

# The Cytoplasmic Domains of Immunoglobulin (Ig) $\alpha$ and Ig $\beta$ Can Independently Induce the Precursor B Cell Transition and Allelic Exclusion

By Fotini Papavasiliou,\* Mila Jankovic,‡ Heikyung Suh,‡  
and Michel C. Nussenzweig‡

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From the \*Laboratory of Molecular Immunology, The Rockefeller University; and ‡The Howard Hughes Medical Institute, New York 10021

## Summary

In mature B cells, signals transduced through membrane immunoglobulin (Ig) produce cellular activation, yet the same receptor can also mediate deletion and silencing of autoreactive B cells. In addition, Ig expression during the antigen-independent phase of B cell development regulates the precursor B (pre-B) cell transition and allelic exclusion. To account for the diverse regulatory functions induced by membrane Ig, it has been proposed that individual receptor components have independent physiologic activities. Here we establish a role for Ig $\alpha$  in the pre-B cell transition and allelic exclusion. We find that the cytoplasmic domain of Ig $\alpha$  contains sufficient information to trigger both of these antigen-independent events. Direct comparisons of the cytoplasmic domains of Ig $\alpha$  and Ig $\beta$  show that the two are indistinguishable in the induction of the pre-B cell transition and allelic exclusion. Our experiments suggest that, despite the reported differences in certain biochemical assays, Ig $\alpha$  and Ig $\beta$  have redundant functions in the developing B cell.

Lymphocyte development progresses through a series of ordered events, ultimately resulting in B and T cells that express clonotypic Ig or TCRs. Although the initial lineage commitment events are independent of Ig and TCR expression, many of the subsequent checkpoints in both the T and B cell pathways are regulated by these receptors. In the bone marrow, regulation of the B cell pathway by membrane Ig begins in the earliest identifiable committed B cell progenitors (pro-B cells).<sup>1</sup> Experiments with transgenic and gene-targeted mice have shown that progression from the pro-B cell to precursor B (pre-B) cell stage is dependent on expression of the membrane-bound form of heavy chain (1–3). In the pre-B cell,  $\mu$  expression regulates a second checkpoint, the allelic exclusion of a second heavy chain locus (4–6). Both the pre-B cell transition and allelic exclusion are believed to be antigen-independent events since they can occur before Ig light chain gene rearrangements, and thus before the assembly of a mature receptor Ig.

After light chain gene rearrangement and completion of cell surface receptor assembly, interactions between surface Ig and antigen largely control cell fate. Binding of self-antigens to the receptor in the newly matured B cell can lead

to receptor editing (7, 8) and deletion of autoreactive cells (9–12). By contrast, binding of antigens to the Ig receptor in mature B cells is one of the key elements in activating clonal expansion and antibody secretion. Thus, a single receptor has a number of apparently distinct antigen-dependent and -independent regulatory functions in the B cell pathway.

Two models have been put forward to account for these distinct activities: Either one receptor produces several qualitatively different signals, or, alternatively, similar signals are interpreted differently in different cellular contexts. Support for the first idea, that the signals are different, comes from structural and biochemical studies of the Ig receptor. The mature receptor is associated through its transmembrane domain with the two signaling molecules, Ig $\alpha$  and Ig $\beta$ , that form a disulfide-linked heterodimer (13). When tested in isolation, both can activate mature B cells, but the two proteins bind to different sets of cellular kinases (14), and several groups have found that the responses activated by Ig $\alpha$  and Ig $\beta$  in transfected cell lines are different (15–18).

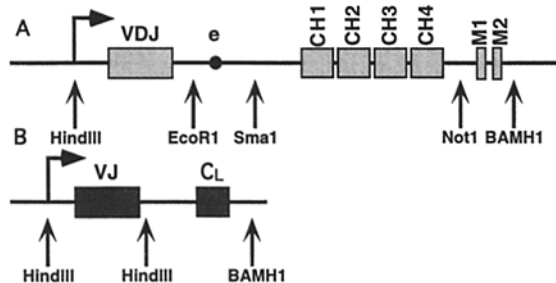
We have recently shown that the cytoplasmic domain of Ig $\beta$  can activate the pre-B transition and allelic exclusion *in vivo* by a mechanism that requires tyrosine phosphorylation (19). Here we examine whether Ig $\alpha$  can mediate these antigen-independent events, and whether Ig $\alpha$  and Ig $\beta$  have different physiologic functions during early B cell development.

<sup>1</sup>Abbreviations used in this paper: pre-B cell, precursor B cell; pro-B cell, B cell progenitor; RAG, recombinase-activating gene.

