B Cell Antigen Receptor Specificity and Surface Density Together Determine B-1 versus B-2 Cell Development

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Summary

Mice expressing the immunoglobulin (Ig) heavy (H) chain variable (V) region from a rearranged V_H12 gene inserted into the IgH locus generate predominantly B-1 cells, whereas expression of two other V_H region transgenes (V_HB1-8 and V_HglD42) leads to the almost exclusive generation of conventional, or B-2, cells. To determine the developmental potential of B cells bearing two distinct B cell antigen receptors (BCRs), one favoring B-1 and the other favoring B-2 cell development, we crossed V_H12 insertion mice with mice bearing either V_HB1-8 or V_HglD42. B cells coexpressing V_H12 and one of the other V_H genes are readily detected in the double IgH insertion mice, and are of the B-2 phenotype. In mice coexpressing V_H12, V_HB1-8 and a transgenic κ chain able to pair with both H chains, double H chain–expressing B-2 cells, and B-1 cells that have lost V_HB1-8 are generated, whereas V_HB1-8 single producers are undetectable. These data suggest that B-1 but not B-2 cells are selected by antigenic stimuli in whose delivery BCR specificity and surface density are of critical importance.

Key words: B-1 cells • B-2 cells • immunoglobulin heavy chain • allelic inclusion • gene targeting

Two major B cell subsets, designated B-1 and B-2, exist in humans and mice. B-1 and B-2 cells can be distinguished by their cell surface phenotypes and anatomical localization. B-1 cells, found mainly in the pleural and peritoneal cavities, express high levels of surface IgM, low levels of B220 and IgD, and moderate levels of CD5. In addition, they do not express CD23. In contrast, conventional, or B-2, cells are the predominant B cells found in the spleen and lymph nodes; they express high levels of B220, IgD, and CD23 and moderate levels of IgM, and lack surface CD5 expression (for review see references 1, 2).

Different V_H genes are preferentially expressed in B-1 and B-2 cells (3, 4). The distinct V_H repertoire that is found in B-1 cells has led to the hypothesis that the specificity of the B cell antigen receptor (BCR)[1] may in fact determine the differentiation of B cells into this subset (5, 6). It is known that antibodies that react with phosphatidyl choline (PtC) are produced largely by B-1 cells (7) and are mostly encoded by either of two H and L chain combinations, namely V_H12 and $V\kappa4$ or V_H11 and $V\kappa9$. Consistent with the view that BCR specificity plays a role in the development of B-1 cells, transgenic mice expressing V_H12 alone, or in combination with $V\kappa4$, generate mostly B-1 cells in all of the peripheral lymphoid organs, including spleens and lymph nodes (8).

We have generated by gene targeting various strains of Ig H chain insertion mice carrying different H chain variable (V) region genes targeted into their physiological position in the IgH locus. The inserted $V_HD_HJ_H$ elements include the segments designated V_H12 (8), V_HB1-8 (9), and V_HglD42 (10). Similar to conventional V_H12 transgenic mice, V_H12 insertion mice develop mainly B-1 cells. In contrast, mice whose IgH alleles were engineered to express V_HB1-8 or V_HglD42 develop mainly B-2 cells. This again suggests that BCR specificity may play a determining role in the differentiation of B-1 cells. If indeed signals transmitted through a BCR of a certain specificity lead to the generation of B-1 cells, then interference with the cell surface expression of that specific BCR may alter the differentiation process of these cells. Here, we generated IgH double (V_H12 and V_HB1-8 or V_H12 and V_HglD42) insertion mice to test whether the expression of a second H chain in V_H12-expressing B cells may act in a dominant negative manner to perturb the generation of B-1 cells.

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[1]*Abbreviations used in this paper:* BCR, B cell receptor; Id, idiotype; PtC, phosphatidyl choline; tg, transgenic.

Materials and Methods

Mice. The B1-8f (11) and glD42i (10) IgH insertion mice were generated in the laboratory in Cologne and in collaboration with D. Eilat's group at Hebrew University (Jerusalem, Israel), and have been described previously (references 10 and 11, as indicated behind each mouse strain). The generation of the V_H12f mice will be described elsewhere (Lam, K.-P., and K. Rajewsky, manuscript in preparation). The conventional $V\kappa4$ transgenic mice (8) were obtained from Stephen Clarke (University of North Carolina, Chapel Hill, NC). Mice used were 2–4 mo old and maintained in a conventional animal facility.

