amounts of type-I interferons by internalising extracellular nucleic acids. Because cathelicidin is processed into several C-terminal peptides, among which LL-37, FR-29, FA-29 and DI-27 directly identified in rosacea lesions, we wondered whether these had a differential ability to activate pDCs. To address this, we isolated human pDCs which we stimulated with DNA-cathelicidin derived peptide complexes. For the same molar concentration, FR-29 activates pDCs to produce IFNα more potently than LL-37, but only in the presence of DNA (Fig.5a). FA-29 and DI-27 are unable to stimulate detectable amounts of IFNα from pDCs *in vitro*. When reducing the concentration of peptides bellow the stimulatory capability of LL-37 to 3µM, FR-29 still retains, albeit drastically reduced, stimulatory capacity. Concurrently, *in vivo* intradermal injection of FR-29 induces more potent interferon α gene expression than LL-37, for the same molar concentration (Fig. 5b). In contrast, *in vivo* pDC infiltration was similar between FR-29 and LL-37 suggesting that difference is due to differential activatory capacity of the cathelicidin peptides

(Fig. 5c). We assessed the DNA-binding capacity by picogreen fluorescence quenching, and found that for the same concentration of DNA, FR-29 binds DNA more potently than LL-37 (Fig.5d). Conversely, FA-29 has greatly reduced and DI-27 has almost indiscernible DNA binding activity. To assess the capacity of FR-29 and LL-37 to condense and internalise DNA into pDCs, we fluorescently tagged purified, undigested DNA, and complexed it with the cathelicidin peptides LL-37 and FR-29. Whereas the proportion of pDCs that could uptake fluorescently labelled DNA in the absence of cathelicidin peptides was near null, LL-37 and FR-29 both allow the uptake in *ca.* 10% of cells (Fig.5e). However, the relative uptake of DNA was more pronounced when complexed with FR-29 than with LL-37. Taken together, these data indicate that several fragments found in rosacea such as FA-29 and DI-27 cathelicidin peptides cannot activate pDCs, whereas others such as FR-29 have a more potent effect than LL-37 due to an increased affinity to nucleic acids.

Cathelicidin peptides kill bacteria associated with rosacea leading to activation of pDCs

To better elucidate the role of pDCs and antimicrobial cathelicidins in the pathogenesis of rosacea, we wanted to address whether there may be a context-specific source of nucleic acids. Rosacea is known to have an altered microbiome and to be infested particularly with bacteria such as *Bacillus oleronius* and *Staphylococcus epidermidis*. Among cathelicidin peptides tested, we find that FR-29 is most potent for killing of skin-associated bacteria *B.oleronius* and *S.epidermidis*, followed by LL-37 and to a lesser extent FA-29 (Fig.6a). FR-29 is less or equally efficient as LL-37 in the bacterial killing of other bacteria often associated to the gut and the lung such as *E.coli*, *P.aeruginosa*, and *K.pneumoniae* (Fig.6b). DI-27 is mostly unable to impair bacterial growth in all conditions tested. Intriguingly, among bacteria tested, *B.oleronius* is most sensitive to cathelicidin-mediated killing, followed by *S.epidermidis* and *P.acnes* (Fig.6c). pDCs are described to be responsive to antimicrobial peptide-killed bacteria,*(8)* we wondered whether LL-37-killed *B.oleronius* could activate pDCs. For this, we isolated human pDCs from multiple donours and put them in culture with live bacteria with or without LL-37. Whereas intact *B.oleronius* alone is not able to activate pDCs, the addition of LL-37 allows for potent activation of pDCs leading to important production of IFNα (Fig.6d). Concurrently with previous results, FR-29 is able to activate pDCs more potently than LL-37 when in the presence of *B.oleronius*. To understand whether *B.oleronius* is able to induce type-I interferon in the context of aberrant overexpression of LL-37 such as is the case in rosacea, we compare LL-37 alone, *B.oleronius* alone, and B.oleronius in the presence of LL-37 *in vivo*. Whereas B.oleronius alone is not able to induce type-I interferon expression, we find that *B.oleronius* pre-incubated with LL-37 induces significantly more type-I interferon expression (Fig. 6e), suggesting that bacterial load alone is not sufficient to engage the type-I interferon pathway. To elucidate whether commensal bacteria are necessary for induction of type-I interferons by exogenous LL-37, we treated mice with Neosporin® containing wide-spectrum antibiotics for 48h, followed by the standard cathelicidin LL-37 mediated inflammation. We find that type-I interferon expression is significantly reduced upon antibiotic treatment of the skin (Fig. 6f). Interestingly, antibiotic treatment followed by injection of *B.oleronius* pre-incubated with LL-37 leads to strong induction of type-I interferons. Taken together, these data suggest that cathelicidin peptides LL-37 and FR-29 found in rosacea, are able to induce type-I interferons potently in a pDC-reliant manner. *B.oleronius*, found in the lesions of rosacea is a potential source of nucleic acids for pDC activation. Moreover, commensal bacteria are required, and *B.oleronius* is sufficient for type-I interferon induction by cathelicidins.

Discussion

We find that type-I interferons are selectively and uniformly upregulated in patients with rosacea specifically during active flare-ups. Intriguingly, downstream interferon-response genes, which are longer lived than type-I interferons, are found upregulated also in stabilised lesions. This suggests previous interferon induction, thus pointing to the cyclical nature of type-I interferon bursting during the progression of the disease. Fuelled by this observation, we set out to investigate the role and origin of the type-I interferon pathway in rosacea flares. We find that pDCs are actively recruited to the site of inflammation and that they are critically required for type-I interferon expression, and of downstream interferon-response genes. We describe a mechanism by which type-I interferons control Th22 and Th17-polarising cytokine expression *in situ* following cathelicidin LL37-mediated inflammation. Moreover, cathelicidins specifically described in rosacea have differential capacities to activate pDCs in vitro and in vivo, suggesting that the imbalance of cathelicidin processing can have pathogenic repercussions. Finally, we find that commensal bacteria are necessary to induce LL37-mediated type-I interferon induction, and that bacteria found in rosacea, in the presence of cathelicidin peptides, are sufficient to drive the type-I interferon pathway.

Our data indicate a primary role of pDCs in the production of type-I interferons during acute flare-ups of rosacea. Recent elegant studies demonstrate that, during chronic inflammation, keratinocytes can be an important source of IFNβ in the skin.*(9)* Intriguingly, we find that, unlike IFNα, IFNβ expression is not entirely abolished upon pDC depletion. Furthermore, certain interferon response genes such as *MX2*, which are known to be strongly expressed particularly within the epidermis following interferon stimulation, are significantly reduced but not abolished in their entirety. This may indicate that keratinocytes could be a potential source of IFNβ also upon acute inflammation.

Commensal bacteria are known to populate normal skin, but also to often be deregulated in cutaneous disorders. It is well regarded that rosacea has a strong microbial component associated to the disease, in particular as to infestation with demodex mites which harbour commensal bacteria such as *B.oleronius* and *S.epidermidis*. We find that among cathelicidins tested, FR-29 is vastly superior to LL-37 and other cathelicidin fragments in killing of certain bacteria, in particular *B.oleronius* and *S.epidermidis*, but not of other bacteria. This differential killing capacity of cathelicidin peptides may reflect an attempt to control the increase in microbial burden observed in rosacea, providing broad-spectrum antimicrobial specificity. It remains to be determined whether there is a specific microbial detection signal which influences protease activity to generate specific fragments, or whether this is stochastic effect dependent on microbial burden.

We and others have observed a pathogenic role for pDCs that gain access to the skin, for diseases such as psoriasis,*(3, 7)* lupus,*(10)* and scleroderma.*(11)* These skin associated disorders share a major common feature which usually is overt autoimmunity. Rosacea is not generally regarded as an autoimmune disease, and for now only few studies have focused on the genetics of the disease.*(12, 13)* Herein, we propose a mechanism by which the pDC-IFN axis is engaged during flare-ups of disease and can induce innate inflammation which can become self-sustained in predisposed individuals. There is increasing molecular evidence for a progression between stages of rosacea*(14)*, and therefore continuous flare-ups of disease may prime a constantly aggravated inflammatory response. As such, targeting type-I interferon signalling during flare-ups may be a viable target for the treatment of rosacea. Though targeting IFNα has not proven to be successful

in the treatment of chronic plaque-type psoriasis,*(15)* it is now postulated to play a role in the acute rather than chronic phases of psoriasis.*(16)* Furthermore, the difficulty of targeting all type-I interferons might also be responsible for the failure of the trial, with IFNβ not being directly targeted. There is considerable success from trials in lupus, both for selective inhibition of IFNα*(17, 18)* and for indiscriminate IFNα/β blockade through the inhibition of the receptor.*(15, 19)* Additionally, newer biologics are currently being trialled, targeting specifically pDCs (NCT02847598), and these may provide wider type-I interferon inhibition without affecting local antiviral immunity. Our data indicate that pDC blockade is sufficient to inhibit Th17-related cytokines and IL22 induction *in situ*, at the site of inflammation, thus making such approaches relevant for the treatment of rosacea.

Several key questions remain to be addressed as to the similarities and potential differences between rosacea, lupus and psoriasis. For one, it is intriguing that similar mechanisms are involved in diseases with rather different histo-pathological outcome. Genetics may define key differences between these, yet whereas there has been considerable focus on the genetics of psoriasis*(20–22)*, little success has been achieved in capitalising novel landmark discoveries for rosacea.*(12)* It is suggested that association with polymorphisms in intergenic HLA-DRA, B, DQA and DQB regions may be in connection with presentation of antigens from extracellular sources, providing a potential link with microbes. Fundamental differences between psoriasis and rosacea might be related to the composition of the microbial flora, which is well known to influence inflammatory processes. We provide evidence that, in the right circumstances, antimicrobial peptides such as FR-29 in conjunction to infestation with hyper-susceptible bacteria, will lead to potent type-I interferon induction. The presence of *B.oleronius* in the demodex mite likely provides it with protection, allowing it to thrive beyond control out of the reach of the immune system. Its shedding following the normal life-cycle of the mites, or environmental stresses such as UV light, could initiate release of the bacterium, thus provide the nucleic acid content required for the triggering of type-I interferons and flare-ups of disease.

In summary, we find that during flare-ups of rosacea, type-I interferons are robustly overexpressed and derived from pDCs. Using a mouse model of rosacea, we identify that both type-I interferon signalling and pDCs maintain a Th17-favourable environment at the site of inflammation. Aberrantly processed antimicrobial cathelicidins display differential bacterial killing and nucleic acid binding capacities, which dictate increased activation of pDCs both *in vitro* and *in vivo*. Finally, we find that among bacteria tested, *B.oleronius* is many fold more susceptible to cathelicidin-mediated killing, and coupled with its increased numbers in lesions of rosacea, this suggests that it may be the source of nucleic acids for pDC activation during flare-ups of disease. Commensal bacteria are needed for induction of type-I interferon, and *B.oleronius* coupled with cathelicidin is sufficient to restore type-I interferon at the site of inflammation.

Materials and Methods

Human samples. Studies were approved by the local institutional review board of Lausanne, Switzerland (ethical approval number 265/12) and Dusseldorf, Germany (ethical approval number 2048). After dermatopathological assessment, punch-biopsies were taken with informed consent from acute flare-ups or stabilised rosacea lesions. Samples were selected where a

Cutaneous Lupus Erythematous diagnosis was unequivocally ruled out by immunopathological assessment. Biopsies from healthy individuals were obtained with informed consent from residual skin from aesthetic surgery of healthy individuals. Buffy coats from healthy donours were obtained from the local transfusion centre and blood bank, with ethical approval from cantonal authorities.

Mouse experimentation. Animal experiments were performed according to institutional and the Swiss Federal Animal Protection Act and cantonal laws on animal protection. Consent was received from the Swiss Federal Food Safety and Veterinary Office. Balb/cByJ (JAX mouse strain) mice were purchased from Charles River Laboratories France and experiments performed on age- and sex-matched animals. Intradermal injections of LL-37 were performed as previously described unless stated otherwise. Briefly, a 50µL volume of sterile saline, or 250µM of LL-37 or FR-29 (Proteogenix, France) was injected intradermally 4 times every 12h and mice euthanized and biopsied at 48h. Antibodies anti-IFNAR (MAR1-5A3, BioXCell) and anti-PDCA1 (BX444, BioXCell) were each injected (200µg) 24h prior to intradermal LL-37 injections. Erythema was scored according to intensity and area of visible redness. *B.oleronius* (10^4 CFU after overnight culture) was incubated for 12h with 250µM of LL-37, or saline, and injected intradermally in a 50µL final volume at 0h and +12h.

Immunohistochemistry. For immunohistochemistry of human skin, samples were fixed in 4% paraformaldehyde and paraffin-embedded. Stainings were performed using anti-CD123 (7G3, BD Pharmingen) followed by visualisation using the horseradish peroxidase technique. The number of positive cells per high magnification field was normalized to the number non-stromal inflammatory cells using automated and standardized counting of round nuclei (ImageJ v1.50b). For immunohistochemistry of mouse skin, samples were snap-frozen in Tissue-Tek optimal cutting temperature compound (VWR) and cryosections stained using anti-CD34 (RAM34, eBiosciences) and visualized using the horseradish peroxidase technique.

Gene expression analyses. For quantitative real-time PCR, samples were homogenized by mechanical disruption using Polytron PT1200E (Kinematica) in Trizol reagent (Invitrogen) and total RNA obtained using phenol/chloroform extraction, and isopropanol followed by ethanol precipitation methods. RNA was reverse transcribed using SuperScript II reverse transcriptase kit (#18064014, Invitrogen). Relative gene expression was determined using TaqMan probes along with TaqMan Gene Expression Mix (Lifetechnologies). Values are expressed as $2^{\Delta-\Delta Ct}$ x 10^4 relative to the endogenous control *GAPDH* and for human samples these were normalized to healthy donour expression.

Flow cytometry. Biopsies of 0.6cm in diameter were taken of the injection site, and skin was mechanically disrupted using a sterile scalpel in PBS containing 2mM EDTA. The resulting cell suspension was stained in 0.5% FBS/PBS 2mM EDTA using anti-B220 (RA3-6B2, BD Pharmingen), anti-CD11c (N418, eBiosciences), anti-CD45 (30-F11, BD Pharmingen), anti-PDCA1 (927, Biolegend), anti-Siglec-H (eBio440c, eBiosciences).

pDC stimulations. pDCs were isolated using the Diamond Plasmacytoid Dendritic Cell Isolation Kit II (Miltenyi), and cultured $5x10^4$ cells/200µL in RPMI 1640 supplemented with 10% FBS and 1% penicillin/streptomycin. Stimulations were performed using 5µg/mL human DNA and 10 µM or 3 µM of the indicated peptides. Live *B.oleronius* was added at 10^5 CFU/mL alone or in combination with 5µM of LL-37. IFNα production was measured by ELISA after 24h of stimulation using the human pan specific IFN-α development kit (Mabtech).

DNA-cathelicidin peptide complex uptake. Isolated pDCs were incubated with 3 μg/mL of Alexa Fluor 488-labeled DNA alone or in combination with 1 μM of the indicated peptide at 37C for 3h in RPMI 1640 (Gibco) supplemented with 10% FBS.

DNA binding. DNA binding and condensation activity of the cathelicidin peptides was determined using a picogreen (Invitrogen) dye fluorescence quenching technique. Peptides were added to 2ug of purified human genomic DNA at the indicated final concentrations for 30 minutes, and picogreen dye added to the samples. Sample fluorescence was determined using 480nm excitation and measured at 520nm using a spectrofluorometer as indicated by the manufacturer. When there is strong peptide-DNA binding, condensed DNA molecules are rendered inaccessible to the dye.

Bacterial killing. Bacterial strains *Bacillus oleronius* (ATCC 700005), *Staphylococcus epidermidis* (ATCC 14990), *Propionibacterium acnes* (ATCC 6919), *Klebsiella pneumoniae* (O1:K2), *Pseudomonas aeruginosa* (ATCC 27853) *Escherichia coli* (O18:K1:H7) were prepared at the indicated working concentrations and incubated with the indicated peptide at working concentrations ranging 0.2-10 μM for 5h in PBS, plated and CFU were counted after overnight incubation at 37°C, except for *P.acnes* which was incubated under anaerobic conditions. Values are expressed as percentage of survival. Killing potency against human cells was assessed by incubating 10^5 PBMCs in PBS with the indicated peptides and concentrations ranging 0.2-50 μM, and determined using SYTO13/SYTOX staining and expressed as percentage of surviving cells.

References and Notes:

1. K. Yamasaki, A. Di Nardo, A. Bardan, M. Murakami, T. Ohtake, A. Coda, R. a Dorschner, C. Bonnart, P. Descargues, A. Hovnanian, V. B. Morhenn, R. L. Gallo, Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea, *Nat. Med.* **13**, 975–980 (2007).

2. K. Yamasaki, R. L. Gallo, Rosacea as a Disease of Cathelicidins and Skin Innate Immunity, *J. Investig. Dermatology Symp. Proc.* **15**, 12–15 (2011).

3. R. Lande, J. Gregorio, V. Facchinetti, B. Chatterjee, Y.-H. Wang, B. Homey, W. Cao, Y.-H. Wang, B. Su, F. O. Nestle, T. Zal, I. Mellman, J.-M. Schröder, Y.-J. Liu, M. Gilliet, Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide, *Nature* **449**, 564–569 (2007).

4. C. Albanesi, C. Scarponi, D. Bosisio, S. Sozzani, G. Girolomoni, Immune functions and recruitment of plasmacytoid dendritic cells in psoriasis, *Autoimmunity* **43**, 215–219 (2010).

5. A. L. Blasius, P. Krebs, B. M. Sullivan, M. B. Oldstone, D. L. Popkin, Slc15a4, a gene required for pDC sensing of TLR ligands, is required to control persistent viral infection., *PLoS Pathog.* **8**, e1002915 (2012).

6. C. Conrad, S. Meller, M. Gilliet, Plasmacytoid dendritic cells in the skin: to sense or not to sense nucleic acids, *Semin Immunol* **21**, 101–109 (2009).

7. N. O. Frank, C. Curdin, T.-K. Adrian, H. Bernhard, M. Gombert, O. Boyman, G. Burg, Y.-J. Liu, M. Gilliet, Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production, *J. Exp. Med.* **202**, 135–143 (2005).

8. S. Meller, J. Di Domizio, K. S. Voo, H. C. Friedrich, G. Chamilos, D. Ganguly, C. Conrad, J. Gregorio, D. Le Roy, T. Roger, J. E. Ladbury, B. Homey, S. Watowich, R. L. Modlin, D. P. Kontoyiannis, Y.-J. Liu, S. T. Arold, M. Gilliet, TH17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26, *Nat. Immunol.* **26**, 1–8 (2015).

9. L. J. Zhang, G. L. Sen, N. L. Ward, A. Johnston, K. Chun, Y. Chen, C. Adase, J. a. Sanford, N. Gao, M. Chensee, E. Sato, Y. Fritz, J. Baliwag, M. R. Williams, T. Hata, R. L. Gallo, Antimicrobial Peptide LL37 and MAVS Signaling Drive Interferon-β Production by Epidermal Keratinocytes during Skin Injury, *Immunity* **45**, 119–130 (2016).

10. S. L. Rowland, J. M. Riggs, S. Gilfillan, M. Bugatti, W. Vermi, R. Kolbeck, E. R. Unanue, M. a. Sanjuan, M. Colonna, Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model., *J. Exp. Med.* **211**, 1977–91 (2014).

11. L. van Bon, A. J. Affandi, J. Broen, R. B. Christmann, R. J. Marijnissen, L. Stawski, G. a Farina, G. Stifano, A. L. Mathes, M. Cossu, M. York, C. Collins, M. Wenink, R. Huijbens, R. Hesselstrand, T. Saxne, M. DiMarzio, D. Wuttge, S. K. Agarwal, J. D. Reveille, S. Assassi, M. Mayes, Y. Deng, J. P. H. Drenth, J. de Graaf, M. den Heijer, C. G. M. Kallenberg, M. Bijl, A.

Loof, W. B. van den Berg, L. a B. Joosten, V. Smith, F. de Keyser, R. Scorza, C. Lunardi, P. L. C. M. van Riel, M. Vonk, W. van Heerde, S. Meller, B. Homey, L. Beretta, M. Roest, M. Trojanowska, R. Lafyatis, T. R. D. J. Radstake, Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis., *N. Engl. J. Med.* **370**, 433–43 (2014).

12. A. L. S. Chang, I. Raber, J. Xu, R. Li, R. Spitale, J. Chen, A. K. Kiefer, C. Tian, N. K. Eriksson, D. a Hinds, J. Y. Tung, Assessment of the Genetic Basis of Rosacea by Genome-Wide Association Study, *2 J. Investig. Dermatology* **00**, 1–8 (2015).

13. A. Egeberg, P. R. Hansen, G. H. Gislason, J. P. Thyssen, Clustering of autoimmune diseases in patients with rosacea, *J. Am. Acad. Dermatol.* **74**, 667–672.e2 (2016).

14. M. Steinhoff, J. Buddenkotte, J. Aubert, M. Sulk, P. Novak, V. D. Schwab, C. Mess, F. Cevikbas, M. Rivier, I. Carlavan, S. Déret, C. Rosignoli, D. Metze, T. A. Luger, J. J. Voegel, Clinical, Cellular, and Molecular Aspects in the Pathophysiology of Rosacea*J. Investig. Dermatol. Symp. Proc.* **15**, 2–11 (2011).

15. R. Bissonnette, K. Papp, C. Maari, Y. Yao, G. Robbie, W. I. White, C. Le, B. White, A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti–interferon-alfa monoclonal antibody, in subjects with chronic psoriasis, *J. Am. Acad. Dermatol.* **62**, 427–436 (2010).

16. L. Grine, L. Dejager, C. Libert, R. E. Vandenbroucke, An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17, *Cytokine Growth Factor Rev.* **26**, 25–33 (2015).

17. M. Khamashta, J. T. Merrill, V. P. Werth, R. Furie, K. Kalunian, G. G. Illei, J. Drappa, L. Wang, W. Greth, CD1067 study investigators, Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study., *Ann. Rheum. Dis.* , 1–8 (2016).

18. K. a. Kirou, E. Gkrouzman, Anti-interferon alpha treatment in SLE, *Clin. Immunol.* **148**, 303–312 (2013).

19. R. Furie, M. Khamashta, J. T. Merrill, V. P. Werth, K. Kalunian, P. Brohawn, G. G. Illei, J. Drappa, L. Wang, S. Yoo, Anifrolumab, an Anti–Interferon-α Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus, *Arthritis Rheumatol.* **69**, 376–386 (2017).

20. a. Tiilikainen, A. Lassus, J. Karvonen, P. Vartiainen, M. Julin, Psoriasis and HLA-Cw6, *Br. J. Dermatol.* **102**, 179–184 (1980).

21. C. D. Veal, F. Capon, M. H. Allen, E. K. Heath, J. C. Evans, A. Jones, S. Patel, D. Burden, D. Tillman, J. N. W. N. Barker, R. C. Trembath, Family-Based Analysis Using a Dense Single-Nucleotide Polymorphism–Based Map Defines Genetic Variation at PSORS1, the Major Psoriasis-Susceptibility Locus, *Am. J. Hum. Genet.* **71**, 554–564 (2002).

22. Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2, A. Strange, F. Capon, C. C. Spencer, J. Knight, M. E. Weale, M. H. Allen, A. Barton, G. Band, C. Bellenguez, J. G. M. Bergboer, J. M. Blackwell, E. Bramon, S. J. Bumpstead, J. P. Casas, M. J. Cork, A. Corvin, P. Deloukas, A. Dilthey, A. Duncanson, S. Edkins, X. Estivill, O. Fitzgerald, C. Freeman, E. Giardina, E. Gray, A. Hofer, U. Hüffmeier, S. E. Hunt, A. D. Irvine, J. Jankowski, B. Kirby, C. Langford, J. Lascorz, J. Leman, S. Leslie, L. Mallbris, H. S. Markus, C. G. Mathew, W. H. I. McLean, R. McManus, R. Mössner, L. Moutsianas, A. T. Naluai, F. O. Nestle, G. Novelli, A. Onoufriadis, C. N. a Palmer, C. Perricone, M. Pirinen, R. Plomin, S. C. Potter, R. M. Pujol, A. Rautanen, E. Riveira-Munoz, A. W. Ryan, W. Salmhofer, L. Samuelsson, S. J. Sawcer, J. Schalkwijk, C. H. Smith, M. Ståhle, Z. Su, R. Tazi-Ahnini, H. Traupe, A. C. Viswanathan, R. B. Warren, W. Weger, K. Wolk, N. Wood, J. Worthington, H. S. Young, P. L. J. M. Zeeuwen, A. Hayday, a D. Burden, C. E. M. Griffiths, J. Kere, A. Reis, G. McVean, D. M. Evans, M. a Brown, J. N. Barker, L. Peltonen, P. Donnelly, R. C. Trembath, A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1., *Nat. Genet.* **42**, 985–90 (2010).

