Modifications of Igα and Igβ Expression as a Function of B Lineage Differentiation*

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Transcription of the mb1 and B29 genes is initiated when lymphoid progenitors enter the B cell differentiation pathway, and their transmembrane Igα and Igβ products constitute essential signaling components of pre-B and B cell antigen receptors. We analyzed Igα/Igβ biosynthesis, heterogeneity, and molecular interactions as a function of human B lineage differentiation in cell lines representative of the pro-B, pre-B, and B cell stages. All B lineage representatives produced a 36-kDa Igβ form and three principal Igα forms, transient 33/40-kDa species and a mature 44-kDa glycoprotein. Deglycosylation revealed a major Igα core protein of 25 kDa and a minor 21-kDa Igα protein, apparently the product of an alternatively spliced mRNA. In pro-B cells, the Igα and Igβ molecules existed primarily in separate unassociated pools, exhibited an immature glycosylation pattern, did not associate with surrogate light chain proteins, and were retained intracellularly. Their unanticipated association with the Lyn protein-tyrosine kinase nevertheless suggests functional potential for the Igα/Igβ molecules in pro-B cells. Greater heterogeneity of the Igα and Igβ molecules in pro-B and B cell lines was attributable to increased glycosylation complexity. Finally, the Igα/Igβ heterodimers associated with fully assembled IgM molecules as a terminal event in B cell receptor assembly.

Membrane-bound immunoglobulin (Ig) molecules are non-covalently bound to the transmembrane Igα (CD79a) and Igβ (CD79b) proteins, respectively the products of the mb1 and B29 genes, to form the B cell antigen receptor (BCR) complex (1). Igα/Igβ heterodimers are also integral components of pre-BCR complexes composed of surrogate light chain (ϕLC) and μ heavy chains (HC) on the surface of pre-B cells (2–8). Ligation of BCR and pre-BCR initiates cytoplasmic signals via the Igα and Igβ molecules whose cytoplasmic domains contain immunoregulatory tyrosine-based activation motifs. BCR aggregation thereby promotes interaction with protein-tyrosine kinases and resultant immunoregulatory tyrosine-based activation motif phosphorylation, hydrolysis of phosphatidylinositol, sustained intracellular calcium elevation (9–12), and the activation of multiple signaling pathways (13–15).

Expression of Igα and Igβ transcripts begins very early in B lineage differentiation prior to the onset of D1H-DJH rearrangements in the μHC locus (16, 17), and Igβ-deficient mice are unable to generate μHC-producing pre-B cells (18). Surprisingly, B cell development in Igβ−/− mice appears to be compromised as early as the pro-B stage when VH-DJH rearrangements are occurring, thereby suggesting an Igβ role in B lymphopoiesis even prior to μHC synthesis. Although pro-B cell lines from humans also produce Igα and Igβ, expression of these as components of cell surface receptors has not been demonstrable (4). Correspondingly, the ϕLC in human pro-B cells were found exclusively in the endoplasmic reticulum and early Golgi compartments, where they transiently associated with 40-, 60-, and 98-kDa proteins before undergoing intracellular degradation (7, 8). In murine pro-B cells, however, Igα/Igβ heterodimers have recently been found on the cell surface, perhaps in association with calnexin (19, 20).

The physiological role of Igα and Igβ during the earliest stages in B lineage differentiation thus remains unclear, and may differ in mice and humans. In this analysis of human B lineage cells, we have compared Igα and Igβ expression, heterogeneity, and molecular association in pro-B cells versus their more mature pre-B and B cell offspring. The results reveal a remarkable progressive complexity of the Igα and Igβ glycoproteins during B lineage differentiation, an unanticipated intracellular association with a Src family protein-tyrosine kinase in pro-B cells, and late stage Igα/Igβ union with assembled IgM molecules to form the BCR on B cells.

