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Immunology

Molecular basis of the cell-surface expression of immunoglobulin μ chain without light chain in human B lymphocytes

(immunoglobulin biosynthesis/protein transport)

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ABSTRACT **Four distinct human B-lymphoid cell lines possess the ability to circumvent the mechanism regulating intracellular transport of immunoglobulin protein. These cells do not produce light chains, yet they express μ heavy chains on the cell surface at comparable levels to B-cell lines that produce native forms of both proteins. The μ-chain mRNA produced in all four cell lines was found to contain an identical deletion of most of the heavy-chain variable (V_H) region (75% of the 3′ portion), with no apparent alteration in constant (C) region structure. The truncated μ (μ*)-chain mRNA in these cells was created through the use of a cryptic splice donor site found within the human V_H gene(s) utilized by these B-cell lines. The truncated μ* chains exhibited a decreased ability to associate with the intracellular transport regulatory protein, heavy-chain binding protein (BiP). This result indicates that V_H region structure, in addition to $C_\mu 1$ region structure, influences the formation of the BiP recognition site on the heavy chain. Furthermore, it suggests that the mechanism allowing for cell-surface expression of the μ* chains in the absence of light-chain pairing is the inability of BiP to bind to the μ* chains and hence prevent their intracellular transport. The high frequency with which the μ-only surface immunoglobulin positive phenotype is present in our collection of human B-cell lines and the isolation of one of the cell lines from a healthy individual also suggest that B cells of this type may represent a significant subpopulation among the normal human B-cell repertoire.**

In the developmental pathway for mammalian B lymphocytes, the immunoglobulin heavy (H)-chain-only phenotype is normally limited to the precursor population known as pre-B cells, which generally synthesize the μ form of H chain (1–3). Certain instances can arise though where more mature B-lymphoid cells can also stably express H chains in the absence of any light (L)-chain synthesis. The best documented examples of this phenomenon are found in plasma cell stage lines isolated from patients with γ H-chain disease (4) or represented by certain variant clones isolated during culture of mouse plasmacytomas (5). The H chain produced by the plasmacytoid cell lines from each of these sources is always found to be structurally altered from the native H-chain protein of the same isotype and is generally secreted by the cell as an H-chain-only multimeric complex. The ability of these altered H chain (H*) to be transported intracellularly in the absence of L-chain association contrasts with the block in intracellular transport for native μ chains in pre-B cells (2).

An explanation to this anomaly has been recently provided by studies showing that H-chain transport in B-lymphoid cells is regulated by an endoplasmic reticulum (ER)-localized protein termed H-chain binding protein (BiP; see ref. 6), which apparently belongs to the glucose-regulated family of proteins (7). BiP appears to regulate transport of several membrane and secreted glycoproteins from the rough ER to the Golgi apparatus by binding to a specific site(s) on the target molecule. In the case of BiP–H-chain association, BiP seems to require a site within the C_H1 domain of H chains and is no longer associated with H chain after the L-chain–H-chain pairing process (6, 8). Consistent with this scheme is the uniform deletion of all or part of the C_H1 domain among the secreted human H* chains (4, 9–12). As all transported H* chains thus far tested fail to interact with BiP with normal affinity (8), it has been suggested that such truncated H* chains can be secreted by virtue of the inability of BiP to recognize them and prevent their movement out of the ER.

Few examples exist that document expression of H chain without L chain at the cell surface in mature B lymphocytes (13, 14). In these two reports, it was suggested that the μ chains synthesized were either normal or lacked proper glycosylation, thereby indicating that the ability of membrane-associated H* chains to be transported to the cell surface was not a function of altered protein structure. To better understand the mechanism behind cell-surface expression of H chain without L chain, we have characterized at both the protein and nucleic acid levels the H* chains produced by four distinct human B-cell lines, all of which express cell-surface μ chain at levels nearly identical to human B-cell lines that express native IgM. Our results indicate that cell-surface expression of μ H chain without L chain in these human B-cell lines is based on production of a uniformly truncated μ chain that lacks most of the H-chain variable (V_H) region, but has normal constant (C) region domains. This alteration in the protein backbone of the μ* H chain prevents its efficient association with BiP and allows these chains to circumvent the normal regulatory mechanism controlling intracellular transport of immunoglobulin molecules.

