CMLS, Cell. Mol. Life Sci. 56 (1999) 5–21
1420-682X/99/020005-17 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Antigen-specific T cells in autoimmune diseases with a focus on multiple sclerosis and experimental allergic encephalomyelitis

B.-G. Xiao* and H. Link

Division of Neurology, Karolinska Institute, Huddinge University Hospital, S-14186 Huddinge, Stockholm (Sweden), Fax + 46 8 585 87080, e-mail: bao-guoxiao@cnsf.ki.se

Received 29 December 1998; received after revision 15 March 1999; accepted 16 March 1999

Abstract. Although the pathogenesis of autoimmune diseases remains poorly understood, the current view is that autoaggresive antigen-specific T cells play a central role in the cascade of events leading to most autoimmune diseases. A major event in the development of autoimmune diseases is the activation of antigen-specific T cells—how, when and where does this activation take place? This review addresses questions concerning the occurrence of unique autoantigens triggering autoimmune diseases, the factors influencing the balance between self-tolerance and autoaggresive immunity, and the mechanisms by which dendritic cells mediate immunity and tolerance to antigen-specific T cells. Knowledge of how antigen-specific T cells are activated is now being used to develop therapeutic approaches to control autoimmune diseases. We discuss tolerance to antigen-specific T cells and tolerance induction as treatment of T-cell-mediated autoimmune diseases. Therapeutic modalities have been established which selectively target the pathogenic T cells, leaving the remainder of the immune system intact.

Key words. Autoimmune disease; multiple sclerosis; experimental allergic encephalomyelitis; antigen-specific T cells; autoantigen; self-tolerance; immunity; immunotherapy.

Introduction

In general, immune responses are subdivided into humoral versus cellular immune responses, and non-specific versus antigen-specific immune responses. Autoimmune diseases are characterized by tissue destruction and functional impairment caused by autoreactive cells or autoantibodies, for example T-cell-mediated multiple sclerosis (MS) and B-cell-mediated myasthenia gravis (MG). Antigen-specific responses rely on antigens and lymphocytes. Lymphocytes entering the thymus undergo two types of selection. Positive selection that allows the differentiation into mature CD4 + CD8 − T cells [if the T cell receptor (TCR) is specific for the peptide class II major histocompatibility complex (MHC)] or CD4 − CD8 + T cells (if the TCR is specific for peptide class I MHC). Negative selection eradicates those lymphocytes that may recognize self cells and induce aggressive autoimmunity. Only approximately 2% of the lymphocytes entering the thymus exit successfully as mature T cells, with the remaining 98% being deleted by selection processes.

The development of T-cell-mediated autoimmune diseases is a complex process that proceeds over several different stages, for example escape from tolerance of autoreactive T cells, repeated stimulation with antigens, activation of autoreactive T cells, dysfunction of immune regulation, infiltration of T cells into a target organ, and tissue destruction. In recent studies, mechanisms have been defined by which some autoreactive T

* Corresponding author.

cells escape tolerance and induce autoimmune diseases. The questions of when, where, and how autoreactive T cells that escape tolerance are activated remain unanswered for most autoimmune diseases.

Autoantigen

Most autoimmune diseases are associated with immune responses directed against a major or a limited number of autoantigens which are the target of the autoimmune process. Expression of these antigens can be restricted to a given organ (typically in organ-specific autoimmune diseases) or ubiquitous (in non-organ-specific autoimmune diseases) [1]. It has become increasingly clear that organ-specific autoimmune diseases like MS, MG, thyroiditis, insulin–dependent diabetes mellitus (IDDM), and rheumatoid arthritis (RA) involve a set of target antigens (table 1). It is, however, still unclear whether these autoimmune responses are driven by these self proteins, or by cross-reacting non-self antigens or both. Four reference disease models, namely IDDM and MS as prototypes of T-cell-mediated organ-specific autoimmune diseases, MG as a prototype of autoantibody-mediated organ-specific autoimmune disease, and system lupus erythematosus (SLE) as a prototype of non-organ-specific autoimmune disease, are all associated with the presence of autoantibodies and/or autoreactive T cells that recognize a large number of antigenic molecules. MS is considered to be mediated by T cells specific for myelin antigens. In this review, we focus mainly on MS and its animal model experimental allergic encephalomyelitis (EAE), describing defined or putative autoantigens, self-tolerance, and autoimmunity, and antigen-specific T cells, and discuss antigen-specific immunotherapy.

Because degradation of the myelin sheath is a central event in the pathogenesis of MS, proteins within the central nervous system (CNS) myelin are considered to represent the candidate autoantigens eliciting the pathogenic self-responses. Myelin basic protein (MBP) has been studied extensively as an autoantigen in both MS and EAE. MBP-specific T cells have been isolated in both diseases and their epitope reactivity, phenotype, functional properties, and TCR gene expression characterized. Both T and B cell responses to native myelin proteolipid protein (PLP) have also been detected in patients with MS [2]. Reactivity to multiple PLP peptides has been demonstrated in the cerebrospinal fluid (CSF) and peripheral blood of patients with MS [3]. These studies confirmed that PLP is a strong encephalitogen in EAE and suggest that PLP is a candidate autoantigen in MS also. Besides MBP and PLP, other minor myelin proteins such as myelin oligodendrocyte glycoprotein (MOG) have become the focus of renewed interest as candidate autoantigens in MS. MOG expression seems to be restricted to the CNS. In contrast to MBP and PLP, MOG is located on the surface of the myelin sheath, rendering it readily accessible to autoreactive T cells. Sun et al. [4] demonstrated that levels of autoreactive T cells producing interferon (IFN)-γ upon stimulation with MOG were elevated in CSF and blood from patients with MS, indicating the presence of MOG-specific T cells in these patients. After stimulation with MBP, PLP, myelin-associated glycoprotein (MAG) or MOG, autoreactive T cells producing interleukin (IL)-2 were also increased in blood from patients with MS, further underlining that autoreactive T cells in MS are directed to several myelin antigens [5]. Kerlero de Rosbo et al. [6, 7] studied primary proliferative responses to several myelin antigens in MS patients and controls. They observed a predominant response to MOG in MS patients, but not in control subjects. Because MOG is the only myelin protein exclusively expressed in the CNS myelin, T cell responses to MOG may play an important role in the pathogenesis of MS.

T cells specific for S100β, a calcium-binding protein that is expressed by astrocytes, can also induce encephalomyelitis in Lewis rats [8]. Interestingly, T cells specific for this non-myelin antigen were class-II-restricted CD4+ Th1 cells, and induced a severe inflammatory response in the nervous system, but only minimal neurological dysfunction. Histopathological analysis also revealed striking differences in the distribution of inflammatory lesions in MBP- and S100β-specific T-cell-mediated disease. In contrast to MBP, S100β-specific T cells induce intense inflammation not only in the spinal cord, but also throughout the entire CNS and in the uvea and retina of the eye, reflecting some characteristics of MS. This new model of S100β-specific T cell-mediated autoimmune CNS disease also exhibits a number of similarities to MS, such as its mild clinical course. Thus, non-myelin antigens may play an unexpectedly important role in the immunopathogenesis of inflammatory diseases of the CNS.

Table 1. Selected examples of antigen clustering in human autoimmune diseases.

Diseases	Antigens
Organ-specific diseases	
Multiple sclerosis	MBP, PLP, MOG
Myasthenia gravis	AChR
Insulin-dependent diabetes mellitus	GAD, insulin, gangliosides
Systemic autoimmune diseases	
Systemic lupus erythematosus	chromatin, dsDNA, histones

Myelin proteins had been thought to be sequestered behind the blood-brain barrier (BBB). It was recently found that both MBP and PLP are expressed at the RNA and protein level in lymph nodes, thymus, and spleen of SJL mice with relapsing EAE [9, 10]. Expression of golli-MBP BG21 mRNA was increased two- to fivefold in lymph nodes from mice with relapsing EAE [9]. Myelin protein expression occurs within T and B cells and macrophages. Further, T cell lines from SJL mice specific for the immunodominant and subdominant epitopes of MBP and PLP can recognize endogenous protein within cells derived from lymphoid tissue. Thus, immunologically relevant myelin proteins are endogenously produced and presented within lymphoid tissues. These observations would explain how myelin-protein-specific T cells become activated outside the CNS, followed by their passage through the BBB to form new CNS lesions during relapses. These considerations should also apply to other non-sequestered antigens. Antigen location may thus regulate immune responses in a dose- and time-dependent fashion [11].

If MBP and PLP are not sequestered antigens, they can cause negative selection. Lehmann et al. [12] proposed a model of a shifting T cell activation threshold to explain how ignorant/naive T cells can become effector cells of autoimmune pathology and why this effector cell repertoire spreads. Assuming that the triggering threshold is constant for naive cells (for example, it could require the simultaneous occupancy of 100 TCRs), the autoantigen-specific repertoire will be negatively selected down to this threshold. If T cells are triggered by a cross-reactive infection or by immunization with peptide 'X,' they upregulate accessory molecules, associated with a lowering of the T cell triggering threshold. The previously ignored endogenous determinant 'X' becomes stimulatory to these cells. An autoimmune response against determinant 'X' leads to upregulation of accessory molecules in the target organ. Based on this theory, the induction of autoimmune responses depends upon antigen reaching and becoming available in lymphoid organs, on the amount of antigen presented, the avidity of antigen binding, timing of antigen presentation and costimulation associated with presented antigens. These interactions among antigen-presenting cells (APCs), T cells, and antigen will form a three-dimensional integral that influences the immunological response.

MBP-specific T cells can be isolated from most individuals, i.e. both patients with MS and control subjects, by repeated stimulation with MBP at limiting-dilution conditions, indicating that MBP-specific T cells are part of the normal T cell repertoire. No substantial differences in epitope reactivity or HLA restriction of MBP-specific T cells were found between patients with MS and healthy subjects [3]. Therefore, the deletion of autoreactive T cells by autoantigen expressed in the thymus is incomplete. Immunologists are considering the possibility that thymic autoantigens 'shape' the immune repertoire positively rather than simply delete the autoreactive T cells [13]. It is proposed that autoreactive T cells that have not been deleted in the thymus are controlled by peripheral mechanisms of tolerance. One mechanism is 'clonal inactivation' that occurs when an autoreactive T cell happens to encounter 'its' autoantigen on a self-MHC molecule in the periphery, but fails to receive costimulatory signals [14]. Another mechanism is active suppression of autoreactive T cells by suppressor T cells and natural killer (NK) cells. Furthermore, some of the autoreactive T cells may not need to be strictly controlled, because their autoantigens are either inaccessible or expressed only at very low levels.

