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Development of Myelin Oligodendrocyte Glycoprotein Autoreactive Transgenic B Lymphocytes: Receptor Editing In Vivo After Encounter of a Self-Antigen Distinct from Myelin Oligodendrocyte Glycoprotein

Tobias Litzenburger,* Horst Blüthmann,† Patricia Morales,‡ Danielle Pham-Dinh,§ André Dautigny,§ Hartmut Wekerle,* and Antonio Iglesias†*

We explored mechanisms involved in B cell self-tolerance against brain autoantigens in a double-transgenic mouse model carrying the Ig H-chain (introduced by gene replacement) and/or the L-chain κ (conventional transgenic) of the mAb 8.18C5, specific for the myelin oligodendrocyte glycoprotein (MOG). Previously, we demonstrated that B cells expressing solely the MOG-specific Ig H-chain differentiate without tolerogenic censure. We show now that double-transgenic (THκMOG) B cells expressing transgenic Ig H- and L-chains are subjected to receptor editing. We show that in adult mice carrying both MOG-specific Ig H- and L-chains, the frequency of MOG-binding B cells is not higher than in mice expressing solely the transgenic Ig H-chain. In fact, in THκMOG double-transgenic mice, the transgenic κMOG L-chain was commonly replaced by endogenous L-chains, i.e., by receptor editing. In rearrangement-deficient RAG-2−/− mice, differentiation of THκMOG B cells is blocked at an immature stage (defined by the B220MOG IgM−IgD− phenotype), reflecting interaction of the autoreactive B cells with a local self-determinant. The tolerogenic structure in the bone marrow is not classical MOG, because back-crossing THκMOG mice into a MOG-deficient genetic background does not lead to an increase in the proportion of MOG-binding B cells. We propose that an as yet undefined self-Ag distinct from MOG cross-reacts with the THκMOG B cell receptor and induces editing of the transgenic κMOG L-chain in early immature B cells without affecting the pathogenic potential of the remaining MOG-specific B cells. This phenomenon represents a particular form of chain-specific split tolerance. *The Journal of Immunology, 2000, 165: 5360–5366.

Myelin oligodendrocyte glycoprotein (MOG) is a brain autoantigen with intriguing properties. First, increasing evidence suggests that MOG-specific T and B cells play a crucial role in inflammatory demyelinating disease of the CNS in both experimental and clinical situations (1–4). Second, MOG has several properties setting it apart from other myelin proteins. It is exclusively localized on the outer surface of the myelin sheath, where it can be readily attacked by specific Ab mediating demyelination (5, 6). In addition, the expression of MOG is restricted to the CNS, and it appears late during development of myelin (7–11).

Several mechanisms of B cell tolerance have been identified in Ig-transgenic animal models (12–17). Clonal deletion and receptor editing occur at distinct developmental stages during B cell development in the bone marrow (18). On contact with self-Ag, immature B cells are preferentially induced to undergo receptor editing, i.e., secondary rearrangements of the Ig genes to alter their self-reactive specificity (16, 17). Cells that fail to edit their autoreactive Ag receptors are prone to apoptotic deletion even before entering the mature B cell pool.

Would any of these mechanisms act during the development of MOG-specific B cells? Alternatively, considering that recirculating MOG-specific Abs remain innocuous as long as the tight endothelial blood-brain barrier prevents them from reaching their target structure on the myelin surface, would MOG-autoreactive B cells be generated in bone marrow and spleen without any self-tolerogenic control?

We have recently generated an Ig H-chain “knock-in” transgenic mouse (denominated TH) to retrace the function and fate of MOG-reactive B cells in vivo (4). The Ig H-chain V region was derived from the MOG-specific mAb 8.18C5, an Ab, which induces strong demyelination and clinical exacerbation in experimental autoimmune encephalomyelitis experiments (1, 6). The TH mice showed a diverse B cell repertoire with about one-third of the B cells binding MOG. Thus, in this transgenic setup, the MOG-specific B lymphocytes were not tolerized and secreted MOG-binding Ab, which, on opening the endothelial blood-brain barrier, caused substantial myelin destruction. The absence of B cell tolerance in this case is possibly due to clonal ignorance, because under normal circumstances MOG is sequestered behind the blood-brain barrier and does not interact with peripheral immune cells.