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Figures:

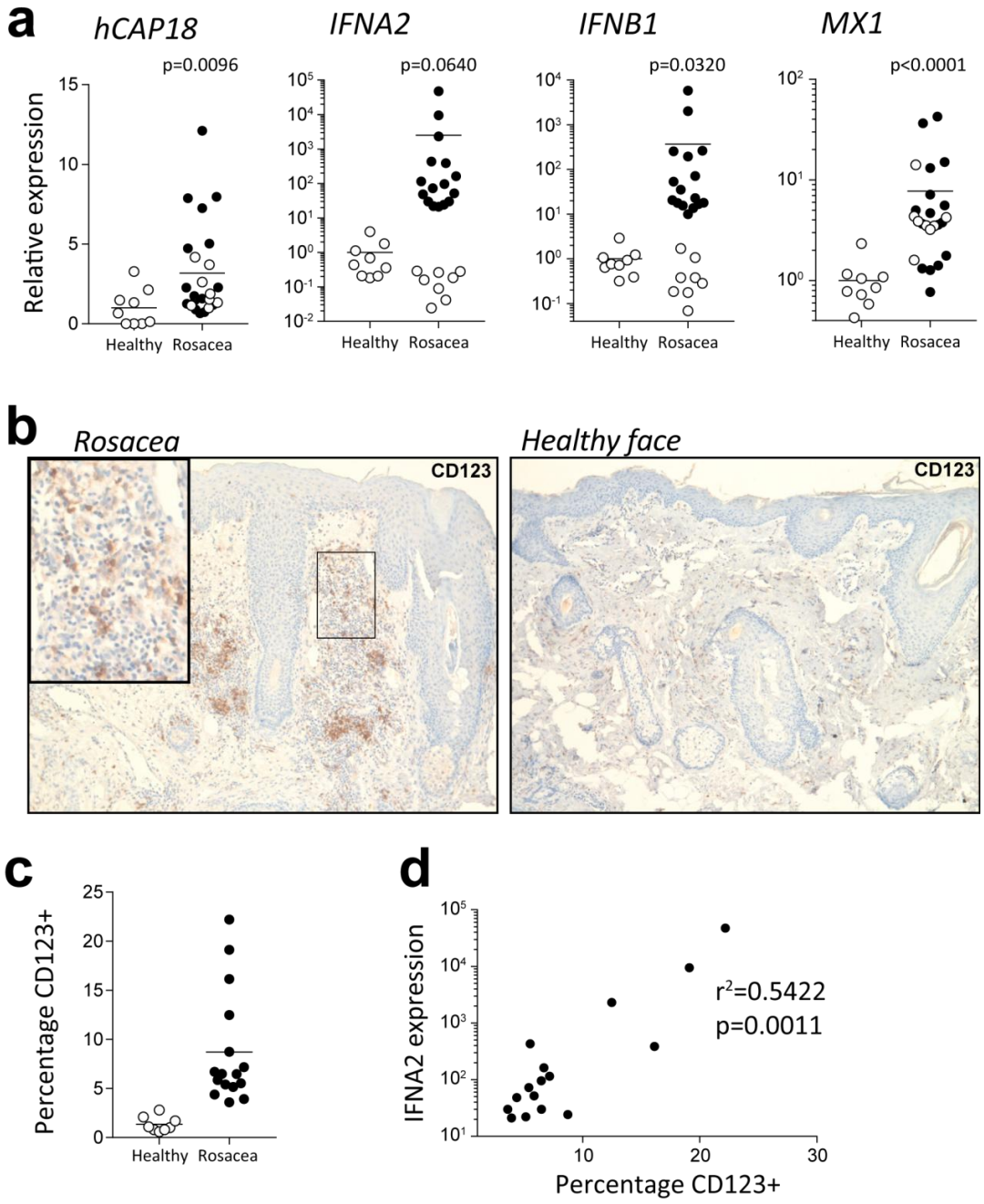
a
hCAP18
IFNA2
IFNB1
MX1
p=0.0096
p=0.0640
p=0.0320
p<0.0001
Relative expression
Healthy
Rosacea
b
Rosacea
Healthy face
CD123
c
Percentage CD123+
d
IFNA2 expression
r²=0.5422
p=0.0011
Percentage CD123+

Fig.1: Type-I interferon expression correlates with pDC infiltration in acute flares of rosacea. (**A**) Gene expression of indicated genes from acute flare-ups (•) and stabilised lesions (○) as compared to healthy skin. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted. (**B**) Rosacea flare-up and healthy skin sections stained for CD123+ pDCs. (**C**) Percentages of CD123+ pDCs were determined in relation to non-stromal inflammatory cells per high magnification field as mean of triplicate measurements per patient sample. (**D**) Percentage CD123+ pDCs pictured in (**C**) plotted against *IFNA2* expression from lesions during flare-ups pictured in (**A**). Non-linear regression with least-squares fit is depicted, with corresponding p-value.

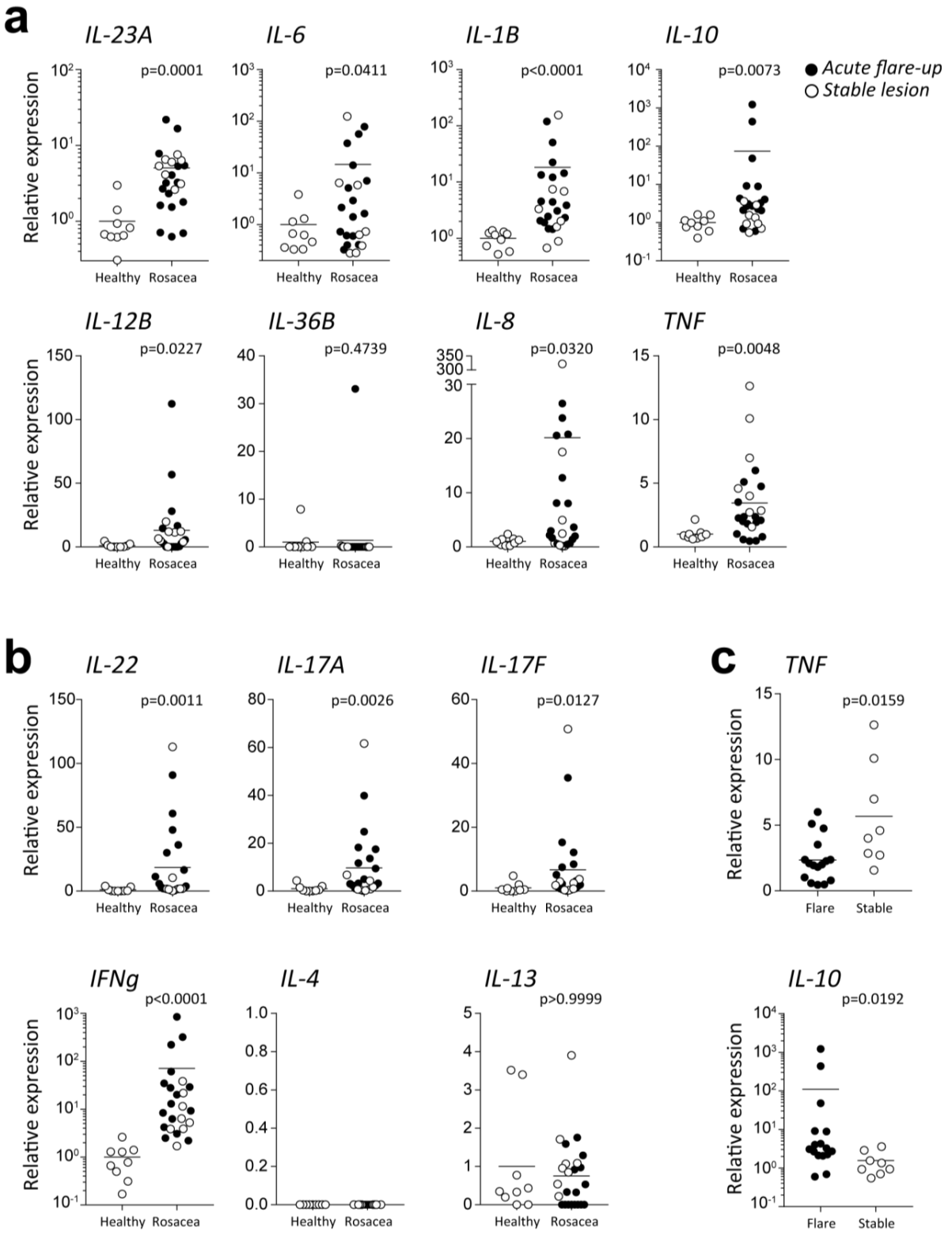
a
IL-23A
p=0.0001
IL-6
p=0.0411
IL-1B
p<0.0001
IL-10
p=0.0073
Acute flare-up
Stable lesion
Relative expression
Healthy
Rosacea
IL-12B
p=0.0227
IL-36B
p=0.4739
IL-8
p=0.0320
TNF
p=0.0048
b
IL-22
p=0.0011
IL-17A
p=0.0026
IL-17F
p=0.0127
IFNg
p<0.0001
IL-4
IL-13
p>0.9999
c
TNF
p=0.0159
IL-10
p=0.0192
Flare
Stable

Fig.2: Inflammatory cytokine profile in acute flare-ups and stabilised lesions of rosacea. (**A**) Gene expression of innate and (**B**) adaptive inflammatory genes from acute flare-ups (•) and stabilised lesions (○) as compared to healthy skin. (**C**) *TNF* and *IL10* expression comparison between flare-ups and stabilised lesions. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted.

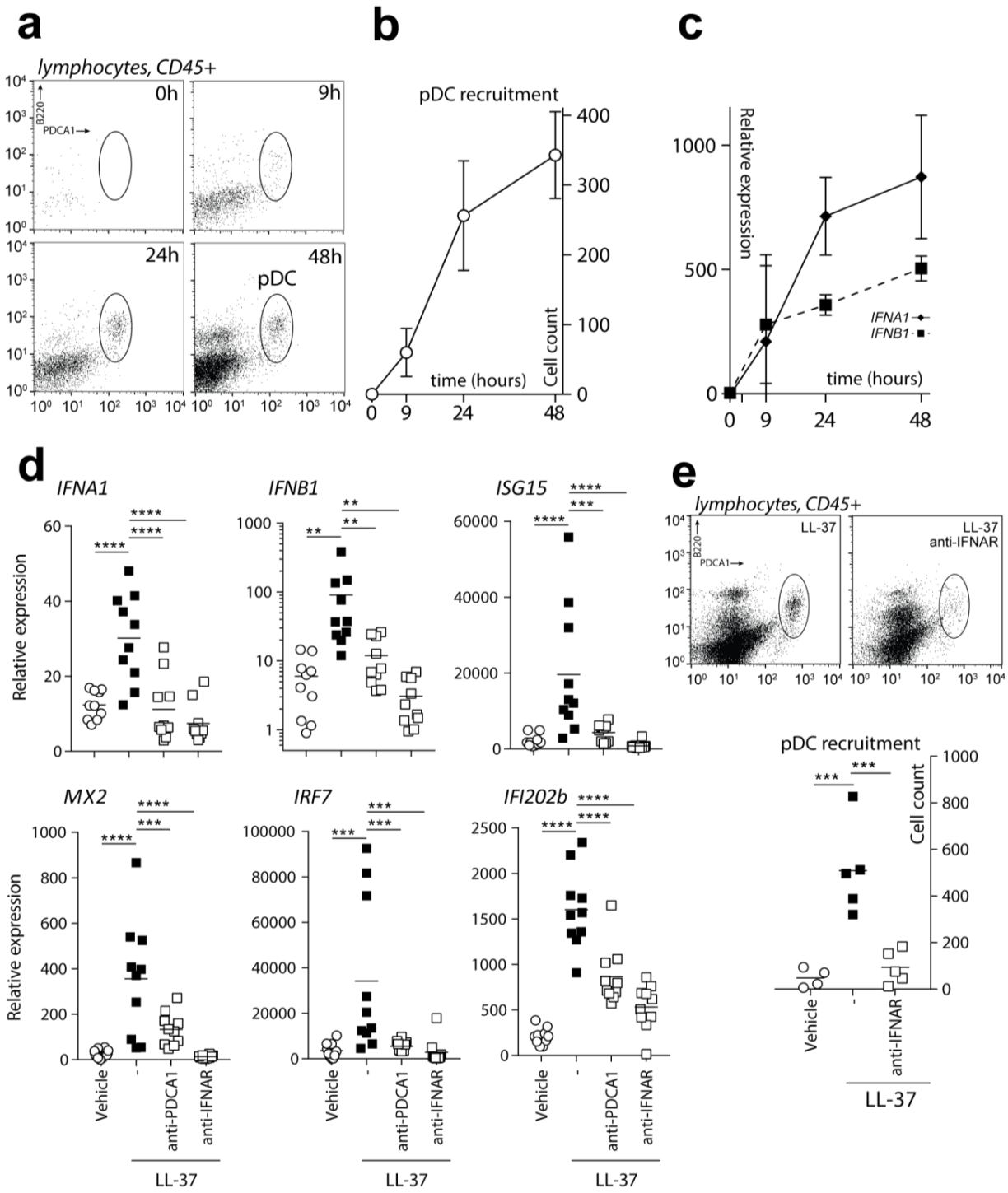
a
lymphocytes, CD45+
0h
9h
24h
48h
pDC
B220
PDCA1
b
pDC recruitment
time (hours)
Cell count
c
Relative expression
IFNA1
IFNB1
time (hours)
d
IFNA1
IFNB1
ISG15
MX2
IRF7
IFI202b
Relative expression
Vehicle
anti-PDCA1
anti-IFNAR
LL-37
e
lymphocytes, CD45+
LL-37
LL-37
anti-IFNAR
pDC recruitment
Cell count
Vehicle
anti-IFNAR
LL-37

Fig. 3: Type-I interferon and downstream response gene expression require pDCs *in vivo*. (**A**) Dotplots from mouse skin biopsies of LL-37 intradermal injections, pre-gated on CD45+ lymphocytes at the indicated timepoints. (**B**) Quantification of pDC infiltration and (**C**) *IFNA1* and *IFNB1* expression, values expressed as means ± SD of 5 mice per group. (**D**) Gene expression from biopsies following LL-37 intradermal injection in mice depleted of pDCs or blockaded of type-I interferon signalling 24h prior to injection. (**E**) Dotplots and quantification from skin biopsies of LL-37 intradermally injected mice, with or without prior antibody-mediated IFNAR blockade. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted (*: $p<0.05$; **: $p<0.01$; ***: $p<0.005$; ****: $p<0.001$).

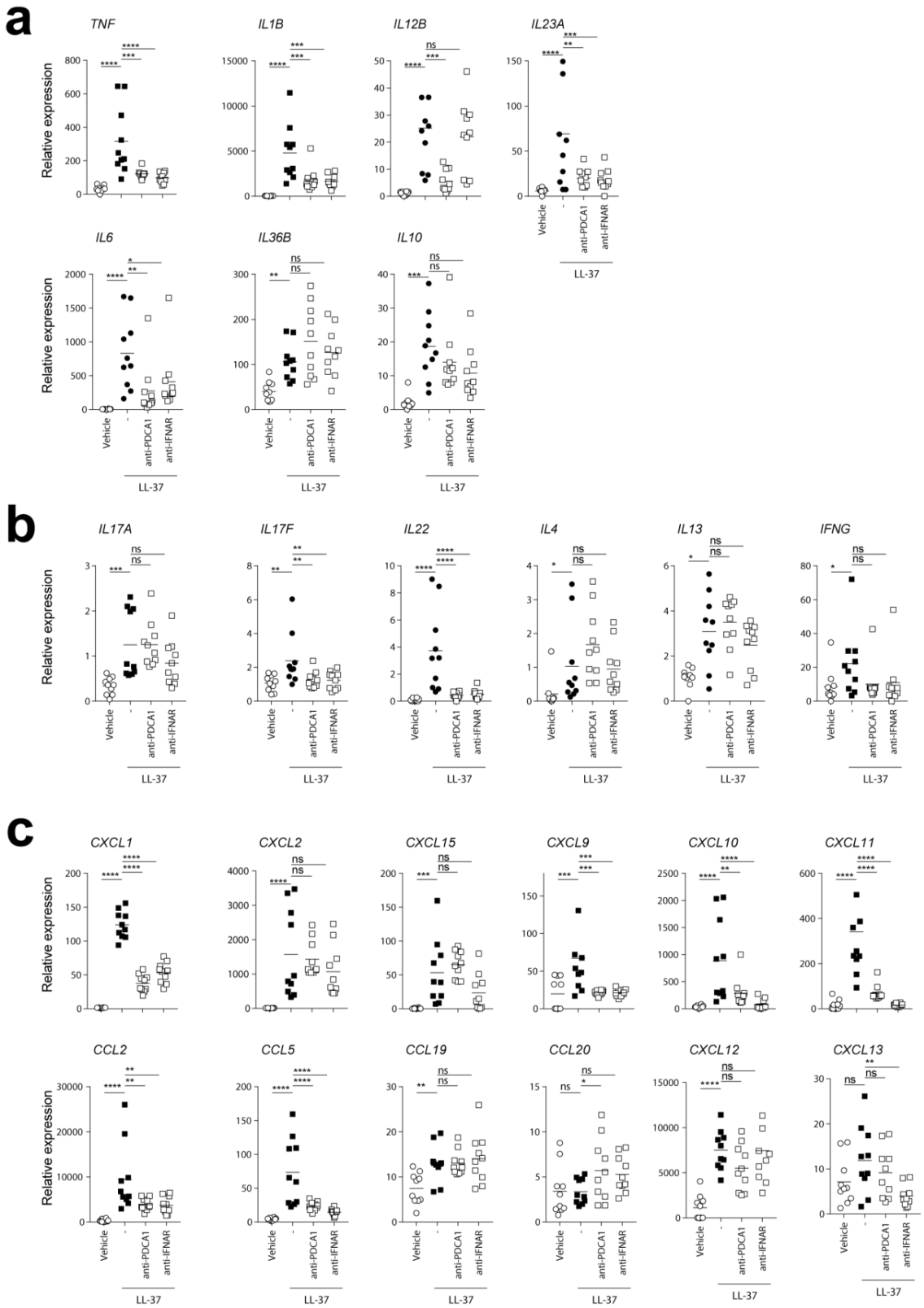
a
TNF
IL1B
IL12B
IL23A
IL6
IL36B
IL10
Relative expression
Vehicle
-
anti-PDCA1
anti-IFNAR
LL-37
b
IL17A
IL17F
IL22
IL4
IL13
IFNG
c
CXCL1
CXCL2
CXCL15
CXCL9
CXCL10
CXCL11
CCL2
CCL5
CCL19
CCL20
CXCL12
CXCL13

Fig. 4: Type-I interferon and pDC contribute to Th1 and Th22, but not Th2, inflammatory cytokine expression *in situ*. (**A**) Innate and (**B**) adaptive cytokine, and (**C**) chemokine expression in biopsies obtained from intradermally injected mice with corresponding pre-treatments. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted (*: $p<0.05$; **: $p<0.01$; ***: $p<0.005$; ****: $p<0.001$).

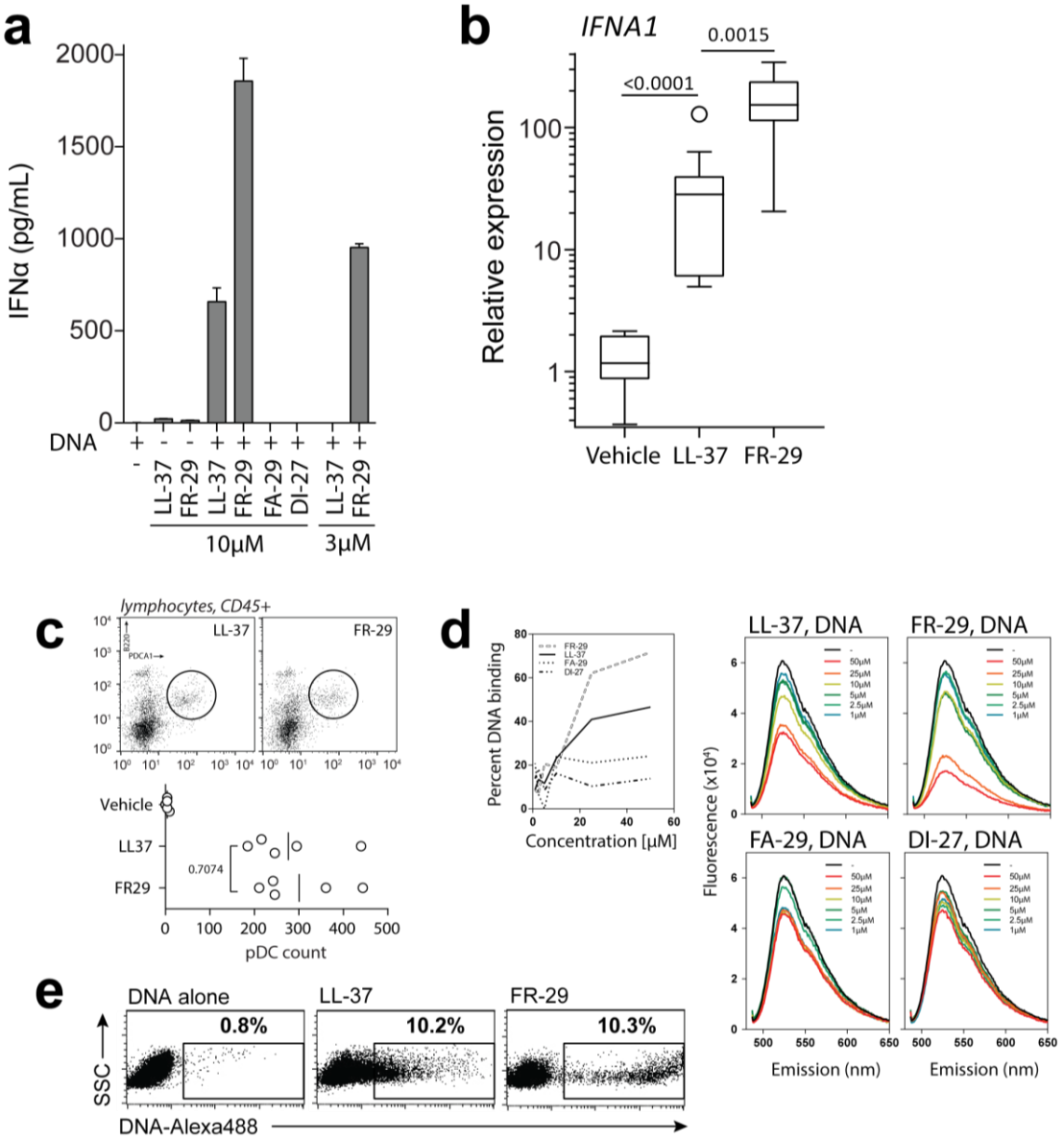
a
IFNα (pg/mL)
2000
1500
1000
500
0
DNA
- + - - + + + + + +
- LL-37 FR-29 LL-37 FR-29 FA-29 DI-27 LL-37 FR-29
10µM
3µM
b
IFNA1
0.0015
<0.0001
Relative expression
100
10
1
Vehicle LL-37 FR-29
c
lymphocytes, CD45+
LL-37
FR-29
B220
PDCA1
Vehicle
LL37
FR29
0.7074
0 100 200 300 400 500
pDC count
d
Percent DNA binding
FR-29
LL-37
FA-29
DI-27
0 20 40 60
Concentration [µM]
LL-37, DNA
FR-29, DNA
FA-29, DNA
DI-27, DNA
Fluorescence (x10⁴)
50µM
25µM
10µM
5µM
2.5µM
1µM
500 550 600 650
Emission (nm)
e
DNA alone
LL-37
FR-29
0.8%
10.2%
10.3%
SSC
DNA-Alexa488

Fig. 5: Cathelicidin peptide FR-29 activates pDCs more potently than LL-37 by binding DNA with more affinity, resulting in more potent internalisation of nucleic acids. (**A**) Isolated human pDCs were stimulated the indicated cathelicidin peptides complexed with DNA, and IFNα was measured from supernatants after a 24h stimulation. (**B**) LL-37, or FR-29, or vehicle control (saline) were injected intradermally twice over a 24h period into wild-type Balb/c mice, and biopsies taken for gene expression analysis and (**C**) pDC quantification by flow cytometry. (**D**) DNA-binding efficacy of the indicated cathelicidin peptides as measured by picogreen assay. (**E**) DNA was labelled with Alexa488 according to manufacturer's instructions, incubated with the indicated cathelicidin peptide, and put in culture with isolated human pDCs for 3h before measuring the fluorescence by flow cytometry.

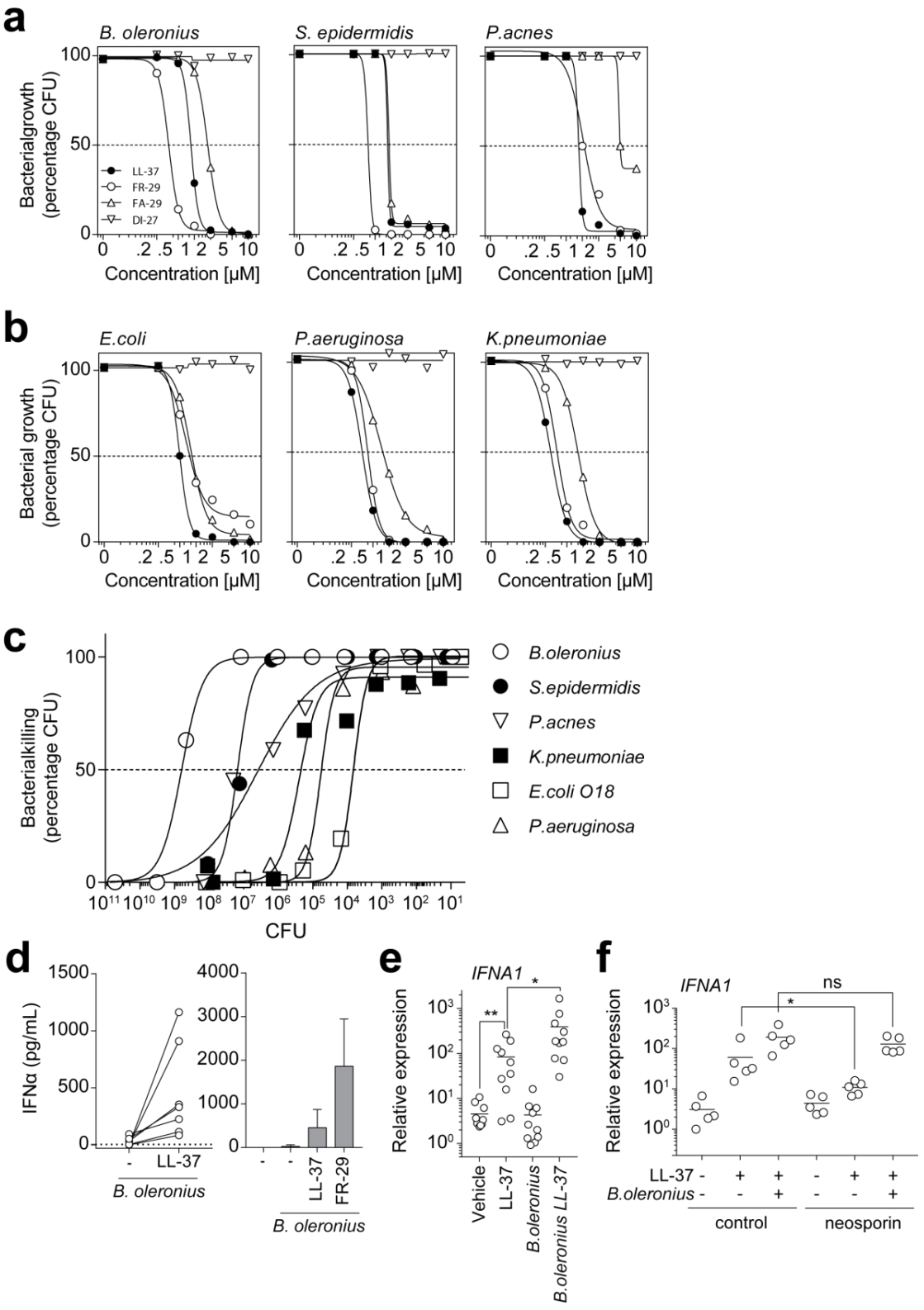
a
B. oleronius
S. epidermidis
P.acnes
Bacterialgrowth (percentage CFU)
LL-37
FR-29
FA-29
DI-27
Concentration [µM]
b
E.coli
P.aeruginosa
K.pneumoniae
Bacterial growth (percentage CFU)
c
Bacterialkilling (percentage CFU)
B.oleronius
S.epidermidis
P.acnes
K.pneumoniae
E.coli O18
P.aeruginosa
CFU
d
IFNα (pg/mL)
LL-37
B. oleronius
LL-37
FR-29
e
IFNA1
Relative expression
Vehicle
LL-37
B.oleronius
B.oleronius LL-37
f
IFNA1
ns
Relative expression
LL-37
B.oleronius
control
neosporin

Fig. 6: Cathelicidin-mediated killing of rosacea-associated bacteria activates pDCs to produce IFNα. (**A**) Skin- and (**B**) gut- and respiratory tract-associated bacteria killing assay by indicated cathelicidins. Bacteria CFU counts were determined after 18h culture in their appropriate culture conditions and the corresponding 10^4-10^5 CFUs were incubated with the indicated peptides at the indicated concentration for 2h, followed by plating and culturing for 18h. CFUs were counted and calculated as relative percentage of control cultures. (**C**) Bacteria at the indicated CFU were incubated with FR-29 at a constant 10μM concentration for 3h, and subsequently CFUs were counted after 18h culture in their appropriate culture conditions. (**D**) Plasmacytoid dendritic cells were isolated from human blood and stimulated with live *B.oleronius*, or *B.oleronius* pre-incubated with either LL-37 or FR-29, or left unstimulated (dotted line) for 24h, and IFNα production was assessed by ELISA. Results are pooled from a total of 7 donours. (**E**) IFNα gene expression from biopsies collected mice injected with LL-37, heat-killed *B.oleronius*, or heat-killed *B.oleronius* pre-incubated with LL-37, (**F**) in either neosporin treated mice or untreated controls. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted (ns: not significant; *: $p<0.05$; **: $p<0.01$).

