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Subtle Effects on Myelin Basic Protein-Specific T Cell Responses Can Lead to a Major Reduction in Disease Susceptibility in Experimental Allergic Encephalomyelitis

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The presence of potentially autoreactive T cells is a necessary, but not sufficient, condition for the development of autoimmune disease. However, the relationship between T cell response and susceptibility to disease is not straightforward. In this report, we use experimental allergic encephalomyelitis as a model to demonstrate that subtle alterations of the T cell response to an encephalitogenic epitope are sufficient to cause a dramatic decrease in disease susceptibility. Transgenic expression of a fusion protein of hen egg lysozyme and an encephalitogenic peptide of myelin basic protein (MBP) residues 84–105, coexpressed with MHC class II, causes profound tolerance to hen egg lysozyme, while maintaining a near normal response to MBP. Detailed analysis of the T cell repertoire of transgenic animals using a panel of T cell hybridomas revealed a highly selective loss of one minor component of the response to the MBP 84–104 region. Despite this, transgenic animals were highly resistant to experimental allergic encephalomyelitis induction with the MBP peptide, indicating that minor changes to the T cell repertoire may result in major alterations in disease susceptibility. Possible reasons for this are discussed.

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the MBP response of the TG mice was sufficient to produce a highly significant reduction in the susceptibility of the mice to MBP-induced EAE.

Materials and Methods

**EH43.69 and E43.30 TG mouse lines**

TG mice that coexpressed residues 84–105 of mouse MBP with MHC class II have been previously described (14). This region of MBP contains the major encephalitogenic epitope in SJL mice (3, 16) and is expressed as a fusion protein using HEL as a “carrier,” with the MBP peptide embedded in one of the two loop regions of HEL (17). Briefly, TG mice expressing either mLM43 (EH43.69) or sLM43 (E43.30) were made as follows. A PvuII/BamHI digest of the pKCR7 plasmid with the LM fusion proteins was excised and replaced with the PvuII/BamHI fragment of pD015, containing the mouse MHC class II Eα promoter (15). The PvuII/BamHI fragment (as shown in Fig. 1) was microinjected into fertilized (C57BL/6 x SJL)F1 oocytes. Potential founders were identified by screening of tail DNA by standard Southern blotting techniques using an Eα promoter probe.

The principle line used in this paper contains the MBP insertion between positions 43 and 44 of HEL and was further modified at the C terminus by addition of the membrane and cytoplasmic domains of influenza A virus (A/PR/8/34) hemagglutinin (HA), amino acid residues 509–549 (18), resulting in a membrane-bound form of the HEL/MBP fusion protein (Fig. 1). EH43.69 contains three copies of the mLM43 transgene construct, shown in Fig. 1, and E43.30 contains a single copy of the sLM43 transgene construct (without the HA tm/cyto). S1-nuclease protection and RT-PCR could detect expression of this transgene. However, cell-surface expression could not be detected by conventional Ab staining and FACS analysis (data not shown).

Lines were continually backcrossed with SJL/J mice (The Jackson Laboratory, Bar Harbor, ME) for the first seven or eight generations and subsequently, for the EH43.69 line only, SJL obtained from Harlan (Olac, U.K.) Initial breeding was in a conventional mouse facility, but lines were rederived into an specific pathogen-free facility for the sixth and subsequent generations. The E43.30 line is not in continuous breeding.

**Peptides**

HEL112–129 (RNRCKGTDVQAWIRGCRL) was synthesized by NeoSys- tems (Strasbourg, France). Proteolipid protein (PLP) (139–151) (HLGK WLGHDPDKF) was synthesized by Research Genetics. MBP84–104 (VH FFKKNI VTPRTPPSQGGR), MBP84–96 (VHFFKNIVTPR), and MBP87–105 (FKNIVTPRTPPSQGGR) had all been synthesized at Laboratoire de Génétique Moléculaire des Eucaryotes (Strasbourg, France) and were >95% pure.

