

A Novel Anti-Vpre-B Antibody Identifies Immunoglobulin-Surrogate Receptors on the Surface of Human Pro-B Cells

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Summary

Vpre-B and $\lambda 5$ genes, respectively, encode V-like and C-like domains of a surrogate immunoglobulin light chain (ΨL). Such ΨL complex is expressed in early progenitor B (pro-B) cells, before conventional immunoglobulin heavy (μH) and light (L) chains are produced. We raised a wide panel of monoclonal antibodies (mAbs) against soluble recombinant Vpre-B proteins to study early events in human B cell development. One of these antibodies, B-MAD688, labeled surrogate Ig-complexes on the surface of μH^- pro-B cell lines and normal bone marrow cells in immunofluorescence assays. Immunoprecipitations using surface-labeled pro-B cells and B-MAD688 mAb indicated that human ΨL is associated with high molecular weight components homologous to the surrogate heavy (ΨH) chains described in mouse. Using B-MAD688 and SLC2 mAbs, we were able to distinguish between $\Psi H\Psi L$ and $\mu H\Psi L$ complexes on the surface of human pro-B and later precursor, pre-B, cells. The finding of $\Psi H\Psi L$ complexes in mouse and man lead us to hypothesize a role for ΨH -containing receptors in B cell development.

B-lymphocyte lineages are best defined by the expression of their clonal receptors for antigen, the immunoglobulins (Ig). Hence, stages in B cell development pathways are drawn in terms of the rearrangement state of Ig heavy (H) and light (L) chain genes. Rearrangement of H and L chain loci is markedly asynchronous, creating H^+L^- or H^-L^+ intermediate receptors that might be implicated in further B cell differentiation (1–4).

Whereas H chains are not transported to the cell surface of mature B cells in the absence of L chains, this is not the case for B cell progenitors (3–6). Best characterized is the association of the proteins encoded by the Vpre-B and $\lambda 5$ genes that constitutes a surrogate L (ΨL) complex, which replaces the requirement for L in the transport of Ig-like complexes to the cell surface (5–7). Mouse mutants deficient in this ΨL complex, created by targeted disruption of $\lambda 5$ gene, show a B cell deficiency phenotype (8). Analyses of ΨH and ΨL expression patterns and function are therefore of interest to know how B cell progenitors differentiate, and to study key molecular events in B cell development.

Mouse models suggest that the ΨL proteins couple first with ΨH on the surface of pro-B cells and later with conventional μH chains on pre-B cells (3–5). Similar steps were proposed in man (9), although none of the monoclonal antibodies (mAbs) reported yielded any immunoprecipitate. Contrary to this hypothesis, other anti-human ΨL mAb recognized $\mu H\Psi L$ receptors in pre-B cells but failed

to detect surface $\Psi H\Psi L$ complexes in human pro-B cells, suggesting that ΨL associates only with μH chains (6).

We have produced human recombinant soluble Vpre-B molecules to characterize novel mAbs against ΨL -containing receptors. One of these mAbs, B-MAD688, labels Vpre-B on the surface of H^-L^- pro-B tumors and also detects equivalent B cell progenitors [i.e., surface μH^- , CD10^{bright} or CD19^{dull}] in human bone marrow. We show that this mAb and the anti- $\lambda 5$ SLC2 mAb (6) can indeed discriminate whether ΨL is associated with either $\Psi H\Psi L$ or $\mu H\Psi L$ Ig-like complexes on human pro-B and pre-B cell lines. The above findings suggest a B cell development scheme. B-MAD688 mAb may facilitate the analyses of Ig-surrogate receptors, and their relationship with the growth and differentiation requirements of B cell progenitors in man.

