A Complex of Glycoproteins Is Associated with \( V_{\text{preB}}/\lambda_5 \) Surrogate Light Chain on the Surface of \( \mu \) Heavy Chain-negative Early Precursor B Cell Lines

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Summary

Monoclonal antibodies (mAbs) have been made specific for the pre-B cell-specific proteins \( V_{\text{preB}} \) and \( \lambda_5 \) which together form the surrogate light (L) chain. mAbs specific for \( V_{\text{preB}} \) protein identified the 16-kD molecule associated on precursor B cell lines with \( \lambda_5 \) protein as the product of the \( V_{\text{preB}} \) gene. Surrogate L chain was detectable even in the absence of \( \mu \) heavy (H) chain on the surface of early precursor cell lines such as pro-B cell lines where all immunoglobulin (Ig) loci are in the germline configuration, as well as early pre-B cell lines where Ig H chain loci are \( D\lambda\mu \) rearranged in reading frame I or III, which does not allow the expression of a \( D\lambda\mu\mu \) protein. A complex of glycoproteins (200, 130, 105, and 65-35 kD) was identified as coprecipitated with the \( V_{\text{preB}}/\lambda_5 \) surrogate L chain in \( \mu \) H chain-negative precursor B cell lines. The 130-kD protein was most strongly labeled with iodine and most consistently detected in noncovalent association with surrogate L chain. This protein turned out to be a N-linked glycoprotein with a 100-kD protein core and isoelectric point 5.8, indicating that it is distinct from CD43 and the BP-1/6C3 antigen. The surface deposition of the surrogate L chain in association with the newly identified glycoproteins suggests that the surrogate L chain may function as a receptor even before the association with \( \mu \) H chain in early precursor B cells.

Two genes, \( V_{\text{preB}} \) and \( \lambda_5 \), are transcribed selectively in precursor B cells (1–3). Mature B cells, Ig-secreting plasma cells, and any other cells in the body so far tested do not express these two genes. The \( V_{\text{preB}} \) gene has sequence homology to V regions of Ig H and L chain genes, whereas part of the \( \lambda_5 \) gene displays homology to the J and C regions of the Ig L chain gene. Therefore, it was expected that the \( V_{\text{preB}} \) and \( \lambda_5 \) proteins might associate with each other to form an Ig L chainlike structure, the so-called surrogate L chain. It was further expected that the \( \lambda_5 \) protein of surrogate L chain could be disulfide linked via its penultimate cysteine at the COOH-terminal end to \( \mu \) H chain to form an Ig-like complex. Indeed, the formation of such a \( \mu \) H chain/\( \lambda_5 \)/\( V_{\text{preB}} \) complex has been proven by gene transfection experiments (4, 5).

Once the \( \lambda_5 \) and \( V_{\text{preB}} \) genes had been discovered, two proteins called \( \omega \) and \( i \) were found associated with \( \mu \) H chains on the surface of some pre-B cell lines (6, 7). One of them (\( \omega \)) was shown to be the product of the \( \lambda_5 \) gene, and the other (\( i \)) was predicted to be the product of the \( V_{\text{preB}} \) gene (7). Subsequently, \( \lambda_5 \) protein was detected with polyclonal \( \lambda_5 \)-specific antibodies on the surface of pre-B cell lines and bone marrow cells, although it remained to be proven that the 16-kD polypeptide coprecipitated with \( \lambda_5 \) protein was, indeed, the product of the \( V_{\text{preB}} \) gene (8, 9).

The \( \mu \) H chain/surrogate L chain complex expressed on pre-B cell lines has been found to transmit biochemical signals to the cells (9, 10), suggesting that this complex could function as a receptor on the surface of pre-B cells. The important role of \( \lambda_5 \) protein in the B cell development was demonstrated by targeted disruption of the \( \lambda_5 \) gene in the germline yielding a \( \lambda_5 \)-deficient mutant mouse strain (11). In these mice, B cell development is impaired at the transition from pre-B I to pre-B II cells, resulting in a depletion of 95% of all CD45R(B200)+ B cell precursors in bone marrow and in a delayed appearance of CD5− B cells in the peripheral lymphoid organs (11, 12).

It has been shown that the 22-kD \( \lambda_5 \) protein and the 16-kD protein (p16)—presumed to be the \( V_{\text{preB}} \) protein—are expressed together on the surface of \( \mu \) H chain-negative precursor B cell lines (9). This has raised the questions (a) is the p16 identical to \( V_{\text{preB}} \) protein, and if so; (b) how can the \( V_{\text{preB}}/\lambda_5 \) surrogate L chain be deposited in the surface membrane in the absence of \( \mu \) H chain?

