Surrogate or Conventional Light Chains Are Required for Membrane Immunoglobulin Mu to Activate the Precursor B Cell Transition

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Summary

To examine the role of light chains in early B cell development we combined RAG-1 and λ5 mutations to produce mice that expressed neither conventional nor surrogate light chains (RAG-1<sup>-/-</sup>, λ5<sup>-/-</sup>). Unique heavy and light chain genes were then introduced into the double and single mutant backgrounds. Membrane immunoglobulin (Igμ) (mlgμ) associated with Igα-Igβ but was unable to activate the pre-B cell transition in RAG-1<sup>-/-</sup> λ5<sup>-/-</sup> mice. Either λ5 or kappa light chains were sufficient to complement this deficiency. Therefore light chains are absolutely required for a functional Ig signaling module in early B cell development. Our data provide direct evidence for the existence of two pathways for induction of early B cell development: one which is activated through surrogate light chains and mlgμ, and an alternative pathway which uses conventional light chains and mlgμ.

lymphocyte development in the bone marrow advances through a series of ordered events, ultimately resulting in B cells that express Ig Ag receptors (1, 2). Although little is known about the initial lineage commitment events, progression along later stages in this pathway is guided by the expression of productively rearranged Ig heavy chains, and the membrane Igμ (mlgμ)-associated Igα and Igβ signal transducers (3, 4).

The earliest event thought to be induced by mlgμ is allelic exclusion (5). Upon a productive VH→DJH rearrangement and subsequent mlgμ expression, further rearrangements at other heavy chain loci are inhibited, resulting in B cells with clonotypic receptors (6–8). Recent work in several laboratories has shown that in addition to mlgμ, Igα, and Igβ, the surrogate light chain λ5 is also required to establish allelic exclusion (9–11). Thus, allelic exclusion is thought to be regulated by a complex formed by mlgμ, λ5, V pre-B, and the Igα-Igβ signal transducer.

After activation of allelic exclusion, mlgμ induces progenitor B (pro-B) cells to progress to the next stage in development and become precursor B cells (pre-B) (6, 12, 13). The pre-B cell transition appears to be activated by the same B cell receptor (BCR) components that trigger allelic exclusion. Mice that cannot produce a functional mlgμ, (8, 14–16), or the Igα-Igβ signaling complex (3, 17, 18) exhibit a profound block in B cell development at the pro-B cell stage. In contrast the requirement for the surrogate light chain in the pre-B cell transition is not absolute and λ5 deficiency results in an incomplete block in development: λ5<sup>-/-</sup> pro-B cells leak through to the pre-B cell stage and ultimately populate the mature B cell compartment (19, 20).

To explain the leaky phenotype found in λ5<sup>-/-</sup> mice, Melchers, Rajewsky, and colleagues have proposed that there are two pathways for B cell development (19, 21). Under normal circumstances mlgμ would be paired with the surrogate light chains, λ5 and Vpre-B, to produce an active pre-B cell receptor. Alternatively, when the surrogate chains are not expressed, or when a light chain rearranges early, a salvage pathway would be activated. This secondary pathway would require that mlgμ pair with conventional light chains to produce the functional pre-B cell receptor complex.

To test this hypothesis we studied B cell development in mice restricted to expressing mlgμ alone, or in combination with either κ light chain, or surrogate light chains. Here we report direct evidence for the existence of two pathways that mediate early B cell development.

Materials and Methods

Constructs and Mice. The transgenic constructs used for the experiments reported here have been described elsewhere (22). The antibodies produced by the heavy and light chain combination is detected with the 54.1 monoclonal antibody (23). RAG-1<sup>-/-</sup> mice were a gift from Dr. David Baltimore (Massachusetts Institute of Technology, Cambridge, MA) and E. Spanopoulou (Mount Sinai Medical School, New York). λ5<sup>-/-</sup> mice were a gift from Dr. F. Melchers (The Basel Institute for Immunology, Basel, Switzerland) and provided by Dr. M.D. Cooper (University of Alabama at Birmingham, Birmingham, AL). All mice were bred and maintained under specific pathogen-free conditions.