MATERIALS AND METHODS

Cell Lines. LBW-2 and LBW-14 are Epstein–Barr virus (EBV)-transformed human B-cell lines derived from peripheral blood lymphocytes of two different patients with common variable immunodeficiency (14). DB is an EBV-transformed human B-cell line derived from a healthy individual (14). LR1 is a B-cell line that spontaneously arose from cultured peripheral blood lymphocytes from a patient with acute lymphocytic leukemia (D.L., unpublished work). F4 is a spontaneously arising human B-cell line from lymphocytes

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Abbreviations: V, C, J, and D regions, variable, constant, joining, and diversity regions of immunoglobulin; H and L chains, heavy and light chains; EBV, Epstein–Barr virus; BiP; heavy-chain binding protein; ER, endoplasmic reticulum.

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of a patient with hairy cell leukemia (15). All five cell lines have been cloned by limiting dilution or in soft agar and each reflects the mature B-cell stage of maturation as all express immunoglobulin μ H chains on their cell surface (refs. 14 and 15; unpublished work).

The four μ^*-producing human B-cell lines were shown to be independent in origin by (*i*) HLA typing—DB and LBW-2 express different class II haplotypes (14), and (*ii*) restriction fragment length polymorphism analysis—all four cell lines exhibited a distinct allelic restriction fragment length polymorphism pattern for the DNA flanking the 5′ side of the C_μ coding region [in the H chain joining region (J_H)–C_μ intron]. *Xba* I-digested DNA probed with pHuC_μ gave these fragments for the cell lines: LR1, 6.8 and 3.5 kilobase pairs (kbp); LBW-2, 3.5 and 3.35 kbp; LBW-14, 3.5 kbp; DB, 5.4 and 3.5 kbp (not shown).

The human pre-B-cell line 697 used as a control in the anti-BiP immunoprecipitation experiment is derived from cultured lymphocytes of a patient with non-T non-B acute lymphoblastic leukemia and produces a native μ chain but no L chain.

Protein Analysis. To biosynthetically radiolabel proteins, 10^7 cells were incubated for 16 hr with 100 μCi of [^{3}H]leucine per ml (1 Ci = 37 GBq; New England Nuclear) in leucine-free RPMI 1640 medium (supplemented with 10% fetal bovine serum). Cells were washed twice before lysis in a nonionic detergent buffer and the postnuclear supernatant was immunoprecipitated using either affinity-purified goat anti-human μ- or goat anti-human L-chain antibodies (κ and λ) conjugated to Affi-Gel beads (Bio-Rad). The precipitated immunoglobulins were analyzed on 10% NaDodSO$_4$/polyacrylamide slab gels under reducing conditions and the gels were impregnated with En^{3}Hance (New England Nuclear), dried, and exposed to Kodak XAR-5 film for autoradiography for 24–72 hr at −70°C.

Cell-surface immunoglobulin was analyzed after labeling washed cell suspensions with a ^{125}I plus lactoperoxidase labeling system (16). After labeling, the cell lysates were treated as described above.

DNA Isolation and Analysis. High molecular weight genomic DNA was isolated from the cells according to the method of Perry *et al.* (17). Restriction enzyme-digested DNA was separated on 0.8% agarose gel (10 μg per lane) and transferred to Nytran (Schleicher & Schuell) after acid cleavage, denaturation, and neutralization of the DNA in the gel. DNA probes used in both Southern blots and RNA blots were radioactively labeled by nick-translation according to the method of Rigby *et al.* (18). Hybridization and washing conditions are as described by Schuler *et al.* (19).

RNA Isolation and Analysis. Cytoplasmic RNA from bulk suspension cultures was isolated as described by Schibler *et al.* (20). Selection of poly(A)-containing RNA was performed by affinity chromatography on oligodeoxythymidylate-cellulose columns.

RNA blot analysis was carried out using a formaldehyde gel system (21), followed by blotting to Nytran (Schleicher & Schuell). The RNA on the filter was hybridized with nick-translated cloned DNA probes of the human immunoglobulin C_μ region or C_κ region or end-labeled oligonucleotide probes corresponding to the 5′ ends of the human $C_\mu 1$ and $C_\mu 4$ exons (22, 23). Hybridization and washing conditions were done as described (20).

Sequencing of the μ-chain mRNA was accomplished using the procedure described by Geliebter *et al.* (24). A 19-mer synthetic oligonucleotide complementary to nucleotides 1–19 ($C_\mu 1.1$) and an 18-mer synthetic oligonucleotide complementary to nucleotides 26–44 ($C_\mu 1.3$) of the human $C_\mu 1$ exon (22) were used as primers to initiate cDNA synthesis (see Fig. 3).