In this paper, we describe the generation of mAbs specific for the \( V_{\text{preB}} \) protein or for \( \lambda_5 \) protein. We use them to study the synthesis, assembly, and surface membrane deposition of \( V_{\text{preB}}/\lambda_5 \) surrogate L chain in a panel of precursor B cell lines. We identify molecules that are associated with the \( V_{\text{preB}}/\lambda_5 \) surrogate L chain and that might take the place of...
Materials and Methods

Cell Lines. The origins and the state of Ig gene loci of pro-
and pre-B cell lines used in this study are listed (see Table 1). The cell lines have been described as the following: 63-12 and 63-24 (13); 38B9, 40E1, 204-1-8, 300-19P, 220-8, 28C9, and 204-3-1 (14, 15); 18-81 (16); 702/3 (17); NF53.5 (18); B3-P8-16-1-19; and 38C13 (20). Clone 18, PAL1, and NP were established as described (21, 22). B cell lymphoma cells used as control were IgM (μ), κ-expressing WEHI231 (23) and IgM (μ, λ)-expressing CH1-1 (24).

All cell lines were maintained in IMDM supplemented with 10% FCS (Northumbria Biologicals Ltd., Cramlington, UK), 100 U/ml of penicillin-streptomycin (Gibco Laboratories, Grand Island, NY), 2 mM L-glutamine (Gibco Laboratories), and 5 × 10⁻⁵ M 2-ME.

Antibodies. Rat mAbs used were M41 (IgG1) specific for murine γ H chain (25), 187.1 (IgG1) specific for murine κ L chain (26), R1-2 (IgG2b) specific for lymphocyte Peyer's patch high endothelial venules (HEV) adhesion molecule 1 [LPAM-1] (murine very late antigen [VLA] 400 (27), M1/9.3.4 (IgG2a) specific for CD45 (28), and Str10 (IgG) specific for MHC class I (Rolin A., unpublished observations).

Vectors and DNA Transformation. BCMGSHygλ5 and BCMGSHygνpri were constructed by insertion of λ cDNA (1) or ν pri cDNA (3), respectively, into the expression vector BCMGSHyg (4). Ig-nonproducing X63.Ag8.653 myeloma cells (29) were transfected by electroporation with either BCMGSHygλ5, BCMGSHygνpri, or BCMGSHygλ5 ν pri (4) and selected with hygromycin B (0.8 mg/ml, Boehringer Mannheim GmbH, Mannheim, Germany) to obtain single or double transfectants. Transfectants expressing high levels of transcripts specific for λ and/or ν pri detected by RNA dot blot were subcloned by limiting dilution. The establishment of Ltk- λL V pri fibroblast Ltk- cells transfected with three genes encoding γ H chain, λ L, and ν pri has been described previously (4).

Preparation of mAbs Specific for λ5 and ν pri. The μ γ chain/ 
λ5 ν pri complex was purified by absorption to M41-conjugated Sepharose beads from culture supernatants of a fibroblast transfectant Ltk- μL V pri which secreted a soluble form of the complex at a concentration of 200-300 ng/ml (4). Female Lewis rats (Institut für Medizinische Forschung AG, Fällinsdorf, Switzerland) were immunized intraperitoneally four times at 2-8 wk intervals, each time with 7 μg of the complex absorbed to the beads. Sera taken from the animals after a fourth immunization contained antibodies to the complex as detected by immunoprecipitation.

One of the animals was given a final boost with intravenous and intraperitoneal injections of 70 μg of the complex eluted from M41-conjugated beads with glycine-HCl buffer (pH 2.7). 3 d after the final injection, spleen cells (3.2 × 10⁹) were fused with murine X63.Ag8.653 myeloma cells (5.7 × 10⁴) using polyethylene glycol 1500 (Boehringer Mannheim) and distributed at a density of 1.6 × 10⁶ spleen cells/well in 1.968 microtiter wells with medium containing HAT and rIL-6 (30). Each well had several colonies of hybridoma in 10 d. Culture supernatants of hybridomas were initially screened by ELISA for reactivity to μL ν pri complex but not to IgM (μκ and μλ). Supernatants of selected cultures were further characterized by cell surface staining as well as immunoprecipitation and cloned twice by limiting dilution. All the mAbs to λ5 and ν pri described here were of the IgG2a subclass and had κ L chains as determined by the isotyping kit (Serotec, Oxford, UK).