Antibodies. The following mAbs used in this study were produced and conjugated to fluorochromes in our laboratory: anti-B220 (RA3-6B2); anti-IgM (R33-24.12); anti-IgD (1.3-5); anti-CD43 (S7); anti-μ^a(RS3.1); anti-μ^b (MB86); anti–V_HB1-8 (Ac146); and anti-V_H12 (5C5). The anti-CD5 and anti-CD23 mAbs were purchased from PharMingen.

FACS® Analyses and Cell Sorting. Tissues and cell preparations for flow cytometric analyses and cell sorting were prepared as previously described (12). In brief, spleen cells were prepared by dissociation between frosted glass slides. Peritoneal cavity and bone marrow cells were obtained by injecting staining medium (PBS containing 3% FCS and 0.1% NaN_3) into the peritoneal cavity and femurs and tibia, respectively, using a 1-ml syringe with a 26-gauge needle. All cells were treated with RBC lysing solution (0.15 M NH_4Cl, 1 mM $KHCO_3$, and 0.1 mM Na_2EDTA) to eliminate erythrocytes. For FACS® analyses, cells were stained with optimal amounts of FITC-, PE-, and biotin-conjugated mAbs for 10 min on ice and washed three times with staining medium. Biotin-conjugated mAbs were revealed with streptavidin-Cychrome. Flow cytometry analyses were performed on a FACScan™ (Becton Dickinson) and cell sorting was done on a FACStar$^{PLUS™}$.

Southern Blot Analysis. Genomic DNA was prepared from mouse livers and sorted splenic B cells (13), digested with BamHI and fractionated on a 1% agarose gel. After capillary transfer, the membrane was hybridized with a random-primed α-P^{32}–labeled specific probe, as shown in Fig. 5.

Results

Different B Cell Populations Are Generated in glD42i, B1-8f, and V_H12f Mice. We used gene targeting to generate a series of IgH insertion mice in which the J_H locus was replaced by distinct $V_HD_HJ_H$ segments (14). These segments were taken from the 4-hydroxy-3-nitrophenyl acetyl–binding antibody, B1-8 (9); the antibody glD42, which is a reduced affinity mutant of the DNA-binding antibody, D42 (10); and the anti-PtC antibody, V_H12 (8). The corresponding mice were designated B1-8f, glD42i, and V_H12f respectively. The B1-8f (11) and glD42i (10) mice have been described previously, whereas the generation of the V_H12f mice will be described elsewhere (Lam, K.-P., and K. Rajewsky, manuscript in preparation).

Flow cytometric characterizations of the B cell populations in the spleens of wild-type, glD42i, B1-8f, and V_H12f mice are depicted in Fig. 1. The majority of the B cells
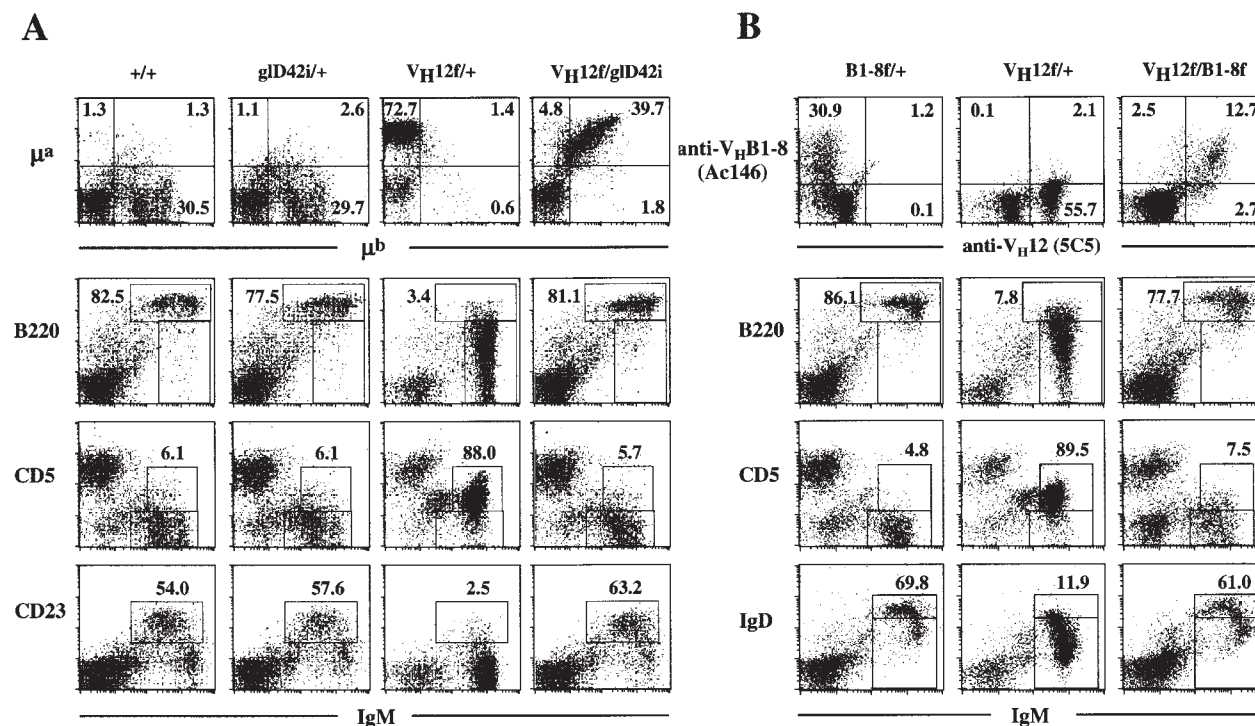