In this study, we show that transgenic B cells expressing both the Ig H- and Ig L-chain of the 8.18C5 mAb are eliminated from the mature B cell repertoire. In Ig H- L-chain-transgenic mice, the transgenic κMOG L-chain is silenced by editing in immature B cells of the B220MOG IgM−IgD− phenotype. In addition, we show that elimination of this particular specificity is not mediated by MOG itself but rather by another cross-reacting self-Ag(s).

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2 Abbreviations used in this paper: MOG, myelin oligodendrocyte glycoprotein; pre-BCR, pre-B receptor complex.
Materials and Methods

Transgenic mice

The variable region of the L-chain gene was cloned from the MOG-specific hybridoma clone 8.18C5 (7) and inserted in an expression vector including the Ck region, the κ intron enhancer, and the κ 3’-enhancer. A 1.7-kb SacI fragment of genomic DNA containing the rearranged V\textsubscript{K}mog gene was subcloned from a larger clone isolated from a genomic λ phage library of the hybridoma 8.18C5. This DNA fragment including 1 kb 5’-regulated and untranslated sequences, the two exons encoding the leader sequence, and the entire V\textsubscript{K}k mog rearranged gene and the intron between them was then inserted 5’ of the Ck intron sequence in transcriptional orientation, followed by a 1.1-kb DNA fragment containing the κ 3’-enhancer. The entire sequence of the V\textsubscript{K}mog gene, including coding exons and the intron between leader sequences and V\textsubscript{K}, as well as 125 bp of the region 5’ of the AUG codon including promoter sequences was determined. The obtained sequence corresponded to the germ-line V\textsubscript{K}k gene joined to J\textsubscript{K}S. On comparison with known Ig genes, the V\textsubscript{K}mog gene was found to be identical with the κ chain gene of the phosphorylcholine-specific hybridoma 253.15D10 (19), except for a single silent point mutation at aa 110 (CTG/Leu→TTG/Leu) in the J\textsubscript{K} region of our V\textsubscript{K}mog gene. This point mutation served for unequivocal identification of the transgenic sequence, where the expression of κ genes was studied in immune organs.

For generation of transgenic mice, a 8.5-kb EcoRI fragment of genomic DNA was separated from vector sequences and injected into fertilized oocytes of (C57BL/6 × CBA) F\textsubscript{1} mice. Three lines of \textsuperscript{κ}mog-transgenic mice were obtained, bearing 2–3, and 8, and 20 copies of the κ transgene. The two κ-transgenic lines with the higher copy numbers of the transgene were crossed back into C57BL/6 and with the TH knock-in mice. The TH knock in mice were derived from doubly transgenic TH and κ\textsuperscript{mog} mice as described previously (4). Both TH- and κ\textsuperscript{mog}-deficient mice were kindly provided by Dr. H. Mossmann (Max-Planck-Institut für Immunobiologie, Freiburg, Germany). The generation and phenotype of MOG knockout mice will be described elsewhere (D. Pham-Dinh, P. Daubas, C. Vizler, C. Delarasse, D. Dimitri, J. Bauer, A. Dietrich, M. LeMeur, G. Roussel, J.-L. Nassbaum, R. Liblau, and A. Dautigny, manuscript in preparation).

Flow cytometric analysis

Single-cell suspensions were prepared from spleen and bone marrow of adult (6- to 8-wk-old) mice or from spleen and liver of first day newborn mice. RBC were lysed by incubation in 0.165 M NH\textsubscript{4}Cl for 10 min. Cells were washed with PBS/1% FCS and stained with the following Ab conjugates: anti-IgM-b-FITC (clone DS-1; PharMingen, San Diego, CA); anti-IgG1-b-FITC (clone AF6-78, PharMingen); goat anti-mouse IgM-biotin (Southern Biotechnology Associates, Birmingham, AL); anti-B220-FITC (PharMingen); anti-IgD-FITC (PharMingen); and biotin-labeled recombinant MOG. The biotinylated mAb 493 (20) was supplied by Ton Rolink (Basel Institute for Immunology, Basel, Switzerland). The rabbit anti-

