Immunoglobulin \( \gamma_2b \) Transgenes Inhibit Heavy Chain Gene Rearrangement, but Cannot Promote B Cell Development

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Summary

Transgenic mice with a \( \gamma_2b \) transgene were produced to investigate whether \( \gamma_2b \) can replace \( \mu \) in the development of B lymphocytes. Transgenic \( \gamma_2b \) is present on the surface of B cells. Young transgenic mice have a dramatic decrease in B cell numbers, however, older mice have almost normal B cell numbers. Strikingly, all \( \gamma_2b \)-expressing B cells in the spleen also express \( \mu \). The same is true for mice with a hybrid transgene in which the \( \mu \) transmembrane and intracytoplasmic sequences replace those of \( \gamma_2b \) (\( \gamma_2b\text{-}\mu\text{mem} \)). The B cell defect is not due to toxicity of \( \gamma_2b \) since crosses between \( \gamma_2b \) transgenic and \( \mu \) transgenic mice have normal numbers of B cells. Presence of the \( \gamma_2b \) transgene strongly enhances the feedback inhibition of endogenous heavy chain gene rearrangement. Light chain genes are expressed normally, and the early expression of transgenic light chains does not improve B cell maturation. When the endogenous \( \mu \) locus is inactivated, B cells do not develop at all in \( \gamma_2b \) transgenic mice. The data suggest that \( \gamma_2b \) cannot replace \( \mu \) in promoting the developmental maturation of B cells, but that it can cause feedback inhibition of heavy chain gene rearrangement. Thus, the signals for heavy chain feedback and B cell maturation appear to be different.

The development of B lymphocytes is intimately linked to the expression of Ig genes (reviewed in reference 1). Production of membrane-bound IgM is required for B cell maturation, as mice whose \( \mu \) gene membrane exons have been inactivated by targeted recombination lack B cells (2). Furthermore, membrane-bound IgM is required in the control of the rearrangement of Ig genes by feedback inhibition (reviewed in reference 3). During B cell development feedback inhibition occurs first at the stage of heavy chain gene rearrangement, followed later by the cessation of Ig gene rearrangement due to the shutoff of V(D)J recombinase. Both \( \mu \) and \( \delta \) heavy chains can promote feedback inhibition at the stage of heavy chain gene rearrangement and, in combination with light chains, at the stage of V(D)J recombinase shutoff (3, 4). It is clear from work with transgenic mice that the membrane form of \( \mu \) (\( \mu \text{mem} \)) is required for both types of feedback inhibition, and that the secreted form (\( \mu \text{s} \)) does not mediate feedback (5, 6). The \( \mu \) and \( \delta \) proteins have related transmembrane domains and identical short cytoplasmic tails (Lys-Val-Lys) (7). The \( \gamma \) heavy chains have a similar degree of homology to \( \mu \) and \( \delta \) in the transmembrane portion, but have a much longer intracytoplasmic domain (7). All three heavy chain classes differ considerably in the CH domains. In light of these structural differences, it was of great interest to compare the effect of \( \gamma_2b \) in B cell development to that of \( \mu \) and \( \delta \). Presumably, if \( \gamma_2b \) did not have one or all of the effects of \( \mu \) and \( \delta \), sequence differences between \( \mu/\delta \) and \( \gamma_2b \) may be implicated in the maturation promoting and/or feedback effects.

We have produced several different transgenic mouse lines with a \( \gamma_2b \) gene, presumably representing different chromosomal insertion sites (8), as well as mice carrying a \( \gamma_2b \) transgene whose transmembrane and intracytoplasmic portions have been replaced by those of \( \mu \) (\( \gamma_2b\text{-}\mu\text{mem} \)). All express the \( \gamma_2b \) transgene as shown by tissue-specific \( \gamma_2b \) RNA synthesis (8 and not shown). Here we describe the analysis of B cells of these transgenic lines in comparison with B cells from normal littermates and \( \mu \) transgenic mice.

Materials and Methods

Transgenic Mice. The 343-1 and 348A \( \gamma_2b \) transgenic lines have been described (8). The \( \gamma_2b\text{-}\mu\text{mem} \) transgene, 1241-5-3 (9), was constructed by ligating the V through the CH3\( \gamma_2b \) region on a 10.6-kb PvuI to KpnI fragment that also includes the majority of pUC13cm' from pPCM to the \( \mu \) membrane exons contained on a 2.6-kb KpnI to PvuI fragment from pV167\( \mu \) (5, 8). The transgene was isolated as an 11.4-kb Sall to PvuI fragment by gel electrophoresis and elution from hydroxyapatite (10). The 243-4 \( \mu \) transgenic line used as a control has been described (5, 11). The \( \mu \) transgene encodes both the secreted and membrane forms of \( \mu \).
and contains the VDJ^ region from myeloma MOPC 167 (11). The μdelS transgenic line contains a transgene that is a deletion derivative of the V^167A transgene used to generate the 243-4 ji line. This transgene was derived by deletion of a 1.3-kb BclI fragment containing the secreted terminus of the μ gene. This transgene produces only the membrane form of A. The xJo knockout line (Amo) was obtained from K. Rajewsky (University of Koln, Koln, Germany) via C. Sidman (University of Cincinnati, Cincinnati, OH). This line contains a disruption of the membrane region encoding exons of the D heavy chain (2). The X2 transgenic line 1275-4 contains a functionally rearranged X2 light chain gene from plasmacytoma MOPC315 and is regulated by the heavy chain enhancer (12).

The founder mice of the y2b-only mice and the μ and μdelS mice were derived from (SJL × C57BL/6)F, and were bred in the first generation with F, mice. Later generations (for 5–7 yr) were backcrossed to C57BL/6. The X2- and y2b-umem transgenic mice were produced and propagated in C57BL/6. The 343-1, 348A, 1241-5-3, and 243-4 lines have the following Brinster designations: Tg(Igh)Bri49, Tg(Igh)Bri50, Tg(Igh)Bri128, and Tg(Igh)Bri35, respectively. Their approximate transgene copy numbers are: 8, 22, 14, and 6, respectively.

Flow Cytometry. Flow cytometry (FACSO; Becton Dickinson & Co., Mountain View, CA) was performed essentially as described using the following antibodies (12). Biotinylated rat anti-mouse CD45R (clone RA3-6132) (PharMingen, San Diego, CA) at 1:100, PE-conjugated monoclonal rat anti-mouse κ (Becton Dickinson & Co.) at 1:50, FITC-conjugated goat anti-mouse γ2b (GAMγ2b; Southern Biotechnology Assoc., Birmingham, AL) at 1:100, PE-conjugated GAMκa (Southern Biotechnology Assoc.) at 1:100, PE-conjugated Streptavidin (Jackson Immunoresearch, Inc., West Grove, PA) at 1:50, FITC-conjugated or biotinylated mouse anti-mouse IgH-6a (μ^), clone DS-1 at 1:100 (kindly provided by Dr. Jim Kenny, Frederick Cancer Center, Frederick, MD), and biotinylated mouse anti-mouse IgH6b (μ^), clone AF6-78 at 1:100 (PharMingen). Samples were analyzed by FACScan® using Consort30, FACScan®, or LYSIS software (Becton Dickinson & Co.). A total of 10^4 lymphocytes as determined by forward and side scatter were analyzed per sample. Where indicated, dead cells were excluded using propidium iodide staining. Just before analysis, 10–20 μl of 1 mg/ml propidium iodide (Sigma Chemical Co., St. Louis, MO) was added to each sample. In these cases, the data collection gate was set on live cells and was regated on lymphocytes upon analysis.