Assay for BiP–μ H-Chain Intracellular Association. Cells (5×10^6) were labeled for 2 hr with 25 μCi of [^{35}S]methionine (Amersham) and cell lysates were prepared as described above. The cell lysates were divided and one half was immunoprecipitated with goat anti-human μ (Southern Biotech, Birmingham, AL) and the other half was immunoprecipitated with rat anti-BiP (6). Immune complexes were precipitated with fixed *Staphylococcus aureus* Cowan strain I and analyzed on 10% NaDodSO$_4$/polyacrylamide gels. The radioactive protein bands were visualized by autoradiography as described above.

RESULTS

Immunoglobulin Protein Expression in the Surface μ-Only B-Cell Lines. Immunofluorescence analysis confirmed the observations of Hendershot and Levitt (14) that the LBW-2, LBW-14, and DB cell lines expressed μ chain on their surface with no detectable presence of κ or λ L chains (data not shown). The LR1 cell line also exhibited only μ chains on the cell surface. To reexamine their finding that the μ H chains produced by these cell lines are smaller, the proteins synthesized by LBW-14 and DB cells were radiolabeled with [^{3}H]leucine and the immunoprecipitated μ chains were reduced and analyzed on NaDodSO$_4$/polyacrylamide gels (Fig. 1*A*). As expected, the μ chains synthesized by both lines exhibited a molecular mass of ≈66 and ≈63 kDa (presumably representing the membrane and secretory forms of the μ^* chain, respectively); these μ-chain molecules are 9 kDa smaller than that of the normal-sized μ chain made by F4 cells [Southern blot analysis of the C_μ region of F4 and sequencing of the V_HDJ_H region (D, diversity) of F4 mRNA has shown it to be of native form; unpublished work].

In addition, this experiment using leucine rather than methionine as the radiolabeling isotope allowed for a more definitive demonstration that L chain does not accumulate intracellularly to a detectable extent in these cell lines (Fig. 1*A*). In a separate experiment, LBW-2 and LR1 lines were also shown to lack L chain and to produce an identical-sized μ^* chain to that of the LBW-14 and DB cells (data not shown).

Cell-surface expression of the truncated μ^* chains was demonstrated by radiolabeling the plasma membrane proteins of LBW-14 and DB cells with ^{125}I. The 66-kDa μ^* species is transported to the surface in these lines, providing evidence that it is the membrane form (μ^*_m) of the truncated μ-chain protein (Fig. 1*B*). The μ^*_m protein is expressed on the surface of LBW-14 and DB cells to an equivalent degree as the native IgM monomers expressed on the surface of F4 cells. This finding was also confirmed by quantitative fluorescence-activated flow cytometry (data not shown). These results indicate that the μ^* chain is transported with roughly equivalent efficiency as native IgM molecules even though no pairing with L chain occurs. As expected, κ L chains are not expressed at the cell surface of these B-cell lines (the faint band at 30 kDa that is inconsistently present in the LBW-14 and DB lanes is due to overflow material from the F4 lane).

RNA Blot Analysis of μ^* mRNA. Cytoplasmic poly(A)-containing RNA from all four μ^*-only human B-cell lines and the F4 IgM (κ) control human B-cell line was assayed for the steady-state level and the size of C_μ-hybridizing and C_κ-hybridizing transcripts (Fig. 2). All four μ^*-producing lines contained C_μ-containing transcripts of 2.4 and 2.1 kb, which represent the membrane and secretory forms of μ^* mRNA, respectively [the 2.4-kb transcript but not the 2.1-kb transcript hybridizes with a probe specific for the human μ_m exon (data not shown)]. Although the μ^*_s mRNA species predominates in these cells, equal quantities of μ^*_m and μ^*_s protein exist intracellularly; this is due to the preferential degradation of μ_s protein, which occurs in nonsecretory B-lymphoid cells (25). The approximate 0.3-kb difference between the corresponding μ^* mRNA and μ^+ mRNA molecules is consistent