Materials and Methods

Production of Human Soluble Vpre-B Proteins. Plasmids pCEH-Vpre-B/mC κ and pCEH-Vpre-B/h $\gamma 1$ were constructed by insertion of a human full-length Vpre-B DNA into two distinct expression vectors containing either mouse C κ or the hinge-CH₂-CH₃ domains of human IgG₁ heavy chain, respectively, kindly provided by Dr. Karjalainen (10). The published sequence for the hVpre-B gene ends prematurely at a PstI site upstream to the termination codon due to the sequencing strategy. To allow for a full-length cloning, we have obtained the last 18 residues sequence from a genomic Vpre-B clone, pHVPB-6 created by

Bauer et al. (11). The missing DNA sequence is GCCAGGACACGTGTCCCT, coding for Ala-Arg-Thr-Arg-Val-Pro amino acids. This information was then used to subclone Vpre-B DNA into the two expression vectors after generating the required complementary overhangs using oligonucleotides and the polymerase chain reaction to introduce EcoRI and HindIII restriction sites at the 5' and 3' ends, respectively. The overhang at the 3' primer also substitutes the termination codon for a splice donor site to create two distinct chimeric Vpre-B soluble proteins, fused to either mouse C κ or human γ 1 domains according to the vector used. (Fig. 1 A). The primers were (sense) 5'-AGACTAGAATTCATG-

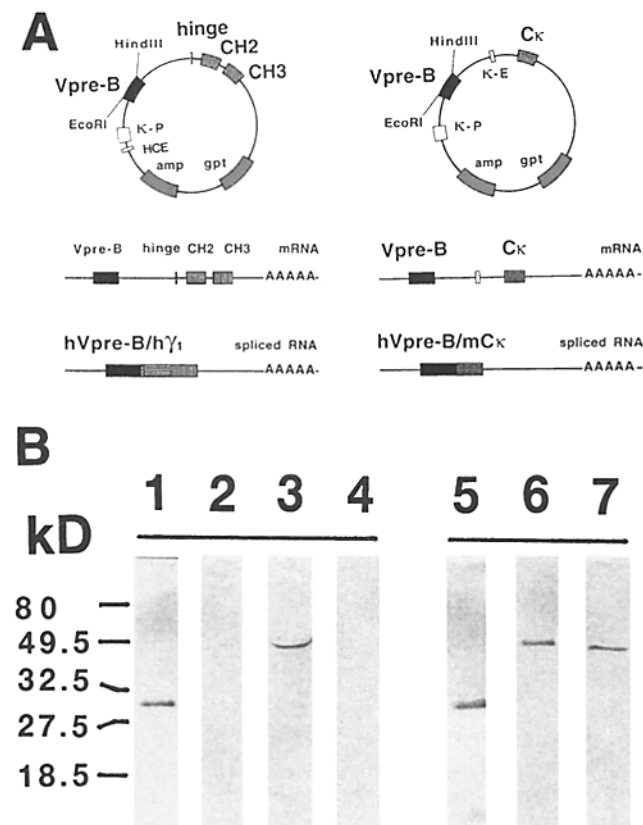


Figure 1. B-MAD688 mAb specifically recognizes soluble Vpre-B recombinant proteins. A palette of single-chain chimeric proteins was created using expression vectors that allow the generation of fusion proteins through the splicing of their coding RNA messages (A). The expression vectors used (10) splice the genes of interest into either the human Ig γ 1 hinge-CH2-CH3 constant domains (h γ 1, left) or the constant domain of mouse κ light chain (mC κ , right). They contain the adequate sequences for the expression of the chimeric proteins in myeloma cells (κ -P, κ -promoter; HCE, heavy chain core enhancer, κ -E, κ -enhancer; polyadenylation) and selection (*amp*, ampicillin; *gpt*, mycophenolic acid resistance). In B, purified recombinant proteins were probed in several independent Western blots with either the B-MAD688 mAb (lanes 1-4), or antisera specific for the fusion domains (lanes 5 and 6, anti-mC κ ; lane 7, anti-h γ 1). The chimeric proteins were hVpre-B/mC κ , lanes 1 and 5; mCD2/mC κ , lanes 2 and 6; hVpre-B/h γ 1, lanes 3 and 7; and hCD4/h γ 1, lane 4. Similar results were obtained for the other B-MAD mAbs reported here. The selected anti-soluble Vpre-B mAb do not bind in parallel assays to several other natural and recombinant proteins that lack Vpre-B but include the same fusion domains (hIL-2/mC κ and hIL-2/h γ 1 chimeras; and, hIgG γ 1 and mIgG κ monoclonals, data not shown). The apparent molecular weights matched the predicted chimeric nature of the purified proteins employed.