Ribonuclease Protection. Ribonuclease protection assay (RPAs) were performed using the RPA and RPAII kits (Ambion Inc., Austin, TX) according to the manufacturer’s instructions. Briefly, 10–30 μg of sample RNA plus yeast tRNA to give a total of 30 μg of RNA was hybridized overnight (>8 h) with 2 × 10^5 cpm of each probe in a 20-μl reaction volume at 53°C. RNase digestion was carried out at 30°C using a 1:100 dilution of solution R (RNaseA and T1) followed by resolution of protected fragments on 5% denaturing acrylamide/urea gels. The T, β-Act plasmid (Ambion Inc.) contains a 250-bp KpnI to Xbal fragment from pAL41 (13) in the USB vector pIγ-1. Restriction with Ddel and in vitro transcription using T7 RNA polymerase results in a 160-nucleotide (nt) protected fragment in RPA. The CH3MA probe was generated by cloning the 458-bp Accl to Mscl fragment containing γ2b CH3 and poly(A) signal for the secreted γ2b message into the Smal site of Bluescript KS+. Linearization with Xbal and in vivo transcription with T7 RNA polymerase generates a ~548-nt transcript that protects 404-nt secreted γ2b and 297-nt membrane γ2b fragments in RPA (see Fig. 3). The CH4AH probe was generated by cloning the 726-bp ApaLI to HindIII fragment containing μ CH4, the secreted μ terminus, and poly(A) signal into Bluescript KS+. Linearization with BamHI and in vitro transcription using T7 RNA polymerase protects a 519-nt secreted ji fragment and a 333-nt membrane μ fragment in RPA. The Cx-2 probe was generated by PCR amplification of a 625-bp fragment containing the last 307 bp of the J-Cx intron and the first 318 bp of the Cx exon (essentially the whole coding region). The fragment was cloned into the Smal site of Bluescript KS+ (Stratagene, La Jolla, CA), and antisense Cx transcripts are generated by linearization with BamHI and transcription using T7 RNA polymerase. Processed Cx mRNAs result in a 318-nt protected fragment.

Abbreviations used in this paper: M, membrane; nt, nucleotides; RPA, ribonuclease protection assay.
Results

Splenic B Lymphocytes at Different Ages. The results from two transgenic lines carrying a functionally rearranged \( \gamma_2b \) transgene and one line containing a hybrid \( \gamma_2b-\mu\)mem transgene are reported here. Fig. 1 shows a scheme of each transgene construct.

Splenic lymphocytes from littermate or age-matched normal and \( \gamma_2b \) transgenic mice were analyzed by FACS\textsuperscript{®} in order to compare their B cell populations. Neonatal mice from the \( \gamma_2b \) 343 line have very few B cells. Less than 1% of splenic lymphocytes are either \( \kappa \) or \( \mu \) positive compared with 11-12% in normal littermates (Table 1). 2-wk-old \( \gamma_2b-\mu\)mem transgenic mice have a similar defect (data not shown). At 4 wk of age, the \( \gamma_2b \) transgenic lines have a significantly lower percentage of \( \kappa^+ \) or \( \mu^+ \) (mIg\textsuperscript{+}) cells compared with normal mice or with \( \mu \) transgenic mice. The defect is most severe in the 343 line, which has only 11% \( \mu^+ \) cells compared with 54% in normal mice (Table 1). The percentage of \( \kappa^+ \) cells matches that of \( \mu \), indicating that there are essentially no B cells that express \( \gamma_2b \) without also expressing \( \mu \). Surprisingly, when the transmembrane and intracytoplasmic portion of \( \mu \) is substituted for that of \( \gamma_2b \), the resulting \( \gamma_2b-\mu\)mem transgenic mouse have the same phenotypes as \( \gamma_2b \) transgenic

Table 1. Flow Cytometric Analysis of Splenic Bone Marrow B Cells

| Mouse Type | \( \kappa \) | \( \mu \) | \( \gamma_2b \) |
|------------|-------------|-------------|---------------|
| Spleen of 6-d-old mice | Normal (\( n = 7 \)) | 12.5 ± 3.0 | 11.5 ± 2.1 | 2.3 ± 1.2 |
| | 343-1 (\( n = 2 \)) | 0.2 ± 0.3 | 0.25 ± 0.5 | 1.0 ± 0.3 |
| | \( \gamma_2b \) | | | |
| Spleen of 4-wk-old mice | Normal (\( n = 13 \)) | 56.1 ± 1.8 | 54.2 ± 2.9 | 3.05 ± 1.5 |
| | 343-1 (\( n = 9 \)) | 11.7 ± 2.7 | 11.4 ± 2.2 | 14.5 ± 4.3 |
| | \( \gamma_2b \) | | | |
| | 348A (\( n = 2 \)) | 39.8 ± 7.3 | 35.6 ± 3.8 | 5.1 ± 3.8 |
| | \( \gamma_2b \) | | | |
| | 1241-5-3 (\( n = 5 \)) | 16.0 ± 3.8 | 16.8 ± 3.9 | 15.7 ± 3.4 |
| | \( \gamma_2b-\mu\)mem | | | |
| | 243-4 (\( n = 4 \)) | 49.8 ± 2.1 | 28.8 ± 7.4 | 2.4 ± 0.4 |
| | \( \mu \) | | | |
| | 343-1 (\( n = 5 \)) | 48.2 ± 8.7 | 21.0 ± 10.7 | 11.1 ± 1.8 |
| Bone marrow cells of adult mice\dagger | Normal (\( n = 5 \)) | 51.1 ± 3.8 | 50.5 ± 5.3 | 10.3 ± 4.2 |
| | 343-1 (\( n = 5 \)) | 18.2 ± 8.7 | 21.0 ± 10.7 | 11.1 ± 1.8 |

| No. of spleen cells* | \( \times 10^6 \) | \( B_{220} \) | \( B_{220^{\text{end}}} \) | \( B_{220^{\text{trg}}} \) |
|----------------------|-----------------|-------------|-------------|-------------|
| Normal (\( n = 7 \)) | 8.5 (3.8–13.2) | 64.3 ± 2.5 | 51 (39–90) | |
| 343-1 (\( n = 2 \)) | 8.6 (7.2–10.0) | 44.4 ± 0.6 | 25 (23–27) | |
| \( \gamma_2b \)     |                 | 44.3 ± 7.2 | 19 (16–22) | |
| Normal (\( n = 13 \)) | 25.6 ± 1.8 | 16 (10–27) | 16 (10–27) | |
| 343-1 (\( n = 9 \)) | 14.5 ± 4.3 | 25.6 ± 1.8 | 16 (10–27) | |
| \( \gamma_2b \)     | 44.4 ± 0.6 | 25 (23–27) | 25 (23–27) | |
| 348A (\( n = 2 \)) | 5.1 ± 3.8 | 44.4 ± 0.6 | 25 (23–27) | |
| \( \gamma_2b \)     | 44.3 ± 7.2 | 19 (16–22) | 19 (16–22) | |
| 1241-5-3 (\( n = 5 \)) | 16.0 ± 3.8 | 44.3 ± 7.2 | 19 (16–22) | |
| \( \gamma_2b-\mu\)mem | 15.7 ± 3.4 | 44.3 ± 7.2 | 19 (16–22) | |
| 243-4 (\( n = 4 \)) | 28.8 ± 7.4 | 44.3 ± 7.2 | 19 (16–22) | |
| \( \mu \)            | 2.4 ± 0.4      | 52.6 ± 4.4 | 42 (31–56) | |
| 343-1 (\( n = 5 \)) | 28.8 ± 10.8 (end) | 52.6 ± 4.4 | 42 (31–56) | |
|                       | 51.1 ± 8.4 (trg) | 51.3 ± 8.4 | 30.3 ± 7.6 | |

Cells were stained for the indicated markers and analyzed by FASCAN\textsuperscript{®}. A total of \( 10^6 \) lymphocytes as defined by forward scatter and side scatter were analyzed per sample. The average percentage of lymphocytes positive for the given marker is indicated at a 90% confidence level as determined by t test.