Supplementary Materials:

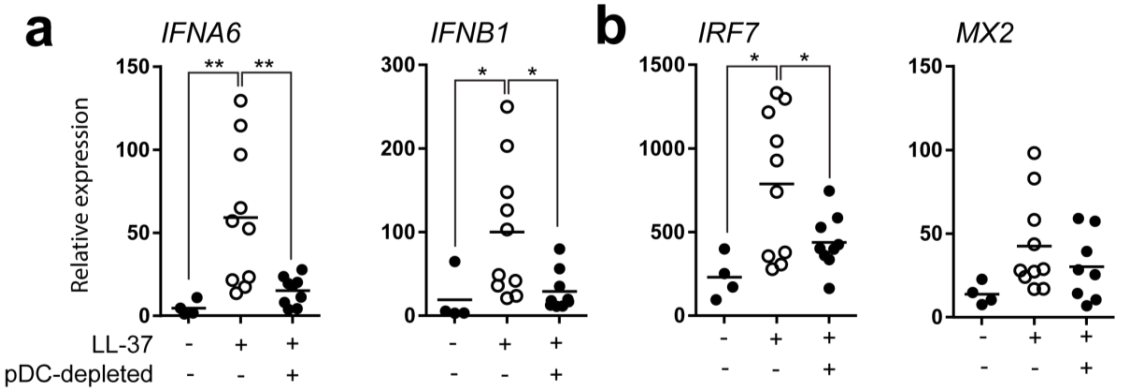

Fig. S1: pDC depletion in BDCA2-DTR mice leads to loss of type-I interferon and downstream response genes during LL37-mediated inflammation. (**A**) *In situ* gene expression of type-I interferon genes and of (**B**) downstream interferon-response genes. Mice were treated with DT or control injections 24h prior to intradermal LL37 injections. Following 4 intradermal injections over 48h, biopsies were taken and gene expression analyses performed. P-values of two-tailed Mann-Whitney nonparametric unpaired t-test are depicted (*: $p<0.05$; **: $p<0.01$).

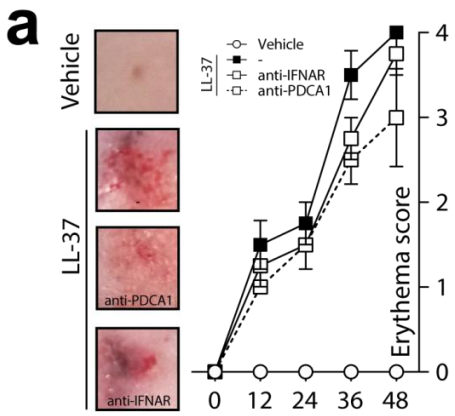

Fig. S2: Neither IFNAR blockade nor pDC depletion lead to differential erythema score in the LL-37 injection model. (**A**) Mice were treated with either anti-IFNAR or depleted of pDCs using anti-PDCA1 antibodies 24h prior to start of intradermal LL-37 injections. Images are representative of each individual group. Erythema scores are defined as mean of two parameters which include shade of redness and size of the area affected around the injection site. Data depicted are of five mice per group, mean ±SD.

JAMA Dermatology | Brief Report

Interleukin 23–Helper T Cell 17 Axis as a Treatment Target for Pityriasis Rubra Pilaris

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IMPORTANCE Treatment of pityriasis rubra pilaris (PRP) is solely based on its resemblance to psoriasis rather than any knowledge of its pathomechanism. Insight into pathogenic mediators of inflammation is essential for targeted and valid treatment options that could replace previous serendipitous therapeutic approaches in refractory PRP.

OBJECTIVE To determine whether blockade of the interleukin 23–helper T cell 17 (IL-23–T_H17) pathway with ustekinumab represents an efficacious and, based on its proinflammatory cytokine profile, targeted treatment option in PRP.

DESIGN, SETTING, AND PARTICIPANTS In this case report, a patient with PRP received outpatient treatment at a university hospital department of dermatology with ustekinumab according to the dosing regimen approved for psoriasis. Lesional skin biopsy samples were taken from this patient and 2 others with refractory PRP. Messenger RNA (mRNA) expression of proinflammatory innate and T-cell–derived cytokines were measured and compared with skin samples from patients with psoriasis and healthy donors. From 1 patient, lesional skin samples were taken before ustekinumab treatment and 4 and 28 weeks after treatment initiation. Follow-up was completed after 6 months.

INTERVENTION Subcutaneous ustekinumab, 45 mg, at weeks 0 and 4 and quarterly thereafter.

MAIN OUTCOMES AND MEASURES The primary outcome was to determine the changes in expression of proinflammatory innate and T-cell–derived cytokines during ustekinumab therapy. The secondary objective was to evaluate the clinical and histopathologic phenotype in relation to the mRNA expression profile of proinflammatory cytokines.

RESULTS In lesional PRP skin samples from a single patient, upregulated expression levels were found for most proinflammatory innate cytokines, including tumor necrosis factor (TNF), IL-6, IL-12, IL-23, and IL-1β. Among adaptive T-cell cytokines, an increase of T_H1 cytokines and, in particular, T_H17 cytokines IL-17A, IL-17F, and IL-22 was seen in PRP. The patient with PRP who received ustekinumab showed regression of skin lesions after 2 weeks and almost complete resolution after 1 month. Clinical and histopathologic improvement paralleled the expression levels of T_H17 cytokines but not of interferon-γ and TNF, which lagged behind the amelioration.

CONCLUSIONS AND RELEVANCE In this case report, a role of the IL-23–T_H17-axis in PRP was identified, suggesting a shared pathogenic inflammatory pathway with psoriasis, despite evident clinical and histopathologic differences. In addition, this report provides a rationale for targeting the IL-23–T_H17-pathway as a treatment option for refractory PRP.

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Pityriasis rubra pilaris (PRP) is a chronic inflammatory skin disease that typically appears sporadically and is acquired in most cases.[1] Pityriasis rubra pilaris is clinically characterized by follicular hyperkeratosis on an erythematous base. These papules show a tendency to coalesce, thereby forming large orange-red plaques with classic demarcated islands of sparing. Pityriasis rubra pilaris frequently involves the palms and soles, leading to palmoplantar orange-red waxy keratoderma. The major clinical differential diagnosis is psoriasis. However, in its most common form, type 1 PRP is typically self-limited and resolves within 3 years in 80% of cases. Based on reported associations with various autoimmune diseases, such as myasthenia gravis, arthritis, and myositis, a pathogenesis driven by an aberrant immune response has been suggested. However, the pathogenesis of PRP remains elusive. Thus, treatment of PRP is mainly empirical and, owing to its resemblance to psoriasis, classic treatments for psoria-

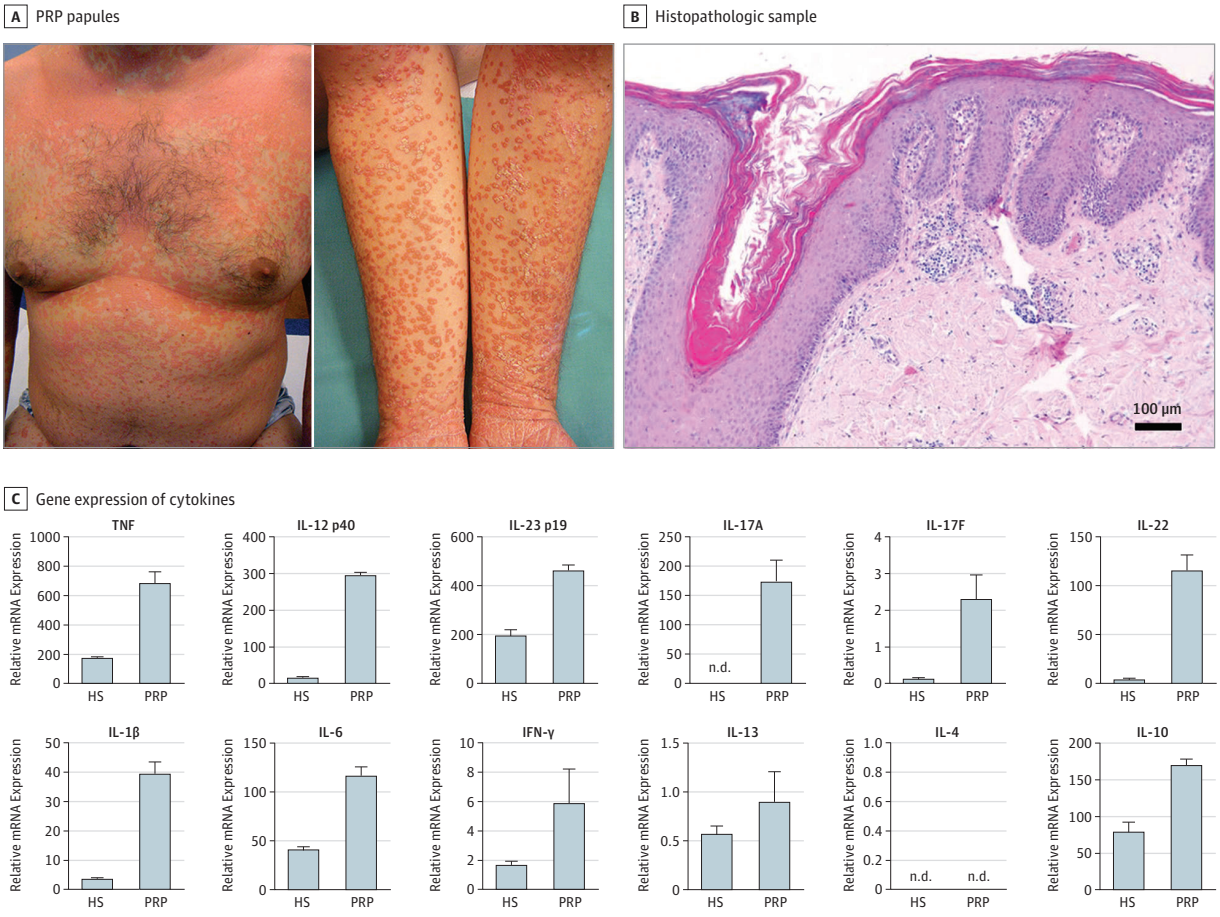
Key Points

Question Does targeting the interleukin 23–helper T cell 17 pathway represent a targeted treatment option for pityriasis rubra pilaris?

Findings In this case report, gene expression analyses of lesional skin samples taken from 3 patients with pityriasis rubra pilaris revealed a preferential helper T cell 17 expression profile. Analyses of samples from 1 patient performed before and during anti–interleukins 12 and 23 treatment with ustekinumab showed that expression levels of helper T cell 17 cytokines, but not of tumor necrosis factor or interferon-γ, paralleled clinical and histologic improvements.

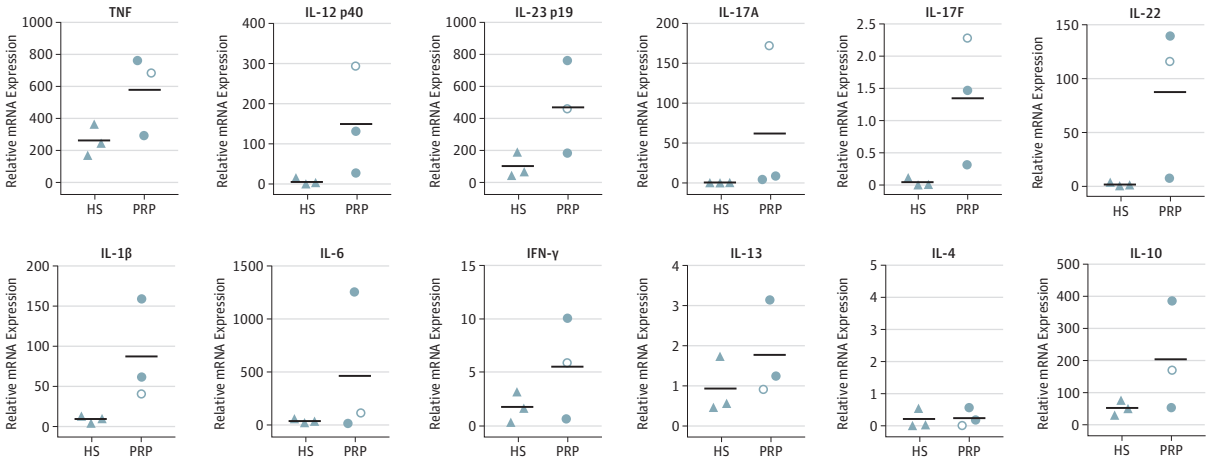
Meaning This report identifies a role of the interleukin 23–helper T cell 17 axis in pityriasis rubra pilaris and provides a rationale for targeting this pathway as a treatment option for refractory pityriasis rubra pilaris.

Figure 1. Expression of Helper T Cell 17 (T_H17) and T_H22 Cytokines in Skin Lesions of Pityriasis Rubra Pilaris (PRP)



A, Clinical image of a patient in his 40s with PRP shows typical orange-red follicular papules with scaly centers progressing to a widespread suberythroderma with islands of normal skin. B, Histopathologic evaluation of lesional skin with PRP shows a psoriasiform acanthosis (thickening of the epidermis), irregular hyperkeratosis (thickening of the corneal layer) with alternating vertical and horizontal orthokeratosis and parakeratosis (characteristic checkerboard pattern), and keratin plugs in the follicles (hematoxylin-eosin, bar indicates 100 µm). C, Relative messenger RNA (mRNA) expression of innate cytokines and adaptive T-cell–derived cytokines in lesional PRP skin compared with healthy skin shows particularly an upregulation of T_H17 cytokines in PRP. Error bars represent range of duplicates. HS indicates healthy skin; IFN-γ, interferon-γ; IL, interleukin; mRNA, messenger RNA; and TNF, tumor necrosis factor.

Figure 2. Preferential Expression of Helper T Cell 17 and 22 (T_H17 and T_H22) Cytokines in Skin Lesions of Pityriasis Rubra Pilaris (PRP)



Messenger RNA (mRNA) expression of innate cytokines and adaptive T-cell–derived cytokines in the lesional skin samples of 3 patients (the patient in Figure 1 and 2 additional patients) with PRP compared with healthy skin (HS) confirms upregulation of proinflammatory innate cytokines and T_H17 cytokines in PRP. Triangles represent individual donor with HS; dots, individual patients with PRP (open symbol corresponds to the patient in Figure 1). Horizontal bar denotes the mean value. IFN-γ indicates interferon-γ; IL, interleukin; and TNF, tumor necrosis factor.

sis are used. Topical corticosteroids, vitamin D analogues, phototherapy, systemic retinoids, methotrexate disodium, cyclosporine, and more recently anti-tumor necrosis factor (TNF) agents have been described.[1] In addition, isolated cases of effective treatment with combined anti-interleukins 12 and 23 (IL-12, IL-23) (ustekinumab) have been published.[2,3] None of these treatments was based on a pathophysiological rationale, but solely on the resemblance of PRP to psoriasis.

Herein we report that the cytokine expression in PRP shows a helper T cell 17 (T_H17) and T_H1 profile and that clinical improvement parallels the decrease in lesional T_H17 cytokines during effective anti-IL-12/IL-23 therapy. These findings suggest a role for T_H17 cytokines in PRP and provide basis for a targeted treatment in blocking the IL-23-T_H17 axis.

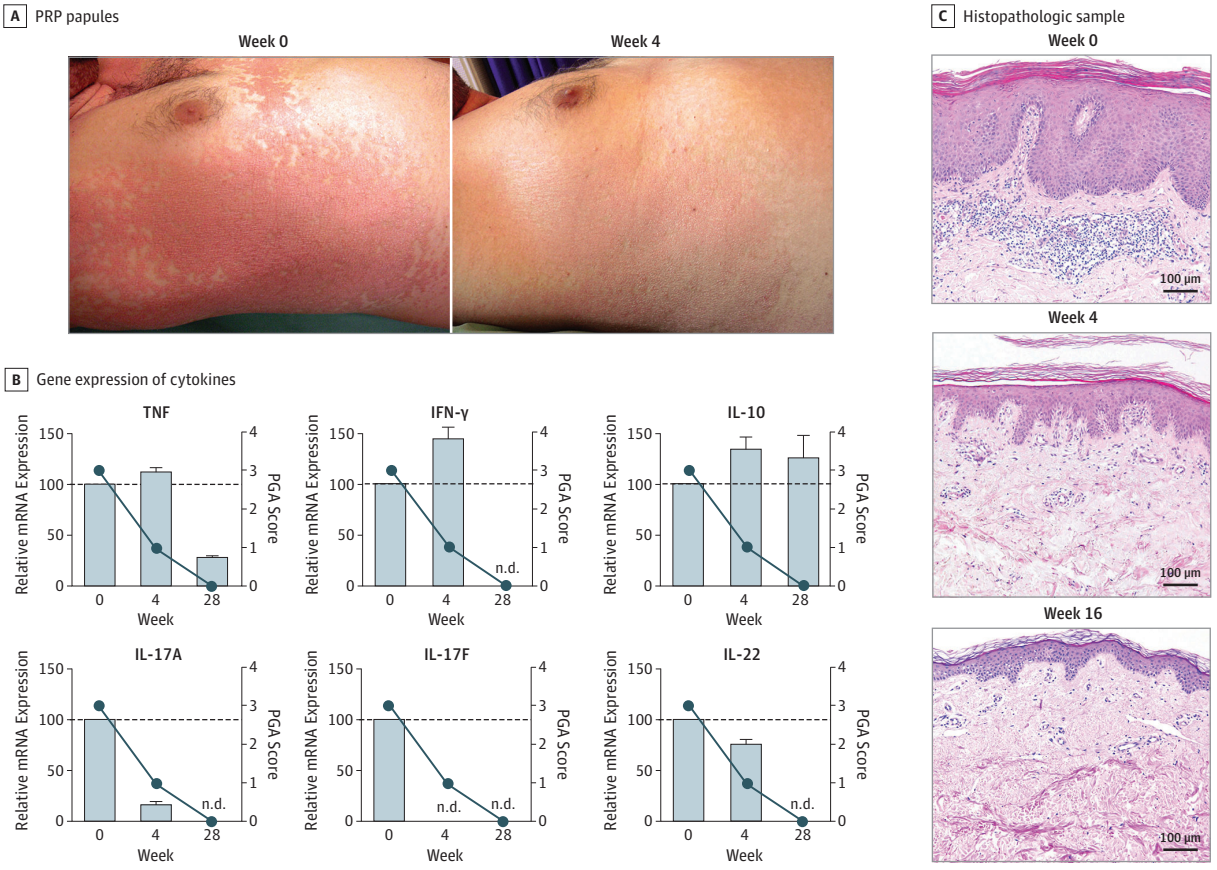
Methods

Skin biopsy samples were obtained from 3 patients with PRP at specified time points. Skin samples were fixed using 4% paraformaldehyde for histopathologic analysis or snap frozen and stored at −80°C for reverse transcription–polymerase chain reaction analysis (RT-PCR). Paraffin-embedded skin sections were deparaffinized and stained using a standard hematoxylin-eosin staining protocol. For quantitative RT-PCR, complementary DNA was synthesized using reverse transcriptase (SuperScript II; Invitrogen), and relative gene expression was quantified using specific probes (*TaqMan*; Life Technologies) and calculated using the comparative C_T method, where $2^{-\Delta\Delta Ct}$ describes the difference in C_T values between the target gene and normalizer gene [(C_T gene of interest − C_T internal control) sample A − (C_T gene of interest − C_T internal control) sample B)] using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous control. Analyses were performed in duplicates, and mean values or mean values plus the range of duplicates are depicted. This study was performed in accordance with the guidelines of the Declaration of Helsinki[4] and was approved by the cantonal ethics committee of Vaud, Switzerland. All patients provided written informed consent.

Report of a Case

A man in his 40s with PRP presented with painful palmoplantar hyperkeratosis, erythema of the face and scalp, and characteristic confluent red to orange follicular papules progressing to suberythroderma (**Figure 1**A). Diagnosis was confirmed by results of histopathologic analysis showing a psoriasiform dermatitis with irregular hyperkeratosis and the typical alternating vertical and horizontal orthokeratosis and parakeratosis (checkerboard pattern), keratin plugs in the follicles, and a sparse lymphohistiocytic, perivascular dermal infiltrate (Figure 1B). Because PRP shares common features with psoriasis, we analyzed messenger RNA (mRNA) expression of innate and adaptive cytokines involved in psoriasis pathogenesis. We found upregulated mRNA expression levels for most proinflammatory innate cytokines, including TNF, IL-6, IL-12, IL-23, and IL-1β in the lesional skin sample of the patient with PRP compared with normal skin (Figure 1C). Among adaptive T-cell cytokines, we found a particular increase of T_H17 cytokines IL-17A, IL-17F, and IL-22 in PRP compared with basal expression in normal skin, suggesting that these cytokines might play a pathogenic role in PRP similar to that in psoriasis. Preferential overexpression of T_H17 cytokines was confirmed in 2 additional patients with PRP, showing a similar profile (**Figure 2**). Furthermore, cytokines of the IL-23-T_H17 axis showed comparable mRNA expression levels in all 3 patients with PRP and in psoriasis.

Figure 3. Change in Helper T Cell 17 (T_H17) Cytokines in Pityriasis Rubra Pilaris (PRP) During Treatment With Ustekinumab



A, Clinical image of the patient with PRP before (week 0) and week 4 after initiation of anti–interleukins 12 and 23 (IL-12/IL-23) treatment (ustekinumab [Stelara]) shows rapid improvement. B, Relative messenger RNA (mRNA) expression of tumor necrosis factor (TNF), interferon-γ (IFN-γ), IL-10, IL-17A, IL-17F, and IL-22 within the skin at weeks 0, 4, and 28. The different cytokines are shown overlapped to the Physician Global Assessment (PGA) score to analyze potential correlation of gene expressions with clinical improvement. PGA scores range from 0 to 4, with higher scores indicating greater severity. Error bars represent range of duplicates. C, Histopathologic images of lesional skin samples with PRP at weeks 0, 4, and 28 show reduction of acanthosis, normalization of hyperkeratosis and parakeratosis toward orthokeratosis, and disappearance of the dermal inflammatory infiltrate (hematoxylin-eosin).

In our patient, topical therapies remained insufficient; his PRP was further aggravated despite 4 months of treatment. Worsening of the dermatosis on sun exposure prevented us from using phototherapy. Acitretin and methotrexate were contraindicated owing to a history of a drug-induced hepatitis with ongoing elevation of liver enzyme levels. Because IL-23 is critical for the differentiation and expansion of T_H17 cells,[5,6] and because we found a preferential overexpression of T_H17 cytokines, we opted for ustekinumab (Stelara), a human anti-IL-12/IL-23 antibody approved for severe psoriasis.[7]

Subcutaneous ustekinumab, 45 mg, was given at weeks 0 and 4 and quarterly thereafter, according to the psoriasis dose regimen. The lesions showed regression after 2 weeks and almost complete resolution after 1 month (**Figure 3**A). After 6 months, the treatment was interrupted and the patient remained in remission. The clinical improvement as reflected by the 4-point Physician Global Assessment (a measure of the mean redness, thickness, and scaling of the lesions, each graded on a scale of 0-4, with higher scores indicating increased severity) nicely paralleled the mRNA expression of IL-17A and IL-17F and, to some degree, expression of IL-22 (Figure 3B). On the other hand, although the expression of TNF and IFN-γ declined eventually, their decrease markedly lagged behind the clinical improvement, whereas IL-1β and IL-10 mRNA expression remained unchanged after 6 months of treatment. Furthermore, the expression of T_H17 cytokines also paralleled the improvement of histopathologic findings, such as normalization of the epidermal thickening and the corneal layer and attenuation of the cellular infiltrate (Figure 3C). This finding suggests a role for T_H17 cytokines in the pathogenesis of PRP and in driving its skin phenotype.