## Materials and Methods

**Constructs and Mice.** Many of the heavy chain vectors used for these experiments have been described elsewhere (19). Briefly, the starting construct for the heavy chain transgenes was a HindIII–BamHI fragment of the human  $\mu$  gene, containing the constant and membrane exons, and modified to direct the synthesis of only the membrane form of human  $\mu$  (20). To this fragment we added a HindIII–EcoRI piece containing a murine V region (9) and murine enhancer (21). Finally, unique BamHI and NotI sites were placed around the membrane exons for rapid replacement of the wild-type sequence with specifically modified transmembrane domains (15) (Fig. 1 A). In addition to the wild-type heavy chain construct, we have created four others. The first construct, which we call YS:VV-IgM, differs from the wild-type in that Tyr-587 and Ser-588 of the wild-type transmembrane domain are replaced by Val. This mutation renders the molecule unable to contact endogenous Ig $\alpha$  and Ig $\beta$  (15). The Ig $\alpha$  and Ig $\beta$  cytoplasmic tails were amplified from plasmids by PCR, and, after sequence verification, they were subcloned in frame at the end of the heavy chain transmembrane domain (15). The addition of the cytoplasmic tail of Ig $\beta$  to the YS:VV  $\mu$  mutant gave rise to the YS:VV-IgM:Ig $\beta$  chimeric molecule (15). Similarly, YS:VV-IgM:Ig $\alpha$  was created by adding the cytoplasmic tail of Ig $\alpha$  onto the YS:VV $\mu$ . Finally, the YS:VV-IgM:Ig $\beta$  Y:F chimera differs from YS:VV-IgM:Ig $\beta$  in the Tyr-195 and Tyr-206 in the cytoplasmic domain of Ig $\beta$  have been replaced with Phe (15). The light chain constructs are composed of a HindIII fragment of the mouse V region (9) added to a HindIII–BamHI piece containing the mouse light chain constant region (22). (Fig. 1 B) Expression of this heavy and light chain pair in vivo results in an antibody that reacts specifically with H2<sup>k/k</sup> but not with H2<sup>d/d</sup> (9). To create transgenic mice, equimolar amounts of purified DNA from heavy and light chain constructs was coinjected into recombination-activating gene (RAG1<sup>-/-</sup>H2<sup>d/d</sup>x CD2F1 H2<sup>d/d</sup> fertilized oocytes (23). Two independent integrants were obtained for each line. The founders were then back-crossed to either RAG1<sup>-/-</sup>H2<sup>d/d</sup> (2, 24) or BALB/c mice.

**Intracellular Human IgM Expression.** The presence of human  $\mu$  heavy chain in the cytoplasm of B cells was verified by FACS<sup>®</sup>. B cells from the bone marrow of 6–8-wk-old mice were first stained with PE-B220 (PharMingen, San Diego, CA) and FITC-CD43 (S7 hybridoma; American Type Culture Collection, Rockville, MD) antibodies for 30 min. The cells were then fixed by adding to them a solution of 4% paraformaldehyde in PBS for 10



**Figure 1.** Diagrammatic representation of the mouse–human hybrid  $\mu$  and the mouse  $\kappa$  light chain transgenes. (A) Heavy chain gene. (B) Light chain gene. Together, the heavy and light chain genes produce an Ig that reacts with the 54.1 antiidiotypic antibody. VDJ and VJ, variable region coding exons; e, mouse heavy chain enhancer; CH1–CH4, human constant region coding exons; M1 and M2, transmembrane domain coding exons; C<sub>L</sub>, constant region coding exons for the mouse light chain.

min. After one wash in PBS/1% FCS solution, the fixed cells were permeabilized with 1% saponin for 30 min and stained with either anti–human IgM (Southern Biotechnology Associates, Birmingham, AL), anti–mouse  $\kappa$  light chain (PharMingen), or anti-idiotypic antibody (hybridoma 54.1 [9] provided by Dr. D. Nemazee, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). Data acquisition was performed with a FACSscan<sup>®</sup> (Becton Dickinson & Co., Mountain View, CA), and appropriate gating and analysis was done with CellQuest software (Becton Dickinson & Co.).

