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Role of B cells in common variable immune deficiency

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Abstract

Common variable immune deficiency is a heterogeneous immune deficiency characterized by reduced serum immunoglobulins and a lack of antibodies. As the name implies, B-cell defects are variably defective. In particular, peripheral blood isotype-switched CD27⁺ memory B cells are reduced in number and have been the basis of several classification schemes. A lack of these B cells has been associated with selected clinical conditions, including immune cytopenias, splenomegaly, granulomatous disease and lymphadenopathy. Genetic defects in *ICOS*, *CD19* and *TACI* have been described. In addition to defects in the production or survival of memory B cells, in most subjects, B cells have defects in Toll-like receptor signaling.

Keywords

antibody; B cell; CVID; memory B cell; primary immune deficiency

Common variable immune deficiency (CVID) is the most common symptomatic primary immune deficiency, with an estimated prevalence of 1 in 25,000 individuals [1]. The characteristic features of this syndrome include reduced serum IgG, and low IgA and/or IgM levels, coupled with an inability to produce protective antibody, and the exclusion of other causes of hypogammaglobulinemia [2]. As a result of a lack of antibodies, most patients have recurrent infections of the upper and lower respiratory tract, especially episodes of pneumonia, bronchitis and sinusitis. However, other harder to explain noninfectious complications arise, including lymphoid hyperplasia, splenomegaly, autoimmunity and granulomatous disease [3,4]. Approximately 90% of CVID patients have normal numbers of peripheral B lymphocytes (PBLs), suggesting that the defects are in the later stages of B-cell differentiation. Although hypogammaglobulinemia is the hallmark of CVID, a number of T-

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cell, cytokine and dendritic cell defects have also been described. In the past two decades, much of the literature on CVID has described functional assays of *in vitro* immunoglobulin (Ig) production; these showed that while the B cells of some individuals could produce variable amounts of Ig in culture, the B cells of others had lost this capacity. As these cultures are time-consuming, not standardized and the results are based on the activators used, more recently CVID subjects have been classified by the phenotype of unstimulated peripheral blood B cells. Here, we discuss the B-cell defects in CVID and the clinical consequences of these abnormalities, and explore how these studies may lead to a better understanding of this complex immune defect.

B lymphocytes arise from hemopoietic stem cells in the bone marrow. Early progenitor B cells are characterized by progressive variable-diversity-joining recombination, first of the Ig heavy chain, followed by the surrogate light chains, to produce a pre-B-cell receptor (BCR) [5]. B cells are characterized by the surface expression of CD19, among other characteristics. Progression to the immature B cell occurs after a mature light chain combines with a heavy chain to produce IgM, which is expressed on the cell surface as an integral part of the BCR. Immature B cells express surface IgD and IgM and, after activation by antigen in germinal centers of lymphoid tissue, in conjunction with various signals, including interaction of B cell CD40 with CD40 ligand on activated T cells, become mature Ig-secreting B cells [6,7]. Along with somatic hypermutation of Ig V genes and Ig class-switching from IgM to IgG or IgA, B cells complete their maturation to memory B cells and plasma cells. Memory B cells are characterized by the activation of the cell surface marker CD27⁺. Those B cells that bear IgM and IgD on their surface are nonisotype-switched memory B cells, and those that bear surface IgG, IgA or IgE but lack IgM and IgD are called class-switched (or isotype-switched) memory B cells. Memory B cells will persist after antigen encounter for years and can differentiate into antibody-secreting plasma cells upon further challenge with antigen or following selected environmental signals.

