Brief Definitive Report

Surrogate Light Chain Expressing Human Peripheral B Cells Produce Self-reactive Antibodies

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Abstract

Human B cells that coexpress surrogate and conventional light chains (V-preB$^+$L$^+$) show an unusual heavy and light chain antibody repertoire that display evidence of receptor editing. However, it is unclear whether V-preB$^+$L$^+$ B cells have been silenced by receptor editing or still express autoreactive antibodies. Here we report that 68% of the antibodies expressed by V-preB$^+$L$^+$ B cells are autoreactive. A majority of these autoantibodies are true antinuclear antibodies (ANA), and 50% of the ANAs are also reactive with a diverse group of antigens that include dsDNA, ssDNA, immunoglobulin, insulin, and bacterial lipopolysaccharide. Such antibodies are rarely encountered among conventional B cells. We conclude that V-preB$^+$L$^+$ B cells are a unique subset of normal circulating human B cells that escape central tolerance mechanisms and express self-reactive antibodies including potentially harmful ANAs.

Key words: surrogate light chains • B lymphocytes • polyreactive • autoantibody

Introduction

A majority of the antibodies initially produced during human B cell development are self-reactive, including antinuclear antibodies (ANAs) and polyreactive antibodies (1). These autoantibodies typically display a long Ig heavy (IgH) complementarity determining region (CDR)3 enriched in positively charged residues, a pattern that is believed to favor self-reactivity especially against DNA (1–5). Most of the polyreactive antibodies and ANAs are removed from the repertoire during B cell development thereby ensuring self-tolerance (1). Three central mechanisms are responsible for self-reactive antibody silencing: receptor editing, anergy, and deletion (for review see reference 6). However, central tolerance is imperfect, and some self-reactive B cells are exported from the bone marrow to the periphery where they can be deleted (7, 8), remain "ignorant" (9), or anergic (10, 11).

The self-reactive B cells that escape deletion are thought to benefit the organism by producing antibodies that play a role in the clearance of apoptotic cells and in the initial immune response to infections (12, 13). In the mouse, these "natural" antibodies are produced by two subsets of peripheral B cells: B1 cells and marginal zone B cells, both of which are positively selected by self-antigens (14–16). Natural antibodies are also found in human serum (17), but the human counterpart of the murine natural antibody producing B cell is not well defined. CD5, a molecule specifically expressed on mouse B-1a B cells, is expressed at variable levels on all human peripheral blood B cells and therefore cannot be used as a marker for the human counterpart of murine B1-a B cells (18, 19). Thus, there is little understanding of the origin of self-reactive antibodies in humans despite the observation that inappropriate autoantibody production is a characteristic of most autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus (20).

We have identified a population of human B cells that coexpress surrogate and conventional light chains, V-preB$^+$L$^+$ B cells, whose antibodies display sequence features that suggested that they might be self-reactive (5, 21). However, Ig kappa light chain sequences showed evidence of receptor editing that may have silenced V-preB$^+$L$^+$ B cells (5). To determine the specificity of the antibodies expressed

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The online version of this article includes supplemental material.

