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Memory B cells in common variable immunodeficiency: Clinical associations and sex differences

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Abstract

Common variable immunodeficiency (CVID) is a heterogeneous syndrome characterized by impaired antibody responses, recurrent infections, inflammatory, autoimmune and malignancy-related conditions. We evaluated the relationship between memory B cell phenotype, sex, age at diagnosis, immunologic and clinical conditions in 105 CVID subjects from one medical center. Reduced numbers of switched memory B cells (cutoff $\leq 0.55\%$ of B cells) were an independent risk factor of granulomas, autoimmune diseases and splenomegaly ($p < 0.001$). Not previously noted, CVID females had significantly more switched memory cells ($p = 0.007$) than males. Splenectomized subjects did not have fewer IgM memory B cells and these numbers were not related to the development of lung disease, as previously proposed. Lower baseline serum IgG was an independent predictor of pneumonia ($p = 0.007$) and severe infections ($p = 0.001$). We conclude that outcomes in CVID depend on an interplay of factors including sex, numbers of switched memory B cells, and baseline serum IgG and IgA levels.

Keywords

CVID; IgM memory B cells; Switched memory B cells; Pneumococcal antibody; Sex differences; IgG; Splenectomy

Introduction

Common variable immunodeficiency (CVID) is a primary immunodeficiency characterized by impaired immunoglobulin production leading to recurrent infections predominantly of the respiratory and gastrointestinal tract. For unclear reasons, the syndrome also incorporates a constellation of autoimmune, inflammatory, and lymphoproliferative manifestations and malignancy [1–3]. From an immunological and clinical perspective, CVID is a highly heterogeneous disease, probably reflecting a diversity of underlying pathogenetic mechanisms. Defects in early and late stages of B cell differentiation to plasma cells [4,5] appear to account for the impaired antibody synthesis, although T cell abnormalities, [6–9] defects of monocytes [10] and myeloid and plasmacytoid dendritic cells [11–13] contribute to immune dysfunction. Mutations of genes involved in B–T cells interactions and B cell homeostasis, encoding

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costimulatory molecules such as the inducible co-stimulator (ICOS) 13 related subjects in one kindred [14,15], BAFF-R in one individual and CD19 in other four individuals with CVID [16,17]. Mutations in transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI) are identified in 7–8% of CVID subjects, but as unaffected relatives can have some of the same mutations, the role of this receptor in this immune deficiency is complex. [18–21]

Several classifications of CVID have been developed in order to define more homogeneous groups and to identify those patients with potentially worse prognosis. Classifications based on *in vitro* functions of B cells are subject to technical difficulties and have unclear clinical relevance [22]. In the last years, several laboratories have proposed classifications of patients based on numbers of memory B and class-switched memory B cells [23,24]. Enumeration of memory cells in CVID has been proposed in both assessment and as prognostic markers in terms of respiratory disease [24,25], splenic enlargement [25,26], autoimmunity [25,27], granulomatous disease [24,25,27] and intestinal involvement [25,26]. The use of these markers was recently amplified in a multi-institutional study which showed that reduced numbers of switched memory B cells were related to splenomegaly and granulomatous disease [28]. Also of particular interest in other studies has been the CD27⁺ IgM⁺ “IgM memory B cell” population, which has been linked to the generation of T cell independent (TI) responses against polysaccharide (Ps) antigens of encapsulated bacteria [29–31]. This cell subset is lacking in hyper-IgM syndrome and may be reduced in asplenic as well as splenectomized patients [32]; this population has been suggested as the circulating counterpart of splenic marginal zone (MZ) B cells [29–31]. Carsetti et al concluded that CVID patients with normal numbers of IgM + memory B cells were less frequently affected by chronic respiratory infections potentially leading to bronchiectasis, than CVID patients with reduced IgM memory B cells [31]. Other authors have not found evidence of such an exclusive ontogeny for IgM memory cells nor for their exclusive role on TI immune responses to Ps antigens and bacterial infections [33]. These latter authors suggest that IgM memory cells, besides having a specific GC-independent ontogeny, share a common pathway of differentiation with conventional B cells, and that they can participate in both TI and T cell dependent (TD) responses [33,34]. Here we examine in a large cohort of CVID patients from one medical center to explore the relationship between memory B cell phenotype, antibody production, immunologic and clinical features.