CVID B-cell defects & classification schemes

One of the essential issues with regard to CVID B cells is that they do not become fully activated, proliferate normally, nor terminally differentiate into plasma cells. One aspect of poor activation is displayed by the impaired upregulation of CD86. Mice that are CD86 deficient fail to respond to antigen challenge, lacking antibody formation and isotype switching, congruent with defects seen in CVID [8,9]. CVID B cells exhibit increased apoptosis, presumably due to increased expression of CD95 (Fas) and reduced expression of CD38 [10–12]. With enhanced apoptosis, B cells could be unable to complete the maturation and differentiation process needed for B-cell development. Over the past three decades, a number of *in vitro* assays have been used to examine the B-cell defects in CVID. The earliest studies showed that the B cells may be functional in some cases, but that CVID T cells could exert a suppressive influence, which if alleviated could lead to enhanced Ig secretion [13]. Later, CVID B cells cultured with *Staphylococcus aureus* Cowan strain I (SAC) showed that CVID B cells of some patients could also secrete Ig; however, in most cases, only IgM was produced [14]. To examine T-cell activation, CVID B cells have been cultured with CD40 ligand and IL-10, a combination that leads to IgG and IgA production in X-linked hyper IgM syndrome. This was also successful in stimulating B cells of some CVID subjects to produce Ig in culture supernatants, while for others this was unsuccessful [15]. Since activated CVID T cells can be deficient in CD40L expression, the lack of Ig production could be based on insufficient T-cell signaling. Based on these observations, a number of attempts have been made to use *in vitro* analyses to classify patients into groups, hopefully leading to a better understanding of the underlying pathogenesis. In 1990, Bryant *et al.* classified subjects on B-cell Ig production after exposure to anti-IgM or pokeweed mitogen (PWM) [16]. One group of patients had very low B-cell numbers and near complete

lack of Ig production. The second group, termed type A patients, had reduced or normal numbers of B cells but they also failed to produce IgG, IgA and IgM upon *in vitro* stimulation. A third group, termed type B patients, had B cells that only produced IgM in cultures. A fourth group, type C patients, had normal numbers of B cells that differentiated *in vitro* into IgG-, IgA- and IgM-producing plasma cells, but evidently failed to do so *in vivo*. *In vitro* culture systems have not been used in large studies to classify CVID patients because they are labor intensive, the results vary according to the activators used, and clinical correlations have not been readily apparent. However, these efforts have been important in trying to better define this heterogeneous disease into biologically relevant groups.

Examining steps in B-cell maturation

Because the most fundamental defect in CVID is a lack of maturation of B cells into functioning plasma cells, other studies have focused on the ability of CVID B cells to undergo somatic hypermutation of Ig genes, a process that normally occurs in the GCs, leading to the selection of long-lived, high-affinity Ig-secreting B cells. In CVID, Ig-secreting cells often lack somatic hypermutation in both the Ig(V) regions and in the light chains, reflecting blocks to B-cell maturation [17–20]. One of the characteristics of effectively activated normal human B cells that have undergone somatic hypermutation and are capable of generating Igs in response to antigen rechallenge is the presence of surface CD27, a type 1 TNF family member, a marker for memory B cells in humans. The differentiation of naive B cells into memory B cells and plasma cells generally occurs within the GCs in secondary lymphoid organs. Here, antigen-activated naive B cells undergo proliferation, somatic hypermutation of Ig V-region genes, isotype switching from IgM to IgG or IgA, and ultimately differentiation into plasma cells [21,22]. The ligand for CD27, CD70, is expressed on activated T and B cells and, for normal B cells, promotes the differentiation of CD27⁺ cells into plasma cells. While cord blood B cells do not express CD27, with age, more and more B cells become CD27⁺, reflective of a growing number of memory B cells committed to selected antigens. B cells of normal adults are approximately 40% CD27⁺ [23]. The significance of CD27 as a particularly relevant biologic marker was solidified by the finding that CD27⁺, especially IgD⁺CD27⁺, memory B cells are reduced or absent in the blood of patients with hyper-IgM syndromes, immune defects characterized by the lack of isotype switching.

Agematsu *et al.* and Brouet *et al.* showed that CD27⁺ B cells, including IgM⁺CD27⁺ memory cells and IgD⁺CD27⁺ switched memory B cells (which in normal subjects have undergone somatic hypermutation), are also decreased in CVID [24,25]. The same authors also showed the CD27⁺IgD⁺ B cells, which for normal subjects have undergone somatic hypermutation, have not done so in CVID, a situation very similar to the autosomal form of hyper IgM, activation-induced cytidine deaminase (AID) defect. Since differentiation into plasma cells predominantly occurs from CD27⁺ B cells, the loss of such cells in tissues in CVID is not unexpected [26].