Figure 1. Phenotype of splenic B cells in (A) wild-type, glD42i/+, V_H12f/+, and V_H12f/glD42i; and (B) B1-8f/+, V_H12f/+, and V_H12f/B1-8f IgH insertion mice. Spleen cells obtained from wild-type and various Ig H chain insertion mice were stained with fluorochrome-conjugated allotypic (A, top) and idiotypic (B, top) antibodies as well as antibodies that recognize various cell surface markers used to define B-1 and B-2 cells. Numbers indicate percentage of total lymphocytes in the top panels and percentage of total B220$^+$ cells in the others.

present in glD42i (Fig. 1 A) and B1-8f (Fig. 1 B) mice are B-2 cells in that they express high levels of CD23 (shown for glD42i mice), IgD (shown for B1-8f mice), and B220, the pan-B cell marker. In addition, they do not express CD5, a marker found on T and B-1 cells. In contrast, V_H12f mice generate cells predominantly of the B-1 phenotype in that they express intermediate levels of CD5, low levels of B220 and IgD, and no detectable CD23. These data are consistent with a previous report that showed the preferential generation of B-1 cells in the lymphoid organs of conventional V_H12-transgenic mice (8). Thus, different V_H gene specificity seems to bias the generation and/or selection of different B cell subsets in the mouse.

V_H12-expressing B Cells Bearing a Second In-frame V Region Gene Develop into Conventional B Cells. B cell development under the condition of H chain allelic inclusion had previously been analyzed in mice bearing V_HB1-8 and V_HglD42 (15). In these double IgH insertion mice, B cells expressing two functional V_H alleles are readily generated in the bone marrow and are not counter-selected in the peripheral lymphoid organs. Here, we cross V_H12f mice with glD42i and B1-8f mice to examine the developmental potential of B cells bearing two distinct BCRs, one that is preferentially expressed in B-1 and the other in B-2 cells.

Expression of the V_H12 and glD42 alleles in B cells can be identified by the expression of their constant regions as the former is of the *a* and the latter, of the *b* allotype. Flow cytometric analyses of the B cells in double V_H12f/glD42i mice revealed that the majority of the cells in the spleen (Fig. 1 A), bone marrow, and lymph nodes (data not shown) of these mice coexpress both V_H genes. Similar results were also obtained when V_H12f mice were crossed with B1-8f mice. Expression of V_H12 and V_HB1-8 can be distinguished by staining with the anti-idiotype (Id) mAb 5C5 (8) and Ac146 (9) respectively. The 5C5 mAb recognizes V_H12 independent of the L chains (8) whereas the Ac146 mAb recognizes V_HB1-8 in association with λ and the majority (~80%) of the κ L chains (9). As shown in Fig. 1 B, the majority of the splenic B cells in V_H12f/B1-8f mice coexpress both Ids, indicating that they are double-IgH expressors. This is also true for the B cells in the bone marrow and lymph nodes of these mice (data not shown). Taken together, these data indicate that V_H12-expressing B cells can coexpress another V_H gene.