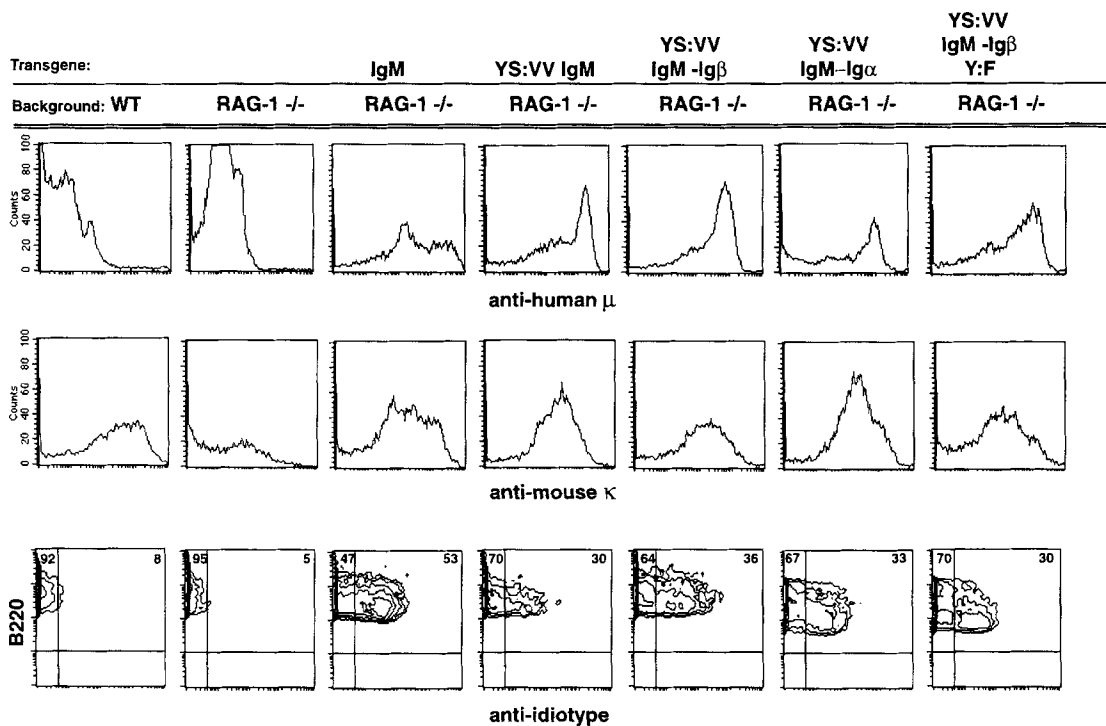
**Flow Cytometric Analysis.** The following antibodies were used for surface staining of B cells: PE-B220; FITC-CD43 (S7 hybridoma); biotin-labeled anti–human IgM (heavy chain–specific DA4.4 clone; Southern Biotechnology Associates); biotin-labeled anti–mouse  $\kappa$  light chain (PharMingen); and biotin-labeled anti-idiotypic antibody (hybridoma 54.1 [9]). Streptavidin-RED670 was the secondary reagent (GIBCO BRL, Gaithersburg, MD) where biotinylated antibodies were used. Data were collected on a FACSorter<sup>®</sup> and were analyzed using CellQuest software.

**VDJ Recombination and Allelic Exclusion.** Ig gene rearrangements in transgenic mice were assayed by PCR as previously described (25). Recombined sequences of VJ558L family or D region segments with J segments were amplified from bone marrow DNA from two mice for each integrant. Amplification of intervening sequences was used as DNA loading control. Primer sequences and PCR conditions were as previously described (25). Amplified DNA was visualized by Southern hybridization with an EcoRI DNA fragment that spans the mouse J region. In this assay, the capability of a transgene to inhibit V to DJ recombination is evident as an absence of recombined VJ558L sequences.

## Results

**Expression of Transgenic Igs.** To compare the signaling functions of Ig $\alpha$  and Ig $\beta$  in vivo, we started with Ig heavy chains that carried mutations in their transmembrane domains (YS:VV-IgM), which have been shown to render the heavy chain unable to interact with Ig $\alpha$ –Ig $\beta$  (15). The cytoplasmic domains of Ig $\alpha$  and Ig $\beta$  were added to the YS:VV-IgM to produce the chimeric molecules YS:VV-IgM:Ig $\alpha$  and YS:VV-IgM:Ig $\beta$ . These mutant heavy chains were combined with cognate light chain genes and introduced into the RAG1<sup>-/-</sup> H2<sup>d/d</sup> background, where B cell development is blocked at the pro–B cell stage (2, 24, 26). An additional modification carried by all of the heavy chain genes described below was the deletion of the first poly-A addition site, resulting in a gene that directs the synthesis of only the membrane form of Ig (4, 20). Strains that carried the wild-type version of the membrane-only Ig transgenes were used as controls. Heavy and light chain gene expression was assayed by staining bone marrow cells with a panel of mAbs (Fig. 2). The levels of intracellular heavy and light chain expression (Fig. 2, top and center) and cell surface idiotype expression were similar in all 10 of the transgenic strains tested in both RAG1<sup>-/-</sup> and wild-type backgrounds (Fig. 2, bottom, and data not shown). Thus, all transgenic strains produced similar amounts of transgenic Ig and expressed the receptor on the surface of developing B cells.

