Expression Levels of B Cell Surface Immunoglobulin Regulate Efficiency of Allelic Exclusion and Size of Autoreactive B-1 Cell Compartment

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Summary

Surface-expressed immunoglobulin (Ig) has been shown to have a critical role in allelic exclusion of Ig heavy (H) and light (L) chains. Although various degrees of suppression of endogenous Ig expression are observed in Ig transgenic (Tg) mice, it was not clear whether this difference is due to different onsets of Tg expression or to different levels of Tg expression, which are obviously affected by integration sites of the transgene. In this study we generated anti-erythrocyte antibody Tg mice that carry tandem joined H and L chain transgenes (H\(^1\)L) and confirmed that homozygosity of the transgene loci enhances the level of transgene expression as compared with heterozygosity. Suppression of endogenous H and L chain gene expression was stronger in homozygous than in heterozygous Tg mice. Similar results were obtained in control Tg mice carrying the H chain only. These results suggest that there is a threshold of the B cell receptor expression level that induces allelic exclusion. In addition, despite the same B cell receptor specificity, the size of Tg autoreactive B-1 cell compartment in the peritoneal cavity is larger in homozygous than in heterozygous mice, although the number of the Tg B-2 cell subset decreased in the spleen and bone marrow of homozygous Tg mice as compared with heterozygous Tg mice. By contrast, homozygosity of the H chain alone Tg line, which does not recognize self-antigens, did not increase the size of the peritoneal B-1 subset. These results suggest that the size of the B-1 cell subset in the Tg mice may depend on strength of signals through B cell receptors triggered by self-antigens.

Key words: transgenic lines • homozygosity • flow cytometry • anti-RBC antibody

Productive V\(_{\mu}D_{\mu}J_{\mu}\) recombination in the Ig H chain locus of one allele of the B cell results in suppression of V\(_{\mu}D_{\mu}J_{\mu}\) recombination in the other allele. Thus, a B cell expresses generally only one H chain gene out of two alleles (1). This phenomenon is called H chain allelic exclusion. Surface expression of the \(\mu\) chain has a critical role in allelic exclusion of the H chain locus because targeted disruption of the \(\mu\) chain membrane exon results in loss of H chain allelic exclusion (2). The essential role of the cell surface \(\mu\) chain expression in allelic exclusion is also supported by studies using Ig transgenic (Tg)\(^3\) mice (3). Mice expressing the membrane-form \(\mu\) chain transgene inhibit expression of the endogenous \(\mu\) chain, whereas Tg mice with the secreted form \(\mu\) chain do not show such inhibition (4–6). In addition, expression of Ig L chain transgenes also exerts suppression on the rearrangement and expression of the endogenous L chain Ig locus (6, 7). However, the suppression of endogenous H and L chain gene expression in Ig Tg mice are generally not complete, and efficiencies of the suppression are variable among different lines of Tg mice (8–17). Variable integration sites of transgenes may influence the onset and level of transgene expression, which may affect the efficiency of allelic exclusion. Since the V(D)J recombination event in each B cell is all or none, it has not been clear whether allelic exclusion can be induced by a small number of surface \(\mu\) chain or if it requires a relatively higher number of surface \(\mu\) chain (a threshold). To examine these possibilities, quantitative comparison between the levels of transgene expression and suppression of endogenous H and L chain gene expression should be carried out using Tg lines with the same integration site to avoid the difference due to the developmental onset of transgene expression.

Abbreviations used in this paper: BCR, B cell receptor; H + L mice, tandem joined H and L chain transgenic mice; H × L mice, double transgenic mice with H and L chain transgenes; Id, idiotype; MFI, mean fluorescence intensity; SA, streptavidin; Tg, transgenic.
We have generated Tg mice that produce an autoantibody (4C8) to RBCs, and have analyzed the mechanisms of B cell tolerance and B-1 cell activation (18-24). Since autoreactive B cells are eliminated at the immature stage in the bone marrow, the number of self-reactive B cells is markedly decreased in the bone marrow, peripheral blood, spleen, and lymph nodes in the Tg mice. In contrast, the peritoneal cavities of the Tg mice contain a normal number of autoreactive B-1 cells that can be eliminated by interaction with RBCs (18, 19). B-1 cells show distinct surface antigen expression and anatomical localization from conventional B (B-2) cells (25-28). Furthermore, the V\textsubscript{H} gene usage, N region diversity, and antigen specificity of B-1 cells are also unique (29-34). B-1 cells are thought to be generated from fetal liver cells, and two independent studies have demonstrated that adult bone marrow cells do not give rise to CD5\textsuperscript{+} B-1 cells after transfer to irradiated hosts (35, 36). In contrast, Wortis and colleagues (37-39) have shown that adult bone marrow contains precursors for CD5\textsuperscript{+} cells and that CD5 expression in splenic B cells is induced by surface IgM cross-linking, suggesting that B-1 cells are generated from common precursors to conventional B cells. Arnold, Clarke, and colleagues (40, 41) have reported that B-1 cells differentiate from B-0 cells after expression of specific antigen receptors. Thus, it is not yet clear whether B-1 cells constitute a B cell lineage distinct from conventional B cells. In addition, even if some B-1 cells are derived from activation of B-2 cells, it is not clear whether a certain threshold level of B cell receptor (BCR) expression is required for B-1 cell differentiation or if the receptor specificity alone plays a major role.