Surprisingly, phenotypic characterization of the IgH "double-producers" in V_H12f/glD42i and V_H12f/B1-8f mice revealed that these B cells express high levels of B220 and IgD (shown for V_H12f/B1-8f mice; Fig. 1 B); and the majority of them are also CD23-positive (shown for V_H12f/glD42i mice; Fig. 1 A). In addition, these double-producers do not express CD5 on their cell surfaces. Thus, in contrast to B cells that express V_H12 only, B cells that coexpress V_H12/V_HglD42, or V_H12/V_HB1-8 assumed a phenotype that is characteristic of B-2 cells.

Development of B Lymphocytes that Coexpress V_H12 and V_HB1-8 into Conventional B-2 Cells Is Not Due to Restricted Light Chain Usage. The specificity of the BCR is determined by the variable regions of the H and L chains. Thus,

the loss of the B-1 phenotype in cells coexpressing V_H12 and V_HB1-8 or V_H12 and V_HglD42 may be due to altered Ig L chain usage. It is conceivable that the L chains that associate with both V_H12 and V_HB1-8 or V_H12 and V_HglD42 under the condition of H chain allelic inclusion are different from those that normally associate with V_H12 alone. This altered L chain usage could affect the specificity of the V_H12 receptor and thus could influence the generation and/or selection of B-1 cells. To examine this possibility, we crossed $V\kappa4$ L chain transgenic (tg) mice (8) with $V_H12f/+$, B1-8f/+, and V_H12f/B1-8f mice. The $V\kappa4$ gene used in the generation of the transgenic mice was initially isolated from a CD5+ B lymphoma cell line that together with V_H12 recognizes PtC (8). In addition, this $V\kappa4$ L chain can also associate with the B1-8 H chain to form a BCR of innocuous specificity. Association of the $V\kappa4$ L chain with either or both V_H12 and V_HB1-8 is demonstrated in Fig. 2. We had previously shown that the bone marrow pre-B cell compartment is absent in Ig transgenic mice whose H and L chains pair to form a BCR of an innocent specificity (16). This probably reflects the fact that precursor cells carrying functional Ig H and L chain transgenes rapidly differentiate into IgM+ B cells. We have used this phenomenon to examine the association of $V\kappa4$ with both V_H12 and V_HB1-8. As shown in Fig. 2, B220+
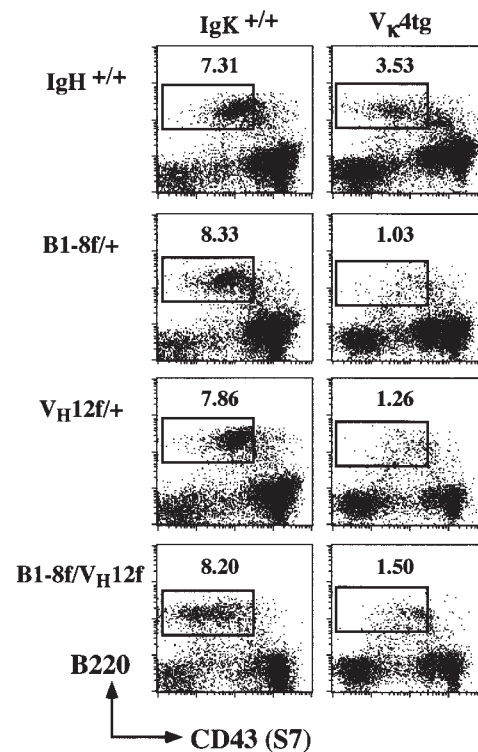