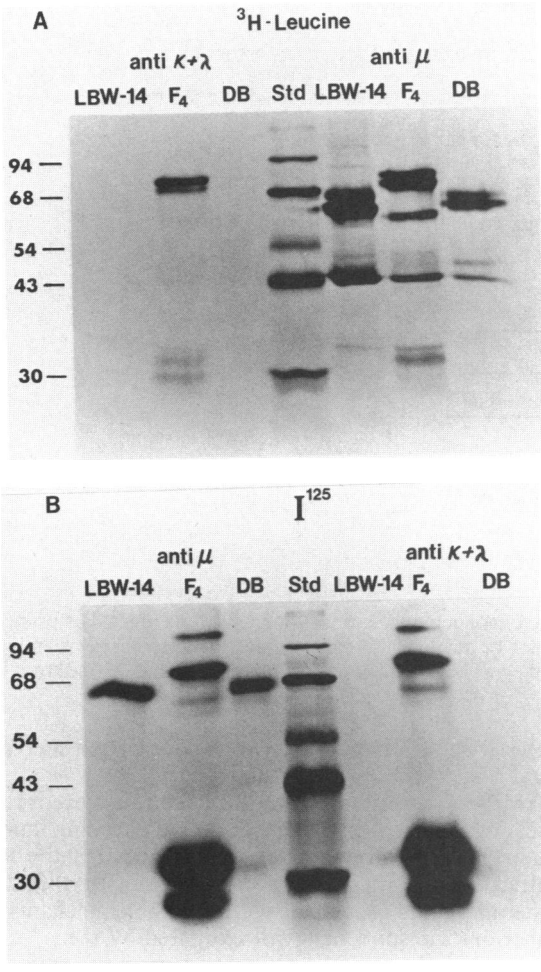

FIG. 1. Immunoglobulin protein expression in human μ-only B-cell lines. (*A*) LBW-14, DB, and F4 human B-cell lines were radiolabeled with [^{3}H]leucine and the anti-μ and anti-L chain (κ and λ) immunoprecipitates were analyzed by NaDodSO$_4$/PAGE under reducing conditions. The μ-chain species at 59 kDa in the F4 lane represents a putative human D_μ chain encoded by the "nonproductively" rearranged second H-chain allele (B.A.P. and D.L., unpublished data). The band at 42 kDa is actin (as shown from the marker lane). (*B*) Cell-surface immunoglobulin was radiolabeled with ^{125}I and was immunoprecipitated and analyzed as in *A*. Only the larger μ-chain species (membrane form) of each cell line is seen, with the μ^*_m chains being truncated to an identical degree as before. The bands for F4 at 30 kDa are κ L chains; the 100-kDa species is as yet undefined.

with a 100-codon deletion in the μ* message, which translates into a roughly 10-kDa calculated decrease in molecular mass for the μ* protein. This value agrees with the empirically derived difference in protein size of 9 kDa between μ* and μ$^+$ proteins (Fig. 1*A*). These results clearly indicate that the μ* chain is created from an alteration in protein structure and not incomplete glycosylation. Reprobing of the blot with a cloned DNA probe of the 3′ end of the human C_μ region (spanning the $C_\mu 4$ exon and the $C_\mu 4$–μ_m intervening sequence) gave μ* RNA transcript bands identical to those in Fig. 2 (data not shown).

All four μ* lines contain mature κ transcripts in their cytoplasm despite their inability to stably express κ-chain protein (Fig. 2). This observation confirms their B-cell rather than a pre-B-cell differentiative status. In addition, none of the lines contained mature λ-chain transcripts (data not shown). The RNA blots shown here were reprobed with two nonoverlapping oligonucleotides specific for the human $C_\mu 1$ exon ($C_\mu 1.1$ and $C_\mu 1.3$). Both oligonucleotides hybridized with the μ* transcripts under stringent conditions (washed at 5°C lower than melting temperature), demonstrating that the μ* mRNA molecules contain sequences of the 5′ end of the C_μ region (data not shown). This region is missing in the protein for a previously described case of human μ H-chain disease (11). Sequences at the 5′ end of the $C_\mu 4$ exon are also present in the μ* transcripts, as determined with a $C_\mu 4$-specific oligonucleotide (spanning C_μ codons 485–490) as a probe in RNA blot (data not shown).