Discussion

Studies of PRP treatment are hampered by the unclear pathogenesis and the low incidence of the disease; therefore PRP therapy is based on the results of small case series and case reports.[8,9] Consequently, the therapy for PRP remains largely

empirical and, owing to its clinical and histopathologic similarities to psoriasis, classic psoriasis treatments are being used.

In psoriasis, epidermal hyperplasia is driven by IL-23 and mediated by IL-17 and IL-22, with IL-22 directly inducing keratinocyte hyperproliferation.[10,11] Furthermore, the blockade of IL-12/IL-23, IL-23 specifically, and IL-17 have all been proven effective in psoriasis.[7,12,13] Epidermal thickening, hyperproliferation and altered differentiation of keratinocytes are also hallmarks of PRP, rendering T_H17 cells an interesting therapeutic target in PRP. Indeed, we found an increased T_H17 expression profile in skin lesions of 3 patients with PRP and showed that the expression levels of T_H17 cytokines, but not of TNF or the T_H1 cytokine IFN-γ, paralleled clinical improvement during anti-IL-12/IL-23 treatment. Pityriasis rubra pilaris is often self-limiting, but the progressive disease course before treatment and the rapid response after a single injection strongly suggest that disease resolution was not spontaneous. Furthermore, previous reports in PRP described equally rapid efficacy on initiation of ustekinumab treatment.[3] Successful anti-IL-17 treatment of a patient with refractory PRP was also recently reported, which further supports an efficacy of the IL-23-T_H17 blockade in PRP.[14]

This study indicates a shared inflammatory pathway in psoriasis and PRP. Interestingly, mutations in the caspase recruitment domain family member 14 gene (*CARD14* [HGNC 16446]) have been identified in both diseases, raising the possibility that *CARD14* is upstream of the IL-23-T_H17 pathway. However, our patient did not display any *CARD14* mutation known in familial[15] or sporadic[16] PRP. Whether the efficacy of blocking the IL-23-T_H17 pathway is linked to a specific genotype and/or clinical subtype remains to be shown.

Despite pathogenic commonalities between psoriasis and PRP, obvious distinctions remain, such as orange-red waxy keratoderma, follicular hyperkeratosis, and absence of neutrophils in PRP. Future studies should identify pathologic mechanisms underlying these differences. Another feature of PRP is its spontaneous resolution. Interestingly, we found higher levels of IL-10 in PRP compared with psoriasis, which persisted on treatment, suggesting a potential anti-inflammatory pathway in PRP.

Limitations

This study has some limitations owing to the restricted number of patients included. Additional studies will need to further corroborate our findings by treating larger cohorts of patients with PRP using ustekinumab, and anti-IL-17– and anti-IL-23–specific antibodies.

Conclusions

This study identifies a role of the IL-23-T_H17-axis in PRP, suggesting a shared pathogenic inflammatory pathway with psoriasis. The findings provide a rationale for targeting the IL-23-T_H17 axis as a treatment option for refractory PRP that could replace previous serendipitous therapeutic approaches.

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Study concept and design: Mylonas, Gilliet, Conrad.
Acquisition, analysis, or interpretation of data: Feldmeyer, Mylonas, Demaria, Mennella, Yawalkar, Laffitte, Hohl, Conrad.
Drafting of the manuscript: Feldmeyer, Mylonas, Conrad.
Critical revision of the manuscript for important intellectual content: Feldmeyer, Mylonas, Demaria, Mennella, Yawalkar, Laffitte, Hohl, Gilliet.
Statistical analysis: Mylonas, Mennella, Conrad.
Administrative, technical, or material support: Mylonas, Demaria, Yawalkar, Laffitte, Hohl.
Study supervision: Feldmeyer, Mylonas, Gilliet, Conrad.

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REFERENCES

1. Ross NA, Chung HJ, Li Q, Andrews JP, Keller MS, Uitto J. Epidemiologic, clinicopathologic, diagnostic, and management challenges of pityriasis rubra pilaris: a case series of 100 patients. *JAMA Dermatol*. 2016;152(6):670-675.

2. Wohlrab J, Kreft B. Treatment of pityriasis rubra pilaris with ustekinumab. *Br J Dermatol*. 2010;163(3):655-656.

3. Di Stefani A, Galluzzo M, Talamonti M, Chiricozzi A, Costanzo A, Chimenti S. Long-term ustekinumab treatment for refractory type I pityriasis rubra pilaris. *J Dermatol Case Rep*. 2013;7(1):5-9.

4. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

5. McGeachy MJ, Chen Y, Tato CM, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17–producing effector T helper cells in vivo. *Nat Immunol*. 2009;10(3):314-324.

6. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. *N Engl J Med*. 2009;361(9):888-898.

7. Leonardi CL, Kimball AB, Papp KA, et al; PHOENIX 1 study investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet*. 2008;371(9625):1665-1674.

8. Ross NA, Chung HJ, Li Q, Andrews JP, Keller MS, Uitto J. Epidemiologic, clinicopathologic, diagnostic, and management challenges of pityriasis rubra pilaris: a case series of 100 patients. *JAMA Dermatol*. 2016;152(6):670-675.

9. Klein A, Landthaler M, Karrer S. Pityriasis rubra pilaris: a review of diagnosis and treatment. *Am J Clin Dermatol*. 2010;11(3):157-170.

10. Rizzo HL, Kagami S, Phillips KG, Kurtz SE, Jacques SL, Blauvelt A. IL-23-mediated psoriasis-like epidermal hyperplasia is dependent on IL-17A. *J Immunol*. 2011;186(3):1495-1502.

11. Conrad C, Gilliet M. Type I IFNs at the interface between cutaneous immunity and epidermal remodeling. *J Invest Dermatol*. 2012;132(7):1759-1762.

12. Gordon KB, Duffin KC, Bissonnette R, et al. A phase 2 trial of guselkumab versus adalimumab for plaque psoriasis. *N Engl J Med*. 2015;373(2):136-144.

13. Langley RG, Elewski BE, Lebwohl M, et al; ERASURE Study Group; FIXTURE Study Group. Secukinumab in plaque psoriasis: results of two phase 3 trials. *N Engl J Med*. 2014;371(4):326-338.

14. Schuster D, Pfister-Wartha A, Bruckner-Tuderman L, Schempp CM. Successful treatment of refractory pityriasis rubra pilaris with secukinumab. *JAMA Dermatol*. 2016;152(11):1278-1280.

15. Fuchs-Telem D, Sarig O, van Steensel MA, et al. Familial pityriasis rubra pilaris is caused by mutations in *CARD14*. *Am J Hum Genet*. 2012;91(1):163-170.

16. Li Q, Jin Chung H, Ross N, et al. Analysis of *CARD14* polymorphisms in pityriasis rubra pilaris: activation of NF-κB. *J Invest Dermatol*. 2015;135(7):1905-1908.

Letters to the Editor

Targeting CD8$^+$ T cells prevents psoriasis development

To the Editor:

In psoriasis, intraepidermal T cells are predominantly CD8$^+$ and represent key effector cells. Here, we show that these T cells produce pathogenic IL-17 and that neutralization of CD8$^+$ T cells effectively prevents psoriasis development *in vivo*.

Psoriasis is a common inflammatory skin disease, resulting from the interaction of genetic and environmental triggers, leading to dysregulated immune response of innate and adaptive immune cells.[1,2] T lymphocytes infiltrating psoriasis skin lesions play key effector roles by driving disease development and maintenance. Traditionally, CD4$^+$ T_H cells producing proinflammatory cytokines, such as IL-17A, IL-22, and IFN-γ, are regarded as the main pathogenic T-cell subpopulation. However, CD8$^+$ T cells, which are present in healthy skin as tissue resident memory T cells (T_{RM}),[3] have been shown to produce a similar profile of proinflammatory cytokines[4]; they are abundantly present in the psoriatic epidermis and potentially recognize peptide antigens presented on MHC class I molecules, such as HLACw6, which is the strongest psoriasis susceptibility allele.[5] Furthermore, we have previously shown that intraepidermal T cells represent key effector cells in psoriasis development and that impeding the entry of T cells into the epidermis, by blocking α1β1-integrin, prevents the development of psoriasis in the clinically relevant AGR mouse model of psoriasis.[6] Thus, we set out to explore the pathogenic relevance of CD8$^+$ T cells in psoriasis.

We first performed a time course experiment using the AGR mouse model. AGR mice are grafted with noninvolved skin from patients with psoriasis, which spontaneously develops a psoriatic phenotype after 4 to 6 weeks.[6] Thus, at days 0, 7, 21, and 35, skin transplants were harvested and processed for histological and immunohistochemical assessment as described previously.[6] In line with earlier findings, while the proliferation of dermal T cells preceded epidermal changes, the numerical expansion of the epidermal T-cell pool temporally coincided with the onset of the psoriatic phenotype, as shown by the papillomatosis index

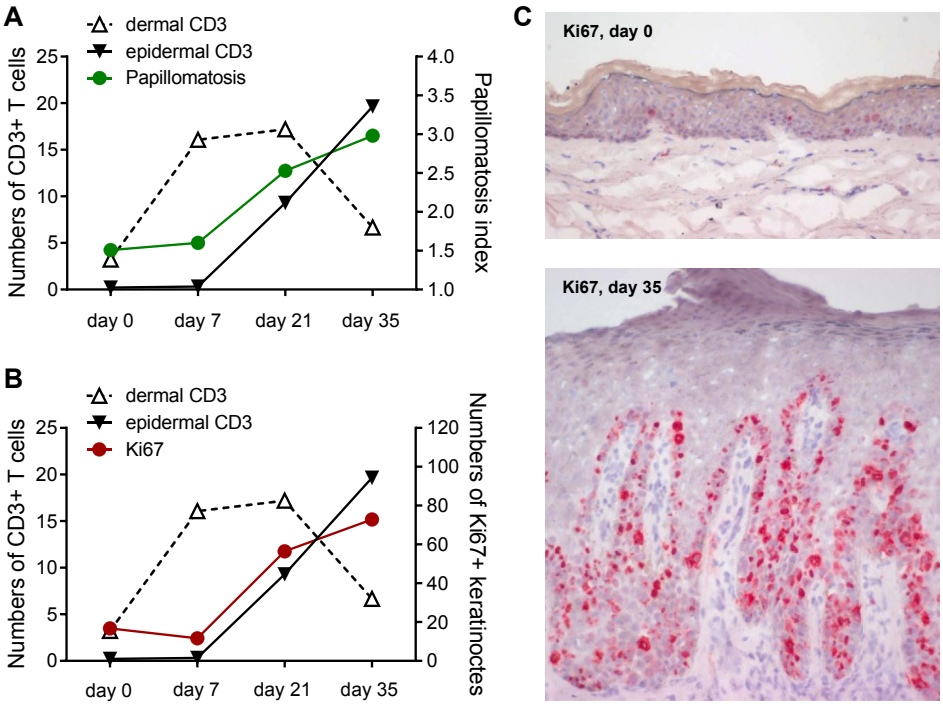

FIG 1. Expansion of epidermal T cells induces epidermal hyperproliferation and onset of a psoriatic phenotype. Quantification of T-cell numbers during psoriasis development in the AGR mouse model (days 0, 7, 21, and 35): dermal (*dashed black line,* **A** and **B**) and epidermal (*solid black line, A* and *B*) T-cell counts compared with papillomatosis index (*green line, A*) and number of Ki67-positive keratinocytes per 100 basal keratinocytes (*red line, B*) during psoriasis development. Microscopic view of nonlesional psoriatic skin stained with an mAb to Ki-67 on the day of transplantation onto AGR mice and after development of fully fledged psoriasis on day 35 **(C)**. Data depicted correspond to mean values and reflect 1 representative experiment of 2 independent experiments with skin from 2 donors (n = 3-4 transplanted mice for every time point). Values of standard error of the mean (SEM) for each parameter and time point are depicted in Table E1 in this article's Online Repository at www.jacionline.org.

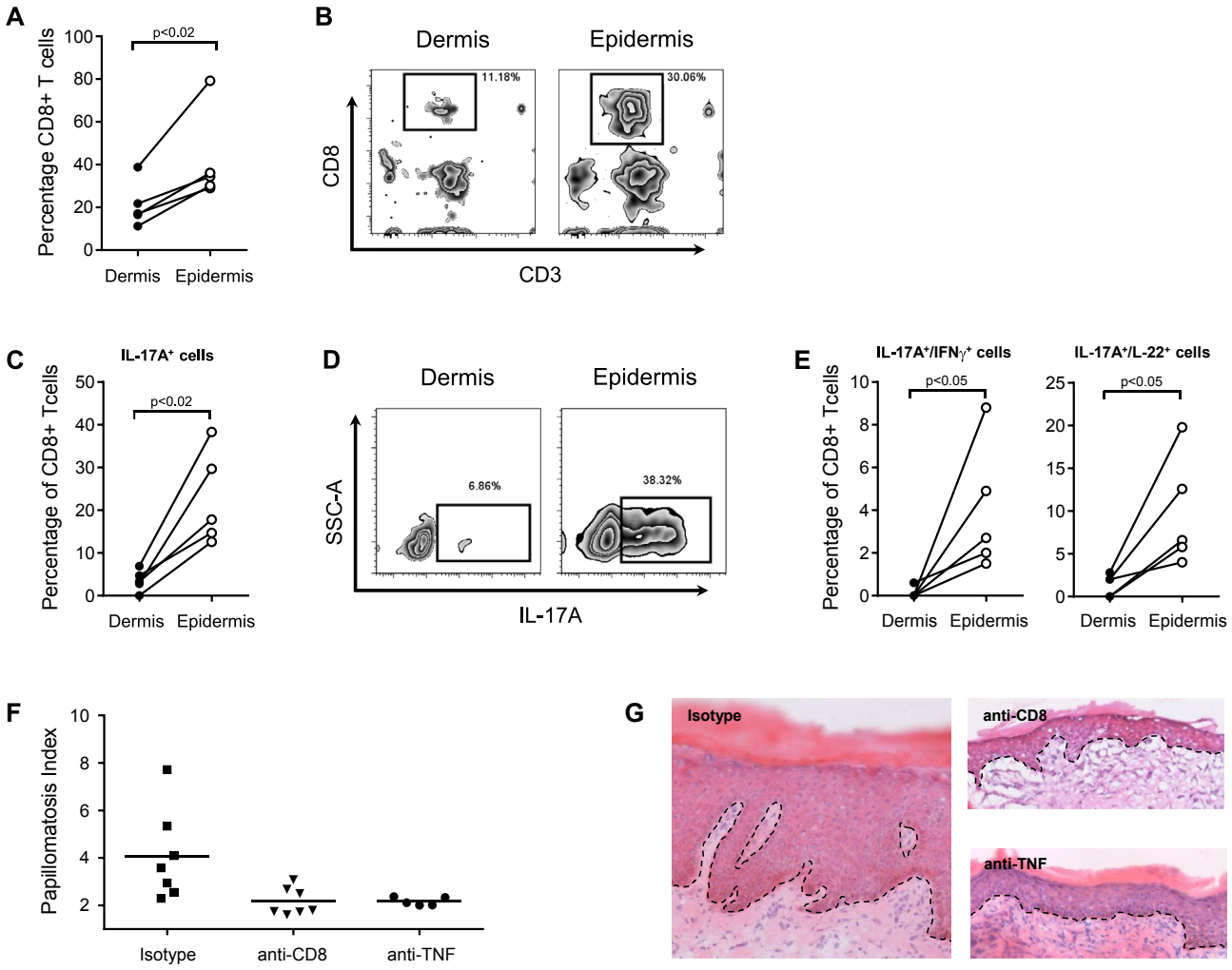

FIG 2. Epidermal $CD8^+$ T cells are highly pathogenic and their blockade prevents the development of psoriasis. Relative frequency **(A)** and representative zebra plot **(B)** of $CD3^+CD8^+$ T cells among live $CD45^+$ cells isolated from the epidermis and the dermis of 5 patients with psoriasis, with each patient denoted by a connecting line. Functional characterization of epidermal and dermal $CD3^+CD8^+$ T cells as $IL\text{-}17A^+$ **(C)**, with representative zebra plot in **(D)** $IL\text{-}17A^+IFN\text{-}\gamma+$ and $IL\text{-}17A^+IL\text{-}22^+$ **(E)** obtained by intracellular cytokine staining after phorbol 12-myristate 13-acetate/ionomycin stimulation. Each line connects epidermal and dermal $CD8^+$ T cells from the same patient. **F,** Microscopic changes of nonlesional psoriatic skin quantified using the papillomatosis index 35 days after transplantation onto AGR mice treated with isotype control mAb (mean ± SEM, 4.07 ± 0.72), mAb to CD8 (2.17 ± 0.22), or anti-TNF mAb (2.17 ± 0.08). **G,** Representative microscopic views of nonlesional psoriatic skin 35 days after transplantation onto AGR mice treated with isotype control mAb, mAb to CD8, or anti-TNF mAb. *B* and *D*, Zebra plot shown is representative of 1 of 5 patients. *F*, Data shown represent pooled results from 2 independent experiments with skin of 2 patients. Each symbol represents a transplanted mouse (n = 5-7). Statistical analyses in *A*, *C*, and *E* were performed by paired *t* test and in *F* with ANOVA followed by Bonferroni correction. All testing was 2-sided, and a *P* value of less than .05 was considered to indicate statistical significance.

(Fig 1, *A* and *C*, and Conrad et al[6]). Moreover, the accumulation of epidermal T cells paralleled the increase in proliferating keratinocytes as identified by positive Ki67-staining (Fig 1, *B* and *C*). Importantly, in the absence of T-cell expansion upon transplantation, which did not occur in one of the experiments we performed, we did not observe any epidermal pathology, in terms of both papillomatosis and frequency of proliferating keratinocytes (see Fig E1 in this article's Online Repository at www.jacionline.org). Thus, the accumulation of epidermal T cells induces both keratinocyte hyperproliferation and onset of papillomatosis, 2 hallmarks of psoriasis, thereby further confirming the role of intraepidermal T cells as key effectors in psoriasis.

In keeping with the classical distribution of $CD4^+$ and $CD8^+$ T cells in human psoriatic lesions, intraepidermal T cells in skin grafts 35 days posttransplant were predominantly $CD8^+$ T cells (see Fig E2 in this article's Online Repository at www.jacionline.org). Psoriatic $CD8^+$ T cells have been previously characterized in terms of their cytokine production; however, little distinction has been made between those residing in the dermis and the epidermis in the absence of post-isolation *in vitro* culture. Thus, to obtain a faithful functional characterization of psoriatic $CD8^+$ T cells, we isolated T cells from the epidermis and the dermis of psoriasis lesions and performed intracellular cytokine staining and fluorescence-activated cell sorting analyses. Among

live CD45$^+$ immune cells, the frequency of CD3$^+$CD8$^+$ T cells was significantly higher in the epidermis than in the dermis (Fig 2, *A* and *B*). Interestingly, the frequency of epidermal CD3$^+$CD8$^+$ T cells producing IL17A (Fig 2, *C* and *D*) or double-producing both IFN-γ and IL-17A or IL-22 and IL-17A, respectively (Fig 2, *E*), significantly exceeded that of dermal CD3$^+$CD8$^+$ T cells. No significant difference was found for IFN-γ$^+$, IL-22$^+$, or IL-22$^+$IFN-γ$^+$ T cells between the dermis and the epidermis (see Fig E3 in this article's Online Repository at www.jacionline.org). Thus, the main factor differentiating the epidermal from the dermal CD8$^+$ T-cell population is an active Tc17 phenotype.

On the basis of these findings, we sought to determine the *in vivo* pathogenic relevance of CD8$^+$ T cells infiltrating psoriasis lesions. Therefore, we treated xenotransplanted mice with either 1 mg mAb to human CD8 (M-T807) or the corresponding isotype control mAb on days 0 and 14, or mAb to TNF (infliximab, 1 mg intravenously on days 7 and 21 after transplantation). Isotype control antibody-treated skin grafts developed fully fledged psoriasis over the course of 35 days (Fig 2, *F* and *G*). Injection of mAb to CD8 resulted in a significantly reduced papillomatosis index and complete blockade of psoriasis development. The effect was equivalent to that obtained with TNF antagonists, a current benchmark in psoriasis treatment (Fig 2, *F* and *G*).

CD8$^+$ T cells and their role in psoriasis are currently under the spotlight (see Fig E4 in this article's Online Repository at www.jacionline.org). CD8$^+$ T cells isolated from patients with psoriasis produce psoriasis-relevant cytokines, they are retained in the epidermis as T_{RM} after successful therapy,[7] and LL-37–specific CD8$^+$ T cells expressing α1β1-integrin, a key molecule for trafficking of T cells into psoriatic epidermis,[6] have been identified in psoriatic blood.[8] The preferential anatomical location in the epidermis makes CD8$^+$ T cells ideally located to engage in a pathogenic cross talk with keratinocytes (Fig E4); a recent mouse model of psoriasiform murine inflammation relying on keratinocyte genetic abnormalities identified CD8$^+$ T cells as critical players.[9] In addition, we show that the accumulation of epidermal T cells, which mainly reflect CD8$^+$ T cells, correlates with the onset of keratinocyte hyperproliferation and papillomatosis, 2 characteristic features of psoriasis. Epidermal CD8$^+$ T cells display highly pathogenic features, and the significantly increased frequency of those producing IL-17A, alone or in combination with IL-22 and IFN-γ, makes them a reasonable primary source for this pivotal cytokine, whose clinical targeting is proving highly successful.[10] Finally, we show that blockade of CD8$^+$ T cells via a neutralizing mAb prevents the development of psoriasis in a clinically relevant xenotransplantation mouse model, thus uncovering a critical role for them in driving pathology. These findings may provide the basis for the design of new strategies targeting CD8$^+$ T cells for the treatment of psoriasis.

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REFERENCES

1. Di Meglio P, Villanova F, Nestle FO. Psoriasis. Cold Spring Harb Perspect Med 2014;4.
2. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol 2014;32:227-55.
3. Watanabe R, Gehad A, Yang C, Scott LL, Teague JE, Schlapbach C, et al. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. Sci Transl Med 2015;7:279ra39.
4. Hijnen D, Knol EF, Gent YY, Giovannone B, Beijn SJ, Kupper TS, et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. J Invest Dermatol 2013;133:973-9.
5. Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012;44:1341-8.
6. Conrad C, Boyman O, Tonel G, Tun-Kyi A, Laggner U, de Fougerolles A, et al. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. Nat Med 2007;13:836-42.
7. Cheuk S, Wiken M, Blomqvist L, Nylen S, Talme T, Stahle M, et al. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. J Immunol 2014;192:3111-20.
8. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, et al. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014;5:5621.
9. Gunderson AJ, Mohammed J, Horvath FJ, Podolsky MA, Anderson CR, Glick AB. CD8(+) T cells mediate RAS-induced psoriasis-like skin inflammation through IFN-gamma. J Invest Dermatol 2013;133:955-63.
10. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, et al. Secukinumab in plaque psoriasis–results of two phase 3 trials. N Engl J Med 2014;371:326-38.

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Human nasal epithelial cells derived from multiple subjects exhibit differential responses to H3N2 influenza virus infection *in vitro*

To the Editor:

Nasal epithelium is the first line of mechanical and immunologic defense in the upper respiratory tract.[1] Upper respiratory

METHODS

Animal studies were approved by the Kantonale Veterinaeramt of Zurich. Human studies, conducted according to the Declaration of Helsinki, were approved by the institutional review boards of the University Hospital of Zurich and Guy's and St Thomas' Hospital and informed patient consents were obtained. Xenotransplantation of noninvolved psoriatic skin, obtained from 3 patients with psoriasis, was performed as previously described using AGR129 mice, which are deficient in type I (A) and type II (G) IFN receptors in addition to being *Rag2*$^{-/-}$ (R). After 4 to 6 weeks, these skin grafts spontaneously develop a psoriatic phenotype including thickening of the epidermis (acanthosis), elongation of the rete ridges (papillomatosis), and increased numbers of dermal and epidermal T cells, closely reflecting the pathology of patient samples.[E1] For fluorescence-activated cell sorting analyses of skin T cells, we obtained 4-mm full-thickness skin biopsies from 5 patients with psoriasis, incubated them in 0.5 mol/L EDTA at 37°C for 3 hours to separate the epidermis and the dermis, and then digested them separately in 0.8 mg/mL collagenase type IV in RPMI + 10%FCS + 1%Pen/Strep (cRPMI) at 4°C overnight. EDTA treatment does not affect the expression of cell surface markers, such as CD4, in contrast to the widely used dispase treatment (data not shown). Subsequently, digested tissue was stimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomicin (1 μg/mL) in the presence of brefeldin A (3 μM) and monensin (3 μM) in cRPMI at 37°C for 5 hours. Dead cells were excluded from the analysis by staining with Live Dead Yellow (Life Technologies, Carlsbad, Calif). Cells were stained for surface markers, fixed and permeabilized, and stained for intracellular cytokines. The following antibodies were used: anti-CD3 APC (SK7, BD Biosciences, Franklin Lakes, NJ), anti-CD4 BV650 (SK3, BD Biosciences), anti-CD8 PE-Texas Red (3B5, Invitrogen, Carlsbad, Calif), anti-CD45 V500 (HI30, BD Biosciences), anti–IL-17A V450 (N49-653, BD Biosciences), anti–IL-22 PerCP-eFluor710 (22URTI, eBiosciences, San Diego, Calif), and anti–IFN-γ A700 (B27, BD Biosciences).

REFERENCE

E1. Conrad C, Boyman O, Tonel G, Tun-Kyi A, Laggner U, de Fougerolles A, et al. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. Nat Med 2007;13:836-42.