Network recognition of antigen-specific TCRs represents an important peripheral mechanism for controlling self-reactive T cells that escape the thymus. Regulatory T and B cells are naturally induced within the 'immune homunculus' to recognize unique determinants, or idiotopes, thought to be located preferentially within the hypervariable regions of the TCR sequence [15, 16]. The immunological homunculus is an internal image of the self acquired by early recognition of self antigens, both in the thymus and in the periphery. The self image involves T and B cells that deal with the dominant self antigens. The autoimmune T or B cells, channeled to respond to the dominant self antigen by the homunculus, may produce an aggressive autoimmune reaction if not properly regulated by the suppressing control elements of the homunculus. Thus, natural autoimmunity is benign because the immunological dominance of the major self antigens comprising the homunculus is encoded by two committees of cells: naturally occurring autoimmune T and B cells and their anti-idiotypic regulatory cells [15].

Two central questions in the pathogenesis of autoimmune diseases are: which is the autoantigen that constitutes the driving force in triggering autoimmune diseases and how are antigen-specific T cell responses generated? Viruses may break peripheral self-tolerance, and induce and maintain autoimmunity via several mechanisms [17]. They may induce chronic inflammation, which promotes naive T cell priming by providing virus-altered antigens and by inducing APC activation. Among the T cells primed during immune responses to virus, chronic inflammation and persisting viruses can synergistically support autoimmunity through other mechanisms: unveiling of cryptic self-epitopes, determinant spreading, activation of dendritic cells (DCs), constant priming of new autoreactive T cells, and efficient generation and restimulation of memory cells [18]. Viral infection of DC may have either enhancing or inhibitory effects on their capacity to present antigen and costimulate T cells,

depending on virus effects on cell viability, protein synthesis, and secretion of cytokines such as IL-12 [19, 20]. If these positively selected T cells encounter sufficient amounts of self-epitopes in the periphery, they may induce autoimmunity. During viral infections, the generation of many cryptic self-epitopes can be upregulated, either directly, because of virus effects on cellular protein expression and processing, or indirectly, by virus-induced inflammation with subsequent release and processing of autoantigens [21]. Thus, virus-induced inflammation provides optimal conditions for T cell priming [18].

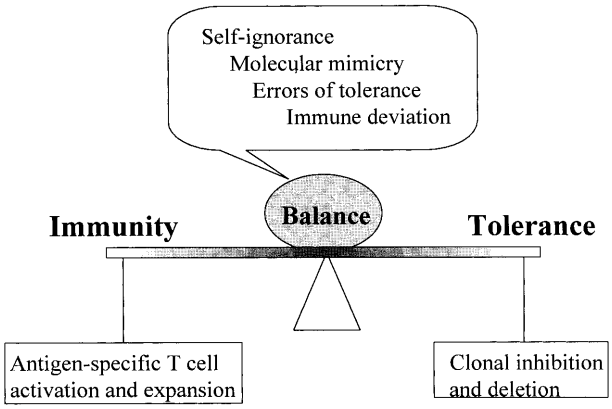

Figure 1. Factors that influence the balance between immunity and tolerance.

Self-tolerance and autoimmunity

Autoimmune diseases represent a failure of control in self-tolerance and a transition from normal to aggressive autoimmunity. Self-tolerance is a state that needs to be actively maintained by various mechanisms. It is also clear that an understanding of these mechanisms is essential for understanding the pathogenesis of autoimmune disorders, and for developing immunotherapies. Self-tolerance at the level of T cells is primarily central, i.e., intrathymic T cell tolerance, and is applicable to locally synthesized antigens presented in association with either MHC class I or class II molecules, or to antigens synthesized in the periphery that are taken up and displayed in the thymus by MHC class II molecules on migrating or resident APCs. The existence of peripheral extrathymic T cell tolerance for some autoantigens has also been suggested. T cell precursors that enter the thymus must first learn to recognize self-MHC molecules. Next, they must learn to tolerate autoantigens present on MHC molecules. In general, antigens associated with peripheral tissue, especially those sequestered behind anatomic barriers, may not come into contact with the developing T cell repertoire and, therefore, tolerance for such antigens may not be needed.

There is ample evidence that immunological tolerance even to abundant myelin antigens is either absent or incomplete. Breakdown in self-tolerance may occur by several mechanisms (fig. 1).

1) The self-ignorance hypothesis [22]. In vitro experiments have shown that mature resting T cells specific for extrathymic antigens can be induced to undergo anergy due to the absence of appropriate 'second signals' or 'costimulatory' factors [23, 24]. This would result in T cells simply ignoring such antigens and remaining quiescent. It follows that, if adequate antigen presentation and costimulation occur through professional APCs, then these autoreactive but quiescent cells may be activated and cause tissue damage. Many studies in TCR-transgenic mice with specificity for peripheral tissue antigens have suggested the absence of active tolerance, and provided evidence of self-ignorance [25].

2) The molecular mimicry hypothesis [26]. Many peptide fragments of infectious agents are homologous with host proteins, including MHC molecules. However, the importance of this mimicry in the pathogenesis of spontaneous autoimmune disease is uncertain. It is similarly unclear why immunological responses to heat shock proteins (HSPs), which are expressed in every cell, could lead to organ-specific autoimmune diseases [27]. Although there are several examples of autoimmune disease following infections with certain microbes, it is somewhat disheartening that, for most human autoimmune diseases, no specific pathogen has been consistently identified.

3) Errors of tolerance. Errors in central or peripheral tolerance at the T or B cell levels have also been suggested as causes for aggressive autoimmunity. Such a mechanism would have more applicability to systemic than to organ-specific autoimmune diseases, since it is difficult to understand how such a defect could be confined to a single, organ-restricted antigen.

4) Immunoregulatory disturbances. Immunoregulatory disturbances have been considered as a primary cause of autoimmune diseases. However, the mechanisms underlying such disturbances have not been delineated. CTLA-4 regulates tolerance induction and T cell differentiation in vivo [28]. The administration of IL-12 stimulates Th1 differentiation, but does not promote antigen-specific T cell proliferation. Conversely, inhibiting CTLA-4 engagement reverses the block in T cell proliferation, but does not promote Th1 differentiation.

The combination of IL-12 antibody and CTLA-4 Ig is sufficient to convert an immunogenic state to a tolerogenic one [29]. IL-12 can reverse established antigen-specific tolerance; these reversing effects of IL-12 were not blocked by anti-IFN-γ monoclonal antibody

(mAb), but were blocked by mAb against B7-1, and more strongly by anti-B7-2 and by both mAbs together [30]. In addition, apoptosis is important for maintaining lymphocyte homeostasis and minimizing the accumulation of autoreactive lymphocytes. Disruption of apoptotic pathways has been linked to breakdown of peripheral tolerance and the development of autoimmunity [31].

Immunological self-tolerance is ensured by eliminating or inhibiting autoreactive lymphocyte clones, creating physical or functional holes in the B and T cell antigen receptor repertoires [32]. The nature and size of these gaps in immune defenses must be balanced against the rapid immune responses to a foreign pathogen. This balance between tolerance and immunity can shift, altering susceptibility to autoimmune disease and to infection as a result of genetic or environmental factors [33].

The role of DCs in immunity and tolerance

Naive T cells can become either tolerant or immune as a result of their first encounter with antigen. It has been suggested that lymphoid and myeloid DCs, respectively, control such decisions [34]. T and B cells are the mediators of immunity, but their function is under the control of DCs. DCs not only activate lymphocytes, they also tolerize T cells to antigens, thereby minimizing autoimmune reactions [35]. Transfer of lymph node DCs from normal mice modulates autoimmunity and prevents diabetes in non-obese diabetic mice by inducing regulatory cells [36], indicating that DCs may play an important role in the regulation of autoimmunity. DCs precultured with IL-10 induced a state of alloantigen-specific anergy in CD4+ T cells and of peptide-specific anergy in influenza-hemagglutinin-specific T cell clones, indicating that IL-10 converts immature DCs into tolerogenic APCs [37]. DCs exposed to IL-10 may play an important role in priming IL-4-secreting cells in early immune responses [38]. Mice treated with Flt3L, a DC growth factor, exhibited more profound systemic tolerance after they were fed soluble antigen [39]. Accordingly, DCs may be capable of presenting many autoantigens to T cells, thus inducing tolerance to self proteins that have no access to the thymus. The mechanism of DC-induced tolerance is likely to depend upon initiation of a complex set of changes in T cell physiology, accompanied by completion of signaling pathways that will induce cell death upon subsequent ligation of the appropriate surface molecules [34].

DCs are the most potent APCs of the immune system and are critically involved in the initiation of primary immune responses and autoimmune diseases. They are not only efficient at presenting peptide antigen but can also process and present soluble protein antigen to antigen-specific T cells and cloned T cell lines. They are very strong stimulators of both allogeneic and syngeneic mixed lymphocyte reactions, and have a unique capacity to stimulate naive T cells. Repetitive injection of the antigen-expressing DCs initiated an autoimmune response leading to destruction of β islet cells [40]. The potent functional capacity of DCs is related to high-level expression of MHC class I or class II molecules and constitutive expression of costimulatory molecules, such as CD80 and CD86, as well as the CD40 and LFA family of adhesion molecules.

Role of autoreactive antigen-specific T cells

EAE is an animal model used in the study of MS. EAE is induced by either immunization with CNS antigens in complete Freund's adjuvant (CFA) or by adoptive transfer of activated CNS antigen-specific T cells. T cells specific for MBP that are able to adoptively transfer EAE recognize encephalitogenic peptides of MBP [3]. EAE can also be induced with PLP-specific T cell lines and clones [3]. Adoptive transfer of autoreactive S100β-specific T cells induces EAE in the Lewis rat, mimicking the distribution of lesions seen in a subset of patients with MS [41]. The resulting inflammatory, demyelinating disease is similar to MS and is manifested by acute, chronic, or relapsing paralysis. Analysis of the cell phenotype demonstrated that EAE is mediated primarily by MHC-class-II-restricted CD4+ T cells that secrete the inflammatory cytokines IL-2 and IFN-γ, and in many cases is inhibited by activation in vivo of Th2 T cells that secrete IL-4 and IL-10. Myelin-autoantigen-reactive T cells can migrate into the CNS, interact with glial cells within the CNS, amplify local immune responses and induce encephalomyelitis.