Materials and methods

Subjects and antibody testing

CVID subjects (62 females, 43 males) of age range 3 to 81 years (mean age, 39.25 ± 17.22) seen between 2003 and 2007 at Mount Sinai Medical Center were studied in an Institutional Review Board approved investigation. All patients fulfilled criteria for CVID based on the ESID/PAGID definition [35,36], which stipulates a marked decrease of IgG (of at least 2 standard deviations (SDs) below the mean for age) and reduced serum IgA and/or IgM, antibody deficiency, age greater than 2 years, and exclusion of other causes of hypogammaglobulinemia. Males with early onset and B cell numbers less than 2% ($n = 6$) or males with preserved or elevated serum IgM ($n = 4$) were examined for mutations in Btk and CD40L. Other potential causes of this phenotype, such as autosomal hyper-IgM syndromes were not specifically excluded. As noted in prior studies, the females were older at the time of diagnosis than males, 35.9 ± 18.1 vs 25.1 ± 15.5 years, respectively ($p = 0.001$). Females were also older at the time of study than males (43.7 ± 16.7 vs 32.8 ± 15.9 , $p = 0.002$). Data on initial serum immune globulins and antibody responses before immunoglobulin replacement to tetanus, diphtheria, measles, mumps and/or pneumococcal vaccine were collected. For protein vaccines, we defined a positive response as at or above the level cited by the laboratory as protective; for IgG pneumococcal polysaccharide vaccine (PPV) responses, a protective

response was defined as $>1.3 \mu\text{g/ml}$ for each serotype as published previously and recently outlined [37,38]. In order to produce uniform criteria for comparison, the values of these responses are given as the ratio between the numbers of serotypes found to be protective/the number of serotypes tested.

Memory B cell phenotype

Blood samples were collected from patients and from 50 healthy adult volunteers and normal blood bank donors (ages 21 to 65), randomly selected and matched for date of sampling and examined for B cell subsets, as previously published [27]. Based on the expression on CD27, IgD and IgM, CD19⁺ B cells were divided in six well-characterized subsets, naive (CD19⁺CD27⁻IgD⁺IgM⁺), IgM memory (CD19⁺CD27⁺IgD⁺IgM⁺), IgD-only (CD19⁺CD27⁺IgD⁺IgM⁻), switched memory (CD19⁺CD27⁺IgD⁻IgM⁻) and IgM-only (CD19⁺CD27⁺IgD⁻IgM⁺) B cells and double negative (DN) (CD19⁺CD27⁻IgD⁻) B cells.

Clinical information

Clinical information was compiled on each CVID subject, including history of pneumonias; chronic lung disease, both obstructive and restrictive patterns, bronchiectasis, pulmonary granulomata or lymphoid interstitial pneumonia; severe infections; autoimmune diseases, in particular autoimmune haemolytic anemia (AIHA), autoimmune thrombocytopenia (ITP), rheumatoid arthritis, pernicious anemia, granulomatous infiltration at any localization; presence of splenomegaly; and splenectomy.

Statistical evaluation

Descriptive data are presented as mean $\pm$ standard deviation (SD). We compared categorical and continuous variables between groups by the chi-square χ^2 or Fisher exact test and Student *t* test or the non-parametric Mann–Whitney *U*-test, respectively, when appropriate. Multiple proportional hazards analysis was performed to determine the independent predictors of any clinical condition studied, after selecting significant variables by univariate analysis, with the inclusion of sex and age as potentially confounding variables. Correlations were assessed using Pearson correlation coefficients. Receiver operating characteristic (ROC) curves were used to select the optimal cutoff values of significant variables for predicting the development of a clinical condition based on the optimum sensitivity and specificity [39]. Data were analyzed with SPSS software (Chicago, Illinois). A *p* value of 0.05 was considered as statistically significant.

Results

Sex differences for memory B cells and immunoglobulins

As previously demonstrated, the CVID subjects examined here had significant lack of isotype switched memory and IgM-only CD27⁺ B cells [23,24]. In contrast, levels of IgM memory B cells (IgM⁺IgD⁺) were actually higher in CVID patients than in controls (Table 1). Indeed, the majority (~85%–90%) of CD27⁺ memory B cells in these subjects were IgM memory B cells since IgD-only, IgM-only and switched memory B cells represented less than 15% of memory B cells. While these results were characteristic of the group as a whole, there were very significant differences between male and female CVID subjects; females had a much greater percentage of switched memory B cells than males ($3.0\pm 4.2\%$ vs $1.1\pm 1.8\%$, $p=0.007$) and also greater numbers of IgM-only B cells ($1.0\pm 1.7\%$ vs $0.3\pm 0.3\%$, $p=0.03$), a difference not investigated previously (Fig. 1A and B). These results were age independent; significant differences in memory B cell numbers were not found for 20 additional male and female controls recruited for additional analysis of sex differences (median percentages of switched memory B cells of 17.90% for control females vs 13.40% in control males, $p=0.3$; median