**Ig $\alpha$  Induces the Pre–B Cell Transition.** Induction of the

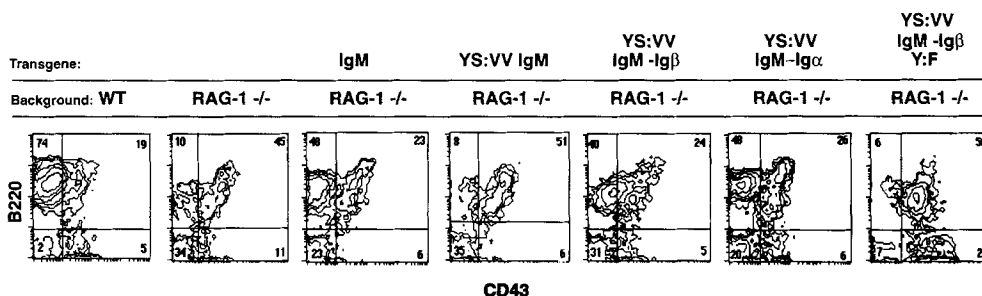


**Figure 2.** Expression of the heavy and light chain transgenes. (*Top*) Intracellular expression of the human  $\mu$  heavy chain. Cells, stained with PE-labeled anti-B220, were fixed, permeabilized, and stained with biotin-labeled anti-human IgM. Gating was on B220<sup>high</sup> cells. (*Center*) Intracellular expression of the mouse  $\kappa$  light chain. Cells were treated as in *A* but were stained with biotin-labeled anti-mouse  $\kappa$  chain instead of  $\mu$ . Gating was on B220<sup>high</sup> cells. (*Bottom*) Surface expression of the fully assembled IgM receptor on B cells from the bone marrow of RAG-1<sup>-/-</sup>-transgenic mice. Bone marrow cells were stained with PE-labeled anti-B220, FITC-labeled anti-CD43, and biotin-labeled antiidiotypic antibody 54.1 (9). Gating was on CD43<sup>+</sup>B220<sup>high</sup> cells. The numbers in each quadrant represent percentages of gated lymphocytes.

pro- to pre-B cell transition was assessed by staining of bone marrow cells with anti-B220 and anti-CD43 antibodies (Fig. 3). Pro-B cells are B220<sup>+</sup>CD43<sup>+</sup>, whereas more mature cells lose expression of CD43 and therefore become B220<sup>+</sup>CD43<sup>-</sup>. Mice that carry complete YS:VV membrane Igs, in contrast to transgenic mice that carry the wild-type membrane Ig, were unable to induce the pre-B cell transition (Fig. 3). Thus, even in the presence of the light chain, the mutant Igs were inactive, suggesting that contact with Ig $\alpha$  and Ig $\beta$  was the essential factor in the induction of the pre-B cell transition, irrespective of light chain expression and antigen receptor assembly.

We have shown that the cytoplasmic domain of Ig $\beta$  was sufficient to activate the pre-B cell transition when ex-

pressed as part of a chimeric heavy chain that includes the membrane and external domains of the inactive YS:VV  $\mu$  (19). To examine the relative activities of Ig $\alpha$  and Ig $\beta$  in the induction of the pre-B cell transition, we compared the previously reported Ig $\beta$  chimera and a similar construct expressing the cytoplasmic domain of Ig $\alpha$ . Both of these chimeric immunoglobulins were coinjected with the cognate light chain gene described above, and at least two independent integrants were tested for each of these new strains. In contrast to the parental YS:VV-IgM mutant Igs, expression of either the YS:VV-IgM:Ig $\alpha$  or the YS:VV-IgM:Ig $\beta$  chimeras in the RAG-1<sup>-/-</sup> background reconstituted the pre-B cell compartment (Fig. 3). Bone marrow cells from RAG-1<sup>-/-</sup> mice and the YS:VV-IgM-transgenic



**Figure 3.** B cell development in the bone marrow of RAG-1<sup>-/-</sup> H2<sup>d/d</sup> IgM-transgenic mice. Bone marrow cells from 6- to 8-wk-old transgenic mice were analyzed by staining with PE-labeled anti-B220 and FITC-labeled anti-CD43. The lymphocyte population was gated according to standard forward- and side-scatter values. The numbers in each quadrant represent the percentages of gated lymphocytes.