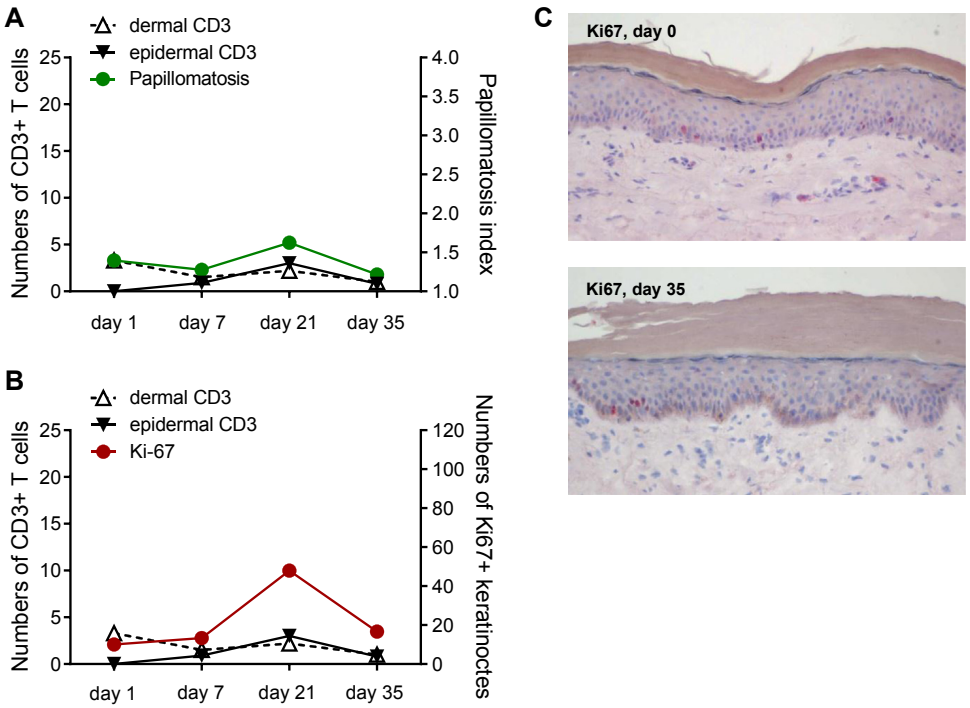

FIG E1. Absence of epidermal pathology after failed T-cell expansion. Quantification of T cells present in skin samples upon transplantation in the AGR mouse model (days 0, 7, 21, and 35): dermal (*dashed black line*) and epidermal (*solid black line*) T-cell counts compared with papillomatosis index (*solid green line*, **A**) and Ki-67 postive keratinocytes (*solid red line*, **B**) during psoriasis development. Microscopic view of nonlesional psoriatic skin stained with an mAb to Ki-67 on the day of transplantation onto AGR mice and on day 35 **(C)**. Data in *A* and *B* reflect 1 experiment with skin from a single donor not showing any relevant T-cell proliferation upon transplantation (n = 2 for every time point).

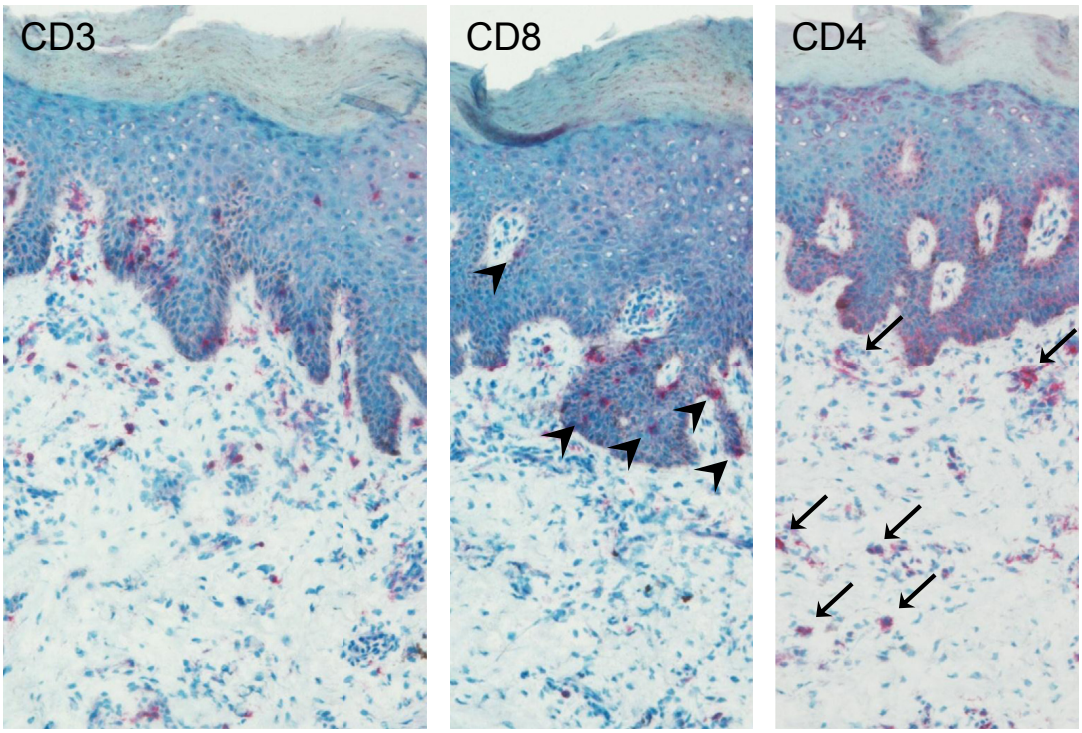

FIG E2. As in classical human psoriasis, intraepidermal T cells in the AGR mouse model represent mostly $CD8^+$ T cells. Microscopic view of representative CD3, CD4, and CD8 immunostaining of nonlesional psoriatic skin upon development of fully fledged psoriasis, 35 days after engraftment onto AGR mice. *Arrowheads* depict intraepidermal $CD8^+$ T cells, and *arrows* depict dermal $CD4^+$ T cells.

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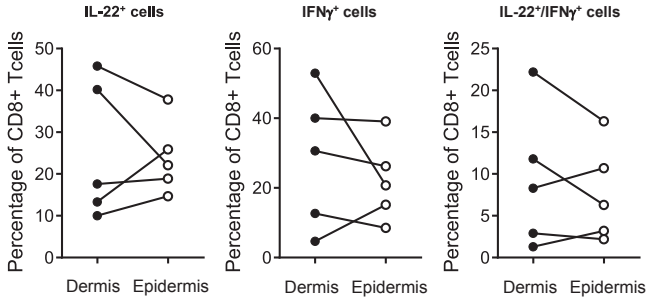

FIG E3. No differences in IL-22 and IFN-γ production between epidermal and dermal $CD8^+$ T cells. Functional characterization of epidermal and dermal $CD3^+$ $CD8^+$ T cells isolated from the epidermis and the dermis of 5 patients with psoriasis. Percentages of IL-22^+, IFN-γ^+, and IL-22^+ IFN-γ^+ $CD8^+$ T cells, as obtained by intracellular cytokine staining upon phorbol 12-myristate 13-acetate/ionomycin stimulation, show no differences between dermal and epidermal $CD8^+$ T cells. Each *line* connects epidermal and dermal $CD8^+$ T cells from the same patient.

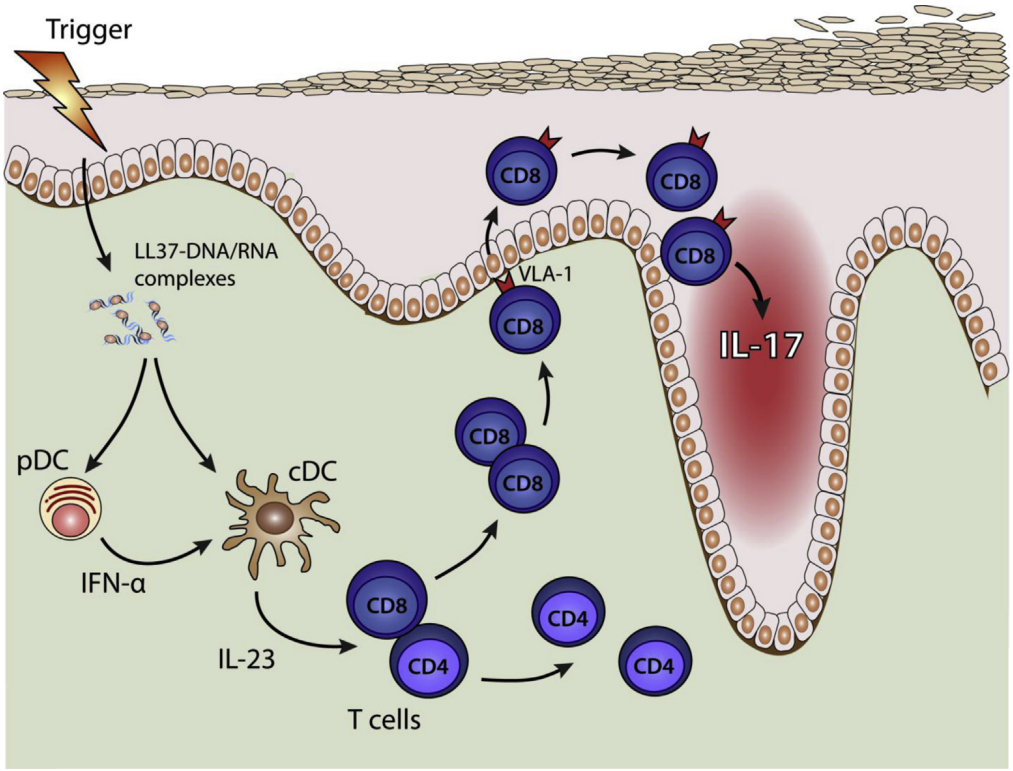

FIG E4. The role of $CD8^+$ T cells in psoriasis immunopathogenesis. Environmental triggers (eg, skin injury, known as Koebner phenomenon) induce the expression of LL37 by keratinocytes, which forms complexes with self-DNA/RNA released by dying cells. These complexes activate skin-infiltrating plasmacytoid dendritic cells (pDCs) to produce IFN-α, which in turn—together with LL37-RNA complexes—promotes maturation and activation of conventional dendritic cells producing IL-23. This leads to expansion and activation of autoreactive $CD8^+$ T cells, as well as $CD4^+$ T cells, in the dermis. Although $CD4^+$ T cells remain principally within the dermis, activated $CD8^+$ T cells acquire expression of very late antigen (VLA)-1 and migrate into the epidermis. Subsequently, potentially upon recognition of autoantigens on keratinocytes via MHC-I, intraepidermal $CD8^+$ T cells release IL-17, which is critically involved in psoriatic inflammation and its pathogenesis.

TABLE E1. Cellular and histologic changes over time during psoriasis development

Experiment depicted in Fig 1	Day 0	Day 7	Day 21	Day 35
Dermal T cells	3.22 (0.71)	16.08 (3.51)	22.30 (8.66)	6.65 (2.11)
Epidermal T cells	0.23 (0.08)	0.33 (0.24)	10.50 (1.62)	16.32 (1.68)
Papillomatosis index	1.507 (0.06)	1.598 (0.04)	2.529 (0.28)	2.978 (0.32)
Ki-67	16.67 (3.83)	11.67 (1.70)	56.39 (9.91)	72.78 (29.51)

Mean and SEM values of experimental data depicted in Fig 1, *A* and *B*. Mean (± SEM) values of dermal and epidermal T cells, papillomatosis index, and Ki-67 positive keratinocytes in skin samples upon xenotransplantation in the AGR mouse model at indicated time points. Values correspond to the data depicted in Fig 1, *A* and *B*.

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6. BIBLIOGRAPHY

1. Owen, J. A., Punt, J., Stranford, S. A., Jones, P. P. & Kuby, J. *Kuby immunology*. (W.H. Freeman, 2013).
2. Burns, T., Breathnach, S., Cox, N. & Griffiths, C. E. M. *Rook's Textbook of Dermatology*.
3. Grice, E. a. & Segre, J. a. The skin microbiome. *Nat. Rev. Microbiol.* **9,** 626–626 (2011).
4. Gallo, R. L. Human Skin Is the Largest Epithelial Surface for Interaction with Microbes. *J. Invest. Dermatol.* **137,** 1213–1214 (2017).
5. Nakatsuji, T., Chen, T. H., Two, A. M., Chun, K. a., Narala, S., Geha, R. S., Hata, T. R. & Gallo, R. L. Staphylococcus aureus Exploits Epidermal Barrier Defects in Atopic Dermatitis to Trigger Cytokine Expression. *J. Invest. Dermatol.* **136,** 2192–2200 (2016).
6. Gregorio, J., Meller, S., Conrad, C., Di Nardo, A., Homey, B., Lauerma, A., Arai, N., Gallo, R. L., Digiovanni, J. & Gilliet, M. Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. *J. Exp. Med.* **207,** 2921–2930 (2010).
7. Yamasaki, K., Di Nardo, A., Bardan, A., Murakami, M., Ohtake, T., Coda, A., Dorschner, R. a, Bonnart, C., Descargues, P., Hovnanian, A., Morhenn, V. B. & Gallo, R. L. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat. Med.* **13,** 975–980 (2007).
8. Yamasaki, K., Schauber, J., Coda, a, Lin, H., Dorschner, R. a, Schechter, N. M., Bonnart, C., Descargues, P., Hovnanian, a & Gallo, R. L. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *Faseb J* **20,** 2068–2080 (2006).
9. Yamasaki, K. & Gallo, R. Antimicrobial peptides in human skin disease. *Eur. J. dermatology* **18,** 11–21 (2008).
10. Demaria, O., Di Domizio, J. & Gilliet, M. Immune sensing of nucleic acids in inflammatory skin diseases. *Semin. Immunopathol.* **36,** 519–29 (2014).
11. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D., Weinrauch, Y. & Zychlinsky, A. Neutrophil Extracellular Traps Kill Bacteria. *Science (80-.).* **303,** 1532–1535 (2004).
12. Yipp, B. G., Petri, B., Salina, D., Jenne, C. N., Scott, B. N. V, Zbytnuik, L. D., Pittman, K., Asaduzzaman, M., Wu, K., Meijndert, H. C., Malawista, S. E., de Boisfleury Chevance, A., Zhang, K., Conly, J. & Kubes, P. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat. Med.* **18,** 1386–1393 (2012).
13. Canesso, M. C. C., Vieira, A. T., Castro, T. B. R., Schirmer, B. G. a., Cisalpino, D., Martins, F. S., Rachid, M. a., Nicoli, J. R., Teixeira, M. M. & Barcelos, L. S. Skin Wound Healing Is Accelerated and Scarless in the Absence of Commensal Microbiota. *J. Immunol.* **193,** 5171–5180 (2014).
14. Rigby, K. M. & DeLeo, F. R. Neutrophils in innate host defense against Staphylococcus aureus infections. *Semin. Immunopathol.* **34,** 237–259 (2012).
15. Tecchio, C., Micheletti, A. & Cassatella, M. a. Neutrophil-derived cytokines: Facts beyond expression. *Front. Immunol.* **5,** 1–7 (2014).
16. Chen, S. C., De Groot, M., Kinsley, D., Laverty, M., McClanahan, T., Arreaza, M., Gustafson, E. L., Teunissen, M. B. M., De Rie, M. a., Fine, J. S. & Kraan, M. Expression of chemokine receptor CXCR3 by lymphocytes and plasmacytoid dendritic cells in human psoriatic lesions. *Arch. Dermatol. Res.* **302,** 113–123 (2010).
17. Di Domizio, J., Belkoudja, C., Chenuet, P., Murray, T., Van Lierop, A., Demaria, O., Conrad, C., Homey, B., Speiser, D., Ryffel, B. & Gilliet, M. Commensal bacteria control plasmacytoid dendritic cell recruitment and activation in injured skin. in *Swiss Soc. Allergol. Immunol.* (2016).
18. Isaacs, A. & Lindenmann, J. Virus Interference. I. The Interferon. *Proc. R. Soc. B Biol. Sci.* **147,** 258–267 (1957).
19. Rönnblom, L., Ramstedt, U. & Alm, G. V. Properties of human natural interferon-producing cells stimulated by tumor cell lines. *Eur. J. Immunol.* **13,** 471–6 (1983).
20. Perussia, B., Fanning, V. & Trinchieri, G. A leukocyte subset bearing HLA-DR antigens is responsible for in vitro alpha interferon production in response to viruses. *Nat. Immun. Cell Growth Regul.* **4,** 120–137 (1985).
21. Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P., Shah, K., Ho, S., Antonenko, S. & Liu, Y.-J. The Nature of the Principal Type 1 Interferon-Producing Cells in Human Blood. *Science (80-.).* **284,** 1835–1837 (1999).
22. Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, a & Colonna, M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* **5,** 919–923 (1999).
23. Nakano, H., Yanagita, M. & Gunn, M. D. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J. Exp. Med.* **194,** 1171–1178 (2001).
24. Asselin-Paturel, C., Boonstra, a, Dalod, M., Durand, I., Yessaad, N., Dezutter-Dambuyant, C., Vicari, a, O'Garra, a, Biron, C., Brière, F. & Trinchieri, G. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat. Immunol.* **2,** 1144–1150 (2001).
25. Kadowaki, N., Ho, S., Antonenko, S., Malefyt, R. W., Kastelein, R. a, Bazan, F. & Liu, Y. J. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* **194,** 863–9 (2001).
26. Izaguirre, A., Barnes, B. J., Amrute, S., Yeow, W., Megjugorac, N., Dai, J., Feng, D., Chung, E. & Pitha, P. M. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. **74,** 1125–1138 (2003).
27. Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. Toll-like Receptor 9–mediated Recognition of Herpes Simplex Virus-2 by Plasmacytoid Dendritic Cells. *J. Exp. Med.* **198,** 513–520 (2003).

28. Krug, a., Luker, G. D., Barchet, W., Leib, D. a., Akira, S. & Colonna, M. Herpes Simplex Virus type 1 (HSV-1) activates murine natural interferon-producing cells (IPC) through toll-like receptor 9. *Blood J.* **103,** 1433–1438 (2004).
29. Krug, A., French, A. R., Barchet, W., Fischer, J. a a, Dzionek, A., Pingel, J. T., Orihuela, M. M., Akira, S., Yokoyama, W. M. & Colonna, M. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* **21,** 107–119 (2004).
30. Lund, J. M., Alexopoulou, L., Sato, a, Karow, M., Adams, N. C., Gale, N. W., Iwasaki, a & Flavell, R. a. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci U S A* **101,** 5598–5603 (2004).
31. Diebold, S. S., Kaisho, T., Hemmi, H., Akira, S. & Reis e Sousa, C. Innate Antiviral Responses by Means of TLR7-Mediated Recognition of Single-Stranded RNA. *Science (80-.).* **303,** 1529–1531 (2004).
32. Macal, M., Lewis, G. M., Kunz, S., Flavell, R., Harker, J. a. & Zúñiga, E. I. Plasmacytoid dendritic cells are productively infected and activated through TLR-7 early after arenavirus infection. *Cell Host Microbe* **11,** 617–630 (2012).
33. Gilliet, M., Cao, W. & Liu, Y.-J. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat. Rev. Immunol.* **8,** 594–606 (2008).
34. Sadler, A. J. & Williams, B. R. G. Interferon-inducible antiviral effectors. *Nat. Rev. Immunol.* **8,** 559–568 (2008).
35. Schoggins, J. W., Wilson, S. J., Panis, M., Murphy, M. Y., Jones, C. T., Bieniasz, P. & Rice, C. M. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* **472,** 481–485 (2011).
36. Hardy, A. W., Graham, D. R., Shearer, G. M. & Herbeuval, J.-P. HIV turns plasmacytoid dendritic cells (pDC) into TRAIL-expressing killer pDC and down-regulates HIV coreceptors by Toll-like receptor 7-induced IFN-alpha. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 17453–8 (2007).
37. Gabrielle Lui, P., Angel, J., Molens, J.-P., Laurence Chaperot, J., Blum, A., Manches, O., Chaperot, L., Lui, G. & Plumas, J. Virus or TLR Agonists Induce TRAIL-Mediated Cytotoxic Activity of Plasmacytoid Dendritic Cells. *J Immunol Ref.* **176,** 248–255 (2006).
38. Swiecki, M., Gilfillan, S., Vermi, W., Wang, Y. & Colonna, M. Plasmacytoid Dendritic Cell Ablation Impacts Early Interferon Responses and Antiviral NK and CD8(+) T Cell Accrual. *Immunity* **33,** 955–966 (2010).
39. Swiecki, M., Wang, Y., Gilfillan, S. & Colonna, M. Plasmacytoid Dendritic Cells Contribute to Systemic but Not Local Antiviral Responses to HSV Infections. *PLoS Pathog.* **9,** 2–11 (2013).
40. Bach, P., Kamphuis, E., Odermatt, B., Sutter, G., Buchholz, C. J. & Kalinke, U. Vesicular stomatitis virus glycoprotein displaying retrovirus-like particles induce a type I IFN receptor-dependent switch to neutralizing IgG antibodies. *J. Immunol.* **178,** 5839–47 (2007).
41. Koerner, I., Kochs, G., Kalinke, U., Weiss, S. & Staeheli, P. Protective role of beta interferon in host defense against influenza A virus. *J. Virol.* **81,** 2025–30 (2007).
42. Steinhoff, U., Müller, U., Schertler, a, Hengartner, H., Aguet, M. & Zinkernagel, R. M. Antiviral protection by vesicular stomatitis virus-specific antibodies in alpha/beta interferon receptor-deficient mice. *J. Virol.* **69,** 2153–2158 (1995).
43. Jego, G., Palucka, A. K., Blanck, J.-P. J., Chalouni, C., Pascual, V. & Banchereau, J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* **19,** 225–234 (2003).
44. Poeck, H., Wagner, M., Battiany, J., Rothenfusser, S., Wellisch, D., Hornung, V., Jahrsdorfer, B., Giese, T., Endres, S. & Hartmann, G. Plasmacytoid dendritic cells, antigen, and CpG-C license human B cells for plasma cell differentiation and immunoglobulin production in the absence of T-cell help. *Blood* **103,** 3058–3064 (2004).
45. Swiecki, M., Gilfillan, S., Vermi, W., Wang, Y. & Colonna, M. Plasmacytoid Dendritic Cell Ablation Impacts Early Interferon Responses and Antiviral NK and CD8+ T Cell Accrual. *Immunity* **33,** 955–966 (2010).
46. Conrad, C., Meller, S. & Gilliet, M. Plasmacytoid dendritic cells in the skin: to sense or not to sense nucleic acids. *Semin Immunol* **21,** 101–109 (2009).
47. Geissmann, F., Manz, M., Jung, S., Sieweke, M., Merad, M. & Ley, K. Development of Monocytes, Macrophages, and Dendritic Cells. **327,** 656–661 (2010).
48. Sandblad, K. G., Jones, P., Kostalla, M. J., Linton, L., Glise, H. & Winqvist, O. Chemokine receptor expression on monocytes from healthy individuals. *Clin. Immunol.* **161,** 348–353 (2015).
49. Chomarat, P., Dantin, C., Bennett, L., Banchereau, J. & Palucka, a. K. Macrophages to Dendritic Cells TNF Skews Monocyte Differentiation from. *J. Immunol.* **171,** 2262–2269 (2003).
50. Farkas, Á. & Kemény, L. Interferon-α in the generation of monocyte-derived dendritic cells: Recent advances and implications for dermatology. *Br. J. Dermatol.* **165,** 247–254 (2011).
51. Lee, P. Y., Li, Y., Kumagai, Y., Xu, Y., Weinstein, J. S., Kellner, E. S., Nacionales, D. C., Butfiloski, E. J., van Rooijen, N., Akira, S., Sobel, E. S., Satoh, M. & Reeves, W. H. Type I Interferon Modulates Monocyte Recruitment and Maturation in Chronic Inflammation. *Am. J. Pathol.* **175,** 2023–2033 (2009).
52. Bauer, J. W., Baechler, E. C., Petri, M., Batliwalla, F. M., Crawford, D., Ortmann, W. a., Espe, K. J., Li, W., Patel, D. D., Gregersen, P. K. & Behrens, T. W. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. *PLoS Med.* **3,** 2274–2284 (2006).
53. Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., Koyasu, S., Locksley, R. M., McKenzie, A. N. J., Mebius, R. E., Powrie, F. & Vivier, E. Innate lymphoid cells — a proposal for uniform nomenclature. *Nat. Rev. Immunol.* **13,** 145–149 (2013).