TCCTGGGCTCCTGTC and (antisense) 5'-TGATACTTACCAGGGACACGTGTCCTGGCTGC. The constructs were sequenced to confirm the fidelity of the inserts, and they were transfected into J558L myeloma cells by the protoplast fusion method (10). Mycophenolic acid resistant clones were selected and their supernatants were checked for the production of fusion proteins by ELISA and Western assays, using antiserum against either mC κ or the h γ 1 domains. The best producer clones were subcloned four times to stabilize high secretion levels. The hVpre-B/h γ 1 and Vpre-B/mC κ fusion proteins were purified after protein A-sepharose and anti-mouse-C κ -Affigel-10 affinity chromatographies from serum-free culture supernatants.

Identification of B Cell Hybridomas that Produce Antibodies Specific against Soluble Human Vpre-B. BALB/c female mice were immunized four times with purified hVpre-B/h γ 1 protein (5 μ g/mouse/d) in the rear left footpad. The antigen administered at day 0 was emulsified in complete Freund adjuvant, and in PBS for days 3, 7, and 14. Samples from tail blood were monitored for anti-hVpre-B/mC κ activity. The left popliteal lymph node from the best responder was obtained 24 h after the final boost, a single cell suspension was prepared, and the lymphocytes fused to the X63 myeloma using the Köhler and Milstein's protocol. Supernatants from the hybridoma clones arising from the fusion were tested for anti-Vpre-B activity using ELISA and Western assays, including hVpre-B/mC κ and hVpre-B/h γ 1 proteins. The mAb specificity was established using in parallel a broad palette of recombinant and natural proteins containing the fusion domains. The palette included hIL-2/mC κ , mCD2/mC κ , hIL-2/h γ 1, hCD4/h γ 1, pure human IgG1 from a myeloma patient and mouse C λ or C κ -containing monoclonals. mAbs recognizing the Vpre-B domain in the two chimeric soluble proteins, that did not cross-react to mouse C κ or the human IgG1 domain were selected for further immunofluorescence screening of binding to surface Vpre-B on several human cells. Four mAbs which reacted specifically and in a dose-dependent fashion on ELISA plates coated with the recombinant Vpre-B proteins are characterized here.

Human Cells. We used cell lines that represented different stages in the development of the B cell lineage. They included REH and 207 pro-B cells, 697 and Nalm-6 pre-B cells, and Daudi (IgM, κ), Ramos (IgM, λ), and JY (IgG, κ) mature B cells (6). Other hematopoietic and non-hematopoietic cell lines included were K562, Jurkat, U937, and HeLa. The origins and the state of Ig gene loci of the cell lines used are listed elsewhere (6, see Table 1). All cells were maintained in RPMI-1640 supplemented with 2 mM L-glutamine, 10% FCS (BioWhittaker, Verviers, B) and 5×10^{-5} M 2-ME (Fluka, Buchs, CH). Samples from bone marrow were obtained after informed consent from healthy donors involved in allogeneic transplants.

Antibodies and Immunofluorescence Assays. Mouse mAb were DA4.4 (IgG γ 1, κ) specific for human μ H chain, SLC1 (IgG γ 1, κ), SLC2 (IgM, κ), and SLC3 (IgM, κ) specific for human Ψ L chain components (6). These were a kind gift of Dr. Lassoued. CD19 and CD10 mAb directly conjugated with FITC were purchased from Becton-Dickinson (MountainView, CA). B-MAD mAbs reported herein (IgM, κ) were purified by size-exclusion chromatography, and DA4.4 was purified by ProtA columns. They were biotin-labeled using standard protocols. In three-color studies with biotinylated-DA4.4 we used streptavidin-TC (CALTAG Labs., South San Francisco, CA). For immunofluorescence staining, cells (1.5×10^5 /test) were incubated with 1 μ g of purified mAb or 50 μ l of culture supernatant in 96-well conical bottom plates at 4°C for 20 min. They were then washed three times with PBS-gelatin. Where necessary, they were incubated with the ap-

