

## Immunology taught by lung dendritic cells

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### Abbreviations

DC	dendritic cell
mDC	myeloid or conventional DC
pDC	plasmacytoid DC
AMDC	airway mucosal DC
LPDC	lung parenchymal DC
AM	alveolar macrophages

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### Summary

Dendritic cells (DCs) are leukocytes specialised in the uptake, processing, and presentation of antigen and fundamental in regulating both innate and adaptive immune functions. They are mainly localised at the interface between body surfaces and the environment, continuously scrutinising incoming antigen for the potential threat it may represent to the organism. In the respiratory tract, DCs constitute a tightly enmeshed network, with the most prominent populations localised in the epithelium of the conducting airways and lung parenchyma. Their unique localisation enables them to continuously assess inhaled antigen, either inducing tolerance to inoffensive substances, or initiating immunity against a potentially harmful pathogen. This immunological homeostasis requires stringent control mechanisms to protect the vital and fragile gaseous exchange barrier from unrestrained and damaging inflammation, or an exaggerated immune response to an innocuous allergen, such as in allergic asthma. During DC activation, there is up-regulation of co-stimulatory molecules and maturation markers, enabling DC to activate naïve T cells. This activation is accompanied by chemokine and cytokine release that not only serves to amplify innate immune response, but also determines the type of effector T cell population generated. An increasing body of recent literature provides evidence that different DC subpopulations, such as myeloid DC (mDC) and plasmacytoid DC (pDC) in the lungs occupy a key position at the crossroads between tolerance and immunity. This review aims to provide the clinician and researcher with a summary of the latest insights into DC-mediated pulmonary immune regulation and its relevance for developing novel therapeutic strategies for various disease conditions such as infection, asthma, COPD, and fibrotic lung disease.

*Key words: dendritic cell; macrophage; T cell; lung; respiratory tract*

### Introduction – DCs in the respiratory tract

With its enormous surface area of approximately 150 m<sup>2</sup>, a volume of 350 Litres per hour of environmental air ventilated at rest, and highly fragile gaseous exchange surfaces, the respiratory tract poses a colossal challenge to the immune system. Anatomically, the respiratory tract can be broadly subdivided into two major compartments, the conducting airways and the lung parenchyma. Both regions are functionally distinct and differ in the composition of their immune cells (fig. 1).

Within the conducting airways, the mucosal surfaces contain ciliated epithelial cells, interspersed goblet cells, macrophages, and a tightly enmeshed network of airway mucosal DCs (AMDCs) that increases in its density after birth [1–3]. The latter mainly consist of myeloid DCs (mDCs) with a fraction of plasmacytoid DCs (pDCs) [4–6]. The phenotypic characteristics of both respiratory tract DC populations have been described in the mouse and human as detailed in table 1. DCs are continuously generated from flt-3-expressing myeloid and lymphoid progenitors produced in bone marrow from haematopoietic stem cells. The final DC type, functional specialisation, and degree of maturation is determined by the tissue environment and cells DCs interact with [7]. In general, AMDCs are endowed with a high capacity for antigen uptake, but a reduced ability to stimulate T cells. AMDCs continuously sample incoming airborne antigen by extending their dendrites through the intact epithelial layer into the airway lumen [8, 9]. Effector and memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as well as B cells are also present in the airway mucosa and may play a role in the constitution of bronchial-associated lymphoid tissue (BALT) [10–13].

The lung parenchyma consists of alveoli that are separated by fine vascularised interstitial tissue. Lung parenchymal DCs (LPDCs), macrophages, and T cells occur in the alveolar space, the alveolar epithelial layer, and the interstitium. Under non-inflammatory, i.e. steady-state conditions, the alveolar space (as reflected by broncho-alveolar lavage fluid composition) consists of 80% macrophages, the remainder being T cells and DCs [5].

