Review

T Cell Vaccination as an Immunotherapy for Autoimmune Diseases

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Immunization with inactivated autoreactive T cells (T cell vaccination) selected from individual's own T cell repertoire provides a unique *in vivo* setting for testing immune regulation that is known to involve interactions of a variety of related surface molecules (1). It induces regulatory immune responses that closely resemble the *in vivo* situation where the immune system is challenged by clonal activation and expansion of given T cell populations in various autoimmune diseases. T cell vaccination provides a powerful means of eliciting natural reactions of the immune system in response to clonal expansion of T cells, which can used as a therapeutic approach to suppress or eliminate specific pathogenic autoreactive T cells in autoimmune conditions. Clinical trials using T cell vaccination to deplete autoreactive T cells in human autoimmune conditions have begun to reveal the pathologic relevance of various autoimmune T cell populations in the disease processes, providing a unique opportunity to test the autoimmune theories in a clinical setting. *Cellular & Molecular Immunology*. 2004;1(5):321-327.

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Role of myelin autoreactive T cells in MS

Multiple sclerosis (MS) is an inflammatory, demyelinating disease involving the white matter of the central nervous system (CNS). Although the exact cause of MS is unknown, the disease has conspicuous features of an autoimmune disease. The autoimmune theory has gained considerable popularity in recent years in light of an increasing body of supporting evidence from both MS and animal model studies (2). It is hypothesized that T cells specific for myelin antigens play an important role in the pathogenesis of MS. These autoimmune T cells may be activated in the periphery potentially through molecular mimicry as a result of microbial infections and migrate into the CNS in MS. There is evidence indicating that MS is associated with certain infectious agents (3, 4) and that peptides derived from a variety of viruses and bacteria can induce activation of myelin autoreactive T cells (5-7).

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Among several candidate myelin antigens implicated in MS, myelin basic protein (MBP) is best studied. MBP represents one of the dominant myelin proteins and is proven to induce experimental autoimmune encephalomyelitis (EAE), an animal model for MS, either by injections of MBP with an adjuvant or by adoptive transfer of T cells sensitized to MBP (8). In humans, MBP-reactive T cells are found at relatively low frequency in the blood circulation of both patients with MS and healthy individuals. However, MBP-reactive T cells found in patients with MS display different functional characteristics, which may be related to their potential pathologic role in the disease. There is evidence that these MBP-reactive T cells undergo *in vivo* activation and accumulate in the brain of patients with MS, as opposed to healthy individuals. This observation has been reported by a number of independent research laboratories using different technical approaches. Allegretta and co-workers used an hprt-mutant assay to define *in vivo* activated T cells from MS patients (9). They reported that a high frequency of mutant T cell clones from the peripheral blood of MS patients showed strong reactivity to MBP, while none the T cell clones grown from blood of normal subjects did. Furthermore, Chou and Zhang demonstrated independently high precursor frequency of *in vivo* activated MBP-reactive T cells in cerebrospinal fluid of MS patients. In these two independent studies, exogenous interleukin-2 (IL-2) was used to pre-select T cells that undergo recent activation *in vivo* and express the IL-2 receptors. High precursor frequencies of MBP-reactive T cells were detected among IL-2 selected T cell population in both peripheral blood and cerebrospinal fluid of MS patients, as opposed to patients with other neurologic diseases, suggesting *in vivo* activation of MBP-reactive T cells (10, 11). Another important feature of MBP-reactive T cells

found in MS patients is related to *in vivo* clonal expansion. MBP-reactive T cell clones generated from MS patients under limiting dilution conditions exhibit a restricted patterns of TCR V gene repertoire as opposed to that of MBP-reactive T cells derived from healthy controls by TCR V-D-J sequence analysis. TCR V gene profile of MBP-reactive T cell clones raised from MS patients is often characteristic of and identical/similar among the T cell clones of given individuals, suggesting that MBP-reactive T cells undergo *in vivo* clonal expansion in an individual-dependent manner (12, 20).

It is hypothesized that if myelin-reactive T cells are associated with the disease processes, they may undergo *in vivo* activation and expansion during acute exacerbation. In a recent study, we demonstrated that T cells recognizing the immunodominant peptides of candidate myelin antigens occurred at a significantly increased precursor frequency during acute exacerbation in patients with relapsing-remitting MS (RR-MS) (21). The T cell responses to MBP focused on the immunodominant regions (residues 83-99 and residues 151-170) during exacerbation, and shifted toward other epitopes of MBP at the time of remission. Furthermore, there was a marked increase in the production of Th1 cytokines among T cell lines obtained during exacerbation compared to those obtained during remission. The findings suggest that myelin-reactive T cells undergo selective activation and expansion during acute MS exacerbation (21). Taken together, these studies strongly suggest that MBP-reactive T cells are activated *in vivo* in MS patients and thus are pathologically relevant and potentially pathogenic.

