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Tolerance and autoimmunity in TCR transgenic mice specific for myelin basic protein

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Summary: T-cell receptor (TCR) transgenic mice provide the ability to follow the maturation and fate of T cells specific for self-antigens *in vivo*. This technology represents a major breakthrough in the study of autoimmune diseases in which specific antigens have been implicated. Proteins expressed within the central nervous system are believed to be important autoantigens in multiple sclerosis. TCR transgenic models specific for myelin basic protein (MBP) allowed us to assess the role of tolerance in providing protection from T cells with this specificity. Our studies demonstrate that T cells specific for the immunodominant epitope of MBP do not undergo tolerance *in vivo* and that TCR transgenic mice are susceptible to spontaneous autoimmune disease. The susceptibility to spontaneous disease is dependent on exposure to microbial antigens. MBP TCR transgenic models expressing TCRs specific for the same epitope of MBP but utilizing different V_α genes exhibit differing susceptibilities to spontaneous disease. These data support the idea that genetic and environmental differences play a role in susceptibility to autoimmunity. MBP TCR transgenic models are playing an important role in defining mechanisms by which infectious agents trigger autoimmune disease as well as defining mechanisms by which tolerance is induced to distinct epitopes within self-antigens.

Multiple sclerosis

Background

Multiple sclerosis (MS) is a neurological disorder whose underlying cause is unknown. It is a primary demyelinating disease characterized by infiltrates of mononuclear cells that are predominantly found in the white matter (1). Patients with MS manifest symptoms of neurological deficit in an acute, chronic relapsing/remitting or chronic progressive course of disease. The disease typically occurs between the ages of 20–45 and females have a relative risk of 2:1 compared to males (2).

An autoimmune pathogenesis is suspected for MS for several reasons (3). First, cellular infiltrates in lesions within the central nervous system (CNS) consist primarily of lymphocytes and monocytes. Second, genetic mapping studies have shown that immune response genes are linked to disease susceptibility. Third, an animal model of demyelinating disease elicited by stimulating autoimmune responses has been established that exhibits many similarities to MS. These observations led to a

proposed model for MS in which self-reactive T lymphocytes are activated and cross the blood brain barrier into the CNS where they attack cells expressing self-antigens.

Triggers of MS

Both genetic and environmental factors influence susceptibility to MS. The importance of both of these factors to disease is reflected in the 25–35% concordance rate for MS in monozygotic twins (4, 5). Variations in prevalence rates of MS throughout the world support a role of environmental factors in influencing MS. There are almost no cases of MS reported in populations living near the Equator and the prevalence significantly increases in populations living in moderate or colder climates (6).

Epidemiological studies suggest that an infectious agent triggers MS (7). Some studies indicate that an individual's relative risk of developing MS is correlated with the environment in which that individual lived prior to the age of twelve and is not associated with the environment inhabited later in life (6). This suggests that exposure to the pathogen occurs much earlier in life than when the disease is clinically manifested. Numerous viruses have been implicated in the etiology of MS and relapses of MS have been associated in some cases with viral infections (3, 8–11). Bacterial infections have been less commonly associated with MS, possibly because it is more difficult to obtain evidence for antecedent bacterial infection in patients. While no specific infectious agent has yet been consistently linked to MS, an infectious etiology remains an attractive hypothesis.

Several theories have been proposed to explain how infection could initiate MS (12). First, an infection in the CNS will produce inflammatory cytokines and chemokines. These factors will cause non-specific recruitment of lymphocytes to the site of infection. Some of the lymphocytes could be specific for CNS antigens and undergo activation *in situ*, initiating an autoreactive response in the target organ. Second, opening of the blood brain barrier due to inflammation may expose the immune system to normally sequestered CNS antigens. Under these circumstances, naïve T lymphocytes that have not undergone tolerance may be activated by self-antigens in the CNS. Third, molecular mimicry of CNS antigens by microbial antigens may result in activation of cross-reactive T lymphocytes (13, 14). In support of the molecular mimicry hypothesis, some T-cell clones and lines established from MS patients demonstrated cross-reactivity for both CNS antigens and peptides derived from various human viruses (15, 16). In addition, a peptide from the bacterium *Pseudomonas aeruginosa* was also able to activate a T-cell clone specific for MBP (15).

Experimental allergic encephalomyelitis

Background

Experimental allergic encephalomyelitis (EAE) is an animal model of autoimmune disease with many similarities to MS (3). EAE is an inflammatory, demyelinating disease that causes acute, chronic or chronic-relapsing paralysis (17). It is characterized by perivascular inflammatory lesions in the white matter of the CNS (18), demonstrating a similar pathology to MS. EAE can be induced in many species either by immunization with CNS antigens in complete Freund's adjuvant or by adoptive transfer of activated CNS-specific T cells into naïve recipients (19, 20). Adoptive transfer experiments demonstrated that MHC class II-restricted $CD4^+$ T cells secreting inflammatory cytokines (Th1 helper cells) are the primary agents mediating EAE (21).

Like MS, susceptibility to EAE is correlated with expression of particular MHC class II genes. Among mice, the strains that have been most commonly employed in studies of EAE are SJL mice that express $H\text{-}2^s$ MHC alleles and B10.PL or PL/J mice that express $H\text{-}2^u$ MHC alleles. These strains demonstrate some interesting differences. B10.PL mice respond primarily to myelin basic protein (MBP). The T-cell response to MBP in this strain is very restricted. There appears to be a single immunodominant epitope consisting of the amino-terminal peptide MBP1-11 (22). There is also little heterogeneity in the primary structures of the T-cell receptors (TCRs) that recognize this epitope. The majority of MBP1-11-specific T cells employ the $V_\beta 8.2$ gene segment paired with either the $V_\alpha 2.3$ or 4.2 gene segment (23, 24). Subdominant responses to MBP31-50 and MBP121-140 have also been reported, representing a minor fraction of the T-cell proliferative response to MBP in these mice (25, 26).