Resident DCs in the respiratory tract are referred to as “immature”, implying a maximal ability to detect, capture, and process inhaled antigen, but an attenuated capability to stimulate T cells. Upon antigen exposure, in the presence of a so-called danger signal, through pathogen-associated molecular patterns (PAMPs) bound to pathogen recognition receptors (PRR), DCs are activated and “matured” [14]. At this stage, DCs shut down their capacity for antigen uptake, and concurrently increase their ability to activate T cells by presenting antigen fragments on MHC molecules in the presence of up-regulated co-stimulatory molecules (fig. 2). DCs migrate into draining lymph nodes along a chemokine gradient and are able to stimulate antigen-specific proliferation of naïve T cells and their differentiation into cytokine-secreting effector T cells (fig. 2). These, in turn, either home to the respiratory tract, where the “danger” situation initially occurred, or enter systemic circulation to generalise the immune response. Depending on the type and dose of antigen, the DC subset, and the regional cytokine microenvironment, different effector T cells are generated. Broadly four different types of effector T cell responses are distinguished at present: The hallmark of T helper 1 (Th1) responses is the T cell-dependent production of IFN- $\gamma$  and TNF- $\beta$  following exposure of DCs to intracellular pathogens such as bacteria and viruses. Th1 responses also occur with delayed hypersensitivity responses, e.g. tuberculin reaction and some autoimmune diseases. Exposure to extracellular pathogens (e.g. parasites) induce T helper 2 (Th2) responses with the production of IL-4, IL-5, and IL-13 that generate eosinophilia, mast cells and IgE antibody production. Allergic reactions to a seemingly innocuous substance are characterised by a Th2 response. Regulatory T cells (Treg) are tolerogenic, secreting IL-10 and TGF- $\beta$  to prevent excessive inflammation to non-pathogenic antigen. A unique T helper cell subset, termed Th17 due to T cell-dependent IL-17 production, has recently been discovered. IL-17 is a strongly inflammatory cytokine and Th17 cells are being recognised to play a role in diverse immunopathological conditions ranging from autoimmune diseases (rheumatoid arthritis, psoriasis, multiple sclerosis, inflammatory bowel disease) to asthma [15].

## Dendritic cells: importance in disease states

### Respiratory tract infections

Tuberculosis: *M. tuberculosis*, *M. africanum*, and *M. bovis* cause tuberculosis through aerosol spread from an infected and contagious individual. In the lung, mycobacteria activate innate immune cells by binding of pathogen-associated molecular patterns (PAMP) to pattern recognition receptors (PRR). Though mycobacteria preferentially infect macrophages, several receptors facilitate uptake by DCs: (1) DC-specific ICAM-3-grabbing non-integrin (DC-SIGN) binding to mycobacterial mannose residues; (2) mannose receptor; (3) DEC205 (CD205), (3) complement receptor (C3R); and (4) scavenger receptors [16–19]. Though mycobacteria affect antigen processing through inhibition of phagosome maturation and improved survival in macrophages, the fate of mycobacteria in DCs is controversial with reports ranging from no growth but survival to unrestricted growth [20–22]. Following endocytosis of mycobacteria, activated DCs migrate to draining lymph nodes, where they prime T cells [23]. Upon mycobacteria exposure, engagement of TLR on DCs, in particular TLR-2, induces the secretion of cytokines IL-1  $\beta$ , IL-12, IL-18, and IFN- $\alpha$ , which stimulate T cells to produce IFN- $\gamma$ , a cytokine essential for the bactericidal activity of DC through reactive oxygen intermediates. With the exception of  $\gamma\delta$  T cells that do not require conventional antigen presentation, DCs are the key antigen-presenting cells in tuberculosis [24]. As DCs express MHC class I, MHC class II, CD1, as well as co-stimulatory molecules CD80, CD86, and CD40, they are capable of priming naïve T cells. Mycobacterial T cell antigens are either lipids presented on CD1 molecules, or proteins such as ESAT-6, CFP-10, Ag85, and lipoprotein p19 [25–27]. Given their powerful antigen-presenting capabilities, DCs have also been termed natural adjuvants and have been used as experimental vaccine vectors in tuberculosis. Several studies employing murine DCs pulsed with whole mycobacteria, CD4, or CD8 T cell Ag85 epitopes induced IFN- $\gamma$  secretion by T cells and protected mice from infection with *M. tuberculosis* [28–31]. Upon primary infection, the specific T cell response restrains the mycobacterial infection in an immunocompetent host and generates a so-called caseating granuloma. In this structure, the necrotic centre is enclosed by macrophage-derived multinucleated giant cells (Langhans cells) and epithelioid cells, which in turn are bounded by a layer of T and B cells [32, 33]. Compared to macrophages, DCs also induce granuloma but contain fewer mycobacteria [34–37]. In most immunocompetent hosts, tuberculosis infection remains clinically latent with only one tenth of infected individuals eventually progressing to full blown disease [38]. If protective mechanisms fail, as in immunosuppression, unrestricted growth of mycobacteria with subsequent lympho- and haematogenic systemic spread to other organs occurs. Though the currently used BCG vaccination protects children against the systemic form of tuberculosis, it does not provide protection against pulmonary tuberculosis in adults. Future efforts to develop a highly efficient vaccination will require in depth understanding of how to enhance immunogenicity of mycobacteria and how these are handled by DCs.