* Average number of nucleated spleen cells. Range of values is in parenthesis.

† \( \mu \) transgenics were analyzed using allotype-specific antibodies. end, endogenous \( \mu \); trg, transgenic \( \mu \).
mice (Table 1). In contrast, in μ transgenic mice, the percentage of κ+ cells is greater than endogenous μ, indicating that ~21% B cells express transgenic μ without endogenous μ (Table 1) (5, 14, 15).

By 16 wk, B cells in the γ2b mice have increased to near normal numbers (not shown). But, unlike in μ and δ transgenic mice (5, 6, 16), B cells of the γ2b mice always coexpress γ2b and endogenous μ (Fig. 2). Again, the γ2b-μmem transgenic mice have the same phenotype as γ2b transgenics (Fig. 2). In the 343 γ2b and γ2b-μmem lines virtually all B cells coexpress μ and γ2b. In the 348A line, a large proportion of μ+ B cells are γ2b+ or γ2b−. It is not known whether in these cells the transgene has been inactivated or whether the endogenous μ H chain preferentially associates with the L chain that happens to be produced by these cells.

Thus, splenic B cells from γ2b mice coexpress μ on all γ2b+ cells. The replacement of the γ2b transmembrane and intracytoplasmic regions with those of μ is not sufficient to permit the development of γ2b-only B cells. In addition, γ2b and γ2b-μmem mice are defective in the formation of B cells, as revealed by the low B cell numbers in young mice. This defect is overcome with age and the severity of the defect varies among transgenic lines. Preliminary analysis of two additional γ2b transgenic lines agrees with these findings (not shown).

The γ2b Transgene Is Expressed Early and at High Levels during Fetal Development. Analysis of lymphoid bone marrow cultures and Abelson murine leukemia virus–transformed bone marrow pre-B cells indicated that the 343 mice express γ2b in the pre-B compartment (17). This implies that the trans-
Figure 3. RPA of fetal liver and adult bone marrow RNAs. (A and B) Schematic representation of μ and γ2b probes for RPA. The indicated regions were cloned into the Bluescript KS+ vector in order to generate in vitro transcripts specific for the μ or γ2b isotypes. Exons are represented by boxes; TGA indicates the termination codon for the secreted form of the indicated Ig; the site of the poly-adenylation signal is indicated, as are splice donor sites for the membrane specific form of the mRNA. The predicted region of identity between the cloned region and the membrane vs. secreted forms of the Ig mRNA are shown. (A) μ-specific probe covering the CH4 exon and the μ-secreted tail. (B) γ2b-specific probe covering the CH3 exon and poly(A) region of secreted γ2b. (In contrast to μ, γ2b does not contain a specific secreted terminus.)

(C–E) Approximately 30 μg of total cellular RNA from individual fetal livers (C and E) or 10 μg from adult bone marrow (D) were analyzed by RPA for expression of γ2b and μ (C and D) or κ (E). (C) Day 12 and 13 fetal liver expression of the membrane and secreted specific forms of the γ2b and μ mRNAs are indicated. Each lane contains mRNA from one individual fetus of either normal (NML) or γ2b (343) transgenic genotype. Lane 5 of the 13-d experiment shows traces of sample carried over from lane 4. (D) μ and γ2b expression in individual samples of adult bone marrow from normal or 343 γ2b transgenic mice is indicated. (E) Day 13 or 17 fetal liver expression of Cκ in individual normal or 343 γ2b fetuses is indicated. In all cases β-actin expression served as a control for RNA loading in order to facilitate comparison between lanes. Labeled 123-nt ladder or pBR322 cut with MspI served as size markers.
Figure 4. FACS® analysis of bone marrow from 343 γ2b transgenic and normal mice. (A) Anti B220 (top) and anti κ (bottom) staining. The x-axis indicates the fluorescence intensity of staining for the indicated marker using PE-coupled antibodies. The y-axis indicates the number of bone marrow lymphocytes positive for each level of staining with the indicated marker. Blackened area, 343 γ2b transgenic; outlined area, normal mouse. (B) B220− bone marrow lymphocytes of 343 γ2b mice. B220−, mlg− bone marrow cells reanalyzed for cell size by forward scatter are indicated by histograms. The percentage of large, less mature cells is indicated in the right-hand region of each panel. The percentage of smaller, more mature cells is indicated in the left-hand region. Three individual adult 343 γ2b mice are shown each in comparison with a normal littermate.

gene is expressed appropriately to mediate allelic exclusion, namely around the time of the first μ expression when developing B cells are beginning to rearrange their endogenous H chain genes (18, 19, 20). In light of the observed lack of allelic exclusion in mature cells described above, we sought to define more precisely the stage at which the γ2b gene is first expressed. We used an RPA for analysis of fetal liver (Fig. 3), because B cell development occurs in a synchronous wave in this organ (20). Individual fetal livers at days 12, 13, 15, and 17 of gestation were analyzed. Expression of γ2b is readily detectable and several-fold higher than that of μ in day 12 fetal liver from 343 γ2b transgenic mice (Fig. 3
Both membrane and secreted \( \gamma_2b \) mRNAs are produced, although the level of secreted mRNA exceeds that of membrane mRNA (Fig. 3 C). This prevalence of secreted \( \gamma_2b \) mRNA is in agreement with data that show an excess of secreted over membrane \( \gamma_2b \) mRNA in lymphoma and myeloma lines (21, 22). The level of \( \gamma_2b \) expression increases through day 17 (days 15 and 17 not shown).

The data indicate that the \( \gamma_2b \) transgene is expressed at an appropriate developmental point to mediate allelic exclusion, namely during the time of endogenous H gene rearrangement. The levels of membrane \( \gamma_2b \) are approximately equivalent to or higher than membrane \( \mu \) levels at every time point. In light of these findings, the lack of H gene allelic exclusion seen in the peripheral B cells of \( \gamma_2b \) transgenic mice does not appear to be due to insufficient or delayed expression of membrane \( \gamma_2b \).

