Characterization of Lymphocyte Subsets in Patients with Common Variable Immunodeficiency Reveals Subsets of Naive Human B Cells Marked by CD24 Expression

Marcela Vlková,* Eva Froňková,†‡ Veronika Kanderová,†‡ Aleš Janda,† Šárka Růžičková,§ Jiří Litzman,* Anna Šedivá,* and Tomáš Kalina†‡

Increased proportions of naive B cell subset and B cells defined as CD27negCD21negCD38neg are frequently found in patients with common variable immunodeficiency (CVID) syndrome. Current methods of polychromatic flow cytometry and PCR-based detection of κ deletion excision circles allow for fine definitions and replication history mapping of infrequent B cell subsets. We have analyzed B cells from 48 patients with CVID and 49 healthy controls to examine phenotype, frequency, and proliferation history of naive B cell subsets. Consistent with previous studies, we have described two groups of patients with normal (CVID-21norm) or increased (CVID-21lo) proportions of CD27negCD21negCD38neg B cells. Upon further analyses, we found two discrete subpopulations of this subset based on the expression of CD24. The B cell subsets showed a markedly increased proliferation in CVID-21lo patients as compared with healthy controls, suggesting developmental arrest rather than increased bone marrow output. Furthermore, when we analyzed CD21pos naive B cells, we found two different subpopulations based on IgM and CD24 expression. They correspond to follicular (FO) I and FO II cells previously described in mice. FO I subset is significantly underrepresented in CVID-21lo patients. A comparison of the replication history of naive B cell subsets in CVID patients and healthy controls implies refined naive B cell developmental scheme, in which human transitional B cells develop into FO II and FO I. We propose that the CD27negCD21negCD38neg B cells increased in some of the CVID patients originate from the two FO subsets after loss of CD21 expression. The Journal of Immunology, 2010, 185: 6431–6438.

Common variable immunodeficiency (CVID) is a clinically heterogeneous group of primary Ab immunodeficiency diseases with an estimated prevalence of 1 in 30,000 with unclear etiology (1). It is characterized by decreased serum levels of IgG, IgA, and occasionally IgM and impaired Ab response to Ag stimulation and/or vaccination as well; it is usually accompanied by recurrent bacterial infections. Splenomegaly, granuloma formation, autoimmune disorders, and increased risk of malignancy are common complications (1, 2).

Although various abnormalities in number and function of T cells (3–7), B cells (8–13), and dendritic cells (14–16) have been described, the immunopathological mechanism leading to disturbed Ab production in CVID patients has not yet been defined.

The online version of this article contains supplemental material.

Abbreviations used in this paper: CVID, common variable immunodeficiency; FC, flow cytometry; FO, follicular; GC, germinal center; KREC, κ-deleting recombination excision circle; MFI, mean fluorescence intensity.

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Previous studies have shown abnormalities in B cell subset composition—diminution in memory B cells and increase in naive follicular (FO) B cells (5). Naive FO B cells IgMposCD24neg (17, 18) constitute a major B cell subset in the peripheral blood of patients with CVID. In some CVID patients, increased numbers of transitional B cells and CD21neg B cells were observed (11, 19, 20). The CD21neg B cell subpopulation was originally described as CD19highCD21neg B cells and has since developed into the present characterization CD21negCD38negCD19highCD27neg to distinguish this population from other CD21neg B cell populations: CD21neg plasmablast, CD21neg transitional B cells. Expansion of the CD21neg B cell subset has been found in patients suffering from systemic lupus erythematosus or HIV infection (21, 22). In patients with systemic lupus erythematosus, the CD21neg subset represents activated B cells, as these cells express activation markers CD86 and CD95 (21, 23). In CVID patients, CD21neg B cells were shown to have increased expression of these activation markers as well as low expression of CD24. Although CD21neg B cells were found among CD27neg naive B cells in CVID patients, recent results showed that CD21neg B cells in CVID patients were preactivated, polyclonal, partially autoreactive B cells that home to peripheral tissues (23).