**Proliferation assays**

Mice were primed with the appropriate peptide or Ag dissolved in PBS, mixed in an emulsion of CFA together with H37-RA (Difco, Detroit, MI), at a final concentration of 4 mg/ml. Mice were primed in two sites s.c., on the dorsum of the foot and the tail base. After 10 days, (HEL, or 11 days (MBP assays), the popliteal lymph node (LN) on the side of injection and inguinal LN were removed under aseptic conditions. LN from individual mice were pooled, disrupted into a single-cell suspension, and reuspended in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with glutamine, penicillin and streptomycin, sodium pyruvate, 5 × 10⁻⁵ M 2-ME, and 1% normal mouse serum. Cells were plated out at 5 × 10⁶ cells per well in flat-bottom 96-well plates in quadruplicate, with the appropriate dilutions of Ag. Assays were incubated for a total of 96 h at 37°C 5% CO₂ with the addition of 1 μCi [³H]TdR for the final 18–20 h of culture, and proliferation was measured. Results are expressed as stimulation index, being the mean of experimental cpm for quadruplicate wells divided by the mean cpm in the absence of Ag. Background counts varied but were normally <15,000 cpm.

For IFN-γ estimation, 48 h supernatants from proliferation assays were tested by standard ELISA using the Quantikine M Mouse IFN-γ Immunoassay (R&D Systems, catalogue no. M1F00). Dilutions of supernatant were prepared at 1:2 and 1:20 in assay diluent. Concentration of IFN-γ was calculated using a standard curve. IL-4 and IL-10 concentrations were established in the same manner.

**T hybridomas**

The HEL-specific I-Aβ-restricted T cell hybridoma SHEL9 was derived from a fusion of an HEL-specific T cell line to the mouse BW58 αβ fusion partner. The cell line was originally grown from draining LN of SJL mice primed with HEL (Sigma, St. Louis, MO) and restimulated in vitro with HEL. This hybridoma recognizes the C-terminal peptide of HEL, residues 112–129, as well as native HEL.

The panel of MBP-specific T cell hybridomas was derived from two female EH43.69 TG mice and two of their non-TG littermates. The mice were primed with 50 μg MBP84–104 peptide (HPLC purified >95% pure) as for a proliferation assay (14). On day 11, draining LN were removed and a conventional proliferation assay set up with a proportion of the cells (Fig. 1A). The remainder of the cells were depleted of IgM-positive cells and adherent cells by panning. Cells for fusion were restimulated in a 24-well tissue culture plate with 2 × 10⁵ responder cells and 3 × 10⁶ mitomycin C-treated SJL spleen cells per well, in the presence of 100 μg/ml bovine MBP (BMBP) (Sigma). Cells were fused to the BW58 αβ fusion partner at day 6 following addition of IL-2 to the cultures at day 3 and plated out in limiting dilution. On an initial screen, 146 of 169, 77 from the TG mice and 69 from the non-TG mice, recognized whole MBP presented on I-Aβ-transfected L cells. Of these, 63 were stable enough to continue growing for further analysis. Vβ usage was determined by standard staining techniques using Vβ-specific Abs. All of these hybridomas were reactive to both MBP and to the longest peptide MBP84–104 and restricted through I-Aβ. Statistics were performed using Arcus Pro-II.

**Ag presentation assays**

T cell hybridomas were plated out in duplicate in 96-well flat-bottom plates at 2–3 × 10⁵ cells/well together with stimulator cells and Ag where appropriate. Stimulator cells were either various L cell transfectants at 2–3 × 10⁵ cells per well, or spleen cells at 3 × 10⁵ cells per well, or at the cell numbers shown for the assay (Fig. 2). After 20–24 h incubation, 50 μl supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay: supernatant was assayed for IL-2 production in a standard assay. Supernatants were tested in triplicate. IL-2 activity was measured by the ability to stimulate 1 × 10⁵ CTLL-2 cells and incubated overnight then pulsed with 1 μCi [³H]TdR per well for the final 4–6 h of incubation. Results are expressed as cpm proliferation of CTLL-2 cells.