In SJL mice, proteolipid protein (PLP) is the predominant autoantigen although T-cell responses to MBP are also generated. The immunodominant epitope of PLP is PLP139-150 and the immunodominant MBP epitope is MBP89-100 (27, 28). SJL mice more commonly exhibit a relapsing/remitting disease than B10.PL mice (29), which may reflect the spreading of T-cell responses from PLP to MBP epitopes (30).

Effector mechanisms in EAE

EAE has been a very useful model to investigate the effector cells contributing to CNS autoimmune disease. Studies of EAE demonstrated that autoreactive, CNS-specific T cells are a normal part of the T-cell repertoire in healthy animals. These cells only become pathogenic following exposure to an exogenous stimulus. Differentiation of CNS-specific T cells into Th1 cells that

secrete inflammatory cytokines is an important requirement for disease induction. Activation of these T cells results in expression of adhesion markers that facilitate their interaction with endothelial cells comprising the blood brain barrier and promote entry into the CNS (31–34). Entry of Th1 T cells into the CNS and secretion of pro-inflammatory cytokines such as interleukin (IL)-12, interferon (IFN)-γ and tumor necrosis factor (TNF)-α elicit the production of chemokines that cause a further influx of monocytes and non-specific T cells (35–40). The toxic effects of TNF-α and nitric oxide produced by T cells and macrophages play an important role in damaging the myelin sheath (41–45). Recovery mechanisms from this inflammatory response are not well understood. They appear to involve in part the production of anti-inflammatory cytokines such as IL-10, IL-4 and transforming growth factor (TGF)-β (46–49). Production of these cytokines may be the result of generating Th2 T cells which have been shown in some cases (50–52) but not in others (53, 54) to suppress EAE. Apoptosis of CNS antigen-specific T cells that infiltrated the tissue has also been implicated in recovery from EAE (55–58).

EAE is a model system to test therapies for MS

In addition to defining many of the events that are critical to the effector stage of disease, EAE has been a valuable model for developing therapeutic strategies. Approaches to inhibit EAE have targeted different steps in the pathogenesis of the disease. Several strategies have used either antibodies directed against pro-inflammatory cytokines, soluble cytokine receptors or inhibitors of specific cytokine activity to prevent EAE (59–65). Direct administration of anti-inflammatory cytokines has also been used to regulate EAE (66–70). Encephalitogenic T cells have been targeted in a number of ways. Antibodies against V genes expressed on encephalitogenic T cells have been used to eliminate T cells mediating disease (71). Vaccination of animals with inactivated encephalitogenic T cells (72) or specific peptides derived from V genes expressed on these cells (73, 74) has also been effective in inhibiting EAE in some systems. Other strategies have utilized knowledge of the antigen specificity of T cells to inhibit disease. Several studies have shown that exposing encephalitogenic T cells to altered peptides, variants of the wild-type peptide sequence normally recognized by the T cell, can change the pattern of cytokine secretion from inflammatory to anti-inflammatory cytokines. This approach has been used effectively to inhibit EAE (75–78). Exposing animals to CNS antigens administered by different routes, such as intravenous, oral or intranasal, also prevents induction of EAE (79–81). Several of these studies have led to the design of therapies and clinical trials that may be useful in the treatment of MS.

Studies using EAE have contributed enormously to our understanding of CNS autoimmune disease. A great deal has been learned about the cell types and effector mechanisms involved in demyelination. This knowledge has laid the foundation for developing therapies to prevent and/or regulate the autoimmune responses. The methods used to induce EAE are artificial, however, and therefore preclude investigation of potential triggers of CNS autoimmune disease that may be relevant to MS. The development of TCR transgenic models of EAE described below offers new potential to investigate these factors.

TCR transgenic models of EAE

Tolerance to MBP

The first TCR transgenic model of EAE utilized genes encoding a TCR specific for MBP1-11 presented by I-A^u isolated from a B10.PL mouse (82). Two transgenic lines were generated, one expressing the transgenic TCR α-chain (V_α2.3 paired with J_α11) and one expressing the transgenic TCR β-chain (V_β8.2 paired with J_β2.6) that together encode the MBP1-11-specific TCR. When these two transgenic lines were bred together, MBP1-11 TCR transgenic mice were generated that allowed us to follow the fate of MBP1-11-specific T cells *in vivo*. We were able to study maturation of these cells in the thymus as well as their ability to survive in the periphery and mediate disease.

It was already clear that some MBP1-11-specific T cells are normally present in the peripheral repertoire in B10.PL mice because these cells are activated by immunization with MBP and are the predominant effector cells in EAE in this strain of mouse. It was not understood, however, why these autoreactive T cells were present in the periphery. One possibility was that these T cells are not subject to the mechanisms of tolerance induction that normally eliminate self-reactive T cells. MBP was believed to be sequestered behind the blood brain barrier and therefore not presented to T cells under conditions that would induce tolerance. Alternatively, exposure to MBP may induce T-cell tolerance, but the MBP1-11-specific T cells may represent a specific subset of T cells that escape tolerance.