The Bone Marrow Compartment Reveals a Block in B Cell Development in \( \gamma_2b \) Transgenic Mice. To assess the stage at which B cell development is inhibited in \( \gamma_2b \) transgenic mice, cells of the B cell lineage were analyzed in the bone marrow. FACS® analysis of bone marrow from normal and 343 \( \gamma_2b \) mice shows two B220 populations (Fig. 4 A and Table 1). In normal mice, the B220\(^{hi} \) population comprises 51% of the lymphoid compartment in bone marrow and the B220\(^{lo} \) population comprises 30% (Table 1). However, in the bone marrow of 343 \( \gamma_2b \) transgenic mice the B220\(^{hi} \) population is decreased to only 11% of the lymphoid cells (Table 1). In addition, in normal mice, 50% of the bone marrow lymphoid cells express IgM on the cell membrane (mlgM) while only 18% of 343 bone marrow lymphoid cells are mlg positive as indicated by \( \kappa \) staining (Fig. 4 A and Table 1). As in the spleen, all 343 bone marrow cells that are \( \gamma_2b \) positive are also \( \mu \) positive (data not shown). While few cells in 343 bone marrow express mlg at the cell surface, mRNA for membrane \( \gamma_2b \) readily can be detected (Fig. 3 D).

In both normal and 343 \( \gamma_2b \) bone marrow, all B220\(^{hi} \) cells are also mlg\(^{+} \), while the B220\(^{lo} \) population consists of both mlg\(^{-} \) and mlg\(^{+} \) populations representing pre-B and B cells, respectively. In addition, the B220\(^{lo} \), mlg\(^{\cdot} \) population contains both large and small pre-B cells that can be distinguished based on forward light scatter. In normal mice, 69–80% of the B220\(^{lo} \) pre-B cells are the small more mature type, while only 44–56% of 343 \( \gamma_2b \) transgenic B220\(^{lo} \) pre-B cells are of this type (Fig. 4 B).

The data suggest that the paucity of B cells observed in the spleens of younger \( \gamma_2b \) mice may result from an inefficiency in generating B cells. This defect manifests itself in the bone marrow as a decrease in the B220\(^{lo} \) population. It appears that the block in B cell development may occur within the B220\(^{lo} \) pre-B cell compartment, which is skewed to contain a greater proportion of larger, less mature cells. The data imply that \( \gamma_2b \) transgenic mice are not able to generate small pre-B (and subsequently B cells) as efficiently as normal mice. All the B220\(^{lo} \) cells that emerge in \( \gamma_2b \) transgenic bone marrow express \( \mu \), thus implying that \( \mu \) may be required for this step of B cell development and that \( \gamma_2b \) cannot function as a substitute.

\( \gamma_2b \) Expression Is Not Toxic to B Cells, but It Inhibits Heavy Chain Gene Rearrangement. The high levels of expression of \( \gamma_2b \) in the 343 fetal liver and bone marrow and the dramatic decrease in the population of more mature B220\(^{hi} \) cells in \( \gamma_2b \) transgenic mice raised the possibility that the \( \gamma_2b \) transgenic heavy chain is toxic to developing B cells. Alternatively, \( \gamma_2b \) may not be able to provide certain physiological signals that are essential for development. To distinguish between these alternatives, a functional \( \mu \) transgene (from mouse line 243-4) was crossed into the 343 \( \gamma_2b \) transgenic background.

Similar to previous findings, 7-wk-old 343 \( \gamma_2b \) mice exhibit a dramatic decrease in splenic B cells (Table 2). The 243-4 \( \mu \) transgenic mice, on the other hand, have almost normal numbers of B cells. Crossing the \( \gamma_2b \) transgenic mice with the \( \mu \) transgenic mice leads to a reversal of the B cell defect characteristic of \( \gamma_2b \) transgenic mice. There is a considerable increase in the population of B220\(^{+} \) and \( \kappa \) \( \times \) cells in \( \gamma_2b \) \( \times \) \( \mu \) double transgenic mice at both 7 and 16 wk in comparison with the 343 \( \gamma_2b \) only littermates (Table 2). These findings suggest that the \( \gamma_2b \) transgene product itself is not inhibitory to B cell development in the presence of a functional \( \mu \) chain.

The \( \mu \) transgenic mice show little feedback inhibition of endogenous \( \mu \) genes. These \( \mu \) transgenic mice have few transgenic \( \mu \)-only cells, but endogenous \( \mu \)-only and endogenous \( \mu \)+ transgenic \( \mu \)-positive B cells represent the majority of B cells in the spleen of the \( \mu \) transgenic mice (Fig. 5 A). There is a striking decrease in the percentage of cells expressing endogenous \( \mu \) in the double transgenic mice in comparison with transgenic \( \mu \)-only mice (Fig. 5 A), showing that \( \gamma_2b \) strongly inhibits endogenous H gene rearrangement. However, all the \( \gamma_2b \) expressing cells also coexpress transgenic \( \mu \) (Fig. 5 B).

Thus, the \( \gamma_2b \) protein does not act in a dominant manner to disrupt B cell development. The exclusion of endogenous \( \mu \) gene expression in \( \gamma_2b \) \( \times \) \( \mu \) double transgenic B cells reveals a pronounced rearrangement feedback effect by the \( \gamma_2b \) transgene. The feedback presumably occurs through inhibition of heavy chain gene rearrangement and does not impede the expression of the \( \mu \) transgene.

The feedback effect by \( \gamma_2b \) is further demonstrated in hybridomas from \( \gamma_2b \) transgenic mice (Table 3). Only 18% of hybridomas from normal mice have either a germline heavy chain or a DJ rearrangement on one of the heavy chain alleles. However, 59% of \( \gamma_2b \) transgenic hybridomas have an unarranged or incompletely rearranged heavy chain allele.

Expression of a Transgenic Light Chain Does Not Improve B Cell Development in \( \gamma_2b \) Transgenic Mice. The data suggest a functional inadequacy of \( \gamma_2b \) compared with \( \mu \) during B cell development. One characteristic attributed to heavy chain expression is the induction of light chain gene rearrangement (23). To test the possibility that the defect in B cell development characteristic of \( \gamma_2b \) transgene expression is due to an inability to induce light chain gene expression and rearrangement, the 343 \( \gamma_2b \) line was crossed to the 1275-4 \( \lambda 2 \) transgenic line. This transgene causes partial feedback on both
Spleencellsfromoffspringof343-1y2b × 243-4
Ft
crosseswerestainedfortheindicatedmarkersandanalyzedbyFACScan®.A totalof104lym-
phocytesasdefinedby forwardscatterandsidescatterwereanalyzedpersample.The percentageofspleniclymphocytesfromeachanimalpositive
forthegivenmarkerisindicated.

* Et
expressionwasanalyzedusingallotype-specificantibodies.A
b, endogenous a;
IA-, transgenic u.

No micewithoutatransgenewerepresentinthislitter.

H and L chaingenerearrangement, presumablybecause it
is expressed in early pre-B cells due to the presence of the
heavy chain enhancer (12).

Table 4 summarizes the analysis of (343 γ2b × 1275-4
X2) offspringfrom threeindependentexperiments. As be-
fore, 343 γ2b transgenic spleen cells express μ and κ as well
as γ2b. The percentage of λ + cells in 343 transgenic spleen
is 5%, equivalent to that in normal mice. Similar to previous
results (12), the λ2 transgenic mice express λ on 33% of splenic
lymphocytes and κ on 26%. In double transgenic γ2b ×
λ2 offspring the percentage of λ expression is increased
compared with γ2b-only mice. Many cells coexpress κ and λ (not
shown). Compared to γ2b transgenic mice the numbers of
Ig + B cells do not increase. Again, all the B cells that
emerge in the spleen express μ, and most also express γ2b.
Thus, the presence of a functionality rearranged light chain
expressed early in B cell development does not result in the
repair of the B cell depletion or the production of B cells
expressing the γ2b transgene alone.