percentages of IgM-only B cells of 0.55% in these females vs 0.60% in males, $p=0.7$). For CVID subjects, the numbers of switched memory B cells in peripheral blood were correlated with the baseline levels of IgA ($r=0.62$, $p<0.0001$) and IgM ($r=0.23$, $p=0.02$), but not serum IgG. In concordance with this, CVID females had significantly higher levels of both baseline serum IgA ($24.8 \text{ mg/dl} \pm 27.6$ vs $14.0 \text{ mg/dl} \pm 21.6 \text{ mg/dl}$, $p=0.02$) and serum IgM ($43.7 \text{ mg/dl} \pm 49.2$ vs $19.4 \pm 26.7 \text{ mg/dl}$, $p=0.001$) as compared to CVID males (Fig. 1C and D).

Memory B cells and antibody responses

For 59 patients, baseline IgG antibody responses to protein and pneumococcal polysaccharide vaccine (PPV) antigens were available; for this group, the number of serotypes to which an IgG protective PPV response was developed was highly correlated with the number of switched memory B cells ($r=0.49$, $p<0.0001$) which we previously noted in a smaller group of subjects [27]. CVID subjects who had positive responses to both protein and polysaccharide vaccines, had greater numbers of switched memory B cells, higher baseline levels of serum IgG and IgA ($p=0.04$, $p=0.01$ and $p=0.01$, respectively), than those subjects with a protective response to either vaccine or no response to either vaccine ($p=0.04$, $p=0.01$ and $p=0.002$, respectively) (Fig. 2).

Memory B cells, granulomatous disease, autoimmunity, splenomegaly, and splenectomy

CVID subjects with granulomatous disease had both lower numbers of switched memory and IgM-only memory B cells than those without this complication ($n=90$) ($p<0.0001$ and $p=0.04$, respectively) (Table 2). In addition, patients with autoimmune diseases or splenomegaly exhibited lower proportions of switched memory B cells than patients without autoimmune diseases or splenomegaly ($p<0.0001$ and $p=0.001$, respectively) (Table 2).

We then examined the memory B cell phenotype of the CVID population for the probability of association with granuloma, autoimmune disease or splenomegaly. Using ROC curves, the statistically chosen cutoff value for switched memory B cells in our population was 0.55% of B cells, with sensitivity for the presence of granuloma, autoimmune disease or splenomegaly: 86%, 70.0% and 71.4%; and specificity: 60%, 64.0% and 59.5%, respectively. Using this level, a greater numbers of subjects with switched memory B cells $\leq 0.55\%$ developed granulomas (69% vs 36%, $p=0.001$), autoimmune diseases (87% vs 40%, $p=0.001$) and splenomegaly (71% vs 37%, $p=0.004$) than those with $>0.55\%$ switched memory B cells. By multiple regression analysis, switched memory B cells were independently associated with increased risk of all three conditions after adjustment for both sex and age. Patients with switched memory B cells $\leq 0.55\%$ had 10.2-fold (hazard ratio, HR 95% CI: 2.2–6.0) higher risk for having granulomatous disease, 3.3-fold (HR 95% CI: 1.6–7.1) higher for having autoimmune diseases and 4.4-fold (HR 95% CI: 1.7–11.6) higher for having splenomegaly than patients with switched memory $>0.55\%$. As noted before [2,28], the occurrence of granulomas was significantly associated with both autoimmune diseases ($p=0.001$) and splenomegaly ($p<0.0001$). The number of males and females with switched memory $\leq 0.55\%$ was similar (25 versus 24), and thus there were no differences in the incidence of these complications between the sexes. While some authors have proposed that splenectomy leads to a loss of IgM+ memory B cells [32], our splenectomized CVID patients ($n=11$) actually had more IgM memory B cells than either non-splenectomized patients or HC ($43.6\% \pm 33.2$ vs $35.2\% \pm 26.1$ and $22.3\% \pm 14.5$).

