Counterselection against Dμ Is Mediated through Immunoglobulin (Ig)α-Igβ

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Summary

The pre-B cell receptor is a key checkpoint regulator in developing B cells. Early events that are controlled by the pre-B cell receptor include positive selection for cells express membrane immunoglobulin heavy chains and negative selection against cells expressing truncated immunoglobulins that lack a complete variable region (Dμ). Positive selection is known to be mediated by membrane immunoglobulin heavy chains through Igα-Igβ, whereas the mechanism for counterselection against Dμ has not been determined. We have examined the role of the Igα-Igβ signal transducers in counterselection against Dμ using mice that lack Igβ. We found that Dμ expression is not selected against in developing B cells in Igβ mutant mice. Thus, the molecular mechanism for counterselection against Dμ in pre-B cells resembles positive selection in that it requires interaction between mDμ and Igα-Igβ.

Materials and Methods

Mice. Igβ−/−, mIgμ, and Bcl-2 transgenic strains have been previously described and were maintained by backcrossing with BALB/c mice under specific pathogen-free conditions (19–21). All experiments were performed with 4–8-wk-old female mice.

Fluorescence Analysis and Cell Sorting. Single cell suspensions prepared from bone marrow or spleen were stained with PE-labeled anti-IgM, and analyzed on a FACScan®. For cell sorting, bone marrow cells from four to six mice were stained with the same reagents and separated on a FACSvantage®. CD43+ cells were collected based on gating with RAG-1−/− controls.

DNA and PCR. Total bone marrow DNA was prepared for PCR as previously described (22). DNA from sorted cells was prepared for PCR in agarose plugs (23). Primers for Vμ–DJμ rearrangement were as in reference 22; these primers are mouse specific and do not detect the human Igμ transgene. All experiments were performed a minimum of three times with two independently derived DNA samples. Nonrearranging Ig gene intervening sequences were amplified in parallel with other reactions and used as a loading control (22). Amplified DNA was visualized after transfer to nylon membranes by hybridization with a 6-kb EcoRI fragment that spans the mouse JH region.

Isolation and Sequencing of Vμ–DJμ and Dμ–JH Joints. A JH4 primer was combined with either a Dμ primer or a VHJ558L primer to

1 Abbreviations used in this paper: BCR, B cell receptor; mIgμ, membrane immunoglobulin heavy chain; RF, reading frame.
amplify DJH and VDJH rearrangements, respectively. The primers were: (a) JH4, AC GGATCCGGTGA CTTGAAGCT; (b) DJH, ACAAGCTTCAAGGCACACTGCCTGG; and (c) VHJS8L, GCCAAGCTTAAGGCCAGCATGCTTAC. PCR amplification for DJH joints was for 35 cycles of 0.5 min at 94°C, and 2 min at 72°C; for VDJH joints, it was for 0.5 min at 94°C, 1 min at 68°C, and 1.5 min at 72°C. PCR products were purified by agarose gel electrophoresis, subcloned into plasmid, sequenced using an Applied Biosystems (Foster City, CA) DNA sequencing kit, and analyzed on a genetic analyzer (ABI-310; Applied Biosystems).

**Results**

**mIgM Cannot Induce the Pre-B Cell Transition or Allelic Exclusion in the Absence of Igβ.** Expression of Igβ is required for B cells to efficiently complete Ig VH to DJH gene rearrangements (19). B cells in Igβ−/− mice fail to express normal levels of mIgM, and B cell development is arrested at the CD43+ B220+ pre-B1 stage (19). A similar celltype specific developmental arrest is also found in mice that carry a mutation in the transmembrane domain of mIgM (24), and mice that fail to complete Ig VH to DJH recombination (25–29). In view of the abnormally low levels of mIgM in Igβ−/− mice, failed pre-B cell development might simply be due to lack of Ig expression.

To determine whether mIgM could induce the pre-B cell transition in the absence of Igβ, we introduced a productively rearranged immunoglobulin gene (20) into the Igβ−/− background (TG.mIgβ−/−). We then measured B cell development by staining bone marrow cells with anti-CD43 and anti-B220 monoclonal antibodies (30). We found that expression of a pre-rearranged Ig transgene was not sufficient to activate the pre-B cell transition in the absence of Igβ (Fig. 1). TG.mIgβ−/− B cells did not develop past the CD43+B220+ pre-B cell stage (Fig. 1). In control experiments, the same mIgM transgene did induce the appearance of more mature CD43+B220+ pre-B cells in a RAG−/− mutant background where B cell development was similarly arrested at the CD43+B220+ stage (20, 25, 26; data not shown). We conclude that in the absence of Igβ, a productively rearranged mIgM is unable to activate the pre-B cell transition.