Classification schemes based on memory B cells

As the *in vitro* and genetic studies are impractical to use for larger-scale analyses, Warnatz *et al.* proposed a scheme based on the numbers of peripheral blood CD27⁺IgM⁺IgD⁺ switched memory B cells to better characterize patients [27]. In this system, Group I comprised those with switched memory B cells below 0.4% of total PBLs and Group II comprised those with normal numbers of switched memory B cells ($\geq 0.4\%$), suggesting a post-GC defect (Table 1). A further correlation was found between having reduced switched memory B cells and an absence of IgG production *in vitro*, based on assays using SAC and

IL-2. Group I was then subdivided into Ia, with increased numbers of CD19⁺CD21^{low} immature B cells ($\geq 20\%$ of CD19⁺ B cells), and Group Ib, with normal numbers of CD19⁺CD21^{low} immature B cells ($<20\%$ of CD19⁺ B cells). An increased proportion of patients in Group Ia had splenomegaly and autoimmune cytopenia as compared with Groups Ib and II. Increased numbers of CD21^{low} B cells appeared to correlate with autoimmunity in CVID, and expansion of CD38⁺⁺IgM^{high} transitional B cells with lymphoid hypertrophy and deleterious lymphoid infiltrations [25]. An expanded number of CD19⁺CD21^{low} have been found in patients with systemic lupus erythematosus (SLE), although how this population of B cells is related to the autoimmune phenomenon remains to be determined [28]. This expansion of CD19⁺CD21^{low} immature B cells in group Ia CVID patients was also confirmed in subsequent studies [29]. In fact, splenomegaly, lymphadenopathy and immune cytopenias were associated with increased CD19⁺CD21⁺, even without a reduction in switched memory B cells [30].

Piqueras *et al.* then proposed a modified classification system. In this scheme, group MB0 had almost no total CD27⁺ B cells ($<11\%$ of B cells) [31], group MB1 had reduced switched memory B cells ($<8\%$ of B cells) and group MB2 had normal numbers of both memory B cells (Table 1). In this study, there was a significant association between being in group MB0 and splenomegaly, lymphoid proliferation and granulomatous disease, although autoimmune disease was found with equal prevalence in all three groups. There was also no difference in CD19⁺CD21^{low} B cells between the MB0 and MB1 groups, unlike the difference Warnatz observed between Group Ia and Ib. Four patients from the MB0 group were analyzed for defects in somatic hypermutation of the VH genes. In these patients, isotype-switched memory B cells were found to have normal somatic mutations when compared with controls. However, transcripts from the IgM gene within CD27⁺ B cells had reduced mutation rates, signifying a defect in antigen-driven selection rather than a defect in the hypermutation mechanism itself.

In 2008, the multicenter EURO class trial involving 303 CVID patients sought to unify the classification schemes, and incorporate transitional B cells along with CD27⁺ and CD19⁺CD21^{low} B cells into the framework [32]. Patients with more than 1% of peripheral B cells were divided into those with 2% or less class-switched memory B cells and those with more than 2% class-switched memory B cells (Table 1). Each of these groups was further subdivided according to whether they had expansion of CD21^{low} B cells ($>10\%$ of CD19⁺ B cells). Splenomegaly and granulomatous disease were associated with the group with decreased switched memory B cells. Expansion of CD21^{low} B cells ($>10\%$ of CD19⁺ B cells) was also strongly correlated with splenomegaly. The group with more than 1% CD19⁺ B cells and 2% or less class-switched memory B cells was further subdivided based on the expansion of transitional B cells ($\geq 9\%$). This group was found to be more likely to have lymphadenopathy. While the EURO class study picked a level of 2% or less as a point of reference, Sanchez-Ramon *et al.*, using receiver operating characteristic curves to select the optimal cut-off value, found that 0.55% or less isotype-switched memory B cells was an independent predictor of granulomas, autoimmune disease and splenomegaly [33]. In addition, this study showed that females had a significantly higher level of switched memory B cells, as well as IgM⁺ only CD27⁺ B cells, not previously noted in the European studies. Although these classification schemes are based on flow cytometry performed on isolated peripheral blood mononuclear cells (PBMCs), results using whole blood correlate very well with PBMCs in the afore-mentioned classifications, making classification of patients easier and more practical. As CD27⁺IgM⁺ B cells decrease with age, and memory B cells of all kinds increase with age, comparisons with age-related standards will be important [34].