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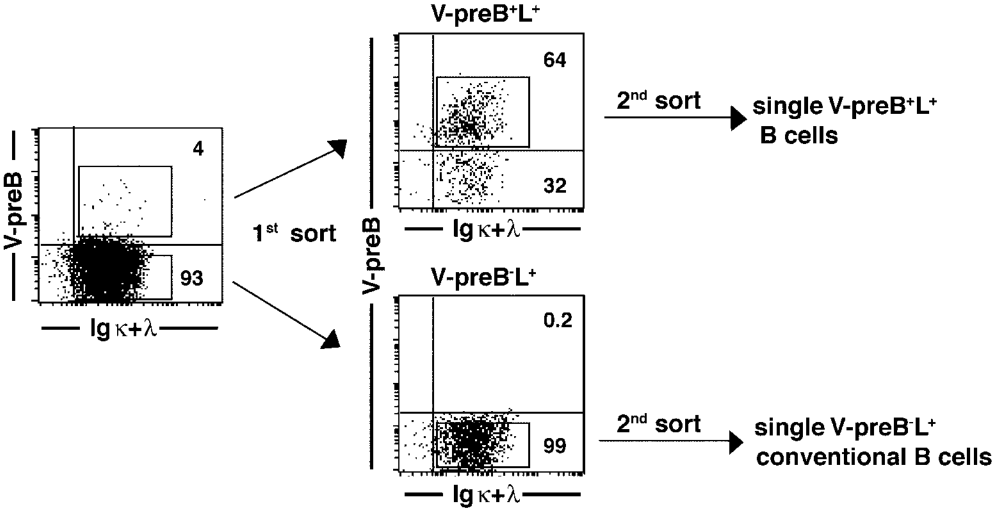


Figure 1. V-preB$^+$L$^+$ B cell purification scheme. Dot plots show V-preB and Igκ+λ expression on B cells preenriched for V-preB expression using magnetic beads (left), and after the first sort for V-preB$^+$L$^+$ (top right) or conventional V-preB$^-$L$^+$ B cells (bottom right). These populations were subsequently sorted into 96-well plates during a second round of cell sorting.

by V-preB$^+$L$^+$ B cells, we sorted single B cells from two unrelated healthy donors, cloned their IgH and Ig light chains, expressed the recombinant antibodies, and tested them for self-reactivity. Here we report that the majority of V-preB$^+$L$^+$ B cells produce self-reactive and polyreactive antibodies.

Materials and Methods

Single Cell Sorting. All samples were collected after signed informed consent in accordance with Institutional Review Board–reviewed protocols. V-preB$^+$L$^+$B cells and conventional V-preB$^-$L$^+$ B cells were purified from the blood of two nonrelated healthy donors using a combination of magnetic bead positive and negative selection, followed by two rounds of cell sorting (5). Enriched B cells were stained with FITC human anti-κ and anti-λ, PE human anti–V-preB (a gift from C. Schiff, Centre d'Immunologie de Marseille-Luminy, Marseille, France), and APC anti-CD19 (BD Biosciences). V-preB$^+$L$^+$CD19$^+$ B cells and V-preB$^-$L$^+$ CD19$^+$ B cells were first bulk sorted on a FACSVantage™ (5). Single cells were obtained by a second sort from the enriched populations directly into 96-well plates containing 4 μl Lysis solution (0.5× PBS containing 10 mM DTT, 8 U RNAsin [Promega]), 0.4 U 5′-3′ RNase Inhibitor (Eppendorf), and immediately frozen on dry ice. All samples were stored at –70°C.

cDNA, RT-PCR, Antibody Production, and Purification. RNA from single cells was reverse transcribed in the original 96-well plate in 12.5-μl reactions containing 100 U of Superscript II RT (GIBCO BRL) for 45 min at 37°C. RT-PCR reactions, primer sequences, cloning strategy, expression vectors, antibody expression, and purification were as described (1). Polyreactive human M55 mAb (a gift from P. Casali, Weill Medical College of Cornell University) was cloned from a CD5$^+$ B cell line as a positive control antibody (22). Ig sequences were analyzed by Ig BLAST comparison with GenBank.

ELISAs and Indirect Immunofluorescence Assays. Antibody concentration, reactivity against specific antigens, and indirect immunofluorescence were as described (1). M55 was used as a positive control in all polyreactivity ELISAs (22).

Online Supplemental Material. Antibody characteristics from conventional and V-preB$^+$L$^+$ B cells are presented in Tables S1 and S2 available at http://www.jem.org/cgi/content/full/jem.20031550/DC1.

