Different sensitivity to receptor editing of B cells from mice hemizygous or homozygous for targeted Ig transgenes

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Ig knock-in mice have been used to study the relative contribution of receptor selection versus clonal selection in the control of autoreactive B cells. The anti-MHC class I 3-83Ig knock-in (3-83Igi) mice manifest extensive receptor editing in the presence of H-2^b. However, receptor editing is also observed on the H-2^d background, although reactivity toward this antigen is below detection and its presence does not affect the generation of 3-83Ig$^+$ mature B cells in classical 3-83Ig transgenic mice. In this study we have analyzed the contribution of genetic background, B cell receptor signaling, and transgene copy number on the initiation and extent of receptor editing in the 3-83Igi;H-2^d mice. Crossing the 3-83Ig insertion into either CD45-deficient H-2^d mice or onto the BALB/c background reduces the extent of receptor editing and increases the fraction of 3-83Ig-expressing B cells, indicating that in the original line editing depends on B cell receptor signaling induced by cross-reacting antigen(s). However, receptor editing is still detectable in hemizygous 3-83Igi mice even on the BALB/c background, on which the 3-83 antibody was originally raised, whereas it is abrogated in homozygous 3-83Igi;H-2^d animals. This latter observation indicates that immature B cells expressing immunoglobulin from single heavy and light chain loci, as they do physiologically, utilize receptor editing for an exquisite quality control of their antigen receptor that may only partly be based on self-reactivity.

The mature B cell population expresses a diversified immunoglobulin (Ig) repertoire able to recognize any foreign antigen. Generation of antigen receptors depends on the combinatorial somatic rearrangement of V (variable), D (diversity), and J (joining) gene segments [the V(D)J recombination process; refs. 1 and 2]. In this way antibodies can be produced that are specific for foreign as well as self antigens. Therefore, the immature B cell repertoire must undergo a selection process to prevent self-reactive cells from distributing throughout the body (3–5).

To analyze the selection process of given Ig specificities, conventional transgenic technology first and ''knock-in'' gene technology later have been used to generate mice whose B cells develop with prerearranged Ig heavy (H) and light (L) chain genes (6). These systems take advantage of the fact that Ig expression from one allele inhibits V(D)J recombination on the second allele, a phenomenon known as allelic exclusion (7). Consequently, expression of the Ig transgenes prevents rearrangement and expression of endogenous Ig genes, promoting the generation of a monospecific B cell population (8–13). The analysis of Ig transgenic mice expressing autoreactive specificities has demonstrated the existence of clonal deletion, clonal anergy, and receptor editing as mechanisms that operate to censor autoreactive B cells (14, 15). In Ig knock-in mice receptor editing results in the efficient elimination of autoreactive specificities and the generation of a diversified B cell population at the same time (16, 17). It mainly involves secondary rearrangements at the Ig L loci, resulting in the deletion of the L chain V region gene initially expressed and expression of novel L chains. The newly expressed L chains associate, in general, with the original H chain to constitute a diversified nonautoreactive B cell repertoire (16–19). Receptor editing temporarily violates allelic exclusion, but this is apparently reestablished at both the genetic and protein level in the resulting B cell population (16–18).

Receptor editing was initially described as a rare event occurring in developing autoreactive B cells of classical Ig-transgenic mice (18, 19). To better understand the physiological contribution of receptor editing to B cell tolerance, we previously generated and characterized an Ig knock-in mouse model, the 3-83 Ig insertion (3-83Igi) mice (16, 20). These mice carry productively assembled VDJ and VJ gene segments encoding the variable parts of 3-83 Ig H and 3-83 Ig κ chains in their genomic Ig loci, in the position where endogenous somatically rearranged Ig genes are located. The 3-83 antibody is specific for the MHC class I antigens H-2K^k and -K^b but not H-2K^d, and cross-reacts with a spectrum of lower affinities with H-2 molecules from other haplotypes (21, 22). As predicted, efficient editing was observed in 3-83Igi B cells developing on an H-2^b background (16). Unexpectedly, however, receptor editing was also active on H-2^d, albeit to a lesser extent (ref. 16 and unpublished observations). We have shown that counterselection of the transgenic receptor specificity is not a general feature of Ig knock-in mice, since it is not seen in mice carrying a different combination of Ig H and L gene insertions (B1-8Hi and 3-83ki), encoding for an innocuous specificity (16). In addition, this phenomenon did not appear to be characteristic of the 3-83 antibody, since receptor editing was observed in mice carrying randomly integrated 3-83Ig classical transgenes on the H-2^b, but not the H-2^d, background (11).