Analyses of the thymocytes in MBP1-11 TCR transgenic mice demonstrated that MBP1-11-specific T cells do not undergo clonal deletion in the thymus. Strong skewing in the thymocyte populations toward mature $CD4^+$ thymocytes expressing the transgenic TCR indicated that these cells undergo very efficient positive rather than negative selection in the thymus (82). Indeed, the selection of a TCR with this particular V_α/V_β combination is so efficient on the B10.PL background that mice expressing only the transgenic β-chain generate a high precursor frequency of T cells paired with endogenous α-chains en-

coded by the same $V_\alpha 2.3$- $J_\alpha 11$ gene segments that are expressed in the parental MBP1-11-specific TCR (83). The frequency of T cells expressing the MBP-specific TCR in the TCR β-chain mice is sufficiently high to allow bulk lymph node cells from unimmunized TCR β-chain mice to proliferate when cultured with MBP1-11 peptide (83, 84). In contrast, T cells from TCR α-chain mice do not proliferate when stimulated with MBP1-11 peptide (T. Brabb, J. Goverman, unpublished observations).

The strong positive selection of transgenic thymocytes on the B10.PL background indicates that these self-reactive T cells are not subject to central tolerance induction. One reason that MBP1-11-specific T cells may escape negative selection is that the MBP1-11 epitope displays a very low affinity for its I-A^u MHC ligand and therefore may be a poor mediator of negative selection (85, 86). Observations from a different MBP1-11-specific TCR transgenic model are consistent with the notion that these cells would not undergo negative selection in the thymus even if they were exposed to their cognate antigen because of the instability of the peptide/MHC complex (87). Thymocytes in these MBP1-11 TCR transgenic mice did not undergo clonal deletion when the wild-type MBP1-11 was administered intraperitoneally to the mice but did exhibit deletion when a variant of MBP1-11 with a much higher affinity for I-A^u (MBPAc1-11[4Y]) was administered. Similar experiments carried out in different MBP1-11 TCR transgenic mice resulted in some deletion of transgenic thymocytes following administration of the native MBP1-11 peptide; however, higher doses of peptide were used in these experiments (88).

In addition to demonstrating that endogenous expression of MBP does not result in negative selection of MBP1-11-specific thymocytes, our analyses of thymocyte maturation in the MBP1-11 TCR transgenic mice revealed an unexpected observation related to the strong positive selection of the thymocytes with this antigen specificity. The MBP1-11 TCR transgenic mice exhibit a disrupted thymic architecture in which the stromal elements do not organize into a central medulla. Instead, small medullary foci are dispersed throughout the thymus surrounded by regions of cortical epithelium (89). Bone marrow-derived cells, dendritic cells and macrophages are still preferentially associated with the medullary foci. Analyses of other TCR transgenic mice revealed that disruption of the thymic architecture is increased in models that exhibit strong positive and negative selection compared to models that exhibit weak positive selection. Thus, the organization of medullary foci into a central medulla is impeded when the normal balance of signals between thymocytes and stromal cells is skewed by high-avidity interactions (89). Despite the lack of organization of the medullary foci into a central medulla, thymocyte maturation and export to the periphery occurs normally.

The MBP1-11-specific T cells exported from the thymus do not undergo tolerance induction in the periphery (82). T-cell numbers in the periphery are comparable to wild-type mice and consist primarily of $CD4^+$ T cells expressing the transgenic TCR. These T cells are not anergic as they respond vigorously to stimulation with antigen *in vitro* and can mediate EAE *in vivo*. Thus, the peripheral T-cell repertoire in the transgenic animals is dominated by fully functional MBP1-11-specific T cells.

In recent studies, we demonstrated that expression of MBP *in vivo* does induce T-cell tolerance and that the lack of tolerance to MBP1-11 is epitope specific (90). We compared the immune response to MBP in both H-2^u wild-type mice and H-2^u MBP-deficient shiverer mice ($MBP^{-/-}$) that do not synthesize intact MBP. Wild-type mice primed with intact MBP responded only to the dominant MBP1-11 epitope and intact MBP *in vitro*. We did not detect significant responses to MBP31-50 or MBP121-140 that had been reported by other investigators as subdominant MBP epitopes (25, 91). In contrast to wild-type mice, immunized $MBP^{-/-}$ mice generated very strong T-cell responses to two distinct epitopes within the MBP121-150 region of the protein. The response to MBP1-11 was very weak in comparison to the MBP121-150 epitopes. These data demonstrate that the true immunodominant epitopes of MBP are within MBP121-150 and that endogenous expression of MBP induces tolerance in T cells responding to this region (90). These observations also exclude the hypothesis that MBP1-11-specific T cells do not undergo tolerance because MBP is a sequestered antigen that is invisible to the immune system. Tolerance to MBP has also been demonstrated by comparing T-cell responses to MBP in C3H $MBP^{-/-}$ and wild-type mice (92).

Further characterization of the MBP121-150-specific T cells found in $MBP^{-/-}$ mice revealed that this response is unexpectedly complex (93). Two distinct, non-overlapping epitopes represented by MBP125-135 and MBP136-146 are present in this region that form very stable complexes with I-A^u MHC molecules *in vitro*. The half-times of dissociation of these peptides are 270 and 180 h respectively. Analyses of the residues that function as TCR contacts in these epitopes demonstrated that the antigenic surfaces of these two peptide/MHC complexes lack any structural similarity. Thus, it was very surprising that most of the T cells that respond to this region of MBP are cross-reactive for both epitopes. Even more intriguing was the observation that recognition by the cross-reactive T cells was lost when functional TCR contacts were interchanged between the two epitopes. Thus, the T cells appear to adopt mutually exclusive conformations to achieve specific re-

cognition of two distinct epitopes that present very different antigenic surfaces (93). Interestingly, the few T cells specific for this region of MBP that escape tolerance in wild-type mice recognize peptides that contain the first but not the second epitope (90).