The heterogeneity of naive CD27neg B cell subpopulations has not been thoroughly studied. The CD21neg B cell subpopulation forms <10% of all B cells in healthy controls (5, 20). The CD21pos naive CD27neg B cell subset can be further divided into two groups, as follows: 1) transitional immature B cells (IgMposCD24highCD38high) making up ~3% of B cells (24), and 2) the remaining CD27neg B cells that are comprised of mature IgMposCD24pos CD38posCD27neg FO B cells, which reside in the spleen and lymph nodes and also circulate through the body. FO B cells are primed by T cells to generate class-switched memory and plasma cells producing high-affinity Abs. Two FO B cell recirculating popu...
FIGURE 1. Scheme for gating B cell subsets. A, Gating of B cells is shown for a representative healthy donor. Lymphocytes were gated on forward and side light scatter and then on CD19 positivity, after which the remaining cells were divided into CD21pos and CD21neg subsets. Furthermore, naive B cells were defined as IgMpos and CD27neg. The CD21posIgMposCD27neg cells were subdivided into transitional and naive subsets. The CD21negIgMposCD27neg subset was increased in some CVID patients. The expression of IgM and CD24 defined two subsets of naive CD21pos (B) and CD21neg (C) B cells. Both subsets were observed in a CVID patient and in a healthy control. Overlays of CD19 histograms show differences between CD21negCD24neg (black) and CD21negCD24pos (gray) cells, whereas no difference was found between CD21posCD24neg (black) and CD21posCD24pos (gray) cells. D, Bar graph presenting the frequency of CD21posCD24neg/CD21negCD24pos cells in individual patients and controls.
CD21neg B cells) patients, as defined by Wehr et al. (20), whereas the CVID-21lo group comprises smB+21norm (normal number of switched memory B cells) and smB+21low (decreased number of switched memory B cells) patients. To assess the developmental relationships between subsets identified by FC, we performed a replication history analysis in these cells by means of k-deleting recombination excision circles (KREC) (27). We describe two phenotypically distinct subpopulations of CD21neg B cells that are expanded in CVID patients, but can be also identified in healthy controls. Furthermore, we identified cells in human peripheral blood that correspond to mouse FO I and FO II B cells. The frequencies, replication history, and mutual proportions of these populations differ between healthy controls and CVID patients. Based on these findings, we propose a revised developmental scheme of human naïve B cell development.

**Materials and Methods**

**Patients and healthy donors**

We examined 48 CVID patients (25 females, 23 males; mean of age 43.4 ± 15.3 y, range 12–74 y) and 49 healthy donors (26 females, 23 males; mean of age 42.6 ± 13.6 y, range 20–78 y). In all CVID patients, B cells constituted >1% lymphocytes in the peripheral blood. The presence of polymorphisms in the TNFR family member transmembrane activator and cyclophilin ligand interactor gene (TNFRSF13B) was analyzed in all patients. The p.C104R polymorphism was found in two patients, and the p.C104S polymorphism was found in one patient.

**Table I.** Characterization of frequencies of basic B lymphocyte subpopulations and expression levels of CD19 for each examined group of CVID patients and healthy controls

| Subpopulations of Naive B cells | Healthy Controls (n = 49) | CVID-21lo Patients (n = 24) | CVID-21norm Patients (n = 24) |
|---------------------------------|--------------------------|-----------------------------|-----------------------------|
| Percentage from CD19pos B cells  |                          |                             |                             |
| IgMposCD27int                   | 44.1 ± 11.1              | 75.0 ± 15.1*                | 71.1 ± 13.9*                |
| CD21negCD27neg                  | 39.9 ± 10.7              | 28.8 ± 13.0***              | 59.8 ± 12.4*                |
| CD21posCD38negCD24pos CD21negCD27neg | 3.8 ± 2.6              | 27.0 ± 15.8***              | 3.7 ± 2.3                   |
| Transitional B cells            | 1.5 ± 1.1                | 5.6 ± 6.8                   | 7.5 ± 7.5*                  |
| Expression level of CD19 (MFI)  |                          |                             |                             |
| CD21negCD27neg                  | 557.1 ± 59.5             | 583.2 ± 119.7               | 571.1 ± 57.2                |
| CD21negCD38negCD24pos          | 554.4 ± 119.9            | 676.9 ± 205.1***            | 649.8 ± 118.6***            |
| CD21negCD38negCD24pos*          | 828.6 ± 186.0*           | 975.5 ± 293.3***            | 1011.6 ± 190.6***           |