Results

Cloning Antibodies from Single V-preB$^+$L$^+$ B Cells. To examine the specificity of antibodies produced by human V-preB$^+$L$^+$ B cells, we cloned antibodies from single IgM-expressing B cells and expressed them as IgG1. The overall efficiency of cloning both heavy and light chain genes from individual B cells was 40–60%. Antibodies composed of heavy chains and V-preB/λ–like with or without conventional light chains were not secreted and could not be assayed.

V-preB$^+$L$^+$ B cells represent only 0.5–1% of all circulating B cells. To obtain pure populations of these cells, we initially enriched V-preB$^+$L$^+$ B cell using magnetic beads and then performed two additional rounds of cell sorting (Fig. 1). We found that the sequence features of Igs cloned from single V-preB$^+$L$^+$ B cells were similar to those obtained from batch-sorted cells (5, 21). IgHs displayed increased J$_H$6 usage (20.7% in V-preB$^+$L$^+$ versus 0% in

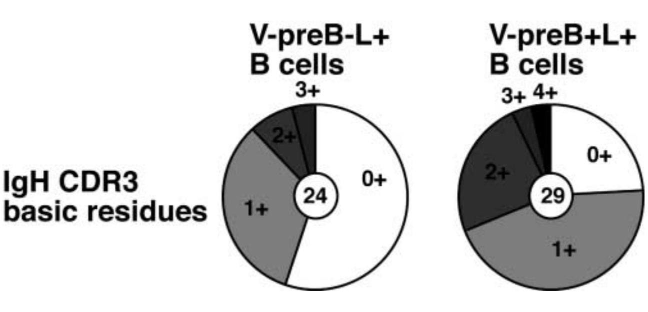


Figure 2. Increased frequency of positively charged amino acids in IgH CDR3s from V-preB$^+$L$^+$ B cells. Pie charts show segment size proportional to the number of clones from V-preB$^-$L$^+$ (left) and V-preB$^+$L$^+$ B cells (right) displaying 0, 1, 2, 3, and 4 or more positively charged amino acids per IgH CDR3. The number of sequences analyzed in each group is indicated in the center.

V-preB^{-}L^{+} B cells) and longer CDR3s (15.3 amino acids in V-preB^{+}L^{+} versus 12.2 amino acids in V-preB^{-}L^{+} B cells, $P = 0.005$). In addition, we found that antibodies from V-preB^{+}L^{+} B cells showed an increased prevalence of basic amino acid residues in IgH CDR3s (Fig. 2). As in batch-sorted cells, Igκ genes from single V-preB^{+}L^{+} B cells showed increased downstream Jκ3, Jκ4, and Jκ5 segment usage compared with Igκs from conventional V-preB^{-}L^{+} B cells (27.5% in V-preB^{+}L^{+} versus 16.5% in V-preB^{-}L^{+} B cells) (Tables S1 and S2, available at http://www.jem.org/cgi/content/full/jem.20031550/DC1) (5). V-preB^{+}L^{+} B cells also displayed increased incidence of Vκ4–1, which has been associated with anti-DNA antibodies (13.6% in V-preB^{+}L^{+} versus 0% in V-preB^{-}L^{+} B cells) (23), and an increase in 11–amino acid–long Igκ CDR3s due to increased N addition (5) (Tables S1 and S2). Finally, 97% of Ig sequences isolated from single V-preB^{+}L^{+} B cells was in germline configuration (Table S2). We conclude that the sequences of the Igs cloned from single V-preB^{+}L^{+} B cells were similar to those obtained from batch-sorted cells and were highly unusual when compared with Ig sequences cloned from conventional B cells (5, 21).