To answer these questions we generated new lines of anti-RBC antibody Tg mice that carried tandem joined H and L chain transgenes, and we compared the levels of allelic exclusion and the size of B-1 cell compartment between homozygous and heterozygous mice. Since expression of specific antigen receptors. Thus, it is not yet clear whether B-1 cells constitute a B cell lineage distinct from conventional B cells (25-28). Furthermore, the V\textsubscript{H} gene expression is required for B-1 cell differentiation or if the receptor specificity alone plays a major role.

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We compared the fluorescence intensity of Ig\(_{1}\) bone marrow cells in homozygous mice to that in heterozygous mice (Fig. 1 C). The mean fluorescence intensities (MFI) of Ig\(_{1}\) cells in heterozygous and homozygous H + L5 mice are 481.70 and 717.42, respectively. Similarly, the MFI of Ig\(_{1}\) cells in heterozygous and homozygous H + L6 mice are 997.76 and 1670.11, respectively. These results indicate that in both H + L5 and H + L6 lines the levels of transgene expression are clearly higher in homozygous than in heterozygous mice. We also examined surface Ig expression on the bone marrow cells of H3 Tg mice that had only H chain transgene. The fluorescence intensity of antiallotypic mAb for IgM\(_{a}\) (Tg) of bone marrow cells was also stronger in homozygous (MFI = 908.77) than in heterozygous mice, which can clearly distinguish IgM of the control mice; Fig. 2, A and B) for IgM\(_{a}\) (Tg) and IgM\(_{b}\) (endogenous). In all heterozygous Tg mice, the numbers of IgM\(_{a}\)IgM\(_{b}\) - cells (allelic inclusion) were small but significant, whereas these cells almost completely disappeared in all homozygous Tg mice. This conclusion is further confirmed by the finding that both bone marrow and spleen B cells with only endogenous H chains (IgM\(_{a}\)IgM\(_{b}\)) decreased in homozygous Tg mice as compared with heterozygous Tg mice (Fig. 2, A and B). In all lines of Tg mice, total numbers of endogenous H chain-
IgM<sup>b</sup> (C57BL/6) spleen cells are shown. The percentages of cells of each gated region in total viable cells are indicated. Total numbers of the cells with endogenous H chains in heterozygous or homozygous H<sup>+</sup>L, H<sup>+</sup>L<sup>6</sup>, and H<sup>3</sup> Tg mice are shown (C). Spleen cells were stained and analyzed from at least three mice of each Tg line. Numbers of indicated cells were calculated by (percentage of indicated cells in viable cells) × (N. o. of viable cells). Results are expressed as mean ± SD.

|                  | Hetero | Homo |
|------------------|--------|------|
| **H<sup>+</sup>L** | 0.77   | 0.02 |
| **H<sup>+</sup>L<sup>6</sup>** | 3.4   | 0.05 |
| **H<sup>3</sup>** | 0.06   | 0.06 |

**Figure 2.** Tg H chain expression in B cells of heterozygous or homozygous H<sup>+</sup>L and H<sup>3</sup> Tg mice. Cells were isolated from bone marrow (A) and spleen (B) of indicated mice at 10–14 wk of age and stained with FITC-conjugated antiallotypic antibody for IgM<sup>b</sup> (Tg) and PE-conjugated antiallotypic antibody for IgM<sup>a</sup> (endogenous). Control stainings of IgM<sup>a</sup> (BALB/c) and IgM<sup>b</sup> (C57BL/6) spleen cells are shown. The percentages of cells of each gated region in total viable cells are indicated. Total numbers of the cells with endogenous H chains in heterozygous or homozygous H<sup>+</sup>L, H<sup>+</sup>L<sup>6</sup>, and H<sup>3</sup> Tg mice are shown (C). Spleen cells were stained and analyzed from at least three mice of each Tg line. Numbers of indicated cells were calculated by (percentage of indicated cells in viable cells) × (N. o. of viable cells). Results are expressed as mean ± SD.