Percentage from CD19pos B cells: Results are expressed as mean ± SD. Statistically different from healthy controls (*p < 0.00001; **p < 0.05). Statistically different from CVID-21norm patients (**p < 0.00003). Expression level of CD19 (MFI): Results are expressed as mean ± SD. Statistically different from healthy controls (**p < 0.0008).

MFI, mean fluorescence intensity.

FIGURE 2. The frequencies (A) and replication history (B) of naïve B cell subpopulations for each examined group of CVID patients and healthy donors. Transitional B cells, IgMposCD27intCD24int, CD21negCD27neg, CD21negCD24neg, CD21negCD38negCD24neg, CD21posCD38posCD24pos B cells: CD21negCD24neg, IgMposCD27intCD24posCD24neg B cells. The number of cell divisions is expressed relative to the transitional B cell value in a healthy control sample (set as 0). Results are expressed as the median, the 25th–75th percentiles, and the outlier range. ○, Outlier values; *, extreme values.
p.I87N polymorphism was found in one patient; all three of the identified polymorphic genes were heterozygous. In 15 patients, ICOS gene mutations were analyzed with negative results.

The patients were on regular i.v. or s.c. Ig substitution. For patients who received i.v. Ig treatment, blood samples were always collected before the treatment. Informed consent was approved by the Medical Ethics Committee of St. Anne’s Faculty Hospital and was obtained before blood sampling.

CVID patients were divided into two groups based on the expression patterns of CD21 and CD38 on the patients’ B cells. Twenty-four patients fell into the CVID-21lo group, which was characterized by an increased proportion of B cells with low expression of CD21 and CD38 (>10%). The remaining 24 patients were placed in the CVID-21norm group, which was characterized by a normal frequency (<10%) of CD21<sub>low</sub>CD38<sub>low</sub> B cells.

The raw FC data from these patients and the control cohort were used in a previously published technical study describing probability binning algorithms for the evaluation of multicolor FC data (28). FC data analysis was performed using the FlowJo 7.5 or 8.8.7 software (Tree Star, Ashland, OR).

**B cell staining and FC**

PBMCs were isolated from 10 ml EDTA blood by density gradient centrifugation using a Ficoll-Paque (GE Healthcare Bio-Sciences, Uppsala, Sweden), and the isolated PBMCs were washed twice with PBS that was supplemented with 1% BSA. The PBMCs were incubated for 15 min in the dark with anti-CD27 Pacific Blue, anti-CD38 Alexa-Fluor 700 (Exbio Praha a.s., Prague, Czech Republic), anti-human IgM FITC or anti-human IgM biotin (BD Pharmingen, San Jose, CA), followed by streptavidin-Qdot 605 (Invitrogen, Carlsbad, CA), anti-CD21 allophycocyanin (BD Pharmingen), anti-CD24 PE, and anti-CD19 PC7 (ImmunoTech, Marseille, France). After a final wash with PBS, two million PBMCs from each sample were acquired using a Cyan ADP flow cytometer (Dako, Glostrup, Denmark), as described in Kalina et al. (28).

**B cell response to a TLR9 agonist or a BCR agonist**

B lymphocytes were isolated from peripheral blood using the RosetteSep Human B Cell Enrichment Cocktail (StemCell Technologies, Rockford, IL) and Ficoll-Paque PLUS (GE Healthcare Bio-Sciences) density gradient centrifugation. Cells were stimulated with the TLR9 agonist CpG oligodeoxynucleotide 2006 at 2.5 µg/ml (Imgenex, San Diego, CA) with recombinant protein L at 10 µg/ml (Thermo Fisher Scientific, Rockford, IL) or with a negative control in RPMI 1640 medium that contained L-glutamine and 25 mM HEPES (Lonza, Basel, Switzerland) and supplemented with 10% heat-inactivated FBS (HyClone, South Logan, UT), 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml Fungizone (Lonza Walkersville), and 10 ng/ml human rIL-4 (R&D Systems, Minneapolis, MN) at a starting concentration of 5 × 10<sup>6</sup> cells/ml.