Viral infections: In recent years, viruses have been shown to be a major cause of COPD and asthma exacerbations. Human rhinoviruses (HRV) are dominant pathogens, but influenza, para-influenza, corona viruses, and respiratory syncytial virus contribute as well [39]. The airway epithelium is the primary target of inhaled viruses, causing epithelial cells to release cytokines and chemokines that attract and activate cells of the immune system, including immature DCs expressing CCR6. Once replicating viral particles are released in the airways, they interact with other cell types, including DCs, to initiate antigen presentation to T cells. A protective influenza-specific CD8 T cell response seems to require interactions with dendritic cells in the lungs [40]. HRV seems to be capable of inhibiting the accessory function of dendritic cells and to evading this immune surveillance. Respiratory dendritic cells in murine models differ in their susceptibility and response to

infections, with plasmacytoid DCs exhibiting the least susceptibility [41, 42]. Recently, mouse models of rhinovirus-induced disease with a pattern of allergic airway inflammation have been generated and should allow major progress in this field [43].

### Allergy and asthma

Allergic asthma, one of the most common chronic diseases in Western countries, is characterised by a chronic eosinophilic inflammation of the airways with intermittent reversible airflow obstruction, mucus hypersecretion, and smooth muscle hypertrophy eventually leading to airway remodelling. Th2 T cells are central to asthma pathogenesis, producing IL-4, IL-5, IL-9, IL-13, and GM-CSF. The cytokine and chemokine microenvironment leads to broad effects, such as recruitment of inflammatory cells, IgE antibody production, bronchial hyperreactivity, and structural changes through effects on epithelial cells, fibroblasts, and smooth muscle cells. Under steady-state conditions, DCs in the respiratory tract constitutively present innocuous antigen in a tolerogenic form that favours Th2 responses [44, 45]. Myeloid DCs induce a protective immune response to an inhaled antigen only if the innate immune system is sufficiently activated through pattern recognition receptors such as TLRs, leading both to Th1 and Th2 responses. As an example, low concentrations of TLR4 agonists matured mDCs to induce a Th2 response without IL-12 secretion, whereas high concentrations led to IL-12 production [46]. Though IL-12 is a key cytokine to generate pulmonary Th1 responses, the Th1 response induced by LPS was not IL-12-dependent. If DCs are able to prime an immune response to an allergen, then why is tolerance the predominant response in the respiratory tract? This phenomenon was well shown in early studies by Holt et al and subsequently by other groups with experiments, in which administration of the model allergen ovalbumin (OVA) by aerosol, nasal, or tracheal administration inhibited the development of airways inflammation after subsequent sensitisation with OVA in adjuvant [47–51]. Despite OVA-loaded DCs inducing a vigorous proliferation of naïve antigen-specific T cells, the outcome of these studies is immunological tolerance and not immunity [48, 52, 53]. There are several possible explanations: When an antigen is encountered under non-inflammatory conditions, it fails to activate DCs sufficiently to induce an effector T cell response [54]. Insufficiently activated DCs primarily induce regulatory T cells that have an inhibitory effect on the immune response [55–57]. An IL-10-secreting, specialised CD8 $\alpha$ <sup>-</sup> mDC subset in thoracic lymph nodes of OVA-“tolerised” mice induced Th2-like regulatory cells that expressed both Fox P3 and GATA-3 and protected against allergic airways disease [47]. Lung pDCs take up non-pathogenic antigen and traffic to lymph nodes, where they induce regulatory T cells [48]. Depletion of pDCs abrogated tolerance to the antigen and led to Th2-dependent airways inflammation, whereas adoptive transfer of pDCs inhibited the development of asthma. A possible explanation for the tolerogenic properties of pDC is their production of indoleamine 2,3-dioxygenase (IDO) that inhibits T cell proliferation and inflammatory airways disease [58]. The above lines of evidence suggest that adaptive regulatory T cells can be generated in the respiratory tract depending on the type of antigen encountered and the DC subset involved.