Although the pathogenesis of MS remains poorly understood, increasing evidence indicates that MS is an autoimmune disease mediated by T helper lymphocytes. The activated T cells traverse the BBB and are locally reactivated when they recognize 'their' antigen on the surface of local APCs. The activated T cells secrete cytokines that stimulate microglia and astrocytes, recruit additional non-specific T cells and macrophages, and induce antibody production by plasma cells. Anti-MOG antibodies and activated macrophages/microglia are thought to cooperate in demyelination.

How do antigen-specific T cells mediate CNS inflammatory and demyelinating processes? Antigen-specific T cells constitute only a small component of the inflammatory infiltrate. However, when studying the biology of inflammatory cells, rare antigen-specific T cells in an inflammatory infiltrate can regulate the behavior of non-specific cells. In vivo administration of native self

peptides or altered self peptide ligands for these rare antigen-specific T cells can lead to the disapperance of an entire autoimmune infiltrate from a target organ over a few hours [42]. Thus, Steinman [43] considered that a few autoreactive cells in an inflammatory infiltrate control a vast population of non-specific cells: a tale of smart bombs and the infantry. Cytokines, adhesion molecules like very late antigen-4 (VLA-4), and matrix metalloproteases are critical in the process of cell penetration [44–46]. Activated T cells are able to bind selectively to inflamed endothelium in the CNS via $\alpha 4$ integrin. Binding was inhibited by antibodies against the integrin molecule $\alpha 4$, but not by antibodies against numerous other adhesion receptors [44]. Surface expression of $\alpha 4$ integrin has been identified as a major pathogenic factor in EAE. Using a cloned Th1 cell line, $\alpha 4$-integrin-high-expressed Th1 cells enter the brain parenchyma in abundance, while $\alpha 4$-integrin-low-expressed Th1 cells do not [45]. Encephalitogenic clones and non-encephalitogenic variants differ by tenfold in their level of surface expression of $\alpha 4$ integrin and in their ability to bind to endothelial cells [45]. The matrix metalloprotease gelatinase B allows the T cells and macrophages to penetrate the extracellular matrix barrier. Inhibition of gelatinase B with hydroxamic acid derivatives blocks the matrix metalloprotease and prevents or reverses ongoing EAE [46]. Gelatinase B can be detected and its activity can be inhibited in CSF obtained from patients with MS with these same hydroxamic acid derivatives [47].

Encephalitogenic-antigen-specific T cells are CD4 + CD8 − T cells and are members of the Th1 phenotype, secreting the cytokines IL-2, IFN-γ and tumor necrosis factor (TNF)-α. Encephalitogenic T cells from various animal strains express a highly restricted TCR gene repertoire. TCR variable (V) gene usage can be studied using monoclonal antibodies directed at a given Vβ or Vα product. Encephalitogenic T cells specific for the Ac1-9 MBP peptide from PL/J mice expressed a TCR composed of Vβ8.2 product associated with either Vα2.3 or Vα4.2 elements [48]. Remarkably, encephalitogenic T cells from the Lewis rat use the same Vα/Vβ pair, even though they recognize a different MBP epitope [49]. What determines the encephalitogenicity of autoreactive T cells? Kuchroo et al. [50] studied several functional properties and phenotypic characteristics of murine PLP-specific T cell clones that were encephalitogenic and other clones with the same antigen reactivity that lacked encephalitogenic potential. They demonstrated that, in addition to the activation status, both the expression of adhesion molecules and the ability to synthesize proinflammatory cytokines determines the encephalitogenicity of the clones [50].

Adoptive-transfer experiments showed that antigen-specific Th1 cells were sufficient to induce EAE. Indeed, MBP- and PLP-specific Th1 cell clones could induce disease, whereas Th2 clones specific for the same peptide-MHC complexes could not [45]. Mucosally derived MBP-specific Th2 clones were able to suppress EAE induced with either MBP or PLP [51]. An analysis of cytokine expression by myelin-antigen-specific T cell clones isolated at different defined stages during the course of MS has suggested that cytokine secretion patterns change with disease status, and that exacerbations and remissions in MS may be characterized by different patterns of cytokine secretion by autoreactive T cells [52]. Thus, when PLP-specific T cell clones isolated from patients with relapsing-remitting MS at various stages during the course of the disease were assessed for secretion of proinflammatory and anti-inflammatory cytokines, the predominant pattern of cytokine secretion during acute attack resembled that of Th1 cells while, during remission, the pattern of secretion of the clones from the same patients included Th0, Th1, and Th2 [7]. These data suggest that not only the presence of autoreactive T cells, but also their nature, are potentially deleterious in MS and need to be taken into consideration in devising therapeutic approaches.

The Th1/Th2 concept remains a useful paradigm for understanding T cell differentiation and regulation, but many immune responses and disease states, including MS and EAE, are more complex and may not be so highly polarized as to depend on only one effector population [53]. Recent data from diverse areas challenge the view that the Th1/Th2 balance regulates autoimmune diseases. Both EAE and experimental diabetes can be induced by adoptive transfer of Th2 cells into immune-deficient hosts [54, 55]. In MS and EAE models, inflammatory lesions can present a Th2-type allergic profile [54]. TNF-α, which has had a long-standing reputation as the typical cytopathic cytokine in MS, can also have anti-inflammatory effects in demyelinating disease [56]. Rational development of experimental therapy has to take into account Th1/Th2 activities of T cells, APCs and/or glial cells and should be based on unequivocal identification of effector cells and the mechanisms by which they differentially contribute to different aspects of CNS damage.

In addition, it is widely believed that autoimmune disease is a developmental process involving a continuous acquisition of new recognition events that maintain the inflammatory condition and thereby cause chronic-progressive disease. The acquired T cell neo-autoreactivity is commonly referred to as epitope or determinant spreading and is presumably due to endogenous priming with new autoantigens presented during the inflammatory destruction of tissue. This phenomenon has been defined in myelin-antigen-induced or Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) [57, 58]. Alternatively, autoreac-

tive T cells may be activated in vivo by self epitopes released secondary to CNS damage resulting either directly from virus-mediated tissue destruction or indirectly from virus-specific T-cell-mediated bystander damage [59]. Several lines of evidence indicate that myelin-peptide-specific responses arising during the chronic stages of TMEV-IDD are mediated by epitope spreading and not as a results of molecular mimicry. The difference in the temporal appearance of T cell reactivity to TMEV (within 7 days post-infection) and responses to myelin epitopes (>50 days post-infection) [58] argues against molecular mimicry as the mechanism for induction of anti-myelin responses in infected mice. There is no apparent T cell cross-reactivity between epitopes on TMEV and MBP, PLP, MOG, or mouse spinal cord homogenate [59]. These findings indicate that, besides molecular mimicry, epitope spreading is an important mechanism to explain the etiology of infection-induced T-cell-mediated autoimmune diseases. Progression of EAE, and perhaps MS, involves a shift in T cell autoreactivity from primary initiating self-determinants to secondary determinants that sustain the inflammatory self-recognition process during disease progression [57]. Three lines of evidence suggest that determinant spreading is pathogenic for disease progression: (i) spreading determinants mediate passive transfer of acute EAE in naive recipients; (ii) an invariant relationship exists between the development of relapse-progression and the spreading of recognition to new immunodominant encephalitogenic determinants [60], and (iii) after EAE onset, the induction of peptide-specific tolerance to spreading but not to non-spreading encephalitogenic determinants prevents subsequent progression of EAE [61].

Activation of antigen-specific T cells

The introduction of foreign or altered antigens dramatically alters the behavior of those lymphocytes in secondary lymphoid tissue that are antigen specific. Antigens that are deposited in tissue may travel passively to the nearest lymph node by draining afferent lymphatic vessels. Alternatively, immature DCs present in the tissue, e.g., Langerhans cells of the skin, may take up the antigen and, in response to inflammatory cytokines, migrate via the afferent lymph to the node [62]. Antigen that is present in the blood will be taken up by red pulp macrophages or diffuse from the red pulp into the white pulp of the lymph node. Although B cells and macrophages probably take up and process free antigen that enters the lymph node and spleen, many studies indicate that antigen-bearing, interdigitating DCs are essential for the initial activation of naive T cells [63]. In addition, the immature DCs that migrate from the tissue may be critical because this type of cell can take up and process large amounts of antigen, a property that is lost in mature DCs [63, 64]. The colocalization of DCs and naive T cells in the paracortex and the periarterial lymphatic sheath increases the chances that naive T cells with the appropriate TCR will find an antigen-bearing APC. The interaction of CD28 expressed by T cells and B7-1 or B7-2 expressed by DCs plays an important role in the clonal expansion of naive T cells in the paracortex. Paracortical DCs express B7-2 molecules in situ, and immature tissue DCs show increased expression of B7 molecules during their migration from tissue to the lymph node [65]. Once activated by antigen-bearing DCs, the specific T cells proliferate in the paracortex and become competent to receive further activation signals from antigen-bearing APCs.

The activation mechanisms of T cells have been extensively analyzed, including recognition of the peptide-MHC complex. The initial activation starts with phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) within CD3 chains by the src tyrosine kinases, Lck and Fyn. This is followed by the recruitment of a second family of tyrosine kinases, ZAP-70 or Syk, to the phosphorylated ITAMs. Then, phosphorylation and activation of these kinases occur to transmit the downstream signal cascades [66]. These downstream signal cascades activate the genes that code for the IL-2 and Fas signaling pathways. The IL-2 and Fas receptors employ additional signaling cascades to mediate their effects on proliferation and apoptosis, respectively (fig. 2). On the cell surface, considerable numbers of other molecules are involved in the initial recognition and activation by the TCR: CD4 and CD8 coreceptors are known to associate with the non-polymorphic region of MHC class II and I, respectively, and are responsible for efficient recruitment of Lck to the TCR complex; CD28 provides costimulation signals by interacting with the ligands B7-1 and B7-2 on APC; several adhesion molecules such as LFA-1 and VLAs play important roles in T cell activation through general cell-cell adhesion between T cells and APCs; and adhesion molecules such as 4-1BB ligand [67], CD47 [68], and CD49 [69] also have a costimulatory function to augment T cell activation. On the other hand, negative regulation of the initial phase of T cell activation appears to be mediated by two distinct mechanisms. The first is the active induction of negative signals through cell surface molecules such as CTLA-4 and killer cell inhibitory receptors (KIRs). The second is the induction of an unresponsive/anergic or suppressive status through various mechanisms including inhibitory cytokines such as IFN-α/β and transforming growth factor (TGF)-β, as well as modulation of the TCR complex and signaling molecules by redox regulation and unresponsiveness induced by antagonist/partial

agonist peptide. A number of signaling components are thus required to stimulate a resting T cell.