>14% λ<sup>+</sup> cells (Fig. 3 A). The total number of cells expressing endogenous L chains (IgM<sup>a</sup>L<sup>Id<sup>−</sup></sup>) in bone marrow (Fig. 3, B and C) and spleen (Fig. 3, D and E) was less in homozygous than in heterozygous Tg lines, indicating that homozygosity of the Tg loci enhances the inhibition of endogenous L chain expression as compared with heterozygosity.

The size of an autoreactive B1 cell compartment is larger in homozygous mice than in heterozygous H<sup>+</sup>L mice. H<sup>+</sup>L mice show almost complete deletion of autoreactive B cells from bone marrow and spleen, whereas a normal level of B-1 cells is found in the peritoneal cavity (18). Bone marrow of both lines of H<sup>+</sup>L mice contained some autoreactive B cells (IgM<sup>a</sup>L<sup>Id<sup>−</sup></sup>), which have B-2 cell phenotypes (CD5<sup>–</sup>B220<sup>hi</sup>IgM<sup>low</sup>), whereas autoreactive B cells in spleen almost completely disappeared in H<sup>+</sup>L mice (Fig. 3, B and D). We examined whether homozygosity of the Ig transgene loci influences the size of the autoreactive B-1 cell compartment in the peritoneal cavities of H<sup>+</sup>L and H<sup>+</sup>L<sup>6</sup> mice. Peritoneal cells of H<sup>+</sup>L mice had a limited number of B220<sup>+</sup>L<sup>−</sup> cells, almost all of which were IgM<sup>a</sup> (Fig. 4, A–C and E–G). Almost all L<sup>+</sup> cells in the peritoneal cavity were Mac1<sup>+</sup> and therefore belong to the B-1 subset (Fig. 4, D and H). Thus, IgM<sup>a</sup>L<sup>Id<sup>−</sup></sup> cells are most likely to be autoreactive B-1 cells (Fig. 4, C and G). These B220<sup>+</sup>L<sup>−</sup> cells in the peritoneal cavity were much more abundant in homozygous H<sup>+</sup>L mice than in heterozygous H<sup>+</sup>L mice, indicating that the size of the autoreactive B-1 cell subset is larger in homozygous than in heterozygous H<sup>+</sup>L mice. The relative size of the B-1 cell compartment is directly proportional to the B-1 cell number because total cell numbers in the peritoneal cavity did not vary between homozygous and heterozygous Tg mice. These results suggest that a higher expression level of the autoantibody facilitates the increase in autoreactive B-1 cells in the peritoneal cavity.

To test whether autoreactivity of Ig expressed on B cells
is crucial to enlargement of the B-1 cell subset, we carried out similar studies on the peritoneal B cells in H3 Tg mice, the vast majority of which are not autoreactive due to the absence of the Tg L chain. The majority of peritoneal B cells expressed the Tg H chain (IgM$\alpha$), in agreement with analysis of bone marrow and spleen cells (Fig. 4, I and J). Indeed, B cells expressing endogenous H chains were less in homozygous than in heterozygous H3 mice (Fig. 4 J), probably because of more efficient allelic exclusion due to stronger expression of the transgene product on homozygous B cells (Fig. 1 C). Nonetheless, the percentage of IgM$\alpha$Mac1$\alpha$ cells in the peritoneal cavity did not increase in homozygous H3 mice as compared with those in heterozygous H3 mice (Fig. 4 K). These results indicate that increased surface Ig expression of non-auto-reactive B cells did not facilitate enlargement of the B-1 cell subset in the peritoneal cavity. Taken together, enhancement of autoreactive surface Ig expression appears to be crucial to increase the B-1 cell subset in the peritoneal cavity.

**Discussion**

In this study, we generated new lines of the anti-RBC antibody Tg mice that carry tandem joined H and L chain transgenes, and compared B cell phenotypes between heterozygous and homozygous Tg mice to evaluate quantitatively the effect of surface Ig level on allelic exclusion and B-1 cell development. Higher levels of surface Tg Ig expression resulted in (a) stronger clonal deletion of B-2 cells from bone marrow and spleen; (b) reduction of B cells expressing endogenous H or L chains; and (c) enlargement of the autoreactive B-1 cell subset in the peritoneal cavity.