V-preB^{+}L^{+} B Cell Antibodies Are Self-reactive. To determine whether the antibodies expressed by V-preB^{+}L^{+} B cells were self-reactive, we expressed 28 antibodies obtained from single V-preB^{+}L^{+} B cells and compared them with 21 antibodies from conventional V-preB^{-}L^{+} B cells. As an initial screen for self-reactivity, we used a commercially available ELISA for antinuclear antibodies (ANA). This assay detects antibodies that recognize antigens in HEp-2 cell lysates, and therefore, reactivity is not restricted to ANAs but includes a broad spectrum of self-antigens. We found that 68% of V-preB^{+}L^{+} antibodies (19 out of

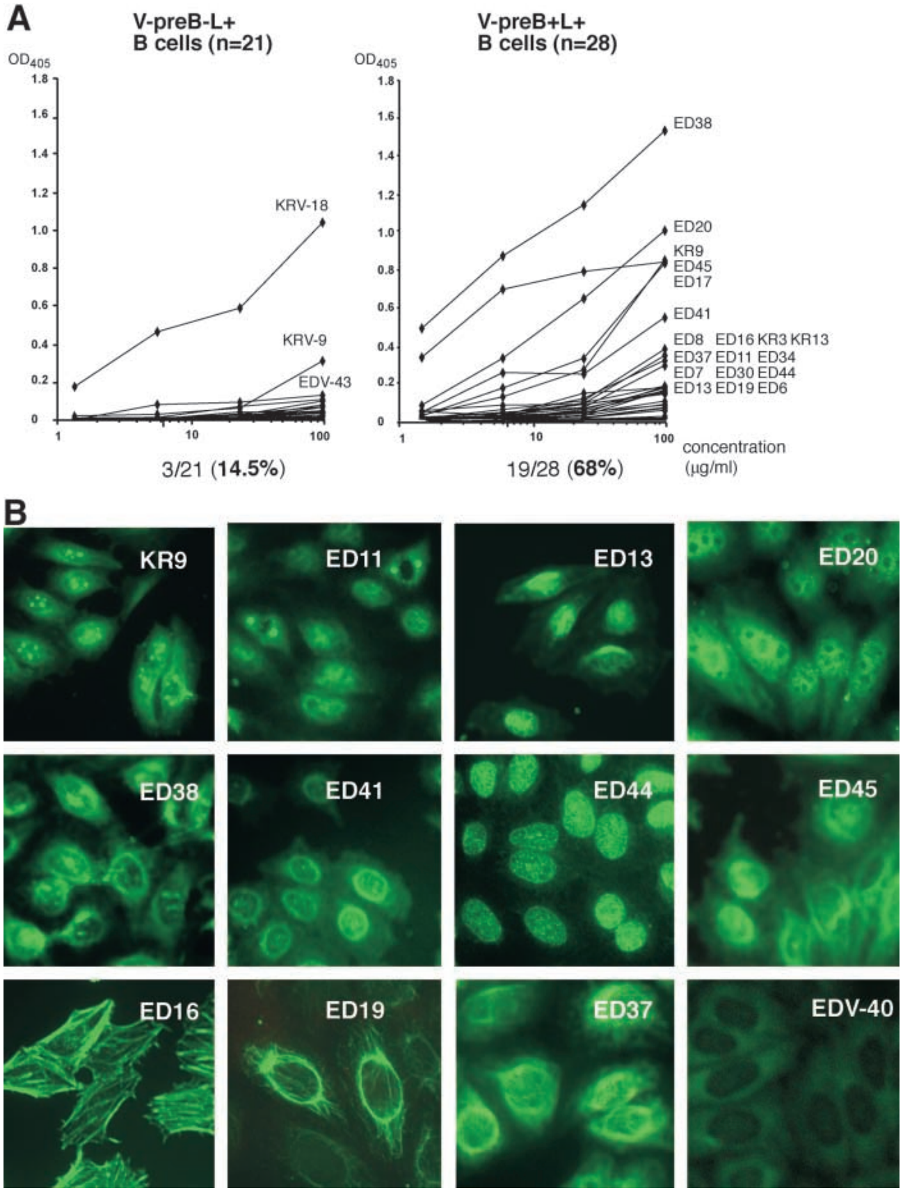


Figure 3. V-preB^{+}L^{+} B cells express self-reactive antibodies. (A) Antibodies from V-preB^{+}L^{+} B cells react against HEp-2 cell lysates. ELISAs for anti-HEp-2 cell reactivity using recombinant antibodies from 21 V-preB^{-}L^{+} (left) and 28 V-preB^{+}L^{+} B cells (right). The percentage of autoreactive clones for each fraction is indicated. (B) V-preB^{+}L^{+} antibodies express ANAs. Antibodies from V-preB^{+}L^{+} B cells show various patterns of ANA including nucleolar (KR9), mitotic spindle apparatus (ED11), speckled (ED20, ED44), and other uncharacterized patterns (ED13, ED38, ED41, ED45), and cytoskeletal reactivity against stress fiber (ED16), vinculin (ED19), and vimentin (ED37). Antibodies isolated from conventional B cells such as EDV-40 do not show ANA staining.

