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B-Cell Subsets in Blood and Lymphoid Organs in *Macaca fascicularis*

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Background: Cynomolgus monkeys (*Macaca fascicularis*) are widely used animal models in biomedical research. However, the phenotypic characteristics of cynomolgus monkey (CM) B cells in peripheral blood (PB) and lymphoid organs are poorly understood.

Methods: FACS analyses of PB-, spleen-, lymph node (LN)-, and bone marrow (BM)-derived B cells were performed.

Results: CM peripheral blood B cells have a smaller fraction of $CD27^-$ (naive) cells (~40%), as compared to human blood samples (~70%). Similar to humans, an early activation marker, CD23, is expressed more on $CD27^-$ CM naive B cells, as compared to $CD27^+$ B cells. The mean fraction of B cells exhibiting a memory phenotype is similar to that seen in human blood. Unlike humans, CM blood contains a subset of $CD20^{++}CD80^+CD21^-IgM^{+/-}CD27^+CD19^+FSC^{++}$BAFF-$R^{low}$ B cells that are likely of germinal center origin. Thus, CM blood contains (i) a higher percentage of B cells that express the co-stimulatory molecule CD80, and (ii) a lower fraction of B cells that are $CD21^+$, as compared to human blood. Due to the relative paucity of information on B-cell subsets in organs of healthy humans, a direct comparison between human and CM lymphoid organ data is limited. The fraction of $CD27^+$ and $CD23^+$ B cells appears to be similar, while the fraction of $CD80^+$ B cells appears to be higher than that seen in human lymphoid organs. CM spleens and to some extent lymph nodes have a distinct subset of $CD21^{++}$ cells that are also $CD80^{+/-}CD23^{low}IgM^{++}CD27^{+/-}FSC^{++}$. This subset is phenotypically similar to the marginal zone B cells present in human spleen and LN samples. We also provide detailed analyses on the fraction of lymphoid organ B cells that express CD21, CD23, CD32, and/or CD80 B-cell markers.

Conclusions: In general, cynomolgus monkey B-cell subsets are similar to those seen in humans, as well as to those seen in other nonhuman primates. However, there are some clear differences between human and cynomolgus monkey B-cell subsets. These findings have direct implications for a variety of in vivo studies in cynomolgus monkeys, ranging from basic research on primate B-cell differentiation to models of infectious diseases and trials of new B-cell targeting therapeutic agents. © 2004 Wiley-Liss, Inc.

Key terms: *Macaca fascicularis*; B-cell subsets; B-cell targeting

The cynomolgus monkey (*Macaca fascicularis*) is widely used as an animal model for analyzing immunity, hematopoiesis, infectious diseases, transplantation, and toxicology, as this macaque species is phylogenetically proximate to human. Flow cytometric analysis of major peripheral blood (PB) and lymphoid organs leukocyte subsets has become an important approach in delineating pharmocodynamics, mechanism of action, safety, and efficacy of a variety of therapeutic agents and procedures in animal models (1–3). Our ability to extrapolate results from cynomolgus monkeys to humans is hindered, at least partially, by the lack of detailed analyses of similarities and differences between human and cynomolgus monkey leukocytes. Recent studies have demonstrated several key phenotypic differences between peripheral blood B-, T-, and natural killer (NK) cell subsets in man and cynomolgus monkey. For example, in contrast to humans, cynomolgus monkey peripheral blood samples have (i) a distinct population of $CD21^-$ B cells, (ii) a substantial population of CD4/CD8 double-positive T cells (1,4); (iii) a lower $CD4^+$: $CD8^+$ T cell ratio (5); (iv) $CD90^+$ lymphocytes (1); and (v) $CD56^+$ monocytes and $CD56^-$ NK cells (1). The differences and similarities between cynomolgus monkey and human B-cell subsets in peripheral blood and lymphoid organs are poorly understood. However, this