Induction of κ Transcription during Fetal Development Is
Normal. To further analyze a possible effect of the γ2b trans-
gene on light chain expression, we determined the levels of
κ mRNA in fetal livers of γ2b mice in comparison with normal
littermates. Using RPA analysis we examined Cκ transcripts
at days 13, 15, and 17 of gestation. The probe used in these
studies detects but does not distinguish between rearranged
and germline κ transcripts (see Materials and Methods). At
day 13, κ transcripts are barely detectable in both normal and
343 γ2b transgenic fetal liver (Fig. 3 E). By days 15 (not
shown) and 17 (Fig. 3 E), the levels of κ expression have
substantially increased. However, no differences are seen in the
relative levels of κ expression between normal and γ2b trans-
genomic mice at any time.

The findingsof normal mice do not initiate κ
transcription earlier nor toa higher level at any of the time
points analyzed in comparison to γ2b transgenic mice. Since
a correlation exists between the rate of transcription at the
κ locus and the rearrangement of κ genes (24), no significant
differences in κ gene rearrangement are likely to be occurring
normal vs. γ2b mice. Thus, the defect in B cell development
characteristic of γ2b transgenic mice does not seem to result
from a failure to induce light chain production.
No Mature B Cells Develop in γ2b Transgenic Mice in the Absence of a Functional Endogenous μ Locus. The production of membrane-bound H chain is an absolute requirement for B cell development (2). To test the hypothesis that γ2b cannot promote B cell development beyond the pre-B cell stage, 343 γ2b transgenic mice were crossed to knockout mice. Spleen and bone marrow from the offspring of these crosses were analyzed at 8 wk of age for expression of B220 and mlg (Fig. 6).

Nontransgenic mice heterozygous for the disruption of the μ membrane domain express B220 and κ on 47% of splenic lymphocytes (Ty2b-, μm+/−; Fig. 6 A). In comparison, only a background staining of ~1% positive cells is seen in homozygous μ knockout mice (Ty2b-, μm−/−). Homozygous μ knockout mice also expressing the γ2b transgene (Ty2b+, μm−/−) do not produce κ-expressing B cells and are phenotypically similar to nontransgenic μ knockout mice (Ty2b−, μm−/−). Thus, the presence of the γ2b transgene does not allow the rescue of B cells in the absence of membrane-bound μ. Heterozygous μ knockout, γ2b transgenic mice, however, are able to produce splenic B cells (Ty2b+, μm+/−; Fig. 6 A).

As expected, the bone marrow of the γ2b−/−; μm−/− mice contains only B220o cells (Fig. 6 B), but also in γ2b transgenic mice, regardless of the μ genotype, very few B220hi cells are seen in the bone marrow (Fig. 6 B). This is consistent with the failure to produce B cells in the presence of the γ2b transgene. These cells are lower in numbers than in the analysis shown in Table 1, presumably because the mice in Fig. 6 B are younger (see Discussion).

As a control, we performed a similar analysis using a μ transgenic line, μdelS. This transgenic line expresses only the membrane form of μ, and exhibits efficient B cell development and feedback inhibition of endogenous μ expression (Fig. 6 C, Kim, J. Y. and P. Roth, unpublished results). In the absence of a functional endogenous μ locus, μdelS transgenic mice (μdelS+; μm−/−) retain the ability to produce a normal composition of splenic lymphocytes (Fig. 6 C). Thus, the peripheral B cell population can be reconstituted with a single type of heavy chain molecules.

Discussion

From the analysis of these γ2b and γ2b-μmem mice, we conclude that the γ2b heavy chain cannot functionally substitute for μ in all aspects of B cell development. While γ2b or γ2b-μmem are able to mediate feedback inhibition of endogenous H gene rearrangement, they do not promote maturation or survival of B cells.

B Cell Development Is Blocked at an Early, Pre-B Cell Stage in γ2b Transgenic Mice. The bone marrow of γ2b transgenic mice shows a marked decrease in the B220hi population.

Figure 5. 343 γ2b × 243-4 μ transgenic mice. (A) Endogenous μ and transgenic μ staining of splenic lymphocytes: endogenous μ (μh-PE) on the y-axis and transgenic μ (μh-FITC) on the x-axis. The genotypes are indicated. (B) Transgenic μ and γ2b staining on splenic lymphocytes. Transgenic μ expression is shown on the y-axis and transgenic γ2b-FITC expression on the x-axis. The mice were 16 wk old.
Table 3. IgH Rearrangement and Ig Secretion in γ2b Transgenic and Normal Hybridomas

| H genes*  | γ2b transgenic hybridomas | Normal mouse hybridomas |
|-----------|---------------------------|------------------------|
|           | No. | Percent | Secreted Ig | No. | Percent | Secreted Ig |
| G         | 6(9) | 22     | 6 γ2b, 3 none | 0 | - | - |
| DJ/DJ     | 0   | -      | -           | 0 | -      | - |
| VDJ       | 8   | 30     | 6 μ and γ2b, 1 γ2b, 1 μ | 18 | 82   | 8 μ, 10 γ2b |
| VDJ/G     | 1   | 4      | 1 μ and γ2b | 1 | 4   | 1 γ2b |
| VDJ/DJ    | 9   | 33     | 8 μ and γ2b, 1 γ2b | 3 | 14  | 2 μ, 1 γ2b |
| VDJ/VDJ   | 3   | 11     | 3 μ and γ2b | 0 | - | - |

Hybridomas were generated from splenic LPS blasts of 4-wk-old mice. Culture supernatants were screened in an ELISA for the presence of secreted Ig. DNA was analyzed by Southern blots for IgH rearrangement.

* Configuration of detectable H genes. G, germline; DJ, D to J rearrangement; VDJ, V to D to J rearrangement. In cases where only a single allele is indicated, the other allele could either have been lost or given an indistinguishable pattern on Southern blots.

† Number of hybridomas containing the indicated gene configurations.

§ Secreted Ig detected in hybridoma culture supernatant by isotype-specific ELISA.

Three hybridomas that did not secrete Ig were excluded from the calculation of percentages since it is not known if they may have arisen from pre-B cells.

This population contains both recirculating and newly produced B cells that express mlg (25, 26). The B220<sup>hi</sup> cells that are present in γ2b transgenic bone marrow exhibit an identical phenotype as peripheral splenic B cells from the same mice. All the γ2b<sup>+</sup> cells also coexpress endogenous μ at the cell surface. This is an important observation because it indicates that γ2b-only B cells are not produced.

In contrast to the defect in the B220<sup>hi</sup> population, the percentage of cells in the immature B220<sup>lo</sup> population appears relatively normal, however, the distribution of cell sizes within this pre-B cell compartment is skewed towards larger, less mature cells in γ2b transgenic bone marrow (Table 1 and Fig. 4 B). Large, B220<sup>hi</sup> early preB cells have begun to rearrange D to J<sub>H</sub> genes at the H loci; these cells transit to the next preB stage and become small, B220<sup>lo</sup>, noncycling cells with rearranged VDJ<sub>H</sub> genes and germline κ genes (26–28).