28) showed reactivity against HEp-2 cell lysates compared with 14.5% of antibodies (3 out of 21) isolated from V-preB$^-$L$^+$ B cells (Fig. 3 A). Finding that 14.5% of the antibodies from conventional B cells reacted with HEp-2 cell lysate was consistent with previous reports that 10–30% of IgMs from peripheral B cells transformed by Epstein-Barr Virus were similarly reactive and that 20% of naive B cells expressed such antibodies (1, 24, 25). To determine whether the HEp-2 ELISA-reactive antibodies were true ANAs, we performed indirect immunofluorescence assays (IFAs). Overall, 54% of V-preB$^+$L$^+$ antibodies tested showed true ANA reactivity in several distinct staining patterns including nucleolar (KR9), mitotic spindle apparatus (ED11), speckled (ED20, ED44), and other uncharacterized patterns (ED13, ED38, ED41, ED45) (Fig. 3 B). Three of the HEp-2–reactive antibodies expressed by V-preB$^+$L$^+$ B cells that were not ANAs displayed reactivity against the cytoskeleton with patterns reminiscent of anti–stress fiber (ED16), antivinculin (ED19), and antivimentin (ED37) antibodies (Fig. 3 B). In contrast, none of the 21 antibodies cloned from conventional V-preB$^-$L$^+$ B cells showed authentic ANA staining. We conclude that a high proportion of V-preB$^+$L$^+$ B cells express ANAs and other self-reactive antibodies, whereas conventional B cells rarely express ANAs.