Several experimental approaches for asthma treatment that interfere with DC function have recently been described: inhalation of CD86 antisense oligonucleotide (interference with co-stimulation), activation of the D prostanoid 1 receptor (modulation of DC phenotype, induction regulatory T cells), inhaled iloprost (reduced DC migration and maturation), and purinergic P2-receptor antagonists (decreased DC-driven inflammation) [59–62]. It will require additional carefully designed studies to understand whether these approaches may provide novel therapeutic strategies to effectively treat asthma in the future.

### COPD

The accumulation of DCs in COPD with increased chemokine ligand 20 (CCL20), one of the most potent chemokines attracting CCR6 positive DCs, has been recently described [63]. Other investigators found an increased number of immature DCs in the small airways, whereas the total number of DCs appears to be reduced in large airways of active smokers. It is hypothesised that this reduction of DCs in number and activity in central airways may favour repeated infections, as previously shown in murine models exposed to smoke inhalation [64, 65]. On the other hand, lung DCs exposed to cigarette smoke produced increased levels of the matrix metalloprotease MMP12, possibly contributing to tissue damage. In support of the pathogenic role of DC in COPD, mice deficient in CCR6, a chemokine receptor required to recruit pulmonary DCs, displayed reduced emphysema formation [66]. In a recent study on broncho-alveolar lavage fluid DCs, Bratke et al showed that smoking up-regulated co-stimulatory molecules CD80 and CD86, but decreased CCR7 expression in smokers with airflow limitation [67]. To date no detailed study has yet addressed how smoking and COPD affects DC function and distribution of sub-populations – an issue that requires clarification before DC-related novel therapeutic strategies are under development.

### Fibrotic lung disease

Recently, DCs have been shown to accumulate in human fibrotic interstitial diseases. Results were similar in idiopathic pulmonary fibrosis (IPF) and non-specific interstitial pneumonia (NSIP) lungs, heavily infiltrated by immature DCs, both in established fibrosis and in areas of epithelial dysplasia, expressing most chemokines involved in DC and lymphocyte recruitment [68]. A further attempt to characterise lung DCs was performed lately through broncho-alveolar lavage, quantifying the presence of plasmacytoid DCs and myeloid DCs either in pneumonia, sarcoidosis, or idiopathic pulmonary fibrosis. Plasmacytoid DCs were increased in pneumonia; patients with idiopathic pulmonary fibrosis had a normal percentage of DC subtypes, which were, however, less mature than in sarcoidosis, which presented paradoxical and contrasted changes [69]. DCs obtained through BAL may, however, not be the most biologically active DCs, due to their interaction with the alveolar milieu. As an example, surfactant in the alveoli may, on the one hand, enhance opsonisation of pathogens, but on the other hand, surfactant proteins SP-A and SP-D can inhibit functions of mononuclear cells by acting on

SIRP $\alpha$  [70]. Alveoli also contain metabolites, such as prostaglandins E2 or oxygen radicals, which may alter cell function in this compartment, without necessarily reaching the interstitium, where DCs have a different phenotype and biological behaviour. In this field of research many questions thus still remain unanswered.

## Conclusions and outlook

An important body of research on DCs has been performed in recent years, showing that DCs occupy a key role in regulating immune responses. Through relentless scrutiny of inhaled antigen, DCs uphold the delicate homeostasis between immune reaction to pathogens and tolerance to harmless substances. A breach of this immunological equilibrium may lead to immune-deficiency, allergic asthma, or autoimmune disease. Stringent control of the immune response is particularly important to maintain vital gaseous exchange in the respiratory tract, where a large mucosal surface area is unremittingly in contact with ambient airborne antigen. Respiratory tract DCs are heterogeneous, endowed with unique biological properties, and constitute different functional subsets within various anatomical compartments. A continuous discovery of new DC markers is to be expected in the next years which, together with advanced technological methods (e.g. multi-colour flow-cytometry), will contribute to establishing an exhaustive taxonomy and elucidating functional properties of specific DC subpopulations. An additional task will be to define which molecular pathways are defective in pulmonary DC during immunopathology.

This expanded understanding of DC biology, intrinsic molecular pathways, and their significance in health and disease will provide the basis to develop novel therapeutic strategies for respiratory disease states such as allergic asthma, cancer, and infections.