Several lines of evidence suggest that surface expression of the $\mu$ chain is critical for H chain allelic exclusion (2, 4–6). In addition, pre-BCR, which is the H chain paired with the surrogate L chain, is suggested to mediate H chain allelic exclusion through downregulation of recombination-activating genes (42–46). Ig$\alpha$ and Ig$\beta$, which associate with surface-expressed H chains, have also been shown to trigger the signals inducing allelic exclusion (47–49). Taken together, expression of the H chain as a pre-BCR on the cell surface may induce signals mediated by Ig$\alpha$ and Ig$\beta$, resulting in H chain allelic exclusion. In this study we have demonstrated that homozygosity of the transgene Ig loci increases surface Ig expression on bone marrow B cells and causes stronger allelic exclusion, as compared with heterozygosity. Since allelic exclusion is all or none in each B cell, the present results suggest that there is a threshold of the pre-BCR signal intensity that induces allelic exclusion.

There are several other possibilities to explain our results. First, B cells with allelic inclusion may be negatively selected. However, Sonoda and Rajewsky (50) have shown that B cells with allelic inclusion can normally expand in the periphery using double Ig knock-in mice, indicating...
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the absence of negative selection against allelic inclusion B cells. In addition, we have shown that endogenous only B cells (IgMa\(^2\)IgMb\(^1\)) are also reduced in homozygotes (Fig. 2). Second, reduction of allelic inclusion B cells could be due to selective expansion of higher surface Ig-expressing cells by a self-antigen. However, the self-antigen (RBC) can kill self-reactive B cells (19). In fact, Id\(^1\)B cells of H\(^1\)L mice decreased in homozygotes as compared with heterozygotes in spleen as well as in bone marrow (Fig. 3, B and D). In addition, we have shown that non–self-reactive (H chain alone expressing) Tg (H3) B cells also show suppression of endogenous Ig expression (Fig. 2). These results cannot be explained by positive selection of self-reactive Ig expressing B cells by the self-antigen. Third, excess Tg Ig expression on surface may inhibit detection of endogenous Ig expression. It is unlikely that endogenous and Tg Ig molecules compete for surface expression in H + L5 and H + L6 spleen cells. This is because the surface Ig levels of homozygous H3, H + L6, and H + L5 cells are 909, 251, and 173 (MFI using the anti-IgMa Ab), respectively (Fig. 1 D), indicating that H + L5 and H + L6 B cells have not hit the ceiling of the surface Ig expression level. Sonoda and Rajewsky (50) have shown that there is no inhibitory mechanism for the H chain on the surface as long as it can associate with the L chain. It is inconceivable that the surface expression efficiency differs between H chains derived from the transgene and endogenous gene. The possibility that a lower detection efficiency by anti-IgM\(^b\) Ab staining in the presence of large amounts of IgM\(^a\) is also unlikely because the FACSCalibur profiles of H3 spleen cells expressing large amounts of IgM\(^a\) clearly show the presence of IgM\(^a\) IgM\(^b\) double-positive cells even in homozygotes (Fig. 2 B). Finally, the suppression efficiency of the endogenous locus may be increased in homozygotes simply because the frequency of silencing the transgene locus is reduced by doubling the number of the transgene locus in homozygotes. Assuming that this is the case, the efficiency of silencing of the transgene locus can be calculated from the endogenous H chain only cells shown in Fig. 2 A: H3, 0.06 / (0.06 + 0.11 + 6.50) = 0.009 in heterozygotes and 0.00144 / (0.01 + 0.03 + 6.9) in homozygotes. The value in homozygotes is \( \approx 18 \) times of the expected value (0.009 \( \times \) 0.009 = 0.000081) based on the simple statistics. Similar discrepancy was seen in the H + L5, H + L6, and H3 spleen cells (Fig. 2 B). In addition, homozygosity alters the number of Tg Id\(^1\)B cells in spleen and peritoneal cavity to the opposite direction. These considerations make the final possibility unlikely.