Allelic exclusion is established as early as the CD43+B220+ stage of B cell development (31–33). This early stage of development is found in the bone marrow of Igβ−/− mice (19). However, we were initially unable to measure allelic exclusion in Igβ mutant mice due to the low efficiency of complete Ig VH to DJH gene rearrangements and absence of surface IgM expression (19). To determine whether expression of mIgM could activate allelic exclusion in TG.mIgβ−/− mice, we measured inhibition of VH to DJH gene rearrangements by PCR (34). In controls, the mIgM transgene inhibited VH to DJH gene rearrangement (22), but the same transgene had no effect in the Igβ−/− background (Fig. 2). We had previously shown that the cytoplasmic domains of Igα and Igβ are sufficient to activate allelic exclusion (20, 35). The finding that mIgM is unable to induce allelic exclusion in the absence of Igβ suggests that Igβ is essential for allelic exclusion.
Igβ Is Required for RF2 Counterselection. Igs with D<sub>H</sub> joined to J<sub>H</sub> in RF2 are rarely found in mature B cells (15–17). Genetic experiments in mice have shown that counterselection against RF2 requires the transmembrane domain of mIg<sub>m</sub> and the syk tyrosine kinase (15, 18). To determine whether counterselection is mediated through Igβ, we sequenced DJ<sub>H</sub> joints amplified from sorted CD43<sup>+</sup>B220<sup>+</sup> pre-B cells from Igβ<sup>−/−</sup> mice and controls. In control samples, only 10% of the DJ<sub>H</sub> joints were in RF2 (Fig. 3), which is in agreement with similar measurements performed in other laboratories (15–17, 31–33). In contrast, there was no counterselection in the bone marrow cells of Igβ<sup>−/−</sup> mice; 13 out of 30 DJ<sub>H</sub> joints were in RF2 with the remainder being distributed in RF1 and 3 (Fig. 3). Thus, in the absence of Igβ, there was no RF2 counterselection at the level of DJ<sub>H</sub> rearrangements in CD43<sup>+</sup>B220<sup>+</sup> cells in the bone marrow.

V<sub>H</sub> to DJ<sub>H</sub> joining and counterselection are normally completed in CD43<sup>+</sup>B220<sup>+</sup> pre-B cells (31–33), but in Igβ<sup>−/−</sup> mice, V<sub>H</sub> to DJ<sub>H</sub> joining is inefficient (19). To determine whether RF2 was counterselected in the few Igβ mutant B cells that completed V<sub>H</sub> to DJ<sub>H</sub> rearrangements, we amplified and sequenced VHJ<sub>558L</sub>-DJ<sub>H4</sub> joints from unfractionated bone marrow cells (Fig. 4). As with the DJ<sub>H</sub> joints, we found no evidence for counterselection against RF2 in VDJ<sub>H</sub> joints in Igβ<sup>−/−</sup> mice were in RF2. By contrast, RF2 was only found in 1 of 11 mature Ig's in the controls. The VDJ<sub>H</sub> and DJ<sub>H</sub>Igβ<sup>−/−</sup> joints otherwise resembled the wild type in the number of N and P nucleotides as well as in the extent of nucleotide deletion (Figs. 3 and 4). We conclude that there was no selection against RF2 in the absence of Igβ, and that the absence of Igβ has no significant impact on the mechanics of recombination as measured by the variability of the joints.

Discussion

The transmembrane domain of mIg<sub>m</sub> is required to produce the signals that mediate several antigen-independent events in developing B cells, including allelic exclusion and the pre-B cell transition (24, 36–39). However, mIg<sub>m</sub> itself is insufficient for signal transduction (40), and it requires the Igα and Igβ signaling proteins to activate B cell responses in vitro and in vivo.

The earliest developmental checkpoint regulated by Igα-Igβ appears to involve either activation of cellular competence to complete V<sub>H</sub> to DJ<sub>H</sub> rearrangements, or positive selection for cells that express mIg<sub>m</sub> (19). In the next phase of the B cell pathway, the same transducers are necessary (Fig. 2) and sufficient to produce the signals that activate allelic exclusion and the pre-B cell transition (19, 20, 35, 41).
In the present report, we show that in addition to these functions, Igα-Igβ transducers are also necessary for negative selection against Dµ.

