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Common Variable Immunodeficiency: molecular pathways and clinical manifestations

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I. Introduction

1. Common variable immunodeficiency (CVID)

1.1 Epidemiology

Common variable immunodeficiency (CVID) is the most prevalent primary immune disorder. Its prevalence is estimated to be 1 in 25 000 to 50000 individuals, both sexes are equally affected [(1)].Compared to other primary immunodeficiencies which are diagnosed before age 2, mean age of onset is in late childhood or early adulthood (mean age 23 to 28 years [(1)]). Diagnosis is often delayed for several years due to the various clinical manifestations [(1)].

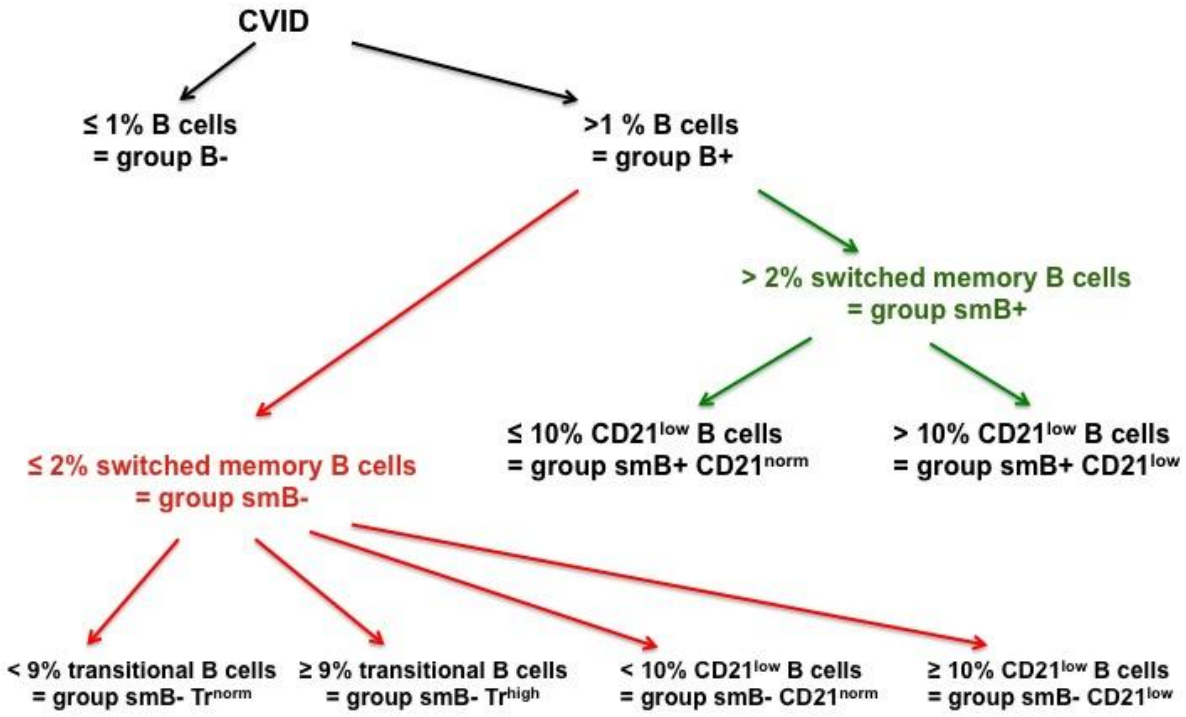
1.2 Classification

CVID is a heterogenous disorder, leading to frequent and severe infections as well as gastro-intestinal dysregulation, auto-immunitiy, lymphoproliferative and granulomatous disease that might degenerate to lymphoproliferative malignancies. Manifestations vary in frequency and severness from one patient to another. In the past few years several studies showed associations between B-cell defects and specific phenotypes [(2)(3)(4)(5)]. According to these studies, splenomegaly, granulomatous disease and auto-immune cytopenia are associated to a reduction of total and / or switched memory B cells, and patients with an increase in $CD21^{low}$ B cells are more frequently subjected to splenomegaly and auto-immune disorders [(2)(3)(4)(5)]. In 2008 the EUROclass trial improved the former Paris- and Freiburg-classifications by developing a new organization, based upon B-cell abnormalities and clinical courses of CVID [(2)]. The following divisions have been established: presence (B+) or absence (B-) of circulating B lymphocytes; the B+ group is sub-divided according to the proportion of switched memory B cells (smB + or -). Further on the possible increase of two B-cell subsets is taken into account: transitional B-cells and $CD21^{low}$ B cells (Figure 1).

The french DEFI-study team performed further investigations including T-cell dysfunction. They first classified their patients using the EURO-class definition. Then they divided patients into four groups based upon the most common symptoms of CVID: infections only (IO), lymphoproliferation, autoimmune cytopenia and chronic enteropathy. They found the following associations: In the infections only group a

decrease in smB cells as well as the plasmablastic population could be shown. In the T-cell compartement a reduction of naive CD4 and CD8 cells associated to an increase in activated T-cells were the major difference compared to healthy controls. In the three remaining clinical groups the abnormalities were more pronounced than in the IO-group, but with a modification in the CD21low population, which was normal in the IO group [6].

Figure 1: The EUROclass classification scheme (adapted from Wehr C et al. [2]) :



1.3 Diagnosis

The diagnosis of CVID is based upon 1) the exclusion of other known immunodeficiency disorders, 2) the presence of hypogammaglobulinemia of at least two classes of immunoglobulins (Igs) (IgG and IgA or IgM), 3) impaired functional antibody responses to protein- or polysaccharide based vaccines (*i.e.* diphtheria, tetanus or *S. pneumoniae* respectively) [(7)].

1.4 Symptoms

Patients present a heterogenous set of symptoms. These symptoms encompass respiratory tract infections, gastro-intestinal diseases, auto-immune disorders, granulomateous disease and patients with CVID are at a higher risk for malignancies [(1)].

Sino-pulmonary disease: The most frequent clinical presentation are upper and lower respiratory tract infections (sinusitis, otitis media, pneumonia, bronchitis), which may be acute, recurrent or chronic [(8)]. They are mainly caused by *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Haemophilus influenzae* (1). Most studies report a prevalence of 91% to 95% of upper and lower respiratory tracts infection [(1)(9)]. Ten to 20% of CVID patients develop granulomatous-lymphocytic interstitial lung disease (GLILD). These non infectious, chronic lung diseases are associated with high morbidity, mortality and poor prognosis [(7)(9)]. The granulomatous disease ressembles sarcoidosis and in addition to the lung, also lymph nodes, the liver, the skin, the spleen, the bone marrow, the gastrointestinal tract, the brain, the kidneys may be affected [(1)].

Chest X-ray or CT show different abnormalities of the lung parenchyma: nodular infiltrates, pulmonary fibrosis, bronchectasis, all associated to either chronic or recurrent infections and lymphocitic infiltration [(8)(7)(8)]. In the same cohort as mentioned above, at time of diagnosis more than 30% presented signs of chronic lung disease at CT-Scan, which increased up to 45% during follow-up [(7)].

Gastro-intestinal disease: As in pulmonary disease, CVID patients present infectious as well as chronic gastro-intestinal abnormalities. The most common symptom is recurrent diarrhea. It is found in 10 to 50% of patients [(1)(7)(9)]. In rare cases infectious

agents are identified: *Gardia lamblia* being the most common organism [7]. These infections are more frequent in patients with an IgA deficiency than in those with a residual production of IgA [10]. Among others, one of the known roles of IgA is the regulation of gut immunity by modulating the interaction between commensal flora and mucosal immunity [11]. It has been observed that IgA deficiency is associated with increased infection frequency as well as with lymphocytic inflammatory changes, due to an excessive B-cell stimulation by expanded commensals [12].

Gut biopsies present lymphoid hyperplasia, villous atrophy, granulomas, inflammatory bowel disease-like lesions, often associated with malabsorption, diarrhea and weight loss [7][9][10]. The underlying causes of these histological changes have not been definitely identified, but seem to correlate with defects in cellular immunity [8]. Gastric complications are discussed in the "neoplasia" section.

Auto-immune disorders: Up to 25% of CVID patients suffer from auto-immune disorders [1][7][13][14]. Immune thrombocytopenic purpura (ITP) and auto-immune haemolytic anaemia (AIHA) are frequently encountered with a prevalence of 5-8% [1][13]. Cytopenias can manifest before the diagnosis of immunodeficiency [13][15]. They are strongly associated with splenomegaly, granulomatous disease and increased risk for hematological malignancy [14][15][16].

Rheumatoid arthritis is present in 1-10% [15]. Cases of other auto-immune disease can be found, such as neutropenia, Sjögren syndrom, thyroiditis, vitiligo, systemic lupus erythematosus, pernicious anemia [9][13][14]. Autoantibodies (rheumatoid factor or antinuclear antibodies) are typically absent [15].

The underlying mechanisms of auto-immune disorders in CVID have been analyzed in several studies [6][14][16][17]. Autoimmune cytopenia are associated with low numbers of class-switched memory B cells, low number of regulatory T cells and expanded $CD21^{low}$ B cells [2][14].

Neoplasia: It is hard to determine the incidence of cancer in CVID. Recent studies estimated an occurence in 15% of subjects with a 5-fold overall increased risk for cancer [7][18]. The group of patients suffering from lymphoid hyperplasia have an up to 30-fold higher risk to develop non-Hodgkin lymphoma [7][19]. The incidence for this

malignancy is also related to female gender and a higher serum level of IgM [2][7][18]. The combined Danish and Swedish study for cancer risk in CVID and their relatives, found no increased risk in close relatives, suggesting that the higher prevalence is more likely due to immunodeficiency than to inherited factors [19].