Western blot analysis

Protein extracts of mouse brain and tMOG were run on a 12.5% polyacrylamide gel and blotted onto a nitrocellulose membrane (Hybond ECL, Amersham International, Arlington Heights, IL). The blot was probed with biotinylated 8.18C5 mAb (1:1000) or anti-actin mouse mAb (1:1000, Amersham) and detected with streptavidin-peroxidase (PharMingen) and peroxidase-conjugated goat anti-mouse Ig (Jackson ImmunoResearch), respectively. The signals were developed with the ECL system according to the instruction of the manufacturer (Amersham).

Results

Generation of \textsuperscript{κ}mog-transgenic mice

We generated Ig-transgenic mice the B cells of which share their specificity with the MOG reactive mAb 8.18C5, an Ig with a highly demyelinating potential (23). We cloned the Ig H- and L-chain genes from the 8.18C5 hybridoma, and the rearranged V\textsubscript{H} region was used for the generation of the V\textsubscript{H} knock-in (TH) mice, as described previously (4). Genomic DNA including the rearranged V\textsubscript{K}mog gene as well as intron and Ck 3’-enhancer was injected into oocytes to produce conventional Igl-transgenic mice (designated \textsuperscript{κ}mog).

Both TH knock-in and \textsuperscript{κ}mog-transgenic mice were crossed into the C57BL/6 strain. To generate double-transgenic animals (TH\textsuperscript{κ}mog), TH and \textsuperscript{κ}mog-transgenic mice of the fourth backcross into C57BL/6 were bred. The functional expression of the transgenic \textsuperscript{κ}mog gene was demonstrated both by the presence of V\textsubscript{K}mog in-frame transcripts in the spleens of transgenic mice and by the association with the transgenic Ig H-chain on the surface of immature B cells (see below).

B cell phenotype of adult TH\textsuperscript{κ}mog mice

In \textsuperscript{κ}mog single-transgenic mice, the \textsuperscript{κ}mog L-chain does not contribute to MOG specificity. There are neither MOG-specific B cells nor Ab detectable in these animals (not shown). In striking contrast, we had shown before (4) that in knock-in single-transgenic TH mice ~30% of their B cells bind MOG (Fig. 1), although the B cell repertoire is diverse. The transgenic Ig H-chain combines with some, but not all, endogenous L-chains to reconstitute MOG specificity. The MOG-binding pattern of specific B cells is heterogenous, ranging from very low to high density by identical surface IgM (Fig. 1), but MOG-reactive B cells are not demonstrably tolerated and seem to undergo normal development (4).

 Unexpectedly, B cells isolated from spleen or bone marrow of double-transgenic TH\textsuperscript{κ}mog mice showed a frequency of MOG-binding cells indistinguishable from that of single-transgenic TH mice (Fig. 1). Furthermore, the titer of MOG-specific Ab in the serum of TH\textsuperscript{κ}mog mice was not higher than in TH mice. The absence of an increased frequency of MOG-reactive B cells cannot be explained by clonal deletion (12, 13, 24), because the total number of (B220\textsuperscript{IgM}+) B cells in the spleen and bone marrow of

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THκmog mice was not reduced, as compared with TH mice or wild-type controls (Fig. 1). We also exclude anergy, given that the concentrations of surface IgM as compared with surface IgD are normal in these mice (Fig. 1, top). Bottom, Profile of IgM-positive MOG-binding B cells. IgMα allotype corresponds to the transgenic κ-chain whereas wild-type (WT) mice carry the IgMβ allotype on both H chain alleles. Data are representative of three experiments with similar results.

Expression of transgenic κmog transcripts was demonstrated by RT-PCR analyses of total mRNA from spleen of single-transgenic adult κmog mice (not shown) and neonatal liver of double-transgenic THκmog mice (see below). Functional expression of transgenic κmog L-chains was also demonstrated on the surface of B cells in the liver of neonatal THκmog and in the bone marrow and the spleen of adult THκmog/RAG-deficient mice (see below).