Memory B-cell phenotypes & clinical correlations

While the aforementioned studies use somewhat different values, an overall conclusion is that a deficiency of switched memory B cells and an expansion of CD19⁺CD21^{low} B cells are associated with selected clinical complications, including splenomegaly, autoimmune disease, lymphadenopathy and granulomatous disease [35]. Table 1 also outlines these associations. The most common autoimmune diseases include immune cytopenias, such as immune thrombocytopenic purpura, autoimmune hemolytic anemia and Evan's syndrome. Other autoimmune diseases have also been described in CVID, including SLE, rheumatoid arthritis, Sjogren's syndrome and inflammatory bowel disease, but the relationships to these and B-cell phenotypes have not been clarified.

Using the Paris classification, Detkova *et al.* also noted significantly more gastrointestinal malabsorption in patients in the MB0 group [36]. Although most studies do not find a significant association between memory B cells and initial serum Igs, one small study of 12 CVID patients did demonstrate a correlation between low CD27⁺ B cells and low serum Ig concentrations, and peripheral blood B-cell counts [37]. However, loss of isotype-switched memory B cells (<0.4%) was found to be correlated with poorer antibody production.

Chronic lung disease is one of the most common complications of CVID, occurring in approximately 20% of patients. It is a concerning complication because the current literature is mixed in opinion regarding the utility of intravenous Ig (IVIG) for reversing or slowing its progression. Reduced isotype-switched memory B cells were significantly associated with chronic lung disease (i.e., bronchiectasis, lymphocytic pneumonitis and diminished forced vital capacity) in some studies but not in others [29,36,38,39]. Non-switched IgM memory B cells have also been shown to be associated with chronic lung disease. Both Carsetti *et al.* and Detkova *et al.* have suggested that patients who have significantly lower numbers of IgM⁺CD27⁺ memory B cells develop chronic lung disease [40]. The concentration of IgM antibodies to antipneumococcal polysaccharides appeared lower in patients with reduced IgM memory B cells; these patients also had a higher prevalence of recurrent lower respiratory tract infections (LRTIs), suggesting that IgM memory B cells might play a crucial role in the response against polysaccharide antigens, reflective of antibacterial immunity. This hypothesis was furthered by the finding that while switched memory B cells were reduced in patients with and without recurrent LRTIs, reduced IgM memory B cells were only found in patients with recurrent LRTIs. This implies that defects in B-cell development and a lack of residual IgM antibody may play a significant role in the pathophysiology of chronic lung disease, although other factors such as T-cell defects, unregulated inflammation and/or impaired viral immunity may be involved.

Aside from infections, autoimmunity, granulomatous infiltrations and other inflammatory complications, patients with CVID also have an increased risk of developing malignancies; these are predominantly lymphomas. In a large study of 248 CVID patients, 7.7% of patients developed non-Hodgkin's lymphoma, mostly extranodal [4]. A study in Scandinavia showed four cases in 176 (2.2%) subjects and a more recent study from the European Society for Immune Deficiency (ESID) contained ten out of 334 (3%) subjects [3]. In most cases, these lymphomas are B cell in origin and well differentiated, suggesting a defect in the later stages of maturation. A cohort of patients with mucosa-associated lymphoid tissue (MALT) lymphomas has been identified, suggesting that MALT lymphomas might develop in response to continuous antigen exposure, leading to clonal B-cell proliferation [41]. Whether these subjects can be identified as at risk prior to the development of lymphoma based on their peripheral blood memory B-cell phenotype is unknown. However, the level of serum IgM may be revealing. In the ESID study, a higher serum IgM was correlated with lymphoid hyperplasias and the development of lymphoid malignancy. For each additional 1 g/l of IgM

there was a 16% increase in the odds that the patient would develop polyclonal lymphocytic infiltration, and a 31% increased odds that the patient would have a lymphoid malignancy [3].