Figure 2. Association of the $V\kappa4$ L chain with the V_H12 and V_HB1-8 H chains. Bone marrow cells from wild-type, $V_H12f/+$, B1-8f/+, or V_H12f/B1-8f mice with or without the $V\kappa4$ L chain transgene were stained with anti-B220, anti-CD43, and anti-IgM mAbs. The figure depicts the surface IgM− cells and the boxed area indicates the pre-B cell compartment in the bone marrow. Numbers indicate percentage of total lymphocytes.
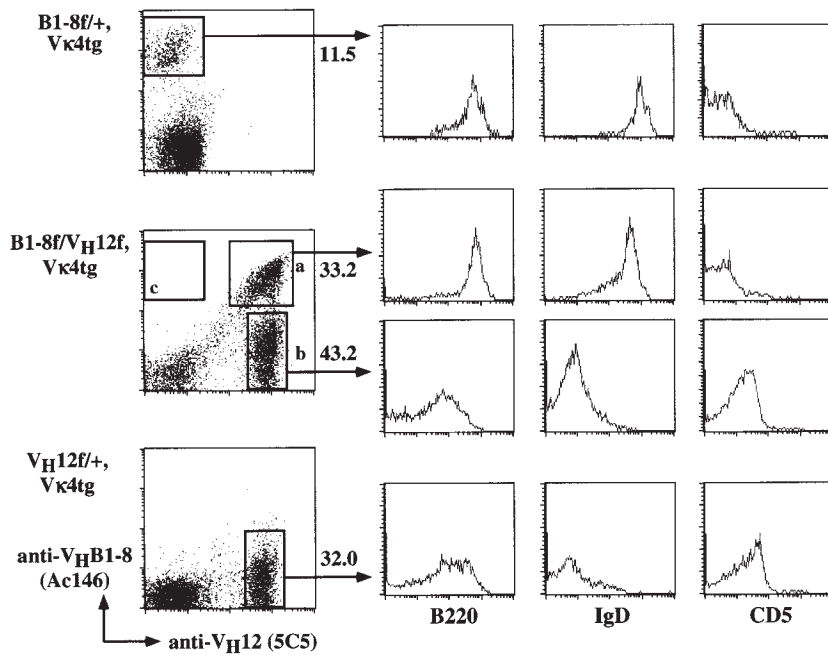
Figure 3. B cells coexpressing V_H12, $V\kappa4$, and V_HB1-8, $V\kappa4$ assume a conventional B cell phenotype. Phenotype of the various splenic B cell populations found in B1-8f/+, $V\kappa4$tg; $V_H12f/+$, $V\kappa4$tg; and $V_H12f/B1-8f$, $V\kappa4$tg mice. Cells were stained with antiidiotypic Ac146 and 5C5 mAbs, and with anti-B220, anti-IgD, and anti-CD5 mAbs. Numbers indicate percentage of total splenic lymphocytes.

$CD43^-$ pre-B cells are present in wild-type and in the various single and double IgH tg mice and represent ~8% of the cells present. However, this population is fivefold reduced in the B1-8f/+, $V_H12f/+$, and B1-8f/V_H12f H chain tg mice that also carry the $V\kappa4$ L chain transgene. This suggests that the $V\kappa4$ L chain can associate efficiently with both V_HB1-8 and V_H12. Association of $V\kappa4$ with V_HB1-8 is also evident in the splenic B cell population of B1-8f/+, Vk4tg mice, as the cells expressing this H and L chain combination are all Ac146 Id^+ (Fig. 3, top).

Phenotypic analyses of splenic B lymphocytes in B1-8f/+, $V\kappa4$tg mice indicate that these cells are predominantly B-2 cells (Fig. 3, top) as they are $B220^{high(hi)}$, IgD^{hi}, and $CD5^+$. In comparison, splenic B cells present in $V_H12f/+$, $V\kappa4$tg mice are of the B-1 phenotype as indicated by their lower levels of B220 and IgD expression. Furthermore, these cells are $CD5^+$ (Fig. 3, bottom), in agreement with previous published data (8).

Interestingly, two populations of B cells are present in the spleens of $V_H12f/B1-8f$, $V\kappa4$tg mice (Fig. 3, middle). The $Ac146^+5C5^+$ population (fraction a) represents double producers that coexpress V_H12, $V\kappa4$, and V_HB1-8, $V\kappa4$ receptors. The $Ac146^-5C5^+$ population (fraction b) seems to have lost the surface expression of the B1-8 H chain and appears to express only V_H12, $V\kappa4$. In contrast, $Ac146^+5C5^-$ cells (fraction c) that express only V_HB1-8, $V\kappa4$ are not seen in these mice.

FACS® analyses of the $Ac146^+5C5^+$ double producers indicate that these cells are conventional B cells in phenotype, as they express high levels of B220 and IgD and lack CD5 expression. They are indistinguishable from the B cells found in B1-8f/+; B1-8f/+, $V\kappa4$tg; or $V_H12f/B1-8f$ and $V_H12f/glD42i$ mice. Thus, the development of allelically included V_H12-expressing B lymphocytes into conventional B cells is not likely to be due to altered L chain usage, as it occurs also in the presence of the $V\kappa4$ transgene.