We have speculated that receptor editing in 3-83Igi;H-2^d mice resulted from a reactivity of 3-83 toward a weakly cross-reacting antigen that is either unrelated to MHC class I or is H-2^d itself, and that the phenotype of the 3-83Ig knock-in mice contrasted with that of the classical transgenic because of a more physiological control of transgene expression. To test this hypothesis, the 3-83Igi mice were backcrossed to BALB/c and to CD45-deficient mice. These latter animals carry a mutation that lowers the intensity of B cell receptor (BCR) signaling initiated upon antigen interaction (23, 24). During this breeding, the 3-83Igi mice were also rendered homozygous at both Ig H and Ig κ loci, either on a pure BALB/c (H-2^d) or a mixed BALB/c $\times$ C57BL/6 $\times$ 129 (either H-2K^d or H-2K^b) background. In this paper we present an analysis of receptor editing in these various mutant strains.

Abbreviations: BCR, B cell receptor; sIgM and sIgD, surface IgM and IgD.

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Materials and Methods

Mice. The generation of 3-83Igi mice has been described previously (16, 20). The animals used for the work carried the Jκ3–5 gene segments downstream of 3-83ki. Mice were bred and maintained in a specific pathogen-free (SPF) facility at the Max Planck Institute. CD45-deficient mice (25) were from a stock maintained in the same SPF facility. Mice analyzed were between 6 and 20 weeks of age. The genotype for the Ig H, Ig κ, and CD45 loci was determined by PCR on tail genomic DNA. Expression of H-2^b and H-2^d alleles was determined by monoclonal antibodies specific for either H-2K^b or H-2K^d.

Flow Cytometric Analysis and Antibodies. Preparation, staining, and flow cytometric analysis of bone marrow and spleen cells was done as previously described (16, 20). The following antibodies were used: R33-24 anti-IgM (26), RA3-6B2 anti-B220 (PharMingen), anti-CD22 (PharMingen), 54.1 anti-3-83 (27), polyclonal goat anti-λ (Southern Biotechnology Associates), 1.3-5 anti-IgD (28), polyclonal goat anti-IgM (Southern Biotechnology Associates), and R33-18 anti-Ig κ L chain (26). For cytoplasmic (+ surface) staining, cells were first surface-stained with anti-B220-PE antibody and then fixed in 2% paraformaldehyde in PBS for 20 min at room temperature. Fixed cells were washed twice with PBS and then incubated with FITC-conjugated antibodies (either anti-μ H chain or anti-κ L chain) in PBS containing 0.5% saponin (Sigma), 0.5% BSA, and 0.05% NaN_3 for 15 min at room temperature. Cells were subsequently washed twice with the saponin-containing PBS and resuspended for the analysis in PBS without saponin.

Stromal Cell Culture. Bone marrow cells were prepared as described (20). Cells were plated on a layer of γ-irradiated ST2 cells (29) in IMDM complete medium supplemented with recombinant IL-7. After 4 days of culture, the nonadherent cells were recovered and plated on a new irradiated ST2 cell layer in the presence or absence of IL-7. Cells were recovered after 3 days and analyzed by flow cytometry. The ST2 cells expressed H-2^d, but not H-2^b, upon staining with anti-H-K^d and anti-H-2K^b antibodies (data not shown).

Results

Receptor Editing Is Not Abolished in 3-83Igi Mice Backcrossed to BALB/c. The 3-83Igi mice were maintained hemizygous for the 3-83 Ig H and Ig κ insertions on the H-2^d background. Such animals were backcrossed for seven generations to BALB/c (H-2^d). The initial purpose of this backcross was to eliminate any possible 3-83-interacting antigen(s) that may have been present in the original mixed (129 × BALB/c × C57BL/6) background (16).