Autoreactive T cells can be activated during immune responses through antigen-independent pathways. T cells activated through an antigen-independent 'alternative' pathway develop precocious sensitivity to Fas-induced apoptosis, which may be important in permitting the elimination of autoreactive bystander cells activated in the course of immune responses [70]. Human naive CD4+ T cells can be sensitized in vitro to certain antigens presented by DCs, and the sensitized cells can be expanded into long-term lines that retain their antigen specificity [71]. CD40L-deficient mice are defective in antigen-specific T cell responses. Adoptively transferred antigen-specific CD4+ T cells lacking CD40L failed to expand upon antigen challenge of the recipients, showing that expression of CD40L on cells is required for in vivo priming of CD4+ T cells and, therefore, for the initiation of specific T cell immune responses [72].

Adoptive transfer of TCR-transgenic T cells uniformly expressing an identifiable TCR of known peptide-MHC specificity can be used to monitor the in vivo behavior of antigen-specific T cells. It has been demonstrated that naive T cells are initially activated within the T cell zones of secondary lymphoid tissue to proliferate in a B7-dependent manner. If adjuvants or inflammatory cytokines are present during this time period, T cells accumulate, migrate into B-cell-rich follicles, and acquire the capacity to produce IFN-γ and help B cells to produce IgG2a. If inflammation is absent, most of the initially activated antigen-specific T cells disappear without entering the follicles, and the production of IL-2 and IFN-γ is poor, indicating that inflammatory mediators play a key role in regulating clonal expansion, survival and cytokine production of antigen-stimulated T cells in vivo [73].

CTLA-4 is a negative regulator of T cell activation and autoreactivity. Direct evidence for a critical physiological role for CTLA-4 in negatively regulating T cell activation and autoreactivity came from the phenotype of mice lacking CTLA-4 (CTLA-4 $-/-$) [74, 75]. These mice rapidly developed a spontaneous lymphoproliferative disease with massive lymphocytic infiltrates and tissue destruction in many organs. Myocarditis was particularly severe, and the mice died by 3–4 weeks of age. They exhibited splenomegaly, lymphadenopathy and elevated serum immunoglobulin levels. The peripheral T cells from these mice were activated, proliferated spontaneously in vitro and produced abundant cytokines.

The critical immunoregulatory function of CTLA-4 has been studied by the effects of anti-CTLA-4 mAb upon immune responses to tumors and autoantigens. In murine relapsing-remitting EAE, administration of anti-CTLA-4 mAb led to accelerated and more severe disease [76]. Anti-CTLA-4 mAb treatment during disease remission resulted in exacerbation of relapses. Other

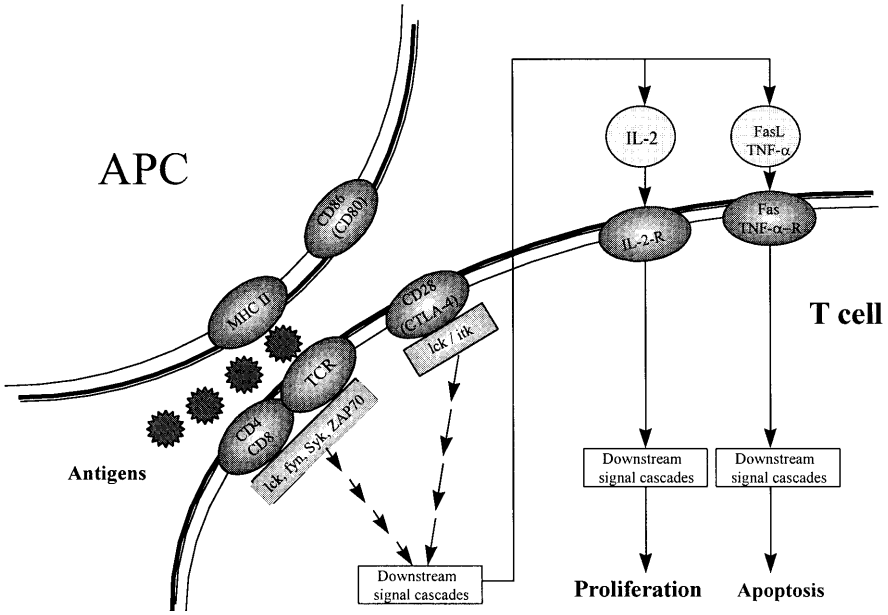

Figure 2. A schematic drawing of T cell activation. The sequence begins (top left) with the antigen bound to MHC class II interacting with the TCR and culminates many hours later (bottom right) in either proliferation or apoptosis.

studies have examined the cellular and molecular mechanisms by which CTLA-4 exerts its effects. CTLA-4 can terminate a T cell response by inducing T cell death by triggering antigen-specific apoptosis [77]. Studies with anti-CTLA-4 mAb also indicate that CTLA-4 signaling can block CD28-dependent IL-2 production, IL-2 receptor expression and cell cycle progression of activated T cells, and arrest cells in G0/G1 [78].

Tolerance of antigen-specific T cells

Our knowledge of how lymphocytes are activated is now being used to develop therapeutic approaches to control autoimmune diseases. The problem is to develop immunosuppressive procedures that will directly target antigen-specific T cells. There are two basic strategies for suppressing such immune responses. One is inhibition of the activity of mature lymphocytes as they mount an immune response. The other seems more suitable since it acts at the onset of an immune response by educating or reprogramming T cells to recognize non-self as self.

Anergy and TCR downregulation

Recent experimental evidence suggests that clonal anergy is one mechanism associated with T cell unresponsiveness induced by antigens or peptides. Typically, after administration of antigen, T cells rapidly enter into the cell cycle and express early activation markers such as CD69 and CD25. One to 4 days post-injection, the T cells exhibit a state of hyporesponsiveness with decreased $Ca2^+$ mobilization, reduced IL-2 production, and diminished cellular proliferation to antigen in vitro [79]. In addition to clonal anergy, the refractory state exhibited by T cells may be the consequence of reduced levels of surface expression of TCR. However, the T cell hyporesponsiveness can be reversed in vivo in the absence of antigen [80], suggesting that the continuous presence of antigen is necessary to maintain a state of anergy.

Anergy is also induced when antigen is recognized in the absence of second signals, the best example being costimulators of the B7 family. An important function of adjuvants is to enhance the expression of the costimulators that determine whether antigen recognition will lead to activation or anergy. T cell tolerance was induced by the administration of protein antigen without adjuvant in normal mice. T cell anergy can also be induced in the presence of costimulation, by using altered peptide ligands (APLs) [81]. Furthermore, antigen presentation by activated MHC class II can lead to a state of anergy in preactivated T cells [82]. It appears, therefore, that the hyporesponsive state which follows soluble-peptide administration is an apparent anergic state which may correlate with altered trafficking properties. If the treated cells are unable to migrate to lymphoid follicles, they will be unable to participate in the normal cell-cell interactions required for their own expansion.

Because the TCR is a marker that distinguishes the pathogenic T cells from other unrelated T cells, the TCR seems to be the most appropriate target structure in designing an effective and specific immunotherapy. Various therapeutic strategies have been designed to target the TCR by monoclonal antibodies to the Vβ gene products and by vaccination with a peptide matching the CDR2 region of the responsible Vβ gene [83, 84]. These studies have shown remarkable success in preventing the development of EAE in sensitized animals and have generated great enthusiasm, since MS may be amenable to a similar treatment. For instance, a peptide corresponding to TCR Vβ 5.2 and Vβ 6.1 is being used in a clinical trial to treat patients with MS [83, 85, 86]. Low doses of peptide induced T cell immunity in 7 of 11 MS patients and no side effects were observed [85]. However, the heterogeneous expression of TCR V gene products among the population of MS patients would considerably complicate the development of TCR-V-gene-based therapeutic strategies. A treatment agent designed to target certain TCR V gene product(s) may be useful in one patient but not suitable for another, which hampers its clinical usefulness.

Peripheral T cell deletion

Upon systemic administration of antigen, CD4 + - and CD8 + -specific T cells that have entered the cell cycle can undergo clonal deletion, the extent of which varies according to the experimental system employed. Antigen-specific T cells can either be completely eliminated with no subsequent development of memory T cells, or deletion can be incomplete. Several studies have provided evidence favoring apoptosis as the mechanism mediating T cell deletion following systemic administration of antigen. Using a TCR-transgenic system, a significant increase in the number of apoptotic cells in lymph nodes and spleen could be detected following intravenous (i.v.) injection of an I-A^d-restricted flu virus hemagglutinin peptide [79]. Using the TUNEL reaction, in situ apoptosis was detectable as early as 12 h post-injection and peaked by 36 h, clearly indicating that apoptosis was involved in the deletion of mature CD4 + T cells in this system [87].

Using two-color flow cytometry, a single intraperitoneal injection of MBP increased the level of apoptosis of the Vβ8.2 + T cell population in the CNS of Lewis rats with EAE induced by inoculation with MBP and CFA compared to saline-treated rats and ovalbumin-treated

rats. In contrast, treatment with MBP did not increase the level of apoptosis of the Vβ8.2+ population in popliteal lymph nodes or spleen. Limiting-dilution analysis revealed that this treatment decreased the frequency of T cells reactive with the major encephalitogenic epitope in the CNS but not in popliteal lymph nodes [88]. Intravenous administration of MBP-coupled syngeneic splenocytes is an extremely efficient method for prevention and treatment of chronic relapsing EAE, in which inhibition of MBP-specific encephalitogenic CD4+ effector T cells is due to the direct induction of anergy/deletion [89]. Although it has been proposed that autoimmune disease could result from the failure of normal deletional mechanisms, this preliminary survey of MBP-reactive mature T cells from MS patients revealed that such cells are more highly susceptible to TCR-induced apoptosis than those of normal subjects, indicating that therapeutic strategies based on antigen-induced apoptosis of T cells may be feasible in humans. However, both Fas ligand (FasL) and TNF-dependent pathways appear to mediate the peripheral CD4+ T cell deletion following i.v. administration of antigen.