In the normal development pathway, the transition from large to small B220<sup>lo</sup> pre B cells is concomitant with the decrease of CD43 and the acquisition of cytoplasmic μ expression (26, 27). This latter population is decreased in γ2b transgenic mice similar to mice with homozygous deletion of the λ5 gene or the μ membrane gene segment (2, 29, 30). The implication is that γ2b transgenic mice are not able to generate small, late stage, pre-B cells as efficiently as normal mice, and subsequently exhibit a depletion of later stages of B220<sup>hi</sup> bone marrow and splenic B cells.

A Model for B Cell Development in γ2b Transgenic Mice. We propose a model for B cell development in γ2b transgenic mice in which the majority of B cell precursors developing in the bone marrow express high levels of γ2b early in the developmental pathway (Fig. 7). In developing pre-B cells the γ2b transgenic product causes cessation of further H gene rearrangement. This effect is similar to that of endogenous μ chains and transgenic μ or δ chains. After this point, the function of γ2b diverges from μ in this model. Large, B220<sup>hi</sup> pre-B cells seem to require a signal generated pri-

Table 4. Flow Cytometric Analysis of Splenic B Cells from 343-1 γ2b Crossed to 1275-4 λ2 Mice

| Mouse type | κ  | λ  | μ  | γ2b | B220 |
|------------|----|----|----|-----|------|
| Normal     | 45.6 ± 5.9 | 4.8 ± 3.0 | 48.0 ± 6.0 | 8.7 ± 5.4 | 47.1 ± 9.4 |
| (n = 9)    |    |    |    |     |      |
| 343-1 γ2b  | 17.4 ± 7.1 | 4.9 ± 1.7 | 20.8 ± 7.4 | 11.9 ± 2.4 | 29.0 ± 2.1 |
| (n = 8)    |    |    |    |     |      |
| 1275-4 λ2  | 25.6 ± 6.8 | 33.0 ± 7.4 | 33.1 ± 6.4 | 7.7 ± 1.6 | 30.7 ± 10.0 |
| (n = 8)    |    |    |    |     |      |
| 343-1 γ2b  | 10.8 ± 5.4 | 16.0 ± 4.6 | 20.5 ± 4.0 | 14.7 ± 2.3 | 29.7 ± 1.0 |
| X 1275-4 λ2|    |    |    |     |      |
| (n = 11)   |    |    |    |     |      |

Spleens from offspring of 343-1 γ2b × 1275-4 λ2 crosses were stained for the indicated markers and analyzed by FACScan®. The data are an average of three experiments. A total of 10<sup>6</sup> lymphocytes as defined by forward scatter and side scatter were analyzed per sample in one experiment. In the other two experiments, propidium iodide was used to eliminate dead cells. The analysis was then regated on lymphocytes. The average percentage of splenic lymphocytes positive for the given marker is indicated at a 90% confidence interval as determined by t-test.
Figure 6. FACS® analysis of 343 γ2b × μ knockout mice (A and B) and μ × μ knockout mice (C). Plots depict expression on splenic (A and C) or bone marrow (B) lymphocytes. (A and B) B220-FITC on the x-axis and κ-PE on the y-axis. (C) Total μ-PE (μ+ + μδ) on the y-axis and transgenic μ-FITC (μ-only) on the x-axis. The genotypes are indicated: Ty2b, transgenic γ2b; Tu delS, transgenic μdelS; μ±/−, endogenous μ membrane exon wild-type/disrupted.
The data argue that the failure to produce \( \gamma \)-only mature B cells in \( \gamma 2b \) transgenic mice does not result from a failure of \( \gamma 2b \) to inhibit feedback of endogenous H gene rearrangement. On the contrary, a striking decrease in endogenous \( \mu \) expression occurs in \( \gamma 2b \times \mu \) double transgenic mice compared with \( \mu \) transgenics. Analysis of spleen B cell hybridomas further confirms that \( \gamma 2b \) promotes strong feedback inhibition of H gene rearrangement.

As conclusive test for the proposed model of B cell development in \( \gamma 2b \) transgenic mice, the 343 \( \gamma 2b \) line was crossed with the \( \mu \)em knockou tline. If our model is correct, in the absence of a functional \( \mu \) chain, no peripheral B cells should be produced in 343 \( \gamma 2b \) mice homozygous for the \( \mu \)em disruption. Indeed, this prediction is borne out in these crosses (Fig. 6).

Thus, the data are consistent with the proposed model that \( \gamma 2b \) cannot replace \( \mu \) in promoting the completion of the B cell developmental pathway. While \( \gamma 2b \) appears to mediate feedback on endogenous H gene rearrangement, no B cells emerge either in the bone marrow or in the spleen that express only \( \gamma 2b \) heavy chains. There is an absolute requirement for expression of \( \mu \) in order for pre-B cells to complete their development.

Why Is \( \gamma 2b \) Unable to Provide a Maturation Signal? One function attributed to \( \mu \) chain expression is the induction of L chain gene rearrangement (23, 31). An inability of \( \gamma 2b \) to induce L gene rearrangement would be compatible with the proposed model. B cells could develop to the pre-B cell stage and would fail to progress past this stage in the absence of a light chain. Evidence to support such a scenario comes from studies with scid mice, which are defective in rearrangement of Ig and TCR genes (32). They exhibit a block in B cell development at a similar stage as \( \mu \)em knockout mice (26). In the presence of a functional \( \mu \) transgene, scid mice develop normal pre-B cell populations, but very few B cells, presumably due to the inability to readily rearrange light chain genes (33-35).

If \( \gamma 2b \) simply fails to induce light chain gene rearrange-
ment, supplying a functionally rearranged light chain transgene expressed in early pre-B cells should relieve the block in B cell development and allow the production of γ2b-only producing B cells at increased frequency in the periphery. However, λ2 × γ2b double transgenic mice do not exhibit an increase in the efficiency of B cell production (Table 4). In addition, all the B cells that populate the spleen express endogenous μ, similar to γ2b-only transgenic mice.

Thus, the block in B cell development is not due to lack of light chain gene rearrangement. This conclusion is strengthened by the fact that no differences in fetal liver κ expression were observed between γ2b transgenic mice and normal littermates.

Others have recently obtained evidence that μ is not required for the induction of light chain gene rearrangement in pre-B cells (2, 29, 30, 36). However, in the presence of μ, L gene rearrangement seems to be upregulated (37). It is intriguing to speculate that γ2b may substitute for μ in the upregulation of light chain gene rearrangement. Perhaps the signals for cessation of heavy chain gene rearrangement, which γ can deliver and upregulation of light chain gene rearrangement are the same.

The signals for heavy chain gene rearrangement feedback and B cell maturation apparently are different. The γ2b molecule can only give the first Ig gene rearrangement feedback signal that stops heavy chain gene rearrangement, but not the B cell maturation signal, which μ and δ can deliver. Since γ2b appears capable of delivering a signal to stop H gene rearrangement, but not a signal for B cell maturation, either different regions of the heavy chains are involved in the different signals, or a given region of μ can perform both functions, whereas the analogous region of γ2b can perform only one function.

Structural differences between γ2b proteins and μ proteins must be responsible for their functional differences. The most obvious structural difference between μ and γ2b is the cytoplasmic tail (7). However, the fact that the γ2b-μ membrane transgenic mice exhibit an identical phenotype as the γ2b transgenic mice argues that the μ transmembrane and cytoplasmic tail regions are not sufficient to confer complete functional competence onto a hybrid γ2b-μ gene. It is possible that in the context of the γ2b-μ hybrid gene the μ transmembrane and cytoplasmic domains may not fold into an appropriate three-dimensional structure. Alternatively, the transmembrane portion may be involved in the H gene feedback only and the membrane domain of most heavy chain isotypes may be able to deliver this signal. The μ and γ proteins differ no more in this protein domain than do μ and δ (7), which both can give a feedback as well as a maturation signal (4). Notably, a tyr-ser doublet within the transmembrane portion, which has been shown to be essential for signal transduction and antigen presentation via mlgM (38), is present in γ2b at an identical position (7).