Clinical trials of T cell vaccination in MS

Rationale and pilot clinical studies

As discussed above, the TCR repertoire of MBP-reactive T cells is rather restricted in a patient-dependent fashion as the consequence of *in vivo* expansion of MBP-reactive T cells of a limited clonal origin(s). Hence, a specific immunotherapy may take advantage of T cell vaccination that utilizes the whole pathogenic autoreactive T cells and a naturally pre-existing regulatory network to specifically regulate pathogenic autoreactive T cells. In this scenario, the distinguishable cellular marker of “disease-related” autoreactive T cells is likely to be the TCR variable region(s) characteristic for the clonally expanded population in a given patient. The immune system is upregulated to respond to the idiotypic determinants in association with other related cellular markers, in their naturally assembled form, to achieve an adequate regulatory response. These considerations have provided the rationale for clinical trials using T cell vaccination in patients with MS.

An initial clinical trial was carried out in four patients with MS by Hafler and co-workers (22). Patients were inoculated with formadehyde-fixed autologous T cell clones isolated by phynoheamatoglutinine (PHA) stimulation from cerebrospinal fluid. The T cell clones used for vaccination were chosen based upon the CD4 phenotype, growth characteristic and the expression of dominant rearranged TCR genes. The study revealed some interesting immunological findings regarding a general inhibition on T cell stimulation *via* CD2 pathway and increases in autologous mixed lymphocyte responses after vaccination (22). In 1992, we conducted a pilot clinical trial in patients with MS, for the first time, using irradiated autologous MBP-reactive T cell clones (23). This clinical trial aimed to address some primary questions regarding the technical feasibility, toxicity, regulatory T cell responses and potential changes in the frequency of circulating MBP-reactive T cells after vaccination. Disease activity including the frequency and severity of clinical exacerbation, neurological examination and the magnetic resonance imaging (MRI) of the brain lesions was also monitored to potentially correlate with the immunological changes in vaccinated patients. In that study, selected MBP-reactive T cell clones were first activated *in vitro* and irradiated subsequently to render them incapable of proliferation. Each recipient received a total of three subcutaneous injections of 2-4 vaccine clones at intervals between 2-4 months. The clinical trial revealed that the procedure used for our vaccination trial was safe and technically feasible. Subcutaneous inoculations of the autologous vaccine clones were well tolerated and caused no adverse effects except skin redness at the injection site (usually after the second and third injection), which was reminiscent of a delayed type hypersensitivity reaction. Vaccination with irradiated MBP-reactive T cells induces T cell responses that coincide reciprocally with a progressive decrease in the frequency of circulating MBP-reactive T cells in all vaccinated patients. After three vaccinations, no circulating MBP-reactive T cells were detected in the recipients, suggesting a depletion of the autoreactive T cells (23). This study has confirmed for the first time in a clinical setting that T cell vaccination can be used to boost anti-idiotypic regulatory network, resulting in the depletion of pathologically relevant autoreactive T cells (24, 26). Patients were monitored over a period of two to three years for various clinical parameters, including exacerbation rate, expanded disability status score (EDSS) and quantitative changes in brain lesions as detected by MRI (27). Each patient was paired with a control MS subject selected before vaccination. These control MS subjects were matched with the treated patients for age, gender, clinical characteristics. The results of the preliminary study revealed a reduction in the rate of relapse and the MRI lesion activity in patients who received T cell vaccination (23). More recently, Correale and co-workers treated four progressive MS patients with irradiated autologous T cells stimulated with whole bovine myelin (25). Each patient received 5-7 injections of 40×10^6 cells at various intervals. The results confirmed that T cell vaccination did not cause adverse reactions, as evident by clinical monitoring and laboratory tests and that it induced anti-idiotypic T cell responses associated with depletion of myelin-reactive T cells. Three of the four patients either stabilized or improved while one patient progressed.

