B LYMPHOCYTE DEVELOPMENT IS GUIDED BY THE mlg RECEPTOR

B lymphocytes are characterized by the synthesis of variable Ig molecules that occur in secreted and membrane-bound forms. Secreted Ig (sIg) are highly specific effector molecules that serve, together with soluble and cellular components of the innate immune system, to eliminate foreign substances that are potentially harmful to the host. Ig-secreting B lymphocytes represent cells that are selected out of a large population of B-lineage-committed progenitors and have proceeded successfully through several maturational stages.1 During these selection and differentiation processes, the membrane form of Ig (mIg) plays a key role.

On mature B cells, mIg functions as the clonotypic receptor for antigen (Fig 1). Only those cells that bind antigen with their unique combination of Ig heavy (H) and light (L) chains will, under appropriate conditions, be activated and start to multiply. In the germinal centres of secondary follicles, the repertoire of these proliferating cell diversifies due to the introduction of point mutations in rearranged variable Ig regions.24 This mechanism of somatic hypermutation, which is an exclusive feature of the antibody response, permits the occurrence of daughter cells that synthesize Ig molecules with augmented affinity for the antigen. In the course of further development, high-affinity receptor-carrying variants preferentially expand most likely as a result of ongoing competition for antigen that is “presented” at the surface of follicular dendritic cells.26-7 At this site, unprocessed antigen is preserved for prolonged periods, which is critical for the induction and maintenance of B-cell memory.8,9 Thus, during the antigen-dependent phase, the processes of clonal expansion, affinity maturation, and B-memory-cell formation all seem to be governed by the qualities of the mlg receptors expressed.

Pre-B cells, by definition, produce μH chains, but no L chains, as they have not yet rearranged the L chain gene. It is known that, in the absence of Ig L chains, membrane μ H chains (μm) are retained intracellularly by the heavy chain binding protein (BiP)10-14 and are destined to rapid degradation.15 However, in pre-B cells, a small fraction of the produced μm chains does appear at the cell surface due to interaction with a pseudo L (ψL) chain complex.16-20 The proteins of the ψL-chain complex, initially called i and ω, are encoded by the VpreB and λ5 (or “λ-like” in humans) genes,21-25 which share homology with variable and constant region λ L-chain genes, respectively (Fig 1). Expression of VpreB and λ5 does not require gene rearrangement and is confined to pre-B cells.26-28 Evidence accumulates that, for advancement of early B-cell maturation, the surface expression of low amounts of the μm-ψL complex is essential. Introduction of membrane forms, but not secreted forms, of rearranged Ig H chain genes in pre-B cells was found to suppress rearrangement of the endogenous H chain loci.29 In addition, μm chain expression in early B cells has been implicated in the promotion of L chain gene joining.30-32 Recently, Kitamura and Rajewsky33 have reported that targeted disruption of the μm exon causes loss of the H chain allelic exclusion mechanism in mice heterozygous for this mutation. In animals that carried the defective μm exon at both chromosomes, no mature B cells evolve.34 Ig κ chain gene rearrangement in bone marrow cells of the homozygous μm knockout mice was clearly reduced, but not absent. Similar, although less absolute, defects were observed as a result of targeted disruption of the λ5 gene.35 Interestingly, pre-B cells producing μH chains without attached VH regions have been found in X-linked agammaglobulinemia patients.36 These findings suggest that the μm-ψ-L complex serves as a variable membrane receptor on pre-B cells, which, upon binding of yet unknown ligands, transmits signals indispensable for further differentiation. Possibly, during both early and late B-cell development, cells are selected on the basis of the same principle in that they require constant signaling via mIg for their survival. This selection of B cells on the functional integrity of their mIg may guarantee that finally antibodies with optimal specificity will be secreted.