Two models have been proposed to explain counterselection against mDm. The first model states that mDm is toxic, and that cells expressing this protein are deleted by a mechanism that involves inhibition of proliferation (31). A second theory postulates that Dm proteins produce the signal for heavy chain allelic exclusion and block the completion of productive heavy chain gene rearrangements (15). According to this second model, cells expressing mDm are then unable to continue along the B cell pathway. Support for the active signaling model comes from three sets of observations: (a) that there is no counterselection in the absence of a Ig transmembrane exon (15); (b) that there is no RF counterselection in the absence of syk (18); and (c) that there is no counterselection in early CD43^-B220^-B cell precursors in the absence of λ5 (33). These experiments partially define the receptor structure for counterselection as composed of mDm associated with λ5. Our observation that negative selection against Dµ does not occur in the absence of Igβ supports the signaling model, and identifies Igα-Igβ as the transducers that activate counterselection possibly by linking mDm to nonreceptor tyrosine kinases.

Why does the expression of the Dµ pre-BCR lead to arrested development, whereas mature mIg in the same complex activates positive selection in early B cells? Both signals are produced in CD43^-B220^- pre-B cells, both require λ5 (33, 39, 42), and the Igα-Igβ coreceptors (19, 41), and both are transmitted through a cascade that induces syk (18, 43). One way to explain the difference between the cellular response to mDm pre-BCR and mIg pre-BCR expression might be an inability of Dµ to pair with conventional κ or λ Ig light chains (14). According to this model, cells expressing mDm should be trapped in the CD43^-B220^- pre-B cell compartment because B cell development can progress to the CD43^-B220^- stage in the absence of conventional light chains (44, 45). However, elegant single cell sorting experiments have shown that mDm-producing cells are selected against before this stage in CD43^-B220^- pre-B cells (33, 42). Thus, the idea that abnormal pairing of mDm with light chains is responsible for counterselection fails to take into account the observation that counterselection normally occurs independently of light chain gene rearrangements.

Two alternative explanations for the disparate cellular responses to the Dµ pre-BCR and the mIg pre-BCR are: (a) that there are qualitative differences between signals generated by a mDµ protein and a mIgµ receptor complex, and (b) pre-B-I cells that contain Dµ rearrangements are in a different stage of differentiation than pre-B-II cells that have completed VDJH and express mIg (8). An example of two qualitatively distinct signals resulting in alternative biologic responses has been found in the highly homologous TCR receptor (46, 47). TCR interaction with ligand can produce either anergy or activation, depending on the affinity of the TCR for the peptide-MHC complex (48). High affinity ligands that produce T cell responses fully activate CD3 tyrosine phosphorylation, whereas peptides that induce anergy bind with low affinity and induce a reduced level of CD3 phosphorylation. The low level CD3 phosphorylation induced by the anergizing peptides is associated with less than optimal ZAP-70 kinase activation (46, 47).

Less is known about the physiologic responses activated by Igα-Igβ in developing B cells, but experiments in transgenic mice have shown that early B cell development requires tyrosine phosphorylation of Igβ (20), and by...
inference, receptor cross-linking. Although the cytoplasmic domains Igα and Igβ appear to have redundant functions in allelic exclusion and the pre-B cell transition (20, 35), neither Igα, (41) nor Igβ (Papavasiliou, N., and M.C. Nussenzweig, manuscript in preparation) alone are able to fully restore B cell development in the bone marrow, suggesting that there are specific functions for Igα and Igβ, or the Igα-Igβ heterodimer. Biochemical support for the idea that individual coreceptors could have unique biologic functions also comes from transfection experiments in B cell lines (49–51) and from the observation that the cytoplasmic domains of Igα and Igβ bind to different sets of nonreceptor tyrosine kinases (52).

We would like to propose that positive and negative selection in developing B cells, like activation and anergy in T cells, may be mediated by differential phosphorylation of Igα and Igβ in the pre-BCR. Given the requirement for cross-linking in pre-BCR activation, the mechanism that produces the proposed differential phosphorylation of the mDμ and mIgμ pre-BCRs may be a function of their affinities for the cross-linker.

We thank members of the Nussenzweig laboratory for their helpful suggestions and advice.

This work was supported by the Howard Hughes Medical Institute, and by National Institutes of Health grants to Dr. Nussenzweig.

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Received for publication 13 September 1996 and in revised form 15 October 1996.