Memory B cell phenotype, severe infections and lung disease

A previous study proposed that a lack of IgM memory B cells was correlated with lung disease in CVID [31]. We did not verify this suggestion as none of the memory B cell subsets were correlated with the occurrence of pneumonia or chronic lung disease ($n=27$), (bronchiectasis, lymphoid interstitial infiltrates, or significant lung impairment on pulmonary function testing.). Comparing patients with (55) or without (50) a history of pneumonia, the only significant

difference was lower serum IgG levels in the former ($225.4 \text{ mg/dl} \pm 178.4$ vs $315.6 \text{ mg/dl} \pm 167.9$, $p=0.009$). There was also no relationship between memory B cell subsets and a history of severe infections (meningitis, sepsis, empyema, septic joint) ($n=14$); for this group, the baseline IgG was also significantly lower than for those who had not had such infections ($160.6 \text{ mg/dl} \pm 96.5$ vs $284.1 \text{ mg/dl} \pm 182.8$, $p=0.01$). By multiple regression analysis, serum IgG was an independent risk factor for pneumonia (below the cutoff IgG 270.5 mg/dl , HR of 2.1, 95% CI:1.2–3.8) and severe infections (below the cutoff IgG 206.5 mg/dl , HR of 5.5, 95% CI:1.4–19.6).

Discussion

CVID is the most frequent clinically important primary immunodeficiency in humans, with a prevalence of ~1 in 30,000. Immunoglobulin replacement therapy transformed the clinical course of the disease in reducing infections [40–42] and possibly the occurrence of some autoimmune complications [43], although managing complications based on immune dysregulation remains clinically challenging. The phenotype of peripheral blood memory B cells has been found a useful tool in the classification of patients into clinically and functionally relevant groups; the overall conclusion from these studies is that the lack of isotype switched memory B cells in peripheral blood is clearly associated with problematic clinical complications and outcomes [23–25,28]. Here we show another essential consideration: females with CVID have much larger numbers of both switched memory B cells and IgM-only B cells than males, a difference not previously noted and not examined in the EUROclass study [28]. As the memory B cell phenotype was related to both serum immune globulin levels and antibody production [27], the fact that CVID females had higher baseline levels of serum IgA and IgM than CVID males may be expected. These benefits could potentially explain why CVID females are diagnosed about a decade later than males with CVID [2,28] and may have longer survival [2]. Possibly because baseline serum IgG levels were not higher in females, we did not find that IgG anti-polysaccharide antibody responses in females were higher than in males. Even though CVID females had larger numbers of switched memory cells, the well-known female sex-bias in autoimmune diseases [44] was not observed in this population, nor in other recently analyzed CVID populations [3,28]. The reasons for this sexual dimorphism are unclear, however, the level of serum IgM, a measure of B cell function, has been known for decades to be linked to the numbers of X chromosomes, as determined in a number of studies in which subjects with 1 to 4 X chromosomes were examined [45,46]. The underlying reasons for this remain unknown, although a number of genes on the X chromosome are known to permit or prevent B cell development, CD40L being the best example [47]. In addition, the expression patterns of immune response genes [48], or the role of female sex hormones in immune modulation [49], may account for some of these differences. However, our data point out the necessity of relating data on memory B cells and clinical outcomes, to the sex of the subject; as further work better defines the pathogenesis of CVID, additional aspects of this dimorphism may become evident.

In the recent multi-center EUROclass study on CVID, reduced numbers of switched memory B cells (arbitrarily chosen as 2% or less) were found in subjects with granulomatous disease and splenomegaly, but were not related to the development of autoimmune disease [28]. However, using standard statistical ROC analyses for our population to define the most optimal cutoff level for switched memory B cells (0.55%), we found that numbers of these cells are highly significant and independent predictors for all three conditions. We suggest that this means of determining optimum laboratory measures may better define patient groups and yield additional useful clinical and genetic correlates for this heterogeneous patient population.