THE CONSTITUENTS OF THE BCR

The BCR is a multichain receptor that comprises a variable mlg glycoprotein and a recently identified heterodimer of nonvariant transmembrane (TM) proteins, referred to as Igs.37 Igs are tetramers constituted by pairs of disulfide-linked H and L chains. Within the Ig H and L chains, NH2-terminal variable regions and C-terminal constant regions can be distinguished. The genes that encode the antigen-binding variable regions have to be somatically assembled from individual members of variable (V), diversity (D), and joining (J) gene segment families.38 Two genes, recombination activation gene (RAG)-1 and RAG-2,39 have recently been identified whose products are essential for the processes of V(D)J joining at the Ig and T-cell antigen receptor (TCR) loci.40 Mice that were made deficient for RAG-141 or RAG-242 via gene targeting show total inability to initiate V(D)J rearrangement and, as a consequence, have...
Fig 1. Diagram of the variable receptors found on mature or pre-B cells. (Left panel) Disulfide-linked H chains and L chains form an mlgM molecule with two identical antigen-binding sites. Within the µ-H chain, intracellular, transmembrane (µm), four constant (CH1-CH4), and variable (VH) domains are discerned. The L chain consists of a constant (CL) and a variable (VL) region. Depicted is the location of the complementary determining regions (CDR1-CDR3) relative to the V, (D), and J segments of the H and L chains. (Right panel) On pre-B cells, the variable µm chains are associated covalently with an α protein and noncovalently with a γ protein. These molecules, collectively referred to as the µL chain complex, are the products of the A5 (A-like in human) and VpreB genes.

no mature B or T lymphocytes. In human B cells, assembled Vµ genes can recombine with nine constant (Cµ) genes13,44 (ordered 5'-Cµ5-Cµ6-Cµγ3-µCµγ1-µCµγ2-µCµγ4-Cµα1-Cµα2-3'); in a similar fashion, Vµ genes unite with Cδ or Cε genes.55,46 Hence, five classes of H chains and two L chain isotypes can be generated by the B-cell population. Depending on the differentiation stage of the B cell, the produced Ig H chains have hydrophobic or hydrophilic COOH-terminal segments, which determines whether Ig occurs as membrane-anchored (mlg) or secreted (slg) molecules. All five types of antibodies (IgM, IgD, IgG, IgE, and IgA), named after the constant region of the H chain, can thus be synthesized in secreted or membrane-bound form. Mature resting B cells characteristically coexpress membrane forms of IgM and IgD, with identical variable domains.57-49 The cytoplasmic tails of µm and δm comprise only three amino acids, KVK (one letter amino acid code).30,51 In the intracellular domains of mlgG32 and mlgE,33-35 25 additional amino acids append C-terminally to the membrane-proximal KVK motif. MlgA possesses a 14 amino-acid tail, unrelated to any of the other isotypes.52,56

The mlg-associated αβ heterodimer has been biochemically defined in both mouse and human by virtue of the use of the mild detergent digitonin in immunoprecipitation studies. Under this condition, the weak noncovalent bonds between the variant and invariant complexes of immunoprecipitated BCR remain unaffected. MlgM, originating from murine or human B cells, was thus found to be associated with disulphide-linked αβ dimers of approximately 34/39 Kd57-4 and 47/37 Kd.55-47 Surprisingly, the mlg-associated molecules had not been defined before immunologically,48 and still the number of Igα- and Igβ-reactive antibodies is limited.63,69-74 Independent of these findings, two B-lineage-specific genes mb-175,76 and B2977 had been isolated from murine (pre-)B-minus T-cell cDNA libraries. By transfection studies,78-80 amino-terminal protein sequencing,72,73,75 and biochemical analyses with antipeptide antibodies,72,73 it was established that murine mb-1 and B29 code for the 34-Kd Igα and 39-Kd Igβ polypeptides, respectively. The human mb-181,82 and B29 genes83,84 have also been identified and code for the 47-Kd and 37-Kd mlgM-associated molecules.68,70,83