Immune deviation

In addition to mediating clonal anergy and deletion, immune deviation is the third dimension of T cell tolerance [90]. Systemic injection of antigen appears to promote 'immune deviation,' in which a functional shift from a Th1 to Th2 response occurs. An APL, generated by a single amino acid substitution (trytophan to glutamine at position 144), inhibits the development of EAE induced with PLP 139–151 peptide. The APL induces T cells to produce Th2 (IL-4 and IL-10) and Th0 (IFN-γ and IL-10) cytokines, indicating that immune deviation may be one of the mechanisms by which APL can inhibit an autoimmune disease [91]. The factors that preferentially promote immune deviation versus clonal anergy and/or deletion are not fully understood. Clonal anergy, clonal deletion and immune deviation from Th1 to Th2 T cell subsets have all been implicated as possible mechanisms in T cell tolerance. Which mechanism predominates depends on antigen dosage, frequency and timing of antigen administration [92]. TGF-β-mediated bystander regulatory mechanisms are thought to be important for antigen-specific tolerance induction [93].

Antigen-specific immunotherapy in autoimmune diseases

The concept of antigen-specific immunotherapy of autoimmune diseases is based, in part, on the fact that the pathogenesis of certain autoimmune diseases may involve specific antigen and activated T cells. Current immunosuppressive therapeutics have important drawbacks. Cyclosporine A and corticosteroids cannot be targeted solely to disease-causing T cells, but impair protective T cells in general. These immunosuppressants also cause metabolic derangements and organ toxicities at therapeutic doses. A rational immunotherapy should target antigen-specific T cells in an antigen-specific manner. Critchfield and Lenardo [94] have proposed the model of propriocidal regulation to explain some of the molecular interactions that contribute to antigen-induced T cell death. A T cell goes through at least three distinct stages on the pathway to death: (i) TCR-mediated activation with cytokine production; (ii) cytokine-mediated cell cycle progression, and (iii) TCR re-engagement during susceptible phases of the cell cycle, which leads to apoptosis. Though these results are very promising, a number of challenges must be addressed for the propriocidal mechanism to be of value in the clinic. Due to the complex nature of autoimmune diseases, multiple antigens may be involved in each disease and may need to be administrated to achieve T cell deletion that is significant enough for a clinical effect. In addition the need for activating T cells prior to deletion raises the prospect that disease manifestations may transiently worsen prior to T cell deletion. Finally, epitope spread, or the clonal diversification of the reactive T cells, which might be expected in these chronic diseases, poses the challenge of identifying antigens that activate various subsets of T cells at different stages of diseases. A major goal in the treatment of T-cell-mediated autoimmune diseases is the establishment of therapeutic modalities that selectively target the pathogenic T cells, leaving the remainder of the immune system intact. Ideally, such therapy should be effective in the treatment of established disease.

Systemic administration of antigen

Systemic administration of antigen is one strategy that has been shown to induce effective antigen-specific T cell tolerance. The route of administration and the dose and nature of antigen appeared to be crucial parameters. Intraperitoneal (i.p.) or i.v. routes were more effective than the subcutaneous route in inducing tolerance [87]. Several studies have demonstrated that EAE can be prevented in an antigen-specific manner. This has been accomplished by treating animals with MBP, PLP, or the corresponding peptides, before induction or prior to the onset of disease. Another successful antigen-specific immunotherapy has been the use of APLs. Work by Brocke et al. [42] provides evidence that APL can in fact be used to treat established EAE. Mice exhibiting paralysis and inflammatory infiltrates in the brain induced by the adoptive transfer of an encephalitogenic T cell clone specific for MBP 87–99 can be treated follow-

Table 2. Mucosal tolerance: effects of peptides administered in different experimental autoimmune diseases.

Peptides	Animal models						Reference
	experimental allergic encephalomyelitis	experimental autoimmune myastheria gravis	experimental autoimmune neuritis	experimental autoimmune uveoretinitis	insulin-dependent diabetes mellitus	collagen-induced arthritis	
MBP							
68–88	+(R)(O)						110
21–40	+(R)(O)						111
71–90	+(R)(O)						111
Acl-9	+(M)(N)						112
68–86	+(R)(N)						113
87–99	+(R)(N)						
AChR							
α61–76		−(R)(N)					114
α100–116		−(R)(N)					
α146–162		−(R)(N)					
δ354–367		−(R)(N)					
P2							
57–81			+(R)(N)				115
S-Ag							
342–355				+(R)(O)			116
Insulin							
B-(9-23)					+(M)(N)		117
Collagen							
814–198						+(R)(N)	117
250–270						+(M)(O)	118

+, inhibition of disease; −, no effect; (R), rat; (M), mouse; (O) oral; (N), nasal.

ing administration of an APL in which the proline at position 96 is replaced with an alanine.

Mucosal tolerance of antigen

A large series of studies have demonstrated that mucosal tolerance induced by oral or nasal antigen administration is effective in prevention and treatment of several experimental autoimmune disease models (Table 2) [95]. Mucosal tolerance affects T-cell-mediated effector functions and signs of inflammation. Antibody levels are also reduced, although this is due to a lack of T cell help rather than direct B cell tolerance. The direct inhibitory effect of orally administered antigens on T lymphocytes has been widely explored with target antigens in experimental models of T-cell-mediated autoimmune inflammatory diseases such as experimental autoimmune uveoretinitis, arthritis and EAE, as well as B cell-mediated experimental autoimmune myasthenia gravis (EAMG) [96]. It is more difficult to study the mechanism of antigen-specific unresponsiveness in vivo, because the frequency of precursor cells is so low. To overcome this problem, various groups have described TCR-transgenic models in which the frequency of specific cells is increased either by using the transgenic mouse itself or using transgenic cells transferred into normal homozygous recipients. For example, peptide administered by the intranasal route may reach the thymus, where peptide of sufficiently high affinity can induce apoptosis of developing thymocytes. Intranasally administered isotope-labeled IL-10 enters the blood about 6 h after administration (unpublished data). Nasal administration is an effective route for induction of immune tolerance, with certain advantages in comparison to the oral route. For example, the antigens do not encounter the acidic and proteolytic environments of the gastrointestinal tract. Therefore, nasal administration seems to be more efficient because much smaller amounts of antigen are required to induce mucosal tolerance. We demonstrated that Torpedo acetylcholine receptor (AChR) given orally in milligram doses or nasally in microgram doses to Lewis rats prior to immunization with AChR were equally effective in preventing clinical signs of EAMG and suppressing AChR-specific T- and B-cell-mediated immune responses [97].

The cellular and molecular mechanisms of the regulatory elements acting in mucosal tolerance are unknown. A recent study demonstrated that mucosal administration of antigens induces antigen-specific TGF-β1-secreting T cells in patients with MS [98]. Studies of oral tolerance in the Lewis rat EAE model have shown that the suppressive effect of oral antigen administration could be attributed to TGF-β, which was released by CD8+ T cells upon antigen-specific stimulation [99, 100]. Our studies revealed that an IL-4 and/or TGF-β-mediated regulatory mechanism is responsible for the

protection of protracted-relapsing EAE in DA rats [101]. Thus, the mechanisms of mucosal tolerance via specific antigen administration are either anergy/deletion of antigen-specific T cells or induction of IL-4- and/or TGF-β-related regulatory mechanisms, depending on the property and dosage of antigens administered (fig. 3).

In human autoimmune diseases, a double-blind phase III trial (515 patients) of bovine myelin in relapsing-remitting MS did not show differences between placebo and treated groups [102]. In RA, a double-blind phase II trial (280 patients) of chicken type II collagen in doses ranging between 20–2500 μg demonstrated statistically significant positive effects in the group treated with the lowest dose [103]. In a phase I/II trial (30 patients) of uveitis, patients orally administered S antigen showed no statistically significantly differences compared to the placebo group [104].

T cell or TCR vaccination therapies

Autoreactive antigen-specific T cells are viewed as pathogens in the induction of T-cell-mediated experimental autoimmune diseases. T cell vaccination is a method to induce resistance to autoimmune disease by priming the immune system with autoreactive T cells, in analogy with traditional microbial vaccination against infectious agents. The immunotherapy has two pillars. First, the healthy immune repertoire must contain potentially self-reactive T cell clones, and these clones must be normally suppressed by counter-regulatory mechanisms. Second, in autoimmune diseases, the equilibrium between autoaggressive T cells and the suppressive mechanisms is lost, but can be re-established by appropriate stimulation of the counter-regulatory mechanisms. Administration of autoreactive T cells as vaccines induces or augments the regulatory network to specifically suppress the eliciting autoreactive T cells. On the one hand, T cell vaccination provides a unique tool to study in vivo the network regulation potentially operative in the normal immune system to control autoreactive T cells. On the other hand, T cell vaccination provides a potential therapeutic option for human autoimmune pathologies to target and deplete autoreactive T cells involved in the pathogenesis of the diseases.