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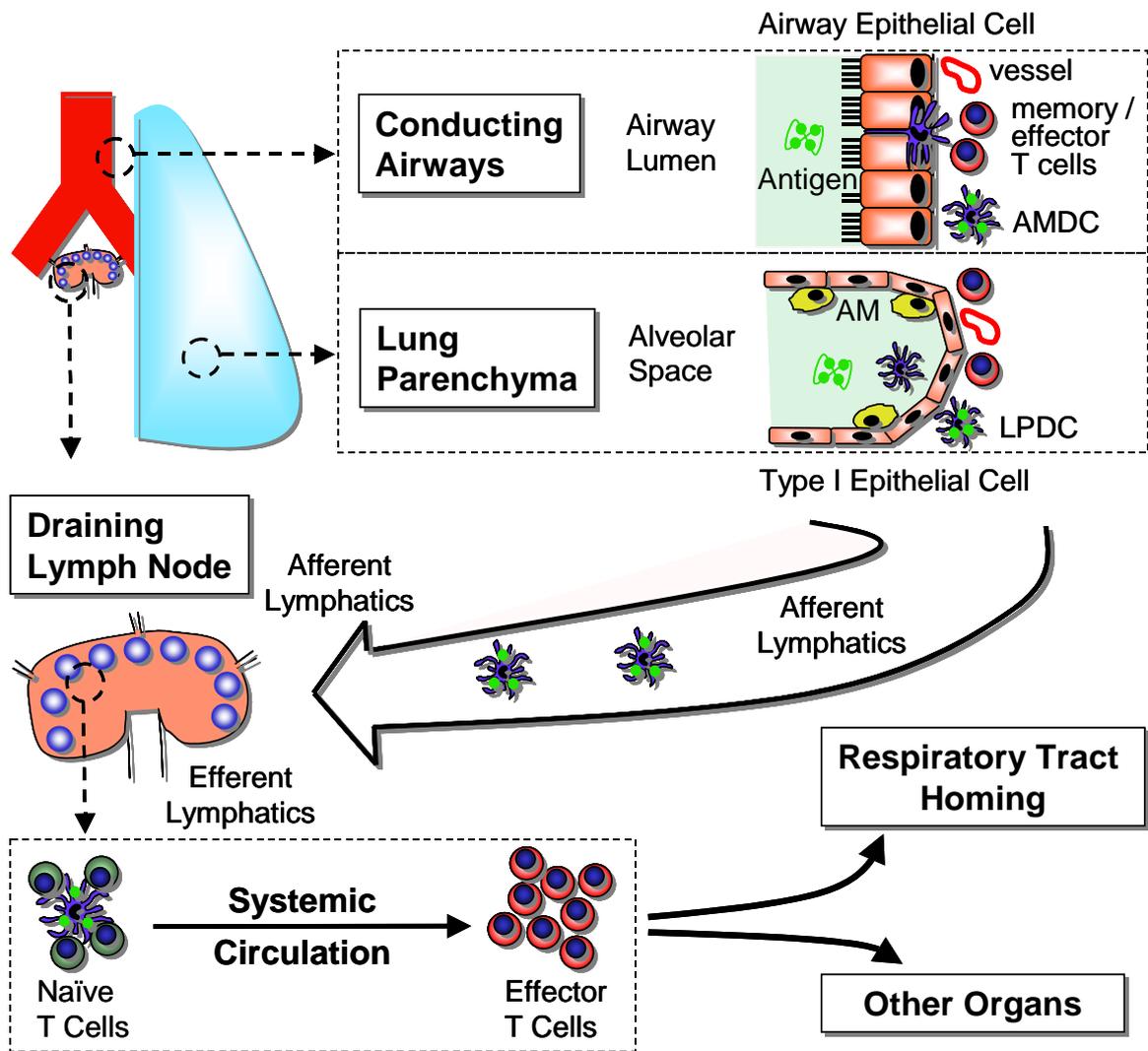
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**Table 1**

Respiratory tract DC described in the mouse and human lung.