References

1. Rajewsky, K. 1996. Clonal selection and learning in the antibody system. Nature (Lond.). 381:751–758.
2. Tonegawa, S. 1983. Somatic generation of antibody diversity. Nature (Lond.). 302:575–581.
3. Alt, F.W., G.D. Yancopoulos, K.T. Blackwell, E. Wood, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangements of immunoglobulin heavy chain variable region segments. EMBO (Eur. Mol. Biol. Organ.) J. 3:1209–1219.
4. Pillai, S., and D. Baltimore. 1987. Formation of disulfide linked μ2α2 tetramers by the 18 KD ω-immunoglobulin light chain. Nature (Lond.). 329:172–174.
5. Karasuyama, H., A. Kudo, and F. Melchers. 1990. The proteins encoded by V pre-B and λ pre-B cell specific genes can associate with each other and with μ heavy chain. J. Exp. Med. 172:969–972.
6. Tsubata, T., and M.G. Reth. 1990. The products of the pre-B cell specific genes (κ and V pre-B) and the immunoglobulin μ chain form a complex that is transported to the cell surface. J. Exp. Med. 172:973–976.
7. Reth, M.G., and F.W. Alt. 1984. Novel immunoglobulin heavy chains are produced from DμH gene segment rearrangements in lymphoid cells. Nature (Lond.). 312:418–423.
8. Rolink, A., and F. Melchers. 1991. Molecular and cellular origins of B lymphocyte diversity. Cell. 66:1081–1094.
9. Nomura, J., T. Matsuo, F. Kubola, M. Kimoto, and N. Sakaguchi. 1991. Signal transmission through the B cell specific MB-1 molecule at the pre-B cell stage. Int. Immunol. 3:117–126.
10. Misener, V., G.P. Downey, and J. Jongstra. 1991. The immunoglobulin light chain related protein λ5 is expressed on the surface of pre-B cell lines and can function as a signaling molecule. Int. Immunol. 3:1–8.
11. Takemori, T., J. Mizuguchi, I. Miyazoe, M. Nakashima, K. Shigemoto, H. Kimoto, T. Shirasawa, N. Maruyama, and M. Taniguchi. 1990. Two types of μ chain complexes are expressed during differentiation from pre-B to mature B cells.

EMBO (Eur. Mol. Biol. Organ.) J. 9:2493–2500.
12. Tsubata, T., R. Tsubata, and M.G. Reth. 1992. Cross-linking 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. 4:637–641.
13. Tsutsumi, A., J. Terajima, W. Jung, and J. Ransom. 1992. Surface μ heavy chain expressed on pre-B lymphomas transduces Ca++ signals but fails to cause arrest of pre-B cell lymphomas. Cell. Immunol. 139:44–57.
14. Horne, M.C., P.E. Roth, and A.L. DeFranco. 1996. Assembly of the truncated immunoglobulin heavy chain Dμ into antigen receptor-like complexes in pre-B cells but not in B cells. Immunity. 4:145–158.
15. Gu, H., D. Kitamura, and K. Rajewsky. 1991. B cell development regulated by gene rearrangement: arrest of maturaion by membrane-bound Dμ protein and selection of Dμ1 element reading frames. Cell. 65:47–54.
16. Meek, K. 1990. Analysis of junctional diversity during B lymphocyte development. Science (Wash. DC). 250:820–823.
17. Kaartinen, M., and O. Makela. 1985. Reading of D genes in variable frames as a source of antibody diversity. Immunol. Today. 6:324–330.
18. Cheng, A.M., B. Rowley, W. Pao, A. Hayday, J.B. Bolen, and T. Pawson. 1995. Syk tyrosine kinase required for mouse viability and B cell development. Nature (Lond.). 378:303–306.
19. Gong, S., and M.C. Nussenzweig. 1996. Regulation of an early developmental checkpoint in the B cell pathway by Igβ. Science (Wash. DC). 272:411–414.
20. Papavasiliou, F., Z. Misulovin, H. Suh, and M.C. Nussenzweig. 1995. The role of Igβ in precursor B cell transition and allelic exclusion. Science (Wash. DC). 268:408–411.
21. Vaux, D.L., S. Cory, and J. Adams. 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature (Lond.). 335:440–442.
22. Costa, T.E.F., H. Suh, and M.C. Nussenzweig. 1992. Chro-
mosomal position of rearranging gene segments influences allelic exclusion in transgenic mice. Proc. Natl. Acad. Sci. USA. 89:2205–2208.

23. Petree, H.P., F. Livak, D. Burtrum, and S. Mazel. 1995. T cell receptor gene recombination patterns and mechanisms: cell death, rescue, and T cell production. J. Exp. Med. 182: 121–127.