EXPERIMENTAL PROCEDURES

Antibodies—Mouse monoclonal antibodies (mAbs) included SA-DA 4.4 (γ1c) anti-human μ chain (21), CB3-1 (γ1c), and CB3-2 (γ1c) anti-human Igβ (4), HM57 (γ1c) anti-human Igα (22), SLC1 (γ1c) anti-human ϕLC (8), 4G10 (γ1c) anti-phosphotyrosine (Upstate Biotechnology Inc., Lake Placid, NY), 4D10 (γ2a) anti-human Syk (Santa Cruz Biotechnology, Inc. CA), and Fyn (γ1c) anti-human Fyn (Santa Cruz Biotechnology). The JH3 (γ1c) anti-human Ig idiotype (23) and CT4 (γ1c) anti-BCR CD4 (24) mAbs were used as controls. In immunoprecipitation experiments, mouse mAbs were either directly coupled to Sepharose 4B beads or incubated with rat anti-mouse ϕLC-coupled beads (Interchim, France). Rabbit antibodies to human Syk (a kind gift of Uriel Blanc, Institut Pasteur, Paris, France) and Lyn (Upstate Biotechnology Inc.) were also employed, with rabbit γ-globulins (Pentex, Miles Laboratory, Kankakee) serving as a control. Phycoerythrin-conjugated goat anti-mouse Ig was obtained from Southern Biotechnology Associates (Birmingham, AL).

Cells—Human cell lines included the RS4;11 (25) and Nalm16 (26) pro-B cells, the 697 (27) and Nalm6 (26) pre-B cells, and Ramos (28) B
cells. These were maintained in stationary culture in RPMI 1640 medium supplemented with l-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 mg/ml), and 10% fetal calf serum at 37 °C in 5% CO2.

Cell Surface Bioirradiation and Biosynthetic Protein Labeling—Viable Nalm16 cells (5 × 107) washed twice in PBS were incubated with 1 mg/ml biotin (NHS-sulfosuccimidyl, Pierce) in PBS for 1 h at 4 °C. Cells incubated with biotinylated cell surface proteins were washed once in chilled RPMI 1640 and twice in PBS before lysis in 1% digitonin or 0.5% Triton X-100 lysis buffer. Cells (1.5 × 107) were also metabolically labeled with [35S]Met (400 Ci/ml) for 6 h, then washed and lysed. For pulse labeling and chase analysis, cells (1–2 × 107) were preincubated in Met- and Cys-free RPMI 1640 for 2 h to deplete internal pools, and then labeled with 300–500 μCi of both [35S]Met and [35S]Cys for 15 min. Labeling was terminated by addition of 100-fold excess of cold Met and Cys. The pulsed cells were incubated for various intervals before harvesting.

Immunoprecipitation—Labeled cells were harvested and lysed in 1% Nonidet P-40, 1% Triton X-100, or 1% digitonin lysis buffer. Nuclei were sedimented at 10,000 × g for 20 min, and the supernatants used for immunoprecipitation. After successive incubations with bovine serum albumin and IgG-coupled Sepharose 4B beads, the precleared lysates were incubated with beads coupled with test or control antibodies. Bound materials were eluted with Laemmli’s sample buffer (29) and were incubated with beads coupled with test or control antibodies. Immunoprecipitates were subjected to two-dimensional gel electrophoretic analysis, immunoprecipitates were separated initially on nonreducing SDS-polyacrylamide gels (10–15%) that were then excised, equilibrated for 45 min in SDS sample buffer containing β-mercaptoethanol, and rotated 90° before electrophoresis in the second dimension on SDS-polyacrylamide (10%) gels under reducing conditions.

Western Blots—Anti-Igα, anti-Igβ, and anti-μ immunoprecipitates were resolved on one-dimensional SDS-PAGE and two-dimensional diagonal gels, blotted onto nitrocellulose membrane (Schleicher & Schuell), before incubation with anti-μ, anti-Igγ, or anti-Igα mAbs. In some experiments, the anti-Igα and anti-Ig-μ immunoprecipitates were submitted to a second immunoprecipitation with an anti-phosphotyrosine mAb; the eluted material was electrophoresed, blotted, and reincubated with the anti-phosphotyrosine mAb. The blots were developed with the ECL chemiluminescence system (Amersham Pharmacia Biotech) using a horse-radish peroxidase-conjugated goat anti-mouse Ig (Sigma). In cell immunoprecipitates, experiments, immunoprecipitates were resolved by SDS-PAGE, blotted as described above, then incubated with a horse-radish peroxidase-conjugated streptavidin (Sigma) and revealed with the ECL method.