Gastric cancer represents an important malignant manifestation of CVID with a 10-fold increased risk of gastric cancer as compared to the general population [7][10][19][20]. In the general population *H. pylori* is listed as a class 1 carcinogen and is associated to a 2- to 9-fold increased risk to develop gastric lesions [20]. One hypothesis could be that gastric cancer risk is increased among CVID patients due to a higher infection rate, which could not be confirmed up to now [20]. The Swedish and Danish study however suggested a direct association of gastric cancer and immunodeficiency after comparing gastric cancer incidence of CVID patients and their relatives [19].

1.5 Genetic

Until now, only 15-20% of CVID cases have been associated to specific genetic defects [26]. In particular, mutations in *TNFRSF13B* (TACI) [21], *ICOS* [22], *CD19* [23], *CD20* [24], *CD81* [25], *BAFF-R* [26] , *CD21* [27] genes were described. Of note, these studies were based on single gene target analyses; therefore, they do not exclude a possible role for other gene mutations. Several studies are assessing a potential genetic defect by whole genome analyses or full exome sequencing.

1.6 Treatment

Hypogammaglobulinemia is defined as plasmatic concentration of IgG lower than 4.9 mg/mL, however, plasmatic IgG concentration of healthy individuals exceeds 7 mg/mL (in 95% of cases). A plasmatic IgG level between 4.9 and 7 mg/mL does not completely rule out the diagnosis. Among those patients assessment of IgG subclasses may be of interest. The current gold-standard treatment is the transcomplementation of Ig administered intravenously every 3-4 weeks (intraveneous immunoglobulins (IVIg)). The goal of the treatment is 1) to prevent bacterial infections and 2) to reduce infection-associated morbidity and mortality [28].

The treatment of secondary affections depends on the manifestation. Breakthrough infections are usually treated with antibiotics, but surgical interventions can be necessary, mainly for sinuses [(1)(29)]. Persistent hematologic auto-immune manifestations despite immunoglobulin substitution are usually treated by cortico-steroids. Use of new biological drugs such as rituximab has been described in management of persistent or refractory cases of autoimmune cytopenia [(7)].

Chronic lung diseases caused by repeated infections can be treated with prophylactic antibiotics, but severe cases may need oxygen therapy or even lung transplantation [(7)(30)].

As there are several etiologies for enteropathy, the treatment varies with the cause. Anti-microbial agents are used for infections, whereas inflammatory bowel disease is treated the same way as in immunocompetent patients, with antibiotics or low dose steroids. In severe cases Infliximab has been used [(7)(31)]. Gut biopsies often show villeous flattening suggesting coeliac sprue, but wheat withdrawel is rarely beneficial [(7)].

Granulomatous disease can be treated with steroids or immuno-modulators, however it is still unclear to which patient, at which moment and whether the immuno-suppressive effect of these drugs is deleterious or not [(7)(17)].

Lymphomas respond to standard chemotherapy and rituximab protocols [(7)].

Splenectomy is not recommended in patients suffering from CVID as severe infections might occur and patient's deaths have been reported [(7)(30)].

II. Aim of the study

At the genetic and clinical level, CVID disorders are heterogenous, however Prof Pantaleo's group hypothesized that they might share a common phenotype. When I started my master as a medical student, the team of Prof Pantaleo had already shown that CVID patients harbored reduced frequencies of bacteria-specific type 1 CD4 T helper (Th1) cells (producing IFN-γ) and Th17 cells (producing IL-17A) (unpublished data). We therefore hypothesized that B-cell defect(s) in CVID patients might lead to CD4 T-cell impairment. Of note, CD4 T-cell functional impairment (*i.e.*

exhaustion) is commonly associated with the expression of co-inhibitory molecules during chronic viral infections [32][33][34][35][36]. In this model, the progressive functional impairment of T cells is initiated by the loss of proliferative capacity, associated with a progressive reduction of IL-2 production capacity and, in the context of CD8 T cells, with a deficient killing capacity.

In this context, the following points were evaluated: 1) the proliferation capacity of CD4 T cells of CVID patients in response to bacteria and viruses, 2) the co-inhibitory molecules (*i.e.* Programmed Death 1 (PD-1), 2B4, CD160, SLAM, CTLA-4, LAG-3, TIM-3) expression levels in both total and antigen-specific CD4 T cells and 3) the restoration of proliferation capacity by the blockade of PD-1/PD-1 ligands (PDL-1, PDL-2 interactions), 4) the levels of LPS (marker of bacterial translocation), together with IgG levels were also assessed in the plasma of CVID patients at various time points.

During my master, I was involved in the collection of the clinical manifestations of our patients in order to characterize and compare our patients to current literature. I also participated in the fundamental research, particularly in the assessment of LPS levels and the expression of co-inhibitory molecules and CD4 T-cell proliferation.

III. Material and methods

Study groups. For this study, 31 CVID patients and 30 healthy individuals were recruited. Blood samples were obtained at the Centre Hospitalier Universitaire Vaudois (CHUV) and the Hôpital Henri Mondor, France or from the Blood Bank of Lausanne, Switzerland, for CVID patients and healthy individuals, respectively. The Institutional Review Board of the CHUV approved this study and informed consent was obtained from each individual.

Cell isolation. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient (GE healthcare). After centrifugation (300g; 20 min), PBMC ring was harvested in PBS-EDTA 2 mM (Laboratorium Dr Bichsel AG, and Ambion for PBS and EDTA, respectively). Cells were either used directly or cryopreserved in liquid nitrogen for future experiments.

Antibodies. The following antibodies were used: APC-H7-conjugated anti-CD3 (clone SK7), FITC-conjugated anti-CD4 (clone RPA-T4), APC-conjugated anti-CD4 (clone RPA-T4), PB-conjugated anti-CD4 (clone RPA-T4), APC-H7-conjugated anti-CD8 (clone SK1), PerCP-Cy5.5-conjugated anti-CD8 (clone SK1), PerCP-conjugated anti-CD69 (clone L78), PECy7-conjugated anti-CD279 (PD-1; clone EH12.1), APC-conjugated anti-TNF-α (MAbII), FITC-conjugated anti-CD25 (OX-39), PE-Cy-conjugated anti-TNF-α (clone MabII) from BD biosciences (CA, USA); ECD-conjugated anti-CD3 (clone UCHT1), ECD-conjugated anti-CD45RA (clone 2H4), ECD-conjugated anti-CD4 (clone T4) from Beckman Coulter (CA, USA); PECy5.5-conjugated anti-2B4 (CD244; clone C1.7), PE-conjugated anti-SLAM (CD150; clone A12), PB-conjugated anti-CD57 (clone HCD57), AlexaFluor 647-conjugated anti-CD160 (clone BY55) from Biolegend (CA, USA); EFluor 625NC-conjugated anti-CD8 (clone RPA-T8), from eBioscience (CA, USA) and FITC-conjugated anti-CCR7 (clone 150503), AlexaFluor 700-conjugated anti-HLA-DR (clone LN3) from R&D systems Inc. (MN, USA).

Flow cytometry. Data were acquired on a LSR SORP four lasers (405, 488, 532 and 633 nm) (Becton Dickinson), analyzed using FlowJo v9.4.11 (Treestar, Inc., Ashland, CR) and SPICE v5.21 (developed by Mario Roederer, National Institute of Health and downloaded from <http://exon.niaid.nih.gov/spice>) [37]. At least 100,000 events were acquired for each sample.

Assessment of CD4 T-cell proliferation. Mononuclear cells were re-suspended at 10^6/ml in PBS and incubated for 7 min at 37°C with 0.25 μM 5, 6-carboxyfluorescein succinimidyl ester (CFSE, Invitrogen) as previously described [38]. The reaction was quenched with one volume of fetal calf serum (FBS; Institut de Biotechnologies Jacques Boy). Subsequently, cells were washed, cultured in 4% human AB serum (Institut de Biotechnologies Jacques Boy) RPMI (Gibco; Life Technologies). Cells were stimulated with *Streptococcus pneumonia, Klebsellia pneumonia, Staphylococcus* enterotoxin B (SEB, Sigma-Aldrich) (positive control) or unstimulated (negative control). After six days of *in vitro* T-cell expansion, dead cells were stained (4°C; 15 min) using the violet LIVE/DEAD stain kit (Invitrogen), and cells were

stained (4°C, 15 min) with anti-CD3-APC-H7, anti-CD4-ECD, anti-CD8-PerCP. Frequencies of proliferating CD4 T cells were assessed by flow cytometry.

Assessment of CD4 T-cell exhaustion and differentiation. PBMCs were re-suspended (10^6 cells/mL and per condition) in complete RPMI medium ((10% FCS, penicillin (100 u/ml) and streptomycin (100 µg/ml) (Bioconcept)). Dead cells were stained using the amcyan LIVE/DEAD stain kit (Life Technologies) (4°C, 15 min). Cells were stained (4°C, 15 min) with anti-CD3-APC H7, anti-CD4-PB, anti-CD8-Efluor625NC, anti-2B4-PeCY5.5, anti-CD160-APC, anti-PD-1-PeCy7, anti-CD45RA-ECD, anti-CCR7-FITC and anti-SLAM-PE. Data were acquired on an LSR SORP.

Assessment of CD4 T-cell activation. PBMCs were re-suspended (10^6 cells/mL and per condition) in complete RPMI medium ((10% FCS, penicillin (100 u/ml) and streptomycin (100 µg/ml) (Bioconcept)). Dead cells were excluded using the amcyan LIVE/DEAD stain kit (4°C, 15 min). Cells were stained (4°C, 15 min) with anti-CD3-ECD, anti-CD4-APC, anti-CD8-APC-H7, anti-CD69-PerCP, anti-CD25-FITC, anti-PD-1-PeCy7, anti-HLADR-AlexaFluor 700 and anti-CD57-PB. Data were acquired on an LSR SORP.