Expression of the mb-1 and B29 genes is, as may be expected on the basis of the isolation strategy, largely confined to the B-cell lineage. mb-1- and B29-derived RNA transcripts75,77,81,82,84 and proteins69,70,72,74,85 have been detected in normal and neoplastic B cells, but not in peripheral T cells. On the other hand, low levels of mb-1 mRNA have been reported for some human pre-T- and T-cell lines,81,82 whereas in human tissue sections, smooth muscle cells and pancreatic acinar cells are stained by anti-mb-1 monoclonal antibody (MoAb).69 During B-cell differentiation, transcription of mb-1 and B29 is initiated before or simultaneously with Ig H chain gene rearrangement (Fig 2). Expression of mb-173 and B2977 mRNA has been reported for two murine B-precursor-cell lines whose Ig genes are in germline configuration. It is not known whether these gene transcripts are also translated, and, if so, whether mb-1 and
germline configuration. It is not known whether these gene transcripts are also translated, and, if so, whether mb-I and B29 protein products can be membrane expressed in the absence of mIg. Mason et al. have reported that the majority of human μH-precursor B-acute lymphoblastic leukemia (B-ALL) cells produce Igα but not Igβ molecules. Those B-ALL that synthesize both Igα and Igβ in most cases express cytoplasmic μ H chains as well, and are therefore to be classified as pre-B-ALL. It was therefore concluded that Igα is, together with CD19, one of the earliest B-lineage-specific molecules expressed during ontogeny. By immunoprecipitation studies of human and murine pre-B-cell lines, it has been shown that Igα and Igβ associate with the μm-ψL chain complexes. Northern analyses of mature human B-cell lines have indicated that the mb-I gene is transcribed in cells expressing mIg of μ/δ, γ, and α iso-types. The presence of mb-I mRNA could not be shown in an mIgE* myeloma line, not even after amplification by the polymerase chain reaction. Evidence for the physical linkage with mb-I/B29-encoded dimers on primary B cells has now been provided for three human (μ, δ, and γ) and two murine (μ and δ) mH-chain classes. Bone marrow-derived human plasma cells and myeloma cells synthesize, like precursor-ALL, Igα in the absence of Igβ. Conversely, most, but not all, murine myeloma cell lines have an mb-I 'B29' phenotype, whereas no data on normal murine plasma cells are available.

The mb-I and B29 genes most likely have a common evolutionary origin. They possess a comparable exon-intron organization and encode type I transmembrane proteins similar in size and overall structure. Nucleotide sequence information indicates that both Igα and Igβ have been well conserved evolutionarily (sequence homology between human and mouse >90%), except for their extracellular (EC) Ig-like regions (42% and 59% for Igα and Igβ, respectively). Human Igα has been synthesized as proteins of 226 and 229 amino acids (aa), including leader (Igα 32 aa/Igβ 30 aa), EC (11/12), TM (22/22), and cytoplasmic (61/48) domains (Fig 3). The EC domain of Igα contains three cysteine residues. Most probably, two of these cysteine residues form an intrachain disulfide bond, thus defining a domain that, deduced from its amino-acid composition, has features in common with Ig- and TCR-V regions. Igβ harbors 5 cysteine residues extracellularly, which in theory offers the opportunity to form two intrachain disulfide bonds. The covalent interaction between Igα and Igβ is presumed to result from disulfide linkage of the most COOH-terminal cysteine residues found in the EC domains of these molecules. Different numbers of potential N-linked glycosylation sites are predicted for human Igα (6, of which 4 are known to be used) or murine Igα (2). This finding is in agreement with biochemical data, which indicated that size differences between human and murine Igα result from an unequal degree of glycosylation. Removal of N-linked sugars from the 47-Kd human Igα and 34-Kd murine Igα molecules reduced their molecular weight to 22.5 Kd and 23.6 Kd, respectively. Human Igβ is known to use three of its four potential N-linked glyco-
The minor size differences between human and murine Igδ (37 Kd v 37 to 39 Kd) may indicate that murine Igδ uses all of the three potential carbohydrate attachment sites. Strong evolutionary conservation has been noted for the membrane-proximal EC and TM regions of Igα (96%) and Igδ (90%), which is not common for transmembrane molecules. Reconstitution studies have shown that the products of mb-l and B29 genes are both required to establish surface expression of mIg in B cells \(^{57,59,78,89}\) and non-B cells.\(^{79,80}\) Within the BCR, the αβ heterodimer interacts with the distal Cε (Cε4 and Cε3, respectively) and TM regions of μ and δm H chains.\(^{59,78,80}\) The TM segments of different mIg isotypes are highly homologous and well conserved between species.\(^{57}\) They contain a number of polar serine and threonine residues that prevent incorporation of incompletely assembled, ie, nonfunctional, antigen receptors in the cell membrane.\(^{90}\) It has been postulated that the TM portions of Igα and Igδ, via interaction of their charged amino acids with these hydrophilic regions, form a hydrophobic sheath around mIg.\(^{57,90,91}\) Thus, analogous to the TCR-CD3 complex,\(^{90}\) only the ensemble of mlg, Igα, and Igδ has the capacity to assimilate with the lipid bilayer.