Cell Labeling and Immunoprecipitation. For biosynthetic labeling, cells (3-5 × 10⁶) were cultured at 37°C for 4 h in 1 ml of methionine-free RPMI-1640 medium or cysteine-free RPMI-1640 medium (RPMI-1640 Select-Amine kit; Gibco Laboratories) supplemented with 10% dialyzed FCS and 0.3-0.5 μCi of [³⁵S]methionine (1,000 Ci/mmol; Amersham International, Amersham, Bucks, UK) or [³⁵S]cysteine (1,300 Ci/mmol, Amersham) and lysed for 30 min on ice in 1 ml of NP-40 lysis buffer (1% NP-40, 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 50 mM iodoacetamide, 0.02% NaN₃, 1 mM PMSF, 2 μg/ml aprotinin, and 1 μg/ml pepstatin). In case of Ltk- μL V pri, cells (2 × 10⁶/0.5 ml/well) were pulsed for 10 h with 0.1 μCi [³⁵S]methionine and culture supernatants were collected.

For cell surface labeling, cells (5 × 10⁶) were suspended in 1 ml PBS and iodinated for 40 min at room temperature by addition of 50 μl 200 mM d-glucose, 1 μCi of Na[¹²⁵I] (15.3 μCi/μg of iodine, Amersham), and 50 μl lactoperoxidase (50 μU/ml, Sigma Chemical Co., St. Louis, MO)/glucose oxidase (10 U/ml, Sigma Chemical Co.) solution, and lysed as above.

Cell lysates and culture supernatants were preincubated with protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) preincubated with normal rat serum. Preincubated samples were incubated with mAbs at 4°C for 1 h followed by incubation with affinity-purified rabbit anti-rat IgG antibodies (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) for 30 min and then with protein A-Sepharose beads for 1 h. Immunoprecipitates were washed as described (4), suspended in 50 μl of reducing or nonreducing Laemmli sample buffer, boiled for 2 min, and subjected to SDS-PAGE. Gels were stained with Coomassie blue for 20 min, destained of 50% of iodine, Amersham), and 50 μl lactoperoxidase (50 μU/ml, Sigma Chemical Co., St. Louis, MO)/glucose oxidase (10 U/ml, Sigma Chemical Co.) solution, and lysed as above.

Cell lysates and culture supernatants were preincubated with protein A-Sepharose beads for 1 h. Immunoprecipitates were washed as described (4), suspended in 50 μl of reducing or nonreducing Laemmli sample buffer, boiled for 2 min, and subjected to SDS-PAGE. Gels were stained with Coomassie blue for 20 min, destained for 4 h, soaked for 30 min in Enlightening (New England Nuclear, Boston, MA) only in the case of [³⁵S]-labeled samples, and dried. Dried gels were autoradiographed at -70°C on X-Omat AR film (Eastman Kodak, Rochester, NY) with intensifying screens. For deglycosylation of proteins, immunoprecipitates were resuspended in 20 μl elution buffer (0.5% SDS, 50 mM Tris-HCl, pH 8) and boiled for 2 min, followed by addition of 3 μl 10% octylglucoside (Boehringer Mannheim) and 3 μl N-glycosidase F (PNGase F, 200 U/ml; Boehringer Mannheim) and incubation at 37°C for 18 h. In two-dimensional nonreducing/reducing SDS-PAGE, precipitates were run in the first dimension under nonreducing conditions using a 7-15% gradient polyacrylamide slab gel. The relevant strips were then cut out, incubated in Laemmli sample buffer with 20 mM dithiothreitol for 30 min, and run in the second dimension on a 7-15% gradient SDS-PAGE gel. Two-dimensional IEF/SDS-PAGE and nonequilibrium pH gradient electrophoresis (NEPHGE)/SDS-PAGE were performed as described (31, 32), using the ampholyte Pharmalyte 2D 3-10 (Pharmacia).

Cell Surface Staining. Cells (10⁴) were incubated with 100 μl of hybridoma supernatant diluted 1:1 in staining buffer (PBS, 0.2% BSA, 0.1% NaNNi) for 30 min on ice. After three washes with staining buffer, cells were incubated for 30 min with 100 μl of 1:100 diluted FITC-conjugated MARK1 (mouse mAb to rat κ L chain; Immunotech S.A., Marseille, France). Immunofluorescence was analyzed on a FACScan® (Becton Dickinson & Co., Mountain View, CA).