**FIGURE 3.** Responses of FO B cells to BCR and TLR9 agonists reveal activated FO I and FO II phenotypes. Enriched B cells were stimulated in vitro with a BCR agonist (protein L) or a TLR9 agonist (CpG oligodeoxynucleotide 2006). A. Activation of B cells led either to a dominant FO II phenotype (IgM high) or to a combined FO II/FO I phenotype (IgM high and low) after 24 h. B. Proliferation was more pronounced in TLR9 agonist-stimulated B cells and was restricted to cells with the FO II phenotype after 3 d. C. Proportion of activated FO I or FO II cells in samples from three healthy donors in the presence of IL-4. No stimulation (CTRL), BCR agonist stimulation (BCR), or TLR9 agonist stimulation (TLR9).
Proliferation was detected with anti-Ki-67 PE (BD Biosciences, San Jose, CA) after permeabilization with BD FACS Lysing Solution and BD FACSCalibur Permeabilizing Solution 2, according to the manufacturer's protocol (BD Biosciences), 3 d after in vitro stimulation.

The apoptosis after stimulation was assessed using annexin V Dy647 (ExBio Praha a.s.), and cell activation was detected by anti-CD69 PerCP-Cy5.5 (clone FN50; BioLegend, San Diego, CA).

Investigation of B cell replication history

B cell subsets were purified from the blood samples of 16 CVID patients (8 CVID-21lo, 8 CVID-21norm). To ensure that we had sufficient numbers of cells belonging to subpopulations that were not expanded in healthy controls, we used cells that were sorted from 11 buffy coats (instead of peripheral blood) as control subpopulations. The cells were sorted using a FACSAria flow cytometer (BD Biosciences) using the same set of mAbs that were described above. DNA from the sorted cell populations was extracted using the QIAamp DNA Blood Micro Kit (Qiagen, Hilden, Germany). We used the KREC detection method that was described previously (27) with in-house modifications, as follows. Unlike the method that was described by van Zelm et al. (27), we used a 8 cycle threshold method with a calibration sample consisting of sorted transitional B cells from a healthy donor that was split into two quantitative PCR reactions. The normalized 8 cycle threshold (KREC) values indicated the number of cell divisions in a given subpopulation in relation to the calibration sample. If the KREC amplification produced no signal within the sensitivity limit of the quantitative PCR reaction (e.g., the KRECs in a given sample were too dilute), we used the lowest possible number of cell divisions that was calculated using the number of intronRSS-KDE rearrangements in the sample.

Statistical analysis

The numerical differences between the subgroups were evaluated using the nonparametric Mann-Whitney U test and Wilcoxon test. In all statistical analyses, p < 0.05 was considered statistically significant. Student’s t test and Fisher’s exact test were used when appropriate. Statistical software STATISTICA (StatSoft, Tulsa, OK) version 7 was used.

Results

Clinical characteristics of the CVID patient groups

The following parameters were assessed for both patient groups: age, age of first symptoms, duration of the disease, presence of bronchiectasis, obstructive lung disease, chronic diarrhea, splenomegaly, lymphadenopathy, granulomatous disease, levels of Igs at the time of diagnosis, and presence of TNFRSF13B polymorphisms (Supplemental Table I). The only significant differences that we found were between the age of the patients (CVID-21lo: mean = 52.5 y, SD = 11.5; CVID-21norm: mean = 35.5 y, SD = 13.9, p < 0.001), the age of the first symptoms (CVID-21lo: mean = 33.5 y, SD = 10.8; CVID-21norm: mean = 18.5 y, SD = 15.5, p < 0.01), and the presence of bronchiectasis (CVID-21lo: 12 patients [52%]; CVID-21norm: 6 patients [26%], p < 0.05). No other differences were found.