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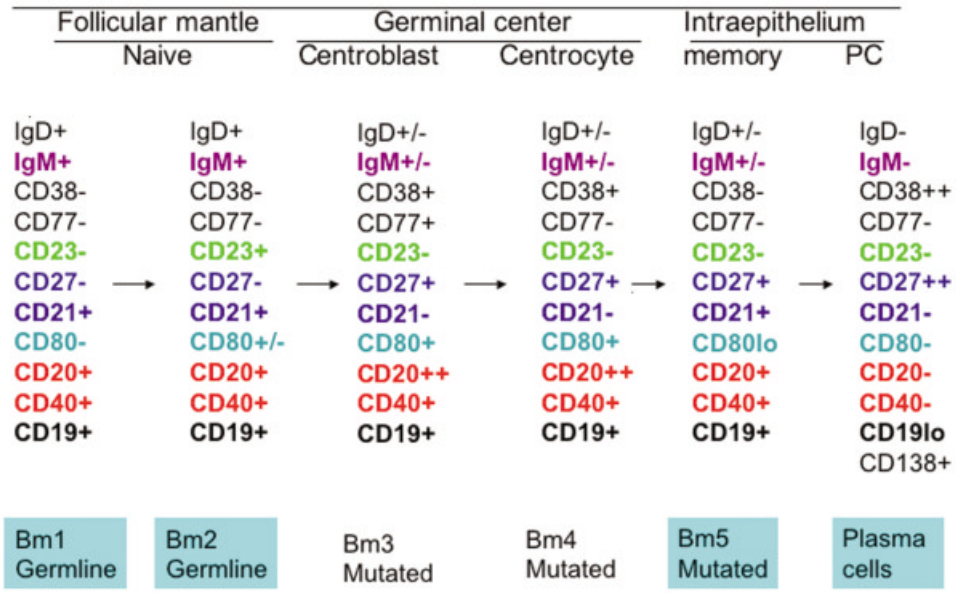

FIG. 1. B-cell subsets and B-cell markers in human lymph nodes. A series of cell surface markers are used to differentiate human bm1–bm5 and plasma B-cell subsets. B-cell subsets that are present in human peripheral blood are color-filled. Some commercially available reagents that are used for phenotypic analyses of human B-cell markers also cross-react with (a subset of) cynomolgus monkey B-cell markers (shown in bold). Human data are compiled from references (6) and (7).

knowledge is of crucial importance for interpretation of data from a variety of in vivo studies, ranging from basic research of primate B-cell differentiation to models of infectious diseases and trials of new B-cell targeting therapeutic agent.

The differentiation of naive B cells into memory B cells is a complex process, driven by the interaction of T cells, follicular dendritic cells, and other cells (6,7). Naive B cells differentiate into effector memory or plasma B cells in germinal centers (GC), which are highly specialized areas of secondary lymphoid organs, such as lymph nodes, spleen, and tonsil. Upon antigen encounter, activated naive B cells undergo proliferation, somatic mutation of Ig V region genes, Ig isotype switching, and affinity maturation. A number of B-cell subsets have been identified in human blood and secondary lymphoid organs. These subsets are believed to represent different stages of development of naive B cells into effector B cells. In human tonsil, at least five distinct subpopulations of mature human B cells (Bm1–Bm5) have been identified (6,7) (Fig. 1). Bm1, Bm2, Bm5, and to a smaller extent plasma cell subsets, were identified in human blood samples. In human spleen, there are two main B-cell subsets: mantle zone B cells ($sIgD^{high}sIgM^{+}CD21^{+}CD23^{+}$), and marginal zone B cells ($sIgD^{+}sIgM^{high}CD21^{high}CD23^{+/-}$) (8–10). It has been suggested that mantle zone B cells are mostly naive B cells, while marginal zone B cells contain mostly memory B cells. Although the details on functional role of marginal zone B cells remain to be established, this subset of B cells is thought to participate in early immune responses, as well as in responses to T-cell-independent antigens (8,9).

In this study, we investigated phenotypic characteristics of PB and lymphoid organ leukocytes in cynomolgus monkeys. In particular, we focused on detailed flow cytometric-based analyses of mature B-cell subsets in spleen, lymph nodes, and bone marrow of cynomolgus monkeys.