B Cells Expressing V_H12 only in $V_H12f/B1-8f$ Mice Have Lost the B1-8f Allele. The $Ac146^-5C5^+$ B cells present in the spleens of $V_H12f/B1-8f$, $V\kappa4$tg mice appear to have lost the surface expression of the B1-8 H chain. Thus, they are de facto single V_H12, $V\kappa4$ expressors and, not surprisingly, have a B-1 phenotype (Fig. 3, middle).

Further analyses revealed that B cells expressing V_H12 only can also be found in the peritoneal cavities of $V_H12f/B1-8f$ (Fig. 4) and $V_H12f/glD42i$ (data not shown) mice that do not carry the $V\kappa4$ L chain transgene. As shown in Fig. 4, the $Ac146^-5C5^+$ cells comprise a large fraction (>90%) of the B cells present in the peritoneal cavity of
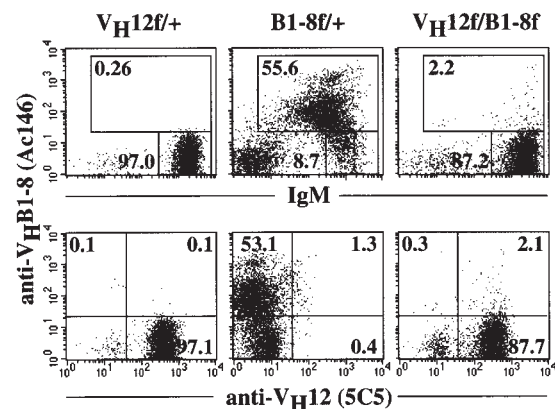

Figure 4. B cell populations found in the peritoneal cavity of B1-8f/+, $V_H12f/+$, and $V_H12f/B1-8f$ mice. Peritoneal cavity cells of the various single and double IgH insertion mice were stained with antiidiotypic Ac146 and anti-IgM (top) and Ac146 and 5C5 (bottom) mAbs. Numbers indicate percentage of total lymphocytes.
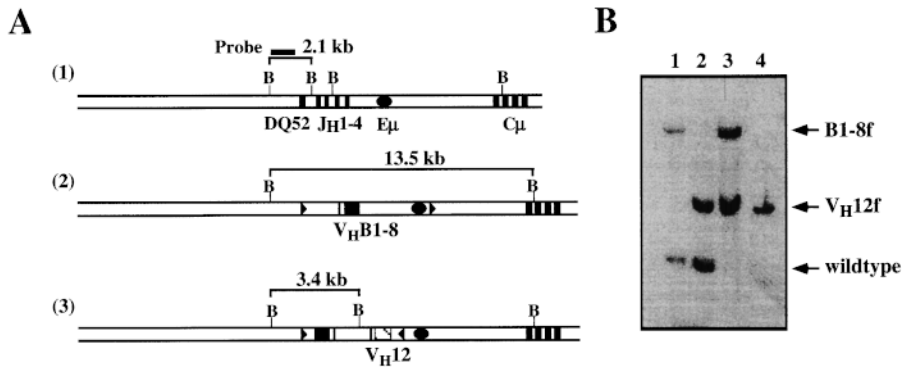
Figure 5. Single V_H12, $V\kappa4$–expressing B cells in V_H12f/B1-8f, $V\kappa4tg$ mice have lost the targeted B1-8f H chain allele. (A) Structures of the wild-type IgH locus (1), the targeted locus bearing the B1-8 VDJ (2), and the targeted locus bearing the V_H12 VDJ (3) are shown together with the size of the respective restriction fragments. Genomic DNA was digested with BamHI and hybridized with the indicated probe. Maps are not drawn to scale. (B) Southern blot analysis of DNA from livers of B1-8f/+, $V\kappa4tg$ (lane 1); V_H12f/+, $V\kappa4tg$ (lane 2); and V_H12f/B1-8f, $V\kappa4tg$ (lane 3) mice; and FACS® sorted $5C5^+Ac146^-$ splenic B cells of V_H12f/B1-8f, $V\kappa4tg$ mice (lane 4).

these mice and possess, as expected, a B-1 cell phenotype (data not shown). Although we cannot exclude the possibility that cells bearing V_HB1-8 but not expressing the Ac146 Id (due to pairing with certain L chains) are also included in this population, such cells should represent a minor fraction. This is supported by FACS® analysis of control B1-8f/+ mice (Fig. 4), which suggests that the majority of the B cells (>80%) in the peritoneal cavity express the Ac146 Id.