Deglycosylation—Immunosorbed proteins were treated with endo-glycosidase H (endo H), endo-glycosidase F (endo F), N-glycanase, O-glycanase, or combinations of these enzymes for 18 h at 37 °C, according to the manufacturer’s instructions (Roche Molecular Biochemicals), before elution with Laemmli’s buffer. Fetoxin protein was used as a control for optimal digestion.

In Vitro Kinase Assay—Nalm16 cells were washed twice in PBS and lysed in 1% digitonin, 25 mM Hepes, pH 7.2, 150 mM NaCl, 5 mM KCl, 5 mM EDTA, 1 mM orthovanadate, and protease inhibitors before lysis immunoprecipitation with anti-Igα, anti-Igγ, or control mAbs. Bound materials were washed three times with lysis buffer and once with kinase buffer (25 mM Hepes, pH 7.2, 150 mM NaCl, 5 mM KCl, 5 mM MnCl2, 5 mM MgCl2, 1% digitonin) before resuspension in 50 μl of kinase buffer containing 1 μCi of γ[32P]ATP (>4000 Ci/mmol, ICN Biomedicals, Orsay, France). After a 20-min incubation at room temperature, beads were washed three times with lysis buffer, then either eluted in 50 μl of SDS sample buffer and boiled for 5 min or submitted to reprecipitation as follows: elution in 50 μl of 0.5% SDS lysis buffer instead of digitonin, incubation for 10 min at 60 °C, 10-fold dilution in 500 μl of 1% Triton X-100, then immunoprecipitation. In one series of experiments, Nalm16 cells were incubated with the CB3–1 anti-Igβ antibody (30 μg/ml) or a control mAb (y1s isotype) for 10 min at 37 °C. Cells were resuspended in Tris exposure buffer containing 5 mM EDTA, 1 mM orthovanadate, and protease inhibitors. Cell lysates were electrophoresed, blotted, and probed with an anti-phosphotyrosine mAb. Alternatively, lysates of the anti-Igβ-stimulated pro-B cells were immunoprecipitated with an anti-phosphotyrosine mAb, and the immunoprecipitate was electrophoresed, blotted, and probed with the anti-phosphotyrosine mAb.

Northern Blots, Reverse Transcription-Polymerase Chain Reactions (RT-PCR), and DNA Sequencing—Total RNA was prepared using the guanidinium isothiocyanate method, electrophoresed on 7% formaldehyde, 1% agarose gels, and blotted onto nitrocellulose filters (Schleicher & Schuell). Northern blots were hybridized with random-primed [32P]dCTP-labeled nucleotide fragments. Results were further analyzed by RT-PCR. The first strand cDNA synthesis employed Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) for 50 min at 42 °C in the presence of 5 μg of total RNA and 100 ng of primers complementary to the coding sequence of Igα mRNA (Igα-R, 5′-CTG-GACATCTCTATGGTTGAG-3′) or Igα mRNA (Igα-R1, 5′-CTCCTGGCC- AGTTGTTCGAC-3′). The cDNAs were amplified in the presence of both the 5′ and 3′ primers with Taq polymerase (Cetus, Emeryville, CA) through 28 cycles involving denaturation at 92 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min. Igα forward and reverse primers were Igα-F (5′-ATGCCCAGGGGTCACAGAC-3′) and Igα-R (5′-AATGTCCAGGGCTCTGCAGG-3′). The RT-PCR protocol was essentially that of Hashimoto et al. (30). Amplified PCR products were analyzed on 2.5% agarose gels. DNA fragments corresponding to the different PCR products were eluted from the agarose gel, cloned in the PCR 2.1 vector (Invitrogen, Carlsbad, CA), sequenced using dye terminator chemistry, and analyzed with an automated ABI DNA sequencer (Perkin Elmer, Foster City, CA).