RAG-1<sup>-/-</sup> mice had few detectable CD43<sup>-</sup>B220<sup>+</sup> cells, whereas the number of CD43<sup>-</sup>B220<sup>+</sup> cells in YS:VV-IgM:Igα<sup>-</sup> and YS:VV-IgM:Igβ RAG-1<sup>-/-</sup>-transgenic mice resembled that found in wild-type mice (Fig. 3). Similar numbers of pre-B cells were found in both sets of YS:VV-IgM:Igα<sup>-</sup> and YS:VV-IgM:Igβ RAG-1<sup>-/-</sup>-transgenic strains. Further, the activation of the pre-B cell transition by Igβ in the chimeric heavy plus light chain RAG-1<sup>-/-</sup> transgenic mice was dependent on the presence of cytoplasmic tyrosine residues in Igβ. Additional strains that carry the light chain and a chimeric heavy chain with a mutation of tyrosine 536 in the cytoplasmic domain of Igβ resulted in an Ig that was no longer able to induce the pre-B cell transition (Fig. 3, right).

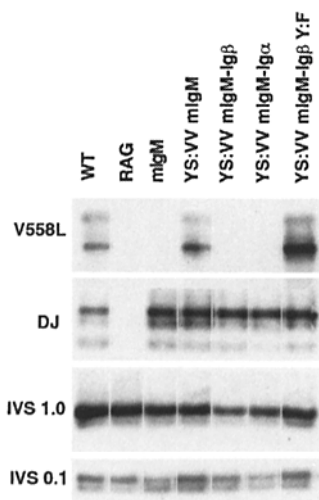
We conclude that the cytoplasmic domain of Igα resembles that of Igβ in that it contains sufficient information to produce the signal that induces the pre-B cell transition.

**Igα Induces Allelic Exclusion.** To examine the role of Igα in allelic exclusion and to compare the activities of Igα with Igβ, we crossed all of the Ig-transgenic mice described above into BALB/c mice that were wild type for RAG-1. Bone marrow DNA samples were then tested for VDJ recombination by PCR. In agreement with previously published experiments, we found that a wild-type membrane IgM transgene was active in allelic exclusion, inhibiting V to DJ rearrangements but not D to J rearrangements (Fig. 4). By contrast, heavy chains bearing the YS:VV mutation that interferes with the ability of μ to pair with Igα-Igβ had no effect on gene rearrangements, even when they were assembled with the appropriate cognate light chain (Fig. 4). Addition of the cytoplasmic domain of Igα to the inactive YS:VV Ig resulted in a chimera that inhibited V to DJ but not D to J recombination (Fig. 4). Side-by-side comparisons of the YS:VV-IgM:Igα<sup>-</sup> and YS:VV-IgM:Igβ-expressing strains showed that the two were indistinguishable in their ability to inhibit recombination. Moreover, as was shown for strains that carry just the heavy chain (19), inhibition of V to DJ rearrangements was dependent on the presence of cytoplasmic tyrosines in Igβ (Fig. 4). Mice that carry the combination of the YS:VV-IgM:Igβ/Y-F and the light chain were unable to induce allelic exclusion (Fig. 4, right lane).

We conclude that the cytoplasmic tail of Igα is sufficient to mediate inhibition of V to DJ recombination, and, therefore, with respect to allelic exclusion, the roles of the Igα and Igβ cytoplasmic domains are functionally equivalent.

## Discussion

The B cell antigen receptor is a complex structure that has a number of diverse regulatory functions in both developing and mature B cells. This receptor is composed of two disulfide-linked heterodimers that are noncovalently associated on the plasma membrane (27–29). The ligand-binding component, membrane IgM, has no signaling activity on its own but requires Igα and Igβ to activate cellular responses such as tyrosine phosphorylation and Ca<sup>2+</sup> flux (15, 16, 18, 30). Although the cytoplasmic domains of Igα and Igβ are



**Figure 4.** Ig gene rearrangements in transgenic mice assayed by PCR. Bone marrow samples were obtained from two individual mice for each of the lines indicated. All mice were of a wild-type (*WT*) genetic background that supports recombination, except for the indicated RAG-1<sup>-/-</sup> control sample. Primer sequences and PCR conditions were as previously described (25). *V558L*, amplified DNA from the synonymous V gene family; *DJ*, amplified DNA from D to J recombined fragments; *IVS*, amplified DNA from intervening sequences.

very different, both have a tyrosine-based SH2 target motif that is essential for signal transduction (15, 16, 30). As first pointed out by Reth (31), the YxxLxxxxxxYxxL motif is also found in a number of other antigen receptor-associated proteins, including CD3 components and FcRγ. In all cases studied to date, signal transduction by this class of receptors requires cross-linking (32) and is believed to involve activation of src family kinases. A second amplification step involves binding to syk family kinases (33), for which both Igα and Igβ have similar affinities (34, 35). However, the cytoplasmic domains of Igα and Igβ have been shown to bind to different sets of src kinases (14), and they appear to have different signaling functions in transfected cell lines (15–17). Given the diversity of cellular responses activated by membrane IgM, it has been proposed that individual receptor subunits may have independent physiologic functions.