Other essential defects of B-cell function

Aside from triggering B cells by the BCR, another method of driving B-cell differentiation involves the Toll-like receptors (TLRs). Prior to the discovery of TLRs, an antisense oligomer to the *rev* gene of HIV-1, specifically the 27-mer phosphorothioate oligodeoxynucleotide, was found to be able to overcome the block in B-cell maturation and stimulate lymphocyte proliferation *in vitro* in eight out of 14 CVID patients [42]. The secreted levels of IgG and IgM from individuals with normal B cells were comparable to those from normal individuals. It is now known that the B-cell activation was due to recognition of the CpG motifs by TLR-9. TLR-9 is an intracellular recognition receptor that detects DNA-containing CpG motifs found on viruses and bacteria. TLR-9 triggering by CpG-DNA activates B cells, upregulates costimulatory molecules, causes secretion of IL-6 and IL-10 and, with or without ligation of the BCR, mediates T-cell-independent isotype switching and specific antibody production [43–49]. CpG-oligodeoxynucleotide with IL-10 activates transcription of AID, C γ 1, C γ 2 and C γ 3 gene transcription, induces IgG class switching and differentiation to antibody-secreting plasma cells. In this process, B cells gain CD27 expression and upregulate levels of TLR-7 and -9, making these cells even more responsive to TLR signals and enabling secondary antibody responses upon antigen rechallenge [23,50,51]. TLR-7, which recognizes ssRNA, can also activate naive human B cells when plasmacytoid dendritic cells or IFN- α are supplied, leading to both differentiation and Ig production [52]. However, B cells of many subjects with CVID have defects in TLR-7 and -9, which potentially defeat the generation of antibody memory. In particular, CVID subjects who have the most severe losses of switched memory B cells appear to be those with the most severe TLR defects. However, B cells of CVID patients have defects in TLR-9 irrespective of a lack of memory B cells, including decreased IFN- α production by plasmacytoid dendritic cells [53]. These defects would theoretically lead to reduced B-cell proliferation, reduced IgG class switching and loss of memory B cells.

Genetic defects in CVID

While the immune defect in 90% of patients with CVID has no known etiology, a small number of genetic defects either lead to or are associated with the CVID phenotype. While efforts are still very much ongoing, the first example of a genetic cause was a recessive mutation in the T-cell activation ligand, inducible costimulator (*ICOS*), found in nine members of one extended kindred [54]. While *ICOS* is normally expressed on activated T cells, the cytokines and cell-adhesion contacts induced by *ICOS* and *ICOS* ligand signaling promote terminal differentiation of B cells into memory B cells and Ig-secreting plasma cells [55]. Patients with *ICOS* deficiency were shown to have reduced B-cell numbers, with a profound reduction of CD27⁺IgM⁺IgD⁺-switched memory B cells and loss of normal germinal centers, while maintaining T-cell proliferation to mitogens and antigens [56]. In contrast to the T-cell defect found leading to a lack of *ICOS* expression in these subjects, homozygous or compound heterozygous mutations found in a few families in *CD19*, a constitutive component of B cells, also lead to hypogammaglobulinemia [57,58]. These mutations impair antigen-dependent signaling of the BCR complex, resulting in reduced numbers of mature B cells and poor responses to antigen stimulation, essentially the phenotype of CVID [59]. More recently, but more complex to understand, are mutations which occur in transmembrane activator and calcium-modulator and cyclophilin ligand inter-actor (*TACI*; TNFRSF13B), a TNF family member located on chromosome 17p. While significantly associated with CVID in approximately 8% of cases, and in some families in an

apparent autosomal-dominant pattern associated with immune deficiency, the same mutations are also commonly found in normal relatives, suggesting that the inheritance is more complex than initially suspected [60–62]. The most common mutations are C104R and A181E; the former extracellular mutation (C104R) leads to a disruption of a region important for binding the ligands, B-cell activating factor (BAFF, also called BLYS) and another soluble ligand, a proliferation inducing ligand (APRIL) [63,64]. Transmembrane (e.g., A181E) or intracytoplasmic *TACI* mutations are presumed to lead to impaired BAFF and APRIL signaling. In transfection studies the heterozygous C104R mutation exerts dominant negative effects [65]. Activation of *TACI* on the B-cell surface normally leads to T-cell isotype switching and independent B-cell responses [66]. In concert with CD40 activation, *TACI* signaling enhances plasma cell differentiation and the production of IgG, IgM and IgA [67–70]. While there does not appear to be a specific peripheral blood B-cell phenotype associated with mutations in *TACI*, several of these mutations are significantly associated with the development of both autoimmunity and lymphoid hyperplasia, two of the most troubling clinical outcomes in CVID [71,72]. Potentially pertinent to the understanding of these clinical problems, the *TACI*^{−/−} mouse, while deficient in the production of antibody to T-cell-independent antigens, has B-cell hyperplasia, splenomegaly, increased Ig production and autoimmunity, and develops B-cell lymphomas, all of which suggest that *TACI* signaling also plays an inhibitory role on B-cell proliferation [73–75].