Restoration of CD4 T-cells proliferation. CFSE-labeled PBMCs (as previously described in methods) were stimulated with replication-deficient adenovirus serotype 5 (Ad5) (1 µg/mL), bacteria pool (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsellia pneumoniae* and *Escherichia coli*, 5 x 10^7 CFU/ml), SEB as positive control or left unstimulated cells as negative control, in presence or in absence of PD-1 ligand 1 and 2 (PD-L1/2) (eBioscience). After 6 days of stimulation, cells were washed and stained with violet LIVE/DEAD stain kit (4°C, 15 min). Then cells were stained (4°C, 15 min) with anti-CD3-APC-H7, anti-CD4-ECD, and anti-CD8-PerCP-Cy5.5. Data were acquired on an LSR SORP.

mTNF-*α* production. Blood Mononuclear cells (10^6 cells/ml) were stimulated (18 hours) with heat-inactivated bacteria pool (5 x 10^7 CFU/mL) or Ad5 (1 µg/mL) in complete RPMI containing TAPI-0 (10 µM; Calbiochem) and APC-conjugated anti-TNF-α (0.2 mg/mL) as described (39). Phytohaemagglutinin (PHA; Sigma) was used

as positive control and unstimulated cells as negative control. At the end of the stimulation period, dead cells were stained using the violet LIVE/DEAD stain kit (4°C, 15 min), cells were stained (4°C, 15 min) with anti-CD3-ECD, anti-CD4-FITC, anti-CD8-APC-H7, and anti-PD-1 PeCY7. Data were acquired on LSR SORP and analyzed using FlowJo.

LPS titration. Plasma were harvested during PBMC isolation or collected in the Immunology and Allergy diagnostic laboratory. Lipopolysaccharides (LPS) were quantified in plasma using limulus assay test (Charles River Laboratories). Briefly, plasma were diluted (1/50) in sterile water (Laboratorium Dr Bichsel AG) and samples were read in Endosafe assay (Charles River Laboratories).

Immunoglobulin quantification. Total immunoglobulin G (IgG) quantification was performed in the Immunology and Allergy diagnostic laboratory by nephelemetry.

Statistical analyses. Statistical significance (*P* values) was obtained using one-way ANOVA (Kruskal-Wallis test) followed by Student's *t* test for multiple comparisons or a Spearman rank test for correlations.

Analysis of patients medical history. Thirteen patients are followed at the Centre Hospitalier Universitaire Vaudois (CHUV), their medical history was analized, based upon the medical doctor's documentation. Specific characteristics *i.e.* age, sex, Ig levels, therapy, pre-and postdiagnostic symptoms, family history were analized and documented in table 1. One patient was excluded, as she had normal levels of total IgG and IgA (#13) a vaccinal response, probably further classified as sub-class deficiency, so only 12 patients could be definitely included. The 19 remainaing patients were included in the fundamental research, but their history could not be analyzed as they are followed at the unit of clinical immunology at the Hôpital Henri Mondor, in France. Considering the small number of patients, the results in „Clinical findings" are only given in numbers and not in percentages.

IV. Results

1. Clinical findings (see table 1 “clinical data”)

1.1 Immunoglobulin Levels

Eight out of 12 patients had total IgG levels under 2 mg/ml by the time of diagnosis, the other 4 had between 3 and 4 mg/ml. IgG subclasses could not be analyzed as they were not tested in all patients. All of them had a deficiency in IgA. IgM levels were too low in 5 out of 12 patients, the remaining 7 subjects were all clearly below 0.7 g/l.

1.2 CD4, CD8 and B-cell count

Two patients could not be taken into account, as only the relative number of cells was known. Only 2 patients had a normal count of all subtypes. Total lymphocyte T-count was normal in 5 patients, 3 only had a dicrease of total CD3 whereas the CD4, CD8 and CD19 count was normal. One patient presented a severe lymphopenia, unfortunately only his CD4 and CD8 T-cell counts were available. One patient had an increased number of CD8 T cells, another one in CD19 B cells. Finally only 2 patients lacked CD4 T cells and only 1 lacked CD8 T cells.

1.3 Vaccine response

The vaccinal response of CVID patients is low for protein- or polysaccharide based vaccines (*i.e.* diphtheria, tetanus or *S. pneumoniae* respectively) Antibody levels were determined in 7 out of 12 patients. Quantities were measured either directely at diagnosis, or one month post-vaccination. Four out of 7 patients had no, or a too low to be protective response. The 3 remaining responded with low levels of antibodies to one out of the three vaccinces tested.

Of note, the assay performed at the division of Immunology and Allergy only detects specific antibodies. Therefore it is not possible to assess affinity maturation of vaccinal responses [40][41].

1.4 Pre-diagnostic elements

Patients documentations were analyzed for symptoms prior to the diagnosis of CVID. Most patients had suffered from their infections several years before the diagnosis of CVID was confirmed and the adequate treatment instored.

All patients suffered from sinusitis, some patients needed antibiotic treatment several times per year. Severe sinusitis leading to meatectomies were found in 2 patients histories. Lower respiratory tract infections were present in 6 out of 12 patients, it was not always clear whether these were pneumonias or bronchitis. Gastro-intestinal problems were relevated in 2 patients. These patients suffered from diffuse abdominal pain, accompanied by diarrhea. An intestinal parasite was found in 1 patient. Two patients had suffered from idiopathic thrombotic purpura in the past, one presented a vitiligo.

1.5 Post-diagnostic elements

After the diagnosis of CVID and the initiation of appropriate treatment, all patients still suffered from manifestations of CVID, but to a lower degree.

Sino-pulmonary infections are highly represented, only 4 patients were symptom free. The others were less frequently infected, but infections could still be severe, needing surgical interventions (amygdalectomie, meatectomie).

Pulmonary infections were reported in 2 patients, one of them with an Aspergilloma and an infection by *Pneumocystis jirovecii*. Interstitial lymphoid pneumopathies with obstructive symptoms were found in 2 patients. Gastro-intestinal manifestations, such as cramps, diarrhea or parasite infections (mainly *Gardia lamblia*) were found in 4 patient treated by IVIgs. One of the patients had 3 episodes of spontanous bacterial peritonitis. Diffuse adenopathies or lymphoid hyperplasia lesions were found in 7 patients. They were located either in the mediastinal compartment, directely on the pulmonary hilum or in the abdominal cavity. One patient presented diffuse polyclonal lymphoid proliferation in the white brain matter, the lungs, the spleen and the liver. Two patients showed adenopathies in the axillary region and both of them suffered from granulomateous interstitial pneumopathies. One patient presented granulomateous uveitis. Hematological auto-immune manifestations were found in 3 patients: one showed an episode of hemolytic anemia, the others thrombocytopenia.

At least 4 patients presented a documented splenomegaly which was operated or not. One patient had a splenectomie after an accident.

2. Impaired bacteria-specific CD4 T-cell proliferative capacity of CVID patients

We previously observed that CVID patients harbored reduced frequencies of IL-2- and IFN-γ-producing bacteria-specific CD4 T cells. In order to assess bacteria-specific CD4 T cells proliferation, blood mononuclear cells were labeled with CFSE and stimulated with heat-killed bacteria (either Gram positive *(Staphylococcus aureus*, *Streptococcus pneumoniae)* or Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsellia pneumoniae*)) and proliferation was measured on CD3-positive T cells at day 6 by flow cytometry (Fig. 2A). Proliferative responses were measured in 30 healthy individuals and 31 CVID patients. We showed that the proliferation capacity of bacteria-specific CD4 T cells in CVID patients was significantly (P<0.0015) reduced as compared to healthy individuals (Fig. 2A and B).

3. CD4 T cells of CVID Patients express high levels of PD-1

Considering that CD4 T cells of CVID patients harbored reduced capacity to produce cytokines and to proliferate (Fig. 2A and B), we postulated that CD4 T cells could be exhausted. To address this issue, the expression of co-inhibitory molecules was assessed by flow cytometry. For that purpose, PBMC of CVID patients (N=21) and healthy individual (N=30) were stained with monoclonal antibodies directed to PD-1, 2B4, CD160, SLAM, CTLA-4, LAG-3, and TIM-3 and the expression of these molecules was evaluated by flow cytometry (Fig. 3A). We observed that CD4 T cells in CVID patients expressed significantly higher levels of PD-1 than in healthy subjects (P<0.0001) (Fig. 3B), suggesting the presence of functional exhaustion. However, we did not detect any other difference in co-inhibitory molecules expression (Fig. 3B). It has been observed that PD-1 expression is prevalent in T cells in effector memory stage of differentiation and is upregulated upon T-cell activation [(42)]. Thus, the proportion of CD4 T cells at the various stages of differentiation (*naïve*, central memory, effector memory and terminally effector

memory) as well as the frequencies of CD4 T cells expressing other activation markers (CD69, CD25 and HLA-DR) were assessed by flow cytometry. No differences were observed in CD4 T-cell differentiation or activation (CD69, CD25 and HLA-DR expression levels) between CVID patients and healthy individuals (Figure 4). Therefore, the possibility that the observed increase in PD-1 was associated to skewing of CD4 T-cell differentiation toward an effector memory phenotype or to activation of CD4 T cells was excluded.