We have sought to resolve this issue by measuring the physiologic activities of Igα and Igβ during the antigen-independent phase of B cell development. For this purpose we produced transgenic mice that express chimeric Ig molecules composed of the extracellular and transmembrane domains of a YS:VV mutant heavy chain and the cytoplasmic domains of Igα and Igβ. Light chains were coinjected with the Igα and Igβ heavy chain transgenes to ensure the assembly of a membrane-bound receptor that could be expressed on the cell surface (Fig. 2, bottom). Previous work has shown that YS:VV mutant heavy chains that do not contact Igα-Igβ cannot support the induction of the antigen-independent phase of B cell development (19). Further, experiments with chimeric Igs showed that the cytoplasmic domain of Igβ was sufficient to trigger the pre-B cell transition and allelic exclusion by a mechanism that requires phosphorylation of the tyrosines of its ARH1 motif (19). The addition of the light chain in the experiments we report here did not diminish the activity of either the wild-type membrane-bound μ chain or the YS:VV-IgM:Igβ chimera in the induction of the pre-B cell transition or allelic exclusion (Figs. 3 and 4). Conversely, the light chain transgene did not rescue the inactive YS:VV-IgM or the

tyrosine-mutant YS:VV-IgM:Ig $\beta$ /Y-F chimera (Figs. 3 and 4). Thus, Ig heavy and light chain assembly is neither required for nor does it significantly affect the antigen-independent events in the B cell pathway.

Analysis of the new chimeric molecules with the cytoplasmic tail of Ig $\alpha$  showed that Ig $\alpha$  is also sufficient to trigger the pro- to pre-B cell transition (Fig. 3). In addition, signaling through the Ig $\alpha$  chimera can lead to inhibition of recombination at endogenous heavy chain loci (Fig. 4). Thus, Ig $\alpha$  resembles Ig $\beta$  in that its cytoplasmic domain can trigger both of these antigen-independent events. Our results are similar to recent reports that examine this question in the context of the TCR (36, 37). The TCR is more complex than the B cell receptor in that it is composed of two independent signaling modules (38–41) (the  $\gamma$ ,  $\delta$ ,  $\epsilon$  module and the  $\zeta$  dimer). Experiments with CD3 $\zeta$ <sup>-/-</sup> mice reconstituted with mutant transgenic  $\zeta$  chains have shown that  $\zeta$  is required for assembly of the TCR, but the signaling function of  $\zeta$  is not required for T cell development (36). In these mice, the  $\gamma$ ,  $\delta$ ,  $\epsilon$  module was sufficient to produce CD4<sup>+</sup>CD8<sup>-</sup> and CD4<sup>-</sup>CD8<sup>+</sup> T cells in the complete absence of  $\zeta$  signaling motifs (36). Similarly, chimeric molecules composed of the external and transmembrane domains of the IL-2 receptor and the cytoplasmic domains of the CD3 $\epsilon$  or CD3 $\zeta$  tails have shown that in vivo cross-linking of either one was sufficient to mediate the transition from the double-negative to the double-pos-

itive cell stage (37). Thus, in developing T cells, as in B cells, the physiologic activity of the antigen receptor homology 1-containing signaling proteins is redundant, and the presence of many molecules containing antigen receptor homology 1 motifs may simply function to amplify the signal. Nevertheless, these conclusions do not invalidate the suggestion that different signals might be delivered through the individual receptor-associated molecules, since only the early segments of the B and T cell pathways have been examined. There is, of course, a possibility that Ig $\alpha$  and Ig $\beta$  have different functions in very early pro-B cells. Both of these proteins are expressed before VDJ recombination (42–44), and it has been suggested that they interact with a surrogate heavy chain to form a pro-B cell receptor (45). This receptor is thought to mediate activation of the transition from the very early pro-B cell to the later stages in development. It is also possible that events regulated by Ig in more mature B cells, such as deletion of autoreactive cells, silencing, or cellular activation, require orchestration of several signals, thus demanding different roles for Ig $\alpha$  and Ig $\beta$ . In our transgenic mice, the heavy and light chain pair produces an anti-H2<sup>k/k</sup> antibody, which results in the deletion of autoreactive B cells in the appropriate genetic background (9). Therefore, the system we have developed will allow us to test for differences between Ig $\alpha$  and Ig $\beta$  at stages subsequent to those of pre-B cell transition and allelic exclusion.

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Address correspondence to Dr. Michel Nussenzweig, Assistant Investigator, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Ave., New York 10021.