Table 1. Reactivity of the B-MAD mAbs with a Panel of Human Cell Lines

Cell Type	Cell Line	Ig gene Rearrangement		mAb reactivity			
		H	L	688	1112	176	792
Pro B (ψ H ψ L)	REH	N	N	+	+	-	-
	207	N	N	+	-	-	-
Pre B (H ψ L)	697	Y	N	+	+	+	+
	Nalm6	Y	N	+	+	+	+
Mature B (HL)	Ramos (μ λ)	Y	Y	-	-	-	-
	Daudi(μ κ)	Y	Y	-	-	-	-
	JY (γ κ)	Y	Y	-	-	-	-
T	Jurkat	N	N	-	-	-	-
Erythroleukemia	K562	N	N	-	-	-	-
Promyelocytic	U937	N	N	-	-	-	-
Epithelial	HeLa	N	N	-	-	-	-

Reactivity was determined by cell surface immunofluorescence as described in Materials and Methods. Y, functional rearrangement/protein available; N, no functional rearrangement/no protein.

appropriate second layer and washed three additional times. In the epitope competition assays, the cells were incubated in criss-cross combinations with 10 μ g/test of the competing mAb (unlabeled and purified) for 20 min. This was followed by 1 μ g/test of the relevant mAb (which was biotinylated or displayed a distinct isotype) for another 20 min. The unbound mAbs were removed by three washes with PBS and the bound relevant mAbs were revealed with the adequate second layer (streptavidin-PE for biotinylated-IgMs and anti-IgG-FITC for IgGs mAbs, respectively). The binding was quantitated using both XL or FACScan[®] cytofluorimeters (Coulter, Hialeah, FL, and Becton-Dickinson, respectively). Flow cytometry was rendered quantitative using QIFIKIT calibrator beads, which bear known amounts of an antigen (5,200 to 480,000) and a FITC-labeled antibody specific for the antigen (BIOCYTEX, Marseille, France).

Surface-labeling of Cells, Immunoprecipitation, and Western Experiments. Cells in culture ($10\text{--}20 \times 10^6$) were washed five times with cold washing buffer (PBS + 1 mM MgCl₂ + 0.1 mM CaCl₂). The cells were then incubated with 0.5 mg/ml of Sulfo-NHS-Biotin (Pierce Chem. Co., Rockford, IL) for 30 min at 4°C on a turning wheel. Biotinylation was stopped washing five times with washing buffer. Aliquots of 10^7 biotin-labeled cells were lysed in 1 ml of 1% NP40, 150 mM NaCl, 1 mM PMSF, 20 mM iodoacetamide and 20 mM Tris, pH 7.5, for 30 min on ice. Cell lysates were precleared four times with Affigel-10 beads (BIO-RAD, UK) preincubated with 5% nonfat dry milk. Precleared samples were incubated overnight at 4°C with 10 μ l of the indicated antibodies coupled to Affigel-10 beads (1 mg/ml). Immunoprecipitates were washed four times with lysis buffer plus 0.5 M NaCl and twice with lysis buffer, suspended in 10 μ l of reducing loading buffer, boiled for 2 min and submitted to SDS-PAGE. The gels were Western-blotted onto nitrocellulose membranes, using a Mini-Protean unit following the manufacturer's recommendations (BIO-RAD). After blocking with 5% nonfat dry

milk in PBST (PBS plus 0.1% Tween 20) the membranes were incubated with a 1:5,000 dilution of streptavidin-HRPO (Southern Biotechnology, Birmingham, AL) in PBST for 30 min at room temperature, and washed three times with PBST. Surface-biotinated proteins were revealed using the chemiluminescence ECL kit (Amersham, UK) after 1–5-s exposures to X-OMAT films (Kodak).