Extended clinical trial

The pilot studies described here have showed excellent safety profile and the potential clinical benefit, which provided the basis for an extended preliminary clinical trial carried out in Houston (26). This open-label clinical trial was undertaken to investigate whether depletion of

circulating MBP-reactive T cells would be clinically beneficial to patients with MS. Fifty-four patients with RR-MS (n = 28) and secondary progressive (SP)-MS (n = 26) were recruited for the study. The protocol employed in the study for the preparation and the administration of T cell vaccine was the same as that in the pilot study. Patients were monitored for changes in the precursor frequency of MBP-reactive T cells, rate of relapse, EDSS and MRI lesion activities over a period of 24 months. The results were compared with pre-vaccination values in a self-paired manner. First, the study affirms that T cell vaccination with selected MBP-reactive T cell clones induces immune responses, resulting in depletion or suppression of MBP-reactive T cells. The preliminary clinical trial suggests a favorable correlation of T cell vaccination with improved clinical variables. The clinical results indicate that depletion of MBP-reactive T cells coincided with a prolonged time to progression in both RR-MS and SP-MS cohorts as compared to the natural history of MS. However, it should be noted that a trend for an accelerated progression was observed 12 months after the last injection. The significance of this apparent accelerated progression is unknown, but it may be associated with a gradual decline of the immunity induced initially by T cell vaccination against MBP-reactive T cells. Indeed, in approx. 10% of the immunized patients, MBP-reactive T cells reappeared around that time, supporting this possibility. In some cases, the reappearing MBP-reactive T cells originated from different clonal populations that were not detected before vaccination, which was also observed in the previous studies (28). The findings suggest that in a minority of patients MBP-reactive T cells may undergo clonal shift (29) potentially associated with the on-going disease processes. If this observation is confirmed, it may indicate the need for additional booster injections with the same or newly appearing T cell clones to maintain adequate immunity.

Annual MRI examinations revealed a slight reduction in MRI lesion activities in the first year and only a 3.3% increase in the second year. The MRI findings may represent a significant stabilization in patients treated with T cell vaccination. The MRI finding is consistent with the initial delay in time to progression that then apparently accelerate in the second year, reinforcing the possibility that the initial effect of T cell vaccination have diminished in the second year. There were favorable changes in other clinical variables, including annual rate of relapse and EDSS in vaccinated patients, suggesting a beneficial effect of T cell vaccination on the clinical course of MS. The results of the study are largely consistent with the findings reported in the pilot clinical trial (27). However, in contrast to other clinical variables, the impact of T cell vaccination on clinical disability as measured by EDSS was minimal in both study groups. It may reflect the lack of sensitivity of the EDSS to measure changes over a relatively short period of time (24 months). The possibility also exists that even after the autoimmune component is removed or suppressed by T cell vaccination, the inflammatory lesions may still take a long time to resolve and some of the existing tissue damage will be permanent. Moreover, in some patients with advanced disease, the inflammatory lesions may not be directly associated with myelin-reactive T cell responses. Consequently, depletion of MBP-reactive T cells may have little impact on the disease processes in these patients. It is hoped that further investigations may provide new insights into our understanding of these fundamental issues.

This preliminary clinical trial confirms that vaccination with self MBP-reactive T cells provides a consistent and powerful means of immunizing patients to deplete circulating MBP-reactive T cells. However, in the absence of placebo controls, the clinical results were compared with the patient's own pre-treatment status as well as an estimate of the natural history of MS as documented in previous MS trials. Such comparisons may introduce biases in the interpretation of the results. The study is also limited by the potential placebo effect associated with the open-label clinical design of the study. It is clear that the treatment efficacy of T cell vaccination must be evaluated in double-blind and placebo-controlled clinical trials.

Immune regulatory mechanisms

Anti-idiotypic T cell regulation

Immunization with irradiated autoimmune T cells induces immune regulation through highly complex mechanisms and involves interactions of multiple molecules and cell types. The regulatory mechanism of action induced by T cell vaccination is still not completely understood. The studies reported so far have pointed to at least three types of immune regulation underlying T cell vaccination in animal models as well as in humans.

First, T cell vaccination induces idiotype anti-idiotypic network in regulating selected T cells used for vaccination. The idiotypic anti-idiotypic network represents one of the peripheral regulatory mechanisms, which forms the internal image through the recognition of the idiotypic determinants of specific antibodies or T cells in regulating the immune responses to both foreign and self antigens (30). It has been established that anti-idiotypic regulatory T cells are part of the normal T cell repertoire and can be identified in healthy human subjects (31). Anti-idiotypic T cell responses can be activated *in vivo* by T cell vaccination. Several lines of indirect evidence suggest that the anti-idiotypic T cell responses induced by T cell vaccination target specifically at the T cells used for immunization in recognition of target TCR. It has been shown in experimental animals that the protective immunity conferred by T cell vaccination is specific for the disease that autoimmune T cells used for vaccination are able to induce (32). The anti-idiotypic T cells isolated from immunized rodents recognize specifically the immunizing T cell clones/lines but not T cells expressing distinct TCR structural features. It is thought that TCR determinants recognized by anti-idiotypic T cells most likely reside within CDR3 or CDR2, as predicted by characteristic sequence diversity within these regions.