Results

Establishment of mAbs Specific for Murine λ5 and ν pri. An Ltk- fibroblast (Ltk- μL ν pri) transfected with the three

1 Abbreviations used in this paper: NEPHGE, nonequilibrium pH gradient electrophoresis; PNGase F, N-glycosidase F.
murine genes coding for μ H chain, λs, and VprB, secretes three proteins (86, 22, and 16 kD, respectively) as a complex (4) that can be precipitated with rat mAb M41 specific for mouse μ H chain (Fig. 1A, lane 2). The complex was purified from culture supernatant of Ltk−μλ5VprB cells by immunoadsorption on M41-conjugated Sepharose beads and used for immunization of rats. Hybridomas were prepared by fusion of spleen cells from an immunized rat. The initial screening of hybridoma supernatants was performed by ELISA using microtiter plates coated with either the μλ5VprB complex, IgM (μκ), or IgM (μλ). 11 mAbs were selected which had reactivity to the complex, but not to IgM (μκ and μλ) (data not shown).

7 out of the 11 mAbs precipitated μλ5VprB complex from culture supernatant of the Ltk−μλ5VprB transfectant as did the μ H chain–specific mAb M41 (two representative mAbs are shown in Fig. 1A, lanes 3 and 4). To identify the reactivity towards antigenic determinants in the complex further, Ig-nonproducing myeloma X63-Ag8.653 cells transfected with either the λs gene or the VprB gene alone, or with the two genes were employed. Five of the mAbs recognized a 22-kD protein present in the λs transfectant but not in the VprB transfectant, whereas two other mAbs detected a 16-kD protein present in the VprB transfectant but not in the λs transfectant (two representatives LM34 and VP245 are shown in Fig. 1B, lanes 2, 3, 5, and 6). This demonstrates that the former five mAbs are specific for the λs protein, whereas the latter two are specific for the VprB protein. For all subsequent experiments, two mAbs, the λs-specific mAb LM34 and the VprB–specific mAb VP245, were used.

λs protein and VprB protein were coprecipitated with each other by either mAb LM34 or VP245 from X63-Ag8.653 cells transfected with both λs and VprB genes (Fig. 1B, lanes 8 and 9). This shows that λs and VprB proteins can be associated with each other to form a surrogate L chain even in the absence of μ H chain. Thus, the established mAbs recognize λs protein or VprB protein as a single or in a form of VprB/λs surrogate L chain, or in the complex of μλ5VprB.

The Synthesis and Cell Surface Expression of λs and VprB Proteins in Pro- and Pre-B Cell Lines. The synthesis of the λs and VprB proteins was monitored in a panel of transformed pro- and pre-B cell lines, as well as nontransformed pre-B cell lines, which are thought to represent various stages of B cell development in fetal liver or bone marrow (Table 1). Cells were biosynthetically labeled with [35S]methionine, and cell lysates were analyzed by immunoprecipitation with specific mAbs. All the cell lines tested were found to produce the 22 kD λs and the 16-kD VprB proteins as a complex, irrespective of whether they produced μ H chain or D,JHCμ protein (33) or none of them (data not shown, but summarized in Table 1).

Next we examined the expression of λs and VprB proteins on the surface of these cell lines by flow cytometry (Fig. 2 and Table 1). All μ H chain–producing pre-B cell lines tested expressed λs and VprB proteins together with μ H chain on their surface (shown for NFS5.3 in Fig. 2, and not shown for others but summarized in Table 1). On the other hand, B cell lines WEHI231 expressing IgM(μκ) and CH-1 expressing IgM(μλ) were not stained with mAbs LM34 nor VP245 (Fig. 2), showing once more that the mAbs do not crossreact with κ or λ L chains. The two cell lines 38C13 and B3-P8-16-1-μ, which appear to be in transit from pre-B to B cells, expressed on the surface κ L chain as well as λs, VprB proteins, and μ H chains (Fig. 2 and Table 1). Proteins

Figure 1. Specificity of mAbs for either λs or VprB protein. (A) Culture supernatant (0.1 ml/lane) of Ltk−μλ5VprB transfectant labeled for 10 h with [35S]methionine was reacted with rat mAbs, and immunoprecipitates were analyzed by 4–20% gradient SDS-PAGE under reducing conditions. Gels were soaked in Enlightning, dried, and autoradiographed. Antibodies used include: control mAb (γ2aκ) (c, lane 1), μ H chain–specific mAb M41 (M, lane 2), mAb LM34 (t, lane 3), and mAb VP245 (v, lane 4). (B) X63-Ag8.653 cells were transfected either with λs gene alone, VprB gene alone, or both genes. Each transfactant (4 × 106 cells/ml) was biosynthetically labeled with [35S]methionine for 4 h and lysed with 1% NP-40 lysis buffer. Detergent-soluble lysate was reacted with either mAb M41 (m, lanes 1, 4, and 7), mAb LM34 (t, lanes 2, 5, and 8), or mAb VP245 (v, lanes 3, 6, and 9), and immunoprecipitates were analyzed by 12.5% SDS-PAGE under reducing conditions.
Table 1.  Synthesis and Cell Surface Expression of V\textsubscript{preB}/\lambda\textsubscript{S} surrogate L chain in Pro- and Pre-B Cell Lines