BAFF itself is a survival factor for B cells, and mutations in the BAFF receptor (BAFF-r), expressed on all peripheral B cells, lead to a decrease in peripheral B cells and an expansion of transitional B cells in the mouse [76]. While defects in BAFF have not been found in CVID, one patient was found to have a mutation in BAFF-r, leading to hypogammaglobulinemia [77]. In mice and possibly in humans, BAFF appears to control the size of the B-cell pool, thus it was of interest to determine if CVID subjects with low B-cell numbers or reduced levels of memory B cells might have increased serum BAFF [78,79]. In fact, high serum levels of both BAFF and APRIL were found in CVID patients, with or without *TACI* mutations (80). These levels, however, were not related to the numbers or phenotypes of peripheral B cells in this group of CVID patients. Although both BAFF and APRIL have been found to be elevated in serum in a number of autoimmune conditions, such as rheumatoid arthritis and SLE, there was no relationship between serum levels of BAFF and APRIL, and autoimmunity, lymphadenopathy or splenomegaly in these subjects with CVID. Thus, the significance of increased BAFF and APRIL in CVID is currently unclear, although immune activation due to low-grade bacterial stimulation would be plausible.

Conclusion

Both intrinsic and extrinsic defects in B-cell differentiation are likely to play a role in the pathophysiology of CVID. As this is a heterogeneous disease, a collection of genetic and potentially also other factors are required for this defect to manifest. Over the last decade, several classification schemes have been proposed; most recently these have been based on clinical phenotypes and/or CD27⁺ isotype-switched memory B cells. As the underlying genetics become more clear, it is likely that further refinement of the classification schemes will evolve, which will better quantify the severity of CVID and predict the disease prognosis.

Key issues

- Most patients with common variable immune deficiency (CVID) have normal numbers of peripheral B cells, suggesting that the defects occur in the later stages of B-cell differentiation.

- B cells in CVID do not become fully activated, do not proliferate normally or terminally differentiate into plasma cells.
- CD27⁺ memory B cells, particularly isotype-switched memory B cells, are reduced in CVID patients.
- Various classification schemes have been developed that center around switched memory B cells, and also note CD19⁺CD21^{low} immature B cells in some.
- Several clinical conditions have been associated with reduced isotype-switched memory B cells, including autoimmunity, splenomegaly, granulomatous disease and lymphadenopathy.
- B cells of CVID patients have defects of Toll-like receptor-7 and -9, which are associated with a loss of switched memory B cells.
- There are a few genetic defects leading to the CVID phenotype, including defects in *ICOS*, *CD19* and *TACI*.

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Table 1

Classification schemes and associated conditions.

Classification	CD27 ⁺ switched memory B cells	CD19 ⁺ CD27 ^{low} immature B cells	Transitional B cells	Autoimmunity [*]	Splenomegaly [*]	Granulomas [*]	Lymph-adenopathy [*]	Ref.
Warnatz <i>et al.</i>	Group Ia	Increased	-	Yes	Yes	-	-	[27]
	Group Ib	Normal	-	No	No	-	-	
	Group II	Normal	-	No	No	-	-	
Piqueras <i>et al.</i>	MB0	No difference MB0/MB1	-	No difference MB0/MB1/MB2	Yes	Yes	Yes	[31]
	MB1	Low	-		Yes	No	No	
	MB2	Normal	-		No	No	No	
EURO class	>1% B cells	≤2.0%	Increased	No	Yes	Yes	Yes	[32]
			Normal	No		No	No	
		>2.0%	Increased	No	Yes	No	No	
Sanchez-Ramon <i>et al.</i>		Normal	-	No	No	No	No	
	≤0.55%	-	-	Yes	Yes	Yes	-	[33]
	>0.55%	-	-	No	No	No	-	

^{*} Represents significant association with a particular subset within a classification.

-: Not mentioned; PBMC: Peripheral blood mononuclear cell.