RESULTS

Characterization of Igα and Igβ Molecules in Pro-B Cell Lines—Four molecules with apparent molecular masses of 44, 40, 36, and 33 kDa were immunoprecipitated with anti-Igα antibodies from the metabolically labeled Nalm16 and RS4;11 pro-B cell lines. In contrast, two molecules of 44 and 36 kDa were precipitated by the anti-Igβ mAb from both pro-B cell lines (Fig. 1A). Analysis of the anti-Igα precipitates by two-dimensional gel electrophoresis indicated the covalent linkage of a minor fraction of the available pools of the 44- and 36-kDa species (Fig. 1C, and data not shown), with the major portion of the 44-kDa molecules remaining on the diagonal. Similarly, two-dimensional gel analysis of anti-Igβ immunoprecipitates indicated that most of the 36-kDa molecules migrated on the diagonal, whereas small fractions were found in off-diagonal positions indicative of their formation of homodimers or heterodimers with the 44-kDa protein (Fig. 1D). Western blot analysis confirmed the Igβ identity of the 36-kDa molecules (Fig. 1B, lanes 3 and 4). It also indicated reactivity of the 44-, 40-, and 33-kDa molecules with the anti-Igα antibody, thus confirming the molecular heterogeneity of Igα proteins (Fig. 1B, lanes 1 and 2). Western blot analysis of anti-Igβ immunoprecipitates resolved on two-dimensional gels indicated that most of the Igβ molecules exist in a free pool, whereas minor fractions exist as covalently linked heterodimers or homodimers in the Nalm16 pro-B cell line (Fig. 1, D and F). A similar two-dimensional analysis of the anti-Igα precipitates showed that only a fraction of the 44-kDa Igα species ran off the diagonal (Fig. 1, C and E), thus indicating that the 40- and 33-kDa Igα forms do not form covalent bonds with Igβ.

Pulse-chase analysis of the RS4;11 and Nalm16 pro-B cells indicated that newly synthesized Igα and Igβ proteins exist initially as completely separate pools with limited Igα and Igβ association (Furrer thereafer, Fig. 2A, and data not shown). The levels of newly synthesized Igα and Igβ transcripts progressively declined with estimated half-lives of less than 30 min, whereas the levels of 44-kDa Igα molecules were maintained over the 3-h observation period (Fig. 2B). Notably, neither the anti-Igα nor the anti-Igβ immunoprecipitates of pro-B cells lysates contained 3′LC proteins, and the Igα and Igβ molecules could not be detected on the surface of the Nalm16 pro-B.
Therefore conducted to determine whether the three Igα species in pro-B cells reflect variably glycosylated forms or other modifications of protein structure. When lysates of metabolically labeled RS4;11 cells were precipitated with anti-Igα or anti-Igβ antibodies and the bound material treated with endo F or with N-glycanase and endo F, anti-Igα precipitates were resolved as two bands of 25 and 21.5 kDa, whereas anti-Igβ precipitates yielded a single band of 24.5 kDa (Fig. 2C, and data not shown). Western blot analysis of the anti-Igα or anti-Igβ precipitates confirmed the Igα nature of the 25- and 21.5-kDa bands, and the single band of 24.5-kDa Igβ forms (Fig. 2C). When the anti-Igα and anti-Igβ immunoprecipitates of unlabeled RS4;11 and Nalm16 pro-B cell lysates were treated with endo H, immunoblot analysis again indicated 25- and 21.5-kDa Igα species and a single 24.5-kDa Igβ band (data not shown). This endo H sensitivity pattern suggests restriction of the Igα and Igβ glycoproteins to the endoplasmic reticulum and early Golgi in human pro-B cells.

Igα and Igβ Transcript Heterogeneity—The fact that the molecular mass of the Igα core protein predicted from complete transcription of the human mb1 gene is 24.5 kDa suggested that the 21.5-kDa deglycosylated molecule could be generated by post-transcriptional modification. Mb-1 (Igα) and B29 (Igβ) transcripts in the Nalm16 and RS4;11 pro-B cell lines were therefore analyzed by RT-PCR. The Igα reactions yielded a major 660-bp DNA fragment and a minor 550-bp DNA fragment (Fig. 3A). Sequence analysis indicated that the 660-bp PCR product corresponds to the full-length Igα transcript, whereas the 550-bp fragment reflects the amplification of an alternatively spliced Igα mRNA. This spliced form could be attributed to the use of a cryptic splice site located 186 bp after the start of the second exon, which is joined to the normal splice acceptor site of the third exon. The resulting transcript, which is devoid of 114 bp of the normal sequence, would maintain the same reading frame as the full-length transcript, and therefore could encode an Igα protein lacking part of the extracellular domain. This truncated protein would not be expected to contain the cysteine residue involved in covalent association with Igβ, but would maintain the transmembrane and cytoplasmic portions as well as four N-linked glycosylation sites.