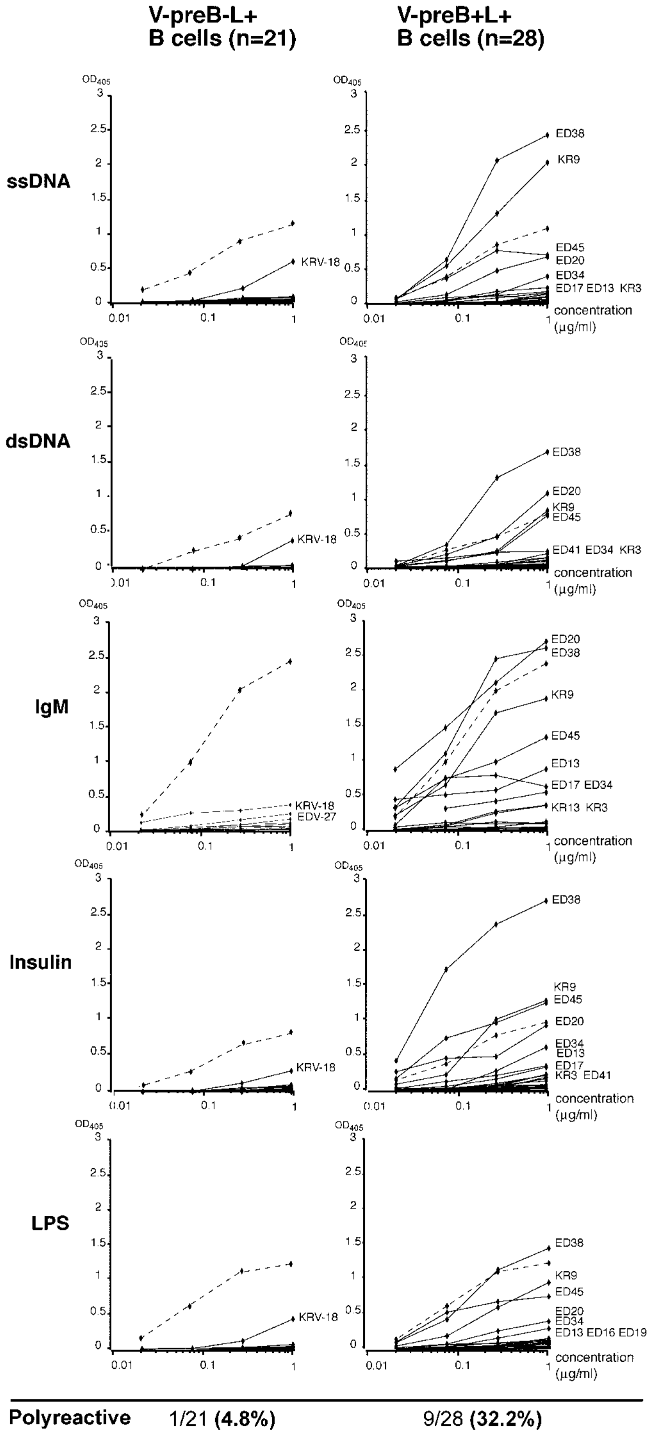
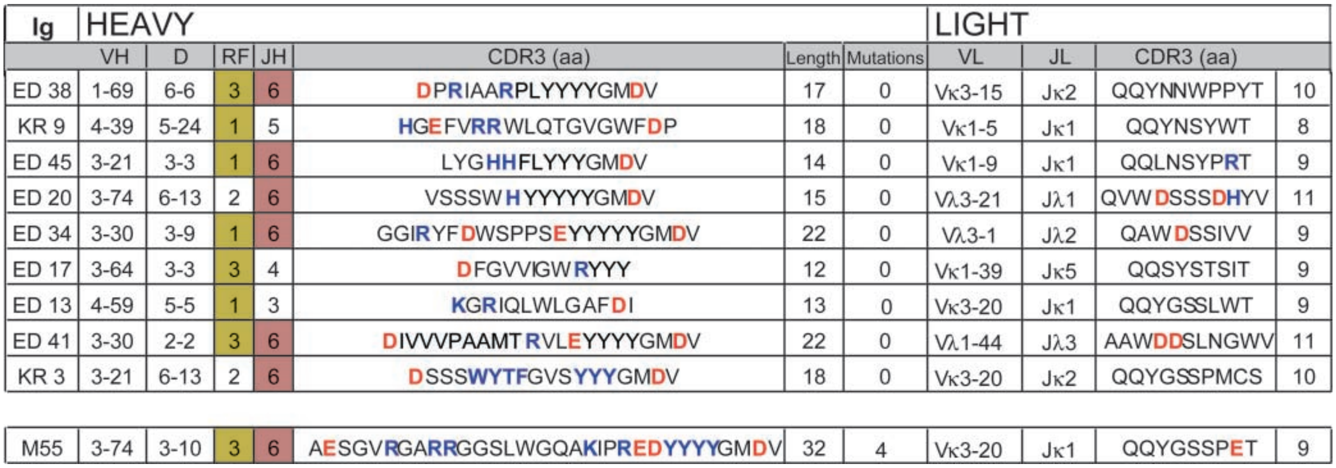


Figure 4. V-preB$^+$L$^+$ antibodies are polyreactive. Recombinant antibodies from conventional V-preB$^-$L$^+$ (left) and V-preB$^+$L$^+$ B cells (right) were tested for anti–single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), IgM, insulin, and LPS reactivity by ELISA. Human M55 mAb (dotted line) was included as a positive control polyreactive antibody (22). The percentage of polyreactive clones for each fraction is indicated.