Before the production of light chain, the heavy chain is expressed in a pre-B cell receptor complex with the surrogate light chains λ5 and VpreB that is also capable of signal transduction (39-42). The importance of this receptor is inferred by the block in early B cell development observed in either μmem or λ5 knockout mice (2, 29). However, λ5 seems not to be required for feedback inhibition of heavy chain gene rearrangement in the few B cell that develop in λ5 knockout mice (29). In analogy, γ2b may not require functional association with λ5 to cause H gene feedback. It is not known whether γ2b can combine with the surrogate light chains. The association between λ5 and μ heavy chains seems to occur through the CH1 domain of μ, as deletions of this domain result in loss of association between μ and these polypeptides (43). Based on the conservation of BiP binding and light chain binding to γ2b via associations within the CH1 domain, it seems reasonable to expect that λ5, which is structurally similar to a light chain, may also associate with γ2b (44-47). However, even if γ2b can bind λ5, a correct signal for B cell maturation may not be created.

mlg is expressed in the context of a cell surface receptor in which the H and L chain polypeptides are associated with Ig-α and Ig-β (48). It is known that all heavy chain isotypes can associate with Ig-α and Ig-β (48-50). This receptor complex participates in signal transduction events resulting from engagement of mlg (51). In addition, multiple protein tyrosine kinases have been found to associate with mlg and to be activated after crosslinking of the mlg receptor (52-54). Many other target substrates have been identified downstream from the mlgM receptor (55). It is likely that certain downstream signaling events may differ between μ- and γ-containing Ig receptors.

The precise signal transduction mechanisms regulating the normal progression of B cell development are beginning to be elucidated. While a number of proteins associated with the pre-B and B cell receptor have been identified, many have not. It appears that γ2b is unable to associate with or efficiently transduce signals through all the pathways in which μ can participate. Further analysis of the γ2b transgenic mice should help to delineate the specific pathways downstream from heavy chain expression that are required to complete B cell development.
References

1. Chen, J., and F. Alt. 1993. Gene rearrangement and B cell development. Curr. Opin. Immunol. 5:194.

2. Kitamura, D., and K. Rajewsky. 1992. Targeted disruption of $\mu$ chain membrane exon causes loss of heavy-chain allelic exclusion. Nature (Lond.). 356:154.

3. Storb, U. 1987. Transgenic mice with immunoglobulin genes. Annu. Rev. Immunol. 5:151.

4. Iglesias, A., M. Lamers, and G. Kohler. 1987. Expression of immunoglobulin delta chain causes allelic exclusion in transgenic mice. Nature (Lond.). 330:482.

5. Manz, J., K. Denis, O. Witte, R. Brinster, and U. Storb. 1988. Feedback inhibition of immunoglobulin gene rearrangement by membrane $\mu$ but not by secreted $\mu$ heavy chains. J. Exp. Med. 168:1363.

6. Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C. Morse III, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin $\mu$. Science (Wash. DC). 236:816.

7. Kabat, E.A., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. Sequences of Proteins of Immunological Interest. U.S. Department of Health and Human Services, Bethesda, MD.

8. Tiang, H., C. Pinkert, J. Hagman, M. Lostrum, R.L. Brinster, and U. Storb. 1988. Cloning of a $\gamma$2b gene encoding anti-<i>Pseudomonas aeruginosa</i> H chains and its introduction into the germ line of mice. J. Immunol. 141:308.

9. Manz, J.T. 1990. Rearrangement of endogenous immunoglobulin genes in mu and kappa transgenic mice. Ph.D. thesis. University of Washington, Seattle.

10. Tábak, H.F., and R.A. Flavell. 1978. A method for the recovery of DNA from agarose gels. Nucleic Acids Res. 5:2321.

11. Storb, U., C. Pinkert, P. Arp, P. Engler, K. Gollahon, J. Manz, W. Brady, and R.L. Brinster. 1986. Transgenic mice with $\mu$ and $\kappa$ genes encoding antiphosphocholine antibodies. J. Exp. Med. 164:627.

12. Hagman, J., D. Lo, L.T. Doglio, J. Hackett, Jr., C.M. Rudin, D. Haasch, R.L. Brinster, and U. Storb. 1989. Inhibition of immunoglobulin gene rearrangement by the expression of a $\alpha2$ transgene. J. Exp. Med. 169:1911.

13. Alonso, S., A. Minty, Y. Boulet, and M. Buckingham. 1986. Comparison of three actin-coding sequences in the mouse: evolutionary relationship between the actin genes of warm-blooded vertebrates. J. Mol. Evol. 23:11.

14. Kenny, J.J., F. Finkelman, F. Macchiarini, W.C. Kopp, U. Storb, and D.L. Longo. 1989. Alteration of the B cell surface phenotype immune response to phosphocholine and the B cell repertoire in M167 $\mu$ plus $\kappa$ transgenic mice. J. Exp. Med. 142:4466.

15. Kenny, J.J., C. O'Connell, D.G. Sieckmann, R.T. Fischer, and D.L. Longo. 1991. Selection of antigen-specific, idiootype-positive B cells in transgenic mice expressing a rearranged M167-$\mu$ heavy chain gene. J. Exp. Med. 174:1189.

16. Okamoto, M., M. Murakami, A. Shimizu, S. Ozaki, T. Tsubata, S. Kumagi, and T. Honjo. 1992. A transgenic model of autoimmune hemolytic anemia. J. Exp. Med. 175:71.

17. Denis, K.A., S. Provost, O.N. Witte, R.L. Brinster, and U. Storb. 1990. Delay of early B-lymphocyte development by gamma 2b immunoglobulin transgene; effect on differentiation-specific molecules. Dev. Immunol. 1:105.

18. Raff, M.C., M. Megson, J.T. Owen, and M.D. Cooper. 1976. Early production of intracellular IgM by B-lymphocyte precursors in mouse. Nature (Lond.). 259:224.

19. Velardi, A., and M.D. Cooper. 1984. An immunofluorescence analysis of the ontogeny of myeloid, T, and B lineage cells in mouse hemopoietic tissues. J. Immunol. 133:672.

20. Strasser, A., A. Rolink, and F. Melchers. 1989. One synchronous wave of B cell development in mouse fetal liver changes at day 1 of gestation from dependence to independence of a stromal cell environment. J. Exp. Med. 170:1973.

21. Milcarek, C., and B. Hall. 1985. Cell-specific expression of secreted versus membrane forms of immunoglobulin gamma 2b mRNA involves selective use of alternate polyadenylation sites. Mol. Cell. Biol. 5:2514.

22. Kobrin, B.J., C. Milcarek, and S.L. Morrison. 1986. Sequences near the 3' secretion-specific polyadenylation site influence levels of secretion-specific and membrane-specific IgG2b mRNA in myeloma cells. Mol. Cell. Biol. 6:1687.

23. Reth, M.G., P. Ammirati, S. Jackson, and F.W. Alt. 1985. Regulated progression of a cultured pre-B-cell line to the B-cell stage. Nature (Lond.). 317:353.