Transgenic Igκmog expression in immature B cells

To track the regulation of the κmog transgene at early stages of differentiation, we analyzed immature B cells in immune organs from neonatal mice. Like other Ig-transgenic mice, our transgenics showed a global acceleration of B cell development, best explained by the presence of preformed Ig (H or L) genes. The ratio of B220+ IgM+ to immature B220+ IgM− pre-B cells was 2-fold higher in the neonatal liver of single-transgenic TH mice than in wild-type littermates (1 vs 0.66, Table I), and this ratio was further elevated in double-transgenic THκmog neonates (2 vs 1, Table I).

More specifically, the proportion of MOG-binding IgM+ B cells in the liver of double-transgenic THκmog neonates is higher than in single-transgenic TH littermates (Table II and Fig. 2). In fact, double staining with IgMα allotype-specific Ab (which detect the TH chain) and biotinylated MOG reveals a tight correlation between IgM surface density and MOG binding intensity in THκmog neonates (Fig. 2, top). This particular staining pattern, which was found in THκmog, but much less in TH pups, indicates a B cell population expressing both transgenic IgH- and L-chains exclusively.

Transgenic Igκmog expression in anergic B cells

The proportion of MOG-binding B cells within this IgM+ MOG+ population is higher in the liver than in the spleen of neonatal THκmog mice (Fig. 2, top, and Table II). This distinctive B cell phenotype prevailed in perinatal THκmog mice, whereas it decreased in the spleen within the first 6 days after birth. By then, the MOG-binding pattern of splenic B cells is indistinguishable in THκmog and TH mice and comparable to the phenotype of B cells in adult transgenic mice (Table II).

To identify the transgenic L-chains during perinatal development, we separated neonatal liver cells into MOG-binding- and -nonbinding B cell fractions and analyzed their L gene transcripts by RT-PCR. As shown in Table III, in MOG-binding B cell populations the transgenic κmog sequence was predominantly expressed (representing 10 of 12 analyzed Ig κ sequences), whereas nonbinding B cells preferentially used endogenous L genes (only 4 κmog sequences found in 36 Ig κ sequences analyzed). The identity of the κmog transcripts was confirmed by sequencing (not shown). These data, together with the Ig κ sequences found in fetal liver B cells of TH mice (Table III), demonstrate that predominant expression of κmog in the MOG+ fraction of THκmog fetal liver is not due to an unspecific preference of similar κ sequences within this B cell compartment. We conclude that in neonatal THκmog mice, the transgenic κmog L-chain actively suppress endogenous L gene expression by allelic exclusion. During further development, however, the transgenic κmog L-chain is replaced by endogenous L-chains, presumably through receptor editing.

**RAG expression in IgM+ B cells from neonatal double-transgenic THκmog liver**

The concept of receptor editing implies that encounter of immature IgM-positive B cells with a self-Ag induces secondary Ig gene rearrangements through reactivation of Ig-recombining genes like...
RAG-1 and RAG-2 (25–27). To learn whether receptor editing was involved in replacing $\kappa^{\text{meg}}$ by endogenous L-chains, we analyzed sorted, IgM$^+$ MOG-binding B cells from neonatal liver of TH$\kappa^{\text{meg}}$ and TH mice for expression of RAG genes (Fig. 3). As in normal B cell differentiation, where the appearance of surface IgM coincides with a shutdown of the rearrangement machinery, we detected neither RAG-1 nor RAG-2 in the IgM$^+$ MOG-binding cells from single-transgenic TH neonatal liver (Fig. 3). In contrast, both RAG-1 and RAG-2 transcripts were detected in the MOG-binding cell fraction from neonatal double-transgenic TH$\kappa^{\text{meg}}$ liver (Fig. 3). Transcription of RAG-1 and RAG-2 was also detected in the excluded fractions from both TH$\kappa^{\text{meg}}$ and TH mice, given that this fraction includes surface Ig-negative pre-B cells, actively rearranging endogenous L-chains. Persistent or reactivated Ig rearrangement in IgM-positive B cells of neonatal TH$\kappa^{\text{meg}}$ mice explains the observed expression of endogenous L genes. Receptor editing in such early TH$\kappa^{\text{meg}}$-transgenic B cells leads to silencing of the transgenic $\kappa^{\text{meg}}$ gene and expression of endogenous L genes. However, like in single-transgenic TH mice, this process does not abrogate MOG specificity totally, because about one-third of endogenous L-chains generate MOG binding on pairing with the TH chain (Fig. 1 and Table II).