As we and others have previously reported, receptor editing in 3-83Ig transgenic mice results in a high frequency of λ^+ and 3-83 idiotype-negative mature B cells (16, 18). These characteristics allow a rapid assessment of the extent of receptor editing in the various mouse strains. By these criteria, backcrossing to BALB/c does not eliminate receptor editing in the hemizygous 3-83Igi mice: Only half of the IgM$^+$ immature (B220^{low}) B cells in the bone marrow of such animals express the 3-83 idiotype. The remaining immature B cells express a BCR that is not recognized by the 3-83-specific 54.1 antibody (27) (Fig. 1 *Lower Middle*). The novel BCRs likely derive in their majority from *de novo* rearrangement and expression of endogenous κ or λ V region genes and the loss of the 3-83k chain, as previously established (16). A selective recruitment and expansion of cells that have replaced the 3-83 with a novel BCR and frequently express λ chains was observed in the mature splenic B cell compartment (Fig. 2 *Center* and Table 1).

The inability of 3-83-expressing B cells to fully develop and expand in the peripheral lymphoid compartment on the BALB/c background indicates either that the 3-83 BCR has intrinsic inability to guide the cells into further development or that it is still interacting with a self-antigen.

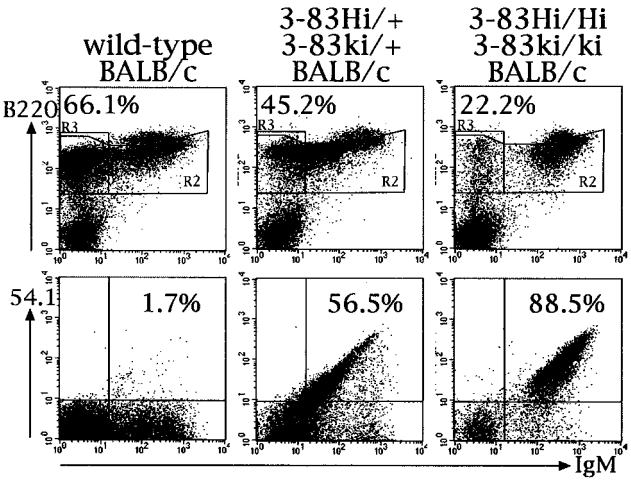

Fig. 1. Flow cytometric analysis of bone marrow cells. Cells were isolated from wild-type, 3-83Igi hemizygous (3-83Hi/+;3-83ki/+), and 3-83Igi homozygous (3-83Hi/Hi;3-83ki/ki) mice, all on a BALB/c background. Cells were stained for B220, IgM, and 3-83Ig (by the 54.1 antibody) in a three-color reaction. (*Upper*) B220 and IgM staining of cells in the lymphocyte gate. Numbers refer to the frequency of B220^{low}/surface IgM (sIgM)$^-$ cells (region R3) in the total B220^{low} cell population (region R2). (*Lower*) The 54.1 and IgM staining of B220^{low} lymphocytes (R2 region in *Upper*). Numbers refer to frequency of 3-83Ig$^+$ cells in the total sIgM$^+$ (B220^{low}) cell population.

Homozygosity of Ig Targeted Alleles Suppresses Receptor Editing and Restores a Monospecific 3-83-Expressing B Cell Population. After seven backcross generations into BALB/c, hemizygous mice were intercrossed to obtain a homozygous 3-83Igi;BALB/c strain to analyze the effect of transgene dosage on the development and selection of Ig-transgenic B cells.

Surprisingly, receptor editing was almost abolished in homozygous 3-83Igi;BALB/c mice. In these mice nearly 90% of the