IgM memory B cells may produce Ig with specificity for polysaccharide antigens [30]; since B cells of this phenotype are found in marginal zone of the spleen, and after splenectomy are

depleted in peripheral blood [32], the possibility that these cells are the equivalent of TI B1 cells in the mouse has been raised [29]. The lack of such cells was suggested as a reason for severe bacterial infections in splenectomized subjects and neonates [32] and was related in one study to the development of lung disease in CVID [31]. Kruetzman et al [32] reported variable data for 6 (non-splenectomized) CVID patients, 3 had undetectable total memory B cells and IgM memory cells and the 3 others had very high IgM memory B cells (around 90%). Here, we found high IgM memory B cell numbers in CVID subjects as a whole, with females again having more of these cells than males. In addition, splenectomized CVID subjects had even higher levels of these cells than those who had not had this procedure. Since CVID is characterized by impaired B cell isotype switch – and potentially those who required a splenectomy were those who were most impaired in this regard – we theorize that circulating B cells may be blocked at the IgM memory B cell stage in this immune defect, and that cells of this B cell phenotype may not arise in or depend upon the presence of a spleen. No memory B cell subset studied here correlated with either episodes of prior pneumonia or severe infections, as reported by other groups [24,50]. For this cohort, the best indicator for susceptibility to pneumonia and severe infections was a reduced baseline serum IgG.

In conclusion, this study underlines the relevance of switched memory B cells for understanding autoimmune and inflammatory conditions in CVID, but we demonstrate that other factors, especially the sex of the subject and baseline serum IgG levels, are essential for an accurate understanding of the clinical diversity and outcome in this immune defect.

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Figure 1.

Sex-specific differences in CVID. CVID females ($n=62$) had higher numbers of switched memory and IgM-only memory B cells and higher baseline levels of IgA and IgM than males ($n=43$).

Figure 2.

Memory B cells and antibody responses. Box plots showing response to protein and polysaccharide (Ps) antigens and A) switched memory B cells; B) serum IgG; and C) serum IgA levels. Vaccine responses were considered as: patients who do not respond to either protein antigens or Ps antigens, labeled as “No resp protein/Ps” in the figure; patients who respond to either protein antigens or Ps antigens or “Single resp Protein/Ps”, and patients who respond to both protein antigens and Ps antigens, or “Both resp Protein/Ps”. Each box plot represents the median (thick band) and the 25th and 75th centiles. The error bars represent the smallest and largest values that are not outliers.

Table 1

Memory B cells

Group	Controls	CVID
No. of subjects	50	105
B cells %		
CD19 ⁺	9.3±3.0	8.5±8.9
Total memory B cells%		
CD19 ⁺ CD27 ⁺	44.9±16.8	46.9±29.4
Naïve B cells%		
CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁺	32.7±15.2	31.2±24.9
IgM memory%		
CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁺	22.3±14.5	36.0±26.9 [*]
IgD-only%		
CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁺	2.9±2.3	5.9±9.9
Switched memory%		
CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁻	15.1±8.9	2.2±3.5 ^{**}
IgM-only%		
CD19 ⁺ CD27 ⁺ IgM ⁺ IgD ⁻	2.0±2.9	0.7±1.4

Data are presented as mean±SD. Statistical differences between any group of CVID patients and healthy controls using Student *t* test:

*
p<0.05;

**
p<0.01.

Table 2
Age, gender, memory B cell phenotype and clinical parameters

	No granuloma (n=90)	Granuloma (n=15)	P value	No autoimmune disease (n=72)	Autoimmune disease (n=33)	P value	No Splenomegaly (n=84)	Splenomegaly (n=21)	P value
Diagnosis	32.1±17.9	28.1±17.5	0.5	32.8±19.1	28.7±14.6	0.4	31.3±19.0	32.4±12.4	0.5
Study (yrs)	39.3±17.6	38.6±15.2	0.9	40.5±18.4	36.4±14.0	0.2	38.7±18.5	41.1±10.7	0.5
Number	52 (83.9)	10 (16.1)	0.5	44 (71.0)	18 (29.0)	0.5	50 (80.6)	12 (19.4)	0.8
Number (%)	38 (88.4)	5 (11.6)	0.5	28 (65.1)	15 (34.9)	0.5	34 (79.1)	9 (20.9)	0.8
%	8.9±0	6.2±8.5	0.08	7.4±7.1	10.8±11.8	0.3	8.7±9.2	7.8±8.0	0.7
and memory%	2.6±0.7	0.2±0.3	<0.0001	2.9±4.0	0.7±1.3	<0.0001	2.7±3.8	0.4±0.5	0.001
%	0.8±0.4	0.2±0.4	0.04	0.8±1.6	0.4±0.7	0.6	0.7±1.3	0.6±1.7	0.2

given as the mean±SD.

(%).