| Cell line   | Origin of cells | Ig gene rearrangements | Synthesis | Surface expression |
|-------------|-----------------|------------------------|-----------|-------------------|
|             | Mouse Tissue    |                        |           |                   |
| 63-12       | RAG-2\textsuperscript{-/-} FL | A-MuLV | H L \lambda\textsubscript{S} V\textsubscript{preB} \muH | L \lambda\textsubscript{S} V\textsubscript{preB} \muH |
| 63-24       | RAG-2\textsuperscript{-/-} FL | A-MuLV | DJ/DJ | G + + - - +* + - - - |
| 38B9        | BALB/c FL      | A-MuLV | DJ/DJ | G + + - - +* + - - - |
| 40E1        | BALB/c FL      | A-MuLV | DJ/DJ | G + + - - + + - - - |
| Clone 18    | BDF\textsubscript{1} FL | Stroma/IL-7 | DJ/DJ | G + + - - + + - - - |
| PAL1        | BDF\textsubscript{1} FL | Stroma/IL-7 | DJ/DJ | G + + - - + + - - - |
| NP          | BALB/c BM      | A-MuLV | DJ/DJ | G + + - - + + - - - |
| 300-19P     | NIH/Swiss BM   | A-MuLV | DJ/DJ | G + + (DJC/\mu) - + + (DJC/\mu) - |
| 204-1-8     | BALB/c BM      | A-MuLV | R | G + + - - + + - - - |
| 220-8       | BALB/c BM      | A-MuLV | VDJ\textsuperscript{-}/VDJ\textsuperscript{-} | G + + - - + + - - - |
| 28C9        | BALB/c FL      | A-MuLV | VDJ\textsuperscript{-}/VDJ\textsuperscript{-} | G + + - - + + - - - |
| 40E1        | BALB/c BM      | A-MuLV | R | G + + - - + + - - - |
| 18-81       | BALB/c BM      | A-MuLV | R | R + + + - + + + - |
| 70Z/3       | BDF\textsubscript{1} BM | Methyl nitrosourea | VDJ\textsuperscript{-}/DJ | R + + + - + + + - |
| NFS5.3      | NFS/N Cas-2SM-MuLV | R | R + + + - + + + - |
| B3-PB-16-1\mu | NIH/Swiss BM | A-MuLV | R | R + + + - + + + - |
| 38C13       | C3H/eB Dimethylbenzanthracene | VDJ\textsuperscript{-}/DJ | R + + + - + + + - |

* Proteins were detected by immunoprecipitation of biosynthetically labeled cell lysates with specific mAbs.
† Proteins were detected by cell surface staining.

\(\lambda\textsubscript{S}\) and V\textsubscript{preB} were also detectable on the surface of the 300-19P cell line which has D\textsubscript{DJ} rearrangements in both alleles of the IgH locus and produces a D\textsubscript{DJ}C/\mu protein (Fig. 2). The level of expression of \(\lambda\textsubscript{S}\) and V\textsubscript{preB} was lower compared with other pre-B cell lines that express a full-size \(\mu\) H chain.

Cell surface expression of \(\lambda\textsubscript{S}\) and V\textsubscript{preB} proteins was also observed on precursor B cell lines that could not, or not yet express D\textsubscript{DJ}C/\mu or normal \(\mu\) H chains. Such cell lines could either be transformed with A-MuLV (38B9 and 40E1 cells) or could be growing on stromal cells in the presence of IL-7 (Clone 18, PAL1, and NP) as shown in Fig. 2 and Table 1. These cell lines have Ig L chain loci in germline configuration and D\textsubscript{DJ} rearrangements on both alleles of the IgH chain locus, but produce no D\textsubscript{DJ}C/\mu proteins (34). The surface expression of \(\lambda\textsubscript{S}\) and V\textsubscript{preB} proteins without \(\mu\) H chain was also detectable on two pro-B cell lines 63-12 and 63-24 derived from the fetal liver of mice in which the RAG-2 gene had been disrupted by targeted integration on both chromosomes (Fig. 2 and Table 1). These cell lines have been shown to have all Ig loci in germline configuration, i.e., are incapable

![Figure 2](image-url)
producing any Ig chain (13). We conclude that the $\lambda_5$ and $V_{\text{preB}}$ proteins can be deposited on the surface membrane not only in $\mu H$ chain–positive precursor B cell lines, but also in progenitor and precursor B cell lines that do not, or do not yet express $\mu H$ chains.