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## References

1. Kitamura, D., and K. Rajewsky. 1992. Targeted disruption of mu chain membrane exon causes loss of heavy-chain allelic exclusion. *Nature (Lond.)*. 356:154–156.
2. Spanopoulou, E., C.A.J. Roman, L.M. Corcoran, M.S. Schlisel, D.P. Silver, D. Nemazee, M.C. Nussenzweig, S.A. Shinton, R.R. Hardy, and D. Baltimore. 1994. Functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1-deficient mice. *Genes. Dev.* 8:1030–1042.
3. Young, F., B. Ardman, Y. Shinkai, R. Lansford, T.K. Blackwell, M. Mendelsohn, A. Rolink, F. Melchers, and F.W. Alt. 1994. Influence of immunoglobulin heavy- and light-chain expression on B-cell differentiation. *Genes. Dev.* 8:1043–1057.
4. Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C.I. Morse, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin  $\mu$ . *Science (Wash. DC)*. 236:816–819.
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.* 168:1363–1381.
6. Kitamura, D., J. Roes, R. Kuhn, and K. Rajewsky. 1991. A B cell deficient mouse by targeted disruption of the membrane exons of the immunoglobulin  $\mu$  chain gene. *Nature (Lond.)*. 350:423–426.
7. Radic, M.Z., J. Erickson, S. Litwin, and M. Weigert. 1993. B lymphocytes may escape tolerance by revising their antigen receptors. *J. Exp. Med.* 177:1165–1173.
8. Tiegs, S.L., D.M. Russell, and D. Nemazee. 1993. Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.* 177:1009–1020.
9. Nemazee, D.A., and K. Bürki. 1989. Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature (Lond.)*. 337:562–566.