V-preB$^+$L$^+$ B Cell Antibodies Are Polyreactive. Autoantibodies reactive against DNA and Ig are prevalent in the serum of patients with systemic lupus erythematosus and rheumatoid arthritis, respectively. To determine whether V-preB$^+$L$^+$ antibodies recognize such antigens, we performed ELISAs for single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), IgM, insulin, and lipopolysaccharide (LPS) (Fig. 4). As a positive control for polyreactivity, we used M55, a well-characterized polyreactive human antibody (22). 43% of antibodies expressed by V-preB$^+$L$^+$ cells (12 out of 28) recognized at least one of the above antigens and 32% (9 out of 28) bound to two or more antigens and were therefore polyreactive (Fig. 4). All of the polyreactive antibodies isolated from V-preB$^+$L$^+$ B cells showed long IgH CDR3s enriched in positively charged, hydrophobic, and aromatic amino acid residues encoded by unusual D reading frames and germline J_H6 segments (Fig. 5). Thus, the polyreactive antibodies showed the typical signature of V-preB$^+$L$^+$ Igs (5, 21). In contrast, only 4.8% (1 out of 21) of the antibodies expressed by conventional B cells were polyreactive, and these antibodies showed lower levels of reactivity than those from V-preB$^+$L$^+$ or M55 controls (Fig. 4). Similar low frequencies of polyreactivity were found in 93 antibodies cloned from naive human B cells (1). The one weakly polyreactive antibody isolated from conventional B cells differed from V-preB$^+$L$^+$ polyreactive antibodies in having a short IgH CDR3 without positively charged residues (KRV-18; Table S1). We conclude that antibodies expressed by V-preB$^+$L$^+$ B cells are frequently polyreactive.

Discussion

V-preB$^+$L$^+$ B cells are a subset of circulating human B cells that express antibodies with sequence features that have been associated with self-reactivity (5, 21). However,



Ig	HEAVY							LIGHT			
	VH	D	RF	JH	CDR3 (aa)	Length	Mutations	VL	JL	CDR3 (aa)	
ED 38	1-69	6-6	3	6	DPRIAARPLYYYYGMDV	17	0	Vκ3-15	Jκ2	QQYNNWPPYT	10
KR 9	4-39	5-24	1	5	HGEFVRRWLQTGVGWFDP	18	0	Vκ1-5	Jκ1	QQYNSYWT	8
ED 45	3-21	3-3	1	6	LYGHHFLYYYGMDV	14	0	Vκ1-9	Jκ1	QQLNSYPRT	9
ED 20	3-74	6-13	2	6	VSSSWHYYYYYGMDV	15	0	Vλ3-21	Jλ1	QVWDSSSDHYV	11
ED 34	3-30	3-9	1	6	GGIRYFDWSPPSEYYYYYGMDV	22	0	Vλ3-1	Jλ2	QAWDSSIVV	9
ED 17	3-64	3-3	3	4	DFGVVIGWRYYY	12	0	Vκ1-39	Jκ5	QQSYSTSIT	9
ED 13	4-59	5-5	1	3	KGRIQLWLGAFDI	13	0	Vκ3-20	Jκ1	QQYGSSLWT	9
ED 41	3-30	2-2	3	6	DIVVVPAAMTRVLEYYYYGMDV	22	0	Vλ1-44	Jλ3	AAWDDSLNGWV	11
KR 3	3-21	6-13	2	6	DSSSWYTFGVSYYYGMDV	18	0	Vκ3-20	Jκ2	QQYGSSPMCS	10
M55	3-74	3-10	3	6	AESGVRGARRGGSLWGQAKIPREDYYYYGMDV	32	4	Vκ3-20	Jκ1	QQYGSSPET	9

Figure 5. Polyreactive antibodies from V-preB$^+$L$^+$ B cells and M55 display similar IgH CDR3 features. Hydrophobic D reading frame and JH6 segments are in olive and brown boxes, respectively. Basic residues are in blue, and acidic amino acids are in red.