Igβ reactions produced major 600-bp and minor 290-bp DNA amplification products (Fig. 3B). The sequence of the larger fragment contained all six CD79b exons, whereas the smaller product reflected a complete loss of the third exon. The 105 codon truncation maintains a correct reading frame in the 3′ portion of the short Igβ transcripts that could encode for an 11-kDa protein. The predicted truncated Igβ protein would lack all N-linked glycosylation sites and the ability to form disulfide-bonded heterodimers with Igα. Antibodies to the C-terminal portion of Igβ are needed to determine whether this truncated Igβ is expressed in pro-B cells. The present RT-PCR and sequence analyses of pro-B cell lines thus agree with those previously reported for human pre-B and B cell lines (41–45).

**Association of Igα and Igβ Molecules with Protein-tyrosine Kinases in Pro-B Cell Lines—**Antigen ligation of the BCR complex on B cells leads to interaction of the Igα/Igβ subunits with Src family kinases (Lyn, Fyn, Blk, Hck, and Lck) and Syk kinase to initiate cell activation (46–56). Pre-BCR interaction with Src kinases has also been demonstrated (12, 57). It was therefore of interest to determine whether interaction of the Igα/Igβ molecules with these protein-tyrosine kinases may occur at the pro-B cell stage. Both Lyn (53/56 kDa) and Syk (70 kDa) tyrosine kinases were identified in digitonin lysates of metabolically labeled RS4;11 pro-B cells, although the level of background radioactivity did not permit clear resolution of their association status with Igα and Igβ molecules.
Fig. 2. Analysis of Igα and Igβ biosynthesis and glycosylation status in pro-B cells. Pulse-chase analysis of Igα and Igβ in RS4;11 pro-B cells (A and B). Cells were pulse-labeled with [35S]Met and [35S]Cys, chased with cold amino acids for various time intervals, and then lysed before incubation with antibodies against Igα or Igβ. Immunoprecipitates were analyzed by SDS-PAGE under reducing conditions. A, Igα and Igβ molecules are unassembled at time 0. B, Analysis of the biosynthetically labeled 44-, 40-, and 33-kDa Igα molecules at later time points, by determination of relative autoradiographic intensities, indicates the relative stability of the 44-kDa Igα form and the transient nature of the 33/40-kDa species. C, 24.5-kDa Igβ core protein and 21.5/25-kDa Igα core proteins are revealed by deglycosylation. Nonlabeled RS4;11 cell lysates were subjected to immunoprecipitation with anti-Igα or anti-Igβ antibodies, the immunoprecipitates treated with endo F, and the deglycosylated Igα and Igβ proteins identified by immunoblots as described in Fig. 1.

Anti-Lyn and anti-Syk eluates were submitted to a second immunoprecipitation with the anti-Igβ antibody, Igβ was detected in the anti-Lyn immunoprecipitate (Fig. 4A, lane 1) but not in the anti-Syk immunoprecipitate (data not shown). A band of 44 kDa, likely Igα, was also coprecipitated with anti-Lyn. Conversely, p55/p56 Lyn was identified in anti-Igβ precipitates (Fig. 7A, lane 2), whereas Syk was not (data not shown).