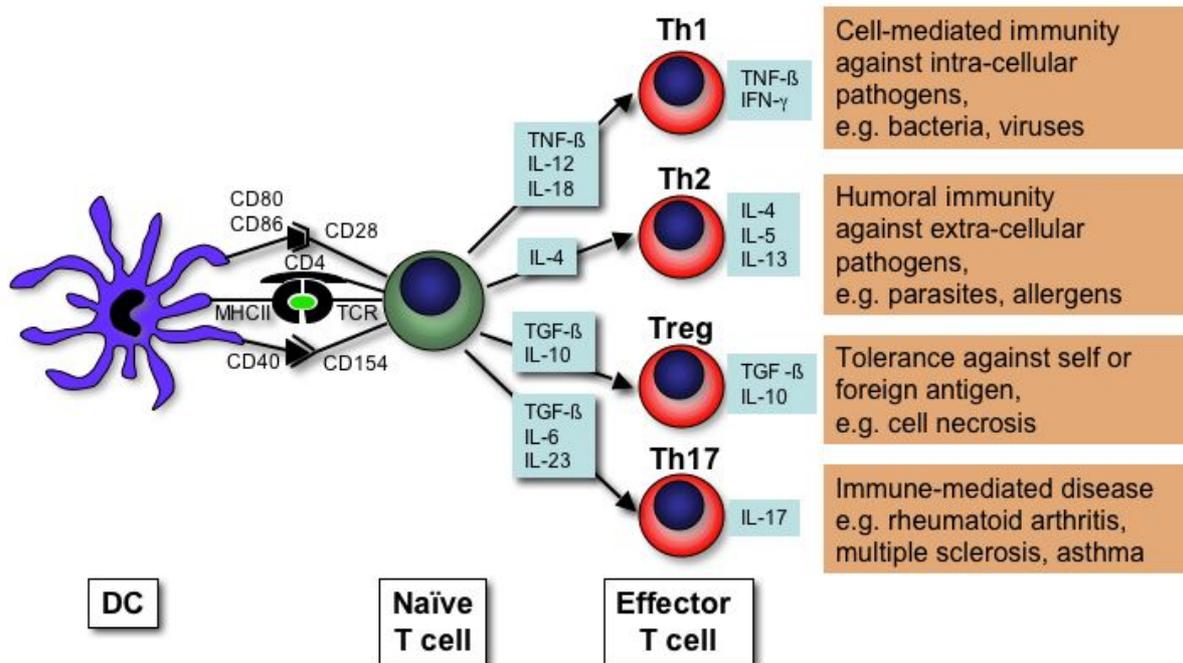
DC type	Mouse [5, 48, 71–74]	Human [8, 65, 69, 75, 76]	
Myeloid (mDC)	CD11c <sup>+</sup> , MHCII <sup>+</sup> , CD11b <sup>+</sup>	DC1	CD11c <sup>+</sup> , HLA-DR <sup>+</sup> , BDCA-1 <sup>+</sup>
		DC2	CD11c <sup>+</sup> , HLA-DR <sup>+</sup> , BDCA-3 <sup>+</sup>
Plasmacytoid (pDC)	CD11c <sup>low</sup> , MHCII <sup>low</sup> , CD11b <sup>-</sup> , 120G8 <sup>+</sup> , Gr-1 (Ly6G/C) <sup>+</sup> , B220 <sup>+</sup>	CD11c <sup>+</sup> , HLA-DR <sup>+</sup> , BDCA-2 <sup>+</sup> , CD123 <sup>+</sup>	

BDCA, blood DC antigen; MHCII, major histocompatibility class II; HLA, human leukocyte antigen



**Figure 1**

Immunological compartments in the respiratory tract. Inhaled antigen is deposited within the main conducting airways or alveolar spaces and taken up by antigen-presenting cells such as DCs or macrophages. Within the main conducting airways, airway mucosal DCs (AMDC) predominate, whereas in the lung parenchyma there is an excess of alveolar macrophages (AM) exerting an inhibitory effect on lung parenchymal DCs (LPDC) to protect vital gaseous exchange surfaces. AMDC and LPDC relentlessly scrutinise the airway lumen and alveolar space for antigen, subsequently migrating to draining lymph nodes to present antigen-derived peptides to naïve T cells, causing their differentiation into memory / effector T cells. Adapted from Holt et al. [8].



**Figure 2**

Differentiation of naïve T cells into effector T cells. In lymph nodes, DCs present antigen-derived peptide fragments to naïve T cells on MHC class II molecules in combination with co-stimulatory molecules and in the presence of cytokines. Following cognate interaction with DCs, naïve T cells may differentiate into several effector T cell populations that play a role in immunity and immune-mediated disease.

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