We and others have identified that T cell vaccination induces similar anti-idiotypic regulation in humans (20, 24, 31). Anti-idiotypic T cell lines isolated from immunized patients were predominantly $CD8^+$ cytotoxic T cells and recognized and lyzed the immunizing T cell clones in the context of MHC class I molecules (23, 25). These cytolytic anti-idiotypic T cells exhibit a highly specific recognition pattern that does not cross-react with autologous T cells of

other antigen specificity. In a recent study, we defined the TCR sequences that are responsible for eliciting anti-idiotypic T cell responses in T cell vaccination. A panel of anti-idiotypic T cell lines was isolated from immunized MS patients using overlapping TCR peptides corresponding to CDR2 and CDR3 of the immunizing MBP-reactive T cell clones. Based on the sequence diversity and immunogenic properties of CDR2 and CDR3, the idiotypic determinants recognized by the anti-idiotypic T cells are most probably localized within these regions. The study provided the experimental evidence, for the first time, indicating that T cell vaccination induces $CD8^+$ cytotoxic anti-idiotypic T cell responses that are preferentially directed at CDR3 sequences of the immunizing MBP-reactive T cell clones (34). On the other hand, CDR2 contains cryptic determinants and is less frequently recognized.

There are several existing models that may explain how the idiotypic determinants of target TCR are presented to and recognized by anti-idiotypic T cells. There is experimental evidence indicating that peptides of cell surface molecules are often presented by MHC class I molecules, and that peptide binding motifs for MHC class I molecules have been identified (35, 36). Several recent studies have demonstrated that endogenous TCR peptides can be presented by self MHC to anti-idiotypic T cells (37, 38). The $CD8^+$ cytotoxic anti-idiotypic T cells seem to be characteristically associated with and elicited by T cell vaccination (23, 28). It is remarkable that the immune system immobilizes the $CD8^+$ cytotoxic anti-idiotypic T cells as a specific regulatory component in restraining clonal activation and expansion of MBP-reactive T cells, an exaggerated *in vivo* condition created by T cell vaccination. Alternatively, the route of administration, the amount of T cells administered and potentially altered biological and immunologic properties of the cell surface molecules by irradiation may all account for selective activation of $CD8^+$ cytotoxic anti-idiotypic T cell responses seen in T cell vaccination. The $CD8^+$ cytotoxic anti-idiotypic T cell responses to MBP-reactive T cells induced by T cell vaccination may favorably alter the clinical course of MS. This is suggested by an inverse correlation of anti-idiotypic T cell responses with depletion of circulating MBP-reactive T cells and with some clinical improvement seen in immunized patients with MS, as described in the preceding section. The findings are important in our understanding of the mechanism underlying *in vivo* idiotypic regulation that can be boosted by T cell vaccination.

Anti-idiotypic antibody responses

In addition to anti-idiotypic T cell responses, T cell vaccination is found to induce anti-idiotypic antibody reactivity to the immunizing T cell clones. In a recent study, we examined the occurrence and functional properties of anti-idiotypic antibodies induced by T cell vaccination in patients with MS. A 20-mer TCR peptide incorporating the common CDR3 sequence expressed by the immunizing MBP-reactive T cell clones was synthesized and used as a pure agent in the initial screening to identify anti-idiotypic antibodies. In the study, we employed a cell culture-based technique combining EBV transformation and limiting dilution to generate antibody-producing B cell lines. The study revealed for the first time that B cells producing anti-idiotypic antibodies occurred at an increased precursor frequency in the blood of MS patients after T cell vaccination (39). These antibodies initially screened for their specific reactivity to the CDR3 peptide bound to and had an inhibitory effect on the immunizing MBP-reactive T cells expressing the CDR3 sequence. The study demonstrated that these anti-idiotypic antibodies induced by T cell vaccination appear to have specific regulatory properties, as evident by the inhibition of the proliferation of MBP-reactive T cells used for vaccination (39). It is likely that the anti-idiotypic antibodies bind specifically to the idiotypic determinants of target T cells, thus blocking the T cell recognition of the MBP peptide. The possibility is consistent with other reports describing similar blocking effects of anti-idiotypic antibodies on T cell recognition and function (40). Therefore, the observed anti-idiotypic antibody responses are likely to contribute to the regulatory effects of T cell vaccination on MBP-reactive T cells.