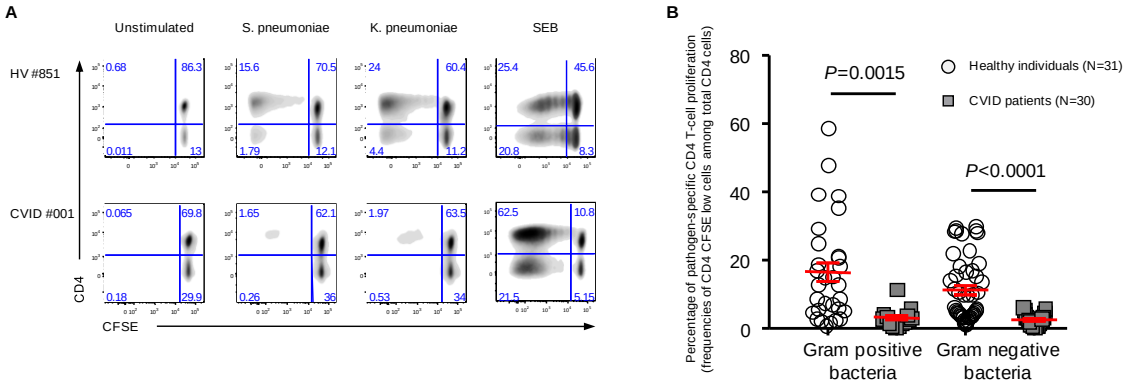


Figure 2. Impaired bacteria-specific CD4 T-cell proliferative capacity of CVID patients. Blood mononuclear cells were labelled with CFSE and stimulated with heat-killed bacteria and proliferation was measured on CD3 positive T-cells at day 6. Proliferative responses were measured in 31 healthy individuals and 30 CVID patients. **(A)** Flow cytometry profiles of CD3 T-cells stimulated with *S. pneumoniae* and *K pneumoniae*. Representative examples of healthy individuals (#851) and CVID patients (#1) are shown. Unstimulated cell cultures (negative control) and cell cultures stimulated with SEB (positive control) are also shown. **(B)** cumulative data representing the percentage of bacteria-specific CD4 T-cell proliferation. *P* values were derived using Student's t-test.

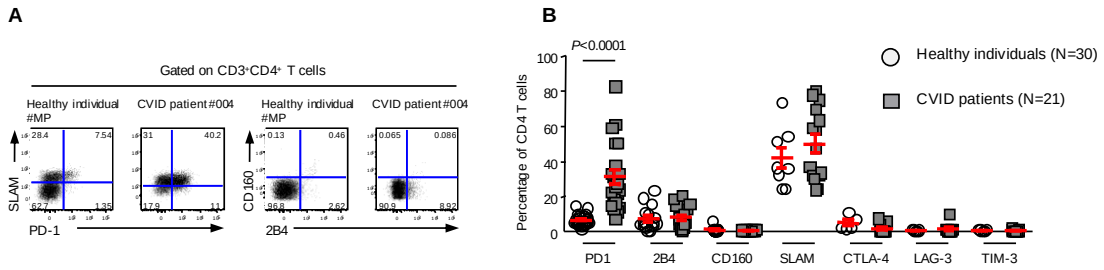


Figure 3. **CD4 T cells of CVID Patients express high levels of PD-1**. Expression of PD-1, SLAM, CD160, 2B4, CTLA-4, LAG-3 and TIM-3 were evaluated in healthy individuals (N=30) and CVID patients (N=21) by flow cytometry. **(A)** Representative example of the expression of PD-1 and SLAM or CD160 and 2B4 in a representative healthy individual (#MP) and in a CVID patient (#3). **(B)** Cumulative data of co-inhibitory molecules expression**.** *P* values were derived using Student's t-test.

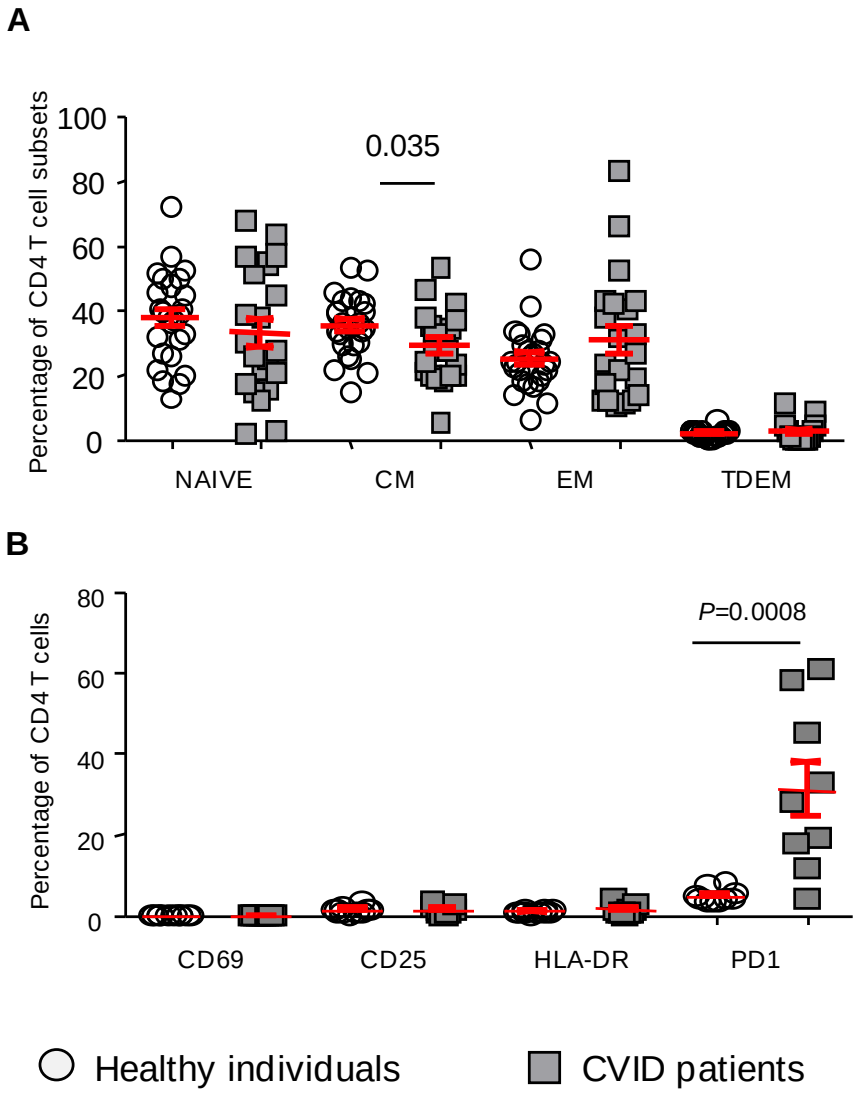


Figure 4. Differentiation and activation profiles of CD4 T cells of CVID patients. **A)** Proportion of naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory (TDEM) in CD4 T cells from CVID patients. **B)** Proportion of CD4 T cells expressing CD69, CD25, HLA-DR and PD-1 in healthy individuals and CVID patients. *P* values were derived from One-way ANOVA (Kruskal-Wallis test) followed by a Student's t-test.

4. Bacteria-specific CD4 T cells of CVID Patients express high levels of PD-1

We showed that PD-1 was increased on total CD4 T cells (P<0.0001) similarly to what was observed in the context of chronic infections such as HIV-1 [33][35]. In this model, HIV-1-specific but not CMV-specific CD8 T cells of viremic HIV patients expressed high levels of PD-1, suggesting that chronic antigen stimulation triggers the up-regulation of PD-1 and consequently functional impairment of HIV-specific CD8 T cells [33][35]. Interestingly, CVID patients suffer from recurrent bacterial infections [1][43][44], while no increase of acute viral infections (*i.e.* influenza) nor reactivation of chronic viral infections (*i.e.* EBV or CMV) was reported [45]. Of note, we previously showed that bacteria-specific but not virus-specific CD4 T cells of CVID patients harbored reduced capacity to produce cytokines and to proliferate (data not shown). Therefore, we assessed whether PD-1 upregulation was restricted to bacteria-specific CD4 T cells. In the context of chronic viral infection, most of the study relies on the use of tetramer staining to identify virus-specific CD8 T cells and therefore evaluate their phenotype directly *ex-vivo* [33][34][35]. However, in the context of bacteria-specific CD4 T-cell responses, MHC-II tetramer are poorly available and technical issues are still unresolved; in addition, virus-specific T-cell responses are usually oligoclonal while bacteria-specific T cell responses are more polyclonal [46][47][48][49][50]. Thus, we took advantage of a recently described technique by haney *et al.*, in *Journal of Immunological Methods* that exploits an inhibitor (TAPI-0) of TNF-α converting enzyme (TACE) that blocks TNF-α release from the membrane, to identify specific CD4 T cells [39].

Using this technique, the expression of PD-1 on bacteria-, virus- and tetanus toxoïd-specific CD4 T cells were evaluated by flow cytometry (Fig. 5A and B). For that purpose, PBMCs of CVID patients (N=7) and healthy individuals (N=9) were stimulated for 18h with a pool of bacteria, or adenovirus (Ad5), or tetanus toxoid or PHA (positive control) in the presence of TAPI-0 and anti-TNF-α-APC monoclonal antibody (MAb). Unstimulated cells were used as negative control. Then, PBMCs were stained with monoclonal antibodies directed to PD-1, CD3, CD4 and CD8 and the expression of these molecules as well as of TNF-α was evaluated by flow cytometry (Fig. 5A). We observed that bacteria-specific but not Ad5/tetanus toxoid-

specific CD4 T cells expressed significantly higher levels of PD-1 in CVID patients compared to healthy individuals (P<0.0001) (Fig. 6B).