MATERIALS AND METHODS

Animals

This study was conducted at the Sierra Biomedical Study Center (NV, USA), according to the SNBL Standard Operating Procedures and in compliance with applicable laws and regulations concerning the care and use of laboratory animals. The present study used 2–4-year-old naive cynomolgus monkeys.

Antibodies and Buffers

Antibodies used in this study are shown in Table 1. Antibodies were raised against human antigens and cross-react with CM antigens. The buffers were ammonium chloride lysing (ACL) reagent (0.15 M NH_4Cl, 10 mM $NaHCO_3$, 80 μM tetrasodium EDTA, pH 7.4), stain buffer (Hank's balanced salt solution (HBSS) with 1% bovine serum albumin (BSA) and 0.1% sodium azide), wash buffer (HBSS with 0.1% sodium azide), and fixative solution (1% formaldehyde).

Spleen and Lymph Node Cell Suspensions and Sample Transport

Sample collection and tissue disruption was performed at the SNBL study center and samples (whole blood, bone marrow aspirates, and tissue cell suspensions) were shipped overnight on cold packs. Tissue samples (~1 cm^3) was trimmed of excess fat and placed in chilled media in Petri dishes. Tissues were mechanically disrupted with sterile plunger from a 10-ml syringe. Suspended cells were aspirated from a Petri dish and placed into pre-chilled 15-ml tube. Cell suspensions were kept on ice until they were packed on cold packs for overnight

Table 1
Anti-Human B-Cell Reagents That Cross-react With Cynomolgus Monkey B Cells

Clone or Catalog No.	Antibody	Source
Clone 2H7	Anti-CD20 FITC	BD Pharmingen
Clone L27	Anti-CD20 APC	BD Pharmingen
Clone 5C3	Anti-CD40 FITC	BD Pharmingen
Clone 5C3	Anti-CD40 APC	BD Pharmingen
Clone B-ly 4	Anti-CD21 PE	BD Pharmingen
Clone B-ly 4	Anti-CD21 APC	BD Pharmingen
Clone 9P25	Anti-CD23 FITC	Immunotech
Clone M-L233	Anti-CD23 PE	BD Pharmingen
Clone L307.4	Anti-CD80 PE	BD Pharmingen
Cat 109-096-129	Anti-IgM FITC	Jackson ImmunoResearch
Cat 109-066-129	Anti-IgM biotin	Jackson ImmunoResearch
Clone KB61	Anti-CD32 FITC	Dako
Clone J4.119	Anti-CD19 PE	Coulter
Clone M-T271	Anti-CD27 PE	BD Pharmingen
Clone 9.1	Anti-BAFF-R-biotin	Avery et al. [19]

transport. This procedure had no significant impact on cell viability as determined by Tryptan Blue exclusion (data not shown).

Flow Cytometry

Samples (shipped overnight) were stained (30 min, room temperature) with a cocktail of antibodies. Each cocktail contained antibodies against either CD20 or CD40 B-cell markers (expressed on all mature B cells but not necessarily plasma cells) plus two additional B-cell markers from Table 1 (expressed on a subset of mature B cells). More than 99% of $CD40^+$ blood, spleen, and LN lymphocytes are $CD20^+$, while there is some small but distinct fraction of $CD20^{dim}$ lymphocytes that are $CD40^-$ and usually express CD3 (reference 11 and data not shown). This $CD20^{dim}$ population was not included for the B-cell subset analyses.

Whole blood samples and bone marrow aspirates (90 μl) were depleted of red blood cells (using ACL reagent), washed (once in the wash buffer and once in stain buffer), and fixed (in the fixative solution) using the Lyse-Wash-Assistant instrumentation (BD Biosciences), while spleen and lymph node suspensions (10^6 cells/100 μl) were washed and fixed without the use of automation.