Cytokine regulation

Another important type of immune regulation induced by T cell vaccination is related to Th2 immune deviation. We demonstrated recently that in addition to $CD8^+$ anti-idiotypic T cell responses, T cell vaccination induced $CD4^+$ Th2 regulatory responses. Although $CD4^+$ regulatory cells shared the same regulatory property with $CD8^+$ anti-idiotypic T cells in the inhibition of MBP-reactive T cells, they differed in cytokine profile, the recognition pattern and MHC restriction (41). In contrast to the immunizing MBP-reactive T cell clones that selectively produced TNF-α and interferon-γ (IFN-γ), the $CD4^+$ regulatory T cell lines displayed a predominant Th2 cytokine profile, producing substantial amounts of IL-4 and IL-10 in response to irradiated immunizing T cell clones (41). The majority of the $CD4^+$ regulatory T cell lines did not produce appreciable amounts of TNF-α and IFN-γ. These Th2 regulatory cells exhibited a selective pattern of reactivity and inhibition on activated T cells, but not the resting T cells derived from the same patients, even though they could not distinguish activated MBP-reactive T cells from autologous PHA-activated T cells. The reactivity to activated T cells could be blocked by an anti-MHC class II antibody but not the antibody directed at MHC class I molecules, indicating that the MHC class II molecules acted as the restriction element for the T-T cell interaction (41). The Th2 regulatory responses induced by T cell vaccination correlated with a significant change of the circulating cytokines profile in immunized patients (41). In the majority of immunized patients, elevated levels of circulating IL-4 and IL-10 were found in serum samples obtained after the second or third immunization as compared to baseline values, while the levels of TNF-α and IFN-γ did not change significantly, suggesting a systemic Th2 immune deviation.

The study provided new evidence suggesting that in addition to the T cell receptors, other T cell surface molecules play an important role in the induction of regulatory responses through different mechanisms. Repeated *in vivo* exposure to a large amount of activated T cells, a condition that mimics *in vivo* T cell activation, may signal the immune system to develop Th2 regulatory immune responses. In this regard, as the T cell receptors

and other surface markers are naturally expressed on immunizing T cells, vaccination with whole T cells offers unique advantages in the induction of effective regulatory responses. By the same token, T cell vaccination may represent an immunologically preferred means of therapeutic regulation of autoreactive T cells by taking advantage of the existing regulatory networks. This mechanism of suppression is of importance in the treatment of MS where the autoimmune injury is associated with a disturbed Th1-Th2 equilibrium and aberrant trafficking of inflammatory cells to the site of pathology. In view of considerable heterogeneity among MBP-reactive T cells in patients with MS and the possibility of "epitope spreading" (42), a combination of specific clonal depletion with more generalized suppression of activated T cells by Th2 cytokine regulation is advantageous. The activation-associated molecule(s) capable of signaling the Th2 regulatory responses remains to be defined. As the signaling molecule(s) is uniquely associated with T cell activation, it may correspond to one of the activation markers, cytokine receptors or adhesion molecules. Further investigations are needed to identify the nature of the signaling molecule(s).

Future perspective

We are still at relative early stage in the development of effective T cell vaccine for MS. The T cell vaccine currently made in various laboratories may not be optimal to achieve the best treatment efficacy. In this regard, although Phase I/II studies are absolutely necessary for the evaluation of the safety profile and the immune regulatory responses, a rush into a double-blind control trials may still be premature. Further investigations and experimentation are needed mainly to (1) understand the underlying mechanism of the disease and the role of defined autoreactive T cell populations in the disease we intend to treat and (2) improve the specificity and potency of T cell vaccine and the treatment regimen. There are major obstacles that can significantly hinder the development of effective T cell vaccine for MS.