**Transgenic TH$\kappa^{\text{meg}}$ B cells on a RAG-2-deficient background**

What would be the destiny of self-reactive transgenic B cells without rescue by receptor editing? We crossed the RAG-2 knockout mutation into the TH$\kappa^{\text{meg}}$-transgenic mice and followed the appearance of B lymphocytes in these animals. As expected, IgM$^+$ B cells are completely absent both in the adult parental RAG-2$^{-/-}$ (28) and in TH/RAG-2$^{-/-}$ mice (Fig. 4). In contrast, in TH$\kappa^{\text{meg}}$/RAG-2$^{-/-}$ mice, a small but significant population (bone marrow) of B220$^+$ IgM$^+$ B cells is reproducibly observed in both bone marrow and spleen (Fig. 4, marked region of upper panels). Interestingly, all of these B220$^{\text{low}}$IgM$^{\text{low}}$IgD$^{-\text{low}}$ B cells bind MOG in the “monospecific” staining pattern also seen in neonatal liver cells of TH$\kappa^{\text{meg}}$ mice (Figs. 2 and 4, middle panels). Judging from their binding of the recently described mAb 493, a further marker of immature B lymphocytes (20) (Fig. 5), excluding the possibility that these cells represent a special form of mature, functionally inactivated, anergic B cells (29). As shown in Fig. 5, IgM$^+$ 493$^+$ mature B cells are not present in the spleen of TH$\kappa^{\text{meg}}$/RAG-2$^{-/-}$ mice and the small population of IgM$^{\text{low}}$493$^+$ B cells is absent in the RAG-2$^{-/-}$ nontransgenic littermates.

In TH$\kappa^{\text{meg}}$/RAG-2$^{-/-}$ mice, in which rearrangement of endogenous Ig genes is blocked, the B cells rely on the transgenic Ig chains to sustain development. In this situation, the transgenic B cells are clearly unable to proceed to full maturation and are blocked at the immature stage defined by the B220$^{\text{low}}$IgM$^{\text{low}}$IgD$^{-\text{low}}$ phenotype. These immature B cells in the bone marrow

| Table III. $\kappa$-chain usage in transgenic neonatal liver B cells |

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<th>Cell Fraction</th>
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**FIGURE 3.** Expression of RAG-1 and RAG-2 in sorted neonatal liver cells. Liver cells were prepared from three to four neonatal (day 0) TH$\kappa^{\text{meg}}$ or TH mice, incubated with biotin-conjugated recombinant MOG, and sorted with streptavidin-coupled magnetic beads. RNA was prepared from the fractions of MOG-binding (+) and nonbinding (−) liver cells, and expression of RAG-1, RAG-2 and β-actin was analyzed by RT-PCR. RNA derived from wild-type bone marrow (BM) cells was used as positive control. The amplified DNA fragments were identified on an ethidium bromide-stained agarose gel.

**FIGURE 4.** Surface phenotype of bone marrow (A) and spleen (B) cells isolated from mice of the indicated phenotype. Cells were stained with FITC-labeled Abs specific for B220, IgM$^+$ (or IgM$^-$ in the case of RAG$^2^{-/-}$ mice, whose genetic background is of IgM allotype b and do not bear transgenic IgM$^+$), and IgD, and with biotinylated anti-IgM Abs or recombinant MOG. Biotin-labeled cells were then stained with streptavidin-PE. B220$^+$IgM$^+$ B cells within the marked region in the upper panels are present only in TH$\kappa^{\text{meg}}$RAG$^+/-$ and TH$\kappa^{\text{meg}}$RAG$^-/-$ mice, but not in TH RAG$^+/+$ or RAG$^-/-$ mice. **Middle panels,** Percentage of MOG$^+$IgM$^+$ B cells in the different mice.
and the spleen of THκmog/RAG-2−/− mice cannot proceed in further differentiation (Figs. 4 and 5). In RAG-2-sufficient THκmog mice, in contrast, expression of the transgenic κmog gene is abolished by editing, and development can reach the fully mature stage of B220+ IgM+IgD− cells found in peripheral immune organs (Figs. 1B and 5).