A FACSCalibur (BD Biosciences) instrument equipped with an automated FACS Loader and CellQuest Software was used for acquisition and analysis of all samples. Cytometer QC and setup included running CaliBrite beads and SpheroTech rainbow beads (BD Biosciences) to confirm instrument functionality and detector linearity. Isotype and compensation controls were run with each assay to confirm instrument settings. Fraction of B cells expressing a certain marker or a combination of markers was obtained by the following gating strategy. The lymphocyte population was marked on the forward scatter/side scatter scattergram to define Region 1 (R1). Using events in R1, fluorescence intensity versus side scatter dot plots were displayed for CD40 or CD20 B-cell markers. Fluorescently labeled isotype controls were used to determine respective cutoff points for CD40 and CD20 positivity and define a region R2. Note, that CD19 marker was not used for B-cell monitoring, since a commercially available anti-CD19 that cross-reacts with all cynomolgus monkey B cells was not identified (see Fig. 2D). Using events in the logical "R1 AND R2" gate, fluorescence intensity dot plots were generated for CD markers expressed on a subset of B cells. Isotype controls

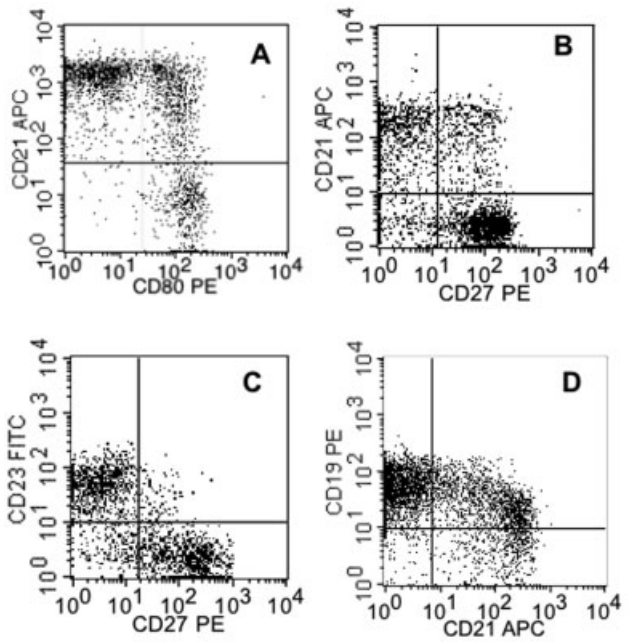

FIG. 2. FACS analyses of cynomolgus monkey peripheral blood B cells. Three-color flow cytometry analysis of whole blood cynomolgus monkeys samples was performed using (**A**) anti-CD40 FITC, anti-CD80 PE, anti-CD21 APC, or (**B**) anti-CD20 FITC, anti-CD27 PE, anti-CD21 APC, (**C**) anti-CD23 FITC, anti-CD27 PE, anti-CD20 APC, or (**D**) anti-CD20 FITC, anti-CD19 PE, anti-CD21 APC staining cocktail. The fluorescence intensity plots were gated on a total B-cell population, as described in Materials and Methods. Note that samples shown were from different donors, analyzed at different times with different instrument settings.