We have shown that H + L Tg mice have B cells expressing endogenous L chains together with the Tg H chain. These cells, expressing endogenous L chains in H + L mice, may be generated either by incomplete allelic exclusion or

Figure 4. Flow cytometric analysis of peritoneal cells of heterozygous or homozygous H + L5, H + L6, H × L, and H3 Tg mice. Cells were isolated from peritoneal cavity of mice at 10–14 wk of age and stained with FITC-anti mouse IgM and Cy-Chrome\(^b\)-conjugated anti–mouse B220 (A, E and I); biotin-conjugated anti-Id antibody followed by FITC-conjugated SA and Cy-Chrome\(^b\)-conjugated anti-B220 antibody (B and F); biotin-conjugated anti-Id antibody followed by FITC-conjugated SA and PE-conjugated antiallotypic antibody for IgMb (C and G); biotin-conjugated anti-Id antibody followed by FITC-conjugated antiallotypic antibody for IgMa (endogenous) and PE-conjugated antiallotypic antibody for IgMa (Tg) (J); and FITC-conjugated goat anti–mouse IgM antibody and PE-conjugated anti–mouse H chain (K). The percentages of the cells of each gated region in total viable cells are indicated. Total peritoneal cell numbers were constant between heterozygous and homozygous Tg mice.

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by receptor editing because the B cells which express the transgenes are self-reactive (51, 52). Nemazee and colleagues (53–55) have suggested that interaction with autoantigens leads IgM low IgD low B cells to undergo receptor editing but IgM high IgD high cells to undergo rapid apoptosis. On the other hand, Rusconi et al. (16) generated B cell hybridomas from anti-trinitrophenol antibody (anti-non-self) Tg mice that carried tandem joined H and L chain transgenomes and demonstrated that the B cells hybridomas secreting the Tg antibody expressed the Tg L chain at about one-tenth of the level of coexpressed endogenous L chains. Endogenous L chain expression in their Tg mice is probably due to incomplete allelic exclusion because these B cells are unlikely to receive BCR stimulation by self-antigens to trigger receptor editing. Although we cannot exclude the possibility that receptor editing is involved in the appearance of B cells with endogenous L chains in our Tg mice, we think it less likely because of the following reason. If the expression of endogenous L chain is due to receptor editing induced by stimulation with the self-antigen, the mechanism to reduce the number of B cells with endogenous L chains by enhanced expression of self-reactive Ig should be clonal deletion. However, when B cells expressed more self-reactive Ig on surface, B cells with both Tg and endogenous L chains (IgM and IgD low) are more efficiently reduced than Tg only B cells (IgM and IgD high), which are most likely eliminated by clonal deletion (Fig. 3). This observation is somewhat opposed to the expected efficiency of clonal deletion by the self-antigen because stronger BCR signaling will be induced in IgM high IgD low cells than IgM high IgD high cells.

Although it is still controversial whether B-1 cells belong to an ontogenetically different B cell lineage from conventional B cells (34–40), B-1 and B-2 cells clearly constitute different subsets of B cells. In this study we have shown that the size of the Tg B-1 cell compartment is larger in homozygous than in heterozygous H + L mice (Fig. 4). Since Tg B-1 cells in heterozygous and homozygous mice show the same antigen specificity, our results suggest that the level of surface Ig expression directly influences the size of the Tg B-1 cell compartment. Our findings are consistent with the previous observations that defects of BCR signaling cause reduction of the B1 cell (56, 57), and loss of a BCR inhibitory molecule, SHP-1, increases the B1 cell number (58–62). It is important to note that increased levels of autoreactive BCR induced augmented clonal deletion of B-2 cells in bone marrow and spleen but expansion of B-1 cells in the peritoneal cavity (Fig. 4). Increased expression of nonautoreactive Ig (H3) enhanced neither clonal deletion of B-2 cells nor expansion of B-1 cells. These results suggest that there are at least three levels of BCR signaling that regulate self-reactive B1 and B2 cell differentiation. At a lower level, self-reactive B-2 cells can be stimulated to induce receptor editing or to become anergic (53–55, 63). At an intermediate signaling level, B-2 cells are clonally deleted and B-0 (40, 41) and/or B-2 cells are induced to differentiate into B-1 cells, which migrate into the peritoneal cavity. At a strong signaling level, B-1 cells are also clonally deleted (19). It is tempting to speculate that at least a sizable fraction of peritoneal B-1 cells originate and expand from autoreactive B cells that are stimulated by self-antigens to a level strong enough to be activated but weak enough to avoid apoptosis.

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References

1. Rajewsky, K. 1996. Clonal selection and learning in the antibody system. Nature. 381:751–758.

2. Kitamura, D., and K. Rajewsky. 1992. Targetted disruption of μ chain membrane exon causes loss of heavy-chain allelic exclusion. Nature. 356:154–156.

3. Storb, U. 1995. Ig gene expression and regulation in Ig transgenic mice. In Immunoglobulin Genes. T. Honjo and W. F. Alt, editors. Academic Press, London. 345–363.