An in vitro kinase assay was conducted using unstimulated and pervanadate-treated Nalm16 pro-B cells. The anti-Igα antibody coprecipitated a faint 53/56-kDa doublet with kinase activity in unstimulated Nalm16 cells, and pervanadate treatment, employed to alter the kinase/phosphatase equilibrium to favor activation of the tyrosine kinases, strongly enhanced the Lyn 53/56 signal (Fig. 7B). The 53/56 doublet was also revealed by an anti-phosphotyrosine mAb in the anti-Igα immunoprecipitates of Nalm16 pro-B cells preincubated with pervanadate (Fig. 4C). An additional 44-kDa band, likely Igα, was also seen. In contrast, Syk could not be identified in anti-Igα/Igβ precipitates of pervanadate-treated pro-B cells. Similarly, Fyn, which was also abundant in pro-B cells, was not detected in association with Igα/Igβ.

The above data suggest that Lyn preferentially associates with the minor population of Igα/Igβ heterodimers within pro-B cells. In order to examine the possibility that an otherwise undetectable level of cell surface Igα/Igβ molecules accounted for the Lyn association, we examined the tyrosine phosphorylation status of proteins in pro-B cells before and after treatment with a known stimulatory antibody against an extracellular Igβ epitope. No differences were observed in the tyrosine phosphorylation status of Lyn or other proteins were observed within 5 min following the anti-Igβ treatment of the pro-B cells (data not shown), thereby mitigating against the possibility of Lyn association with pro-B cell surface Igα/Igβ.

Comparison of Igα and Igβ Expression in Pre-B and B Cell Lines—Two-dimensional gel analysis of anti-Igα and anti-Igβ immunoprecipitates indicated a progressive increase in heterogeneity of the Igα/Igβ heterodimers in pre-B and B cells (Figs. 5 and 6). Additional minor Igα and Igβ forms of lower molecular weights were also revealed by this Western blot analysis. Igα and Igβ existed in major and minor heterodimeric forms in pre-B cell lines as well as in a B cell line. Comparative two-dimensional gel analysis of the Ramos B cell line also revealed prominent fan-like extensions of the major Igα and Igβ units (Fig. 5, C–E), the identity of which was confirmed by Western blot analysis (Fig. 6, C and D). In both the pre-B and B cells, μHC were coprecipitated with the Igα and Igβ molecules. Comparative analysis of the anti-μHC precipitate (Fig. 5E) with the anti-Igα and anti-Igβ precipitates (Fig. 5, C and D) indicates that the (μHC)/μ/LC complexes could be coprecipitated with antibodies against the Igα and Igβ molecules, while the μHC/μ/LC subunits could not.

EndoF digestion of the anti-Igα precipitates from pre-B and B cells revealed core proteins similar to those observed for pre-B cells, and truncated mb1 and B29 transcripts were likewise identified in pre-B and B cells (Fig. 3). A minor population of Igβ homodimers was also detected in pre-B and B cells, but these Igβ homodimers were not seen in the anti-μ precipitates (Fig. 5E), anti-Igα precipitates (Fig. 5, A and C), or on the cell surface (Fig. 7C). Pulse-chase analysis of anti-Igα precipitates from metabolically labeled cells indicated that Igα/Igβ association is initiated immediately after their biosynthesis in pre-B and B cells (Fig. 7A). However, completion of the complex Igα and Igβ glycosylation process required more than 2 h after biosynthesis, as illustrated in pre-B cells in Fig. 7B. The final heterogeneity of cell surface Igα/Igβ components was found to be indistinguishable in the pre-BCR on pre-B cells and BCR on B cells (Fig. 7C).
DISCUSSION

In these studies, we observed a remarkable diversity of the Igα and Igβ glycoproteins, the nature of which is altered as a function of B lineage progression. Igα species of 44, 40, and 33 kDa were produced by pro-B, pre-B, and B cell lines, whereas Igβ was found in a single 36-kDa form. The 40- and 30-kDa Igα species represented transient immature forms that did not associate with Igβ, whereas the 44-kDa Igα and 36-kDa Igβ were found to be relatively stable and to associate with each other to form heterodimers. The formation of Igα/Igβ heterodimers and the complexity of their glycosylation patterns were shown to increase dramatically as a function of B cell differentiation.