24. Schlissel, M.S., and D. Baltimore. 1989. Activation of immunoglobulin kappa gene rearrangement correlates with induction of germline kappa gene transcription. Cell. 58:1001.

25. Forster, I., and K. Rajewsky. 1990. The bulk of the peripheral B-cell pool in mice is stable and not rapidly renewed from the bone marrow. Proc. Natl. Acad. Sci. USA. 87:4781.

26. Hardy, R.R., C.E. Carmack, S.A. Shintod, J.D. Kemp, and K. Hayakawa. 1991. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J. Exp. Med. 173:1213.

27. Rolink, A., and F. Melchers. 1993. Generation and regeneration of cells of the B-lymphocyte lineage. Curr. Opin. Immunol. 5:207.

28. Osmond, D.G. 1991. Proliferation kinetics and the lifespan of B cells in central and peripheral lymphoid organs. Curr. Opin. Immunol. 3:179.

29. Kitamura, D., A. Kudo, S. Schaal, W. Muller, F. Melchers,
and K. Rajewsky. 1992. A critical role of λ5 protein in B cell development. Cell. 69:823.

30. Ehlich, A., S. Schaas, H. Gu, D. Kitamura, W. Muller, and K. Rajewsky. 1993. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. Cell. 72:695.

31. Reth, M., E. Petrac, P. Wiese, I. Lobel, and F.W. Alt. 1987. Activation of Vκ gene rearrangement in pre-B cells follows the expression of membrane-bound immunoglobulin heavy chains. EMBO (Eur. Mol. Biol. Organ.) J. 6:3299.

32. Schuler, W., I.J. Weiler, A. Schuler, R.A. Phillips, N. Rosenberg, T.W. Mak, J.F. Kearney, R.P. Perry, and M.J. Bosma. 1986. Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. Cell. 46:963.

33. Fried, M., R.R. Hardy, and M.J. Bosma. 1989. Transgenic SCID mice with a functionally rearranged immunoglobulin heavy chain gene. Curr. Top. Microbiol. Immunol. 152:107.

34. Era, T., M. Ogawa, S.-I. Nishikawa, M. Okamoto, T. Honjo, K. Akagi, J.-I. Miyazaki, and K.-I. Yamamura. 1991. Differentiation of growth signal requirement of B lymphocyte precursor is directed by expression of immunoglobulin. EMBO (Eur. Mol. Biol. Organ.) J. 10:337.

35. Reichman-Fried, M., M.J. Bosma, and R.R. Hardy. 1993. B-lineage cells in μ-transgenic scid mice proliferate in response to II-7 but fail to show evidence of immunoglobulin light chain gene rearrangement. Int. Immunol. 5:303.

36. Chen, J., M. Trounstine, F.W. Alt, F. Young, C. Kurahara, J.F. Loring, and D. Huszar. 1993. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the Jκ locus. Int. Immunol. 5:647.

37. Tsutbata, T., R. Tsutbata, and M. Reth. 1992. Crosslinking of the cell surface immunoglobulin (μ-surrogate light chain complex) on pre-B-cells induces activation of V gene rearrangements at the immunoglobulin κ locus. Int. Immunol. 4:637.

38. Shaw, A., R. Mitchell, Y. Weaver, J. Campos-Torres, A. Abbas, and P. Leder. 1990. Mutations of immunoglobulin transmembrane and cytoplasmic domains: effects on intracellular signaling and antigen presentation. Cell. 63:381.

39. Nishimoto, N., H. Kubagawa, T. Ohno, G.L. Gartland, A.K. Stankovic, and M.D. Cooper. 1991. Normal pre-B cells express a receptor complex of μ heavy chains and surrogate light-chain proteins. Proc. Natl. Acad. Sci. USA. 88:6284.

40. Nomura, J., T. Matsu, E. Kubota, M. Kimoto, and N. Sakaguchi. 1991. Signal transmission through the B cell-specific Mb-1 molecule at the pre-B cell stage. Int. Immunol. 3:117.

41. Tsutbata, T., and S. Nishikawa. 1991. Molecular and cellular aspects of early B cell development. Curr. Opin. Immunol. 3:186.

42. Melchers, F., H. Karasuyama, D. Haasner, S. Bauer, A. Kudo, N. Sakaguchi, B. Jameson, and A. Rolink. 1993. The surrogate light chain in B-cell development. Immunol. Today. 14:60.

43. Tsutbata, T., R. Tsutbata, and M. Reth. 1991. Cell surface expression of the short immunoglobulin μ chain (Dμ protein) in murine pre-B cells is differently regulated from that of the intact μ chain. Eur. J. Immunol. 21:1359.

44. Hendershot, L., D. Bole, G. Kohler, and J.F. Kearney. 1987. Assembly and secretion of heavy chains that do not associate posttranslationally with immunoglobulin heavy chain-binding protein. J. Cell Biol. 104:761.

45. Pollok, B.A., R. Anker, P. Eldridge, L. Hendershot, and D. Levitt. 1987. Molecular basis of the cell-surface expression of immunoglobulin μ chain without light chain in human B lymphocytes. Proc. Natl. Acad. Sci. USA. 84:9199.

46. Bachhawat, A.K., and S. Pillai. 1991. Distinct intracellular fates of membrane and secretory immunoglobulin heavy chains in a pre-B cell line. J. Cell Biol. 115:619.

47. Sitia, R., M. Neuberger, C. Albertini, P. Bet, A. Fra, C. Valetti, G. Williams, and C. Milstein. 1990. Developmental regulation of IgM secretion: The role of the carboxy-terminal cysteine. Cell. 60:781.

48. Reth, M. 1992. Antigen receptors on B lymphocytes. Annu. Rev. Immunol. 10:97.

49. Venkitaraman, A.R., G.T. Williams, P. Darivach, and M.S. Neuberger. 1991. The B-cell antigen receptor of the five immunoglobulin classes. Nature (Lond.). 352:777.

50. Webb, C.F., C. Nakai, and P.W. Tucker. 1989. Immunoglobulin receptor signaling depends on the carboxyl terminus but not the heavy-chain class. Proc. Natl. Acad. Sci. USA. 86:1977.

51. Sefton, B.M., and M.-A. Campbell. 1991. The role of tyrosine protein phosphorylation in lymphocyte activation. Annu. Rev. Cell Biol. 7:257.

52. Burkhardt, A., M. Brunswick, J. Bolen, and J. Mend. 1991. Anti-immunoglobulin stimulation of B lymphocytes activates src-related protein-tyrosine kinases. Proc. Natl. Acad. Sci. USA. 88:7410.

53. Gold, M.R., L. Matsuschi, R.B. Kelly, and A.L. DeFranco. 1991. Tyrosine phosphorylation of components of the B-cell antigen receptor following receptor crosslinking. Proc. Natl. Acad. Sci. USA. 88:3436.

54. Campbell, M., and B. Sefton. 1992. Association between B-lymphocyte membrane immunoglobulin and multiple members of the Src family of protein tyrosine kinases. Mol. Cell. Biol. 12:2315.

55. Gold, M.R., M.T. Crowley, G.A. Martin, F. McCormick, and A.L. DeFranco. 1993. Targets of B lymphocyte antigen receptor signal transduction include the p21°′ GTase-activating protein (GAP) and two GAP-associated proteins. J. Immunol. 150:377.