Results and Discussion

Production of Human Soluble Vpre-B Proteins and Isolation of Hybridoma Clones that Produce Specific Antibodies to Vpre-B. To raise anti-human Vpre-B specific antibodies we produced two distinct soluble Vpre-B proteins. Vpre-B was fused to Ig-constant domains to create hVpre-B/h γ ₁ or hVpre-B/mC κ recombinant single-chain molecules (Fig. 1 A). It is similar to the strategy we have used to map the subunit specificity of mAbs against the human CD3 transduction subunits of the T cell receptor for antigen (12).

Using purified hVpre-B/h γ ₁ protein as immunogen, we produced a panel of mAbs that indeed recognizes Vpre-B, as they specifically bind to the hVpre-B/h γ ₁ and hVpre-B/mC κ protein chimeras, as demonstrated in ELISA and Western assays (see Materials and Methods and Fig. 1 B).

We next screened the panel of anti-soluble Vpre-B mAbs for binding to Vpre-B on the surface of B cell precursors. Four mAbs were selected and further characterized in immunofluorescence and quantitative flow-cytometry assays. These anti-Vpre-B mAb, the B-MAD 176, 688, 792, and 1112 mAbs, label precursors of B lymphocytes but neither mature B cell lines nor cells from other lineages (Table 1 and Fig. 2 A).

Interestingly, the analysis of the staining patterns of two pro-B (REH and 207 IgH⁻L⁻ cells) and two pre-B (Nalm6 and 697 IgH⁺L⁻ cells) human lines revealed that the surface expression of Vpre-B emerges already in μ H⁻ pro-B cells, as shown using the B-MAD688 mAb (Fig. 2 A). The SLC1 and SLC2 mAbs rendered a distinct pattern (Fig. 2 A), which is the reported pre-B cell-confined staining (6). In this regard, the ability of anti-soluble Vpre-B mAbs to bind their ligand on the surface of both pro-B and pre-B cells was rare, whereas the pre-B cell-restricted cluster (i.e., SLCs (6), B-MAD176 or 792) is large and appears immunodominant. Other specificities however occurred, like the B-MAD1112 mAb that binds to the pre-B cells and the REH pro-B cell but not to the 207 pro-B cell (Fig. 2 A).

Vpre-B Is Expressed on the Surface of B Cell Progenitors from Normal Human Bone Marrow. We examined whether B-MAD688 mAb might be a tool for fluorescence-activated cell-sorting of equivalent Vpre-B⁺ precursor populations from bone marrow.

Two-color flow cytometry analyses showed that B-MAD688 mAb labels well discrete populations of bone marrow cells which are either CD10⁺ or CD19⁺ (Fig. 2 B). As B cell precursors mature they lose CD10 and gain CD19 (9). In this regard, the Vpre-B⁺ subsets were predominantly CD10^{bright} and CD19^{dull} cells. Triple-color studies showed that Vpre-B expression emerges in cells that