Very few T cells that recognize the epitopes within MBP121-150 escape tolerance in wild-type mice. To understand how tolerance to the immunogenic epitopes of this self-antigen occurs, we turned again to the powerful system of antigen-specific TCR transgenic mice. Using two sets of genes that encode TCRs cross-reactive for both MBP125-135 and MBP136-146, we generated several lines of TCR transgenic mice. Our preliminary data indicate that the transgenic T cells may undergo some tolerance during maturation in the thymus but a number of transgenic T cells are exported to the periphery (E. Huseby, J. Goverman, unpublished observations). In preliminary experiments, the transgenic T cells on a heterozygous H-$2^{u/k\ or\ b}$ background retain the ability to respond to one epitope within MBP121-150 but not to the MBP131-150 peptide that contains the second epitope. This response is similar to the reactivity of the few remaining MBP121-140-specific T cells that escape tolerance in wild-type mice (90). These models will be very valuable in defining the mechanisms of tolerance induced by endogenous expression of MBP *in vivo*.

Dissecting CNS autoimmune disease in TCR transgenic mice

Our studies of the MBP1-11 TCR transgenic mice showed that these mice are highly susceptible to induction of EAE using the standard protocol of immunization with MBP peptide and injection of pertussis toxin (82). This result was expected given the high precursor frequency of functional MBP1-11-specific T cells in the periphery. Immunization of the TCR β-chain transgenic mice with MBP1-11 peptide also induced EAE, consistent with the high precursor frequency of MBP1-11-specific T cells in these mice (84). Surprisingly, administration of pertussis toxin alone to the MBP1-11 TCR transgenic mice without immunization with MBP also resulted in a high incidence of EAE (82). The mechanism by which pertussis toxin induces EAE in the transgenic mice is not known. One reported function of pertussis toxin is to promote access of lymphocytes to the CNS by increasing permeability of the blood brain barrier (94, 95). The ability of pertussis toxin alone to trigger EAE in the transgenic mice suggested that entry into the CNS might be the critical checkpoint in the initiation of autoimmune disease. We tested this idea by determining whether injection of transgenic MBP1-11-specific T cells directly into the cerebral spinal fluid (CSF) of non-transgenic recipient mice was sufficient to induce EAE. Injection of transgenic splenocytes that had been activated *in vitro* by exposure to MBP1-11 induced a high incidence of EAE (96). Surprisingly, injection of non-stimulated transgenic splenocytes as well as non-stimulated purified T cells also induced EAE although at a lower frequency. These results suggested that the presence of high numbers of $CD4^+$ MBP-specific T cells behind the blood brain barrier where MBP is expressed might be sufficient to trigger disease (96).

These experiments also demonstrated that EAE induced by injection of pertussis toxin is manifested differently than EAE induced by intrathecal injection of MBP1-11-specific transgenic T cells into the CSF. EAE induced by injection of pertussis toxin in MBP1-11 TCR transgenic mice was significantly more severe and demonstrated fewer relapses than EAE induced by intrathecal injection of activated or resting transgenic T cells (96). A difference in the course of disease induced by these two methods would not be expected if the only effect of pertussis toxin was to facilitate access of T cells to the CNS. Thus, exposure to pertussis toxin appears to influence EAE in multiple ways.

MBP TCR transgenic mice develop spontaneous EAE

The lack of T-cell tolerance that was observed in the MBP1-11 TCR transgenic mice *in vivo* suggested that these mice have the potential to develop spontaneous autoimmune disease. Spontaneous EAE was observed in some transgenic mice as early as one backcross to B10.PL mice when the mice were heterozygote for H-2^u MHC class II molecules (82). We continued to observe cases of spontaneous EAE as the mice were further backcrossed onto the B10.PL background. A few cases of spontaneous EAE have also been observed in TCR β-chain mice, consistent with the idea that these mice are enriched for TCRs that are specific for MBP1-11 compared to non-transgenic mice (T. Brabb, J. Goverman, unpublished observations). The incidence of spontaneous EAE in the β-chain transgenic mice is very low but has not been precisely determined because we have not monitored them consistently for symptoms of EAE.

The window of age in which MBP1-11 TCR transgenic mice are susceptible to spontaneous EAE is similar to MS in that the disease is manifested primarily during adolescence and early adulthood (96). *Fig. 1* shows the age of mice at the onset of symptoms in a colony of 228 conventionally housed transgenic mice in which 107 developed EAE spontaneously. No cases of EAE were seen at less than 5 weeks of age and few cases were observed in mice older than 12 weeks of age. Unlike MS, however, the MBP1-11 TCR transgenic males have a higher relative risk (1.871) than females (96). While females have a higher relative risk for MS than males, gender differences in both the human disease and the transgenic mouse model sup-

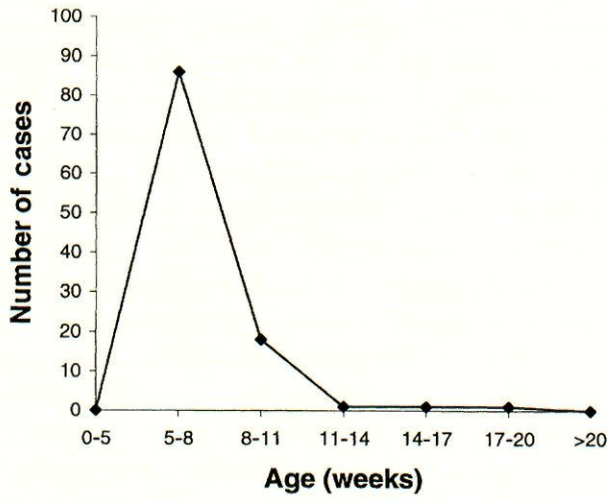

Fig. 1. Age of onset of spontaneous EAE in MBP1-11 TCR transgenic mice. MBP1-11 TCR transgenic mice housed in a conventional animal facility were monitored at least three times/week for clinical symptoms of spontaneous EAE. Severity of symptoms were graded according to the following scale: Grade 0, no symptoms; Grade 1, limp tail; Grade 2, uneven gait; Grade 3, hindquarter paralysis; Grade 4, fore and hindquarter paralysis; Grade 5, moribund. Two hundred twenty-eight transgenic mice were monitored from December 1994 to present; of these 107 exhibited symptoms of EAE.