Although transfection experiments in murine cells have indicated that all five mH-chain isotypes can potentially interact with the protein products of mb-l and B29,\(^{79}\) it has been questioned whether in primary B cells the various mlg isotypes, which differ significantly in their terminal Ig-like Cε regions, associate with the same αβ heterodimer. Particularly, isotype-specific forms of Igα have been suspected on basis of observed differences in molecular weight between mlgM- and mlgD-associated Igα molecules \(^{58,59}\) and the finding that transfected mlgD and mlg can appear at the surface of mb-l cells.\(^{58,59,93}\) However, it is now established that Igα and Igδ molecules, originating from human B-cell lines carrying different mlg classes, are derived from the same mb-l and B29 transcripts, respectively.\(^{58,59,93}\) Biochemical dissimilarities between αβ heterodimers thus result from different posttranslational modifications\(^{52,73,83}\) dictated by the adjacent Ig H chain.\(^{59}\) In addition, the reported mb-l-independent membrane form of IgD\(^{58,59,93}\) has now been shown to represent a phosphatidylinositol (PI)-linked species.\(^{94}\) Selection of PI-linked variants, which most likely carry intrachain mutations, has been described before also for transfected human mlgM.\(^{95}\) The significance of this finding for B-cell physiology seems limited in view of the fact that normal B cells, without exception, have an mb-l+B29+ phenotype.\(^{69,70}\) Indeed, we were unable to release mlgD, or any other mlg class, from the surface of human tonsillar or splenic B cells nor from a variety of B-cell lines with PI-specific phospholipase C (Lankester et al, manuscript submitted).

Recently, biochemical evidence has been obtained for a direct physical interaction of human μm, δm, and γm H chains with their respective Igδ (Van Noesel et al, manuscript submitted). This, combined with the reported absence of Igδ in progenitor and terminally differentiated human B cells,\(^{70}\) may indicate that the appearance and
disappearance of the BCR is regulated via B29 gene expression.

**SIGNAL TRANSDUCTION THROUGH THE BCR**

It is generally believed, but as yet not proven, that the αβ heterodimer plays a prominent role in BCR signaling. The cytoplasmic tails of neither Igα nor Igβ have kinase- or phosphatase-consensus motifs. In these regions, Igα and Igβ contain conserved serine (Igα 2/Igβ 1), threonine (1/5), and tyrosine (4/2) residues that may be targets for phosphorylation. Two of the threonine residues found in Igβ may serve as substrates for protein kinase C because they are preceded by a basic amino acid. Moreover, both Igα and Igβ incorporate a conserved protein motif of six precisely spaced amino acids. This "antigen receptor tail motif", of the composition D-(X)2-E/D-(X)2-Y-(X)2-L-(X)2-Y-(X)2-L/I, is found also in constituents of other immunologic receptors (Fig 4).

Nomura et al showed that an Igα-specific MoAb caused a transient increase in intracellular free Ca\(^{2+}\) and inhibition of proliferation of a murine pre-B-cell line. However, no experimental proof has been delivered for a direct interaction of any of the BCR components with a G-protein.

Prototypes of the so-called receptor-kinase family, such as the platelet-derived growth factor and the epidermal growth factor receptors, possess intracellular tyrosine kinase domains whose activation coincides with ligand-induced receptor oligomerization. As a result, tyrosine residues that are found within the same intracellular domains become auto- or cross-phosphorylated. These phospho-tyrosine residues, together with their unique peptide environment, constitute high-affinity binding sites for specific src-homology 2 (SH2) domains. Significantly, the noncatalytic SH2 domains are contained within a series of signaling proteins such as PLCγ, Ras GTPase-activating protein (GAP), PI 3-kinase, and PTK of the src family.