**EAE induction**

Mice for EAE induction had been backcrossed at least nine times to SJL. Mice were between 8–12 wk of age. On day one, mice were challenged with MBP84–104 (HPLC purified >95% pure) in CFA, 100 μg/mouse, or PLP84–104 (as for a proliferation assay). Then, 24 and 72 h after challenge, mice were given an i.v. injection of pertussis toxin (catalogue no. 180, List Biological Laboratories, Campbell, CA) 0.125 μg/100 μl PBS per injection per mouse.
Mice were assessed for general health status, weight, and EAE score from day 9 (day 6 for PLP peptide), every 1–2 days. EAE was scored as follows: 0, normal; 1, flaccid tail; 2, evidence of hind limb paralysis; 3, complete hind limb paralysis; 4, fore and hind limb paralysis. Animals were sacrificed 60 days after induction or earlier if severe symptoms developed. Before the commencement of experiments, tail DNA from experimental animals was stored, untyped. At the termination of the experiment, animals were sacrificed, and a further DNA sample was taken. Both sets of DNA were analyzed for the presence of the transgene by standard Southern blotting techniques, to assign the genetic status of each animal. Statistics were performed using arcsin Pro-II. Where histological sections were required, animals was stored, untyped. At the termination of the experiment, animals were sacrificed, and a further DNA sample was taken. Both sets of DNA were analyzed for the presence of the transgene by standard Southern blotting techniques, to assign the genetic status of each animal. Statistics were performed using arcsin Pro-II. Where histological sections were required, animals were removed and fixed in 4% paraformaldehyde. Paraffin-embedded sections were stained with hematoxylin and eosin and analyzed by light microscopy.

Results

Presentation of the endogenously synthesized transgene protein by splenic APC

Spleen cells from a number of the TG lines previously described (14) were able to present MBP or HEL, originating from the transgene protein, to selected T cell hybridomas without addition of exogenous Ag. Fig. 2A shows the recognition of the EH43.69 TG spleen cells by one of a subset of MBP-specific I-A<sup>+</sup>-restricted T cell hybridomas. For comparison, recognition of spleen cells from another transgenic line, E43.30, which carries very low copy numbers of the transgene construct with the secreted form (no HA transmembrane region) of the same protein, is also shown. Fig. 2B shows recognition of the secretor line spleen cells only by the I-A<sup>+</sup>-restricted, HEL-specific T hybridoma SHE9. Spleen cells from all the lines were equally efficient at presentation of OVA to an OVA-specific T cell hybridoma (data not shown).

HEL/MBP TG mice have dramatically reduced responses to HEL

To study the effect of the transgene on development of the T cell repertoire, mice were challenged with native HEL and the draining LN proliferative responses assessed. Fig. 3, A and B shows responses to either native HEL or a synthetic peptide, HEL residues 112–129 (day 9 for PLP peptide). HEL 112–129 shows a profound reduction in response to native HEL, while retaining some anti-peptide response. In two separate experiments, animals were primed in vivo with HEL 112–129 50 µg/mouse in CFA. Draining LN cells were restimulated in vitro with either HEL (C and E) or HEL 112–129 peptide (D). Filled symbols are from TG mice, and open symbols are from non-TG mice. All mice responded to purified protein derivative (or H37RA) restimulation with a stimulation index of >8.

Anti-MBP T cell responses in HEL/MBP TG mice

MBP contains a number of epitopes recognized in SJL mice (19). Therefore, to ensure that we were looking only at responses to our chosen region, animals were primed in vivo with peptide MBP<sub>84–104</sub> followed by an in vitro challenge with native MBP. Fig. 4, A–C shows results of three separate experiments comparing
the anti-MBP response of the TG animals with that of non-TG animals. Although there was a trend to a higher response in the non-TG mice in some assays, this result was inconsistent. Fig. 4D shows IFN-γ release in supernatants of proliferation assays of the animals depicted in 4C. The levels of IFN-γ released were highly variable, but there was no correlation seen with transgene status. There was virtually no IL-4 and little IL-10 secretion in TG and non-TG mice (data not shown). Fig. 5A shows the combined results of stimulation indexes of seven experiments, using a total of 16 TG and 15 non-TG mice, looking at responses to MBP after priming with MBP peptide. Although there is a trend to a higher response in the non-TG animals, this does not reach significance for these numbers. In contrast, looking at the equivalent comparison in the HEL response following priming with HEL peptide, there is a highly significant difference between TG and non-TG animals (Fig. 5B).