54. Neill, D. R., Wong, S. H., Bellosi, A., Flynn, R. J., Daly, M., Langford, T. K. a., Bucks, C., Kane, C. M., Fallon, P. G., Pannell, R., Jolin, H. E. & McKenzie, A. N. J. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* **464,** 1367–1370 (2010).
55. Balzola, F., Bernstein, C., Ho, G. T. & Lees, C. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology: Commentary. *Inflamm. Bowel Dis. Monit.* **11,** 79 (2010).
56. Sawa, S., Cherrier, M., Lochner, M., Satoh-Takayama, N., Fehling, H. J., Langa, F., Di Santo, J. P. & Eberl, G. Lineage relationship analysis of RORgammat+ innate lymphoid cells. *Science* **330,** 665–9 (2010).
57. Walker, J. a, Barlow, J. L. & McKenzie, A. N. J. Innate lymphoid cells--how did we miss them? *Nat. Rev. Immunol.* **13,** 75–87 (2013).
58. Constantinides, M. G., McDonald, B. D., Verhoef, P. A. & Bendelac, A. A committed precursor to innate lymphoid cells. *Nature* **508,** 397–401 (2014).
59. Gordon, S. M., Chaix, J., Rupp, L. J., Wu, J., Madera, S., Sun, J. C., Lindsten, T. & Reiner, S. L. The Transcription Factors T-bet and Eomes Control Key Checkpoints of Natural Killer Cell Maturation. *Immunity* **36,** 55–67 (2012).
60. Takatori, H., Kanno, Y., Watford, W. T., Tato, C. M., Weiss, G., Ivanov, I. I., Littman, D. R. & O'Shea, J. J. Lymphoid tissue inducer–like cells are an innate source of IL-17 and IL-22. *J. Exp. Med.* **206,** 35–41 (2009).
61. Mjösberg, J. M., Trifari, S., Crellin, N. K., Peters, C. P., van Drunen, C. M., Piet, B., Fokkens, W. J., Cupedo, T. & Spits, H. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat. Immunol.* **12,** 1055–1062 (2011).
62. Kim, B. S., Siracusa, M. C., Saenz, S. A., Noti, M., Monticelli, L. A., Sonnenberg, G. F., Hepworth, M. R., Van Voorhees, A. S., Comeau, M. R. & Artis, D. TSLP Elicits IL-33-Independent Innate Lymphoid Cell Responses to Promote Skin Inflammation. *Sci. Transl. Med.* **5,** 170ra16–170ra16 (2013).
63. Sonnenberg, G. F. & Artis, D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat. Med.* **21,** 698–708 (2015).
64. Jiang, X., Clark, R. a., Liu, L., Wagers, A. J., Fuhlbrigge, R. C. & Kupper, T. S. Skin infection generates non-migratory memory CD8+ TRM cells providing global skin immunity. *Nature* **483,** 227–231 (2012).
65. Zhu, J., Peng, T., Johnston, C., Phasouk, K., Kask, A. S., Klock, A., Jin, L., Diem, K., Koelle, D. M., Wald, A., Robins, H. & Corey, L. Immune surveillance by CD8αα+ skin-resident T cells in human herpes virus infection. *Nature* **497,** 494–497 (2013).
66. Ariotti, S., Hogenbirk, M. a., Dijkgraaf, F. E., Visser, L. L., Hoekstra, M. E., Song, J.-Y., Jacobs, H., Haanen, J. B. & Schumacher, T. N. Skin-resident memory CD8+ T cells trigger a state of tissue-wide pathogen alert. *Science (80-.).* **346,** 101–105 (2014).
67. Mackay, L. K., Rahimpour, A., Ma, J. Z., Collins, N., Stock, A. T., Hafon, M.-L., Vega-Ramos, J., Lauzurica, P., Mueller, S. N., Stefanovic, T., Tscharke, D. C., Heath, W. R., Inouye, M., Carbone, F. R. & Gebhardt, T. The developmental pathway for CD103+CD8+ tissue-resident memory T cells of skin. *Nat. Immunol.* **14,** 1294–1301 (2013).
68. Gebhardt, T., Wakim, L. M., Eidsmo, L., Reading, P. C., Heath, W. R. & Carbone, F. R. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat. Immunol.* **10,** 524–530 (2009).
69. Glennie, N. D., Yeramilli, V. a., Beiting, D. P., Volk, S. W., Weaver, C. T. & Scott, P. Skin-resident memory $CD4^+$ T cells enhance protection against *Leishmania major* infection. *J. Exp. Med.* **212,** 1405–1414 (2015).
70. Malhotra, N., Yoon, J., Leyva-Castillo, J. M., Galand, C., Archer, N., Miller, L. S. & Geha, R. S. IL-22 derived from γδ T cells restricts Staphylococcus aureus infection of mechanically injured skin. *J. Allergy Clin. Immunol.* **138,** 1098–1107.e3 (2016).
71. Shiow, L. R., Rosen, D. B., Brdičková, N., Xu, Y., An, J., Lanier, L. L., Cyster, J. G. & Matloubian, M. CD69 acts downstream of interferon-α/β to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* **440,** 540–544 (2006).
72. Kolumam, G. a., Thomas, S., Thompson, L. J., Sprent, J. & Murali-Krishna, K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J. Exp. Med.* **202,** 637–650 (2005).
73. Havenar-Daughton, C., Kolumam, G. a & Murali-Krishna, K. Cutting Edge: The direct action of type I IFN on CD4 T cells is critical for sustaining clonal expansion in response to a viral but not a bacterial infection. *J. Immunol.* **176,** 3315–3319 (2006).
74. Crouse, J., Kalinke, U. & Oxenius, A. Regulation of antiviral T cell responses by type I interferons. *Nat. Rev. Immunol.* **15,** 231–242 (2015).
75. Speiser, D. E., Ho, P.-C. & Verdeil, G. Regulatory circuits of T cell function in cancer. *Nat. Rev. Immunol.* **16,** 599–611 (2016).
76. Kurosaki, T., Kometani, K. & Ise, W. Memory B cells. *Nat. Rev. Immunol.* **15,** 149–159 (2015).
77. Cabanillas, F., Liboy, I., Pavia, O. & Rivera, E. High incidence of non-neutropenic infections induced by rituximab plus fludarabine and associated with hypogammaglobulinemia: A frequently unrecognized and easily treatable complication. *Ann. Oncol.* **17,** 1424–1427 (2006).
78. Gea-Banacloche, J. C. Rituximab-Associated Infections. *Semin. Hematol.* **47,** 187–198 (2010).
79. Fried, A. J. & Bonilla, F. a. Pathogenesis, diagnosis, and management of primary antibody deficiencies and infections. *Clin. Microbiol. Rev.* **22,** 396–414 (2009).

80. Winkelstein, J. a, Marino, M. C., Lederman, H. M., Jones, S. M., Sullivan, K., Burks, a W., Conley, M. E., Cunningham-Rundles, C. & Ochs, H. D. X-linked agammaglobulinemia: report on a United States registry of 201 patients. *Medicine (Baltimore).* **85,** 193–202 (2006).
81. Hausser, C., Virelizier, J. L., Buriot, D. & Griscelli, C. Common variable hypogammaglobulinemia in children. Clinical and immunologic observations in 30 patients. *Am. J. Dis. Child.* **137,** 833–7 (1983).
82. Plebani, A., Soresina, A., Rondelli, R., Amato, G. M., Azzari, C., Cardinale, F., Cazzola, G., Consolini, R., De Mattia, D., Dell'Erba, G., Duse, M., Fiorini, M., Martino, S., Martire, B., Masi, M., Monafo, V., Moschese, V., Notarangelo, L. D., Orlandi, P., Panei, P., Pession, A., Pietrogrande, M. C., Pignata, C., Quinti, I., Ragno, V., Rossi, P., Sciotto, A. & Stabile, A. Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. *Clin. Immunol.* **104,** 221–230 (2002).
83. Cunningham-Rundles, C. & Bodian, C. Common Variable Immunodeficiency: Clinical and Immunological Features of 248 Patients. *Clin. Immunol.* **92,** 34–48 (1999).
84. DiSanto, J. P., Bonnefoy, J. Y., Gauchat, J. F., Fischer, a. & de Saint Basile, G. CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM. *Nature* **361,** 541–3 (1993).
85. Armitage, R. J., Fanslow, W. C., Strockbine, L., Sato, T. a., Clifford, K. N., Macduff, B. M., Anderson, D. M., Gimpel, S. D., Davis-Smith, T. & Maliszewski, C. R. Molecular and biological characterization of a murine ligand for CD40. *Nature* **357,** 80–2 (1992).
86. Choi, Y. S., Kageyama, R., Eto, D., Escobar, T. C., Johnston, R. J., Monticelli, L., Lao, C. & Crotty, S. ICOS Receptor Instructs T Follicular Helper Cell versus Effector Cell Differentiation via Induction of the Transcriptional Repressor Bcl6. *Immunity* **34,** 932–946 (2011).
87. Xu, H., Li, X., Liu, D., Li, J., Zhang, X., Chen, X., Hou, S., Peng, L., Xu, C., Liu, W., Zhang, L. & Qi, H. Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature* **496,** 523–527 (2013).
88. Morita, R., Schmitt, N., Bentebibel, S. E., Ranganathan, R., Bourdery, L., Zurawski, G., Foucat, E., Dullaers, M., Oh, S. K., Sabzghabaei, N., Lavecchio, E. M., Punaro, M., Pascual, V., Banchereau, J. & Ueno, H. Human Blood CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion. *Immunity* **34,** 108–121 (2011).
89. Vinuesa, C. G. & Cyster, J. G. How T cells earn the follicular rite of passage. *Immunity* **35,** 671–680 (2011).
90. Ansel, K. M., Ngo, V. N., Hyman, P. L., Luther, S. a., Förster, R., Sedgwick, J. D., Browning, J. L., Lipp, M. & Cyster, J. G. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406,** 309–314 (2000).
91. Le Bon, A., Schiavoni, G., D'Agostino, G., Gresser, I., Belardelli, F. & Tough, D. F. Type I Interferons Potently Enhance Humoral Immunity and Can Promote Isotype Switching by Stimulating Dendritic Cells In Vivo. *Immunity* **14,** 461–470 (2001).
92. Gujer, C., Sandgren, K. J., Douagi, I., Adams, W. C., Sundling, C., Smed-Sörensen, A., Seder, R. a., Hedestam, G. B. K. & Loré, K. IFN-α produced by human plasmacytoid dendritic cells enhances T cell-dependent naïve B cell differentiation. *J. Leukoc. Biol.* **89,** 811–821 (2011).
93. Park, C. O. & Kupper, T. S. The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat. Med.* **21,** 688–697 (2015).
94. Schenkel, J. M. & Masopust, D. Tissue-Resident Memory T Cells. *Immunity* **41,** 886–897 (2014).
95. Nihal, M., Mikkola, D. & Wood, G. S. Detection of Clonally Restricted Immunoglobulin Heavy Chain Gene Rearrangements in Normal and Lesional Skin. *J. Mol. Diagnostics* **2,** 5–10 (2000).
96. Perera, G. K., Di Meglio, P. & Nestle, F. O. Psoriasis. *Annu. Rev. Pathol. Mech. Dis.* **7,** 385–422 (2012).
97. Mueller W, H. B. Cyclosporin A for psoriasis. *N. Engl. J. Med.* **301,** 355–358 (1979).
98. Griffiths, C., Powles, A., Leonard, J., Fry, L., Baker, B. & Valdimarsson, H. Clearance of psoriasis with low dose cyclo. *Br. Med. J.* **293,** 731–2 (1986).
99. Cyclosporine for plaque-type psoriasis. *J Exp Med* **324,** 277–284 (1991).
100. Tiilikainen, a., Lassus, A., Karvonen, J., Vartiainen, P. & Julin, M. Psoriasis and HLA-Cw6. *Br. J. Dermatol.* **102,** 179–184 (1980).
101. Veal, C. D., Capon, F., Allen, M. H., Heath, E. K., Evans, J. C., Jones, A., Patel, S., Burden, D., Tillman, D., Barker, J. N. W. N. & Trembath, R. C. Family-Based Analysis Using a Dense Single-Nucleotide Polymorphism–Based Map Defines Genetic Variation at PSORS1, the Major Psoriasis-Susceptibility Locus. *Am. J. Hum. Genet.* **71,** 554–564 (2002).
102. Gudjonsson, J. E., Karason, A., Runarsdottir, E. H., Antonsdottir, A. a, Hauksson, V. B., Jónsson, H. H., Gulcher, J., Stefansson, K. & Valdimarsson, H. Distinct clinical differences between HLA-Cw*0602 positive and negative psoriasis patients--an analysis of 1019 HLA-C- and HLA-B-typed patients. *J. Invest. Dermatol.* **126,** 740–5 (2006).
103. Boyman, O., Hefti, H. P., Conrad, C., Nickoloff, B. J., Suter, M. & Nestle, F. O. Spontaneous Development of Psoriasis in a New Animal Model Shows an Essential Role for Resident T Cells and Tumor Necrosis Factor- ⌴ The Journal of Experimental Medicine. **199,** (2004).
104. Conrad, C., Boyman, O., Tonel, G., Tun-Kyi, A., Laggner, U., de Fougerolles, A., Kotelianski, V., Gardner, H. & Nestle, F. O. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat. Med.* **13,** 836–842 (2007).
105. Menssen, a, Trommler, P., Vollmer, S., Schendel, D., Albert, E., Gürtler, L., Riethmüller, G. & Prinz, J. C. Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. *J. Immunol.* **155,** 4078–83 (1995).

106. Chang, J. C., Smith, L. R., Froning, K. J., Schwabe, B. J., Laxer, J. a, Caralli, L. L., Kurland, H. H., Karasek, M. a, Wilkinson, D. I. & Carlo, D. J. CD8+ T cells in psoriatic lesions preferentially use T-cell receptor V beta 3 and/or V beta 13.1 genes. *Proc. Natl. Acad. Sci. U. S. A.* **91,** 9282–6 (1994).
107. Gudjonsson, J. . E., Thorarinsson, A. M., Sigurgeirsson, B., K.G, K. & Valdimarsson, H. Clinical and Laboratory Investigations Streptococcal throat infections and exacerbation of chronic plaque psoriasis : a prospective study. *Br. J. Dermatol.* 530–534 (2003).
108. Baker, B. S., Brown, D. W., Fischetti, V. A., J.M., O., Porter, W., Powless, A. & Fry, L. Skin T cell proliferative response to M protein and other cell wall and membrane proteins of group A streptococci in chronic plaque psoriasis. *Clin. Exp. Immunol.* 516–521 (2001).
109. Johnston, A., Gudjonsson, J. E., Sigmundsdottir, H., Love, T. J. & Valdimarsson, H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing CD8 + T cells. (2004). doi:10.1111/j.1365-2249.2004.02600.x
110. Brown, D. W., Baker, B. S., Fischetti, J. O. V. A., Hardman, C., Powles, A. V & Fry, L. Non-M protein (s) on the cell wall and membrane of group A streptococci induce (s) IFN- γ production by dermal CD4 + T cells in psoriasis. 165–170 (2001).
111. Baker, B. S., Ovigne, J., Fischetti, V. A., Powles, A. & Fry, L. Selective Response of Dermal Th-1 Cells to 20 – 50 kDa Streptococcal Cell-Wall Proteins in Chronic Plaque Psoriasis. 335–341 (2003).
112. Besgen, P., Trommler, P., Vollmer, S. & Prinz, J. C. Ezrin, Maspin, Peroxiredoxin 2, and Heat Shock Protein 27: Potential Targets of a Streptococcal-Induced Autoimmune Response in Psoriasis. *J. Immunol.* **184,** 5392–5402 (2010).
113. Arakawa, A., Siewert, K., Stöhr, J., Besgen, P., Kim, S. M., Rühl, G., Nickel, J., Vollmer, S., Thomas, P., Krebs, S., Pinkert, S., Spannagl, M., Held, K., Kammerbauer, C., Besch, R., Dornmair, K. & Prinz, J. C. Melanocyte antigen triggers autoimmunity in human psoriasis. 2203–2212 (2015). doi:10.1084/jem.20151093
114. Lande, R., Botti, E., Jandus, C., Dojcinovic, D., Fanelli, G., Conrad, C., Chamilos, G., Feldmeyer, L., Marinari, B., Chon, S., Vence, L., Riccieri, V., Guillaume, P., Navarini, A. A., Romero, P., Costanzo, A., Piccolella, E., Gilliet, M. & Frasca, L. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. *Nat. Commun.* **5,** 1–15 (2014).
115. Kristensen, M., Chu, C. Q., Eedy, D. J., Feldmann, M. & Brennan, F. M. Localization of tumour necrosis factor-alpha (TNF-a) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. *Clin. Exp. Immunol.* **94,** 354–362 (1993).
116. Lowes, M. A., Chamian, F., Abello, M. V., Fuentes-duculan, J., Lin, S., Nussbaum, R., Novitskaya, I., Carbonaro, H., Cardinale, I., Kikuchi, T., Gilleaudeau, P., Sullivan-whalen, M., Wittkowski, K. M., Papp, K., Garovoy, M., Dummer, W., Steinman, R. M. & Krueger, J. G. Increase in TNF-a and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc. Natl. Acad. Sci.* **102,** 19057–19062 (2005).
117. Hansel, A., Gunther, C., Ingwersen, J., Starke, J., Schmitz, M., Bachmann, M., Meurer, M., Rieber, E. P. & Schakel, K. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17 / T H1 T-cell responses. *J. Allergy Clin. Immunol.* **127,** 787–794 (2011).
118. Wang, H., Peters, T., Kess, D., Sindrilaru, A., Oreshkova, T., Rooijen, N. Van, Stratis, A., Renkl, A. C., Sunderkötter, C., Walschek, M., Haase, I. & Scharffetter-Kochanek, K. Activated macrophages are essential in a murine model for T cell – mediated chronic psoriasiform skin inflammation. *J. Clin. Invest.* **116,** 2105–2114 (2006).
119. Austin, L. M., Ozawa, M., Kikuchi, T., Walters, I. B. & Krueger, J. G. The majority of epidermal T cells in psoriasis vulgaris lesions can produce type 1 cytokines, interferon-γ, interleukin-2, and tumor necrosis factor-α, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: A type 1 differentiation bias is al. *J. Invest. Dermatol.* **113,** 752–759 (1999).
120. Toussirot, É., Aubin, F. & Dumoulin, G. Relationships between adipose tissue and psoriasis , with or without arthritis. *Front. Immunol.* **5,** 1–7 (2014).
121. Kanemaru, K., Matsuyuki, A., Nakamura, Y. & Fukami, K. Obesity exacerbates imiquimod-induced psoriasis-like epidermal hyperplasia and interleukin-17 and interleukin-22 production in mice. *Exp. Dermatol.* **24,** 436–442 (2015).
122. Tan, J. K., Aphale, A., Malaviya, R., Sun, Y. & Gottlieb, A. B. Mechanisms of action of etanercept in psoriasis. *J. Investig. Dermatol. Symp. Proc.* **12,** 38–45 (2007).
123. Zaba, L. C., Cardinale, I., Gilleaudeau, P., Sullivan-Whalen, M., Suárez-Fariñas, M., Fuentes-Duculan, J., Novitskaya, I., Khatcherian, A., Bluth, M. J., Lowes, M. a & Krueger, J. G. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J. Exp. Med.* **204,** 3183–3194 (2007).
124. Zaba, L. C., Suárez-Fariñas, M., Fuentes-Duculan, J., Nograles, K. E., Guttman-Yassky, E., Cardinale, I., Lowes, M. a. & Krueger, J. G. Effective treatment of psoriasis with etanercept is linked to suppression of IL-17 signaling, not immediate response TNF genes. *J. Allergy Clin. Immunol.* **124,** (2009).
125. Gunderson, A. J., Mohammed, J., Horvath, F. J., Podolsky, M. a, Anderson, C. R. & Glick, A. B. CD8(+) T cells mediate RAS-induced psoriasis-like skin inflammation through IFN-γ. *J. Invest. Dermatol.* **133,** 955–63 (2013).
126. Leonardi, C. L., Kimball, A. B., Papp, K. A., Yeilding, N., Guzzo, C., Wang, Y., Li, S., Dooley, L. T., Gordon, K. B. & Investigators, for the P. 1. Efficacy and safety of ustekinumab, a human interleukin-12 / 23 monoclonal antibody, in patients with psoriasis : 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet* **371,** 1675–1684 (2008).

127. Papp, K. A., Langley, R. G., Lebwohl, M., Krueger, G. G., Szapary, P., Yeilding, N., Guzzo, C., Hsu, M. C., Wang, Y., Li, S., Dooley, L. T., Reich, K. & Investigators, for the P. 2. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* **371,** 1675–1684 (2008).
128. Langley, R. G., Elewski, B. E., Lebwohl, M., Reich, K., Griffiths, C. E. M., Papp, K., Puig, L., Nakagawa, H., Spelman, L., Sigurgeirsson, B., Rivas, E., Tsai, T.-F., Wasel, N., Tyring, S., Salko, T., Hampele, I., Notter, M., Karpov, A., Helou, S. & Papavassilis, C. Secukinumab in Plaque Psoriasis — Results of Two Phase 3 Trials. *N. Engl. J. Med.* **371,** 326–338 (2014).
129. Papp, K. A., Blauvelt, A., Bukhalo, M., Gooderham, M., Krueger, J. G., Lacour, J.-P., Menter, A., Philipp, S., Sofen, H., Tyring, S., Berner, B. R., Visvanathan, S., Pamulapati, C., Bennett, N., Flack, M., Scholl, P. & Padula, S. J. Risankizumab versus Ustekinumab for Moderate-to-Severe Plaque Psoriasis. *N. Engl. J. Med.* **376,** 1551–1560 (2017).
130. Papp, K., Thaci, D., Reich, K., Riedl, E., Langley, R. G., Krueger, J. G., Gottlieb, A. B., Nakagawa, H., Bowman, E. P., Mehta, A., Li, Q., Zhou, Y. & Shames, R. Tildrakizumab (MK-3222), an anti-interleukin-23p19 monoclonal antibody, improves psoriasis in a phase IIb randomized placebo-controlled trial. *Br. J. Dermatol.* **173,** 930–939 (2015).
131. Chiricozzi, a, Guttman-Yassky, E., Suarez-Farinas, M., Nograles, K. E., Tian, S., Cardinale, I., Chimenti, S. & Krueger, J. G. Integrative responses to IL-17 and TNF-alpha in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. *J Invest Dermatol* **131,** 677–687 (2011).
132. Krueger, J. G., Fretzin, S., Suárez-Fariñas, M., Haslett, P. a., Phipps, K. M., Cameron, G. S., McColm, J., Katcherian, A., Cueto, I., White, T., Banerjee, S. & Hoffman, R. W. IL-17A is essential for cell activation and inflammatory gene circuits in subjects with psoriasis. *J. Allergy Clin. Immunol.* **130,** (2012).
133. Hartupee, J., Liu, C., Novotny, M., Li, X. & Hamilton, T. IL-17 enhances chemokine gene expression through mRNA stabilization. *J. Immunol.* **179,** 4135–4141 (2007).
134. Johnston, A., Guzman, A. M., Swindell, W. R., Wang, F., Kang, S. & Gudjonsson, J. E. Early tissue responses in psoriasis to the antitumour necrosis factor-?? biologic etanercept suggest reduced interleukin-17 receptor expression and signalling. *Br. J. Dermatol.* **171,** 97–107 (2014).
135. Yamada, D. H., Osokine, I., Yamada, D. H., De la Fuente, J. R., Elsaesser, H. J. & Brooks, D. G. Overcoming CD4 Th1 Cell Fate Restrictions to Sustain Antiviral CD8??T Cells and Control Persistent Virus Infection. *Cell Rep.* **16,** 3286–3296 (2016).
136. Stefan H. E. Kaufmann. Immunity to Intracellular Bacteria. *Immunol Rev* (1993). at <http://www.annualreviews.org/doi/pdf/10.1146/annurev.iy.11.040193.001021>
137. Curtis, M. M. & Way, S. S. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* **126,** 177–185 (2009).
138. Oppmann, B., Lesley, R., Blom, B., Timans, J. C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., Zonin, F., Vaisberg, E., Churakova, T., Liu, M., Gorman, D., Wagner, J., Zurawski, S., Liu, Y., Abrams, J. S., Moore, K. W., Rennick, D., Waal-malefyt, R. De, Hannum, C., Bazan, J. F. & Kastelein, R. A. Novel p19 Protein Engages IL-12p40 to Form a Cytokine , IL-23 , with Biological Activities Similar as Well as Distinct from IL-12. **13,** 715–725 (2000).
139. Aggarwal, S., Ghilardi, N., Xie, M., Sauvage, F. J. De & Gurney, A. L. Interleukin-23 Promotes a Distinct CD4 T Cell Activation State Characterized by the Production of Interleukin-17 *. **278,** 1910–1914 (2003).
140. Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M. & Weaver, C. T. Interleukin 17 – producing CD4 + effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. **6,** 1123–1132 (2005).
141. Acosta-rodriguez, E. V, Napolitani, G., Lanzavecchia, A. & Sallusto, F. Interleukins 1 b and 6 but not transforming growth factor- b are essential for the differentiation of interleukin 17 – producing human T helper cells. **8,** 942–949 (2007).
142. Wilson, N. J., Boniface, K., Chan, J. R., Mckenzie, B. S., Blumenschein, W. M., Mattson, J. D., Basham, B., Smith, K., Chen, T., Morel, F., Lecron, J., Kastelein, R. A., Cua, D. J., Mcclanahan, T. K., Bowman, E. P. & Malefyt, R. D. W. Development , cytokine profile and function of human interleukin 17 – producing helper T cells. **8,** (2007).
143. Segura, E., Touzot, M., Bohineust, A., Cappuccio, A., Chiocchia, G., Hosmalin, A., Dalod, M., Soumelis, V. & Amigorena, S. Human Inflammatory Dendritic Cells Induce Th17 Cell Differentiation. *Immunity* **38,** 336–348 (2013).
144. Zielinski, C. E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., Monticelli, S., Lanzavecchia, A. & Sallusto, F. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. *Nature* **484,** 514–518 (2012).
145. Volpe, E., Servant, N., Zollinger, R., Bogiatzi, S. I., Hupe, P., Barillot, E. & Soumelis, V. A critical function for transforming growth factor- b, interleukin 23 and proinflammatory cytokines in driving and modulating human TH-17 responses. *Nat. Immunol.* **9,** 650–657 (2008).
146. Sallusto, F., Zielinski, C. E. & Lanzavecchia, A. Th17 Review Series Human Th17 subsets. 2215–2220 (2012). doi:10.1002/eji.201242741
147. Huber, M., Heink, S., Pagenstecher, A., Reinhard, K., Ritter, J., Visekruna, A., Guralnik, A., Bollig, N., Jeltsch, K., Heinemann, C., Wittmann, E., Buch, T., Da Costa, O. P., Brüstle, A., Brenner, D., Mak, T. W., Mittrücker, H. W., Tackenberg, B., Kamradt, T. & Lohoff, M. IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis. *J. Clin. Invest.* **123,** 247–260 (2013).