First, the selection of relevant autoreactive T cells relies on better understanding of myelin autoantigens potentially involved in MS. Although there is experimental evidence suggesting the role of certain myelin antigen(s), it is not proven as to what myelin antigens (e.g., myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein) are definitively associated with MS. This represents a major challenge in the attempt to select relevant autoreactive T cells. Alternatively, multiple myelin-derived peptides corresponding to the immunodominant regions of various myelin proteins of encephalitogenic potential may be used to select myelin autoreactive T cells, which may be superior to the use of single myelin protein/peptide. Second, there are many technical issues that directly affect the specificity and potential potency of T cell vaccine. It remains to be determined whether T cells derived from cerebrospinal fluid would be a better source of T cells for T cell vaccination. The issue stems from the speculation and some evidence that cerebrospinal fluid may contain pathogenic T cell populations highly relevant to the disease processes in CNS because of their vicinity to the site of pathology (43, 44). However, the major challenge in preparing T cell vaccine from cerebrospinal fluid is the selection of relevant T cell clones from relatively heterogeneous T cell populations. Although myelin-reactive T cells can be detected in cerebrospinal fluid of MS patients, they often represent a small fraction of all T cells. It is technically difficult to clone and grow these T cells for the purpose of T cell vaccination. Alternatively, relevant T cell populations may be selected based on *in vivo* clonal expansion of certain T cells. There is evidence indicating oligoclonal activation and expansion of T cells in cerebrospinal fluid of patients with MS. Although it is unclear whether expansion of these T cells of oligoclonal origins is driven by myelin antigens or other unknown antigens, they are more likely to bear pathologic relevance to the disease processes. These clonally expanded T cell populations can be identified by TCR CDR3 DNA sequence analysis or "immunoscope" technology and expanded *in vitro* by alternative T cell stimuli, such as PHA and anti-CD3 antibodies, without the knowledge of their true antigen specificity. There are on-going clinical studies designed to explore the feasibility of T cell vaccination in MS patients using oligoclonal T cells derived from cerebrospinal fluid. However, it remains debatable that CSF-derived T cells would be any advantageous over the blood-derived autoreactive T cells as peripheral autoreactive T cells are easily accessible and are able to migrate into the central nervous system (20).

As stated elsewhere in this review, it is clear that T cell vaccination is of great scientific value in our understanding of *in vivo* immune regulation of autoimmune T cells and provides a unique prove-concept opportunity to test the role of certain autoimmune T cell populations in the disease processes. However, it remains a concern as to how practical is T cell vaccination as a treatment for MS and perhaps in other human autoimmune conditions. One possible solution to simplify T cell vaccination is to develop a peptide-based approach, in which a peptide(s) corresponding to the target molecule(s) of autoimmune T cells is used to induce the same regulatory immune responses. Obviously, the key to this approach lies in the identification of the target molecule(s) responsible for signaling and eliciting regulatory immune responses. The finding that T cell vaccination induces anti-idiotypic immune responses makes TCR an obvious target molecule. Vandenbark, et al. and Howell, et al. demonstrated independently that immunization with TCR peptides corresponding to both CDR2 and CDR3 sequences of encephalitogenic T cells can induce anti-idiotypic immune responses, resulting in protection of experimental animals from developing EAE (45, 46). These pioneer studies provided a clear demonstration that TCR is instrumental in eliciting anti-idiotypic immune responses and had generated new excitement that such an approach may be applicable to MS. Vandenbark and co-workers later conducted a limited clinical trial in MS patients. In this study, synthetic peptides corresponding to V-β 5.1 and V-β 6.2 that were predominantly expressed among MBP-reactive T cells in a Portland-based MS cohort were used for immunization. The study demonstrated some interesting findings, supporting the role of TCR peptides in eliciting

anti-idiotypic immune responses and the production of IL-10 (47). However, with the increasing understanding of the structural features of TCR among myelin-reactive T cells in MS, new doubts are raised about the clinical usefulness of TCR peptide immunization in a large group of MS patients. The major hurdle is related to the heterogeneous usage of TCR among MBP-reactive T cells in different patients with MS. It now becomes clear that MBP-reactive T cells derived from different MS patients utilize a variety of V gene families. The CDR3 sequence is even more diverse among the T cell clones derived from different individuals (13). Therefore, the TCR sequence diversity among MBP-reactive T cells in the general MS population significantly limits the applicability and practicality of the peptide-based immunization approach for the treatment of MS.

However, the TCR V gene usage is not completely random. For example, AV3 and AV8 are relatively over-represented among MBP-reactive T cell clones derived from MS patients (18). Interestingly, the two AV genes are highly restricted in T cell clones that are derived from different MS patients and recognize the 83-99 immuno-dominant peptide of MBP in the context of DRB1*1501 (18). The finding suggests that restricted V gene usage can be found in selected T cell clones that share the same restriction element(s) and epitope recognition. Even more interesting is our recent finding that common CDR3 sequence motifs exist in MBP-reactive T cells derived from different individuals with MS (21). The identified common CDR3 motif (LGRAGLTY) was found in peripheral T cells of > 31% randomly selected MS patients and present in the majority of T cell lines recognizing the immunodominant 83-99 region of MBP isolated from different MS patients (21). It is hoped that the understanding of the regulatory mechanisms induced by T cell vaccination will help to develop a more practical peptide-based vaccination approach by selecting most relevant TCR sequences that can be used to target clonally dominant MBP-reactive T cells in a subset of patients with MS. These TCR peptides have been shown to have additional regulatory properties (e.g., induction of the IL-10 production) (47), which may overcome potential disadvantage of depleting only a subset(s) of MBP-reactive T cells in MS patients. Studies are currently underway in our laboratory to explore the regulatory properties and clinical usefulness of the selected common TCR peptides.