Phosphorylation experiments, in vivo and in vi-
tro,56,66,67,83,87 have shown that mIgM, mIgD, and mIgG are physically linked to phosphotransferases with specificity for serine, threonine, and tyrosine residues of Igα and Igβ. In transformed human B cells, the invariant members of the BCR are constitutively phosphorylated on serine and threonine residues.65 In these cells, as well as in murine splenic B cells, ligation of mlg induces rapid tyrosine phosphorylation of the αβ dimer and a variety of other, as yet unidentified, proteins.115-119 Specific inhibitors of PTK completely suppress antigen-receptor–induced inositol-lipid hydrolysis, intracellular Ca2+ mobilization, and cellular activation.100,120,121 Conversely, mlg-mediated protein-tyrosine phosphorylation is unimpaired in PKC-depleted B cells.119 This suggests that activation of PTK is a proximal event that is required for the coupling of the BCR to PLCγ. It has recently been shown that, in T cells, the CD3-ζ complex, after ligation by CD3 MoAb, associates with an active form of the PLCγ1 enzyme.122 Most likely, phosphorylated tyrosine residues of CD3γ, δ, ε, or ζ are sites recognized by the PLCγ1 SH2 domain. This implies that, in view of the structural similarities as defined by the “antigen receptor tails” (Fig 4), tyrosine residues of Igα and Igβ could have a similar function. Indeed, the signaling apparatus in T and B cells must be alike, because artificially expressed BCR have been shown to function in T cells.123 Several investigators have reported a physical and functional association of the BCR with src PTK-family members blk, lck, and fyn.87,124-127 and a newly defined B-cell–specific kinase, PTK72.128-130 Murine PTK72 is structurally related to the 70-Kd ζ-associated protein (ZAP-70), whose expression is limited to T and natural killer cells.131,132 Also, in human B cells, a 70-Kd phosphoprotein has been found in association with the BCR.85 Recently, atk134 or BPK,135 a novel src-like kinase has been identified that, in mutated form, has been implicated in the pathogenesis of human X-linked agammaglobulinemia (XLA). The atk gene is normally expressed during all stages of B-cell maturation, whereas it is suggested that the absence or defectiveness of its products in XLA patients provokes a block in early B-cell development. It is unclear whether atk is receptor-associated and if there is a functional connection with the BCR. At present, it is known that BCR engagement enhances the activity of the previously mentioned src-family members, PI 3-kinase,40,126 microtubule-associated protein-2 kinase (MAP-2K),136 p21ras GTase-activating protein (GAP),137 and the product of the proto-oncogene Vav.138,139 An important issue to be clarified is which of the protein kinases, coisolated with the BCR, can phosphorylate the αβ heterodimer and which of them represent phospho-tyrosine–“recruited” enzymes with different specificity.

THE BCR ON NEOPLASTIC HUMAN B CELLS

In lymphoma or leukemia patients, CD19+ tumor cells with rearranged Ig genes in almost all cases express mlg at the cell surface, even after years of disease. This is significant because mutations that arise in the Ig locus during the numerous replication cycles of the neoplastic cells40,141 will include stop codons and nonfunctional frameshifts. Still, selective forces seem to prevent the outgrowth of Ig− mutants. This notion is supported by the finding that relapse tumors of a follicular lymphoma, after treatment with anti-idiotypic antibodies, possessed extensively modified V regions rather than having lost mlg expression.142 In addition, an oligoclonal precursor-B acute lymphoblastic leukemia has recently been described in which subclones had been generated by V-gene replacement processes.143 Whereas in a transformed B-cell population that would be able to expand independently of mlg, two-thirds of such recombinational events would expected to be nonfunctional, in this patient, the vast majority (>90%) of tumor clones carried in-frame Ig rearrangements. These combined observations suggest that, although B-cell neoplasia are considered to be “frozen” in a particular maturational stage, they may, like normal B cells, still require BCR signals for their survival. In vitro activation studies have indicated that mlg complexes of B-cell neoplasia indeed have the potential to transmit growth regulatory signals. Antibodies against mlg have been shown to induce mobilization of free Ca2+ in freshly isolated B-CLL cells, which, in some cases, is accompanied by the initiation of cellular proliferation.144,145 Similar results have been obtained with prolymphocytic leukemias146,147 and various types of B-cell lymphomas.146,148-150