Flow Cytometry. The following antibodies were used for surface staining of bone marrow B cells: PE-B220 (Pharmingen, San...
Diego, CA), FITC-CD43 (57 hybridoma; American Type Culture Collection, Rockville, MD), biotin-labeled anti-human IgM (heavy chain-specific DA4.4 clone; Southern Biotechnology Associates, Inc., Birmingham, AL), and biotin-labeled antiidiotype (54.1 hybridoma; a gift from Dr. D. Nemazee, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). Biotin-labeled antibodies were visualized with streptavidin-RED670 (GIBCO-BRL, Gaithersburg, MD). Data were collected on a FACScan® (Becton Dickinson and Co., Mountain View, CA) and were analyzed using CellQuest software (Becton Dickinson).

Intracellular Human IgM Expression. The presence of human IgM heavy chain in the cytoplasm of B cells was verified by FACS®, as previously described (24). Briefly, B cells from the bone marrow of 6-8-wk-old mice were first stained with PE-B220 and FITC-CD43 (57 hybridoma) antibodies. The cells were then fixed with 4% paraformaldehyde in PBS and permeabilized with 1% saponin. Anti-human IgM was then used to stain the treated B cells. Data acquisition was performed with a FACScan® and appropriate gating and analysis was done with CellQuest software.

Abelson Virus Transformation of Bone Marrow Cells. Bone marrow from 6-8-wk-old mice was transformed with Abelson MuLV (A-MuLV) in accordance with published protocols (25). Briefly, 2 × 10⁷ bone marrow cells were suspended in 6 ml of complete medium (RPMI 1640 supplemented with 10% FCS, 50 U/ml penicillin, 50 µg/ml streptomycin, and 50 µM 2-ME), treated with 2 ml of virus stock, and plated onto 100-mm plastic petri dishes (Coming Inc., Corning NY). The dishes were left at 37°C in a humidified 5% CO₂ atmosphere for 2 h, and then 4 ml of medium was added. Transformed B cells were maintained in complete medium.

Immunoprecipitation and Immunoblotting. A-MuLV-transformed bone marrow cells were lysed in 1% n-dodecyl-B-D-maltoside...
Transgene: Igμ
Background: RAG-1-/-

Figure 2. Intracellular expression of the human μ heavy chain transgene. Cells, stained with PE-ann-B220 and FITC-ann-CD43, were fixed, permeabilized, and subsequently stained with bmtm-antl-human IgM. Gating was on CD43+ cells.

Results

Light Chains Are Necessary for the Pro-B to Pre-B Cell Transition. In the bone marrow, induction of the pre-B cell transition coincides with loss of expression of CD43, and so pre-B cells are B220+CD43- whereas pro-B cells are B220+CD43+ (Fig. 1 A and [27]). To determine whether light chains are required for early B cell development we first bred together RAG-1-/- and k5-/- targeted mice to create double-deficient animals (RAG-1-/-k5-/-). In this background there are no heavy or light chain gene rearrangements because of the RAG-1 deficiency (15), and k5 is not expressed (19, 20). The phenotype of the RAG-1-/-k5-/- animal resembles that of the RAG-1-/- mice, in that B cells are arrested at the B220+CD43+ pro-B cell stage (Fig. 1 A).

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The developmental defect observed in the RAG-1-/- mice is strictly due to their inability to perform Ig rearrangements, and it can be overcome by the addition of a pre-rearranged mIgμ transgene (12, 13). The same human mIgμ transgene also facilitated the pre-B cell transition in k5-/- mice (Fig. 1 B). However, it had no effect in the double-mutant background: FACS® analysis of the bone marrow of TG.mIgμ RAG-1-/- mice revealed only B220+CD43+ pro-B cells (Fig. 1 B). These cells expressed human Igμ but did not transport it to the cell surface: human mIgμ was entirely intracellular in TG.mIgμ RAG-1-/-k5-/- pro-B cells (Fig. 2) as well as in TG.mIgμ RAG-1-/-k5-/- A-MuLV-transformed B cell lines (data not shown). Since the only difference between the TG.mIgμ RAG-1-/- and the TG.mIgμ RAG-1-/-k5-/- mice is the expression of k5 in the former, we conclude that in the absence of conventional light chains the surrogate chains are absolutely required to deliver the signal for B cell development.