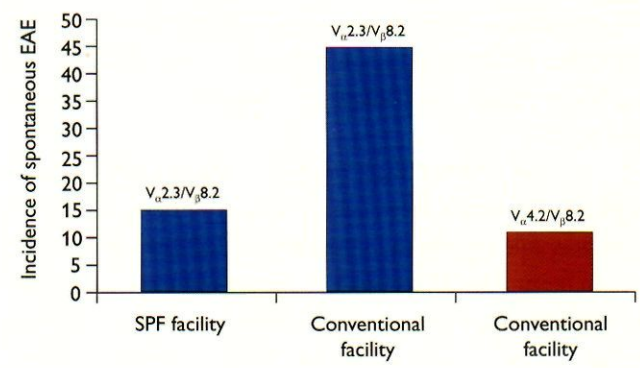

Fig. 2. Incidence of spontaneous EAE in MBP1-11 TCR transgenic mice. Genetically equivalent MBP1-11 TCR transgenic mice expressing the $V_\alpha 2.3/V_\beta 8.2$ TCR (82) were housed in microisolators in either an SPF or conventional animal facility with the same water, food and bedding. MBP1-11 TCR transgenic mice expressing the $V_\alpha 4.2/V_\beta 8.2$ TCR (97) were housed in the same conventional facility as the $V_\alpha 2.3/V_\beta 8.2$ TCR transgenic mice. All mice were monitored for symptoms of EAE from weaning until 12 weeks of age. For the $V_\alpha 2.3/V_\beta 8.2$ TCR transgenic mice, six cases among 40 mice were observed in the SPF facility and ten cases among 24 mice in the conventional facility. For the $V_\alpha 4.2/V_\beta 8.2$ TCR transgenic mice, five cases among 45 mice were observed in the conventional facility.

port the idea that hormonal changes play a role in susceptibility to CNS autoimmune disease.

One of the most interesting observations made with this TCR transgenic model is that spontaneous EAE was seen only in mice that were housed under conventional conditions and was not observed when the TCR transgenic mice were housed under specific-pathogen-free conditions (SPF) (82). The observation that the incidence of spontaneous EAE depended on environmental conditions under which the mice were housed represents a potentially important parallel with MS. Therefore, we sought to determine if spontaneous EAE was triggered by differences in microbial exposure or some other environmental factor. In our initial studies, several environmental differences in addition to microbial flora, such as food, water and caging, existed between the two colonies. To investigate specifically the role of microbes in triggering EAE in the transgenic mice, we established two cohorts of genetically equivalent MBP1-11 TCR transgenic mice that had been backcrossed onto B10.PL for ten generations and had either conventional or limited microbial flora. Mice in both cohorts were housed in microisolators with identical food, water and bedding and were monitored over a 12-week period for symptoms of EAE. The incidence of spontaneous EAE shown in Fig. 2 was significantly higher in the conventional mice (43%) compared to the SPF mice (15%, p=0.017), indicating that exposure to microbes facilitates the induction of EAE (96). This observation is consistent with the hypothesis that MS is triggered by exposure to pathogens.

During our studies of the incidence of spontaneous EAE in the MBP1-11 TCR transgenic mice that we had established, we introduced MBP1-11 TCR transgenic mice into our conventional colony that had been developed independently and utilized a different TCR specific for MBP1-11 (97). These mice express a transgenic TCR specific for MBP1-11 utilizing $V_\alpha 4.2$ and $V_\beta 8.2$ obtained from PL/J mice (98). We backcrossed these MBP1-11 TCR transgenic mice 11 generations onto the B10.PL background and compared spontaneous disease in a cohort of these conventionally housed mice to our conventionally housed MBP1-11 TCR transgenic mice that had been backcrossed for ten generations onto B10.PL. Surprisingly, the data in Fig. 2 show that the incidence of spontaneous EAE was significantly lower (11.1% versus 41.78%, p = 0.003) in the MBP1-11 TCR transgenic mice expressing the $V_\alpha 4.2/V_\beta 8.2$ transgenic TCR than the MBP1-11 TCR transgenic mice expressing the $V_\alpha 2.3/V_\beta 8.2$ TCR. Because these mice had both been backcrossed extensively onto B10.PL and were housed in the same