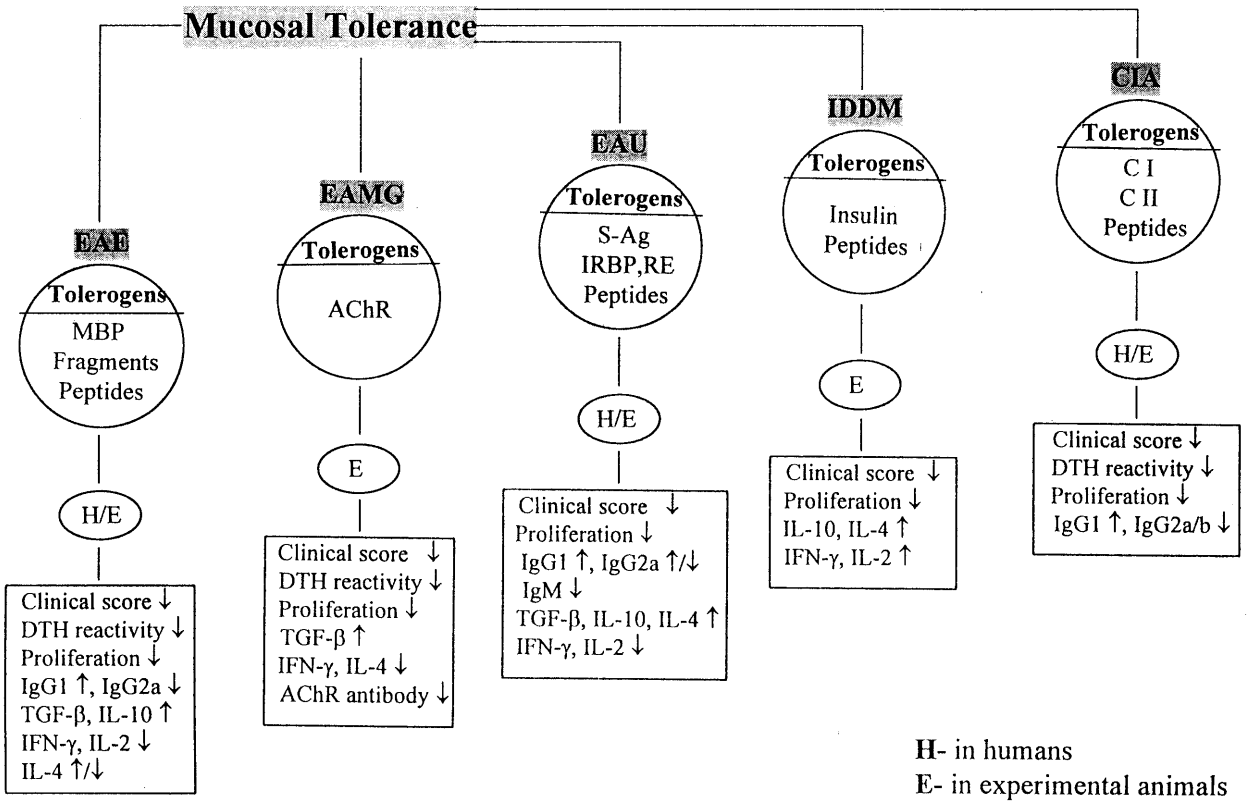

Figure 3. Mechanism of mucosal tolerance induced by orally or nasally administered autoantigens or peptides in several experimental models.

Table 3. Antigen-specific immunotherapy in autoimmune diseases.

Immunotherapy	Model/disease	Antigen	Effect	Reference
Animal models				
Systemic administration	EAE	MBP peptide	+	119
	EAU	IRBP peptide	+	120
	EAT	thyroglobulin	+	121
	Diabetes (NOD mice)	GAD-65	+	122
Muscosal tolerance	EAE	MBP, peptide	+	101, 102
	EAU	S-Ag, IRBP	+	123, 124
	EAT	tyroglobulin	+	125
	Diabetes (NOD mice)	insulin peptide	+	126
	EAN	P2 peptide	+	115
	EAMG	AChR	+	97
TCR vaccination	EAE	Vβ8.2-39-59	+	127
	CIA	VA11.1-JA17 gene	+	128
T cell vaccination	EAE	autoreactive T cells	+	108
	Diabetes (NOD mice)	CD4+Vβ8+ T cells	+	129
	EAU	autoaggressive T cells	+	130
Human disease trials				
Systemic administration	MS	bovine MBP	AB↓	131
Oral tolerance	MS (phase III trial)	bovine MBP	−	102
	RA (phase II trial)	chicken CII	+	103
	Uveitis	bovine S-Ag	+	104
TCR vaccination	MS	Vβ5.2 peptide	+	109
	MS (phase I trial)	Vβ6-39-58	?	132
	RA	Vβ3+Vβ14+Vβ17 peptide	+	133
T cell variation	MS	irradiated T cells	+	107
	RA	synovial T cells	?	134

EAE, experimental allergic encephalomyelitis; EAU, experimental autoimmune uveoretinitis; EAT, experimental autoimmune thyroiditis; EAN, experimental autoimmune neuritis; EAMG, experimental autoimmune myasthenia gravia; CIA, collagen-induced arthritis; MS, multiple sclerosis; RA, rheumatoid arthritis; GAD, glutamic acid decarboxylase; IRBP, interphotoreceptor retinoid-binding protein; NOD, non-obese diabetic; AChR, acetylcholine receptor; CII, collagen type II; Ab, antibody; S-Ag, S antigen; +, effect; −, no effect.

Several variants of successful T cell vaccination against autoimmune diseases have been reported. In these animal models, all encephalitogenic T cell clones use the TCR Vβ8.2 element. It appears that immunization with TCR peptides stimulates regulatory anti-Vβ8.2-specific T cells that inhibit the encephalitogenic target T cells. Inhibition seems to be mediated, at least partially, by soluble factors, raising the possibility that the presence of regulatory TCR-specific T cells in the CNS might inhibit not only the stimulating Vβ8.2 T cells but also 'bystander T cells' expressing different V genes [105].

In human pilot trials of T cell vaccination, MS patients were vaccinated with MBP-reactive T cells cloned from their blood. Selected MBP-reactive T cell clones were activated in vitro and irradiated to render them incapable of proliferation. Based on the potential pathological role of MBP-specific T cells in MS and encouraged by the successful treatment of EAE by T cell vaccination, a small group ($n = 8$) of patients with MS was immunized with irradiated autologous MBP-specific T cell clones. The T cell clones were selected on the basis of their reactivity with the two immunodominant regions of human MBP, which dominated T cell responses to MBP in these individuals. Administration of the vaccines was accompanied by a specific depletion of circulating MBP-reactive T cells [106]. Magnetic resonance imaging showed a mean 8% increase in brain lesion size in the vaccinated patients compared to a 39.5% increase in the controls [107]. At the present time, the information is not sufficient to predict whether T cell vaccination will remain a 'personalized' treatment or may be generalized using peptide(s) in a category of patients whose targeted autoreactive T cells share a common TCR structural feature. Borghans et al. [108] developed a mathematical model to study how the interactions between autoreactive T cells, self epitopes and regulatory cells can explain T cell vaccination, and which predicts a qualitative difference between the two vaccination methods. Vaccination with normal autoreactive cells should give rise to a steady-state of long-term protection, whereas vaccination with attenuated cells should only confer transient resistance. A TCR peptide vaccine has been produced based on Vβ5.2 that is expressed in MS plaques and on MBP-specific T cells. Patients with chronic progressive MS ($n = 22$) receiving this vaccine showed a reduced MBP response to the TCR peptide vaccine, and remained clinically stable without side effects during 1 year of therapy [109]. Taken together, T cell and TCR vaccination in MS is complicated by the remarkable complexity and diversity

of the human autoimmune T cell response. A further problem is that the role of MBP and other potential autoantigens is still unknown.

Antigen-specific immunotherapies use the exquisite specificity of T cell antigen recognition to eliminate selectively the pathogenic T cells. In most studies, it appears that antigen-specific immunotherapy is effective when applied to well-characterized animal models of autoimmunity (table 3). A major question is whether such an approach is feasible in the prevention or treatment of complex spontaneous autoimmune diseases in humans. Knowledge gained in understanding the basic mechanisms of T cell tolerance induction and maintenance, as well as the dissection of the effector mechanisms of pathogenic autoimmunity, should provide rational grounds for further clinical investigations.