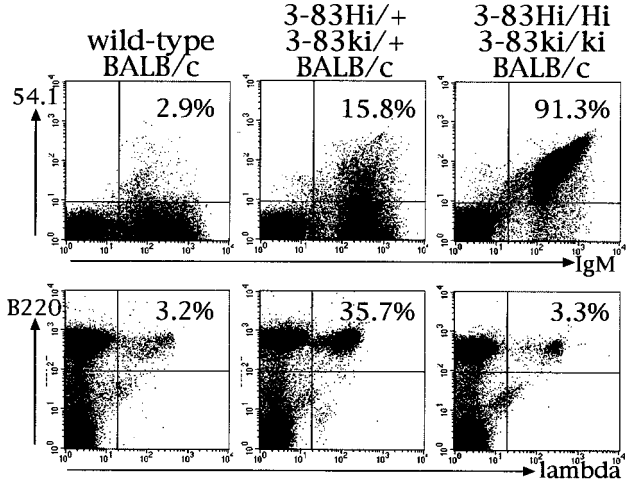

Fig. 2. Flow cytometric analysis of spleen cells. Cells were isolated from wild-type, 3-83Igi hemizygous, and 3-83Igi homozygous mice on a BALB/c background. (*Upper*) The 54.1 and IgM staining of cells in the lymphocyte gate. Numbers refer to frequency of 3-83Ig$^+$ cells in the total sIgM$^+$ cell population. (*Lower*) The B220 and λ staining in cells of the lymphocyte gate. Numbers refer to frequency of λ^+ cells in the total B220$^+$ cell population.

Table 1. Frequencies of 3-83Ig$^+$ and λ^+ spleen B cells

Genotype	% 3-83Ig$^+$*	% λ^+*
3-83Hi/+; 3-83ki/+; H-2^{d†}	22.1 ± 7.3 (n = 4)	31.4 ± 4.0 (n = 7)
3-83Hi/+; 3-83ki/+; H-2^d; CD45−/−‡	76.2 ± 21.7 (n = 3)	13.4 ± 4.2 (n = 3)
3-83Hi/Hi; 3-83ki/ki; H-2^d; BALB/c§	90.6 ± 5.5 (n = 6)	3.8 ± 0.7 (n = 4)
3-83Hi/Hi; 3-83ki/ki; H-2^d; BALB/c × C57BL/6 × 129	71.9 ± 6.3 (n = 6)	17.8 ± 7.6 (n = 6)
3-83Hi/Hi; 3-83ki/ki; H-2^b; BALB/c × C57BL/6 × 129	8.8 ± 3.2 (n = 4)	52.9 ± 8.3 (n = 4)
Wild-type BALB/c × C57BL/6	3.7 ± 2.7 (n = 8)	4.0 ± 0.2 (n = 7)

*Frequencies of 3-83$^+$ and λ^+ cells are calculated, respectively, in the IgM$^+$ and B220$^+$ (CD22$^+$ for CD45−/− mice) populations.
†Mice on BALB/c or mixed (BALB/c × C57BL/6 × 129) background.
‡Mice on mixed (BALB/c × C57BL/6 × 129) background.
§Mice with or without Jκ3-5 segments downstream of the 3-83k insertion.

B220^{low} bone marrow B cells express sIgM of the 3-83 specificity (Fig. 1 *Right*). We have previously reported that the B220^{low}/sIgM$^-$ population contains pre-B-like cells undergoing receptor editing in mice expressing an autoreactive transgenic BCR, whereas such cells are absent in the case of a nonautoreactive transgenic BCR (16). As shown in Fig. 1, the frequency of B220^{low}/sIgM$^-$ cells is 2-fold reduced in homozygous as compared with hemizygous mice. In addition, most of the homozygous B cells represent IgM^{high} immature cells, whereas in the hemizygous strain most B cells are IgM^{low} (Fig. 1). Inhibition of receptor editing in the homozygous mice was confirmed in mature splenic B cells. More than 90% of these cells express the 3-83 idiotype and only 3.8%, on average, express λ chains in this strain (Fig. 2 *Right* and Table 1). These data demonstrate that B cells expressing identical BCRs are not equivalent with respect to their development, depending on whether they carry one or two rearranged Ig alleles.

The ability of homozygous B cells to suppress endogenous Ig L rearrangements on the H-2^d background could be due to increased levels of BCR expression. Therefore, we next compared Ig expression in B cells of hemizygous and homozygous 3-83Igi mice by flow cytometry.

Hemizygous and Homozygous 3-83Igi B Cells Differ in Their Capacity to Express 3-83Ig on the Cell Surface. The dosage of Ig transgenes can influence the level of Ig expression and, consequently, the development and selection of the transgenic B cells. To assess whether this applies to the 3-83Ig insertions, analysis of sIgM, sIgD, and of total (surface + cytoplasmic) μ and κ chain levels was performed by flow cytometry in immature and mature B cells of hemizygous and homozygous 3-83Igi;BALB/c mice. Relative amounts of Ig proteins were compared by the mean fluorescence intensities, which are proportional to the amount of stained protein.