24. 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.

25. 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.

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. Ehlich, A., S. Schaal, 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. 72:695–704.

28. Nussenzweig, A., C. Chen, V. Soares, M. Sanchez, K. Sokol, M.C. Nussenzweig, and G.C. Li. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. Nature (Lond.). 383:551–555.

29. Zhu, C., M.A. Bogue, D. Lim, P. Hasty, and D.B. Roth. 1996. Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. Cell. 86:379–389.

30. Hardy, R.R., C.E. Carmack, S.A. Shinton, 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. 173:1213–1225.

31. Haasner, D., A. Rolink, and F. Melchers. 1994. Influence of surrogate light chain on D\(_{HJH}\)-reading frame 2 suppression in mouse precursor B cells. Int. Immunol. 6:21–30.

32. Ehlich, A., V. Martin, W. Muller, and K. Rajewsky. 1994. Analysis of the B cell progenitor compartment at the level of single cells. Curr. Biol. 4:573–583.

33. Loffert, D., A. Ehlich, W. Muller, and K. Rajewsky. 1996. Surrogate light chain expression is required to establish immunoglobulin heavy chain allelic exclusion during early B cell development. Immunity. 4:133–144.

34. Schlissel, M.S., L.M. Corcoran, and D. Baltimore. 1991. Virus-transformed pre-B cells show ordered activation but not inactivation of immunoglobulin gene rearrangement and transcription. J. Exp. Med. 173:711–720.

35. Papavasiliou, F., M. Jancovic, H. Suh, and M.C. Nussenzweig. 1995. The cytoplasmic domains of Ig\( \alpha \) and Ig\( \beta \) independently induce the pre-B cell transition and allelic exclusion. J. Exp. Med. 182:1389–1394.

36. Nussenzweig, M.C., E.V. Schmidt, A.C. Shaw, E. Sinn, J. Campos-Torres, B. Mathew-Prevot, P.K. Pattengale, and P. Leder. 1988. A human immunoglobulin gene reduces the incidence of lymphomas in c-Myc-bearing transgenic mice. Nature (Lond.). 336:446–450.

37. Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C. Morse, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin \( \mu \). Science (Wash. DC). 236:816–819.

38. 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 (erratum published 169:2269). J. Exp. Med. 168:1363–1381.

39. 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.

40. Costa, T.E., R.R. Franke, M. Sanchez, Z. Misulovin, and M.C. Nussenzweig. 1992. Functional reconstitution of an immunoglobulin antigen receptor in T cells. J. Exp. Med. 175:1669–1676.

41. Torres, R.M., H. Flaswinkel, M. Reth, and K. Rajewsky. 1996. Aberrant B cell development and immune response in mice with a compromised BCR complex. Science (Wash. DC). 272:1804–1808.

42. Loffert, D., S. Schaal, A. Ehlich, R.R. Hardy, Y.R. Zou, W. Muller, and R. Rajewsky. 1994. Early B-cell development in the mouse: insights from mutations introduced by gene targeting. Immunol. Rev. 137:135–153.

43. Turner, M., J.P. Mee, P.S. Costello, O. Williams, A.A. Price, L.P. Duddy, M.T. Furlong, R.L. Geahen, and V.L. Tybulewicz. 1995. Perinatal lethality and blocked B-cell development in mice lacking the tyrosine kinase Syk. Nature (Lond.). 378:298–302.

44. Spanopoulou, E., C.A.J. Roman, L.M. Corcoran, M.S. Schlissel, D.P. Silver, D. Nemeaze, 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.

45. Young, F., B. Arndman, 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.

46. Sloan-Lancaster, J., A.S. Shaw, J.B. Rothbard, and P.M. Allen. 1994. Partial T cell signaling: altered phospho-zeta and lack of zap-70 recruitment in API induced T cell energy. Cell. 79:913–922.

47. Madrenas, J., R.L. Wange, J.L. Wang, N. Isakov, L.E. Samelson, and R.N. Germain. 1995. Zeta phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. Science (Wash. DC). 267:515–518.

48. Lyons, D.S., S.A. Lieberman, J. Hampl, J.B. Bolen, and M. Nussenzweig. 1993. The B cell antigen receptor complex: association of Ig-alpha and Ig-beta with distinct cytoplasmic effectors. J. Exp. Med. 171:1213–1220.

49. Allen. 1994. Partial T cell signaling: altered phospho-zeta and lack of zap-70 recruitment in API induced T cell energy. Cell. 79:913–922.

50. 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.