1 Bach J. F., Koutouzov S. and Endert P. M. van (1998) Are there unique autoantigens triggering autoimmune diseases? Immunol. Rev. **164:** 139–155
2 Sun J. B., Olsson T., Wang W. Z., Xiao B. G., Kostulas V., Fredrikson S. et al. (1991) Autoreactive T and B cells responding to myelin proteolipid protein in multiple sclerosis and controls. Eur. J. Immunol. **21:** 1461–1468
3 Stinissen P., Raus J. and Zhang J. (1997) Autoimmune pathogenesis of multiple sclerosis: role of autoreactive T lymphocytes and new immunotherapeutic strategies. Crit. Rev. Immunol. **17:** 33–75
4 Sun J. B., Link H., Olsson T., Xiao B. G., Andersson G. and Ekre H. P. (1991) T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. J. Immunol. **146:** 1490–1495
5 Lu C. Z., Fredrikson S., Xiao B. G. and Link H. (1993) Interleukin-2 secreting cells in multiple sclerosis and controls. J. Neurol. Sci. **120:** 99–106
6 Kerlero de Rosbo N., Milo R., Lees M. B., Berger D., Bernard C. C. and Ben-Nun A. (1993) Reactivity to myelin antigen in multiple sclerosis: peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J. Clin. Invest. **92:** 2602–2608
7 Kerlero de Rosbo N. and Ben-Nun A. (1998) T-cell responses to myelin antigens in multiple sclerosis: relevance of the predominant autoimmune reactivity to myelin oligodendrocyte glycoprotein. J. Autoimmunity **11:** 287–299
8 Kojima K., Berger T., Lassmann H., Hinze-Selch D., Zhang Y., Gehrmann J. et al. (1994) Experimental autoimmune panencephalitis and uveoretinitis transferred to the Lewis rat by T lymphocytes specific for the S100 beta molecules, a calcium binding protein of astroglia. J. Exp. Med. **180:** 817–829
9 Mackenzie-Graham A., Pribyl T. M., Kim S., Porter V. R., Campagnoni A. T. and Voskuhl R. R. (1997) Myelin protein expression is increased in lymph nodes of mice with relapsing experimental autoimmune encephalomyelitis. J. Immunol. **159:** 4602–4610
10 Voskuhl R. R. (1998) Myelin protein expression in lymphoid tissues: implication for peripheral tolerance. Immunol. Rev. **164:** 81–92
11 Zinkernagel R. M., Ehl S., Aichele P., Oehen S., Kundig T. and Hengartner H. (1997) Antigen location regulates immune response in a dose- and time-dependent fashion: a geographical view of immune reactivity. Immunol. Rev. **156:** 199–209
12 Lehmann P. V., Targoni O. S. and Forsthuber T. G. (1998) Shifting T-cell activation thresholds in autoimmunity and determinant spreading. Immunol. Rev. **164:** 53–62
13 Wekerle H., Bradl M., Linington C., Kaab G. and Kojima K. (1996) The shaping of the brain-specific T lymphocyte repertoire in the thymus. Immunol. Rev. **149:** 231–243
14 Schwartz R. H. (1996) Models of T cell anergy: is there a common molecular mechanism? J. Exp. Med. **184:** 1–8
15 Cohen I. R. and Young D. B. (1991) Autoimmunity, microbial immunity and the immunological homunculus. Immunol. Today **12:** 105–111
16 Cohen I. R. (1992) The cognitive paradigm and the immunological homunculus. Immunol. Today **13:** 490–494
17 Steinman L. and Conlon P. (1997) Virus damage and the breakdown of self-tolerance. Nat. Med. **3:** 1085–1087
18 Rosa F. D. and Barnaba V. (1998) Persisting viruses and chronic inflammation: understanding their relation to autoimmunity. Immunol. Rev. **164:** 17–27
19 Fugier V. I., Servet D. C., Rivailler P., Rissoan M. C., Liu Y. J. and Rabourdin C. C. (1997) Measles virus suppresses cell mediated immunity by interfering with the survival and functions of dendritic and T cells. J. Exp. Med. **186:** 813–823
20 Grosjean I., Caux C., Bella C., Berger I., Wild F., Banchereau J. et al. (1997) Measles virus infects human dendritic cells and blocks their allostimulatory properties for CD4+ T cells. J. Exp. Med. **186:** 801–812
21 Barnaba V. (1996) Viruses, hidden self-epitopes and autoimmunity. Immunol. Rev. **152:** 47–66
22 Elson C. J., Barker R. N., Thompson S. J. and Williams N. A. (1995) Immunologically ignorant autoreactive T cells, epitope spreading and repertoire limitation. Immunol. Today **16:** 72–76
23 Schwartz R. H. (1990) A cell culture model for T lymphocyte clonal anergy. Science **248:** 1349–1356
24 Janeway C. A. Jr. and Bottomly K. (1994) Signals and signs for lymphocyte responses. Cell **76:** 275–285
25 Theofilopoulos A. N. (1995) The basis of autoimmunity. I. Mechanisms of aberrant self-tolerance. Immunol. Today **16:** 90–98
26 Fujinami R. S. and Oldstone M. B. (1985) Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science **230:** 1043–1045
27 Georgopoulos C. and McFarland H. (1993) Heat shock proteins in multiple sclerosis and other autoimmune diseases. Immunol. Today **14:** 373–375
28 Walunas T. L. and Bluestone J. A. (1998) CTLA-4 regulates tolerance induction and T cell differentiation in vivo. J. Immunol. **168:** 3855–3860
29 Van Parijs L., Perez V. L., Biuckians A., Maki R. G., London C. A. and Abbas A. K. (1997) Role of interleukin 12 and costimulation in T cell anergy in vivo. J. Exp. Med. **186:** 1119–1128
30 Ushio H., Tsuji R. F., Szczepanik M., Kawamoto K., Matsuda H. and Askenase P. W. (1998) IL-12 reverse established antigen-specific tolerance of contact sensitivity by affecting costimulatory molecules B7-1 (CD80) and B7-2 (CD86). J. Immunol. **160:** 2080–2088
31 Koh J. S. and Levine J. S. (1997) Apoptosis and autoimmunity. Curr. Opin. Nephrol. Hypertens. **6:** 259–266
32 Goodnow C. C. (1996) Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. Proc. Natl. Acad. Sci. USA **93:** 2264–2271
33 Limmer A., Sacher T., Alferink J., Kretschmar M., Schönrich G., Nichterlein T. et al. (1998) Failure to induce organ-specific autoimmunity by breaking of tolerance: importance of the microenvironment. Eur. J. Immunol. **28:** 2395–2406
34 St. Groth B. F. de (1998) The evolution of self-tolerance: a new cell arises to meet the challenge of self-reactivity. Immunol. Today **19:** 448–454
35 Banchereau A. and Steinman R. M. (1998) Dendritic cells and the control of immunity. Nature **392:** 245–252

36 Clare-Salzler M. J., Brooks J., Chai A., Van Herle K. and Anderson C. (1992) Prevention of diabetes in nonobese diabetic mice by dendritic cell transfer. J. Clin. Invest. **90:** 741–748

37 Steinbrink K., Wölfl M., Jonuleit H., Knop J. and Enk A. H. (1997) Induction of tolerance by IL-10-treated dendritic cells. J. Immunol. **159:** 4772–4780

38 Liu L., Rich E. E., Inobe J. I., Chen W. and Weiner H. L. (1998) Induction of Th2 cell differentiation in the primary immune response: dendritic cells isolated from adherent cell culture treated with IL-10 prime naive CD4+ T cells to secrete IL-4. Int. Immunol. **10:** 1017–1026

39 Viney J. L., Mowat A. M., O'Malley J. M., Williamson E. and Fanger N. A. (1998) Expanding dendritic cells in vivo enhances the induction of oral tolerance. J. Immunol. **160:** 5815–5825

40 Ludewig B., Odermatt B., Landmann S., Hengartner H. and Zinkernagel R. M. (1998) Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. J. Exp. Med. **188:** 1493–1501

41 Kojima K., Reindl M., Lassmann H., Wekerle H. and Linington C. (1997) The thymus and self-tolerance: coexistence of encephalitogenic S 100 beta-specific T cells and their nominal autoantigen in the normal adult rat thymus. Int. Immunol. **9:** 897–904

42 Brocke S., Gijbels K., Allegretta M., Ferber I., Piercy C., Blankenstein T. et al. (1996) Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. Nature **379:** 343–346

43 Steinman L. (1996) A few autoreactive cells in an autoimmune infiltrate control a vast population of nonspecific cells: a tale of smart bombs and the infantry. Proc. Natl. Acad. Sci. USA **93:** 2253–2256

44 Yednock T., Cannon C., Fritz L., Sanchez-Madrid F., Steinman L. and Karin N. (1992) Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature **356:** 63–66

45 Baron J. L., Madri J. A., Ruddle N. H., Hashim G. and Janeway C. A. Jr. (1993) Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J. Exp. Med. **177:** 57–68

46 Gijbels K., Galardy R. E. and Steinman L. (1994) Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J. Clin. Invest. **94:** 2177–2182

47 Gijbels K., Masure S., Carton H. and Opdenakker G. (1992) Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. J. Neuroimmunol. **41:** 29–34

48 Heber-Katz E. and Acha-Orbea H. (1989) The V-region hypothesis: evidence from autoimmune encephalomyelitis. Immunol. Today **10:** 164–169

49 Burns F. R., Li X., Shen N., Offiner H., Chou Y. K., Vandenbark A. A. et al. (1989) Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar Vα and Vβ chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. J. Exp. Med. **169:** 27–39

50 Kuchroo V. K., Martin C. A., Greer J. A., Ju S. T., Sobel R. A. and Dorf M. E. (1993) Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clines to mediate experimental allergic encephalomyelitis. J. Immunol. **151:** 4371–4382

51 Chen Y., Kuchroo V. K., Inobe J. I., Hafler D. A. and Weiner H. L. (1994) Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science **265:** 1237–1240

52 Correale J., Gilmore W., McMillan M., Li S., McCarthy K., Le T. et al. (1995) Patterns of cytokine secretion by autoreactive proteolipid protein-specific T cell clones during the course of multiple sclerosis. J. Immunol. **154:** 2959–2968

53 Laman J. D., Thompson E. J. and Kappos L. (1998) Balancing the Th1/Th2 concept in multiple sclerosis. Immunol. Today **19:** 489–490

54 Lafaille J. J., Van de Keere F., Hsu A. L., Baron J. L., Haas W. and Raine C. S. (1997) Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J. Exp. Med. **186:** 307–312

55 Pakala S. V., Kurrer M. O. and Katz J. D. (1997) T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. J. Exp. Med. **186:** 299–306

56 Liu J., Marino M. W., Wong G., Grail D., Dunn A., Bettadapura J. et al. (1998) TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. Nat. Med. **4:** 78–83

57 Tuohy V. K., Yu M., Yin L., Kawczak J. A., Johnson J. M. and Mathisen P. M. (1998) The epitope spreading cascade during progression of experimental autoimmune encephalomyelitis and multiple sclerosis. Immunol. Rev. **164:** 93–100

58 Vanderlugt C. L., Begolka W. S., Neville K. L., Katz-Levy Y., Howard L. M. and Eagar T. N. (1998) The functional significance of epitope spreading and its regulation by co-stimulatory molecules. Immunol. Rev. **164:** 63–72

59 Miller S. D. (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat. Med. **3:** 1133–1136

60 Miller S. D., Vanderlugt C. L., Lenschow D. J., Pope J. G., Dal Canto M. C. and Bluestone J. A. (1995) Blockade of CD28/B7-1 interaction prevents epitope spreading and clinical relapses of murine EAE. Immunity **3:** 739–745

61 Yu M., Johnson J. M. and Tuohy V. K. (1996) A predictable sequential determinant spreading cascade invariably accompanies progression: a basis for peptide-specific therapy after onset of clinical disease. J. Exp. Med. **183:** 1777–1788

62 Cumberbatch M. and Kimber I. (1995) Tumor necrosis factor-alpha is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. Immunology **84:** 31–35

63 Steinman R. M. (1991) The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. **9:** 271–296

64 Sallusto F., Cella M., Danieli C. and Lanzavecchia A. (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J. Exp. Med. **182:** 389–400

65 Larsen C. P., Ritchie S. C., Pearson T. C., Linslev P. S. and Lowry R. P. (1992) Functional expression of the costimulatory molecules, B7/BB1, on murine dendritic cell populations. J. Exp. Med. **176:** 1215–1220

66 Weiss A. and Litman D. R. (1994) Signal transduction by lymphocyte antigen receptor. Cell **76:** 263–274

67 DeBenedette M. A., Shahinian A., Mak T. W. and Watts T. H. (1997) Costimulation of CD28− T lymphocytes by 4-IBB ligand. J. Immunol. **158:** 551–559

68 Reinhold M. I., Lindberg F. P., Kersh G. J., Allen P. M. and Brown E. J. (1997) Costimulation of T cell activation by integrin-associated protein (CD7) is an adhesion-dependent, CD28-independent signaling pathway. J. Exp. Med. **185:** 1–11