Comparison of the anti-MBP-specific T cell response in EH43.69 TG animals and non-TG littermates

Although there was little quantitative difference between the two sets of mice in response to MBP, we wanted to determine whether there was a qualitative response in terms of the range of epitopes seen. To do this, the fine specificity of a panel of T cell hybridomas from two animals of the TG mouse line EH43.69 was compared with that from two of their non-TG littermates (14). On an initial screen, 146 of 169, 77 from the TG mice and 69 from the non-TG

FIGURE 4. EH43.69 TG mice do not show a significantly reduced response to MBP compared with their non-TG littermates. Animals were challenged in vivo with 50 μg MBP_84–104 peptide in CFA as detailed in Materials and Methods. A–C. Draining LN cells from separate mice were restimulated with BMBP in a standard proliferation assay. TG mice, ●; non-TG mice, ○. Results are shown as stimulation index (proliferation with Ag/proliferation in the absence of Ag) in A and B; in C, results are expressed as cpm proliferation. D. Supernatants from the proliferation assay shown in C were assayed for IFN-γ by ELISA. Results are shown as specific IFN-γ release; 100 μg/ml BMBP, □; 10 μg/ml BMBP, □; MBP_84–104 peptide 10 μg/ml, ■. Stimulation index responses to H37RA were >11 in the A and B and 4.3–9.2 in the C. Three representative experiments are shown.

FIGURE 5. A nonsignificant reduction in MBP response is found in TG animals vs a highly significant decrease in HEL response. A. Shows the combined results of seven separate experiments (including the three experiments depicted in Fig. 4) where mice were challenged in vivo with MBP_84–104 peptide; the in vitro response is shown to whole MBP. There is a trend toward a lower response to MBP in the TG (●) vs non-TG mice (○), which does not reach significance with these numbers (p = 0.06, Mann Whitney U test, at 100 μg/ml). B. Combined results of the two experiments shown in Fig. 3, C and E. There is a highly significant difference in the TG vs non-TG responses at all concentrations of HEL (**, p < 0.01, Students t test).
mice, recognized whole MBP presented on I-A\(^d\)-transfected L cells (data not shown). Of these, 65 were stable enough to continue growing for further analysis. All of these hybridomas were reactive to both MBP and to the longest peptide MBP\(_{84-105}\) and restricted through I-A\(^d\). To assess the specificity of the hybridomas, each was tested on three different shorter peptides within the 84–105 region, MBP\(_{87-105}\), MBP\(_{84-96}\), and MBP\(_{84-95}\). In addition, hybridomas were tested for direct recognition of the HEL/MBP fusion protein (mLM43), either as presented by an L cell transfectant cotransfected with I-A\(^d\) and mLM43 or by EH43.69 TG spleen cells. A summary of the results is shown in Table I. The hybridomas are grouped according to their pattern of reactivity to the various agents. TCR V\(\beta\) usage is also shown and does not necessarily correlate with specificity. Hybridomas from both TG and non-TG animals were able to recognize each of the combinations of peptide. Interestingly, roughly equal proportions of the TG- and non-TG-derived T hybridomas were able to recognize the transgene protein (mLM43) when presented by transfected L cells, 15/28 TG and 21/37 non-TG. This is in contrast to the absence of TG-derived T hybridomas capable of recognizing TG spleen, without the addition of exogenous Ag. We have previously shown a major effect on the range of epitopes presented from this region of MBP depending on whether the APC also expresses the MHC class II-associated invariant chain (Ii) (14). It is probable that we are seeing the same type of effect here in comparing recognition of mLM43 from TG spleen (Ii\(^+\)) and the L I-A\(^d\) cells, which are Ii low or negative. The L I-A\(^d\) cells are unable to present native HEL to an I-A\(^d\)-restricted HEL-specific T hybridoma (data not shown).