The CD27negCD21posCD38neg B cell subset is composed of two cell types that are defined by CD24 expression

For cytometric analysis, all B cells were divided into naive and memory B cell populations, based on the expression of CD27 (Fig. 1A). We confirmed the previously described increased frequency of naive IgMposCD27neg B cells that was observed in subgroups of CVID patients (Fig. 1C, Table I). In CVID-21lo patients, the total B cell population contained an increased percentage of CD21posCD38pos B cells, which was expected, given that this patient group was defined by this characteristic (Table I).

Unlike other groups (23), we found that the CD27negCD21negCD38neg B cell subset is composed of two cell types that are defined by CD24 expression: CD27negCD21negCD38negIgMposCD24neg cells (referred to in this study as CD21negCD24neg cells) and CD27negCD21negCD38neggMposCD24pos cells (Fig. 1C). Both of these cell types contributed to the increased frequency of CD27negCD21negCD38neg B cells, but their relative contributions varied among patients (Fig. 1D).

Previous studies found elevated expression levels of CD19 in the CD21pos naive B cell subset, compared with total B cells (11, 19, 20, 23). Our findings show that this elevation was restricted to the CD21posCD24neg subset in both healthy controls and CVID patients, although the elevation was more prominent in CVID patients (Fig. 1C, Table I).

Although CVID-21lo patients displayed relatively increased frequencies of naive IgMposCD27neg B cells, the increase was caused primarily by higher numbers of CD21negCD38neg B cells. The frequency of naive B cells with the typical CD21pos phenotype was actually decreased in CVID-21lo patients compared with the frequency of naive CD21pos B cells in healthy controls, whereas naive CD21pos B cells were significantly increased in CVID-21norm patients (Table I). The proportion of transitional B cells was higher in both groups of CVID patients compared with healthy controls (Fig. 2A), as already reported (20).

Replication history of the naive B cell subset (excluding transitional B cells) showed a higher number of cell divisions in CVID patients

The analysis of KRECs in transitional B cells showed that the number of cell divisions in both of the CVID subgroups was similar to that in healthy donors. However, in CVID-21lo patients, the CD21posCD24neg subset was found to undergo a higher number of divisions than in controls (Fig. 2B). The same pattern (although not significant) was found for the CD21negCD24pos B cell subset.

Subsets of naive B cells corresponding to mouse FO I and FO II were found in humans and were underrepresented in CVID-21lo patients

Two FO B cell populations, CD21negIgMlowIgDhighCD24pos and CD21negIgMhighIgDhighCD24pos, that were designated FO I and FO II were identified in healthy controls, although they were found in only a minority of patients in CVID-21lo patients. Unlike other groups (23), we found that the CD27negCD21negCD38neg B cell subset is composed of two cell types that are defined by CD24 expression: CD27negCD21negCD38negIgMposCD24neg cells (referred to in this study as CD21negCD24neg cells) and CD27negCD21negCD38neggMposCD24pos cells (Fig. 1C). Both of these cell types contributed to the increased frequency of CD27negCD21negCD38neg B cells, but their relative contributions varied among patients (Fig. 1D).
II, respectively, were recently described in mice. Cariappa et al. (25) distinguished mouse FO II cells from FO I cells according to the enhanced expression of IgM in FO II B cells. Both subpopulations showed increased expression of CD24. When we analyzed CD21^{hi}CD27^{neg}CD38^{int} B cells, we found two subpopulations of cells: IgM^{hi}CD21^{hi}CD27^{neg}CD38^{int}CD24^{pos} and IgM^{int}CD21^{pos}CD27^{neg}CD38^{int}CD24^{int}. We observed both subpopulations in human healthy donors and in CVID patients. The FO I/FO II difference was marked by enhanced CD24 expression on FO II-like cells in CVID patients, whereas in healthy individuals, the CD24 expression was only moderately increased on FO II cells (Fig. 1B). In mice, the distinction between FO subsets from marginal zone B cells is possible because of the enhanced expression of CD21 on marginal zone B cell subsets. Both subpopulations that we found in human peripheral blood had identical CD21 expression levels. The expression of CD21 was higher than it was in marginal zone-like B cells (IgM^{pos}CD27^{pos}CD38^{neg}CD24^{high}) (Supplemental Fig. 1). Because we observed two subpopulations with uniform expression of CD21, we assumed that the IgM^{hi}CD27^{neg}CD38^{int}CD24^{pos} subset corresponds to FO II B cells and that the IgM^{int}CD27^{neg}CD38^{int}CD24^{int} subset corresponds to FO I B cells.