4. 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 μ. Science. 236:816–819.

5. Nussenzweig, M. C., A. C. Shaw, E. Sinn, J. Campos-Torres and P. Leder. 1988. Allelic exclusion in transgenic mice carrying mutant human IgM genes. J. Exp. Med. 167:1969–1974.

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6. Manz, J., K. Denis, O. Witte, R. Brinster, and U. Storb. 1988. Feedback inhibition of immunoglobulin gene rearrangement by membrane µ, but not by secreted µ, heavy chains. J. Exp. Med. 168:1363–1381.

7. Ritchie, K.A., R.L. Brinster, and U. Storb. 1984. Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in κ transgenic mice. Nature. 312:517–520.

8. Stall, A.M., F.G.M. Kroese, F.T. Gadus, D.G. Sieckmann, L.A. Herzenberg, and L.A. Herzenberg. 1988. Rearrangement and expression of endogenous immunoglobulin genes occur in many murine B cells expressing transgenic membrane IgM. Proc. Natl. Acad. Sci. USA. 85:3546–3550.

9. Müller, W., U. Rüther, P. Vieira, J. Hombach, M. Reth, and K. Rajewsky. 1989. Membrane-bound IgM obstructs B cell development in transgenic mice. Eur. J. Immunol. 19:923–928.

10. Kenny, J.J., F. Finkelstein, M. Acchiarini, 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 M 167 µ, plus κ transgenic mice. J. Immunol. 142:4465–4474.

11. Rath, S., J. Durdik, R.M. Gerstein, E. Selsing, and A. Nisonoff. 1989. Quantitative analysis of idiotypic mimicry and allelic exclusion in mice with a µ transgenic. J. Immunol. 143:2074–2080.

12. Forni, L. 1990. Extensive splenic B cell activation in IgM− transgenic mice. J. Immunol. 145:2364–2372.

13. Grandien, A., A. Coutinho, and J. Andersson. 1990. Selection and peripheral expansion and activation of B cells expressing endogenous immunoglobulin in µ transgenic mice. Eur. J. Immunol. 20:991–998.

14. Iacomini, J., N. Yannoutsos, S. Bandyopadhay, and T. Imanishi-Kari. 1991. Endogenous immunoglobulin expression in µ transgenic mice. Int. Immunol. 3:185–196.

15. Lam, K.P., L.A. Herzenberg, and A.M. Stall. 1993. A high frequency of hybridomas from M 54 µ heavy chain transgenic mice initially co-express transgenic and rearranged endogenous µ genes. Int. Immunol. 5:1011–1022.

16. Rusconi, S., and G. Köhler. 1985. Transmission and expression of a specific pair of rearranged immunoglobulin µ and κ genes in a transgenic mouse line. Nature. 314:330–334.

17. Carmack, C.E., S.A. Camper, J.J. Mackle, W.U. Gerhard, and M.G. Wiegert. 1991. Influence of a Vκ8.8 chain transgene on endogenous rearrangements and the immune response to the HA(Sb) determinant on influenza virus. J. Immunol. 147:2024–2033.

18. Okamoto, M., M. Urakami, A. Shimizu, S. Ozaki, T. Tsun, S. Kumagai, and T. Honjo. 1992. A transgenic model of autoimmune hemolytic anemia. J. Exp. Med. 175:71–79.

19. Urakami, M., T. Tsun, S. Okamoto, A. Shimizu, S. Kumagai, H. Imura, and T. Honjo. 1992. Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. Nature. 357:77–80.

20. Nisitani, S., T. Tsun, M. Urakami, M. Okamoto, and T. Honjo. 1993. The bcl-2 gene product inhibits clonal deletion of self-reactive B lymphocytes in the periphery but not in the bone marrow. J. Exp. Med. 178:1247–1254.

21. Urakami, M., T. Tsun, R. Shinkura, S. Nisitani, M. Okamoto, H. Yoshioka, T. Usubi, S. Miyawaki, and T. Honjo. 1994. Oral administration of lipopolysaccharides activates B-1 cells in the peritoneal cavity and lamina propria of the gut and induces autoimmune symptoms in an autoanti-body transgenic mouse. J. Exp. Med. 180:111–121.

22. Nisitani, S., T. Tsun, M. Urakami, and T. Honjo. 1995. Administration of interleukin-5 or -10 activates peritoneal B-1 cells and induces autoimmune hemolytic anemia in anti-erythrocyte autoantibody-transgenic mice. Eur. J. Immunol. 25:3047–3052.