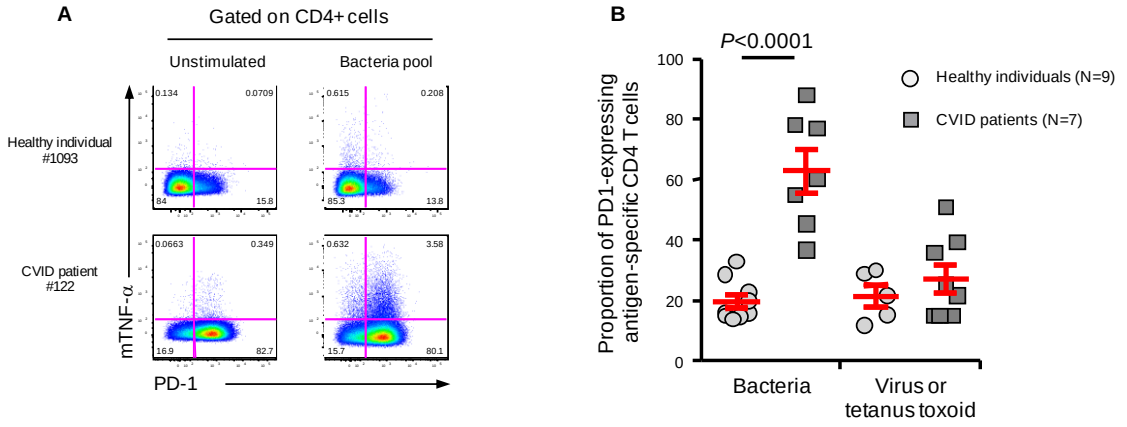


Figure 5. **Bacteria-specific CD4 T cells of CVID patients express high levels of PD-1**. Expression of PD-1 by CD4 T cells were evaluated in healthy individuals (N=9) and CVID patients (N=7) by flow cytometry. Blood mononuclear cells were stimulated during 18h with heat-killed bacteria, Ad5, Tetanus Toxoid or PHA in the presence of TAPI-0 and anti-TNF-α-APC. **(A)** Representative flow cytometry profiles of bacteria-specific CD4 T-cells of one healthy individual (#1093) and in a CVID patient (#7) are shown. **(B)** Proportion of antigen-specific CD4 T cells expressing PD-1. *P* values were derived using Student's t-test.

5. PD-1 blockage restores bacteria-specific CD4 T-cell proliferation of CVID patients

Programmed Death 1 (PD-1) is a T-cell regulator. Upon CD4 T-cell activation, the PD-1 expression is increased in order to negatively regulate immune response when the pathogen is cleared. PD-1 has two ligands, PD-L1 and PD-L2, which differ in localization: PD-L1 is expressed on hematopoietic and non hematopoietic cells, while PD-L2 expression is limited to DCs, macrophages and actif B cells [51]. As described above, PD-1 expression can be related to T-cell activation, T-cell differentiation and T-cell exhaustion [42]. We first showed that PD-1 over expression on CD4 T cells of CVID patients was not related to both T-cell activation and T-cell differentiation (Figure 4). Thus, we investigated the role of PD-1 in T-cell exhaustion. To assess the involvement of PD-1/PD-L1/2 axis in the impairment of bacteria-specific CD4 T-cell proliferation in CVID patients, mononuclear cells from CVID patients (N=6) were labeled with CFSE and stimulated or not with heat-killed bacteria, virus-derived antigens (internal control) or SEB (positive control) in presence or in absence of PD-L1/2. The proliferation levels were measured on $CD3^+CD4^+$ T cells at day 6. Representative flow cytometry profiles (Fig. 6A) and cumulative data (Fig. 6B), showed that bacteria-specific but not virus-specific CD4 T-cell proliferation is restored by the blockade of PD-1/PD-L1/2 axis (P=0.026) (Fig. 6A and B).

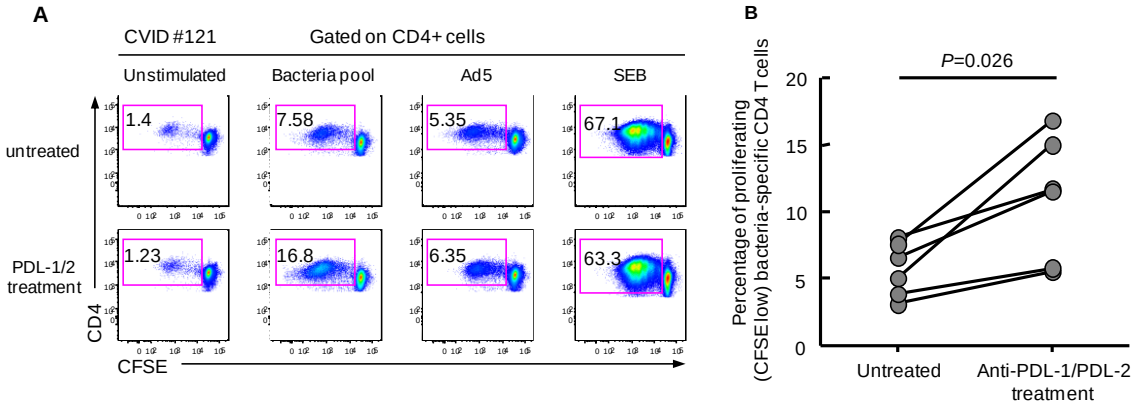


Figure 6. **PD-1 blockage restore CD4 T-cell proliferation of CVID Patients**. Blood mononuclear cells were labelled with CFSE and stimulated with heat-killed bacteria or SEB in presence or in absence of anti-PD-L1/2 MAbs and proliferation was measured on CD4 positive T cells at day 6. Proliferative responses were measured in 6 CVID patients. **(A)** Flow cytometry profiles of bacteria-specific CD4 T-cells proliferation of one representative CVID patient (#4) are shown. Unstimulated cell cultures (negative control) and cell cultures stimulated with SEB (positive control) are also shown. **(B)** Percentage of bacteria-specific CD4 T-cell proliferation in presence or in absence of anti-PD-L1/2 MAbs. *P* values were derived using Student's t-test.

6. Endotoxin levels in CVID patients correlate with IgG levels

We postulated that bacteria-specific CD4 T-cell exhaustion might be due to bacteria antigens persistency. To address this hypothesis, the presence of endotoxins in plasma of CVID patients and healthy individuals was evaluated by an enzymatic reaction based on the amebocyte limulus assay. Briefly, the presence of endotoxins in the plasma cleaves a pro-enzyme (inactive) in an enzyme (active) that converts a chromogenic substrate in para-nitro-aniline detected at 405 nm. The proportion of individuals with endotoxemia and the levels of endotoxin were assessed in CVID patients transcomplemented or not with intravenous IgG (IVIG), subdivided in 3 groups *i.e.* IgG titers < 4.9 mg/mL, between 4.9 and 7 mg/mL and > 7 mg/mL. The proportion of CVID patients harboring detectable levels of entotoxins reached 100%, 50% and 22% for CVID patients with IgG < 4.9 mg/mL, between 4.9 and 7 mg/mL and > 7 mg/mL, respectively. Of note, none of the healthy individuals tested were positive for endotoxin (Fig. 6A). In addition, endotoxin levels in plasma were also dependent on IgG titers (Fig. 6B). Of note, both the proportion and the level of entotoxins were significantly higher in untreated CVID patients than in treated (IgG >7 mg/mL) CVID patients (P=0.0008 and P<0.0001, respectively). Furthermore, the endotoxemia inversely correlated with the concentration of IgG (R=-0.5769, P<0.0001) (Fig. 7C). Finally, we monitored the presence of endotoxin in CVID patients (N=5) before and after IVIG treatment (IgG titers were increasing from <4.9 to >4.9) (Fig. 7D). In all cases, endotoxin levels significantly decreased upon initiation of treatment indicating that immunoglobulin transcomplementation reduces bacterial translocation in CVID patients (P=0.005) (Fig. 7D).