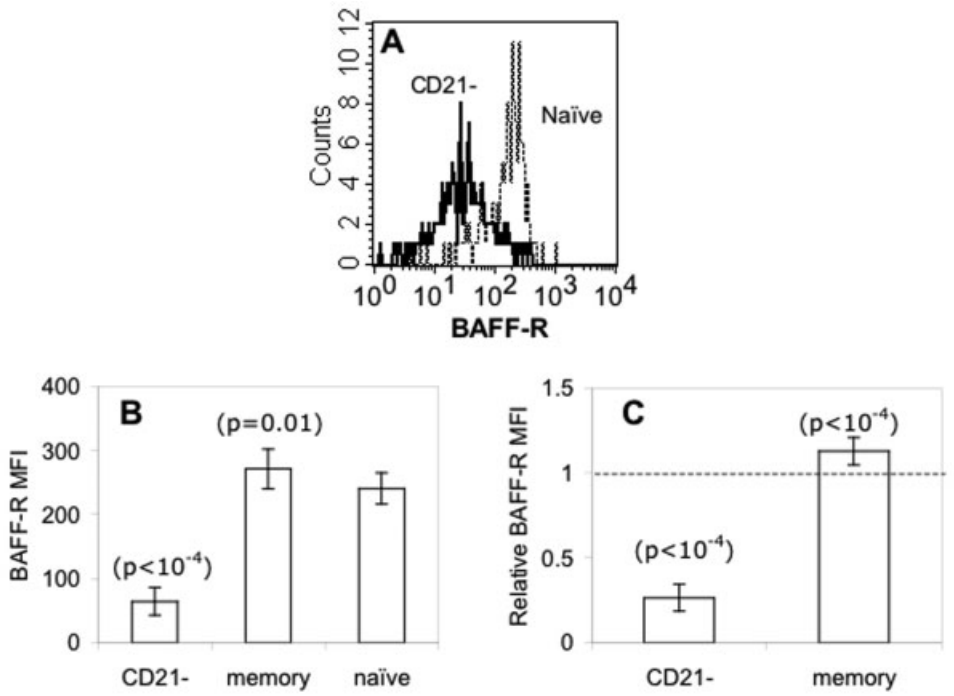

FIG. 3. BAFF-R expression on cynomolgus monkey peripheral blood B cells. Four-color flow cytometry analysis of whole blood cynomolgus monkeys samples was performed using anti-CD20 FITC, anti-CD27 PE, anti-BAFF-R-biotin + streptavidin PerCP, and anti-CD21 APC. BAFF-R Mean Fluorescence Intensity (MFI) was determined for $CD21^{-}CD20^{+}$ ($CD21^{-}$), $CD21^{+}CD27^{-}CD20^{+}$ (naive), and $CD21^{+}CD27^{+}CD20^{+}$ (memory) B cells. **A:** Example of BAFF-R expression on $CD21^{-}$ (solid line) vs naive (dotted line) B cells. **B:** Average BAFF-R MFI was determined for 13 samples. Error bars show standard deviations from the mean MFI value. P values are based on a Student t test with a null hypothesis that population average for either memory or $CD21^{-}$ B cells is identical to that for naive cell population average. **C:** BAFF-R MFI on memory/BAFF-R MFI on naive and BAFF-R MFI on $CD21^{-}$/BAFF-R MFI on naive ratios (referred to as "relative BAFF-R MFI") were determined for each of 13 blood samples; then means for the relative BAFF-R MFI were calculated. Error bars show standard deviation from the mean. P values are based on a Student t test with a null hypothesis that population average for relative BAFF-R MFI on memory or on $CD21^{-}$ B cells is equal to 1.

were used to determine cutoff points to define quadrants or regions of events that are positive for a given marker or a combination of markers. Quadrant or region statistics were used to determine percentages of B cells that express a given marker combination.