148. He, D., Wu, L., Kim, H. K., Li, H., Elmets, C. a & Xu, H. CD8+ IL-17-producing T cells are important in effector functions for the elicitation of contact hypersensitivity responses. *J. Immunol.* **177,** 6852–6858 (2006).
149. Gudjonsson, J. E., Johnston, A., Dyson, M., Valdimarsson, H. & Elder, J. T. Mouse models of psoriasis. *J. Invest. Dermatol.* **127,** 1292–308 (2007).
150. Suárez-Fariñas, M., Fuentes-Duculan, J., Lowes, M. A. & Krueger, J. G. Resolved psoriasis lesions retain expression of a subset of disease-related genes. *J. Invest. Dermatol.* **131,** 391–400 (2011).
151. Frank, N. O., Curdin, C., Adrian, T.-K., Bernhard, H., Gombert, M., Boyman, O., Burg, G., Liu, Y.-J. & Gilliet, M. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J. Exp. Med.* **202,** 135–143 (2005).
152. Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y.-H., Homey, B., Cao, W., Wang, Y.-H., Su, B., Nestle, F. O., Zal, T., Mellman, I., Schröder, J.-M., Liu, Y.-J. & Gilliet, M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449,** 564–569 (2007).
153. Ganguly, D., Chamilos, G., Lande, R., Gregorio, J., Meller, S., Facchinetti, V., Homey, B., Barrat, F. J., Zal, T. & Gilliet, M. Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. *J. Exp. Med.* **206,** 1983–1994 (2009).
154. Dao, T., Iwaki, K., Takeuchi, M., Ohashi, K., Fukuda, S. & Kurimoto, M. *Natural human interferon-alpha inhibits the adhesion of a human carcinoma cell line to human vascular endothelium. J. Interferon Cytokine Res.* **15,** (1995).
155. Ray, S. J., Franki, S. N., Pierce, R. H., Dimitrova, S., Koteliansky, V., Sprague, A. G., Doherty, P. C., Fougerolles, A. R. De, Topham, D. J., York, N., De Fougerolles, A. R. & Topham, D. J. The Collagen Binding a1b1 Integrin VLA-1 Regulates CD8 T Cell-Mediated Immune Protection against Heterologous Influenza Infection. *Immunity* **20,** 167–179 (2004).
156. Pasparakis, M., Alexopoulou, L., Episkopou, V. & Kollias, G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J. Exp. Med.* **184,** 1397 LP – 1411 (1996).
157. Liu, J., Marino, M. W., Wong, G., Grail, D., Dunn, A., Bettadapura, J., Slavin, A. J., Old, L. & Bernard, C. C. A. TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat. Med.* **4,** 78–83 (1998).
158. Marino, M. W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., Richards, E., Jungbluth, A., Wada, H., Moore, M., Williamson, B., Basu, S. & Old, L. J. Characterization of tumor necrosis factor-deficient mice. *Proc. Natl. Acad. Sci.* **94,** 8093–8098 (1997).
159. Bartelds, G. M., Krieckaert, C., Nurmohamed, M., van Schouwenburg, P., Lems, W. F., Twisk, J., Dijkmans, B. A. C., Aarden, L. & Wolbink, G. J. Development of Antidrug Antibodies Against Adalimumab and Association. *JAMA Intern. Med.* **305,** 1460–1468 (2011).
160. Van Schouwenburg, P. A., Rispens, T. & Wolbink, G. J. Immunogenicity of anti-TNF biologic therapies for rheumatoid arthritis. *Nat. Rev. Rheumatol.* **9,** 164–172 (2013).
161. Williams, E. L., Gadola, S. & Edwards, C. J. Anti-TNF-induced lupus. *Rheumatology* **48,** 716–720 (2009).
162. Bardazzi, F., Odorici, G., Virdi, A., Antonucci, V. A., Tengattini, V., Patrizi, A. & Balestri, R. Autoantibodies in psoriatic patients treated with anti-TNF-α therapy. *J. Dtsch. Dermatol. Ges.* **12,** 401–6 (2014).
163. Zettlitz, K. A., Lorenz, V., Landauer, K., Münkel, S., Herrmann, A., Scheurich, P., Pfizenmaier, K. & Kontermann, R. E. ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody. *MAbs* **2,** 639–647 (2010).
164. McCann, F. E., Perocheau, D. P., Ruspi, G., Blazek, K., Davies, M. L., Feldmann, M., Dean, J. L. E., Stoop, A. A. & Williams, R. O. Selective Tumor Necrosis Factor Receptor I Blockade Is Antiinflammatory and Reveals Immunoregulatory Role of Tumor Necrosis Factor Receptor II in Collagen-Induced Arthritis. *Arthritis Rheumatol.* **66,** 2728–2738 (2014).
165. Dapavo, P., Vujic, I., Fierro, M. T., Quaglino, P. & Sanlorenzo, M. The infliximab biosimilar in the treatment of moderate to severe plaque psoriasis. *J. Am. Acad. Dermatol.* **75,** 736–739 (2016).
166. Park, W., Hrycaj, P., Jeka, S., Kovalenko, V., Lysenko, G., Miranda, P., Mikazane, H., Gutierrez-Ureña, S., Lim, M., Lee, Y.-A., Lee, S. J., Kim, H., Yoo, D. H. & Braun, J. A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. *Ann. Rheum. Dis.* **72,** 1605–1612 (2013).
167. Dörner, T. & Kay, J. Biosimilars in rheumatology: current perspectives and lessons learnt. *Nat. Rev. Rheumatol.* **11,** 713–724 (2015).
168. Durez, P., Vandepapeliere, P., Miranda, P., Toncheva, A., Berman, A., Kehler, T., Mociran, E., Fautrel, B., Mariette, X., Dhellin, O., Fanget, B., Ouary, S., Grouard-Vogel, G. & Boissier, M. C. Therapeutic vaccination with TNF-Kinoid in TNF antagonist-resistant rheumatoid arthritis: A phase II randomized, controlled clinical trial. *PLoS One* **9,** (2014).
169. Weinblatt, M., Schiff, M., Goldman, A., Kremer, J., Luggen, M., Li, T., Chen, D. & Becker, J.-C. Selective costimulation modulation using abatacept in patients with active rheumatoid arthritis while receiving etanercept: a randomised clinical trial. *Ann. Rheum. Dis.* **66,** 228–234 (2006).
170. Fischer, J. A. A., Hueber, A. J., Wilson, S., Galm, M., Baum, W., Kitson, C., Auer, J., Lorenz, S. H., Moelleken, J., Bader, M., Tissot, A. C., Tan, S. L., Seeber, S. & Schett, G. Combined inhibition of tumor necrosis factor α and interleukin-17 as a therapeutic opportunity in rheumatoid arthritis: Development and characterization of a novel bispecific antibody. *Arthritis Rheumatol.* **67,** 51–62 (2015).

171. Grine, L., Dejager, L., Libert, C. & Vandenbroucke, R. E. An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17. *Cytokine Growth Factor Rev.* **26,** 25–33 (2015).
172. Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., Ghrayeb, J. & Woody, J. N. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis Rheum.* **36,** 1681–1690 (1993).
173. Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J. S., Leeb, B., Breedveld, F. C., Macfarlane, J. D. & Bijl, H. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet (London, England)* **344,** 1105–1110 (1994).
174. Balzola, F., Cullen, G., Hoentjen, F., Ho, G. T. & Russell, R. Adalimumab: Long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and Crohn's disease. *Inflamm. Bowel Dis. Monit.* **13,** 162 (2013).
175. Ali, T., Kaitha, S., Mahmood, S., Ftesi, A., Stone, J. & Bronze, M. S. Clinical use of anti-TNF therapy and increased risk of infections. *Drug. Healthc. Patient Saf.* **5,** 79–99 (2013).
176. Bongartz, T., Sutton, A. J., Sweeting, M. J., Buchan, I., Matteson, E. L. & Montori, V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* **295,** 2275–2285 (2006).
177. Wu, C.-Y., Chen, D.-Y., Shen, J.-L., Ho, H. J., Chen, C.-C., Kuo, K. N., Liu, H.-N., Chang, Y.-T. & Chen, Y.-J. The risk of cancer in patients with rheumatoid arthritis taking tumor necrosis factor antagonists: a nationwide cohort study. *Arthritis Res. Ther.* **16,** 449 (2014).
178. Breedveld, F. C., Weisman, M. H., Kavanaugh, A. F., Cohen, S. B., Pavelka, K., Van Vollenhoven, R., Sharp, J., Perez, J. L. & Spencer-Green, G. T. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previo. *Arthritis Rheum.* **54,** 26–37 (2006).
179. Costenbader, H. K. REPLY Risk of Serious Infections and Malignancies With Anti-TNF Antibody Therapy in RheumatoidArthritis. **296,** 2201–2204 (2006).
180. Beuthien, W., Mellinghoff, H.-U. & von Kempis, J. Skin reaction to adalimumab. *Arthritis Rheum.* **50,** 1690–1692 (2004).
181. Fréling, E., Baumann, C., Cuny, J.-F., Bigard, M.-A., Schmutz, J.-L., Barbaud, A. & Peyrin-Biroulet, L. Cumulative Incidence of, Risk Factors for, and Outcome of Dermatological Complications of Anti-TNF Therapy in Inflammatory Bowel Disease: A 14-Year Experience. *Am. J. Gastroenterol.* **110,** 1186–1196 (2015).
182. Cleynen, I. & Vermeire, S. Paradoxical inflammation induced by anti-TNF agents in patients with IBD. *Nat. Rev. Gastroenterol. Hepatol.* **9,** 496–503 (2012).
183. Denadai, R., Teixeira, F. V., Steinwurz, F., Romiti, R. & Saad-Hossne, R. Induction or exacerbation of psoriatic lesions during anti-TNF-α therapy for inflammatory bowel disease: a systematic literature review based on 222 cases. *J. Crohns. Colitis* **7,** 517–24 (2013).
184. Toussirot, É. & Aubin, F. Paradoxical reactions under TNF-α blocking agents and other biological agents given for chronic immune-mediated diseases: an analytical and comprehensive overview. *RMD Open* **2,** (2016).
185. Moustou, A.-E., Matekovits, A., Dessinioti, C., Antoniou, C., Sfikakis, P. P. & Stratigos, A. J. Cutaneous side effects of anti-tumor necrosis factor biologic therapy: a clinical review. *J. Am. Acad. Dermatol.* **61,** 486–504 (2009).
186. Hepworth, M. R., Monticelli, L. a., Fung, T. C., Ziegler, C. G. K., Grunberg, S., Sinha, R., Mantegazza, A. R., Ma, H.-L., Crawford, A., Angelosanto, J. M., Wherry, E. J., Koni, P. a., Bushman, F. D., Elson, C. O., Eberl, G., Artis, D. & Sonnenberg, G. F. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature* **498,** 113–117 (2013).
187. Balzola, F., Cullen, G., Hoentjen, F., Ho, G. T. & Russell, R. Anti-TNF antibody-induced psoriasiform skin lesions in patients with inflammatory bowel disease are characterised by interferon-γ-expressing Th1 cells and IL-17A/IL-22-expressing Th17 cells and respond to anti-IL-12/IL-23 antibody treatment. *Inflamm. Bowel Dis. Monit.* **13,** 166 (2013).
188. Sfikakis, P. P., Iliopoulos, a., Elezoglou, a., Kittas, C. & Stratigos, a. Psoriasis induced by anti-tumor necrosis factor therapy: A paradoxical adverse reaction. *Arthritis Rheum.* **52,** 2513–2518 (2005).
189. Ko, J. M., Gottlieb, a B. & Kerbleski, J. F. Induction and exacerbation of psoriasis with TNF-blockade therapy: a review and analysis of 127 cases. *J Dermatolog Treat* **20,** 100–108 (2009).
190. Torres, J., Buche, S., Delaporte, E. & Colombel, J. F. Skin side effects of inflammatory bowel disease therapy. *Inflamm Bowel Dis* **19,** 1086–1098 (2013).
191. Rahier, J.-F., Buche, S., Biroulet, L. P., Bouhnik, Y., Duclos, B., Louis, E., Papay, P., Allez, M., Cosnes, J., Cortot, A., Laharie, D., Reimund, J.-M., Lémann, M., Delaporte, E., Colombel, J.-F. & Peyrin-Biroulet, L. Severe Skin Lesions Cause Patients with Inflammatory Bowel Disease to Discontinue Antitumor Necrosis Factor Therapy. *Clin. Gastroenterol. Hepatol.* **8,** 1–8 (2010).
192. Baumgart, D. C., Grittner, U., Steingräber, A., Azzaro, M. & Philipp, S. Frequency, phenotype, outcome, and therapeutic impact of skin reactions following initiation of adalimumab therapy: Experience from a consecutive cohort of inflammatory bowel disease patients. *Inflamm. Bowel Dis.* **17,** 2512–2520 (2011).

193. Seneschal, J., Lepreux, S., Bouyssou-Gauthier, M. L., Heliot-Hosten, I., Economu, A., Dehais, J., Schaeverbeke, T. & Ta??eb, A. Psoriasiform drug eruptions under anti-TNF treatment of arthritis are not true psoriasis [3]. *Acta Derm. Venereol.* **87,** 77–80 (2007).
194. Seneschal, J., Lepreux, S., Milpied, B., Schaeverbeke, T. & Taıeb, A. Psoriasiform Eruptions During Anti–TNF-? Treatment: Psoriasis or Not? *J. Allergy* **143,** 1593–1595 (2007).
195. Dixon, W. G., Watson, K., Lunt, M., Hyrich, K. L., Silman, a. J. & Symmons, D. P. M. Rates of serious infection, including site-specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: Results from the British Society for Rheumatology Biologics Register. *Arthritis Rheum.* **54,** 2368–2376 (2006).
196. Thielen, a. M., Barde, C., Janer, V., Borradori, L. & Saurat, J. H. Reiter syndrome triggered by adalimumab (Humira) and leflunomide (Arava) in a patient with ankylosing spondylarthropathy and Crohn disease. *Br. J. Dermatol.* **156,** 188–189 (2007).
197. Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2, Strange, A., Capon, F., Spencer, C. C., Knight, J., Weale, M. E., Allen, M. H., Barton, A., Band, G., Bellenguez, C., Bergboer, J. G. M., Blackwell, J. M., Bramon, E., Bumpstead, S. J., Casas, J. P., Cork, M. J., Corvin, A., Deloukas, P., Dilthey, A., Duncanson, A., Edkins, S., Estivill, X., Fitzgerald, O., Freeman, C., Giardina, E., Gray, E., Hofer, A., Hüffmeier, U., Hunt, S. E., Irvine, A. D., Jankowski, J., Kirby, B., Langford, C., Lascorz, J., Leman, J., Leslie, S., Mallbris, L., Markus, H. S., Mathew, C. G., McLean, W. H. I., McManus, R., Mössner, R., Moutsianas, L., Naluai, A. T., Nestle, F. O., Novelli, G., Onoufriadis, A., Palmer, C. N. a, Perricone, C., Pirinen, M., Plomin, R., Potter, S. C., Pujol, R. M., Rautanen, A., Riveira-Munoz, E., Ryan, A. W., Salmhofer, W., Samuelsson, L., Sawcer, S. J., Schalkwijk, J., Smith, C. H., Ståhle, M., Su, Z., Tazi-Ahnini, R., Traupe, H., Viswanathan, A. C., Warren, R. B., Weger, W., Wolk, K., Wood, N., Worthington, J., Young, H. S., Zeeuwen, P. L. J. M., Hayday, A., Burden, a D., Griffiths, C. E. M., Kere, J., Reis, A., McVean, G., Evans, D. M., Brown, M. a, Barker, J. N., Peltonen, L., Donnelly, P. & Trembath, R. C. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat. Genet.* **42,** 985–90 (2010).
198. Okada, Y., Wu, D., Trynka, G., Raj, T., Terao, C., Ikari, K., Kochi, Y., Ohmura, K., Suzuki, A., Yoshida, S., Graham, R. R., Manoharan, A., Ortmann, W., Bhangale, T., Denny, J. C., Carroll, R. J., Eyler, A. E., Greenberg, J. D., Kremer, J. M., Pappas, D. a., Jiang, L., Yin, J., Ye, L., Su, D.-F., Yang, J., Xie, G., Keystone, E., Westra, H.-J., Esko, T., Metspalu, A., Zhou, X., Gupta, N., Mirel, D., Stahl, E. a., Diogo, D., Cui, J., Liao, K., Guo, M. H., Myouzen, K., Kawaguchi, T., Coenen, M. J. H., van Riel, P. L. C. M., van de Laar, M. a. F. J., Guchelaar, H.-J., Huizinga, T. W. J., Dieudé, P., Mariette, X., Louis Bridges Jr, S., Zhernakova, A., Toes, R. E. M., Tak, P. P., Miceli-Richard, C., Bang, S.-Y., Lee, H.-S., Martin, J., Gonzalez-Gay, M. a., Rodriguez-Rodriguez, L., Rantapää-Dahlqvist, S., Ärlestig, L., Choi, H. K., Kamatani, Y., Galan, P., Lathrop, M., the RACI Consortium, the GARNET Consortium, Eyre, S., Bowes, J., Barton, A., de Vries, N., Moreland, L. W., Criswell, L. a., Karlson, E. W., Taniguchi, A., Yamada, R., Kubo, M., Liu, J. S., Bae, S.-C., Worthington, J., Padyukov, L., Klareskog, L., Gregersen, P. K., Raychaudhuri, S., Stranger, B. E., De Jager, P. L., Franke, L., Visscher, P. M., Brown, M. a., Yamanaka, H., Mimori, T., Takahashi, A., Xu, H., Behrens, T. W., Siminovitch, K. a., Momohara, S., Matsuda, F., Yamamoto, K. & Plenge, R. M. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506,** 376–381 (2014).
199. Liu, J. Z., van Sommeren, S., Huang, H., Ng, S. C., Alberts, R., Takahashi, A., Ripke, S., Lee, J. C., Jostins, L., Shah, T., Abedian, S., Cheon, J. H., Cho, J., Daryani, N. E., Franke, L., Fuyuno, Y., Hart, A., Juyal, R. C., Juyal, G., Kim, W. H., Morris, A. P., Poustchi, H., Newman, W. G., Midha, V., Orchard, T. R., Vahedi, H., Sood, A., Sung, J. J. Y., Malekzadeh, R., Westra, H.-J., Yamazaki, K., Yang, S.-K., Barrett, J. C., Franke, A., Alizadeh, B. Z., Parkes, M., B K, T., Daly, M. J., Kubo, M., Anderson, C. a & Weersma, R. K. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* **47,** 979–989 (2015).
200. Cabaleiro, T., Prieto-Pérez, R., Navarro, R., Solano, G., Román, M., Ochoa, D., Abad-Santos, F. & Daudén, E. Paradoxical psoriasiform reactions to anti-TNFα drugs are associated with genetic polymorphisms in patients with psoriasis. *Pharmacogenomics J.* 1–5 (2015). doi:10.1038/tpj.2015.53
201. Sherlock, M. E., Walters, T., Tabbers, M. M., Frost, K., Zachos, M., Muise, A., Pope, E. & Griffiths, A. M. Infliximab-induced psoriasis and psoriasiform skin lesions in pediatric Crohn disease and a potential association with IL-23 receptor polymorphisms. *J. Pediatr. Gastroenterol. Nutr.* **56,** 512–8 (2013).
202. Di Meglio, P., Di Cesare, A., Laggner, U., Chu, C. C., Napolitano, L., Villanova, F., Tosi, I., Capon, F., Trembath, R. C., Peris, K. & Nestle, F. O. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. *PLoS One* **6,** 1–10 (2011).
203. Charles, P. J., Smeenk, R. J., De Jong, J., Feldmann, M. & Maini, R. N. Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum.* **43,** 2383–90 (2000).
204. Alessandri, C., Scrivo, R., Spinelli, F. R., Ceccarelli, F., Magrini, L., Priori, R. & Valesini, G. Autoantibody Production in Anti-TNF- -Treated Patients. *Ann. N. Y. Acad. Sci.* **1110,** 319–329 (2007).
205. Atzeni, F. & Sarzi-Puttini, P. Autoantibody production in patients treated with anti-TNF-alpha. *Expert Rev. Clin. Immunol.* **4,** 275–280 (2008).

206. Wesche, B., Jaeckel, E., Trautwein, C., Wedemeyer, H., Falorni, a, Frank, H., von zur Muhlen, a, Manns, M. P. & Brabant, G. Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C. *Gut* **48,** 378–383 (2001).
207. Palucka, a K., Blanck, J.-P., Bennett, L., Pascual, V. & Banchereau, J. Cross-regulation of TNF and IFN-alpha in autoimmune diseases. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 3372–7 (2005).
208. Conrad, C. & Gilliet, M. Type I IFNs at the interface between cutaneous immunity and epidermal remodeling. *J. Invest. Dermatol.* **132,** 1759–62 (2012).
209. Seneschal, J., Milpied, B., Vergier, B., Lepreux, S., Schaeverbeke, T. & Taïeb, a. Cytokine imbalance with increased production of interferon-α in psoriasiform eruptions associated with antitumour necrosis factor-α treatments. *Br. J. Dermatol.* **161,** 1081–1088 (2009).
210. Ma, H. L., Napierata, L., Stedman, N., Benoit, S., Collins, M., Nickerson-Nutter, C. & Young, D. a. Tumor necrosis factor alpha blockade exacerbates murine psoriasis-like disease by enhancing Th17 function and decreasing expansion of treg cells. *Arthritis Rheum.* **62,** 430–440 (2010).
211. Spoendlin, J., Voegel, J. J., Jick, S. S. & Meier, C. R. A study on the epidemiology of rosacea in the U.K. *Br. J. Dermatol.* **167,** 598–605 (2012).
212. Kyriakis, K. P., Palamaras, I., Terzoudi, S., Emmanuelides, S., Michailides, C. & Pagana, G. Epidemiologic aspects of rosacea. *J. Am. Acad. Dermatol.* **53,** 918–919 (2005).
213. Lazaridou, E., Apalla, Z., Sotiraki, S., Ziakas, N. G., Fotiadou, C. & Ioannides, D. Clinical and laboratory study of rosacea in northern Greece. *J. Eur. Acad. Dermatology Venereol.* **24,** 410–414 (2010).
214. Tan, J., Ortonne, J. P., Wilhelm, K., Marticou, L., Baltas, E., Rivier, M., Petit, L., Martel, P. & Tan, J. An observational cross-sectional survey of rosacea : clinical associations and progression between subtypes. 555–562 (2013). doi:10.1111/bjd.12385
215. Georgala, S. Increased density of Demodex folliculorum and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. 441–444 (2001).
216. Zhao, Y. E., Wu, L. P., Peng, Y. & Cheng, H. Retrospective Analysis of the Association Between Demodex Infestation and Rosacea. *Arch. Dermatol* **146,** 896–903 (2010).
217. Schwab, V. D., Sulk, M., Seeliger, S., Nowak, P., Aubert, J., Mess, C., Rivier, M., Carlavan, I., Rossio, P., Metze, D., Buddenkotte, J., Cevikbas, F., Voegel, J. J. & Steinhoff, M. Neurovascular and Neuroimmune Aspects in the Pathophysiology of Rosacea. *J. Investig. Dermatology Symp. Proc.* **15,** 53–62 (2011).
218. Powell, F. C. Rosacea. 793–803 (2005).
219. Trumbore, M. W., Goldstein, J. A. & Gurge, R. M. Treatment of papulopustular rosacea with sodium sulfacetamide 10%/sulfur 5% emollient foam. *J. Drugs Dermatol.* **8,** 299–304 (2009).
220. Del Rosso, J. Q. Evaluating the role of topical therapies in the management of rosacea: focus on combination sodium sulfacetamide and sulfur formulations. *Cutis* **73,** 29–33 (2004).
221. Dahl, M. V., Jarratt, M., Kaplan, D., Tuley, M. R. & Baker, M. D. Once-daily topical metronidazole cream formulations in the treatment of the papules and pustules of rosacea. *J. Am. Acad. Dermatol.* **45,** 723–730 (2001).
222. Thiboutot, D., Thieroff-Ekerdt, R. & Graupe, K. Efficacy and safety of azelaic acid (15%) gel as a new treatment for papulopustular rosacea: Results from two vehicle-controlled, randomized phase III studies. *J. Am. Acad. Dermatol.* **48,** 836–845 (2003).
223. Akamatsu, H., Komura, J., Asada, Y., Miyachi, Y. & Niwa, Y. Inhibitory effect of azelaic acid on neutrophil functions: a possible cause for its efficacy in treating pathogenetically unrelated diseases. *Arch. Dermatol. Res.* **283,** 162–166 (1991).
224. Narayanan, S., Hùnerbein, a, Hünerbein, A., Getie, M., Jäckel, A. & Neubert, R. H. H. Scavenging properties of metronidazole on free oxygen radicals in a skin lipid model system. *J. Pharm. Pharmacol.* **59,** 1125–30 (2007).
225. Fowler, J., Jarratt, M., Moore, A., Meadows, K., Pollack, A., Steinhoff, M., Liu, Y. & Leoni, M. Once-daily topical brimonidine tartrate gel 0.5% is a novel treatment for moderate to severe facial erythema of rosacea: Results of two multicentre, randomized and vehicle-controlled studies. *Br. J. Dermatol.* **166,** 633–641 (2012).
226. Stein Gold, L., Kircik, L., Fowler, J., Jackson, J. M., Tan, J., Draelos, Z., Fleischer, A., Appell, M., Steinhoff, M., Lynde, C., Sugarman, J., Liu, H. & Jacovella, J. Long-term safety of ivermectin 1% cream vs azelaic acid 15% gel in treating inflammatory lesions of rosacea: results of two 40-week controlled, investigator-blinded trials. *J. Drugs Dermatol.* **13,** 1380–1386 (2014).
227. Sneddon, I. B. A Clinical Trial of Tetracycline in Rosacea. *Br. J. Dermatol.* **78,** 649–652 (1966).
228. Hersle, K. & Gisslen, H. Minocycline in acne vulgaris: a double-blind study. *Curr. Ther. Res. Clin. Exp.* **19,** 339–342 (1976).
229. Schauber, J., Dorschner, R. A., Coda, A. B., Büchau, A. S., Liu, P. T., Kiken, D., Helfrich, Y. R., Kang, S., Elalieh, H. Z., Steinmeyer, A., Zügel, U., Bikle, D. D., Modlin, R. L. & Gallo, R. L. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D – dependent mechanism. **117,** 803–811 (2007).
230. Yamasaki, K., Kanada, K., Macleod, D. T., Borkowski, A. W., Morizane, S., Nakatsuji, T., Cogen, A. L. & Gallo, R. L. TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J. Invest. Dermatol.* **131,** 688–697 (2011).
231. Rupec, R., Korting, H. C., Ruzicka, T., Koller, B. & Mu, A. S. Chitin Modulates Innate Immune Responses of Keratinocytes. **6,** 1–7 (2011).
232. Sotiropoulou, G., Pampalakis, G. & Diamandis, E. P. Functional Roles of Human. **284,** 32989–32994 (2009).