Two Igα protein backbones of approximately 25 and 21.5 kDa were revealed in deglycosylation studies, and two types of mb1 (Igα) gene transcripts were identified that could account for these. The smaller, less-abundant variant, which lacked 114 bp as a consequence of alternative splicing of the Igα mRNA, maintains the same reading frame as the full-length transcript. It has the potential to give rise to a 4-kDa deleted protein that likely corresponds to the truncated Igα detected in B lineage cells. The predicted product of this small mb1 transcript would...
lack the cysteine residue involved in forming disulfide-linked heterodimers with \( \text{Ig} \beta \), but would maintain many \( N \)-linked glycosylation sites. Similarly, two B29 (\( \text{Ig} \beta \)) transcripts were identified. The smaller one has the potential to encode a 11-kDa protein that could not covalently bind \( \text{Ig} \alpha \) and would lack glycosylation sites. All of the representative pro-B, pre-B, and B cell lines expressed identical patterns of \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) transcripts, and deglycosylation with \( N \)-glycanase revealed the truncated 21-kDa \( \text{Ig} \alpha \) molecule in cells representative of each differentiation stage. In mature human B cells, a post-transcriptional regulation of \( \text{mb}1 \) and B29 gene expression has been suggested. In particular, activation of mature B cells with anti-IgM antibody, interleukin 4, or lipopolysaccharide was shown to induce alternative splicing of \( \text{mb}1 \) and B29 (45).

The molecular interactions and functional potential of the truncated \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) molecules are poorly understood. The predicted amino acid sequences of the truncated human \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) proteins suggest they would not covalently associate with each other, and therefore would not be incorporated into either the BCR or pre-BCR complexes. Accordingly, fibroblast co-transfection of human \( \mu \text{HC} \) and \( \kappa \text{LC} \) genes with \( \text{mb}1 \) and

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**FIG. 5.** Analysis of the \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) molecules produced by pre-B and B cell lines. Anti-\( \text{Ig} \alpha \) (A and C), anti-\( \text{Ig} \beta \) (B and D), and anti-\( \mu \) (E) immunoprecipitates of metabolically labeled 697 pre-B cells (A and B) and Ramos B cells (C–E) were assessed by two-dimensional gel electrophoresis. Note that the \( \mu \text{HC} \) and \( \text{LC} \) components representing the unassembled \( \mu \text{HC}/\text{LC} \) subunits (small arrows) are seen in the anti-\( \mu \text{HC} \) and anti-\( \text{Ig} \beta \) immunoprecipitates of pre-B and B cells. The surrogate light chain components (16/18-kDa Vpre-B, and 22-kDa \( \lambda 5/14.1 \)) in the pre-B cells are not shown in A and B.

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**FIG. 6.** Analysis of \( \text{Ig} \alpha/\text{Ig} \beta \) association and heterogeneity in human pre-B and B cells. Anti-\( \text{Ig} \alpha \) (A and C) and anti-\( \text{Ig} \beta \) (B and D) immunoprecipitates of lysed, unlabeled 697 pre-B (A and B) and Ramos B cells (C and D) were resolved by two-dimensional SDS-PAGE and blotted with antibodies against \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) as described in Fig. 1. Note the greater heterogeneity of \( \text{Ig} \alpha \) (C) and \( \text{Ig} \beta \) (D) molecules in the B cells, and the subfractions of smaller \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) species present in both the pre-B and B cells.