RESULTS AND DISCUSSION

Peripheral Blood $CD21^{-}$ B Cells

We have recently reported that unlike humans, cynomolgus monkey peripheral blood samples have a significant fraction of $CD21^{-}$ $CD20^{++}CD40^{low}FSC^{high}$ B cells (11). Absence of CD21 expression and high CD20 expression are phenotypic characteristics of human germinal center (GC) B cells; therefore, we performed additional analyses of cell surface markers to clarify the matter. Here we report that $CD21^{-}$ B cells are also $CD27^{+}CD80^{+}IgM^{+/-}$ (Fig. 2A,B and data not shown), consistent with the hypothesis that the $CD21^{-}$ B-cell subset is GC-derived. Human GC tonsil B cells have somewhat lower expression of BAFF-R, as compared to other B-cell subsets (Sutherland et al., in preparation). BAFF, the ligand for BAFF-R is an important B-cell survival and maturation factor (reviewed in ref. 12). In accord, we report that cynomolgus monkey $CD21^{-}$ peripheral blood B cells have ~4-fold decrease in BAFF-R expression, as compared to $CD21^{+}$ B cells (Fig. 3). Another recent study has demonstrated that GC- derived B cells but not naive or memory B cells have low expression of CD32, the FcγRIIb receptor that serves as negative feedback regulator for B-cell Ag receptor-elicited activation of B cells (13,14). To this end, we did detect a $CD27^{+}CD32^{-}$ B-cell population in cynomolgus monkey peripheral blood, consistent with the existence of GC-derived B cells in the blood compartment (Table 2 and data not shown). However, not all the putative GC-derived B cells were $CD32^{-}$ (data not shown). Future studies aimed at detection of frequency of somatic mutations and isotype switching will provide supportive evidence for the hypothesis that $CD21^{-}$ cynomolgus monkey B cells are of GC origin. Interestingly, most of the $CD21^{-}$ cynomolgus monkey B cells stain positively with the anti-human CD19 reagent, which has been used to track cynomolgus monkey B cells in some animal studies (15,16), while only about 75% of $CD21^{+}$ cynomolgus monkey B cells stain positively with this reagent (Fig. 2D). The $CD21^{-}$ $CD20^{++}CD80^{+}$ B cells have been also detected in another nonhuman primate species, rhesus monkeys (17). Although expression of CD27 and IgM markers has not been examined in this rhesus monkey B-cell subset, it seems plausible that these rhesus B cells are similar to the putative GC-derived B cells seen in cynomolgus

Table 2
B-Cell Subsets in Cynomolgus Monkeys

	Likely stage of differentiation	Spleen	LN-ing[a]	LN-man[b]	BM[c]	Blood
$CD27^{+}$[d]	Memory and GC[e]	39.2 ± 4.2	45.8 ± 5.0	36.2 ± 4.3	13.5 ± 3.8	58.9 ± 1.9
$CD21^{+}$	Memory and naive	76.6 ± 2.9	86.9 ± 1.6	ND	72.5 ± 3.3	73.2 ± 1.8
$CD27^{+}CD21^{+}$	Memory	14.8 ± 4.2	36.7 ± 5.0	ND	ND	31.1 ± 1.7
$CD27^{-}(CD21^{+})$	Naive	61.8 ± 4.2	54.2 ± 5.0	63.8 ± 4.3	ND	41.1 ± 1.9
$CD21^{++}$	Marginal zone	8.74 ± 0.8	5.94 ± 0.78	2.66 ± 0.45	NA	NA
$CD23^{+}$	Mostly naive	55.4 ± 3.9	64.3 ± 2.3	56.9 ± 5.9	55.0 ± 5.6	37.0 ± 2.8
$CD80^{+}$	NA	50.8 ± 4.1	40.6 ± 3.7	ND	18.5 ± 3.9	58.6 ± 2.2
$CD32^{+}$	NA	67.6 ± 7.5	ND	69.5 ± 4.3	74.8 ± 8.3	82.1 ± 6.4
$CD21^{+}CD23^{+}$	Mostly naive	36.4 ± 3.7	56.8 ± 2.4	ND	55.0 ± 5.6	ND
$CD21^{+}CD23^{-}$	Memory and naive	23.2 ± 1.7	33.6 ± 2.3	ND	17.0 ± 3.3	ND
$CD21^{-}CD23^{-}$	Mostly GC	16.9 ± 2.2	3.18 ± 0.5	ND	27.5 ± 3.3	ND
$CD21^{+}CD80^{+}$	Mostly memory	27.8 ± 3.0	32.0 ± 3.7	ND	12.8 ± 2.8	40.1 ± 1.7
$CD21^{-}CD80^{+}$	GC	16.8 ± 2.6	2.7 ± 0.4	ND	6.3 ± 1.4	18.5 ± 1.3
$CD21^{+}CD80^{-}$	Mostly naive	44.8 ± 4.4	54.9 ± 3.5	ND	60.4 ± 6.3	33.1 ± 2.6

ND, not determined; NA, not applicable.
[a]Inguinal lymph node.
[b]Mandibular lymph node.
[c]Bone marrow.
[d]Data show fraction (mean ± SE) of total B cells; N ≥ 8, except for data on CD32 expression in organs, where N = 4.
[e]Germinal center origin.

monkeys. Also note that, the existence of a putative GC-derived B-cell subset in monkey blood resulted in (i) a higher percentage of B cells expressing the co-stimulatory molecule CD80 and (ii) a lower fraction of $CD21^{+}$ B cells, as compared to human samples (Table 2).