In the bone marrow sIgM, total μ and κ levels were 2-fold higher in homozygous versus hemizygous immature (B220^{low}) B cells (Fig. 3*a*). This indicates that the presence of two copies of the Ig knock-in genes results in the expression of twice the amount of protein. Note, however, that the bone marrow B cells of hemizygous and homozygous mice belong to different populations. Pre-B-like editing cells are present only in the hemizygous strain (Fig. 1), and Ig expression is necessarily reduced during the receptor editing process, because of deletion of the 3-83ki gene by secondary rearrangements and BCR downmodulation. Furthermore, the reduction of IgM levels in the hemizygous versus the homozygous cells is seen only for the 3-83 specificity, for which only a few cells reach high levels in the former animals (Fig. 1). In contrast, BCRs of other specificities, composed of the 3-83 H and endogenous L chains (16), are expressed at levels comparable to those observed in wild-type immature B cells and also to 3-83 expression in the homozygous mutants (Fig. 1). This observation is confirmed in the splenic compartment, where most of the B cells express non-3-83 BCRs in the hemizygous strain. Here, equal expression of total μ and κ was observed in the hemizygous and homozygous cells (Fig. 3*b*). Nevertheless, expression of sIgM is on average 20% lower in the hemizygous mature B lymphocytes, suggesting that these cells have an increased cytoplasmic pool of μ chains compared with the homozygous cells. Finally, in contrast to sIgM, expression of sIgD is 40–50% higher in hemizygous bone marrow and spleen B cells (Fig. 3).

In summary, although hemizygous B cells have problems in expressing or maintaining 3-83Ig on the cell surface, they seem to be able to express BCRs composed of the 3-83H chain and endogenous L chains at levels comparable to those of 3-83Ig in the homozygous cells.

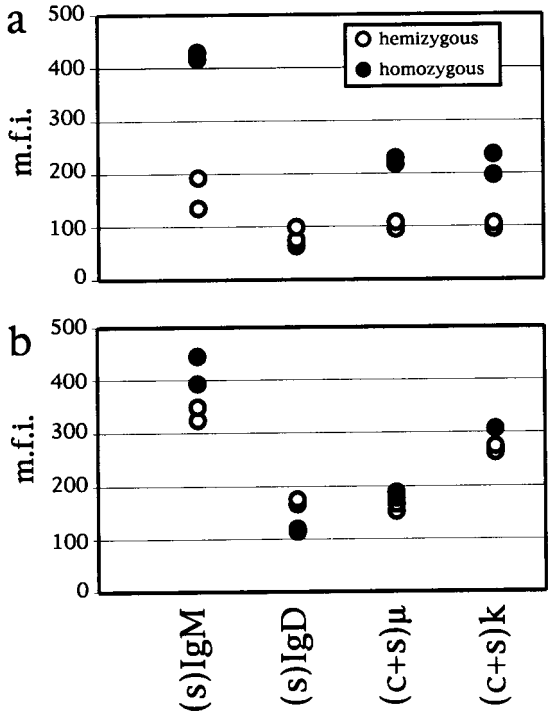

Fig. 3. Analysis of Ig protein levels by flow cytometry. Cells were isolated from either bone marrow (*a*) or spleen (*b*) of hemizygous (○) and homozygous (●) 3-83Igi mice of BALB/c genetic background. Cells were stained for B220 and additionally stained for surface (s) IgM (R33-24 antibody), sIgD, total cytoplasmic and surface (c+s) μ (goat anti-mouse IgM antibody) or total (c+s) κ. Staining of total Ig was performed in the presence of saponin. Arithmetic mean florescence intensity (m.f.i.) values for the stainings reported were calculated on, respectively, B220^{low} (immature B) or total B220$^+$ cells in the bone marrow or spleen cells. Each circle corresponds to a mouse.