All of the non-TG hybridomas able to recognize TG spleen have the same peptide specificity, recognition of MBP\(_{84-95}\) and MBP\(_{84-96}\) (Table I and Fig. 2). Interestingly, there are TG-derived cells capable of recognizing this combination of peptides, but none of them are able to recognize the L cell transfected. This appears to be the only difference in repertoire between the TG and non-TG animals in recognition of the MBP\(_{84-105}\) region of MBP. Thus, a similar repertoire of response was obtained from both TG and non-TG animals. The only evident difference between them was the lack of T cells capable of recognizing the endogenously produced MBP epitope from TG spleen. This was not the major responding population of T cells in the non-TG mice, although it did result in an excess of non-TG hybridomas recognizing the shortest peptide, MBP\(_{84-95}\). This same subset of hybridomas was also able to recognize whole MBP, expressed as a transgene and presented by spleen cells, whereas the majority of T cells specific for this region of MBP could not (A. Kruisbeek, unpublished observation).

Susceptibility to EAE

Finally, we wanted to test the susceptibility of TG mice to induction of EAE with a peptide fragment of the insert of the transgene. Mice were challenged according to the stated protocol with MBP\(_{84-104}\) peptide in CFA, followed by two injections of pertussis toxin, 24 and 72 h after peptide priming. Table II shows the results of three experiments of induction in TG and non-TG mice of the EH43.69 line. Surprisingly, TG animals proved remarkably resistant to the induction of EAE by this regime when compared with their non-TG littermates. Over the three experiments, only four of a total of 34 TG mice developed any signs of EAE, while half of their non-TG littermates were affected (14 of 28). Of the four TG animals that did develop signs of EAE, three had tail paralysis only and one had quite severe signs with total hind limb paralysis. In these experiments, animals were monitored for a period of 60 days to look for signs of development of EAE; therefore, no histology was performed. In another experiment, animals were left for 12 days following EAE induction and then sacrificed for histology. In the absence of clinical signs, three of six TG and four of six non-TG mice had minimal histological signs of EAE, with no apparent difference in distribution between them (data not shown). Thus, although TG animals are susceptible to EAE and like their non-TG littermates can develop histological signs in the absence of clinical signs, there is a highly significant difference in the incidence of clinical EAE in the TG animals.

Expression of the transgene under the control of the MHC class II promoter does, by definition, direct expression to MHC class II-positive cells, including the professional APC, which would be critical for induction of the relevant T cell response. Thus, there is a theoretical possibility that expression of the transgene could effect the function of these cells. We have already shown that TG animals are equally efficient at mounting an immune response against an unrelated Ag (see above, and data not shown); however, this does not test the in vivo function of the cells. To test this, a peptide from a different protein of myelin, PLP residues 131–155, was used with the same induction protocol. This peptide has been shown to be encephalitogenic in SJL mice (20), inducing a severe form of the disease. Table II shows that TG mice were equally as susceptible to disease as their non-TG littermates.

Discussion

EAE is a model of a T cell-mediated autoimmune disease that is dependent on the persistence of T cells specific for a self-Ag. Although these T cells are necessary for the development of disease, they are not sufficient as it is possible to find T cells specific for myelin proteins in relatively resistant strains of mice (21, 22). In addition to the animal studies, MBP-specific responses have been well characterized in humans and are not confined to patients with multiple sclerosis but also occur in normal controls (7, 23, 24). It is not clear why these MBP-specific T cells escape tolerance and so are readily susceptible to stimulation on antigenic challenge, particularly in view of the reported expression of an embryonic form of MBP expressed early in thymic development (5). However, the cell type expressing this form of MBP has not been clearly defined; therefore, it is not known whether it could lead to deletion of potentially reactive cells.