MOG gene expression does not affect differentiation of MOG-specific transgenic B cells

The present results suggest that the transgenic, MOG-specific B cells are affected by self-tolerizing processes that result in receptor editing. However, they do not reveal the nature of the censoring self-determinant involved in tolerization. To explore the possible participation of the MOG gene products, we crossed TH and THκmog mice into a MOG knockout mutant, i.e., completely devoid of MOG protein (Fig. 6A). In the absence of MOG, the phenotype of MOG binding B cells in TH and THκmog mice is identical with that observed in MOG-sufficient animals (Fig. 6B, top). This experiment also excludes a possible editing of the TH gene, as the number of Id-positive B cells in the MOG−/− backcross is comparable with that found in MOG-expressing mice (Fig. 6B, bottom). Thus, MOG per se cannot be responsible for the tolerization of the transgenic specificity in THκmog mice.

Discussion

We have conducted experiments designed to explore whether (and which) mechanisms of self-tolerance could affect the development of B lymphocytes specific for a brain myelin autoantigen, MOG. The paradigm used was a transgenic gene replacement mouse carrying in its genome the rearranged Ig genes of the MOG-reactive hybridoma 8.18C5. Previous work had demonstrated that in single-transgenic mice the 8.18C5-derived IgH-chain is expressed along with endogenous κ-chains without any apparent impairment and that this IgH chain alone confers MOG specificity in about one-third of the B cells (4). We now report that, in striking contrast, in double-transgenic mice carrying both the IgH and L genes of 8.18C5 (THκmog), the expression of the transgenic κmog gene is not permitted but is replaced by endogenous L-chains.

Ig receptor editing on contact with self-determinants is one of the cardinal mechanisms to generate and maintain immunological self-tolerance on the B cell level. To date, it has been mainly been studied in immature B cells arising in the bone marrow of adult mice, leaving neonatal B cell tolerance less well characterized (30). In our system, in neonatal liver and spleen of double-transgenic THκmog mice, a high proportion of B cells expressed the transgenic B cell receptor, as indicated by the Igκ/MOG double staining pattern characteristic of monospecific B lymphocytes. As may be expected, the MOG-binding B cells almost exclusively expressed κmog gene transcripts, showing that at this developmental stage the cells are not yet completely tolerized. However, the expression of RAG-1 and RAG-2 genes in this cell subset points to an ongoing process of receptor editing. In contrast, neonatal B cells, which did not bind MOG, predominantly expressed endogenous L-chains, indicating replacement of the transgenic κmog chain as a result of editing.

The molecular nature of the mechanism(s) responsible for obliteration of the transgenic κmog chain in THκmog mice remains speculative. In an anti-DNA Ig transgenic model, Gay et al. (16) proposed that editing would occur at the level of IgH- and L-chain pairing. Our system seems to rely primarily on transcriptional down-regulation, as indicated by the predominance of endogenous Igκ transcripts over transgenic κmog transcripts in edited THκmog−/− transgenic B cells. Editing of the Ig receptor in THκmog mice affects only the κmog L-chain, although replacement inactivation of the knocked-in TH gene would also alter the transgenic specificity, and can be demonstrated upon immunization of TH mice (31). Yet the Id encoded by the TH transgene is maintained in double-transgenic THκmog B cells in a proportion similar to that in single-transgenic TH B cells (Fig. 6 and Ref. 4). This type of secondary rearrangement has been described also in other IgH knock-in mice.
(32–35). In fact, some of these transgenic models suggest that the \( k \)-chain locus is the preferential target for receptor editing (25, 26).