To determine whether κ light chains could substitute for surrogate light chains in the double-mutant mice we combined a κ light chain transgene with mIgκ transgene (mIgκ+κ) in the RAG-1-/-k5-/- double-deficient mice. Addition of the light chain was found to rescue B cell development in this background: the bone marrow of TG.mIgκ+κ RAG-1-/-k5-/- mice contained B220+ CD43+ pre-B cells, which carried transgenic IgM on their surface and stained positive for the antidiotypic antibody 54.1 (Fig. 1 B). Therefore, the κ light chain is able to reconstitute B cell development by substituting for k5 in the RAG-1-/-k5-/- double-deficient mice.

The Pre-BCR Is Assembled but Does Not Function in the Absence of Light Chains. One explanation for the observation that mIgμ is inactive in the RAG-1-/-κ5-/- background might be that light chains are required for assembly of the early BCR. To determine whether mIgκ was associated with Igα-Igβ in the absence of light chains we performed immunoprecipitation experiments on A-MuLV-transformed cells derived from TG.mIgκ RAG-1-/-κ5-/- bone marrow. We found no difference in the amount of Igα and Igβ that was associated with the heavy chain in the presence or absence of light chains (Fig. 3). However, it was only in the TG.mIgκ RAG-1-/-κ5-/--derived lines that we saw the fully glycosylated surface form of Igβ coprecipitate with human IgM (Fig. 3). The two glycosylated Igβ bands

(Anatrace, Maumee, OH), 50 mM Tris, pH 6.8, 100 mM NaCl. Insoluble material was removed by centrifugation. The supernatant was incubated with rabbit antisera to human IgM (SFB) (Cappel Research Products, Durham, NC) and protein A agarose (Pierce Chemical Co., Rockford, IL) for 2 h at 4°C. Immune complexes were collected, washed in lysis buffer, separated by 10% reducing SDS-PAGE, and transferred to Immobilon-P (Millipore Corp., Bedford, MA) in a semi-dry system (Owl Scientific, Cambridge, MA). After incubation with anti-human IgM (Cappel), anti-Igκ, or anti-Igβ (26) blotting antibodies, labeled proteins were visualized with 125I-protein A (New England Nuclear, Boston, MA) using x-ray film.
nopreclpltauons were done with protein A- coupled rabblt anu-human
Ig~ and Ig~ can associate with mlgt* in thmr absence.
formed B cells derived from the bone marrow of transgenlc nuce. Imnm-
IgM and the membranes were subsequently blotted with anu-human
f,
were absent in the lanes which represent precipitates from
is hght chain independent, but cell surface transport re-
bands collapse into one upon digestion with endoglycosi-
lg receptor complexes were immunopreopltated from A-MuLV trans-
express conventional light chains (not shown). M1 four Ig[3
and only visible after prolonged exposure in TG.mlgb~
TG.mlgb~ R.AG-1-/-R5-/--derived B cell lines (Fig. 3),
light chains we introduced a mlgb~:Ig[3 chimeric transgene
to “7, “7 
into the RAG-1-/-R5 -/- background (TG.mlgl.~:Ig[3
were absent in the lanes which represent precipitates from
were the subject of debate. For example, results from
gene-targeting experiments (19, 20) as well as experiments
with transformed t3 cell lines (39) suggested that the surro-
gate light chains A5 and VpreB are important components
of the early B cell receptor. In addition, signaling by the
early B cell receptor appears to be dependent on A5 in pro-
B cells, as there is no allelic exclusion in these cells in h5 /-
but despite the lack of allelic exclusion in
pro-B cells in A5 -/- mice, the few B cells that do mature
in this mutant background express a single heavy and light
chain combination (19). One way to account for the differ-
ences in allelic exclusion between A5 -/- pro-B cells and
A5 -/- mature B cells would be if the combination ofmlgl,
with light chains could also produce a functional pre-B cell
receptor. According to this model the mlgl-Ig-Lg~ signal-
ing module of the pre-B cell receptor would be recog-
nized and triggered when combined with either a conven-
tional or a surrogate light chain.
Consistent with this idea, targeting of the A5 gene does
not abrogate B cell development completely (19), and so
A5 is not indispensable for either allelic exclusion or B cell
development. Our finding that a heavy chain transgene
could facilitate the pre-B cell transition in A5 -/- mice is in
agreement with Gravunder et al. (40) though not with
Corcos et al. (11). Both our results and those reported by
Gravunder (40) support the idea that the leaky phenotype
in A5 -/- mice can be explained by stochastic V(D)J rear-
Figure 3. Light chains are not necessary for proper assembly of the
early BCR, since lga and lgb can associate with mlgl in their absence.
Ig receptor complexes were immunoprecipitated from A-MuLV trans-
formed B cells derived from the bone marrow of transgenic mice. Immunu-
precipitations were done with protein A- coupled rabbit anti-human
IgM and the membranes were subsequently blotted with anti-human
IgM, anti-mouse IgM (control), anti-lga and anti-lgb antibodies (26).