Peripheral Blood Memory and Naive B Cells

The mean fraction of B cells exhibiting a memory B-cell phenotype ($CD21^{+}CD27^{+}CD80^{+}CD40^{+}CD20^{+}$) in cynomolgus monkey blood samples (31% on average) appeared to be similar to that seen in human blood samples (~30%). However, cynomolgus monkey PB B cells had a smaller fraction of naive ($CD27^{-}$) cells (41% on average, N = 44, SD = 12.7, SE = 1.9), as compared to human blood samples (~70%). Note that (i) since CM blood contains a subset of $CD21^{-}$ B cells, combination of naive plus memory B cells is less than 100% of total B cells, and (ii) future studies (such as detection of frequency of somatic mutations and immunohistochemistry) are needed for definite proof that $CD21^{+}CD27^{+}$ B cells in CM are memory, while $CD21^{+}CD27^{-}$ are naive B cells. Similar to humans, early activation/maturation marker CD23 (FcεR) was expressed more on $CD27^{-}$ cells (Fig. 2C). However, there was a detectable transitional $CD23^{+}CD27^{+}$ subset (10% of all B cells on average). The fraction of CD23-expressing cells was ~40% of all B cells, with ~65% of naive $CD27^{-}$ B cells and 18% of $CD27^{+}$ B cells expressing CD23. The human data on expression of CD23 on peripheral blood B cells is conflicting, ranging from ~7% of $CD23^{+}$ B cells (18) to 64% of $CD23^{+}$ B cells (19). This implies an even larger extent of uncertainty for the average fraction of naive human B cells that express CD23 (10–95% of naive human B cells). Some of the apparent discrepancies in human data may be attributed to the fact that there is an age dependency in the expression of CD23 antigen on human B cells, with the fraction of $CD23^{+}$ B cells nearly doubling from birth to adulthood (19). Thus, comparative analysis of CD23 expression on cynomolgus monkey versus human B cells is limited by the inconsistencies in human data.

Interestingly, cynomolgus monkey memory B cells appear to have a small (~13%) but statistically significant increase (N = 13, p = 1.064E-05, based on Student t test) in the expression of BAFF-R, as compared to naive B cells (Fig. 3). This result may not be explained exclusively by 10% size (proportional to forward side scatter, FSC) difference between memory and naive B cells, as there was little correlation (correlation coefficient ~0.2) between FSC_{memory}/FSC_{naive} and BAFF-R MFI_{memory}/BAFF-R MFI_{naive} in the set of 13 samples examined.

Lymphoid Organ B Cells

First, we characterized a splenic B-cell subset that is phenotypically similar to the marginal zone B cells reported in humans (8–10). This subset was $CD21^{++}CD80^{low}CD23^{lowlow}CD27^{+/-}FSC^{++}$ (Fig. 4 and data not shown) and represented a relatively small fraction (~8% on average) of splenic B cells. Consistent with reports from both human and mouse system, the phenotypically identical ($CD21^{++}$) subset was also present, albeit to a smaller extent, in cynomolgus monkey lymph nodes (Table 2). There appeared to be no significant correlation between the fraction of marginal zone B cells in the spleen and that in the lymph node (correlation coefficient = −0.5, data not shown). Note that detailed immunohistochemical studies are required for definite proof of origin of these $CD21^{++}$ B cells in cynomolgus monkeys. As mentioned in the introduction, the function of splenic marginal zone B cells remains to be detailed, especially in primate systems. Since there are considerable