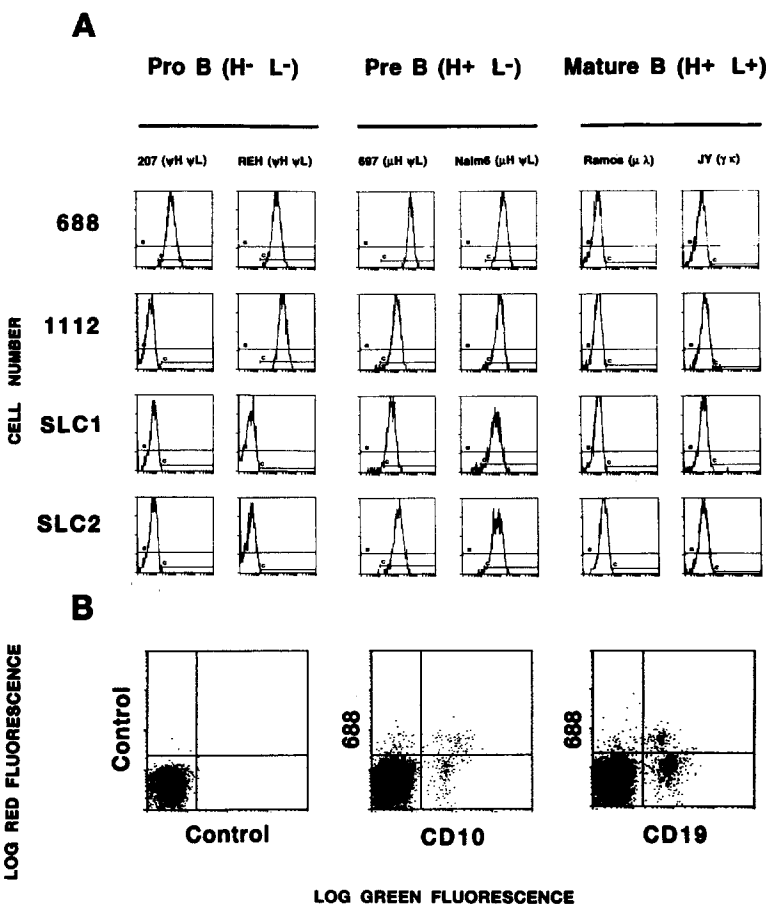


Figure 2. FACSTM analyses of Vpre-B/ λ 5 surrogate L (Ψ L) chains expressed on the surface of human pro- and pre-B cell lines, and normal bone marrow cells as detected by specific mAbs. In *A*, pro-B (207 and REH), pre-B (697 and Nalm6) and mature B (Ramos and JY) cell lines were incubated first with either control mAb, soluble Vpre-B specific mAbs B-MAD688 and 1112, Ψ L-specific mAb SLC1 or λ 5-specific mAb SLC2 (6), and then with FITC-conjugated anti-mouse Ig antiserum. Immunofluorescence was quantitated on an EPICS XLTM analyzer. Histograms depict the fluorescence distribution curves in a four decade logarithmic scale. In the case of control staining more than 99% of cells were below the C (right) statistic analysis bar. SLC3 rendered similar results to SLC1 and 2 mAb (not depicted in the sake of clarity). In *B*, Vpre-B-bearing cells in normal bone marrow were phenotyped by two-color flowcytometry in a FACScanTM analyzer. The lymphoid population was identified by its light scatter features and gated for these analyses. Cells were stained with control mAbs or anti-soluble Vpre-B mAb B-MAD688 (using an orange-red PE-label) and either CD10 or CD19 mAb (green FITC-labeled). In control stainings more than 99% of the cells were in the lower-left quadrant defined by the crossed statistics bars. In the dot plots depicted, 36% of the CD10⁺ cells (which are 3.5% of total) and 29% of the CD19⁺ cells (which are 8% of total) bound the B-MAD688 mAb.

lack surface μ H and are CD34⁺. These cells are the major Vpre-B⁺ bone marrow subset detected (i.e., 3/4 of the 688⁺ cells express CD34). No CD3⁺, CD14⁺, CD16⁺, CD33⁺, or CD56⁺ cells expressed Vpre-B (not shown). Thus, in normal cells, surface expression of Vpre-B emerges early in B cell development.

Surface Vpre-B Is Associated with Surrogate-Heavy Chains (Ψ H) in Human Pro-B Cells. The existence of human Ψ H Ψ L complexes has remained elusive due to the lack of antibodies able to immunoprecipitate surface Ψ L before μ H chain expression (6, 9). We sought to reassess this issue using the B-MAD688 mAb. Immunoprecipitations were carried out after surface biotin-labeling of lines arrested at distinct stages in B cell development. The precipitates were resolved by SDS-PAGE, Western blot and chemiluminescence.