Discussion

The antigen receptor found on mature B cells is a com-
plicated structure. It is composed of an Ig heavy and light
chain heterodimer, and the lgo~-lgb signal transducers
which are noncovalently associated with mlgl (28). The
heavy and light chains bind antigen but have no signaling
capacity on their own, and require lgo and lgb to induce
the phosphorylation of non-receptor tyrosine kinases which
give rise to physiologic responses (26, 29-33).
A receptor similar to the mature BCR is thought to me-
diate several key developmental events in early B cells. As
in the mature BCR, the signaling module of the pre-BCR
is the lg~-lgb heterodimer: both lgo and lgb are expressed
very early in B cell development (34-36), and they associ-
ate with mlgl as soon as it is produced (this paper, and
[28]). Furthermore, the lgo and lgb proteins are necessary
and sufficient to induce early B cell development (17, 18,
22, 24), and the mechanism by which the lgo-lgb het-
erodimer activates early B cell transitions is analogous to
that used by the mature BCR (31-33). Both have a re-
quirement for receptor tyrosine phosphorylation (22, 24),
syk kinase signaling (37, 38) and receptor cross-linking.

However, the molecular identity of the pre-BCR ligand, as
well as the receptor components required for recognition,
have been the subject of debate. For example, results from
gene-targeting experiments (19, 20) as well as experiments
with transformed B cell lines (39) suggested that the surro-
gate light chains A5 and VpreB are important components
of the early B cell receptor. In addition, signaling by the
early B cell receptor appears to be dependent on A5 in pro-
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rangements. Producing in-frame VDJκ and VJλ combinations in any single developing B cell is a rare event, yet it would be essential for pre-B cell development to proceed in the absence of λ5. Thus it would be expected that introducing an already rearranged heavy chain into the λ5−/− background would increase the number of pre-B cells by reducing the requirement for activation of the transition to successful completion of light chain gene rearrangements. Our finding that a transgenic κ light chain was required for mlgκ to activate the pre-B cell transition in RAG-1−/−λ5−/− mice provides further support for this idea, because it shows that in the absence of the surrogate light chains, conventional light chains are indeed essential for pre-BCR function in early B cell development. When neither surrogate light chains nor conventional light chains are expressed, B cell development cannot proceed past the pro-B cell stage.

What is the role of the light chain in developing B cells? Our experiments show that light chains are not simply facilitators for assembly of the mlgκ, lγκ-λκ complex. The mlgκ transgene that was inactive in RAG-1−/−λ5−/− pro-B cells, was associated with lγκ-λκ. Further, a chimeric mlgκ,λκ transgene, that encodes a preassembled BCR, activated the pre-B cell transition in RAG-1−/−λ5−/− R5−/− B cells supports the hypothesis that one of the functions of the light chain in early B cell development is to transport the receptor to the cell surface. Nevertheless, light chains may also contribute directly to receptor activation as functional components which are recognized by the putative cross-linker either on the cell surface or inside the developing B cell. In this model the cross-linker must see a structural element shared by all conventional light chains as well as surrogate light chains. The availability of RAG-1−/−λ5−/− pro-B cell lines which can be complemented with combinations of altered Ig transgenes should facilitate the elucidation of the molecular mechanisms that control early BCR function.

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