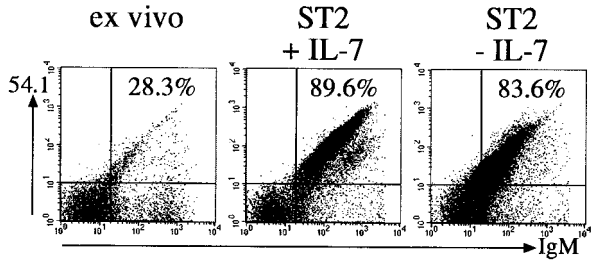

Fig. 4. Comparative analysis of *in vivo* and *in vitro* B cell development. Bone marrow B cells isolated from a mouse carrying hemizygous 3-83Hi and homozygous 3-83ki genes on a mixed (BALB/c × C57BL/6 × 129) H-2^d background were analyzed *ex vivo* (freshly isolated) or after culture on the ST2 stroma cell line in the presence or absence of IL-7. Flow cytometric analysis of 3-83 (54.1) and IgM staining of $B220^{low}$ (immature) gated cells is shown. Numbers refer to frequency of 3-83Ig^+ cells in the total $sIgM^+$ ($B220^{low}$) cell population.

Influence of Genetic Background on 3-83Ig Expression and Onset of Receptor Editing. Homozygous 3-83Igi;H-2^d mice were also analyzed on a mixed (BALB/c × C57BL/6 × 129) background. Here, different phenotypes were observed in different mice. Thus, in five of the six mice analyzed the fraction of λ-expressing and 3-83 idiotype-expressing B cells was 5-fold increased and 25% less, respectively, compared with the homozygous BALB/c strain (data not shown). In contrast, one mouse was phenotypically similar to homozygous mice on the BALB/c genetic background, maintaining 80.5% 3-83Ig^+ and 5.7% λ^+ B cells (data not shown). Average values are given in Table 1.

Strain variability was also observed in hemizygous 3-83Igi;H-2^d mice, but only in the immature bone marrow compartment. Here, backcross to BALB/c resulted in a 1.7-fold increase of the number and frequency of 3-83Ig^+ B cells and a 41% reduction of pre-B cells (data not shown).

All these results can be interpreted to mean that on the mixed genetic background 3-83-reactive self antigens different from H-2^d could promote receptor editing in the developing 3-83$^+$ B cells. To investigate this possibility further, we studied the development of such cells in culture, on the stromal cell line ST2 (29).

In vitro cultivation of bone marrow on a layer of stromal cells in the presence of IL-7 results in the selective growth of B cell precursors at the expense of other cell types (30). Developmental progression to the $sIgM^+$ immature B cell stage requires IL-7 withdrawal in wild-type cultures (31), whereas it is independent of IL-7 in Ig-transgenic cells (32). Bone marrow cells isolated from a mouse hemizygous for 3-83Hi and homozygous for 3-83ki on a mixed BALB/c × C57BL/6 × 129 H-2^d background were cultivated on ST2 cells, which express the H-2^d haplotype (data not shown), in the presence or absence of IL-7. In this mouse, most of the B cell precursors developed into immature B cells expressing endogenous BCRs, as demonstrated by the analysis of freshly isolated cells (Fig. 4, *ex vivo*). This finding is indicative of extensive receptor editing because of the presence of a 3-83-reacting antigen in the animal. In contrast, the same precursors in the ST2 culture generated mostly B cells expressing the 3-83 specificity, irrespective of the presence or absence of IL-7 (Fig. 4, ST2 +/− IL-7). Moreover, addition of H-2^b-expressing cells to the culture caused down-regulation of the 3-83-BCR (data not shown), demonstrating that the sensitivity to antigen is maintained *in vitro*, in accord with published data (33). Thus, no 3-83-specific antigen able to down-regulate 3-83 expression is present in the ST2 culture. It follows that neither H-2^d nor other ST2 or B cell-derived antigens cross-react with the 3-83 BCR.