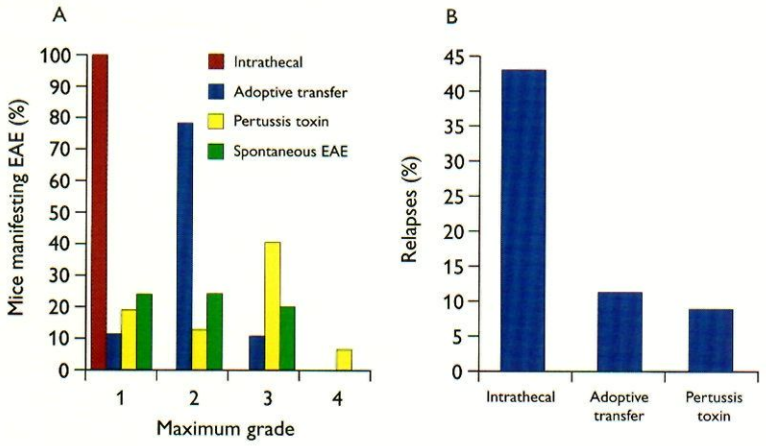

Fig. 3. Manifestation of EAE induced by different methods in MBP1-11 TCR transgenic mice or non-transgenic mice. A. The percentage of mice exhibiting different maximum grades. Data represent 41 transgenic mice that exhibited spontaneous EAE, 17 transgenic mice in which EAE was induced by administration of pertussis toxin, 22 non-transgenic mice in which EAE was induced by intrathecal injection of activated transgenic T cells and nine non-transgenic mice in which EAE was induced by intravenous adoptive transfer of activated transgenic T cells. **B. The percentage of mice that exhibited relapses when EAE was induced by intrathecal injection of activated transgenic T cells, intravenous adoptive transfer of activated transgenic T cells or administration of pertussis toxin.**

animal facility, the difference in the incidence of spontaneous EAE appears to be the consequence of expressing MBP1-11-specific TCRs with different primary structures. Purified T cells isolated from both types of MBP1-11 TCR transgenic mice proliferate similarly and generate similar levels of IFN-γ and IL-4 when stimulated *in vitro* with MBP1-11 peptide (J. Goverman, unpublished results). Thus, the basis for the difference in susceptibility to spontaneous EAE in the two strains of MBP1-11 TCR transgenic mice is not clear. It is possible that the $V_\alpha 2.3/V_\beta 8.2$ TCR is more cross-reactive for microbial antigens than the $V_\alpha 4.2/V_\beta 8.3$ TCR; however, this has not yet been determined.

Immunoregulation in MBP1-11 TCR transgenic mice

We compared the characteristics of EAE that occurs in MBP1-11 TCR transgenic mice either spontaneously or following administration of pertussis toxin, as well as EAE that is induced by intrathecal injection of transgenic T cells into non-transgenic recipients (96). In both spontaneous EAE and EAE induced by pertussis toxin, the maximum severity of symptoms is fairly high, with over half of the cases reaching grade three severity (Fig. 3A). The disease is usually chronic, with improvement observed in some cases, but complete recovery seen less often. Relapses are infrequently observed in either spontaneous or pertussis toxin-induced EAE (Fig. 3B). Manifestation of EAE induced by intrathecal injection, however, is strikingly different. The incidence of disease is high, but the severity of EAE induced by this method never exceeded grade one. In addition, relapses were observed in almost half of the cases. Thus, EAE that is mediated by MBP-specific transgenic T cells is manifested very differently depending on how these T cells are triggered and/or how they enter the CNS.

These observations suggest that the different methods of disease induction using transgenic T cells stimulate different mechanisms of immune regulation. Interestingly, the CNS lesions in mice with intrathecally induced EAE were much more severe than lesions seen in mice with either spontaneous or pertussis toxin-induced EAE despite the very mild clinical symptoms (96). On a scale of 0 to 5, the average severity of cellular infiltrates was 4.3 in intrathecally-injected mice and 1 in mice with spontaneous EAE. A lack of correlation between severity of clinical symptoms and pathology in EAE has been noted in other studies (99, 100).

One of the most intriguing observations regarding spontaneous EAE in the TCR transgenic mice is that, despite a very large population of functional MBP-specific T cells in the periphery, disease incidence does not reach 100% even in mice that are housed together in a conventional facility (96). This suggests that peripheral tolerance mechanisms function in these mice to prevent the occurrence of autoimmune disease and that these mechanisms fail with some stochastic frequency in a percentage of the mice.

The idea that immunoregulatory cells exist *in vivo* that function to prevent EAE was proposed in studies of a separate line of MBP1-11 TCR transgenic mice bred onto the $Rag^{-/-}$ background (97). A low incidence of spontaneous EAE was observed in these transgenic mice under SPF conditions but the incidence increased to 100% when the transgenic TCR was crossed onto the $Rag^{-/-}$ background. Recently, two studies of this MBP1-11 TCR transgenic model have implicated $CD4^+$ T cells expressing endogenous TCRs as the cells responsible for providing protection against the development of spontaneous EAE in MBP TCR transgenic mice on the $Rag^{+/+}$ background (101, 102). Crosses of the transgenic TCR to mice deficient in

B cells, $CD8^+$ T cells, NK-T cells or γδ T cells did not increase the incidence of spontaneous EAE. However, crossing the TCR onto a $Rag^{-/-}$, $C_\alpha^{-/-}$ or $C_\beta^{-/-}$ background significantly increased spontaneous EAE. Adoptive transfer of $CD4^+$ T cells from non-transgenic mice into MBP1-11 TCR transgenic mice on the $Rag^{-/-}$ background decreased the incidence of spontaneous EAE. These adoptive transfer experiments also indicated that TCR transgenic $Rag^{-/-}$ mice had to receive the $CD4^+$ T cells from non-transgenic mice at an early age (40 days old or younger) to be fully protected from spontaneous EAE (101, 102). The mechanism by which these regulatory T cells protect MBP1-11 TCR transgenic mice from EAE is not known.

A different form of immunoregulation of MBP-specific T cells has recently been demonstrated using the TCR β-chain transgenic mice. In these studies, it was found that the TCR β-chain mice produce a population of T cells that are specific for determinants expressed on the transgenic β-chain itself (103). Immunization with recombinant rat (but not mouse) $V_\beta 8$ protein in incomplete Freund's adjuvant was observed to protect TCR β-chain transgenic mice from the subsequent induction of EAE (84). Tolerization of TCR β-chain mice by neonatal administration of recombinant rat $V_\beta 8$ protein caused more severe EAE in adult transgenic animals immunized with MBP (104). Similar findings have been observed in non-transgenic rat and mouse models of EAE (73, 74, 105). The $V_\beta 8$-specific regulatory T cells appear to influence the activity of MBP1-11-specific T cells in the TCR β-chain mice by a cytokine-driven mechanism (84). $CD8^+$ $V_\beta 8$-specific T cells have been proposed also to play a role in affecting the activity of MBP1-11-specific T cells (106); however, these cells have not been investigated in MBP1-11 TCR transgenic models.