**Association of $\lambda_5$ and $V_{\text{preB}}$ Proteins on the Surface of $\mu H$ Chain–negative Precursor B Cell Lines.** To identify a possible partner(s) of the $\lambda_5$ and $V_{\text{preB}}$ proteins which might carry them onto the surface of the $\mu H$ chain–negative progenitors and precursors, the pro-B cell lines 63-12 and the early pre-B cell line 38B9 were surface labeled with $^{125}$I, and the cell lysates were subjected to immunoprecipitation with the specific mAbs (Fig. 3, A and B, respectively). Whereas no $\mu H$ chain was detectable, as expected, the 22-kD $\lambda_5$ protein and the 16-kD $V_{\text{preB}}$ protein were coprecipitated with each other by either LM34 or VP245 (Fig. 3, A and B, lanes 3 and 4). This indicates that $\lambda_5$ and $V_{\text{preB}}$ proteins are associated with each other to form a surrogate L chain in the absence of $\mu H$ chain on the cell surface.

The $\lambda_5$ protein in association with $\mu H$ chain and $V_{\text{preB}}$ protein expressed on the surface of the $\mu H$ chain–positive pre-B cell line NFS5.3 has always been found to be poorly iodinated so that it could hardly be seen in autoradiography (Fig. 3 C, lanes 2–4), as was also noted by others (8). In contrast, the $\lambda_5$ protein was iodinated fairly well on the $\mu H$ chain–negative cell lines (Fig. 3, A and B, lanes 3 and 4). This indicates a differential accessibility of tyrosine residues within the $\lambda_5$ protein, which is apparently dependent on the presence or absence of $\mu H$ chain. This observation is consistent with a recent report by others (9).

The band of 22 kD corresponding to $\lambda_5$ protein was not detectable in the nonreducing gels (in Fig. 3 A, lanes 7 and 8 and Fig. 3 B, lanes 10 and 11), suggesting that $\lambda_5$ protein exists in disulfide-linked complexes on the cell surface. Therefore, the immunoprecipitates with mAb LM34 were further analyzed by two-dimensional SDS-PAGE, in the first dimension under nonreducing conditions, and then in the second dimension under reducing conditions. Monomeric proteins as well as oligomeric proteins not linked by disulfide bonds will migrate identically in both dimensions and can thus be found on the diagonal of gels. In contrast, a disulfide-linked oligomer will migrate as a complex in the first dimension and then will dissociate into separate subunits in the second dimension, therefore appearing as spots below the diagonal. $V_{\text{preB}}$ protein was found on the diagonal whereas most of $\lambda_5$ protein was detected below the diagonal (Fig. 4, A).

Some of $\lambda_5$ protein was detectable as an off-diagonal spot of 38 kD in first dimension and of 22 kD in second dimension. There was no off-diagonal spot detectable above or below this $\lambda_5$ spot. The rest of the $\lambda_5$ proteins were found in poorly defined complexes of higher mol wt, making a "streak" rather than distinct spots.

The immunoprecipitates with LM34 were further characterized by two-dimensional NEPHGE/SDS-PAGE analysis (Fig. 5 A). The $\lambda_5$ protein migrated with a molecular mass of 22 kD and at a fairly basic pH. There was no other spot of 22 kD detectable besides $\lambda_5$ protein. These results suggested two possibilities: (a) the $\lambda_5$ protein in the off-diagonal spot is disulfide-linked to a protein with the same mol wt and the same charge, most likely to itself to form a homodimer; and (b) the $\lambda_5$ protein is disulfide-linked to another protein that is hardly labeled with $^{125}$I. To identify a disulfide-linked partner of $\lambda_5$ protein, cells were biosynthetically labeled with $[^{35}]$S)cysteine, and the immunoprecipitates...
with LM34 were analyzed by two-dimensional nonreducing-reducing SDS-PAGE (Fig. 4 B). In contrast to cell surface \( \lambda_5 \) proteins labeled with \(^{125}\)I, the majority of \( \lambda_5 \) proteins labeled with \(^{35}\)S-cysteine were found on the diagonal, indicating that they were not in disulfide-linked complexes. On the other hand, a small fraction of \( \lambda_5 \) proteins was detectable below the diagonal as a spot and a streak in the same position as observed in the \(^{125}\)I-labeled material. There was again no off-diagonal spot detectable above or below the off-diagonal spot of \( \lambda_5 \). This is consistent with the first possibility suggested above, that is, that the \( \lambda_5 \) protein of the off-diagonal spot is a disulfide-linked homodimer. Similar results were obtained when cells were labeled with \(^{35}\)S-methionine (data not shown). Thus, some of the \( \lambda_5 \) protein expressed on the cell surface appears to exist as a disulfide-linked homodimer, whereas most of the \( \lambda_5 \) protein was found in poorly defined complexes with higher mol wt under the condition employed in this study.