**Different responses to activation stimuli by FO I and FO II cells in human B cells**

The mouse model that was reported by Cariappa et al. (25, 26) suggests that FO I B cells develop from FO II B cells after strong BCR stimulation, and that FO II B cells can give rise to MZ-like cells. To support the phenotype distinction between FO I and FO II B cells in human B cells using functional data, we stimulated human B cells from healthy donors in vitro using a TLR9 agonist and a BCR agonist. We chose the *Peptostreptococcus magnus* protein L superantigen that binds to conserved κ L chains (29) as the BCR agonist because anti-IgM stimulation does not allow for a surface IgM-based definition of the responding B cells. The FO I and FO II definition was based solely on surface IgM in this experiment because CD24 is rapidly downregulated after activation by BCRs (30).

Both agonists triggered cell activation leading to CD69 upregulation and cell proliferation (Fig. 3A, 3B), whereas apoptosis was always below 9% and did not differ between stimulated or control cells at 2 h, 24 h, 48 h, or 3 d after stimulation (Supplemental Fig. 2). In line with the data for the mouse model, the BCR agonist led to the activation of ∼55% of the naive B cells (corresponding to the proportion of κ L chain-positive B cells) that had acquired a predominantly FO I phenotype (Fig. 3A, 3C). In contrast, the TLR9 agonist activated all naive B cells and forced ∼40% of cells to proliferate by day 3 after stimulation. Of note, all proliferating B cells displayed the FO II phenotype (Fig. 3B), which is in line with the lack of cell divisions that was documented in the FO I compartment ex vivo (Fig. 3C).

**The FO I compartment is disrupted in CVID patients**

The naive CD21^{pos} B cell compartment is composed of FO I, FO II, and transitional B cells. The *CVID-21lo* patients had an...
increased percentage of CD21pos B cells by definition, and thus had reciprocally decreased percentages of CD21neg B cells. We also found an elevated frequency of transitional B cells, but the frequency of FO II cells was similar to that in healthy controls. Fig. 4A shows that the FO I subset of cells formed a significantly lower proportion of B cells in CVID-21lo patients than in healthy donors. Thus, the decrease of FO I B cells accounts for the diminution of CD21pos B cells in CVID-21lo patients.

CVID-21norm patients presented with a higher frequency of CD21pos B cells, and both of the FO subsets were relatively increased (Fig. 4A).

The number of cell divisions for FO I and FO II cells in healthy controls was similar to those of transitional B cells (Fig. 4B). However, in CVID-21norm patients, the FO I and FO II cells divided more often than the FO B cells from healthy controls. FO II B cells also divided more often in CVID-21lo patients.

Discussion
Based on the combined analyses of healthy donors and CVID patients, we identified new subsets of human naive B cells that are defined by IgM and CD24 expression. First, we found subsets corresponding to mouse FO I and FO II cells (25, 31) in both healthy donors and CVID patients. Second, when analyzing the expanded CD27negCD21negCD38neg B cell subset in CVID-21lo patients, we were able to distinguish between two distinct populations, based on CD24 expression. A detailed analysis of peripheral blood using sensitive six-color FC also revealed that both CD21negCD24pos and CD21negCD24neg subsets were present in healthy donors, although they were present in low numbers. This finding was in contrast to a previous study by Rahmanov et al. (23), which showed that the entire CD21neg population was also CD24neg. This was presumably caused by the fact that in that study, CD21neg cells were gated as CD19high, whereas we observed that the expression of CD19 in CD21negCD24pos B cells was similar to that of naive and memory B cells, that is, lower than in CD21negCD24neg B cells. CD24 represents the first pan-B cell molecule, and this GPI-anchored protein belongs to the heat-stable protein family. CD24 is downregulated after BCR activation, but its complete physiological function in B cells is unknown (32). Further studies will be needed to determine whether this molecule plays an essential role in naïve B cell homeostasis. Currently, CD24 is used in FC as a marker of the different stages of naïve B cell development.