Analysis of C_μ Gene Structure. Genomic DNA samples from DB, LBW-2, LBW-14, and LR1 cells were analyzed by Southern blotting using cloned DNA probes from the 5′ and 3′ C_μ gene regions (the 5′ C_μ probe is a subcloned *Eco*RI fragment spanning exons 1–3, and the 3′ C_μ probe is a subcloned *Eco*RI fragment spanning exon 4 and the C_μ–μ_m intron). Using restriction enzyme digests that cut within the nonpolymorphic areas of the C_μ region (26), the digested DNA of all four μ* lines when probed with either C_μ clone showed a germ-line restriction pattern (data not shown). The absence of any detectable (>0.05 kb) structural alterations in the C_μ gene region indicated that significant internal deletions or insertions have not occurred in these four lines and are not responsible for creating the μ*-encoding gene.

Sequencing of the μ* Transcript. We next examined whether the μ* transcripts contained a normal V_H sequence. Using two synthetic oligonucleotides complementary to nonoverlapping sequences in the 5′ end of the $C_\mu 1$ exon, the μ* mRNAs in all four lines were sequenced in the 5′ direction (Fig. 3). Because of the abbreviated nature of the 5′ region of the μ* transcript, extension of the primer under Sanger sequencing conditions permitted reading of the complete 5′ nucleotide sequence of the μ* mRNA (complete sequence not shown). All four μ* mRNA samples had near-identical sequences, suggesting that a single V_H gene or a set of closely related V_H genes is utilized by these B-cell lines. Although only two of the μ* mRNAs could be reproducibly and accurately sequenced across the splice junction, the up-

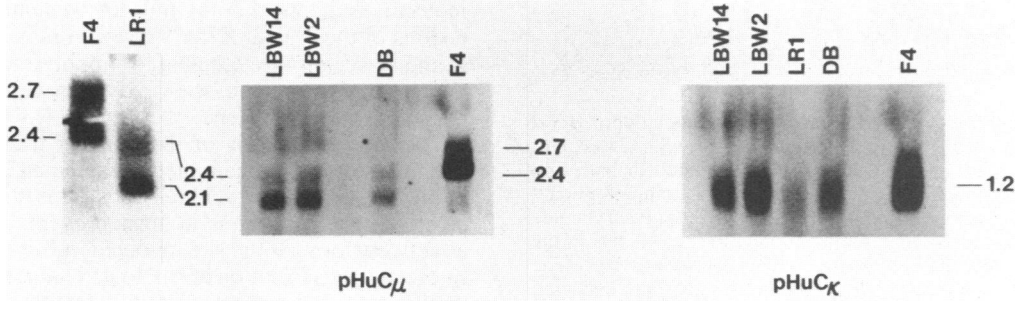

FIG. 2. RNA blot analysis of μ and κ transcripts in μ* cells. Size-fractionated (on formaldehyde gels) poly(A)$^+$ cytoplasmic RNA was probed with a human C_μ-specific DNA clone or a human C_κ-specific DNA clone to determine the steady-state levels of each message. The size demarcations on the sides of the blots are in kb.