Proteins Associated with V\(_{prb}/\lambda_5\) Surrogate L Chain on the Surface of \( \mu \) H Chain-negative Precursor B Cell Lines. A complex of proteins of 200, 130, and 105 kD, and several between 65 and 35 kD, was found coprecipitated with V\(_{prb}/\lambda_5\)
surrogate L chain from the μ H chain–negative cell lines 63-12 and 38B9 (Fig. 3 A, lanes 3, 4 7, and 8; and Fig. 3 B, lanes 3, 4, 10, and 11). These proteins were not detectable in immunoprecipitates with LPAM-1 (murine VLA-4α)–specific, CD45-specific or MHC class I–specific mAbs (Fig. 3 B, lanes 5–7 and 12–14). Therefore, the coprecipitation of these proteins is specific for Vpreh/λ5 surrogate L chain. The strongly labeled 130-kD protein (p130) was detectable on the diagonal when analyzed by two-dimensional nonreducing—reducing SDS-PAGE (Fig. 4 A). This indicates that p130 is associated noncovalently with Vpreh/λ5 surrogate L chain on the cell surface. The p130 migrated in pI 5.8 on reducing—reducing SDS-PAGE (Fig. 4 A). This indicates that the molecular contacts between Vpreh and λ5 are strong enough to allow the formation of a trimolecular complex, even though potential contact sites between Vpreh and Vn are missing.

It is intriguing that Vpreh/λ5 surrogate L chains are detectable without μ H chain on the cell surface of pre-B cell lines where all Ig loci are in the germline configuration, as well as on the surface of pre-B cell lines where Ig H chain loci are DμJμ rearranged in reading frames that do not allow the expression of DμJμ protein. This indicates that the synthesis of Vpreh/λ5 surrogate L chain does not necessarily require the rearrangements of Ig H chain loci. Although this is true for transformed cell lines or for long-term proliferating stromal cell/IL-7–dependent cell lines, it will have to be investigated whether normal precursor cells ex vivo show the same pattern of expression of surrogate L chain.

Discussion

All μ H chain–producing transformed pre-B cell lines tested so far express the Vpreh/λ5 surrogate L chain on the surface. We show that the 16-kD protein, also called ϵ, is detectable by Vpreh-specific mAbs. These mAbs, in fact, coprecipitate λ5 protein and μ H chain. This finally proves that the product of the Vpreh gene is expressed on the surface of pre-B cells, and that it is the 16-kD protein described previously (8, 9, 35). Vpreh protein is also detectable on the surface of 300-19P cells in association with DμJμ protein and λ5. This indicates that the molecular contacts between Vpreh and λ5 are strong enough to allow the formation of a trimolecular complex, even though potential contact sites between Vpreh and Vn are missing.

The intensity in autoradiography of the additional proteins (200, 105, and 65–35 kD) coprecipitated with the surrogate L chain varied in different preparations, compared with that of p130, λ5, and Vpreh proteins. It suggests that these molecules may associate loosely with p130 and surrogate L chain. The 200-kD protein and most of the proteins around 35–65 kD were found on the diagonal in the nonreducing—reducing two-dimensional gel (Fig. 4 A), indicating that they are bound noncovalently to the p130/Vpreh/λ5 complex. The 50-kD protein migrated faster under nonreducing conditions than under reducing conditions (Fig. 3 B, compare lanes 3 and 10), finding itself above the diagonal on the two-dimensional gel (Fig. 4 A). This suggests that the 50-kD protein may contain an intramolecular disulfide bond(s). The 105-kD protein appears to form a disulfide-linked homodimer with an apparent mol mass of 200 kD, since it was detectable as one single off-diagonal spot on the nonreducing/reducing two-dimensional gel (Fig. 4 A). Though some materials between 65 and 50 kD appeared to be below the diagonal (Fig. 4 A), it remains to be investigated with better methods whether they are disulfide-linked to each other or to the λ5 protein or to other proteins. In two-dimensional NEPHGE/SDS-PAGE (Fig. 5 A) and IEF/SDS-PAGE analysis (Fig. 5 B), proteins around 45–65 kD resolved into several spots around pI 5.4–6.0, indicating considerable structural heterogeneity of these surrogate L chain–associated proteins.