Low numbers of cell divisions in the FO II (IgMhighCD24pos) and FO I (IgMintCD24int) subpopulations from healthy control individuals indicate that these cells mature from transitional B cells in the absence of proliferation, whereas the CD21negCD24pos and CD21negCD24neg subsets showed a higher number of cell divisions (Fig. 5). The median numbers of divisions in the latter subsets correspond to the number of divisions in the population of naïve B cells, defined as CD19posIgDposCD27neg by van Zelm et al. (27). In contrast to healthy donors, the CVID-21lo and CVID-21norm patients displayed not only an altered distribution of naïve B cells, but also elevated numbers of cell divisions in this compartment (Figs. 2, 5). We also found that the percentage of FO I B cells was strongly reduced in CVID-21lo patients. This may be explained by deficient responses to BCR signaling.

A marked decrease or absence of switched memory B cells and an increased frequency of naïve B cells (e.g., CD21negCD38neg in a subgroup of patients), together with an increased division rate, suggests a developmental arrest in normal bone marrow migration. Recent studies of a large cohort of patients showed that there was an increase in autoimmune phenomena in the CVID-21lo patient group (20). This may be a consequence of an oligoclonal expansion in the naïve B cell compartment occurring during prolonged homeostatic proliferation or during proliferation in response to non-BCR stimuli, such as the TLR9 agonist that is exemplified in Fig. 3.

In contrast to the proportions of the examined B cell subsets in CVID-21lo patients, the proportions in CVID-21norm patients were inflated in all naïve CD21pos B cell compartments. FO I and FO II B cell subsets were found to undergo a higher number of divisions than controls. This result may be due to prolonged homeostatic proliferation, as described above.

Of note, both groups of CVID patients had an increased proportion of transitional B cells compared with healthy donors (20). However, these cells did not show significantly increased numbers of cell divisions compared with control transitional B cells. Thus, we speculate that increased bone marrow migration represents an attempt to compensate for the reduced numbers of mature B cells in the periphery of these patients.

Replication history data from healthy donors, together with the findings from Cariappa et al. (25), point to a sequence of subset development from transitional B cells to FO II (IgMhighCD24pos) cells, and later, to FO I (IgMintCD24int) cells in the absence of cell division. The two later stage cell types can presumably give rise to the CD21negCD24pos and CD21negCD24neg subsets, respectively, as indicated by the fact that there are more cell divisions at these stages (Fig. 2B). Naïve B cells in CVID patients fail to undergo the germinal center (GC) reaction and fail to enter the switched memory pool. These naïve B cells were, however, shown to react to stimulation with anti-IgM plus IL-2, as demonstrated by the downregulation of CD24 (33), but the upregulation of the co-stimulatory molecules CD70 and CD86 was impaired. It is reasonable to suggest that the physiological (and likely repeated) stimulation of naïve B cells by Ag and/or proinflammatory signals in CVID patients may lead to proliferation, after which these cells lose CD21 and become CD21negCD24pos or CD21negCD24neg cells (with added cell divisions) instead of undergoing the GC reaction. CD21neg cells cannot participate in the GC reaction because CD21 expression is essential for GC formation, B cell FO dendritic cell contact, and B cell survival during the GC reaction (34).

In conclusion, we used IgM and CD24 surface expression patterns to refine our knowledge of naïve peripheral B cell development, which is disrupted in CVID patients. We propose that cell proliferation without developmental progression is responsible for the accumulation of CD21negCD38pos cells in a subset of CVID patients.

Disclosures
The authors have no financial conflicts of interest.

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