In this paper, we have presented a TG mouse model designed to test the nature of T cell tolerance to an encephalitogenic peptide of MBP. We have made use of the MHC class II Eae promoter (15) to target expression to all MHC class II-expressing cells, some at least of which should be competent to cause deletion of autoreactive cells. As the transgene protein is a fusion between MBP\(_{84-105}\) and HEL, it is possible to compare T cell responses to the two Ags. Thymic expression of Ags, particularly in association with MHC class II-positive cells, would normally have been expected to result in the deletion of potentially self-reactive developing thymocytes. In our system, we have shown a profound loss of T cell response to HEL in mice that express the HEL/MBP fusion protein as a transgene. This finding is in agreement with other studies demonstrating a high degree of tolerance to the dominant epitopes of HEL, when expressed as a transgene (25, 26), in comparison to their non-TG littermates. However, when we looked at the T cell response to the MBP portion of the transgene, we found very little effect on the overall response to this region of MBP. That this is unlikely to be due to the site of expression of the transgene is evidenced by the effect on HEL responses in these animals.

A further analysis of the MBP-specific T cell responses from the EH43.69 TG line revealed a remarkably similar pattern of T cell specificity between the transgenic animals and their non-TG littermates, with almost the full range of specificities found in the TG mice (Table I). The principle effect on T cell responses to
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*Summary of results of analysis of T cell hybridoma specificities of MBP-specific T cells from TG and non-TG mice. Hybridomas were derived as described in Materials and Methods. T cell hybridomas, individually listed, were tested for recognition of the peptides shown at a concentration of 5 µg/ml presented by LAS3.1 (I-A<sup>+</sup> cells transfected with I-A<sup>+</sup>) and assessed by CTLL-2 proliferation on supernatants. Recognition was very distinctive (normally >5-fold background in at least two experiments). In addition, they were tested for recognition of the LAS3.1 cell line transfected with the transgene protein mLM43, or spleen cells from an EH43.69 TG mouse, in the absence of added Ag. Recognition of the transfected was defined as CTLL-2 proliferation on supernatants of at least 3-fold greater than untransfected LAS3.1 cells in at least two experiments. Hybridomas are grouped according to those with similar specificity. +, recognition; −, no recognition. TCR Vβ use for each of the hybridomas is indicated. ND, Not reactive with available Abs.
MBP\(^{84–104}\) appears limited to the epitope or epitopes that are efficiently presented on the TG APC themselves, probably due to processing of the endogenous transgene-encoded protein. This group of T cells is represented by the T cell hybridoma 3B3 in Fig. 2A. A T cell hybridoma with a similar specificity, 3A1, (14), also recognizes whole MBP expressed as a transgene presented endogenously by spleen cells, while most other MBP\(^{84–104}\)-specific T cells, with differing fine specificities, do not recognize the endogenously presented molecule (A. Kruisbeek, unpublished observations). The lack of tolerance to the transgene protein is not necessarily due to its inability to be presented in our in vitro assays. Neither a HEL-specific I-As-restricted T cell line (not shown) nor T cell hybridomas specific for HEL, such as SHEL9 (Fig. 2B), are able to recognize the transgene protein presented by splenic APC, yet the mice are still unresponsive. Other workers have found that, in general, a lower amount of self-Ag may be required for tolerance induction than for eliciting a T cell response (26–28). Thus, it may be that there is insufficient presentation of the HEL epitope for detection of T cell recognition in vitro, while there is sufficient presentation for deletion of potentially reactive thymocytes during T cell development. However, this does not explain the persistence of response to the MBP peptide, where recognition of a single specificity is lost, while responses to other epitopes within the MBP\(^{84–104}\) region remain relatively intact. We have previously shown major differences in the epitopes presented from this region, depending on the presenting cell phenotype (14), indicating that in this situation the preserved specificities are not presented in the thymus or peripheral APC. This is not a function of the fusion protein itself, as recombinant Escherichia coli-produced protein is recognized by a wide spectrum of the T cell hybridomas representing all the peptide specificity groups (14). This raises an interesting question in relation to susceptibility to EAE. It is thought that MBP is presented not by the brain oligodendrocytes, which while they synthesize the MBP do not express MHC class II (29, 30), but by other cell types within the brain using an exogenous pathway of presentation.