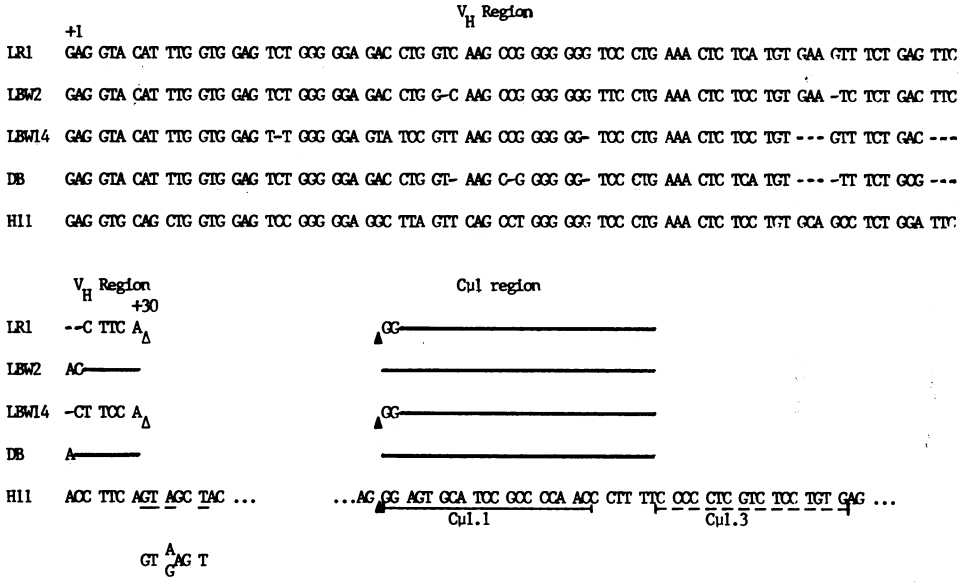

FIG. 3. Sequence of the μ^* transcripts in the region 5′ to $C_\mu 1$. Two synthetic oligonucleotides, $C_\mu 1.1$ and $C_\mu 1.3$, were used as primers for the sequencing reactions. Solid lines denote regions where sequences could not be read, while dashed lines indicate bases of ambiguous composition. Open and solid triangles are splice donor and acceptor sites, respectively. The consensus splice donor sequence (27) is shown below the genomic H11 sequence, with the matching bases underlined.

stream sequences of all four transcripts aligned exactly when run on the same gel, indicating that V_H^* to C_μ splicing occurs at the same site in all four cell lines. The most striking characteristic of the μ^* transcript sequences is the identically arranged juxtaposition of the most 5′ portion of the V_H region to the normal 5′ end of the $C_\mu 1$ exon such that the 3′ three-fourths of the V_H region is missing in the μ^* mRNA. While the 5′ untranslated and leader regions appear to exist intact in these messages, only the first 29 codons plus 1 nucleotide from codon 30 of the V_H region are present. When μ^* mRNA sequences are compared to the germ-line human V_H segment H11 (28), the extent of the V_H region present and the existence of a putative cryptic splice donor site can be defined (Fig. 4).

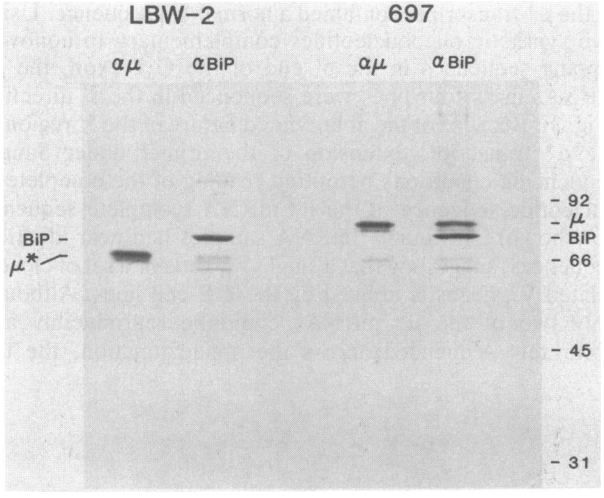

FIG. 4. Assay for μ^* chain–BiP intracellular association. LBW-2 and 697 cells (5×10^6) were labeled for 2 hr with 25 μCi of [^{35}S]methionine. Cell lysates were prepared, divided in half, and immunoprecipitated with anti-human μ or anti-BiP. Immunoprecipitated material was analyzed on a 10% NaDodSO$_4$/polyacrylamide gel under reducing conditions. The band migrating at 79 kDa in the anti-BiP-precipitated material (lanes αBiP) represents BiP. The bands representing native μ H chains and μ^* H chains are indicated in the margins (the membrane form predominates in both lines). The 66-kDa band immunoprecipitated in the 697 line represents a truncated form of μ chain produced by these cells; the 64-kDa band brought down with anti-BiP is undefined.

Interaction of μ^* Chains with BiP. With the recent results implicating BiP as a regulatory protein controlling immunoglobulin H-chain transport (6, 8), we wished to test the degree of intracellular association of μ^* chains with BiP to see whether this could provide us with a possible mechanism for the aberrant transport behavior exhibited by the μ^* chains (Fig. 4). Co-immunoprecipitation experiments using anti-μ chain or anti-BiP antibodies evidenced that the intracellular association of μ^* with BiP is dramatically decreased compared to native μ-chain–BiP intracellular association. While the anti-BiP monoclonal antibody is able to coprecipitate the majority of the μ_m and μ_s chains synthesized by the human pre-B (μ^+, LC$^-$) cell line 697, only small amounts of the μ_m^* and μ_s^* heavy chains from the LBW-2 cell line were coprecipitated with BiP.