To determine the nature of the lack of B1-8 gene expression in these V_H12-only cells, we first sorted $5C5^+Ac146^-$ cells from the spleen of V_H12f/B1-8f, $V\kappa4tg$ mice and analyzed the targeted IgH loci by Southern blotting using a probe located 5′ of the DQ52 region. The wild-type, targeted V_H12f and B1-8f alleles should yield fragment sizes of 2.3, 3.4, and 13.5 kb (Fig. 5, A and B, lanes 1–3), respectively. In the $5C5^+Ac146^-$ B cells sorted from the spleen of V_H12f/B1-8f, $V\kappa4tg$ mice, the band corresponding to the targeted B1-8f allele is missing (Fig. 5 B, lane 4), suggesting that the gene has been replaced. Similar results were also obtained from $5C5^+Ac146^-$ B cells isolated from the peritoneal cavities of V_H12f/B1-8f mice (data not shown). Thus, $5C5^+Ac146^-$ B cells in the spleens and peritoneal cavity of V_H12f/B1-8f, $V\kappa4tg$ or in the peritoneal cavity of V_H12f/B1-8f have lost V_HB1-8 and consequently express only V_H12. Loss of the B1-8f allele could occur by rearrangement of upstream V or D gene segments into the B1-8 VDJ and resulting in a nonfunctional allele (17–19). It is not known presently at which developmental stage the replacement of the B1-8f allele occurs. FACS® analyses of the bone marrow of V_H12f/B1-8f or V_H12f/B1-8f, $V\kappa4tg$ mice suggest that the immature and mature B lymphocytes in this compartment are predominantly double producers (data not shown).

Reduced Expansion of B Lymphocytes in Mice Coexpressing V_H12 and Another H Chain. Assessment of the number of B cells in wild-type and various IgH insertion mice revealed that V_H12-expressing B lymphocytes undergo pronounced cellular expansion. As shown in Table I, V_H12f/+ mice kept in a conventional animal facility generally have 2- and 20-fold more B cells in the spleen and peritoneal cavity respectively, compared with wild-type, B1-8f/+, or

glD42i/+ mice. Interestingly, the number of splenic B cells in mice coexpressing V_H12 and either V_HB1-8 or $V_H$glD42 is similar to that in wild-type, B1-8f/+, or glD42i/+ mice, suggesting that the expansion of V_H12-expressing B cells is curtailed in these mice. However, the number of peritoneal B cells in V_H12f/B1-8f or V_H12f/glD42i mice is similar to that in V_H12f/+ mice. This probably reflects the fact that the B cells that accumulate in the peritoneal cavities of the double IgH insertion mice are mainly V_H12 expressors (see Fig. 4) that have lost expression of the other H chain (see Fig. 5).

The presence of a $V\kappa4$ L chain transgene leads to an even greater increase in the number of splenic B cells as V_H12f/+, $V\kappa4tg$ mice have threefold more cells than do V_H12f/+ mice and eightfold more cells than do either $V\kappa4tg$ or B1-8f/+, $V\kappa4tg$ mice. This is probably due to

Table I. *Number of B Cells in the Spleen and Peritoneal Cavity of Wild-type and Various Ig tg Mice*

Genotype		Spleen ($\times 10^7$)	Peritoneal cavity ($\times 10^6$)
+/+		2.45 ± 0.83	0.85 ± 0.28
V_H12f/+		5.76 ± 0.26	15.40 ± 6.72
B1-8f/+		2.47 ± 0.42	0.24 ± 0.11
glD42i/+		1.08 ± 0.47	0.76 ± 0.26
V_H12f/B1-8f		2.00 ± 0.13	11.63 ± 0.38*
V_H12f/glD42i		1.60 ± 0.21	10.58 ± 0.32*
+/+	$V\kappa4tg$	2.20 ± 0.69	0.95 ± 0.33
B1-8f/+	$V\kappa4tg$	1.60 ± 0.40	0.31 ± 0.10
V_H12f/+	$V\kappa4tg$	15.19 ± 1.71	13.20 ± 3.70
V_H12f/B1-8f	$V\kappa4tg$	6.96 ± 1.90‡	11.20 ± 2.53*