The lack of an enhanced MOG-binding fraction of B cells in TH\( k^{mog} \) double-transgenic mice, as compared with TH single-transgenic animals, could alternatively be interpreted to indicate imperfect fit of the transgenic \( k^{mog} \) L-chain with the knock-in TH chain. This could result in insufficient allelic exclusion of endogenous L-chains and/or lack of positive selection of the transgenic Ig pair. Our experimental observations, however, clearly contradict this interpretation. First, in the MOG\( ^+ \) fraction of fetal liver and in the RAG-deficient background, the transgenic \( k^{mog} \) is able to form disulfide bonds with the TH chain and be efficiently anchored onto the surface of IgM\( ^+ \) B cells (Figs. 2 and 4). The concentrations of surface IgM on these cells is comparable with that of normal cells at the same developmental stage (Figs. 2 and 4). Second, we show that early B cells in fetal liver of double-transgenic mice include a large fraction (the MOG-binding one) in which the transgenic \( k^{mog} \) chain is expressed almost exclusively. As shown in Table III, \( \geq80\% \) of expressed L-chains correspond to the transgenic \( k^{mog} \) chain. This percentage is reduced to \( \leq11\% \) in the MOG-nonbinding fraction, as a result of the action of receptor editing on the MOG-binding fraction. Taken together, 14 of 48, or \( \leq30\% \), of all \( k \) chains expressed in TH\( k^{mog} \) fetal liver are of transgenic origin. Interestingly, in adult spleens of \( k^{mog} \) single-transgenic mice, we find a similar proportion of \( k^{mog} \) transcripts (32%), as revealed in similar RT-PCR analysis of L-chain usage, whereas in TH\( k^{mog} \) adult spleens this proportion is further reduced to 7% (data not shown). These data reinforce the interpretation that the combination of TH and \( k^{mog} \) chains generates a nonpermitted autospecificity, without excluding the possibility that the \( k^{mog} \) chain by itself be autoreactive.

Third, during B cell development, two obligate checkpoints have been defined, at which B cells are positively selected based on structural requirements. In early, so-called pre-B II cells, newly formed \( \mu \) H-chains are tested for appropriate binding to surrogate L-chains and formation of the pre-B receptor complex (pre-BCR). Successful pre-BCR formation is followed by inhibition of H gene rearrangement, extensive proliferation, and, once proliferation ceases, by rearrangement of L-chain genes (see Ref. 36 for review). Now the surrogate L-chains are substituted by newly generated L-chains, and B cells must pass the second check point for selection, as H + L chain (IgM) complexes are checked for adequate binding and surface deposition. Only B cells are now selected that express a good fitting pair of Igs composed of \( \mu \) and a L-chain and able to form membrane-bound IgM. B cells unable to express a BCR on their surface undergo rapid apoptosis (Ref. 36 and references therein). Such “immature,” IgM\( ^+ \)IgD\(^-\) B cells can now leave the bone marrow and colonize the spleen, where they develop into IgM\( ^+ \)IgD\(^-\) mature B cells, provided that they do not react against self-components. This last transition is thus governed by negative rather than positive selection principles. On the basis of this model, we interpret the appearance of IgM\( ^+ \)IgD\(^-\)-low MOG\( ^+ \) B cells in the spleen of TH\( k^{mog} \)-RAG\(^-/-\) mice as demonstration of successful positive selection of the transgenic BCR and its subsequent negative selection at the stage of “transitional” B cells. The principle governing the transition from IgM\( ^+ \)IgD\(^-\) into IgM\( ^+ \)IgD\(^+\) B cells is not yet understood. Thus far, no experimental evidence exists to invoke a positive principle as being active at this stage.