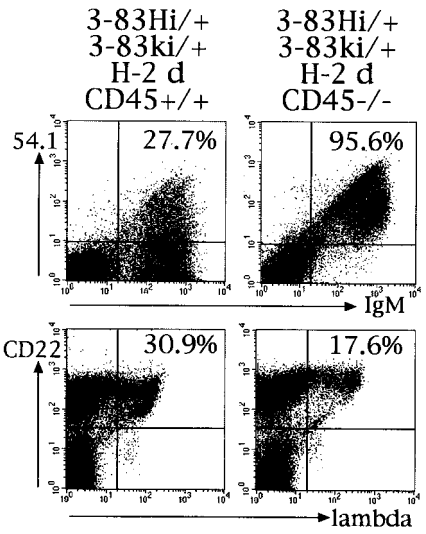

Fig. 5. Influence of a CD45-null mutation on receptor editing. Representative flow cytometric analysis of hemizygous 3-83Igi;H-2^d mice with or without CD45. (*Upper*) The 54.1 and IgM staining of cells in the lymphocyte gate. Numbers refer to frequency of 3-83Ig^+ in the total IgM^+ cell population. (*Lower*) The CD22 and λ staining of cells in the lymphocyte gate. Numbers refer to frequency of λ^+ in the total $CD22^+$ cell population.

Receptor Editing in B Cells from Hemizygous 3-83Igi;H-2^d Mice Is the Result of BCR Signaling. The tyrosine phosphatase CD45 is required for efficient BCR signal transduction (23, 24, 34–38). As a consequence, CD45 deficiency promotes survival and accumulation of self-reactive B cells *in vivo* (38).

The 3-83Igi mice were backcrossed to the CD45-deficient (C57BL/6) strain (25, 37) to test whether variation in the signal intensity of the BCR influences receptor editing in 3-83Igi;H-2^d mice. The breeding was designed to obtain hemizygous 3-83Igi;H-2^d mice carrying the CD45-null mutation. These mice were maintained on a mixed (BALB/c × C57BL/6 × 129) genetic background. Flow cytometric analysis of spleen cells from such animals shows a significant increase of 3-83Ig-expressing and a reduction of λ-expressing B cells relative to 3-83Igi hemizygous mice expressing CD45 (Fig. 5 and Table 1). The B cell population of 3-83Igi CD45-deficient mice appears to be composed of cells expressing the idiotype at either proportional or lower level relative to that of IgM (Fig. 5). The idiotype low (IgM^{high}) cells are perhaps cells expressing an endogenous BCR in addition to the 3-83. Thus, in the absence of CD45 the extent of receptor editing is reduced and 3-83Ig-expressing cells are efficiently recruited into the mature B cell pool, indicating that receptor editing is promoted by BCR signaling.

Receptor Editing Efficiently Removes Autoreactive Receptors Produced from Homozygous Targeted Ig Alleles on the H-2^b Background. We have shown that endogenous Ig expression is suppressed in homozygous 3-83Igi mice on the H-2^d background. To determine whether the targeted loci are still subject to receptor editing in the presence of the nominal antigen (22), we analyzed these mice on an H-2^b background.

Flow cytometric analysis showed that tolerance induction in general, and receptor editing in particular, is not prevented in 3-83Ig-expressing B cells developing in this situation. Thus, expression of 3-83Ig on the cell surface is totally abrogated in immature bone marrow B cells (data not shown). Indeed, approximately 80% of the $B220^{low}$ cells appear as $sIgM^-$ pre-B cells in these mice, in contrast to the 20% observed in homozygous 3-83Igi;H-2^d mice, whereas the IgM^+ cells express a BCR

of a different specificity. Receptor editing in the homozygous 3-83Igi;H-2^b mice results in expression of λ L chains in half of the mature B cell population (Table 1). The bulk of the remaining κ chain-expressing B cells presumably derives from secondary rearrangements at the targeted Ig κ alleles, which carry Jκ4 and Jκ5 gene segments downstream of the inserted 3-83 VκJκ joint (16). Interestingly, expression of the 3-83 idiotype was still observed in approximately 9% of the splenic B cells (Table 1). It is presently unknown whether these cells are functionally competent and indeed recognize self MCH.

Discussion

We have previously reported that receptor editing of 3-83Ig-expressing B cells occurs on both the H-2^b and H-2^d background in the 3-83Igi knock-in mice, whereas it was observed only on the former background in 3-83Ig classical transgenics. This was surprising because the 3-83 antibody was shown to bind H-$2K^b$ but not H-$2K^d$ (22). In separation, both the 3-83Hi and the 3-83ki knock-in gene mediate allelic exclusion at their respective Ig loci and support normal development of B cells (ref. 16 and unpublished observations). This fact indicates that receptor editing in mice carrying both 3-83Hi and 3-83ki is the result of the 3-83 antibody specificity. We therefore speculated that the editing seen in 3-83Igi mice homozygous for H-2^d is caused by some cross-reacting antigen(s) provided by the genetic background.