The number of B lymphocytes is estimated by anti-B220 and anti-IgM staining of splenic and peritoneal cavity cells in flow cytometry analyses. Mice analyzed were kept in a conventional animal facility and were 2–4 mo old. Groups of more than three mice were analyzed for each genotype.
*Greater than 90% of the B cells express only V_H12 (see Fig. 4).
‡Approximately 50% of the B cells are single V_H12 expressors (see Fig. 3).

ligand-mediated clonal expansion, as V_H12 together with $V\kappa4$ recognizes PtC (8). Again, this expansion is modulated in $V_H12/B1$-8f, $V\kappa4$tg mice (Table I). The three- to fivefold increase in the number of splenic B cells in this mouse strain compared with $V\kappa4$tg or B1-8f/+, $V\kappa4$tg mice is probably due to the fact that >50% of these cells in $V_H12f/B1$-8f, $V\kappa4$tg mice are single V_H12 expressors (Fig. 3).

Discussion

V_H12 insertion mice, like conventional V_H12 transgenic mice (8), generate mostly B-1 cells, whereas V_HB1-8 and V_HglD42 insertion mice produce predominantly conventional, or B-2, cells. This is in line with the concept that BCR specificity is a major determinant in B-1 versus B-2 cell development. The novel findings in this study are that the coexpression of V_H12 with either of the two other V_H region genes in double IgH insertion mice (which express wild-type κ chains or a $V\kappa4$ transgene) results in the generation of a population of double-producing B-2 cells. In addition, in such animals a population of single-producing B cells appears, namely, B-1 cells expressing only V_H12.

Why do V_H12-expressing B cells that coexpress a second H chain not differentiate into B-1 cells? This can perhaps, best be explained by postulating that signaling via a BCR of a certain specificity, expressed at the cell surface at high density, is required to drive the differentiation of B cells into the B-1 subset. In our experiments, the provision of a second H chain of a different specificity presumably acts in a "dominant-negative" manner to dilute out the V_H12-containing BCR complexes on the cell surface. Assuming equivalent production of H chains from the various inserted $V_HD_HJ_H$ segments and equivalent pairing of the H and L chains involved, only 25% of the Ig molecules on the cell surface of double-producing cells would carry V_H12 regions on both H chains. This reduced density of B-1–specific BCRs may not provide sufficient signal for the development of B-1 phenotype.

The hypothesis that BCR signaling is responsible for the development of the B-1 cell phenotype has been supported by experiments that show that under certain conditions the cross-linking of sIg on splenic B cells may lead to development of a B-1 cell phenotype on B-2 cells (20). The skewed development of B-1 and B-2 cell subsets in many gene-targeted mice with mutations in specific signaling molecules is also consistent with this hypothesis. For example, CD19-deficient mice (21, 22) and *xid* (23) mice that have a mutation in the *btk* gene have reduced numbers and a lack of B-1 cells, respectively. In contrast, lyn-deficient mice (24, 25) and *motheaten* mice that have a mutation in SHP-1 (26) have increased numbers of B-1 cells. More significantly, the introduction of the *xid* defect into V_H12 conventional transgenic mice leads to the predominance of V_H12-expressing B cells with a B-2 cell phenotype (27), compared with wild-type V_H12 transgenic mice that generate mostly B-1 cells.

The appearance of large numbers of V_H12-expressing B-1 cells in the double mutants that have lost expression of the second H chain is of particular interest. Such loss variants are rare in V_HB1-8/V_HglD42 mice (15), emphasizing the stability of these targeted IgH loci in B cell development. This suggests that the V_H12-only cells in the present system are strongly selected, in accord with the concept that B-1 cell development is driven by BCR signaling. It will be of interest to determine at which stage of development the loss of the second IgH allele occurs in these cells; and whether its loss in mature (B-2) double producers will change their phenotype to that of B-1 cells.

It is apparent that B-2 cell development depends to a lesser extent than that of B-1 cells on density of BCRs of certain specificities at the cell surface (reference 15 and the data presented here). This might reflect a lesser dependence of B-2 cells on positive selection by (self)-antigens. The requirement of BCR expression for B-2 cell survival (11) would then largely be a cell-autonomous phenomenon.

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