Most Proteins Associated with Surrogate L Chain on the Surface of μ H Chain–negative Precursor B Cell Lines Are Glycoproteins

The treatment of the immunoprecipitates with PNGase F, as expected, did not change the apparent mol wt of the Vpreh and λ5 proteins. However, it reduced the apparent mol wt of the 200-kD protein (to 190 kD), the p130 (to 100 kD), and the proteins between 43 and 55 kD (to 35–45 kD) as shown in Fig. 3 B, lanes 15 and 16. The two-dimensional nonreducing/reducing SDS-PAGE analysis revealed that the 105-kD subunit of disulfide-linked homodimer reduced its apparent mol wt to 90 kD by treatment with PNGase F (data not shown). We conclude that most of the proteins associated with surrogate L chain on μ H chain–negative precursor B cell lines contain N-linked oligosaccharides and are, therefore, glycoproteins.
has no tyrosine residue, so that the protein would hardly be iodinated with lactoperoxidase, whereas the p130 is well iodinated.

p130 is also unlikely to be the BP-1/6C3 aminopeptidase. The BP-1/6C3 antigen, a disulfide-linked homodimer formed by 140-kD N-linked glycoprotein subunits with a protein backbone of 110 kD, also has been shown to be expressed on early B-lineage cells (39, 40). However, the cell surface expression of the BP-1/6C3 and that of p130 do not correlate to each other. The 3B9 pre-B cell line displays p130 but not the BP-1/6C3 on the surface (40). Moreover, p130 is not a disulfide-linked homodimer. Thus, p130 does not appear to be the BP-1/6C3 antigen.

CD22 is a B-lineage-specific N-linked glycoprotein of 130/140 kD in humans and 150 kD in mice with a protein core of 100–110 kD. It has been suggested that CD22 plays a role in B cell–B cell, B cell–monocyte, and B cell–T cell interaction (41–43). We have employed the mAb specific for murine CD22 to examine whether surrogate L chain was coprecipitated with CD22. However, the mAb precipitated a 150-kD protein without Vpr~B/ls surrogate L chain from the 63-12 pro-B cell line, indicating that the p130 is not CD22 (data not shown). It remains to be determined whether the p130 is identical to other known N-linked glycoproteins with a molecular mass of ~130 kD expressed in B-lineage cells, such as CD21 (molecular mass 140 kD with a 120-kD core protein in humans) (41), the IL-6 signal transducer gp 130 (molecular mass 130 kD with a 100-kD core protein) (44), and the common β chain of IL-3R, IL-5R, and GM-CSF-R (45).

Besides p130, several glycoproteins (200, 105, and 65–35 kD) were found coprecipitated with surrogate L chain, even though they appeared to be bound less strongly to surrogate L chain (or to p130). In this study, 1% NP-40 was used to solubilize membrane proteins. It remains to be investigated how different protocols of cell lysis (variations of detergent, salt, pH, etc.) might change the composition of the noncovalently associated protein complex.

When the immunoprecipitates with Vpr~B or λ3-specific mAbs were prepared from surface-iodinated μ H chain–negative precursor B cell lines and analyzed by SDS-PAGE under nonreducing conditions, a substantial amount of radioactivity was found in poorly defined high mol wt complexes when the immunoprecipitates were analyzed under nonreducing condition by SDS-PAGE. It needs to be analyzed by better methods which resolve these aggregates of λ3 protein in order to clarify how many of these complexes are really disulfide bonded and which proteins participate in such covalent bonds. It also remains to be investigated whether these macromolecular forms represent naturally occurring complexes or whether they are artificially generated during preparation of samples.

In this study, we have shown the surface deposition of Vpr~B/λ3 surrogate L chain in association with the glycoprotein complexes in μ H chain–negative precursor B cell lines. This suggests that the surrogate L chain may function as a receptor on the cell surface even before the association with μ H chain in early precursor B cells. The mAbs established in this study should be useful in elucidating the structure of Ig-like receptor molecules expressed in the early stage of normal B cell differentiation. We are in the process of analyzing the progenitor and precursor B cell compartments in fetal liver and bone marrow of normal and mutant mice to see whether the pattern of surrogate L chain expression and the association with the newly identified partners is the same in normal cells ex vivo. The mAbs may also be useful in our search for a possible ligand(s) of the surrogate L chain complex and in the characterization of developmentally regulated signal transduction in different precursor B cell subpopulations. They should also prove useful in identifying and purifying progenitor and precursor B cells from different lymphoid organs of mice at different times in ontogeny, and in identifying possible defects in B cell development that might arise as a consequence of an abnormal expression of surrogate L chain.

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