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References

1. Ben-Nun A, Wekerle H, Cohen IR. Vaccination against autoimmune encephalomyelitis with T lymphocyte line cells reactive against myelin basic protein. Nature. 1981;292:60-61.
2. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell. 1996;85:299-302.
3. Hafler D. The distinction blurs between an autoimmune versus microbial hypothesis in multiple sclerosis. J Clin Invest. 1999;104:527-529.
4. Soldan SS, Berti R, Salem N, et al. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. Nat Med. 1997;3:1394-1397.
5. Wucherpfennig K, Strominger J. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell. 1995;80: 695-705.
6. Hemmer B, Fleckenstein BT, Vergelli M, et al. Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. J Exp Med. 1997;9:1651-1659.
7. Kozovska M, Zang YC, Aebischer I, et al. T cell recognition motifs of an immunodominant peptide of myelin basic protein in patients with multiple sclerosis: structural requirements and clinical implications. Eur J Immunol. 1998;6:1894-1901.
8. Zamvil S, Nelson P, Trotter J, et al. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. Nature. 1985;317:355-358.
9. Allegretta M, Nicklas JA, Sriam S, Albertini RJ. T cells responsive to myelin basic protein in patients with multiple sclerosis. Science. 1990;247:718-721.
10. Chou YK, Bourdette DN, Offner H, et al. Frequency of T cells specific for myelin basic protein and myelin proteolipid protein in blood and cerebrospinal fluid in multiple sclerosis. J Neuroimmunol. 1992;38:105-114.
11. Zhang J, Markovic S, Lacet B, Raus J, Weiner HL, Hafler DA. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med. 1994;179:973-984.
12. Hafler D, Saadeh MG, Kuchroo VK, Milford E, Steiman L. TCR usage in human and experimental demyelinating disease. Immunol Today. 1996;17:152-159.
13. Vandervyver C, Mertens N, van den Elsen P, Raus J, Zhang J. Clonal expansion of myelin basic protein-reactive T cells in patients with multiple sclerosis: restricted T cell receptor V gene rearrangements and CDR3 sequence. Eur J Immunol. 1995;25:958-968.
14. Wucherpfennig KW, Zhang J, Witek C, et al. Clonal expansion and persistence of human T cells specific for an immunodominant myelin basic protein peptide. J Immunol. 1994;152:5581-5592.
15. Kotzin BL, Karuturi S, Chou YK, et al. Preferential T-cell receptor β-chain variable gene use in myelin basic protein-reactive T-cell clones from patients with multiple sclerosis. Proc Natl Acad Sci U S A. 1991;20:9161-9165.
16. Garcia KC, Degano M, Stanfield RL, et al. An αβ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science. 1996;274:209-219.
17. Zang YCQ, Kozovska M, Aebischer I, et al. Restricted TCR V-α gene rearrangement in T cells recognizing an immuno-dominant peptide of myelin basic protein in DR2 patients with multiple sclerosis. Int Immunol. 1998;10:991-998.
18. Wucherpfennig KW, Ota K, Endo N, et al. Shared human T cell receptor V usage to immunodominant regions of myelin basic protein. Science. 1990;248:1016-1019.
19. Oksenberg JR, Panzara MA, Begovich AB, et al. Selection for T cell receptor V-D-J gene rearrangements with specificity for a myelin basic protein peptide in the brain lesions of multiple sclerosis. Nature. 1993;362:68-70.
20. Hong J, Zang Y, Tejada-Simon M, et al. A common TCR