23. Murakami, M., K. Nakajima, K. Yamazaki, T. Uruguchi, T. Serikawa, and T. Honjo. 1997. Effects of breeding environments on generation and activation of autoreactive B-1 cells in anti-red blood cell autoantibody transgenic mice. J. Exp. Med. 185:791–794.

24. Nisitani, S., T. Sakiyama, and T. Honjo. 1998. Involvement of IL-10 in induction of autoimmune hemolytic anemia in anti-erythrocyte Ig transgenic mice. Int. Immunol. 10:1039–1047.

25. Hayakawa, K., R.R. Hardy, D.R. Parks, and L.A. Herzenberg. 1983. The “Ly-1 B” cell subpopulation in normal immunodeficient, and autoimmune mice. J. Exp. Med. 157:202–218.

26. Walschmidt, T.J., F.G. Kroese, L.T. Tygrett, D.H. Conrad, and R.G. Lynch. 1991. The expression of B cell surface receptors III. The murine low-affinity IgE Fc receptor is not expressed on Ly 1 or “Ly 1-like” B cells. Int. Immunol. 3:305–315.

27. Hayakawa, K., R.R. Hardy, A.M. Stall, L.A. Herzenberg, and L.A. Herzenberg. 1986. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. Eur. J. Immunol. 16:1313–1316.

28. Marcos, M.A., F. Huetz, P. Pereira, J.L. Andreu, A.C. Martinez, and A. Coutinho. 1989. Further evidence for coelomic-associated B lymphocytes. Eur. J. Immunol. 19:2031–2035.

29. Hayakawa, K., R.R. Hardy, M. Honda, L.A. Herzenberg, A.D. Steinberg, and L.A. Herzenberg. 1984. Ly-1 B cells functionally distinct lymphocytes that secrete IgM autoantibodies. Proc. Natl. Acad. Sci. USA. 81:2494–2498.

30. Lator, P.A., and G. Morahan. 1990. The peritoneal Ly-1 (CD5) B cell repertoire is unique among murine B cell repertoires. Eur. J. Immunol. 20:485–492.

31. Mercolino, T.J., L.W. Arnold, and G. Haughton. 1986. Phosphatidylcholine is recognized by a series of Ly-1+ murine B cell lymphomas specific for erythrocyte membranes. J. Exp. Med. 163:155–165.

32. Su, S.D., M.M. Ward, M.A. Apicella, and R.E. Wood. 1991. The primary B cell response to the O/ core region of bacterial lipopolysaccharide is restricted to the Ly-1 lineage. J. Immunol. 146:327–331.

33. Forster, I., and K. Rajewsky. 1987. Expansion and functional activity of Ly-1+ B cells upon transfer of peritoneal cells into allotype-congenic, newborn mice. Eur. J. Immunol. 17:521–528.

34. Tornberg, U.C., and D. Holnberg. 1995. B-1a, B-1b and B-2 B cells display unique V HDJH repertoires formed at different stages of ontogeny and under different selection pressures. EMBO J. (Eur. Mol. Biol. Org an.). 14:1680–1689.

35. Hayakawa, K., R.R. Hardy, L.A. Herzenberg, and L.A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J. Exp. Med. 161:1554–1568.

36. Kantor, A.B., A.M. Stall, S. Adams, and L.A. Herzenberg. 1992. Differential development of progenitor activity for three B-cell lineages. Proc. Natl. Acad. Sci. USA. 89:3320–3324.

37. Cong, Y.Z., E. Rabin, and H.H. Wortis. 1991. Treatment of...
murine CD5- B cells with anti-Ig, but not LPS, induces surface CD5: two B-cell activation pathways. Int. Immunol. 3:467–476.

38. Hwang, C.A., C. Henry, J. Iacomini, T. Imanshi-Kari, and H.H. Wortis. 1996. Adult bone marrow contains precursors for CD5+ B cells Eur. J. Immunol. 26:2537–2540.

39. Berland, R., and H.H. Wortis. 1998. An NFAT-dependent enhancer is necessary for anti-IgM-mediated induction of murine CD5 expression in primary splenic B cells. J. Immunol. 161:277–285.

40. Arnold, L.W., C.A. Pennell, S.K. Mccray, and S.H. Clarke. 1998. B-1 cell development: evidence for an uncommitted immunoglobulin (Ig)M+ B cell precursor in B-1 cell differentiation. J. Exp. Med. 187:1325–1334.

41. Clarke, S.H., and L.W. Arnold. 1998. B-1 cell development: evidence for an uncommitted immunoglobulin (Ig)M+ B cell precursor in B-1 cell differentiation. J. Exp. Med. 187:1325–1334.