10. Goodnow, C., J. Crosbie, S. Adelstein, T. Lavoie, S. Smith-Gill, R. Brink, H. Pritchard-Briscoe, J. Wotherspoon, R. Loblay, and K.E.A. Raphael. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature (Lond.)*. 334:676–682.
11. Okamoto, M., M. Murakami, A. Shimizu, S. Ozaki, T. Tsubata, S. Kumagai, and T. Honjo. 1992. A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med.* 175: 71–79.
12. Chen, C., Z. Nagy, M.Z. Radic, R.R. Hardy, D. Huszar, S.A. Camper, and M. Weigert. 1995. The site and stage of anti-DNA B-cell deletion. *Nature (Lond.)*. 373:252–255.
13. Reth, M. 1992. Antigen receptors on B lymphocytes. *Annu. Rev. Immunol.* 10:97–121.
14. Clark, M.R., K.S. Campbell, A. Kazlauskas, S.A. Johnson, M. Hertz, T.A. Potter, C. Pleiman, and J.C. Cambier. 1992. The B cell antigen receptor complex: association of Ig $\alpha$  and Ig $\beta$  with distinct cytoplasmic effectors. *Science (Wash. DC)*. 258:123–126.
15. Sanchez, M., Z. Misulovin, A.L. Burkhardt, S. Mahajan, T. Costa, R. Franke, J.B. Bolen, and M. Nussenzweig. 1993. Signal transduction by immunoglobulin is mediated through Ig $\alpha$  and Ig $\beta$ . *J. Exp. Med.* 178:1049–1056.
16. Kim, K.-M., G. Alber, P. Weiser, and M. Reth. 1993. Differential signalling through the Ig $\alpha$  and Ig $\beta$  components of the B cell antigen receptor. *Eur. J. Immunol.* 23:911–916.
17. Williams, G.T., C.J.G. Peaker, K.J. Patel, and M.S. Neuberger. 1994. The  $\alpha/\beta$  sheath and its cytoplasmic tyrosines are required for signaling by the B-cell antigen receptor but not for capping or for serine/threonine-kinase recruitment. *Proc. Natl. Acad. Sci. USA*. 91:471–478.
18. Choquet, D., G. Ku, S. Cassard, B. Malissen, H. Kom, W.H. Fridman, and C. Bonnerot. 1994. Different patterns of calcium signaling triggered through two components of the B lymphocyte antigen receptor. *J. Biol. Chem.* 269:6491–6497.
19. Papavasiliou, F., Z. Misulovin, H. Suh, and M.C. Nussenzweig. 1995. The role of Ig $\beta$  in precursor B cell transition and allelic exclusion. *Science (Wash. DC)*. 268:408–411.
20. Danner, D., and P. Leder. 1985. Role of an RNA cleavage/poly(A) addition site in the production of membrane-bound and secreted IgM mRNA. *Proc. Natl. Acad. Sci. USA*. 82: 8658–8662.
21. Grosschedl, R., D. Weaver, D. Baltimore, and F. Costantini. 1984. Introduction of a mu immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibody. *Cell*. 38:647–658.
22. Max, E.E., J.V. Maizel, and P. Leder. 1981. The nucleotide sequence of a 5.5kb DNA segment containing the mouse kappa immunoglobulin J and C region genes. *J. Biol. Chem.* 256:5116–5120.
23. Palmiter, R.R., and R.L. Brinster. 1986. Germ-line transformation of mice. *Annu. Rev. Genet.* 20:465–499.
24. Mombaerts, P., J. Iacomini, R.S. Johnson, K. Herrup, S. Tonegawa, and V.E. Papaioannou. 1992. RAG-1 deficient mice have no mature B and T lymphocytes. *Cell*. 68:869–877.
25. Costa, T.E.F., H. Suh, and M.C. Nussenzweig. 1992. Chromosomal position of rearranging gene segments influences allelic exclusion in transgenic mice. *Proc. Natl. Acad. Sci. USA*. 89:2205–2208.
26. Shinkai, Y., G. Rathbun, K.-P. Lam, E.M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, A.M. Stall and F.W. Alt. 1992. RAG-2 deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell*. 68:855–867.
27. Gold, M.R., and A.L. DeFranco. 1994. Biochemistry of B lymphocyte activation. *Adv. Immunol.* 55:221–295.
28. Pleiman, C.M., D. D'Ambrosio, and J.C. Cambier. 1994. The B-cell antigen receptor complex: structure and signal transduction. *Immunol. Today*. 15:393–398.
29. Reth, M. 1994. B cell antigen receptors. *Curr. Opin. Immunol.* 12:196–201.
30. Grupp, S.A., K. Campbell, R.N. Mitchell, J.C. Cambier, and A.K. Abbas. 1993. Signaling-defective mutants of the B lymphocyte antigen receptor fail to associate with Ig $\alpha$  and Ig $\beta/\gamma$ . *J. Biol. Chem.* 268:25776–25779.
31. Reth, M. 1989. Antigen receptor tail clue. *Nature (Lond.)*. 338:383–384.
32. Metzger, H. 1992. Transmembrane signaling: the joy of aggregation. *J. Immunol.* 149:1477–1487.
33. Weiss, A., and D.R. Littman. 1994. Signal transduction by lymphocyte antigen receptors. *Cell*. 76:263–274.
34. Kong, G.H., J.Y. Bu, T. Kurosaki, A.S. Shaw, and A.C. Chan. 1995. Reconstitution of Syk function by the ZAP-70 protein tyrosine kinase. *Immunity*. 2:485–492.
35. Bu, J.Y., A.A. Shaw, and A.C. Chan. 1995. Analysis of the interaction of ZAP-70 and syk protein-tyrosine kinases with the T-cell antigen receptor by plasmon resonance. *Proc. Natl. Acad. Sci. USA*. 92:5106–5110.
36. Shores, E.W., K. Huang, T. Tran, E. Lee, A. Grinberg, and P.E. Love. 1994. Role of TCR zeta chain in T cell development and selection. *Science (Wash. DC)*. 266:1047–1050.
37. Shinkai, Y., A. Ma, H.-L. Cheng, and F.W. Alt. 1995. CD3 $\epsilon$  and CD3 $\zeta$  Cytoplasmic domains can independently generate signals for T cell development and function. *Immunity*. 2: 401–411.
38. Wegner, A.-M.K., F. Letourneur, A. Hoeveler, T. Brocker, F. Luton, and B. Malissen. 1992. The T cell receptor/CD3 complex is composed of at least two autonomous transduction molecules. *Cell*. 68:83–96.
39. Romeo, C., and B. Seed. 1991. Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. *Cell*. 64:1037–1046.
40. Letourneur, F., and R.D. Klausner. 1992. Activation of T cells by a tyrosine kinase activation domain in the cytoplasmic tail of CD3 epsilon. *Science (Wash. DC)*. 255:79–82.
41. Irving, B.A., and A. Weiss. 1991. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction. *Cell*. 64:891–901.
42. Sakaguchi, N., S. Kashiwamura, M. Kimoto, P. Thalmann, and F. Melchers. 1988. B lymphocyte lineage-restricted expression of mb-1, a gene with CD3-like structural properties. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3457–3464.
43. Hermanson, G.G., D. Eisenberg, P.W. Kincade, and R. Wall. 1988. B29: a member of the immunoglobulin gene superfamily exclusively expressed on B-lineage cells. *Proc. Natl. Acad. Sci. USA*. 85:6890–6894.
44. Hombach, J., L. Leclercq, A. Radbruch, K. Rajewsky, and M. Reth. 1988. A novel 34-kd protein co-isolated with the IgM molecule in surface IgM-expressing cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3451–3456.
45. Melchers, F., D. Haasner, U. Grawunder, C. Kalberer, H. Karasuyama, T. Winkler, and A.G. Rolink. 1994. Roles of IgH and L chains and of surrogate H and L chains in the development of cells of the B lymphocyte lineage. *Annu. Rev. Immunol.* 12:209–225.