sequence motif shared by myelin basic protein-reactive T cells in patients with multiple sclerosis. J Immunol. 1999;163: 3530-3538.
21. Tejada-Simon MV, Zang YC, Yang D, et al. Aberrant T cell responses to myelin antigens during clinical exacerbation in patients with multiple sclerosis. Int Immunol. 2000;12:1641-1650.
22. Hafler D, Cohen IR, Benjamine D, Weiner HL. T cell vaccination in multiple sclerosis: a preliminary report. Clin Immunol Immunopath. 1992;62:307-312.
23. Zhang J, Medaer R, Stinissen P, Hafler DA, Raus J. MHC restricted clonotypic depletion of human myelin basic protein-reactive T cells by T cell vaccination. Science. 1993; 261:1451-1454.
24. Zhang J, Raus J. T cell vaccination trial in multiple sclerosis, in T cell vaccination and autoimmune disease. In: Zhang J, Raus J, eds. Autoimmune disease and T cell vaccination. Austin: Springer-Verlag, Landes Medical Publishers; 1995: 135-160.
25. Correale J, Lund B, McMillan M, Ko DY, McCarthy K, Weiner LP. T cell vaccination in secondary progressive multiple sclerosis. J Neuroimmunol. 2000;107:130-139.
26. Zhang J, Raus J. T cell vaccination in human autoimmune diseases. From laboratory to clinic. Hum Immuno. 1993;38: 87-96.
27. Medaer R, Stinissen P, Truyen L, Raus J, Zhang J. Depletion of myelin basic protein autoreactive T cells by T cell vaccination: pilot trial in multiple sclerosis. Lancet. 1995;346: 807-808.
28. Zhang J, Vandevyver C, Stinissen P, Raus J. *In vivo* clonotypic regulation of human myelin basic protein-reactive T cells by T cell vaccination. J Immunol. 1995;155:5808-5877.
29. European Study Group on interferon β-1b in secondary progressive MS. Placebo-controlled multicentre randomized trial of interferon β-1b in treatment of secondary progressive multiple sclerosis. Lancet. 1998;352:1491-1497.
30. Tuohy VK, Yu M, Yin L, Kawczak JA, Kinkel RP. Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. J Exp Med. 1999;189:1033-1042.
31. Cohen IR. Natural Id-anti-Id networks and the immunological homunculus. In: Atlan H, Cohen IR, Eds. Theories of Immune Networks. Berlin: Springer-Verlag; 1989:6-12.
32. Saruham-Direskeneli G, Weber F, Meinl E, et al. Human T cell autoimmunity against myelin basic protein: $CD4^+$ cells recognizing epitopes of the T cell receptor β chain from a myelin basic protein-specific T cell clone. Eur J Immunol. 1993;23:530-540.
33. Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science. 1988; 239:181-184.
34. Zang Y, Hong J, Rivera V, Killian J, Zhang J. Preferential recognition of hypervariable region sequence by anti-idiotypic T cells induced by T cell vaccination in patients with multiple sclerosis. J Immunol. 2000;164:4011-4017.
35. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu Rev Immunol. 1999;17:51-88.
36. Pamer E, Cresswell P. Mechanism of MHC class I-restricted antigen processing. Annu Rev Immunol. 1998;16:323-358.
37. Broeren CP, Lucassen MA, van Stipdonk MJ, et al. CDR1 T-cell receptor β-chain peptide induce major histocompatibility complex class II restricted T-T cell interactions. Proc Natl Acad Sci U S A. 1994;91:5997-6001.
38. Kumar V, Sercarz E. The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. J Exp Med. 1993;178: 909-916.
39. Hong J, Zang Y, Rivera V, Zhang J. Reactivity and regulatory properties of anti-idiotypic antibodies induced by T cell vaccination in patients with multiple sclerosis. J Immunol. 2000;165:6858-6864.
40. Ito K, Tanaka T, Tsutsumi N, Obata F, Kashiwag N. Possible mechanisms of immunotherapy for maintaining pregnancy in recurrent spontaneous aborters: analysis of anti-idiotypic antibodies directed against autologous T cell receptor. Hum Reprod. 1999;14:650-655.
41. Zang Y, Hong J, Tejada-Simon M, et al. Th2 immune regulation induced by T cell vaccination in patients with multiple sclerosis. Eur J Immunol. 2000;30:908-913.
42. Lehman P, Forsthuber T, Miller A, Sercarz EE. Spreading of T cell autoimmunity to cryptic determinants of an autoantigen. Nature. 1992;358:155-157.
43. Saeki Y, Mima T, Sakoda S, et al. Transfer of multiple sclerosis into severe combined immunodeficiency mice by mononuclear cells from cerebrospinal fluid of the patients. Proc Natl Acad Sci U S A. 1992;89:6157-6161.
44. Fujimura H, Nakatsuji Y, Sakoda S, et al. Demyelination in severe combined immunodeficient mice by intracisternal injection of cerebrospinal fluid cells from patients with multiple sclerosis: neuropathological investigation. Acta Neuropathol (Berl). 1997;93:567-578.
45. Vandenbark AA, Hashim G, Offner H. Immunization with a synthetic T cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature. 1989; 341:541-544.
46. Howell MD, Winters ST, Olee T, Powell HC, Carlo DJ, Brostoff SW. Vaccination against experimental encephalomyelitis with T cell receptor peptides. Science. 1989;246:668-670.
47. Vandenbark AA, Chou YK, Whitham R, et al. Treatment of multiple sclerosis with T-cell receptor peptides: results of a double-blind pilot trial. Nat Med. 1996;2:1109-1115.