Surprisingly, given the modest reduction in MBP responses in the E4H3.69 TG mice, we found a considerable degree of resistance to the development of EAE. The difference between TG and non-TG animals was highly significant (\(p = 0.001\)), in contrast to the nonsignificant reduction on overall T cell proliferative response to MBP following in vivo challenge with the peptide, MBP\(^{84–104}\) also used for EAE induction. In contrast, TG and non-TG mice are equally susceptible to EAE induction with an unrelated myelin-derived peptide, PLP\(^{131–155}\). This demonstrates that there is no intrinsic defect in function induced in the TG mice due to transgene expression.

There are a number of possibilities that could account for the observed discrepancy between EAE susceptibility and T cell response. The most obvious is that the development of EAE is dependent on the presence of T cell response capable of recognition of MBP when presented as an endogenously synthesized protein. As this T cell response appears to be missing in the TG animals, it could contribute to their resistance. However, the resistance of the TG mice was not complete; four mice developed EAE, including one with a severe disease score of 3. An alternate explanation could be an alteration in the cytokine profile of responses in the TG animals. In proliferative T cell responses to MBP, after priming with MBP\(^{84–104}\) peptide, we could find no gross differences in the level of IFN-\(\gamma\) secretion between the two sets of mice by cytokine ELISA (Fig. 4D) or RT-PCR (data not shown). In addition, we found little secretion of IL-4 and low levels of IL-10 in these cultures by either of the two methods (data not shown). Although this does not exclude the possibility of local in vivo effects, we were unable to uncover any gross differences between the TG and non-TG animals. The third possibility is related to the level of response to MBP. Although there is no significant difference between the TG and non-TG response to MBP with the numbers of mice studied, there is nonetheless a trend toward a higher response in the non-TG animals. In our EAE induction studies, half of the non-TG animals developed EAE following challenge with the MBP peptide, while there was no response in the TG animals. This could be an alteration in the cytokine profile of responses in the TG animals.

### Table II. EAE induction in E4H3.69 TG mice and non-TG littersmates

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<th>Induction with</th>
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<th>Average Day of Onset</th>
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* Summary of results of three experiments to compare induction of EAE in TG and non-TG animals. EAE was induced as detailed in Materials and Methods.
* Values of \(p\) shown are calculated using a Fisher’s exact test (Arcus Pro-II). Individual days of onset and maximum scores are shown in parentheses where there were three or less animals affected.
* In this experiment, all mice reached the maximum severity level permitted and were culled as a consequence.
response to the encephalitogenic region of MBP. We have demonstrated loss of only one minor epitope within this region; however, this may again be sufficient to put the majority of the TG animals below a response threshold for disease and therefore to protect them from disease induction.

Susceptibility to autoimmune diseases is well known to be dependent not only on the MHC type of the responding individual, but also on a wide variety of other non-MHC genes (31). SJL/J and B10.S mice share the same H-2 haplotype, but there is a big difference in the susceptibility of the two strains to the development of EAE (32–34). In our system, we are looking at the difference in susceptibility to EAE between mice with the same genetic background, following at least nine generations backcross to SJL (Table II, Expt. 1) or eleven generations (Expt. 2). The effect of transgene expression is the deletion of a relatively minor epitope in the encephalitogenic region of MBP, resulting in a relatively minor effect on the T cell response to the inducing Ag. Thus, we are able to demonstrate that a subtle modulation of the immune response to MBP can have a major effect on the susceptibility to disease induction in the absence of any other genetic effects. A further implication of this finding is that in populations susceptible to autoimmune disease, the incidence of disease may be greatly reduced by only a modest reduction in immune response to relevant target autoimmune Ags.

Acknowledgments

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References