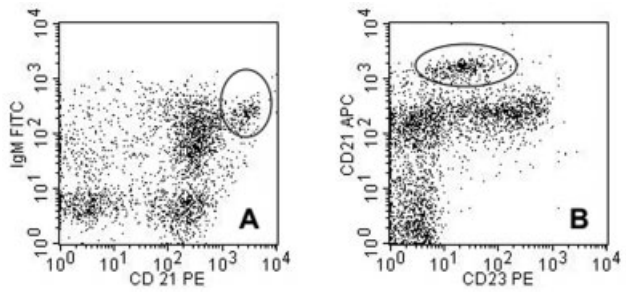

FIG. 4. FACS analyses of cynomolgus monkey splenic B cells. Three-color flow cytometry analysis of single cell suspensions of cynomolgus monkeys spleens was performed using (**A**) anti-IgM FITC, anti-CD21 PE, anti-CD20 APC, or (**B**) anti-CD20 FITC, anti-CD23 PE, anti-CD21 APC. The fluorescence intensity plots were gated on a total B-cell population, as described in Materials and Methods. A B-cell subset that is phenotypically similar to human marginal zone B cells is circled.

differences in the microanatomy between human and rodent spleen (9), it is not possible to extrapolate murine data to humans. The role of marginal-zone-like B cells in primate lymph nodes is not known. As marginal zone B cells are not detected in peripheral blood, it is extremely difficult logistically to study their role in a human system. Thus, nonhuman primate studies of this subset may be a more practical alternative. Phenotypic characterization of cynomolgus monkey marginal zone B cells is likely to be a valuable tool for such studies.

Detailed information on the expression of CD21, CD27, CD23, CD80, and/or CD32 is shown Table 2. Because of the relative paucity of quantitative information on B-cell subsets in organs of healthy humans, a direct comparison between human and cynomolgus monkey lymphoid organ data is limited. The expression of CD27 and CD23 B-cell markers appeared to be similar to that seen in human secondary lymphoid organs. (10). The fraction of $CD80^+$ B cells appeared to be higher in cynomolgus monkey spleen, as compared to human spleen (10). In addition, similar to results in rhesus monkeys (17), the mean percentage of B cells expressing CD80 was higher in the blood (59%) than in the lymph node (41%), while the mean percentage of B cells expressing CD21 was lower in the blood (87%) than in the lymph node (73%). However, the difference between blood and lymph node expression of these antigens appears to be less pronounced for cynomolgus monkeys, as compared to rhesus monkeys.

The differences and similarities between human and cynomolgus monkey peripheral blood and lymphoid organ B-cell subsets should be taken into account when monkey data are extrapolated to humans. These differences are especially important for studies of therapeutics agents, where B-cell numbers are used as a pharmacodynamic marker. B-cell targeting monoclonal antibodies (mAbs), such as anti-CD20 (15,16), anti-CD40 (20), and anti-BAFF, or fusion proteins affecting B-cell survival, such as TACI-Fc, BCMA-Fc, and BAFF-R-Fc (21,22), are examples of such therapeutics. Since mAbs are often engineered with a human Fc region, they are not equivalently recognized by murine or rat effector cells (3). Thus, nonhuman primates may be the most informative animal models. In this regard, $CD21^-$ cynomolgus monkey B-cell subset was shown to be relatively more susceptible to rituximab (11,23), an anti-CD20 mAb approved for the treatment of non-Hodgkin's lymphoma (24).

In conclusion, cynomolgus monkey B-cell subsets are, in general, similar to those seen in humans, as well as to those seen in other monkey species. However, there are some clear differences between human and cynomolgus monkey B-cell subsets in blood, as well as in lymphoid organs. The ability to discriminate between B-cell subsets in blood and lymphoid organs of cynomolgus monkeys is likely to be instrumental for generation and interpretation of data from a variety of in vivo studies, ranging from basic research of primate B-cell differentiation to models of infectious diseases and trials of new B-cell targeting therapeutic agents.

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