DISCUSSION

A departure from the coordinated regulation of immunoglobulin H- and L-chain expression at the cell surface can occur in certain human B-lymphoid cells where μ-chain-only cell-surface immunoglobulin is expressed. Similar to H-chain-only secretion in human H-chain disease and some variant mouse plasmacytomas, cell-surface expression of μ-chain-only molecules involves an alteration in protein structure. The deletion in protein-coding sequences in the μ^* mRNA molecule is identical in the four lines examined such that 69 codons of the 3′ end of the V_H region plus all codons of the D and J_H regions are eliminated. Importantly, no changes in the primary structure of the C_μ regions were found in the μ^* mRNA or in the μ^* gene.

The molecular basis of the unusual transport properties exhibited by the μ^* chain then is explained by its loss of 75% of the V region amino acid residues. This internal deletion of most of the V_H region in turn prevents μ^* chains from effectively forming the site involved in BiP–H-chain binding. Intracellular association of BiP to μ^* chains is largely, but not totally, inhibited by this structural change. This is similar to what was previously observed for truncated mouse μ chains having deletions in the $C_\mu 1$ domain (8). Together these results indicate that the loss of either V_H or $C_\mu 1$ domain primary

sequences in turn leads to aberrant secondary structure in this region of these truncated μ chains such that the BiP recognition site is improperly formed. Because of the impaired ability of BiP to recognize the μ* chains in the ER, L-chain pairing is not required to displace BiP and allow for the transport of μ* H chain. Intracellular transport of the μ* chains is thus unencumbered from the normal mechanism governing transport of native H chains, leading to the expression of the unusual μ-chain-only phenotype for cell-surface immunoglobulin. While it is known that both mouse and human $C_\mu 1$ domain-deleted μ chains can be secreted without being paired with L chain (8–11), the ability of these truncated μ chains to be expressed at the cell surface is currently unknown.

The creation of the μ* mRNA occurs via an alternative pattern for V_H to C_μ RNA splicing. In these B-cell lines, a switch in the splice donor site utilized in the V_H–C_μ splicing event has occurred; presumably, the donor splice site at the 5′ end of the J_H intron normally utilized in producing μ-chain messages has been inactivated either through somatic mutation of the J_H gene or by aberrant V_H to DJ_H recombination with accompanying deletion of the J_H splice site at the DNA level. The cryptic splice donor site activated by this event may exist only within one or several closely related human V_H genes since (*i*) a high degree of sequence identity exists between the four μ* mRNA molecules for the V_H leader and 5′ untranslated regions (>91%), and (*ii*) the site of transcriptional initiation for the μ* transcripts appears to be identical (at nucleotide −73, where nucleotide +1 represents the translational start). The human V_H gene families I and II (27) do not contain any sequence resembling a splice donor site, while the human V_HIII gene family members have a sequence at codon 30 that matches the splice donor site consensus sequence (29), for 5 of 7 nucleotides, with the critical G·T pair present at the exon–intron boundary (see Fig. 3). Whether the V_H gene(s) used by the μ* lines also belongs to the V_HIII gene family, or whether it is a member of a separate V_H gene family is as yet unknown.

All four cell lines that produced μ* protein also synthesized κ L-chain transcripts yet failed to express κ protein. The basis for the inability in stable κ protein expression in these cells is unclear but likely involves a post transcriptional regulatory mechanism since a very low level of κ protein expression is occasionally observed by cytoplasmic immunofluorescence in a small minority of cells in the DB and LBW14 lines (2–5% of control levels in 10–15% of the cells as measured by flow cytometry). Whether rapid posttranslational degradation of the κ-chain protein is occurring [similar to what happens in a truncated H-chain-producing variant of the MOPC21 plasmacytoma line (30)], or if a translational-based regulatory mechanism is responsible is currently being studied.

It was surprising to discover four independent human cell lines of the μ* phenotype present in a collection of only 47 human B-lymphoid cell lines originally screened for immunoglobulin expression. Since one of the lines described here (DB) was derived from a healthy individual, it seems likely that B cells carrying the μ* phenotype normally exist *in vivo*. It is entirely possible that the μ* L-chain-negative phenotype occurs relatively frequently among untransformed B cells, but that such cells are unstable and are rapidly eliminated *in vivo*. If these cells are more susceptible to transformation by EBV or malignancy-inducing events, their increased occurrence as cell lines would be explained. The isolation of two of four μ*-producing B-cell lines from patients with a form of congenital immunodeficiency may also suggest that such clones have a higher frequency of occurrence in certain immunodeficiency disease states. It therefore will be important to define and quantitate H* and L-chain-negative cells in normal and in immunodeficient human B-lymphocyte populations.

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