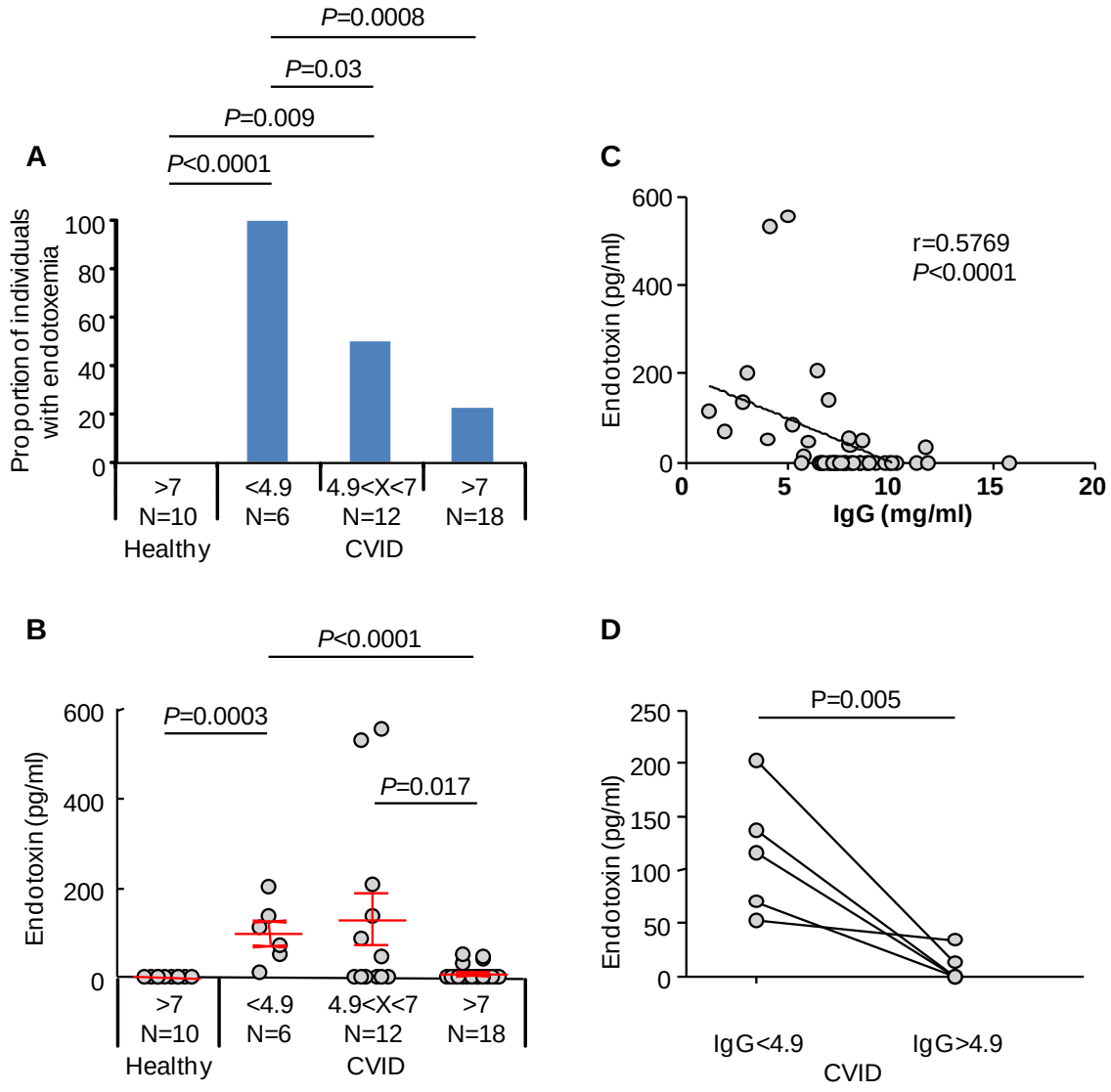


Figure 7. Endotoxin levels in CVID patients correlate with IgG levels. **(A)** Proportion of individuals with endotoxemia. **(B)** Levels of endotoxin in plasma of healthy individuals and CVID patients. **(C)** Endotoxin levels correlate with IgG levels. **(D)** Immunoglobulin transcomplementation reduces endotoxin levels in CVID patients. *P* values were derived from either Chi-square analysis for comparison of positive proportions, or One-way ANOVA (Kruskal-Wallis test) followed by a Student's t-test in the context of multiple comparisons.

V. Discussion

In this study we looked at a complex disease from different angles. We examined our cohorts clinical status and at the same time tried to get a better understanding of underlying molecular mechansims.

Clinically CVID is a heterogenous disease. Symptoms and manifestations vary between individuals and correlations can be established only with great difficulty. One of our aims was to analyze patient's medical histories to compare our findings to current literature. We realised that even in a cohort as small as 12 patients, all known clinical manifestations of the disease, except for lymphoid malignancies, were present. We could confirm that following IVIg treatment, CVID manifestations are usually less frequent, but complications such as auto-immunity, inflammatory disease or gastro-intestinal symptoms may remain [29][52][53].

From a phenotypical point of view all patients harbor an Ig deficiency. At the genetic level in only 15-20% of patients an association with a mono-genetic defect in genes involved in B-cell costimulation or B-cell receptor (BCR) signaling *i.e.* TACI, ICOS, CD19, CD20 or CD81 [17][22][23][24][25] could be identified. For the remaining 80% several mechanisms have been proposed, but no common underlying cause was found so far.

We were able to show other homogenous aspects of CVID. We demonstrated that bacteria-specific but not virus-specific CD4 T cells in CVID patients harbored reduced capacities to produce cytokines (IL-2, IL-17A and IFN-γ) and to proliferate. This loss of CD4 T-cell function might be the consequence of 1) T-cell differentiation to the terminal stage (senescence) or 2) exhaustion of antigen-specific CD4 T cells. Both mechanisms can be caused by a chronic bacteria-derived antigen load, which could be present due to the defect of Ig production or another mechanism, in CVID patients.

We could eliminate 1) T-cell senescence by investigating the level of differentiation (using CCR7 and CD45RA markers) and senescence (using CD57 marker [34]) of CD4 T cells in CVID patients compared to healthy individuals. No differences were observed in 1) the proportion of CD4 T cells in the three populations of antigen-

experienced CD4 T cells *i.e.* central memory, effector memory and terminally differentiated effector memory CD4 T cells, and 2) the proportion of CD4 T cells expressing CD57 (data not shown).

We then assessed the expression of co-inhibitory molecules, classically involved in T-cell exhaustion. This process takes place in order to avoid excessive inflammatory response and the consequent tissue damage. T-cell exhaustion leads to a progressive loss of T-cell functions, such as cytokine production. So far, this mechanism has been observed in chronic viral infections [(54)]. Therefore, we assessed the expression of co-inhibitory receptors associated to T-cell exhaustion *i.e.* PD-1, 2B4, CD160, SLAM, CTLA-4, LAG-3, and TIM-3 and showed that only PD-1 was up-regulated on total CD4 T cells of CVID patients. To determine whether the increase of PD-1 was a general feature of CD4 T cells in CVID patients or if it was linked to specific antigen stimulation, we compared PD-1 expression levels on virus-specific CD4 T cells *versus* bacteria-specific CD4 T cells. We demonstrated that PD-1 was increased preferentially in bacteria-specific CD4 T cells, consistently to the functional defects previously described. These evidences suggest that the increased expression of PD-1 might be induced by chronic exposure to bacterial antigens, leading to functional impairment of bacteria-specific CD4 T cells. The involvement of PD-1 in the impairment of CD4 T cells was further assessed by proliferation assay in presence or in absence of blocking agents of the PD-1/PD-1 ligands pathway. This test demonstrated the involvement of PD-1/PD-1 ligands in the impairment of bacteria-specific CD4 T cells.

Our next step was to determine whether an antigen load was present or not. In the context of chronic viral infections, PD-1 expression levels directly correlate with viral loads [(33)(35)]. We therefore postulated that the levels of PD-1 expression in CVID patients could be correlated to endotoxemia (marker of bacterial translocation). As patients with CVID are unable to protect themselves against extra-cellular bacteria and therefore suffer from chronic bacterial infections, it is possible that bacterial antigens persist, which leads to T-cell exhaustion. In order to confirm our hypothesis, we measured the presence of endotoxins in CVID patients plasma and we showed that 1) the levels of endotoxin inversely correlated with IgG concentration, 2) IVIg

treated CVID patients harbored reduced endotoxemia and 3) IgG concentration exceeding 7 mg/mL strongly reduced both, the proportion of CVID patients with detectable endotoxemia and the concentration of endotoxins in plasma. We showed that transcomplementation with immunoglobulins allows the reduction of bacterial translocation in CVID patients. To further confirm our hypothesis, we are currently longitudinally evaluating PD-1 expression level (on total and bacteria-specific CD4 T cells), in parallel with endotoxemia in plasma of CVID patients treated by IVIG. Preliminary results suggest that in addition to a strong reduction of the endotoxin levels in plasma, PD-1 levels on CD4 T cells decrease over-time under therapy.

Taken together, our observations suggest that primary B-cell defect(s) in CVID patients leads to recurrent bacterial infections. These are associated to an acquired (secondary) impairment of CD4 T cells, which may in turn exacerbate the lack of protection against extracellular bacteria.

All patients share the same basic treatment, which should prevent severe infections and decrease general inflammation leading to a lower morbidity and mortality rate [7][52][53]. The standard dose of IVIg is 400-600 mg/kg body weight per month [7]. At diagnosis, some patients present initial IgG levels of 1 mg/ml, where as others still produce 3-4mg/ml. Unfortunately it is impossible to determine whether these antibodies are functional or not. For this reason as well as for the findings explained above, we would suggest an IgG target level of “initial level +6 mg/ml”, instead of a standard target level of 7mg/ml (valid at the University hospital of Lausanne, Switzerland).

As for the diagnostic approach, different things should be taken into account: when a new diagnosis is made, global Ig levels should be measured, B-and T-cell quantitation should be assessed by flow-cytometry and B-cell subsets should be determined [1]. This would allow to classify patients and to determine their clinical phenotype associated to B- and T-cell specificities. In this case, possible risk factors for later complications would be known from the beginning of treatment. Also, genetic testing for known gene defects could be contributive.

LPS detection in CVID patients is not useful to detect infectious or inflammatory processes. Erythrocyte sedimentation rate and C-reactive-protein are sensitive markers for this purpose. However, these laboratory tests are not specific to indicate bacterial translocation. Therefore, LPS detection can be of value to confirm bacterial translocation to observe specific cellular mechanisms encountered in this condition.

Although up to now it is not possible, it would be of interest to determine whether residual IgG and IgA produced by a patient are functional or not. This would allow to administrate more precise doses of IVIG therapy.

VI. Perspectives

We would like to perform genome wide comparisons between CVID patients, to eventually find other genetic defects. Our hypothesis is that multiple genetic defects lead to similar phenotypes.

We would like to develop a cytofluometric panel to assess B-cell phenotypes as a standard at the University hospital in Lausanne. This panel would allow to further characterize CVID and other disorders, as described in the EUROclass trial (2). One further investigation could target the detection of IgG and IgA in the oro-pharyngeal mucosa in CVID patients versus healthy individuals. Indeed, IgG diffusion through mucosae might depend on the amount of plasmatic IgG. As patients have different levels of IgG, this could allow to determine the minimal IgG level needed to diffuse. Also, plasmatic IgA does not necessarily represent the amount of IgA dimers secreted in the mucosae. Taken together, these additional tests could help to further characterize CVID and contribute to explain different clinical phenotypes.