Interestingly, the anti-Vpre-B B-MAD688 mAb does not co-precipitate μ H among Ψ L-associated proteins, but other components of higher molecular weight, from pre-B and pro-B cell lysates (Fig. 3). A major band of 125-kD substitutes for the absent μ H in the pro-B line REH, and was consistently detected in all nine experiments performed. Other weaker bands (roughly p200, p100, and p70-40) were evident upon longer exposure in some experiments. In contrast, analyses using the SLC2 mAb revealed the known subset of surface Ψ L that coprecipitates with the surface μ H but does not associate with Ψ H. The identity of conventional μ H was readily determined using the

DA4.4 anti- μ mAb in parallel tracks. Both anti- Ψ L mAbs did not recognize mature B cells (Fig. 3). The proteins coprecipitated by B-MAD688 mAb resemble the Ψ H chains found by Karasuyama, Rolink, and Melchers associated to

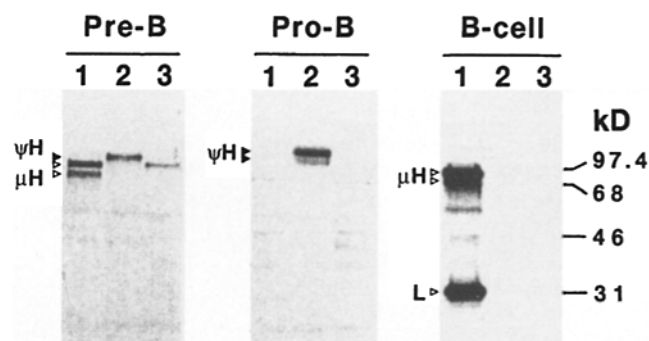


Figure 3. Vpre-B/ λ 5 surrogate L (Ψ L) chains are associated to both conventional (μ H) and surrogate (Ψ H) heavy chains on the surface of B-cell precursors in immunoprecipitation assays. Cells representative of distinct stages of B-lymphocyte development, pre-B (697), pro-B (REH) and mature B (Ramos) cell lines, were surface labeled with biotin and lysed in 1% NP40 lysis buffer. Detergent-soluble lysates were reacted with either anti- μ H mAb DA4.4 (lanes 1), anti-VpreB mAb B-MAD688 (lanes 2) or anti- λ 5 mAb SLC2 (lanes 3), immunoprecipitates were resolved by 10% SDS-PAGE under reducing conditions, Western blotted, and revealed by chemiluminescence. Open arrowheads point to conventional heavy (H) and light (L) chains, filled arrowheads point to surrogate heavy chains (Ψ H). Apparent molecular mass, in kD, were estimated using a set of biotin-conjugated protein standards.

Ψ L in mouse μ H⁻ pro-B cells (5, 7, 13). The finding of p125 as the major Ψ H component parallels an independent report also using mouse pro-B tumor lines (14).

Human Vpre-B is not readily available during selective, vectorial labeling of the cell surface proteins using either ¹²⁵I (6) or biotin. Aliquots of pro-B and pre-B cell lysates were precipitated with B-MAD688 mAb, submitted to electrophoresis and revealed by Western blot using B-MAD688 mAb. The anti-Vpre-B antibody reacted with a 18-kD band (i.e., native Vpre-B size), but not with the 125-kD protein or other higher molecular weight bands (not shown).

We studied whether anti-Vpre-B mAbs B-MAD688 and 1112, and SLC mAbs recognize different epitopes on surface Ψ L chains from pre-B cells, as suggested by the immunofluorescence clusters and immunoprecipitation results. The SLC1, SLC2, and SLC3 mAb epitopes are overlapping (6). Our results of criss-cross competition analyses evidenced that SLC1 showed a clear but partial (30%) competition with B-MAD1112 mAb, again indicative of overlapping or neighbor, but not identical, epitopes. This competition pattern was distinct to the observed for the B-MAD688 mAb and for the anti- μ DA4.4 mAb, that did not show any cross-blocking of their binding by the mAb tested (data not shown).

Since B-MAD688 mAb recognizes VpreB and SLC2 mAb binds to λ 5 (6), it is not surprising that they could define distinct epitopes in human Ψ L. Our results however underscore that these two mAbs discriminate among Ψ H Ψ L and μ H Ψ L due to a differential display of Ψ L epitopes in those complexes. As B-MAD688 mAb recognizes only the former receptor, it defines a novel anti- Ψ L specificity (6, 9, 13).