42. Kitamura, D., A. Kudo, S. Schaal, W. Müller, F. Melchers, and K. Rajewsky. 1992. A critical role of murine CD5 expression in primary splenic B cells. J. Immunol. 149:467–476.

43. Grawunder, U., T.M. Leu, D.G. Schatz, A. Werner, A.G. Löffert, D., A. Ehlich, W. Müller, and K. Rajewsky. 1996. Surrogate light chain expression is required to establish immunoglobulin heavy chain allelic exclusion during early B cell development. Immunity. 3:601–608.

44. Shaffer, A.L., and M.S. Schlissel. 1997. A truncated heavy chain protein relieves the requirement for surrogate light chains in early B cell development. J. Immunol. 159:1265–1275.

45. ten Boekel, E., F. Melmers, and A.G. Rolink. 1998. Precursor B cells showing H chain allelic inclusion display allelic exclusion at the level of pre-B cell receptor surface expression. Immunity. 8:199–207.

46. Papavasiouliou, F., Z. Misulovin, H. Suh, and M.C. Nussenweig. 1995. The role of Igβ in precursor B cell transition and allelic exclusion. Science. 268:408–411.

47. Papavasiouliou, F., M. Jankovic, H., M.C. Nussenweig. 1995. The cytoplasmic domains of immunoglobulin (Ig)α and Igβ can independently induce the precursor B cell transition and allelic exclusion. J. Exp. Med. 182:1389–1394.

48. Téh, Y.M., and M.S. Nuberger. 1997. The immunoglobulin (Ig)α and Igβ cytoplasmic domains are independently sufficient to signal B cell maturation and activation in transgenic mice. J. Exp. Med. 185:1753–1758.

49. Teh, Y.M., and M.S. Nuberger. 1997. The immunoglobulin (Ig)α and Igβ cytoplasmic domains are independently sufficient to signal B cell maturation and activation in transgenic mice. J. Exp. Med. 185:1753–1758.

50. Sonoda, E., Y. Pewzner-Jung, S. Schwers, S. Taki, S. Jung, D. Ellat, and K. Rajewsky. 1997. B cell development under the condition of allelic inclusion. Immunity. 6:225–233.

51. 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.

52. Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp. Med. 177:999–1008.

53. M. elamed, D., and D. Nemazee. 1997. Self-antigen does not accelerate immature B cell apoptosis, but stimulates receptor editing as a consequence of developmental arrest. Proc. Natl. Acad. Sci. USA. 94:9267–9272.

54. Hertz, M., and D. Nemazee. 1997. BCR ligation induces receptor editing in IgM-IgD+ bone marrow B cells in vitro. Immunity. 6:429–436.

55. M. elamed, D., R. Benschop, J.C. Cambier, and D. Nemazee. 1998. Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection. C. el. 92:173–182.

56. Zhang, R., F.W. Alt, L. Davidson, S.H. Orkin, and W. Swat. 1995. Defective signaling through the T- and B-cell antigen receptors in lymphoid cells lacking the vav proto-oncogene. Nature. 374:470–473.

57. Leitges, M., C. Schmedt, R. Guinamard, J. Davoust, S. Schaal, S. Stabel, and A. Tarakhovsky. 1996. Immunodeficiency in protein kinase Cβ-deficient mice. Science. 273:788–791.

58. Sidman, C.L., L.D. Shultz, R.R. Hardy, K. Hayakawa, and L.A. Herzenberg. 1986. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 232:1423–1425.

59. Shultz, L.D., P.A. Schweitzler, T.V. Ryan, T. Yi, J.N. Ihle, R.J. Matthews, M.L. Thomas, and D.R. Beler. 1993. Mutation at the murine motheaten locus are within the hematopoietic cell phosphatase gene. Cell. 73:1445–1454.

60. Tsi, H.W., K.A. Siminovitch, L. de Souza, and F.W.L. Tsiu. 1993. Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase (Hcph) gene. C. el. 73:1445–1454.

61. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Smith-Gill. 1995. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 232:1423–1425.

62. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Smith-Gill. 1995. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 232:1423–1425.

63. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Smith-Gill. 1995. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 232:1423–1425.

64. Pani, G., M. Kozlowski, J.C. Cambier, G.B. Mills, and K.A. Smith-Gill. 1995. Production of immunoglobulin isotypes by Ly-1+ B cells in viable motheaten and normal mice. Science. 232:1423–1425.