ID	Date of Birth	Sex	Ig (g/l)							CD3 Normal values Abs (1140-3380)		CD4 Normal values Abs (490-1640)		CD8 Normal values Abs (170-880)		CD19 Normal values Abs (80-490)		Diagnosis	Therapy (IVIg) in (grams / week)	Vaccin respons es	Prediagnostic elements	Postdiagnostic elements	Other known pathologies	Family history
			IgG tot (7-14.5)	IgG1 (5.2-12.7)	IgG2 (1.43-5.6)	IgG3 (0.28-1.05)	IgG4 (0.011-1.04)	IgA (0.71-4.07)	IgM (0.34-2.41)	%	Abs	%	Abs	%	Abs	%	Abs							
#1	05.05.1976	F	1.75	1.26	0.29	0.16	0.006	0.09	0.56	77.5	1518	48	941	28	552	11	221	9.2006	11.2006 (15g/5w)	2006: NR to DiTe; LR to Pvx	1996: PTI; 1999:Br, cSi ; 2004:TE	2010: EE ; 2011: Lp, GI	PHA, severe myopia	paternal Biermer; familial vitiligo
#2	15.12.1962	F	4.97	4.89	0.16	0.17	<0.02	<0.06	0.19	66.2	1159	31.9	559	33.2	581	12.1	221	4.2003	5.2003 (25g/3w)	NT	2001: GI + Di, cSi 2002: Br, Cy	2003: Br; 2010: Si, HSM, Adp	AII; erythrocytary microcytosis	
#3	24.10.1973	M	3.63	4.08	0.5	0.32	<0.01	<0.06	0.66	64.6	2655	37	1463	23.2	917	27.7	1180	12.2004	7.2005 (30g/3w)	NR	cSi child; 1994: Me; 2003: 3 Pn; 2004: Pn	since 2006: Adp p,a + Di, Br; 2010: SLL p, a, cu		
#4	30.05.1971	F	1.34					<0.13	<0.15	70		60		15		10		6.2005	2011 (10g/3w)	NT	2004: rSi, 2 Pn, Ot	2007 to 2009: rSi; 2006: TE SM, Adp a		child with DM1
#5	11.11.1965	F	0.15	0.12	<0.09	0.07	<0.003	<0.05	0.05	84.5	2251	32	793	46	1141	6	175	6.2007	7.2011 (25g/3w)	NR	2006: rSi +SE	rGlp	1990: post-accidental SME; cTc	
#6	30.06.1982	M	3.67	3.72	1.15	0.55	0.1	0.17	0.21	91.4	1378	49.5	746	40	603	4.2	91	12.1996	12.1996 (40g/3w)	NR	rENT since child	1994: TPI; 1996/1998: AIHA; 2001: SM; 2004: Adp p; 2006: Adp p, a cu,b		
#7	19.08.1977	F	1.12	1	0.12	0.29	0.02	<0.06	1.03	70.8	1021	50.1	723	19.6	283	9.3	138	3.2000	12.2002 (25g/3w)	NT	1981:SM, TPI, LP; rENT, rSi, rPn as	2004: TPI; Adp cu; 2005: Adp p; 2007: AIHA; 2008: GI, rCy	CHD, deficiency in CD16 CD56, C3, C4, Treg, CD25, prot S	
#8	04.07.1948	F	<0.5					<0.06	<0.04	21								2.1981	1981 (21g/3w)	2008: PR to DiTe; Pvx NT		1996 to 2003: 22 Si/Cy; 2005: Glp 2005 to 2008: rSi		CRC
#9	09.06.1958	M	0.83	1.88	ND	ND	0.08	<0.1	0.19					25.3	48	1.9		4.1986	12.1987 (30g/3w)	NT	1987: Si + SE, then rSi	2010: Pn A+PJ; rSBP; HSVIO; rENT	PTM; HSM; DM2	paternal DM2
#10	04.10.1949		1.04	1.22	<0.09	0.03	<0.01	0.1	1.63	56.5	1096	36.6	710	21.2	411	12	217	5.2005	6.2005 (30g/4w)	PR to Te, NR to Di/Pvx	1996 and 2005: Pn, rSi	no ENT; 2005: Adp rp, p	presbycusis	
#11	17.01.1992		1.94	1.75	0.1	0.05	0.019	<0.06	0.43	51	1104	23.3	505	24.2	524	15.4	332	2.2001	3.2001	NR	2008 to 2010: rSi, Br, Ast	2011: GI + Di; Adp a		
#12	28.08.1934	F	7.02					1.7	0.66	53.7	877	38.2	624	15.4	252	4.8	78	09.2000	10.2000 (24g/3w)	PR to DiTe	r ENT for 10 years	no more symptoms	moderate OP; primary Raynaud	Sister: OP + HGG

Table 1. Clinical data.

Abbreviations:

ND : not detectable; **PR** : protective response; **NR** : no response; **LR** : low response; **Di** : Diphteria; **Te** : Tetanos; **Pvx** : Pneumovax; **NT** : not tested; **PT** : thrombopenia; **I** : idiopathic; **M** : multifactorial; **Tc** : thrombocytosis; **Si** : sinusitis; **c** : chronic; **r** : repeated; **Pn** : pneumonia; **A** : aspergilloma; **PJ** : *Pneumocystis jirovecii*; **Br** : bronchitis; **GI** : gastro-intestinal symptoms; **Di** : diarrhea; **p** : parasital; **Cy** : cystitis; **Me** : meningitis; **Ot** : otitis; **TE** : tonsillectomia; **SE** : ablation of part of the sinusis; **EE** : ethmoidectomia; **ENT** : ear-nose-throat infections; **SM** : splenomegalia; **SME** : splenectomia; **HSM** : hepato-splenomegalia; **Ast** : Asthma; **Adp** : Adenopathia; **p** : pulmonary; **a** : abdominal; **cu** : cuntaneous; **rp** : retro-peritoneal; **b** : brain; **rSBP** : repeated spontaneous bacterial peritonitis; **HSV** : Herpes simplex virus; **iO** : oesophagal infection; **DM1** : diabetes type; **DM2** : diabetes type 2; **AII** :

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IX. References

1. Park MA, Li JT, Hagan JB, Maddox DE, Abraham RS. Common variable immunodeficiency: a new look at an old disease. Lancet. 2008 Aug 9;372(9637):489–502.

2. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008 Jan 1;111(1):77–85.

3. Warnatz K, Denz A, Dräger R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27+IgM−IgD−) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1;99(5):1544–51.

4. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J. Clin. Immunol. 2003 Sep;23(5):385–400.

5. Sánchez-Ramón S, Radigan L, Yu JE, Bard S, Cunningham-Rundles C. Memory B cells in common variable immunodeficiency: Clinical associations and sex differences. Clin Immunol. 2008 Sep;128(3):314–21.

6. Mouillot G, Carmagnat M, Gérard L, Garnier J-L, Fieschi C, Vince N, et al. B-cell and T-cell phenotypes in CVID patients correlate with the clinical phenotype of the disease. J. Clin. Immunol. 2010 Sep;30(5):746–55.

7. Cunningham-Rundles C. How I treat common variable immune deficiency. Blood. 2010 Jul 8;116(1):7–15.

8. Evidence-Based Clinical Decision Support at the Point of Care | UpToDate [Internet]. [cited 2012 Dec 29]. Available from: http://www.uptodate.com/home

9. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J. Clin. Immunol. 2007 May;27(3):308–16.

10. Oksenhendler E, Gérard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, et al. Infections in 252 patients with common variable immunodeficiency. Clin. Infect. Dis. 2008 May 15;46(10):1547–54.

11. Cerutti A, Cols M, Gentile M, Cassis L, Barra CM, He B, et al. Regulation of mucosal IgA responses: lessons from primary immunodeficiencies. Ann. N. Y. Acad. Sci. 2011 Nov;1238:132–44.

12. Cerutti A, Chen K, Chorny A. Immunoglobulin responses at the mucosal interface. Annu. Rev. Immunol. 2011;29:273–93.

13. Sève P, Bourdillon L, Sarrot-Reynauld F, Ruivard M, Jaussaud R, Bouhour D, et al. Autoimmune hemolytic anemia and common variable immunodeficiency: a case-control study of 18 patients. Medicine (Baltimore). 2008 May;87(3):177–84.

14. Boileau J, Mouillot G, Gérard L, Carmagnat M, Rabian C, Oksenhendler E, et al. Autoimmunity in common variable immunodeficiency: correlation with lymphocyte phenotype in the French DEFI study. J. Autoimmun. 2011 Feb;36(1):25–32.

15. Agarwal S, Cunningham-Rundles C. Autoimmunity in common variable immunodeficiency. Curr Allergy Asthma Rep. 2009 Sep;9(5):347–52.

16. Salzer U, Grimbacher B. Common variable immunodeficiency: The power of co-stimulation. Semin. Immunol. 2006 Dec;18(6):337–46.

17. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin. Immunol. 2009 Nov;133(2):198–207.

18. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012 Feb 16;119(7):1650–7.

19. Mellemkjaer L, Hammarstrom L, Andersen V, Yuen J, Heilmann C, Barington T, et al. Cancer risk among patients with IgA deficiency or common variable immunodeficiency and their relatives: a combined Danish and Swedish study. Clin. Exp. Immunol. 2002 Dec;130(3):495–500.