TCR transgenic mice as tools for designing new therapies

MBP1-11 TCR transgenic mice offer a unique opportunity to investigate potential therapies for CNS autoimmune disease because the generation and subsequent fate *in vivo* of effector T cells that mediate disease can be directly monitored in these models. In the first example of this approach, we examined the mechanism by which high antigen dose treatment blocks MBP1-11-specific T cells from mediating EAE (107). Boehme & Lenardo demonstrated that apoptosis occurs when exposure to high concentration of antigen causes re-engagement of TCRs expressed on activated T cells that were already cycling in response to IL-2 (108). This system was used to show that exposure of MBP1-11-specific transgenic T cells to high concentration of antigen could induce apoptosis *in vitro* and specifically eliminate transgenic T cells *in vivo* (107). Transgenic T cells were activated *in vitro* and adoptively transferred into the periphery of non-transgenic B10.PL or PL/J mice. In untreated recipients, transfer of the activated transgenic T cells induced severe EAE. Multiple intravenous injections of soluble MBP or MBPAc1-11 peptide, however, were able to delete the transgenic T cells from the periphery of the recipients and inhibit induction of EAE. These experiments provided a foundation for antigen-specific therapy in T-cell-mediated autoimmune disease.

The effect of administering soluble antigen *in vivo* has subsequently been studied in several other MBP1-11 TCR transgenic mouse models. Pearson and colleagues examined the effect of injecting soluble rat MBPAc1-11 and variants of this peptide that bind the $I\text{-}A^u$ molecules with higher affinity into MBP1-11 TCR transgenic mice (88). Their results showed that all soluble peptides were able to induce transient activation of transgenic T cells *in vivo* as measured by the expression of several activation markers. Peptides with high affinity for the MHC molecule then induced downregulation of the TCR as well. Apoptosis of transgenic T cells was also observed in this system, with the extent of apoptosis correlating with the affinity of the injected peptides for $I\text{-}A^u$. Both proliferative T-cell responses *in vitro* and deletion of T cells *in vivo* increased in mice that received higher affinity peptides. The simultaneous expansion and deletion *in vivo* had compensatory effects such that no net loss of T cells was observed following injection of soluble peptide in transgenic PL/J mice. Interestingly, peripheral deletion of T cells expressing the same transgenic TCR appeared to be higher on the B10.PL background compared to PL/J mice. In these MBP1-11 TCR transgenic mice, injection of the peptide with the highest affinity for $I\text{-}A^u$ MHC molecules, MBPAc1-11[4Y], induced differentiation *in vivo* of the transgenic T cells into a protective Th2 phenotype, while the native, low affinity MBPAc1-11 peptide stimulated Th1 cytokine responses.

In contrast to induction of tolerance by deletion of transgenic T cells or induction of Th2 T cells, other investigators have reported that injection of soluble peptide into different MBP1-11-specific TCR transgenic mice induces non-responsiveness. In one model, chronic administration of soluble mouse MBP Ac1-9[4Y] peptide was required to achieve profound non-responsiveness in transgenic T cells (87). In studies of a different MBP1-11 TCR transgenic model, intraperitoneal injection of mouse MBP Ac1-17 in either incomplete or complete Freund's adjuvant resulted in non-responsiveness or anergy of the transgenic T cells (109). Proliferative responses were not restored by the addition of IL-2 *in vitro*. The ability to achieve tolerance when complete Freund's adjuvant, a potent stimulant of the immune system, was used with the injected peptide is not understood. Tolerance occurred in both $Fas^{-/-}$ and $IL\text{-}4^{-/-}$ mice in this system, suggesting that the non-

responsiveness did not involve activation-induced cell death or induction of Th2 T cells.

MBP TCR transgenic mice have also been used to investigate the mechanisms of oral tolerance in inhibiting EAE. Oral tolerance is defined as the antigen-specific suppression of the immune response following oral administration of antigen. Several mechanisms have been proposed as the basis for induction of oral tolerance. Low doses of fed antigen appear to induce antigen-specific regulatory cells that secrete TGF-β, IL-4 and IL-10. These regulatory cells may then mediate suppression of Th1 responses occurring in other tissues including the CNS. High doses of fed antigen appear to induce T-cell anergy and/or deletion. Feeding low doses of MBP to MBP TCR transgenic mice developed by Lafaille et al. (97) resulted in production of TGF-β, IL-4 and IL-10 by transgenic splenocytes and adoptive transfer of these splenocytes inhibited induction of EAE in recipient animals (110). In a separate study, feeding high doses of MBP to MBP1-11 TCR transgenic animals developed by Goverman et al. (82) resulted in a rapid depletion of transgenic T cells from peripheral lymphoid organs and an increase in the transgenic T cells in the intestine (111). This depletion resulted in a decrease in proliferative responses to MBP by T cells in lymphoid organs and protected the transgenic mice from subsequent EAE induction.

MBP TCR transgenic mice will no doubt continue to serve as powerful tools to test new therapies for EAE. Many questions remain concerning protocols involving antigen-specific therapies, including the effectiveness of exposure to specific peptide variants in inhibiting EAE. Data regarding the existence of regulatory T cells in controlling autoimmune responses in the CNS open new areas of investigation using these models. Thus, the potential of these models to test novel therapeutic strategies in treating autoimmune disease is just beginning to be realized.