Recently, Melamed et al. (18), studying B cell development in cultures of adult bone marrow, have identified the B220\(^{low} \) IgM\(^{low} \)IgD\(^-\) cells as the subset of immature B cells, where receptor editing occurs during B cell differentiation. According to this model, immature B220\(^{low} \)IgM\(^{low} \)IgD\(^-\) B cells that fail to alter their self-reactive specificity by editing during a limited time window will be eliminated by apoptotic deletion at the following B220\(^{low} \)IgM\(^{high} \)IgD\(^-\)-stage. Because B cells in TH\( k^{mog} \) mice develop into IgM\(^+\)IgD\(^{low} \) B lymphocytes and because we observe editing of the \( k^{mog} \) chain at this stage in fetal liver, we conclude that receptor editing abolishes a self-reactive specificity distinct from MOG, as given by the combination of the two transgenic Ig chains.

Why are MOG-binding B cells that simultaneously express both transgenic specific Ig H- and L-chains censored while at the same time MOG-binding B cells using the same Ig H-chain but different L-chains develop normally? It appears that an unknown self-structure present in the B cell compartments controls initiation of editing. MOG is not expressed outside the CNS (9). Although we assume that in wild-type as well as in double transgenic TH\( k^{mog} \) mice, the formation of the MOG-specific repertoire occurs in the absence of the nominal Ag, this is definitely the case in MOG knockout mice (Fig. 6). There is no difference in MOG-specific B cell development between MOG-expressing and MOG-deficient mice. Indeed, all our data indicate that the MOG-specific B cell receptor in TH\( k^{mog} \) double-transgenic B cells is confronted with a self-Ag distinct of MOG and that this encounter is responsible for the induction of tolerance. The nature of the self-Ag(s) cross-reacting with MOG is unknown. Preliminary attempts to detect putative autoantigen(s) in tissue prepara-tions using the 8.18C5 Ab failed. However, it is known that B cell receptor editing can be induced by very low affinity interactions that may be difficult to measure (37, 38). We are currently testing butyrophilins, a large protein family that, apart from MOG, contains numerous milk proteins and costimulatory molecules of the B7 series (39–41).

Considering the highly efficient tolerization of double-transgenic TH\( k^{mog} \) B lymphocytes in vivo, the derivation of the original hybridoma 8.18C5 appears enigmatic. At least in theory, the hybridoma precursor B cell should have been edited in the donor mouse, just as the double-transgenic B cells in our experiments are edited. Alternatively, the original B cell may have acquired its MOG/self specificity at a mature stage, after immunization. However, as stated before, the V region of the \( k^{mog} \) transgene represents an unmutated Vx\( ^c \)-gene, identical with a Vx\( ^c \) gene previously found in a phosphorylcholine-specific hybridoma (19). In addition, the only bona fide somatic mutation found in the V region of the TH gene corresponds to a silent point mutation (ACT-Thr\( \rightarrow \)ACG-Thr) within the rearranged DSP2.7 element (4). Therefore, extensive somatic mutation seems not to explain the heteroclitic specificity associated with the nominal MOG specificity of 8.18C5.

Alternatively, the autoreactive B cell clone could have been derived from the B-1 subset of lymphocytes, a population of mainly peritoneal B cells enriched for self-reactivity. Our finding that TH (and TH\( k^{mog} \)) mice are depleted of peritoneal B-1 cells and that the few B-1 cells left in these mice express preferentially endogenous Ig’s may argue against this possibility (Ref. 4 and data not shown). Finally, some B cells expressing autoreactive IgG but not IgM are viable and provoke autoimmunity in transgenic mice (42, 43). There is the possibility that under the conditions of repeated immunization, rare, autoreactive, MOG-specific B cell clones, like 8.18C5, sneaking through tolerogenesis in bone marrow were recruited to undergo isotype switch and escape tolerance.

Our data show that the developing immune system is able to distinguish between self-specificity defined by the TH chain alone (MOG), which is not recognized and censored outside the CNS, and a second self-specificity given by the combination of the TH chain with this particular \( k^{mog} \) L-chain. We propose that through this second specificity TH\( k^{mog} \) B cells bind to an autoantigen cross-reactive with MOG and become efficiently tolerized via editing of their transgenic L-chain without eliminating the MOG.
binding potential. This particular form of chain-specific split tolerance within B cells results in divergent tolerance and persistence of an organ-specific, potentially pathogenic autoreactive clone.

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References