As shown in this paper, the extent of receptor editing in 3-83Igi;H-2^d mice indeed depends on the genetic background, and also on the threshold of BCR signaling. An increase in the frequency of 3-83Ig-expressing B cells was observed in both hemizygous and homozygous 3-83Igi mice on a BALB/c, relative to a mixed BALB/c × C57BL/6 × 129 background. These results are in accord with the idea that receptor editing in 3-83Igi;H-2^d mice at least in part results from the interaction of the 3-83 BCR with cross-reacting antigen(s) provided by the genetic background of the animals. This idea also goes along with the finding that in the absence of the CD45 phosphatase receptor editing is inhibited and 3-83Ig^+ cells are allowed to expand. CD45 acts as a positive regulator of BCR signaling by dephosphorylating the inhibitory tyrosine of the Src-family tyrosine kinases (39, 40). B cells that become anergic and short-lived by developing in the presence of a soluble self antigen expand and survive longer when they lack CD45 (38). This observation indicates that the threshold at which BCR signaling prevents immature bone marrow B cells from moving into and surviving in the peripheral compartment is set at a lower level in the absence of CD45. We conclude, therefore, that the extent of receptor editing in the 3-83Igi;H-2^d mice depends on signaling through the BCR.

We have shown that hemizygous 3-83Igi;H-2^d B cells are impaired in the surface expression of 3-83, but not of other Ig specificities in the animals analyzed. This is the phenotype expected from the interaction of the 3-83 BCR with self antigens during B cell development. The stromal cell culture data suggest that if cross-reacting antigens are indeed involved, they are not expressed by the B cells themselves and are not H-2^d class I molecules as such, because those are expressed on the ST2 stromal cell line used in this work. However, the 3-83 antibody, isolated from H-2^d mice immunized with H-2^k cells (21), exhibits some degree of cross-reactivity toward class I molecules of almost all haplotypes tested (22). This cross-reactivity may well extend to other cell surface molecules of the Ig superfamily.

The 3-83 antibody has acquired its specificity during affinity maturation in an immune response, through somatic hypermutation. It is quite possible that at that stage of differentiation B cells tolerate an extent or type of self-reactivity that they could not tolerate in early development. However, the possibility should also be kept in mind that apart from the problem of autoreactivity, receptor editing could be seen as a mechanism by which BCRs that are functionally inappropriate for whatever reason are eliminated to generate a population of naive B cells expressing a diverse repertoire of fully functional antigen receptors.

Receptor editing is almost abolished in mice homozygous for 3-83Hi and 3-83ki on the H-2^d background. This phenotype is similar to that of mice carrying multiple integrations of classical 3-83Ig transgenes (18). Clearly, the hemizygous 3-83Igi targeted mice, which carry one rearranged and one germ-line Ig H and Ig κ allele, respectively, resemble wild-type mice more closely than their homozygous counterparts. Therefore, we argue that homozygous 3-83Igi and classical 3-83Ig transgenic B cells escape receptor editing on H-2^d by virtue of the nonphysiological make-up of their Ig loci.

Indeed, homozygous and hemizygous 3-83Igi B cells develop with significant differences. Homozygous B cell progenitors, like those from the classical 3-83Ig transgenics (18, 41), seem to develop directly into $sIgM^{high}$ B cells. This could be due to an increased level of IgM expression early on, because of the unphysiological gene dosage, or because progenitors carrying V gene rearrangements in both Ig H and Ig κ loci progress more rapidly in development than others. An accelerated differentiation to the stage of the so-called transitional B cells—i.e., IgM^{high} immature cells (42)—would imply a rapid loss of V(D)J-recombination-activating gene (RAG) expression (43), making the cells less prone to undergo receptor editing (44). This fact could explain the higher signaling threshold discriminating autoreactive from nonautoreactive B cells that is observed in the homozygous 3-83Igi and the conventional 3-83 transgenic mice as compared with the hemizygous 3-83Igi animals, namely the induction of receptor editing on H-2^b but not H-2^d in the former, and on both H-2^b and H-2^d in the latter (refs. 16 and 18 and this paper).

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