Two other reactivities were established in the mouse (5, 13). First, mAbs that selectively bind to Ψ L when assembled in μ H Ψ L complexes (i.e., do not detect Ψ H Ψ L complex although may recognize free Vpre-B or λ 5, as shown before for SLC mAbs in man [6]). Second, mAbs that recognize Ψ L subunits but do not discriminate whether Vpre-B and λ 5 are associated to Ψ H or μ H chains; perhaps similar to some anti- Ψ L mAbs in man (9). The staining pattern of B-MAD 1112 mAb is yet difficult to interpret because the mAb does not render immunoprecipitates from pro-B or pre-B cell lysates.

Light and Enigmas on the Use of Ig-Surrogate Complexes and on the Definition of Human B Cell Developmental Pathways. Our results indicate that Vpre-B is a subunit shared by two Ig-like surface complexes, homologous in mouse and man. First, it emerges in Ψ H Ψ L receptors on pro-B cells. Second, it is component of μ H Ψ L complexes on pre-B cells. The existence of Ψ H has been controversial in man (6, 9).

The fact that SLC2 mAb binds to surface Ψ L chains only when they are associated with μ H chains might explain why Ψ H Ψ L complexes escaped detection by other authors (6).

The emphasis in the analyses of Ig-surrogate chains has been placed on human pre-B cells that are H⁺L⁻, on which μ H Ψ L pre-B receptors would play a developmental role (6, 9). Less attention was paid to a reciprocal H⁻L⁺ human pre-B cell pathway, defined by Kubagawa et al. (1). The finding of surface Ψ H chains in man opens the possibility that Ψ H may assemble with L into putative surface Ψ HL receptors on H⁻L⁺ pre-B cells in the Kubagawa's pathway. By analogy to the major pathway (15, 16), Ψ HL pre-B receptors could serve to sustain the rare cycling pre-B cells that bear only productive V-J-C_L rearrangements. The Ψ HL receptors might allelically exclude the L loci in pre-B cells at the minority pathway, while μ H loci attempts recombination and surface μ H can replace Ψ H in the successful B cell progeny. Two B cell differentiation pathways also occur in mouse but only the Ψ L-containing pre-B receptors were considered in the schemes (2, 3). Other authors pointed that a Ψ L-independent pathway predominates early in ontogeny when B-1a/CD5⁺ cells preferentially develop (17). To test whether the Ψ L-independent pathway uses Ψ H pre-B receptors requires the availability of probes for Ψ H chain components, especially considering that H⁻L⁺ cells are infrequent (2, 18).

Another intriguing observation, divergent from previous models (2-4, 6, 9), concerns the coexpression of Ψ H Ψ L and μ H Ψ L surrogate receptors on pre-B cell tumors. We could not readily detect a similar B-MAD688^{bright}, SLC1^{bright} bone marrow subpopulation (not shown). Similarly a "transition" μ H Ψ L⁺, μ H κ L⁺ step was shown in human tumors, but its in vivo counterpart was undetectable to the same mAb (6). It is worthy of note that, using similar methods in man (6) or mouse (7, 13), faint levels of surface μ H were reported in normal μ H Ψ L⁺ pre-B cells. Also, Ψ H Ψ L⁺ cells are readily shown ex vivo, whereas a majority of cytoplasmic μ H⁺ cells lack surface μ H Ψ L complexes (4, 6, 7). A sensitive analysis of surrogate chains expression and immunoglobulin loci status in single cells will be required to order the B cell development steps.

In summary, we raised mAbs against soluble human Vpre-B. We show that Vpre-B is expressed associated to Ψ H chains on the surface of pro-B cell tumors, and that the Vpre-B⁺/ Ψ H Ψ L complex detected by B-MAD688 mAb is a good marker to identify early B cell progenitors in human bone marrow. We propose that Ψ H-containing receptors might also participate in a second class of Ψ HL pre-B receptors devoted to drive the development of rare precursors that rearrange L-chains first.

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