antibody sequences alone cannot predict autoreactivity (1). Indeed, transgenic mouse B cells that express authentic self-reactive heavy chains usually produce innocuous antibodies because of light chain receptor editing (26). To determine whether V-preB$^+$L$^+$ B cells express self-reactive antibodies, we cloned antibodies from purified single cells. We report herein that most V-preB$^+$L$^+$ B cells express self-reactive antibodies. Antibodies produced by V-preB$^+$L$^+$ B cells differ from low affinity self-reactive or polyreactive antibodies produced by 10–20% of all naive peripheral B cells in several respects (1, 24, 25). V-preB$^+$L$^+$ antibodies show higher affinities for self-antigens, and most V-preB$^+$L$^+$ antibodies are true ANAs, whereas these are infrequent (~4%) among conventional B cells (1). In addition, V-preB$^+$L$^+$ antibodies display specific sequence features that are associated with autoantibodies (5, 21). They frequently have long CDR3s enriched in aromatic and positively charged amino acids, a combination that favors anti-DNA reactivity (2–5). Positively charged amino acids can interact with the negatively charged phosphate backbone in nucleic acids, and long CDR3s enriched in aromatic residues may facilitate the interaction by stacking (27, 28). These features may also favor polyreactivity by creating a flexible binding site (29).

55% of early immature B cells found in the bone marrow of normal humans express ANAs and polyreactive antibodies. The ontology of V-preB$^+$L$^+$ B cells is unknown, but their surface features differ from early immature B cell precursors that also frequently produce self-reactive antibodies (1). For example, V-preB$^+$L$^+$ B cells do not express immature B cell markers such as CD38 and CD10 (5). Most importantly, early immature B cells do not express surface B cell receptor (1). However, the antibodies expressed by V-preB$^+$L$^+$ B cells resemble those found in early immature B cells in that they are polyreactive antibodies with long IgH CDR3s and increased frequency of positively charged residues (1). Most of these self-reactive antibodies are removed from the repertoire during the immature B cell stage in the bone marrow and in the transition between the immature B cell and naive B cell stage in the periphery (1). Thus, very few B cells producing highly polyreactive antibodies and ANAs are found in the mature B cell compartment, but they are highly enriched in the V-preB$^+$L$^+$ B cell subpopulation. How tolerance is maintained by V-preB$^+$L$^+$ B cells is not known, but these cells do not show low B cell receptor surface levels, and therefore, they do not resemble anergic B cells (10).

Since expression of surrogate light chains encoded by λ-like and V-preB genes is normally extinguished in immature B cells, why do V-preB$^+$L$^+$ B cells retain V-preB protein expression after completing B cell development? One clue to a selective advantage for V-preB expression in these cells may be that a large number of the V-preB antibodies bind to DNA. In the mouse, DNA binding antibodies that carry positively charged residues in IgH CDR3 are silenced or "edited" by light chains that have CDRs with low isoelectric points (26). Similarly, in transgenic mice that carry anti-DNA–reactive antibodies, self-reactive B cells are normally deleted in early B cell development, but coexpression of an additional Ig light chain with a low isoelectric point allows continued development, possibly by dilution of autoreactive receptors (30). These dual receptor-expressing B cells then accumulate in the marginal zone B cell compartment. Human V-preB with an isoelectric point of 5.67 may act as an "editor" and substitute conventional editing by light chain gene replacement by neutralizing positively charged IgH CDR3s expressed by V-preB$^+$L$^+$ B cells. In contrast, mouse V-preB1 and V-preB2 proteins have CDRs with isoelectric points of 9.37 and would therefore be unable to edit antibodies with positively charged IgH CDR3s. We propose that sustained expression of V-preB proteins is similar to dual receptor expression in autoreactive mouse B cells (30) and may rescue V-preB$^+$L$^+$ B cells from central deletion.

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