New directions in the study of CNS autoimmune disease using TCR transgenic mice

Triggers of CNS autoimmune disease

The unique properties of the MBP TCR transgenic mice allowed us to investigate several issues of CNS autoimmune disease that could not be addressed in classic models of EAE. The observation that exposure to microbes elicited spontaneous EAE in MBP1-11 TCR transgenic mice suggested that this model may be useful to investigate mechanisms by which pathogens trigger disease in susceptible animals.

Our initial experiments focused on the ability of different bacteria to trigger EAE in the MBP TCR transgenic mice. We tested the ability of *Streptococcus pneumoniae* to induce EAE when the bacteria were injected directly into the CSF. Our results indicate that intrathecal injection in the lumbar spinal cord of either live or heat-killed bacteria induced EAE in the transgenic mice at a high incidence (T. Brabb, J. Goverman, unpublished observations). Interestingly, injection of the same bacteria into the CSF in the cisterna magna at the base of the brain induced EAE at a significant but lower incidence than intrathecal injection in the spinal cord, even though bacteria were injected into the CSF in both protocols. The reason for this difference in incidence of EAE is not yet clear. The ability of the bacteria to induce EAE following introduction into the CNS was not entirely surprising. Exposure to bacteria triggers the innate immune system, resulting in infiltration of white blood cells, including lymphocytes, into the CNS. Thus, a large number of transgenic T cells will cross the blood brain barrier and enter a CNS in which the concentration of inflammatory cytokines is already increased. These conditions are likely to result in activation of the transgenic MBP-specific T cells.

The experiments described above indicate that a bacterial infection in the CNS can trigger EAE in TCR transgenic mice. It is not clear, however, that this method exhibits any parallels to the conditions under which MS is initiated in humans. Therefore, we were also interested in determining whether a peripheral infection of bacteria could trigger EAE in the MBP TCR transgenic mice. Our preliminary experiments indicate that intraperitoneal injection of either *S. pneumoniae* or Group B *Streptococcus* induces EAE in the MBP TCR transgenic mice. We are currently investigating the mechanisms responsible for triggering EAE with these bacteria. The MBP1-11-specific transgenic T cells do not proliferate in response to Group B *Streptococcus in vitro*, and injection of heat-killed bacteria does not induce EAE. These data argue against a molecular mimicry mechanism in which the transgenic TCR is stimulated by a cross-reactive antigen present in the bacteria. Interestingly, preliminary results indicate that injection of *Escherichia coli* does not trigger EAE in the MBP TCR transgenic mice, although comparable numbers of bacteria have not been used in these experiments (T. Brabb, J. Goverman, unpublished observations). Despite the lack of disease in transgenic mice injected with *E. coli*, MBP1-11-specific transgenic T cells appear to proliferate *in vitro* in response to *E. coli* antigens. Future experiments will investigate the mechanisms by which bacterial infection triggers EAE in the MBP1-11 TCR transgenic mice and also address the ability of viral infections to induce EAE in this mouse model.

Lymphocyte trafficking in the CNS

Other questions that the MBP1-11 TCR transgenic model is uniquely suited to address are whether naïve transgenic T cells

can traffic to the CNS and whether MBP-specific T cells that have not been previously activated can be stimulated by encountering endogenous MBP in this tissue. In analyzing T lymphocytes isolated from the CNS of healthy MBP TCR transgenic and non-transgenic mice, we first made the surprising observation that comparable numbers of T cells are present in this tissue in transgenic and non-transgenic mice. This finding was unexpected because only activated T cells are believed to traffic to the CNS, and there are significantly fewer activated T cells in the periphery of TCR transgenic mice that do not have EAE compared to non-transgenic mice. TCR transgenic mice have fewer activated T cells in the periphery because the TCR repertoire is very restricted and therefore the chance that environmental antigens can activate T cells is reduced. Also unexpected was the observation that, while the majority of T cells in the CNS in non-transgenic mice exhibit an activated phenotype, most of the T cells isolated from the CNS of young MBP TCR transgenic mice exhibited a naïve phenotype (T. Brabb, J. Goverman, manuscript in preparation). We have now analyzed several other TCR transgenic models that were not specific for CNS antigens. We found that the number of T cells in the CNS of these TCR transgenic mice was also comparable to the number in non-transgenic mice and that their phenotype was predominantly naïve. These data suggest that there is a steady state number of T cells that traffic through the CNS in a healthy animal. While activated T cells appear to have a preferential advantage in trafficking to this tissue, naïve T cells can traffic to the CNS in the absence of "competition" from activated T cells in the periphery. One of the most interesting observations from these studies is that increased numbers of memory MBP-specific T cells are found in the CNS of older MBP TCR transgenic mice that do not exhibit any signs of clinical disease. Thus, an increase in the number of activated and memory T cells in the CNS of MBP1-11 TCR transgenic mice does not appear to be sufficient for the induction of EAE.

The future

Studies of MBP TCR transgenic mice have been invaluable in answering questions about the induction of tolerance to CNS antigens, as well as the events that lead to the breakdown in tolerance and the induction of autoimmune disease. Studies with these models are continuing as the answers to many questions typically lead to new areas of inquiry. New transgenic models specific for epitopes of MBP that undergo tolerance *in vivo* as well as for MHC class I-restricted epitopes of MBP will also be very informative. These models, as well as TCR transgenic mice specific for different CNS antigens, will be valuable tools in the future to understand the pathogenesis of CNS autoimmune disease and to test new therapies for the treatment of MS.

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