20. Dhalla F, Da Silva SP, Lucas M, Travis S, Chapel H. Review of gastric cancer risk factors in patients with common variable immunodeficiency disorders, resulting in a proposal for a surveillance programme. Clin. Exp. Immunol. 2011 Jul;165(1):1–7.

21. Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat. Genet. 2005 Aug;37(8):829–34.

22. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat. Immunol. 2003 Mar;4(3):261–8.

23. Van Zelm MC, Reisli I, Van der Burg M, Castaño D, Van Noesel CJM, Van Tol MJD, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. N. Engl. J. Med. 2006 May 4;354(18):1901–12.

24. Kuijpers TW, Bende RJ, Baars PA, Grummels A, Derks IAM, Dolman KM, et al. CD20 deficiency in humans results in impaired T cell-independent antibody responses. J. Clin. Invest. 2010 Jan;120(1):214–22.

25. Van Zelm MC, Smet J, Adams B, Mascart F, Schandené L, Janssen F, et al. CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. J. Clin. Invest. 2010 Apr;120(4):1265–74.

26. Kutukculer N, Gulez N, Karaca NE, Aksu G, Berdeli A. Three Different Classifications, B Lymphocyte Subpopulations, TNFRSF13B (TACI), TNFRSF13C (BAFF-R), TNFSF13 (APRIL) Gene Mutations, CTLA-4 and ICOS Gene Polymorphisms in Turkish Patients with Common Variable Immunodeficiency. J. Clin. Immunol. 2012 Dec;32(6):1165–79.

27. Thiel J, Kimmig L, Salzer U, Grudzien M, Lebrecht D, Hagena T, et al. Genetic CD21 deficiency is associated with hypogammaglobulinemia. J. Allergy Clin. Immunol. 2012 Mar;129(3):801–810.e6.

28. Cunningham-Rundles C, Siegal FP, Smithwick EM, Lion-Boulé A, Cunningham-Rundles S, O'Malley J, et al. Efficacy of intravenous immunoglobulin in primary humoral immunodeficiency disease. Ann. Intern. Med. 1984 Oct;101(4):435–9.

29. Favre O, Leimgruber A, Nicole A, Spertini F. Intravenous immunoglobulin replacement prevents severe and lower respiratory tract infections, but not upper respiratory tract and non-respiratory infections in common variable immune deficiency. Allergy. 2005 Mar;60(3):385–90.

30. Chapel H, Cunningham-Rundles C. Update in understanding common variable immunodeficiency disorders (CVIDs) and the management of patients with these conditions. Br. J. Haematol. 2009 Jun;145(6):709–27.

31. Chua I, Standish R, Lear S, Harbord M, Eren E, Raeiszadeh M, et al. Anti-tumour necrosis factor-alpha therapy for severe enteropathy in patients with common variable immunodeficiency (CVID). Clin. Exp. Immunol. 2007 Nov;150(2):306–11.

32. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006 Feb 9;439(7077):682–7.

33. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006 Sep 21;443(7109):350–4.

34. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. J. Exp. Med. 2006 Oct 2;203(10):2281–92.

35. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nat. Med. 2006 Oct;12(10):1198–202.

36. McMahan RH, Golden-Mason L, Nishimura MI, McMahon BJ, Kemper M, Allen TM, et al. Tim-3 expression on PD-1+ HCV-specific human CTLs is associated with viral persistence, and its blockade restores hepatocyte-directed in vitro cytotoxicity. J. Clin. Invest. 2010 Dec;120(12):4546–57.

37. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry A. 2011 Feb;79(2):167–74.

38. Harari A, Cellerai C, Enders FB, Köstler J, Codarri L, Tapia G, et al. Skewed association of polyfunctional antigen-specific CD8 T cell populations with HLA-B genotype. Proc. Natl. Acad. Sci. U.S.A. 2007 Oct 9;104(41):16233–8.

39. Haney D, Quigley MF, Asher TE, Ambrozak DR, Gostick E, Price DA, et al. Isolation of viable antigen-specific CD8+ T cells based on membrane-bound tumor necrosis factor (TNF)-α expression. J. Immunol. Methods. 2011 Jun 30;369(1-2):33–41.

40. Bonhomme D, Hammarström L, Webster D, Chapel H, Hermine O, Le Deist F, et al. Impaired antibody affinity maturation process characterizes a subset of patients with common variable immunodeficiency. J. Immunol. 2000 Oct 15;165(8):4725–30.

41. Levy Y, Gupta N, Le Deist F, Garcia C, Fischer A, Weill JC, et al. Defect in IgV gene somatic hypermutation in common variable immuno-deficiency syndrome. Proc. Natl. Acad. Sci. U.S.A. 1998 Oct 27;95(22):13135–40.

42. Cellerai C, Perreau M, Rozot V, Enders FB, Pantaleo G, Harari A. Proliferation capacity and cytotoxic activity are mediated by functionally and phenotypically distinct virus-specific CD8 T cells defined by interleukin-7R{alpha} (CD127) and perforin expression. J. Virol. 2010 Apr;84(8):3868–78.

43. Hong R, Agrawal S, Gollapudi S, Gupta S. Impaired pneumovax-23-induced monocyte-derived cytokine production in patients with common variable immunodeficiency. J. Clin. Immunol. 2010 May;30(3):435–41.

44. Jc V der H, Bw S, Jw van der M. Hypogammaglobulinaemia: cumulative experience in 49 patients in a tertiary care institution. The Netherlands journal of medicine. 2002 Apr;60(3):140.

45. Raeiszadeh M, Kopycinski J, Paston SJ, Diss T, Lowdell M, Hardy GAD, et al. The T cell response to persistent herpes virus infections in common variable immunodeficiency. Clin. Exp. Immunol. 2006 Nov;146(2):234–42.

46. Price DA, Asher TE, Wilson NA, Nason MC, Brenchley JM, Metzler IS, et al. Public clonotype usage identifies protective Gag-specific CD8+ T cell responses in SIV infection. J. Exp. Med. 2009 Apr 13;206(4):923–36.

47. Price DA, Brenchley JM, Ruff LE, Betts MR, Hill BJ, Roederer M, et al. Avidity for antigen shapes clonal dominance in CD8+ T cell populations specific for persistent DNA viruses. J. Exp. Med. 2005 Nov 21;202(10):1349–61.

48. Mizoguchi A, Mizoguchi E, Tonegawa S, Bhan AK. Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacterial antigens in TCR alpha mutant mice with inflammatory bowel disease. Int. Immunol. 1996 Sep;8(9):1387–94.

49. Di Tommaso A, Xiang Z, Bugnoli M, Pileri P, Figura N, Bayeli PF, et al. Helicobacter pylori-specific CD4+ T-cell clones from peripheral blood and gastric biopsies. Infect. Immun. 1995 Mar;63(3):1102–6.

50. Kersten CM, McCluskey RT, Shaw Warren H, Kurnick JT. Responses of human T cells to dominant discrete protein antigens of Escherichia coli and Pseudomonas aeruginosa. Scand. J. Immunol. 1994 Aug;40(2):151–7.

51. Ito T, Yang M, Wang Y-H, Lande R, Gregorio J, Perng OA, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J. Exp. Med. 2007 Jan 22;204(1):105–15.

52. Kaveri SV, Maddur MS, Hegde P, Lacroix-Desmazes S, Bayry J. Intravenous immunoglobulins in immunodeficiencies: more than mere replacement therapy. Clin. Exp. Immunol. 2011 Jun;164 Suppl 2:2–5.

53. Salehzadeh M, Aghamohammadi A, Rezaei N. Evaluation of immunoglobulin levels and infection rate in patients with common variable immunodeficiency after immunoglobulin replacement therapy. J Microbiol Immunol Infect. 2010 Feb;43(1):11–7.

54. Viganò S, Perreau M, Pantaleo G, Harari A. Positive and negative regulation of cellular immune responses in physiologic conditions and diseases. Clin. Dev. Immunol. 2012;2012:485781.

Abstract

Common variable immunodeficiency (CVID), is a disease that is characterized by hypogammaglobulinemia as well as a defect in T, B and dendritic cells. This leads to recurrent bacterial infection mainly caused by *Streptococcus pneumoniae, Klebsiella pneumoniae and Haemophilus influenzae*, as well as inflammatory manifestations, *i.e.* granulomateous disease, gastro-intestinal disorders and chronic lung disease. Intravenous Immunoglobulin (IVIg) therapy reduces CVID susceptibility to bacterial infections to some extend. We analyzed clinical aspects of patients from our database.

We recently showed that bacteria-specific CD4 T cells of CVID patients were impaired. We therefor postulated that CVID patients may harbor an acquired T-cell deficiency also called exhaustion.

To test this hypothesis, we performed a comprehensive investigation of the functional profiles of bacteria-specific CD4 T cells isolated from 31 healthy individuals and 30 CVID patients.

In the present study, we demonstrated that bacteria-specific but not virus-specific CD4 T cells in CVID patients harbored reduced proliferation capacity and expressed high level of PD-1. Interestingly, the blockade of PD-1/PD-1 ligands interactions restored partially bacteria but not virus-specific CD4 T-cell proliferation. Finally, we showed that 1) the level of endotoxins inversely correlates with IgG concentration, 2) IVIG treated CVID patients harbored reduced endotoxemia and 3) IgG concentration exceeding 7 mg/mL strongly reduces both the proportion of CVID patients with detectable endotoxemia and the concentration of endotoxins in plasma.

Taken together our observations, suggest that primary B-cell defect(s) in CVID patients leads to recurrent bacterial infections that are associated to an acquired (secondary) impairment of CD4 T cells which may in turn exacerbate the lack of protection against extracellular bacteria.

Key words: CVID, hypogammaglobulinemia, T cells, exhaustion, endotoxemia