233. Jang, Y. H., Sim, J. H., Kang, H. Y., Kim, Y. C. & Lee, E. Immunohistochemical expression of matrix metalloproteinases in the granulomatous rosacea compared with the non-granulomatous rosacea. 544–548 (2011). doi:10.1111/j.1468-3083.2010.03825.x
234. Muto, Y., Wang, Z., Vanderberghe, M., Two, A., Gallo, R. L. & Nardo, A. Di. Mast Cells Are Key Mediators of Cathelicidin-Initiated Skin Inflammation in Rosacea. *J. Invest. Dermatol.* **134,** 2728–2736 (2014).
235. Parks, W. C., Wilson, C. L., López-boado, Y. S. & Forest, W. MATRIX METALLOPROTEINASES AS MODULATORS OF INFLAMMATION AND INNATE IMMUNITY. **4,** (2004).
236. Chavanas, S., Rochat, A., Ali, M., Alan, D., Bonafé, J., Taïeb, A., Harper, J. I., De, Y. & Hovnanian, A. Mutations in SPINK5 , encoding a serine protease inhibitor , cause Netherton syndrome. **25,** 141–142 (2000).
237. Deraison, C., Bonnart, C., Lopez, F., Besson, C., Robinson, R., Jayakumar, A., Wagberg, F., Brattsand, M., Hachem, J. P., Leonardsson, G. & Hovnanian, A. LEKTI Fragments Specifically Inhibit KLK5 , KLK7 , and KLK14 and Control Desquamation through a pH-dependent Interaction □. **18,** 3607–3619 (2007).
238. Descargues, P., Deraison, C., Bonnart, C., Kreft, M., Kishibe, M., Ishida-yamamoto, A., Elias, P., Barrandon, Y., Zambruno, G., Sonnenberg, A. & Hovnanian, A. Spink5 -deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat. Genet.* **37,** 56–65 (2005).
239. Furio, L., de Veer, S., Jaillet, M., Briot, A., Robin, A., Deraison, C. & Hovnanian, A. Transgenic kallikrein 5 mice reproduce major cutaneous and systemic hallmarks of Netherton syndrome. *J. Exp. Med.* **211,** 499 LP – 513 (2014).
240. Furio, L., Pampalakis, G., Michael, I. P., Nagy, A., Sotiropoulou, G. & Hovnanian, A. KLK5 Inactivation Reverses Cutaneous Hallmarks of Netherton Syndrome. *Plos Genet.* **11,** 1–20 (2015).
241. Reinholz, M., Ruzicka, T. & Schauber, J. Cathelicidin LL-37: An antimicrobial peptide with a role in inflammatory skin disease. *Ann. Dermatol.* **24,** 126–135 (2012).
242. Meyer-Hoffert, U. & Schröder, J.-M. Epidermal Proteases in the Pathogenesis of Rosacea. *J. Investig. Dermatology Symp. Proc.* **15,** 16–23 (2011).
243. Ushio, H., Nagaoka, I., Ogawa, H. & Alerts, E. The Human β-Defensins (-1, -2, -3, -4) and Cathelicidin LL-37 Induce IL-18 Secretion through p38 and ERK MAPK Activation in Primary Human Keratinocytes. *J. Immunol.* 1776–1784 (2005). doi:10.4049/jimmunol.175.3.1776
244. Li, N., Yamasaki, K., Saito, R., Shimada-omori, R., Aiba, S. & Alerts, E. Alarmin Function of Cathelicidin Antimicrobial Peptide LL37 through IL-36 γ Induction in Human Epidermal Keratinocytes. 5140–5148 (2014). doi:10.4049/jimmunol.1302574
245. Zhang, Z., Cherryholmes, G., Chang, F., Rose, D. M., Schraufstatter, I. & Shively, J. E. Evidence that cathelicidin peptide LL-37 may act as a functional ligand for CXCR2 on human neutrophils. 3181–3194 (2009). doi:10.1002/eji.200939496
246. Koczulla, R., Degenfeld, G. Von, Kupatt, C., Krötz, F., Zahler, S., Gloe, T., Issbrücker, K., Unterberger, P., Zaiou, M., Lebherz, C., Karl, A., Raake, P., Pfosser, A., Boekstegers, P., Welsch, U., Hiemstra, P. S., Vogelmeier, C., Gallo, R. L., Clauss, M. & Bals, R. An angiogenic role for the human peptide antibiotic LL-37 / hCAP-18. **111,** 1665–1672 (2003).
247. Yamasaki, K. & Gallo, R. L. Rosacea as a Disease of Cathelicidins and Skin Innate Immunity. *J. Investig. Dermatology Symp. Proc.* **15,** 12–15 (2011).
248. Peric, M., Koglin, S., Kim, S.-M., Morizane, S., Besch, R., Prinz, J. C., Ruzicka, T., Gallo, R. L. & Schauber, J. IL-17A Enhances Vitamin D(3)-Induced Expression of Cathelicidin Antimicrobial Peptide in Human Keratinocytes. *J. Immunol.* **181,** 8504–8512 (2008).
249. De Witt, S. M., Verdoold, R., Cosemans, J. M. E. M. & Heemskerk, J. W. M. Insights into platelet-based control of coagulation. *Thromb. Res.* **133,** S139–S148 (2014).
250. Two, A. M., Hata, T. R., Nakatsuji, T., Coda, A. B., Kotol, P. F., Wu, W., Shafiq, F., Huang, E. Y. & Gallo, R. L. Reduction in Serine Protease Activity Correlates with Improved Rosacea Severity in a Small, Randomized Pilot Study of a Topical Serine Protease Inhibitor. *J. Invest. Dermatol.* **134,** 8–10 (2013).
251. Zhang, T., Finn, D. F., Barlow, J. W. & Walsh, J. J. Mast cell stabilisers. *Eur. J. Pharmacol.* **778,** 158–168 (2016).
252. Nestle, F. O., Curdin, C., Tun-Kyi, A., Homey, B., Gombert, M., Boyman, O., Burg, G., Liu, Y.-J. & Gilliet, M. Plasmacytoid predendritic cells initiate psoriasis through interferon- production. *J. Exp. Med.* **202,** 135–143 (2005).
253. Steinhoff, M., Buddenkotte, J., Aubert, J., Sulk, M., Novak, P., Schwab, V. D., Mess, C., Cevikbas, F., Rivier, M., Carlavan, I., Déret, S., Rosignoli, C., Metze, D., Luger, T. A. & Voegel, J. J. Clinical, Cellular, and Molecular Aspects in the Pathophysiology of Rosacea. *J. Investig. Dermatol. Symp. Proc.* **15,** 2–11 (2011).
254. Cohen, P. R. & Prystowsky, J. H. Pityriasis rubra pilaris: a review of diagnosis and treatment. *J. Am. Acad. Dermatol.* **20,** 801–7 (1989).
255. Wohlrab, J. & Kreft, B. Treatment of pityriasis rubra pilaris with ustekinumab. *Br. J. Dermatol.* **163,** 655–656 (2010).
256. Nestle, F. O., Kaplan, D. H. & Barker, J. Psoriasis. *N. Engl. J. Med.* **361,** 496–509 (2009).
257. Becher, B. & Pantelyushin, S. Hiding under the skin: Interleukin-17–producing γδ T cells go under the skin? *Nat. Med.* **18,** 1748–1750 (2012).
258. Van Belle, A. B., de Heusch, M., Lemaire, M. M., Hendrickx, E., Warnier, G., Dunussi-Joannopoulos, K., Fouser, L. A., Renauld, J.-C. & Dumoutier, L. IL-22 Is Required for Imiquimod-Induced Psoriasiform Skin Inflammation in Mice. *J. Immunol.* **188,** 462–469 (2012).

259. Mabuchi, T., Singh, T. P., Takekoshi, T., Jia, G., Wu, X., Kao, M. C., Weiss, I., Farber, J. M. & Hwang, S. T. CCR6 Is Required for Epidermal Trafficking of γδ-T Cells in an IL-23-Induced Model of Psoriasiform Dermatitis. *J. Invest. Dermatol.* **133,** 164–171 (2013).
260. Mabuchi, T., Takekoshi, T. & Hwang, S. T. Epidermal CCR6 $^{+}$ γδ T Cells Are Major Producers of IL-22 and IL-17 in a Murine Model of Psoriasiform Dermatitis. *J. Immunol.* **187,** 5026–5031 (2011).
261. Cai, Y., Shen, X., Ding, C., Qi, C., Li, K., Li, X., Jala, V. R., Zhang, H. ge, Wang, T., Zheng, J. & Yan, J. Pivotal Role of Dermal IL-17-Producing γδ T Cells in Skin Inflammation. *Immunity* **35,** 596–610 (2011).
262. Koenecke, C., Chennupati, V., Schmitz, S., Malissen, B., Föster, R. & Prins, I. In vivo application of mAb directed against the γδ TCR does not deplete but generated 'invisible' γδ T cells. *Eur. J. Immunol.* **39,** 372–379 (2009).
263. Khamashta, M., Merrill, J. T., Werth, V. P., Furie, R., Kalunian, K., Illei, G. G., Drappa, J., Wang, L., Greth, W. & CD1067 study investigators. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann. Rheum. Dis.* 1–8 (2016). doi:10.1136/annrheumdis-2015-208562
264. Furie, R., Khamashta, M., Merrill, J. T., Werth, V. P., Kalunian, K., Brohawn, P., Illei, G. G., Drappa, J., Wang, L. & Yoo, S. Anifrolumab, an Anti–Interferon-α Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. *Arthritis Rheumatol.* **69,** 376–386 (2017).
265. Furie, R., Werth, V. P., Merola, J., Wang, W., Rabah, D., Barbey, C., Carrillo-Infante, C., Reynolds, T., Stevenson, L., Martin, D. & Franchimont, N. *BIIB059, a monoclonal antibody targeting BDCA2, shows evidence of biological activity and early clinical proof of concept in subjects with active cutaneous SLE*. *Arthritis Rheumatol. Conf. Am. Coll. Rheumatol. Rheumatol. Heal. Prof. Annu. Sci. Meet. ACR/ARHP 2016. United states. Conf. start 20161111. Conf. end 20161116* **68,** (2016).
266. Gerber, P. A., Buhren, B. A., Steinhoff, M. & Homey, B. Rosacea: the Cytokine and Chemokine Network. *J. Investig. Dermatology Symp. Proc.* **15,** 40–47 (2011).
267. Webb, L. M., Lundie, R. J., Borger, J. G., Cartwright, A. N., Cook, P. C., Brown, S. L., Jackson-Jones, L., Phythian-Adams, A. T., Davis, D. M. & MacDonald, A. S. A central role for Type I IFN in the induction of Th2 responses by dendritic cells. *J. Immunol.* **196,** 46.13 LP – 46.13 (2016).
268. Webb, L. M., Lundie, R. J., Borger, J. G., Brown, S. L., Connor, L. M., Cartwright, A. N., Dougall, A. M., Wilbers, R. H., Cook, P. C., Jackson-Jones, L. H., Phythian-Adams, A. T., Johansson, C., Davis, D. M., Dewals, B. G., Ronchese, F. & MacDonald, A. S. Type I interferon is required for T helper (Th) 2 induction by dendritic cells. *EMBO J.* **36,** 2404–2418 (2017).
269. Duerr, C. U., McCarthy, C. D. a, Mindt, B. C., Rubio, M., Meli, A. P., Pothlichet, J., Eva, M. M., Gauchat, J.-F., Qureshi, S. T., Mazer, B. D., Mossman, K. L., Malo, D., Gamero, A. M., Vidal, S. M., King, I. L., Sarfati, M. & Fritz, J. H. Type I interferon restricts type 2 immunopathology through the regulation of group 2 innate lymphoid cells. *Nat. Immunol.* **17,** 65–75 (2015).
270. Ruiz-Genao, D. P., Lopez-Estebaranz, J. L., Naz-Villalba, E., Gamo-Villegas, R., Calzado-Villarreal, L. & Pinedo-Moraleda, F. Pityriasis rubra pilaris successfully treated with infliximab. *Acta Derm. Venereol.* **87,** 552–553 (2007).
271. KF, D., JJ, W., JE, M., FR, R., EP, S. & Meshkinpour, A. Clinical improvement of pityriasis rubra pilaris with combination etanercept and acitretin therapy. *Arch. Dermatol.* **143,** 1589–1603 (2007).
272. Hijnen, D., Knol, E. F., Gent, Y. Y., Giovannone, B., Beijn, S. J. P., Kupper, T. S., Bruijnzeel-Koomen, C. A. F. M. & Clark, R. A. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J. Invest. Dermatol.* **133,** 973–979 (2013).
273. Tsoi, L. C., Spain, S. L., Knight, J., Ellinghaus, E., Stuart, P. E., Capon, F., Ding, J., Li, Y., Tejasvi, T., Gudjonsson, J. E., Kang, H. M., Allen, M. H., McManus, R., Novelli, G., Samuelsson, L., Schalkwijk, J., Ståhle, M., Burden, A. D., Smith, C. H., Cork, M. J., Estivill, X., Bowcock, A. M., Krueger, G. G., Weger, W., Worthington, J., Tazi-Ahnini, R., Nestle, F. O., Hayday, A., Hoffmann, P., Winkelmann, J., Wijmenga, C., Langford, C., Edkins, S., Andrews, R., Blackburn, H., Strange, A., Band, G., Pearson, R. D., Vukcevic, D., Spencer, C. C. A., Deloukas, P., Mrowietz, U., Schreiber, S., Weidinger, S., Koks, S., Kingo, K., Esko, T., Metspalu, A., Lim, H. W., Voorhees, J. J., Weichenthal, M., Wichmann, H. E., Chandran, V., Rosen, C. F., Rahman, P., Gladman, D. D., Griffiths, C. E. M., Reis, A., Kere, J., Duffin, K. C., Helms, C., Goldgar, D., Li, Y., Paschall, J., Malloy, M. J., Pullinger, C. R., Kane, J. P., Gardner, J., Perlmutter, A., Miner, A., Feng, B. J., Hiremagalore, R., Ike, R. W., Christophers, E., Henseler, T., Ruether, A., Schrodi, S. J., Prahalad, S., Guthery, S. L., Fischer, J., Liao, W., Kwok, P., Menter, A., Lathrop, G. M., Wise, C., Begovich, A. B., Onoufriadis, A., Weale, M. E., Hofer, A., Salmhofer, W., Wolf, P., Kainu, K., Saarialho-Kere, U., Suomela, S., Badorf, P., Hüffmeier, U., Kurrat, W., Küster, W., Lascorz, J., Mössner, R., Schürmeier-Horst, F., Ständer, M., Traupe, H., Bergboer, J. G. M., Heijer, M. den, van de Kerkhof, P. C., Zeeuwen, P. L. J. M., Barnes, L., Campbell, L. E., Cusack, C., Coleman, C., Conroy, J., Ennis, S., Fitzgerald, O., Gallagher, P., Irvine, A. D., Kirby, B., Markham, T., McLean, W. H. I., McPartlin, J., Rogers, S. F., Ryan, A. W., Zawirska, A., Giardina, E., Lepre, T., Perricone, C., Martín-Ezquerra, G., Pujol, R. M., Riveira-Munoz, E., Inerot, A., Naluai, Å. T., Mallbris, L., Wolk, K., Leman, J., Barton, A., Warren, R. B., Young, H. S., Ricano-Ponce, I., Trynka, G., Pellett, F. J., Henschel, A., Aurand, M., Bebo, B., Gieger, C., Illig, T., Moebus, S., Jöckel, K.-H., Erbel, R., Donnelly, P., Peltonen, L., Blackwell, J. M., Bramon, E., Brown, M. A., Casas, J. P., Corvin, A., Craddock, N., Duncanson, A., Jankowski, J., Markus, H. S., Mathew, C. G., McCarthy, M. I., Palmer, C. N. A., Plomin, R., Rautanen, A., Sawcer, S. J., Samani, N., Viswanathan, A. C., Wood, N. W., Bellenguez, C., Freeman, C., Hellenthal, G., Giannoulatou, E., Pirinen, M., Su, Z., Hunt, S. E., Gwilliam, R., Bumpstead, S. J., Dronov, S., Gillman, M., Gray, E., Hammond, N., Jayakumar, A., McCann, O. T., Liddle, J., Perez, M. L., Potter, S. C., Ravindrarajah, R.,

Ricketts, M., Waller, M., Weston, P., Widaa, S., Whittaker, P., Nair, R. P., Franke, A., Barker, J. N. W. N., Abecasis, G. R., Elder, J. T. & Trembath, R. C. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* **44,** 1341–1348 (2012).

274. Kawashima, T., Kosaka, A., Yan, H., Guo, Z., Uchiyama, R., Fukui, R., Kaneko, D., Kumagai, Y., You, D. J., Carreras, J., Uematsu, S., Jang, M., Takeuchi, O., Kaisho, T., Akira, S., Miyake, K., Tsutsui, H., Saito, T., Nishimura, I. & Tsuji, N. Double-Stranded RNA of Intestinal Commensal but Not Pathogenic Bacteria Triggers Production of Protective Interferon-β. *Immunity* **38,** 1187–1197 (2013).
275. McNab, F., Mayer-Barber, K., Sher, A., Wack, A. & O'Garra, A. Type I interferons in infectious disease. *Nat. Rev. Immunol.* **15,** 87–103 (2015).
276. Teles, R. M. B., Graeber, T. G., Krutzik, S. R., Montoya, D., Schenk, M., Lee, D. J., Komisopoulou, E., Kelly-scumpia, K., Chun, R., Iyer, S. S., Sarno, E. N., Rea, T. H., Hewison, M., Adams, J. S., Popper, S. J., Relman, D. A., Stenger, S., Bloom, B. R., Cheng, G. & Modlin, R. L. Type I Interferon Suppresses Type II Interferon–Triggered Human Anti-Mycobacterial Responses. *Science (80-.).* **339,** 1448–1454 (2013).
277. Auerbuch, V., Brockstedt, D. G., Meyer-Morse, N., O'Riordan, M. & Portnoy, D. a. Mice Lacking the Type I Interferon Receptor Are Resistant to *Listeria monocytogenes*. *J. Exp. Med.* **200,** 527–533 (2004).
278. Wilson, E., Yamada, D. H., Elsaesser, H. J., Herskovitz, J., Deng, J., Cheng, G., Aronow, B., Karp, C. & Brooks, D. G. Blockade of Chronic Type I Interferon Signaling to Control Persistent LCMV Infection. **340,** 202–207 (2013).
279. Teijaro, J. R., Ng, C., Lee, A. M., Sullivan, B. M., Sheehan, K. C. F., Welch, M., Schreiber, R. D., Carlos, J., Torre, D. & Oldstone, M. B. a. Persistent LCMV Infection Is Controlled by Blockade of Type I Interferon Signaling. *Science (80-.).* **340,** 207–211 (2013).
280. Diamond, M. S., Kinder, M., Matsushita, H., Mashayekhi, M., Dunn, G. P., Archambault, J. M., Lee, H., Arthur, C. D., White, J. M., Kalinke, U., Murphy, K. M. & Schreiber, R. D. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J. Exp. Med.* **208,** 1989–2003 (2011).
281. Demaria, O., De Gassart, A., Coso, S., Gestermann, N., Di Domizio, J., Flatz, L., Gaide, O., Michielin, O., Hwu, P., Petrova, T. V., Martinon, F., Modlin, R. L., Speiser, D. E. & Gilliet, M. STING activation of tumor endothelial cells initiates spontaneous and therapeutic antitumor immunity. *Proc. Natl. Acad. Sci.* **112,** 15408–15413 (2015).
282. Fuertes, M. B., Kacha, A. K., Kline, J., Woo, S.-R., Kranz, D. M., Murphy, K. M. & Gajewski, T. F. Host type I IFN signals are required for antitumor CD8 $^{+}$ T cell responses through CD8α $^{+}$ dendritic cells. *J. Exp. Med.* **208,** 2005–2016 (2011).
283. Palucka, K. & Banchereau, J. Cancer immunotherapy via dendritic cells. *Nat. Rev. Cancer* **12,** 265–277 (2012).
284. Elkon, K. B. & Stone, V. V. Type I interferon and systemic lupus erythematosus. *J. Interferon Cytokine Res.* **31,** 803–12 (2011).
285. Crow, M. K. Type I Interferon in the Pathogenesis of Lupus. *J. Immunol.* **192,** 5459–5468 (2014).
286. Banchereau, J. & Pascual, V. Type I Interferon in Systemic Lupus Erythematosus and Other Autoimmune Diseases. *Immunity* **25,** 383–392 (2006).
287. Mauri, C. & Menon, M. The many faces of type I interferon in systemic lupus erythematosus. *J. Clin. Invest.* **125,** 2562–4 (2015).
288. Bissonnette, R., Papp, K., Maari, C., Yao, Y., Robbie, G., White, W. I., Le, C. & White, B. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti–interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. *J. Am. Acad. Dermatol.* **62,** 427–436 (2010).
289. Harden, J. L., Johnson-Huang, L. M., Chamian, M. F., Lee, E., Pearce, T., Leonardi, C. L., Haider, A., Lowes, M. a. & Krueger, J. G. Humanized anti-IFN-g (HuZAF) in the treatment of psoriasis. *J. Allergy Clin. Immunol.* **135,** 553–556 (2015).
290. Tsokos, G. C. Systemic lupus erythematosus. *N. Engl. J. Med.* **365,** 2110–2121 (2011).
291. Mylonas, A., Demaria, O., Meller, S., Hawerkamp, H., Homey, B., Di Domizio, J., Gilliet, M. & Conrad, C. *584 Plasmacytoid dendritic cell-derived type I interferon drives flares of rosacea*. *J. Invest. Dermatol.* **137,** (2017).
292. Mylonas, A., Demaria, O., Meller, S., Friedrich, H., Homey, B., Navarini, A., Di Domizio, J., Gilliet, M. & Conrad, C. 257 Plasmacytoid dendritic cell-derived type I interferon drives flares of rosacea. *J. Invest. Dermatol.* **136,** S205 (2016).
293. Yung, S., Yap, D. Y. & Chan, T. M. Recent advances in the understanding of renal inflammation and fibrosis in lupus nephritis. *F1000Research* **6,** 874 (2017).
294. Celhar, T. & Fairhurst, A. M. Modelling clinical systemic lupus erythematosus: similarities, differences and success stories. *Rheumatology (Oxford).* **56,** i88–i99 (2017).
295. Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D. W. & Schmitz, J. BDCA-2, BDCA-3, and BDCA-4: Three Markers for Distinct Subsets of Dendritic Cells in Human Peripheral Blood. *J. Immunol.* **165,** 6037–6046 (2000).
296. Van Kooyk, Y. & Rabinovich, G. a. Protein-glycan interactions in the control of innate and adaptive immune responses. *Nat. Immunol.* **9,** 593–601 (2008).
297. Geijtenbeek, T. B. H. & Gringhuis, S. I. Signalling through C-type lectin receptors: shaping immune responses. *Nat. Rev. Immunol.* **9,** 465–479 (2009).
298. Dzionek, a, Sohma, Y., Nagafune, J., Cella, M., Colonna, M., Facchetti, F., Günther, G., Johnston, I., Lanzavecchia, a, Nagasaka, T., Okada, T., Vermi, W., Winkels, G., Yamamoto, T., Zysk, M., Yamaguchi, Y. & Schmitz, J. BDCA-2, a

novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J. Exp. Med.* **194,** 1823–34 (2001).
299. Cao, W., Zhang, L., Rosen, D. B., Bover, L., Watanabe, G., Bao, M., Lanier, L. L. & Liu, Y. J. BDCA2/FceRIg complex signals through a novel BCR-like pathway in human plasmacytoid dendritic cells. *PLoS Biol.* **5,** 2190–2200 (2007).
300. Pellerin, a., Otero, K., Czerkowicz, J. M., Kerns, H. M., Shapiro, R. I., Ranger, a. M., Otipoby, K. L., Taylor, F. R., Cameron, T. O., Viney, J. L. & Rabah, D. Anti-BDCA2 monoclonal antibody inhibits plasmacytoid dendritic cell activation through Fc-dependent and Fc-independent mechanisms. *EMBO Mol. Med.* **7,** 464–476 (2015).
301. Davidson, S., Crotta, S., McCabe, T. M. & Wack, A. Pathogenic potential of interferon αβ in acute influenza infection. *Nat. Commun.* **5,** (2014).
302. Sapadin, A. N. & Fleischmajer, R. Tetracyclines: Nonantibiotic properties and their clinical implications. *J. Am. Acad. Dermatol.* **54,** 258–265 (2006).
303. Peng, B., Su, Y. Bin, Li, H., Han, Y., Guo, C., Tian, Y. M. & Peng, X. X. Exogenous Alanine and/or Glucose plus Kanamycin Kills Antibiotic-Resistant Bacteria. *Cell Metab.* **21,** 249–261 (2015).
304. Bhargava, P. & Collins, J. J. Boosting bacterial metabolism to combat antibiotic resistance. *Cell Metab.* **21,** 154–155 (2015).
305. Theriot, C. M., Koenigsknecht, M. J., Carlson, P. E., Hatton, G. E., Nelson, A. M., Li, B., Huffnagle, G. B., Z. Li, J. & Young, V. B. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nat. Commun.* **5,** (2014).
306. Antunes, L. C. M., Han, J., Ferreira, R. B. R., Lolić, P., Borchers, C. H. & Finlay, B. B. Effect of antibiotic treatment on the intestinal metabolome. *Antimicrob. Agents Chemother.* **55,** 1494–1503 (2011).
307. Vega, N. M., Allison, K. R., Samuels, a. N., Klempner, M. S. & Collins, J. J. Salmonella typhimurium intercepts Escherichia coli signaling to enhance antibiotic tolerance. *Proc. Natl. Acad. Sci.* **110,** 14420–14425 (2013).
308. Feldmeyer, L., Mylonas, A., Demaria, O., Mennella, A., Yawalkar, N., Laffitte, E., Hohl, D., Gilliet, M. & Conrad, C. Interleukin 23–Helper T Cell 17 Axis as a Treatment Target for Pityriasis Rubra Pilaris. *JAMA Dermatol.* **153,** 304 (2017).
309. Buhl, T., Sulk, M., Nowak, P., Buddenkotte, J., McDonald, I., Aubert, J., Carlavan, I., Déret, S., Reiniche, P., Rivier, M., Voegel, J. J. & Steinhoff, M. Molecular and Morphological Characterization of Inflammatory Infiltrate in Rosacea Reveals Activation of Th1/Th17 Pathways. *J. Invest. Dermatol.* **135,** 2198–208 (2015).
310. Conrad, C., Domizio, J. Di, Mylonas, A., Belkhodja, C., Demaria, O., Navarini, A., Lapointe, A.-K., French, L., Vernez, M. & Gilliet, M. *Paradoxical psoriasis – Unabated type I IFN production induced by TNF blockade*. *Cytokine* **76,** (Elsevier Ltd, 2015).