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**FIG. 7.** Pulse-chase analysis of \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) biosynthesis and association in pre-B and B cell lines and comparative analysis of \( \text{Ig} \alpha/\text{Ig} \beta \) heterogeneity in the pre-BCR and BCR. A, Pre-B and B cell lines, 697 and Ramos, were pulse-labeled with \( ^{35}\text{S} \)Met and \( ^{35}\text{S} \)Cys and chased with cold Met and Cys for varying time intervals before cell lysis and analysis of anti-\( \text{Ig} \alpha \) or anti-\( \text{Ig} \beta \) immunoprecipitates by SDS-PAGE under reducing conditions. The different molecular forms of \( \text{Ig} \alpha/\text{Ig} \beta \) seen in pro-B cells (Figs. 1 and 2) are also present in the pre-B and B cells, in which \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) association is already evident at time 0. B, two-dimensional gel analysis of anti-\( \mu \text{HC} \) immunoprecipitates at various intervals following pulse labeling of the 697 pre-B cells. Note the relative homogeneity of \( \text{Ig} \alpha \) and \( \text{Ig} \beta \) molecules as late as 2 h after biosynthesis. C, comparable heterogeneity of \( \text{Ig} \alpha/\text{Ig} \beta \) molecules on the surface of 697 pre-B cells and Ramos B cells. Cells were surface-biotinylated, lysed in digitonin, then incubated with an anti-\( \mu \) antibody. Immunoprecipitates were resolved by two-dimensional SDS-PAGE.
B29 variants failed to reconstitute the BCR (45). In the mouse, a C-terminally truncated Igβ product was detectable only in activated B cells (38). A truncated murine Igα product has been found to be preferentially associated with IgD, whereas the predicted full-length Igα product associated primarily with IgM (39). In late stage murine pre-B cells, a truncated 23 kDa protein has been identified with a monoclonal antibody against an extracellular Igα epitope (5). This suggests that truncated Igα and Igβ proteins may be differentially expressed according to the stage of B lineage differentiation in the mouse. Truncated Igα variants may also be secreted by bovine B lymphocytes (58).

A functional potential for the Igβ homodimers that we observed in human B lineage cells was not revealed in these studies. The Igβ homodimers were not found to associate with either μHC or Igα, nor were they detected on the surface of pre-B and B cells. It is theoretically possible that Igβ homodimers play an undefined role inside the cell or, less likely, on the cell surface in levels below our detection threshold.

The Igα and Igβ interactions and glycosylation heterogeneity increased progressively in representative pro-B, pre-B, and B cell lines. The Igα and Igβ molecules in pro-B cells were found largely in separate pools, with only a minor fraction forming disulfide-bonded Igα/Igβ heterodimers. In contrast to the Igα/Igβ status in pro-B cells, Igα readily associated with Igβ in pre-B cells and in B cells. Variable glycosylation of the Igα/Igβ heterodimers occurred during their progression through the Golgi to reach the cell surface in association with μHC to form pre-B receptors (pre-BCR) and B cell receptors (BCR), respectively. While the final Igα/Igβ glycosylation spectra in the cell surface pre-BCR and BCR were indistinguishable, a restricted fraction of mature glycosylated molecules was observed in the Igα/Igβ pool in pre-B cells relative to that seen in B cell lines. This striking feature, which is also evident in data obtained in prior studies (4, 6), reflects the relative inefficiency of pre-BCR assembly in pro-B cells compared with BCR assembly in B cells.

Igα/Igβ heterodimers are essential elements in pre-B and B receptor signaling (1, 9–15). They mediate B cell activation by interaction with Syk and Syc family tyrosine kinases and also serve as pre-BCR signal-transducing components to promote pre-B cell differentiation and allelic exclusion (59–62). Much less is known about functional Igα/Igβ potential before VDJH rearrangement. One important clue, however, is provided by the demonstration that mice lacking in Igα exhibit a block at the pro-B cell stage in differentiation prior to the completion of VDJH rearrangements (18). In contrast, VDJH rearrangement proceeds normally in mice that have a c-mut mutation that prevents membrane-bound μHC expression (63). These observations suggest that the Igα/Igβ molecules may play an important biological role during the pro-B cell stage in differentiation before μHCs are expressed. A recent study suggests that Igα/Igβ heterodimers may be expressed with calnexin on the surface of pro-B cells from RAG-2-deficient mice (20). Ligation of this Igα/Igβ cell surface complex induced rapid, transient phosphorylation of Igα and associated tyrosine kinase to promote pro-B differentiation. However, these findings in mice may not be directly applicable to humans, given that Igα/Igβ heterodimers apparently do not reach the cell surface of human pro-B cells. Instead, our analysis of human pro-B cells indicates the intracellular association of Igα/Igβ with Lyn, a member of the Syc tyrosine kinase family. The possibility that the association of Igα/Igβ with Lyn is artificial, occurring after pro-B cell lysis, is unlikely since Igα/Igβ binding to other phosphoproteins was not observed in the pro-B cells. Specifically, phosphorylated Fyn and Syk, which are also abundant in pro-B cell
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