The assumption that for the expansion of malignant B cells mlg-mediated signals are also essential raises the question as to what extent the specificity of their variable receptor matters. Recently, a murine B-lymphoblastic cell line NYC has been described that depended for its growth on a cognate interaction of mlg with an endogenously produced retroviral antigen.151 Based on the finding that FACS-sorted Ig-loss mutants of NYC could not be cultured, it was concluded that continuous antigenic stimulation accounted for the tumorigenic properties of these cells. Several investigators have reported that CD5+B-CLL are often committed to the production of autoantibodies.152,153 This finding may be etiologically significant, as continuous engagement of the BCR by autoantigens might provide agonistic signals resulting in the persistence and accumulation of genetically altered cells in the periphery. More direct evidence for a role of antigenic stimulation in tumorigenesis in vivo has been found in lymphoma patients. Follicular lymphomas are known to have the property to somatically mutate their Ig,140-142 which, in principle, provides the possibility to study the genealogic relationship between transformed cells. Bahler and Levy155 have recorded a follicular lymphoma patient whose cells, in the course of disease, exhibited a sequential and nonrandom pattern of VH gene mutations, strongly suggesting that clonal selection processes had influenced tumor progression. In addition, a longitudinal study of an autospecific B-cell lymphoma, performed by Friedman et al.,156 showed that minor tumor clones at diagnosis became predominant in relapse samples. Markedly, the investigators were able to show that, despite this clonotypic shift, the autospecificity and idiotype of the tumor cells had been preserved. In conclusion, genetic events causing cellular transformation do not necessarily imply that cells function autonomously, in the sense that BCR signals still appear vitally important for the progression of at least some B-cell malignancies.

In view of the fact that most lymphomas and leukemias are derived from B-lineage–committed cells, which in ma-
iority are mIg^+, it is surprising that virtually no data exist on the configuration of their BCR. However, despite the scarcity of data on this subject, there is no reason to assume that the requirements for surface expression of mlg are different on malignant B cells. In this respect, it should be noted that much of the information regarding the structure of the human BCR is based on experiments with malignant B-cell lines.55,66,68,85,137,157 We have also performed biochemical studies on the configuration of mlg-complexes of human B-cell chronic leukemias (B-CLL) and non-Hodgkin lymphomas, and observed no major differences with normal B cells (unpublished observations). However, detailed studies on the expression and function of mb-1 and B29 in the various types of B-cell neoplasia will have to await the availability of reagents specific for the extracellular domains of Igα and Igβ.

CONCLUDING REMARKS

The identification of the invariant mlg-linked heterodimer allows us to study, for the first time, antigen-evoked transmembrane signaling processes in normal and neoplastic B cells at the molecular level. One of the major points to be noted is that, throughout B-cell ontogeny, the overall structure of the variable mlg receptors, but also of their putative signal transduction units, is unchanged. This finding supports the idea that B-cell differentiation progresses on the basis of repeated generation of the same stimuli. It must then be assumed that these signals are able to induce distinct transcriptional events in cells at different developmental stages. On the other hand, evidence exists that differential signals are elicited by different antigen receptors. For example, mature B cells coexpress mlgM and mlgD receptors whose constituents have identical cytoplasmic domains. Cross-linking of either of these antigen receptor classes on a monoclonal cell population is accompanied by mobilization of intracellular Ca^{2+}158,159 and rapid tyrosine phosphorylation of only those heterodimers that are associated with the ligated isotype.148,160 However, proliferation of the mlgM^+mlgD^+ cells is strongly inhibited by the anti-IgM MoAb but is not affected by the IgD-specific MoAb.158,159,160,162 Obviously, these disparate responses cannot, despite the newly obtained insights in the architecture of the BCR, be explained. In fact, they strongly suggest that additional, most likely membrane-associated, components contribute to the BCR. This is in agreement with observations that transfected mlg/Igα/Igβ complexes are, at best, partially functional.80,163,164 The putative new members of the BCR may be constitutive associates of the antigen receptor or molecules that are assembled upon activation of the cells. In this respect, the multimeric CD19/CD21 complex,165,166 whose members have been shown to associate with the BCR after mlg cross-linking,167,168 is a good candidate. We have recently argued why this complex might, both at the extracellular and intracellular level, offer an essential contribution to antigen-related signal amplification.169

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