69 Tai X. G., Yashiro Y., Abe R., Toyooka K., Wood C. R., Morris J. et al. (1996) A role for CD9 molecules in T cell activation. J. Exp. Med. **184:** 753–758

70 Bonay M., Bouchonnet F., Lecossier D., Boumsell L., Soler P., Grodet A. et al. (1997) Activation of T cells through an antigen-independent alternative pathway induces precocious sensitivity to Fas-induced apoptosis. Immunol. Lett. **59:** 107–113

71 Mehta-Damani A., Markowicz S. and Engleman E. G. (1995) Generation of antigen-specific CD4+ T cell lines from naive precursors. Eur. J. Immunol. **25:** 1206–1211

72 Grewal I. S., Xu J. and Flavell R. A. (1995) Impairment of antigen-specific T cell priming in mice lacking CD40 ligand. Nature **378:** 617–620
73 Pape K. A., Kearney E. R., Khoruts A., Mondino A., Merica R., Chen Z. M. et al. (1997) Use of adoptive transfer of T-cell-antigen-receptor-transgenic T cells for the study of T-cell activation in vivo. Immunol. Rev. **156:** 67–78
74 Tivol E. A., Borriello F., Schweitzer A. N., Lynch W. P., Bluestone J. A. and Sharpe A. N. (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity **3:** 541–547
75 Waterhouse P., Penninger J. M., Timms E., Wakeham A., Shahinian A., Lee K. P. et al. (1995) Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science **270:** 985–988
76 Karandikar N., Vanderlugt C. L., Walunas T. L., Miller S. D. and Bluestone J. A. (1996) CTLA-4: a negative regulator of autoimmune disease. J. Exp. Med. **184:** 783–788
77 Gribben J. G., Freeman G. J., Boussiotis V. A., Rennert P., Jellis C. L., Greenfield E. et al. (1994) CTLA-4 mediated costimulation induces apoptosis of activated human T lymphocytes. Proc. Natl. Acad. Sci. USA **92:** 811–815
78 Krummel M. F. and Allison J. P. (1996) CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J. Exp. Med. **183:** 2533–2540
79 Liblau R., Tisch R., Shokat K., Yang X., Dumont N., Goodnow C. C. et al. (1996) Intravenous injection of soluble antigen induces thymic and peripheral T-cell apoptosis. Proc. Natl. Acad. Sci. USA **93:** 3031–3036
80 Alferink J., Schittek B., Schonrich G., Hammerling G. J. and Arnold B. (1995) Long life span of tolerant T cells and the role of antigen in maintenance of peripheral tolerance. Int. Immunol. **7:** 331–336
81 Sloan-Lancaster J., Evavild B. D. and Allen P. M. (1993) Induction of T cell anergy by altered T cell receptor ligand on live antigen-presenting cells. Nature **363:** 156–159
82 LaSalle J. M., Tolentino P. J., Freeman G. J., Nadler L. M. and Hafler D. A. (1992) Early signaling defects in human T cells anergized by T cell presentation of autoantigen. J. Exp. Med. **176:** 177–186
83 Howell M. D., Winters S. T., Olee T., Powell H. C., Carlo D. J. and Brostoff S. W. (1989) Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. Science **246:** 668–670
84 Vandenbark A. A., Hashim G. A. and Offner H. (1989) Immunization with a synthetic T cell receptor V region peptide protects against experimental autoimmune encephalomyelitis. Nature **341:** 541–544
85 Bourdette D. N., Whitham R. H., Chou Y. K., Morrison W. J., Atherton J., Kenny C. et al. (1994) Immunity to T cell receptor peptides in multiple sclerosis. I. Successful immunization of patients with synthetic Vβ5.2 and Vβ6.1 CDR2 peptides. J. Immunol. **152:** 2510–2519
86 Chou Y., Morrison W. J., Weinberg A. D., Dedrick R., Whitham R., Bourdette D. N. et al. (1994) Immunity to T cell receptor peptides in multiple sclerosis. II. T cell recognition of Vβ5.2 and Vβ6.1 CDR2 peptides. J. Immunol. **152:** 2520–2529
87 Liblau R., Tisch R., Bercovici N. and McDevitt H. O. (1997) Systemic antigen in the treatment of T-cell-mediated autoimmune diseases. Immunol. Today **18:** 599–604
88 Ishigami T., White C. A. and Pender M. P. (1998) Soluble antigen therapy induces apoptosis of autoreactive T cells preferentially in the target organ rather than in the peripheral organs. Eur. J. Immunol. **28:** 1626–1635
89 Tan L. J., Kennedy M. K. and Miller S. D. (1992) Regulation of the effector stages of experimental autoimmune encephalomyelitis via neuroantigen-specific tolerance induction. II. Fine specificity of effector T cell inhibition. J. Immunol. **148:** 2748–2755
90 Rocken M. and Shevach E. M. (1996) Immune deviation – the third dimension of nondeletional T cell tolerance. Immunol. Rev. **149:** 175–194
91 Nicholson L. B., Greer J. M., Sobel R. A., Lees M. B. and Kuchroo V. K. (1995) An altered peptide ligand mediates deviation and prevents autoimmune encephalomyelitis. Immunity **3:** 397–405
92 Jewell S. D., Gienapp I. E., Cox K. L. and Whitacre C. C. (1998) Oral tolerance as therapy for experimental autoimmune encephalomyelitis and multiple sclerosis: demonstration of T cell anergy. Immunol. Cell. Biol. **76:** 74–82
93 Link H. and Xiao B. G. (1998) Transforming growth factor beta. In: Encyclopedia of Immunology, vol. 4, 2nd edn, pp. 2392–2398, Delves P. J. and Roitt I. (eds), Academic Press, London
94 Critchfield J. M. and Lenardo M. J. (1995) Antigen-induced programmed T cell death as a new approach to immune therapy. Clin. Immunol. Immunopathol. **75:** 13–19
95 Xiao B. G. and Link H. (1997) Mucosal tolerance: a two-edged sword to prevent and treat autoimmune diseases. Clin. Immunol. Immunopathol. **85:** 119–128
96 Wang Z. Y., Qiao J. and Link H. (1993) Suppression of experimental autoimmune myasthenia gravis by oral administration of acetylcholine receptor. J. Neuroimmunol. **44:** 209–214
97 Ma C. G., Zhang G. X., Xiao B. G., Wang Z. Y., Link H. and Olsson T. (1996) Mucosal tolerance to experimental autoimmune myasthenia gravis is associated with down-regulation of AChR-specific IFN-γ-expressing Th1-like cells and up-regulation of TGF-β mRNA in mononuclear cells. Ann. NY Acad. Sci. **778:** 273–287
98 Hafler D. A., Kent S. C., Pietrusewicz M. J., Khoury S. J., Weiner H. L. and Fukaura H. (1997) Oral administration of myelin induces antigen-specific TGF-β1 secreting T cells in patients with multiple sclerosis. Ann. NY Acad. Sci. **835:** 120–131
99 Miller S. D., Tan L. J., Kennedy M. K. and Dal Canto M. C. (1991) Specific immunoregulation of the induction and effector stages of relapsing EAE via neuroantigen-specific tolerance induction. Ann. NY Acad. Sci. **636:** 79–94
100 Miller S. D., Tan L. J., Pope L., McRae B. L. and Karpus W. J. (1992) Antigen-specific tolerance as a therapy for experimental autoimmune encephalomyelitis. Int. Rev. Immunol. **9:** 203–222
101 Bai X. F., Shi F. D., Xiao B. G., Li H. L., Meide P. H. van der and Link H. (1997) Nasal administration of myelin basic protein prevents relapsing experimental autoimmune encephalomyelitis in DA rats by activating regulatory cells expressing IL-4 and TGF-β mRNA. J. Neuroimmunol. **80:** 65–75
102 Weiner H. L. (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol. Today **18:** 335–343
103 Barnett M. L., Kremer J. M., St. Clair E. W., Clegg D. O., Furst D., Weisman M. et al. (1998) Treatment of rheumatoid arthritis with oral type II collagen: results of a multicenter, double-blind, placebo-controlled trial. Arthritis Rheum. **41:** 290–297
104 Nussenblatt R. B., Gery I., Weiner H. L., Ferris F. L., Shiloach J., Remaley N. et al. (1997) Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. Am. J. Ophthalmol. **123:** 583–592
105 Vandenbark A. A., Vainiene M., Ariail K., Miller S. D. and Offiner H. (1996) Prevention and treatment of relapsing autoimmune encephalomyelitis with myelin peptide-coupled splenocytes. J. Neurosci. Res. **45:** 430–438
106 Zhang J., Medaer R., Stinissen P., Hafler D. and Raus J. (1993) MHC restricted clonotypic depletion of human myelin basic protein-reactive T cells by T cell vaccination. Science **261:** 1451–1454
107 Medae R., Stinissen P., Truyen L., Raus J. and Zhang J. (1995) Depletion of myelin basic protein autoreactive T cells by T-cell vaccination: pilot trial in multiple sclerosis. Lancet **346:** 807–808

108 Borghans J. A., De Boer R. J., Sercarz E. and Kumar V. (1998) T cell vaccination in experimental autoimmune encephalomyeliltis: a mathematical model. J. Immunol. **161:** 1087–1093

109 Vandenbark A. A., Chou Y. K., Whitham R., Mass M., Buenafe A., Liefeld D. et al. (1996) Treatment of multiple sclerosis with T-cell receptor peptides: results of a double-blind pilot trial. Nat. Med. **2:** 1109–1116

110 Javed N. H., Gienapp I. E., Cox K. L. and Whitacre C. C. (1995) Exquisite peptide specificity of oral tolerance in experimental autoimmune encephalomyelitis. J. Immunol. **155:** 1599–1605

111 Miller A., Al-Sabbagh A., Santos L. M. B., Das M. O. and Weiner H. L. (1993) Epitopes of myelin basic protein that trigger TGF-β release after oral tolerance are distinct from encephalitogenic epitopes and mediate epitope-driven bystander suppression. J. Immunol. **151:** 7307–7315

112 Metzler B. and Wraith D. C. (1992) Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. Int. Immunol. **5:** 1159–1165

113 Liu J. Q., Bai X. F., Shi F. D., Xiao B. G., Li H. L., Levi M. et al. (1998) Inhibition of experimental autoimmune encephalomyelitis in Lewis rats by nasal administration of encephalitogenic MBP peptides: synergistic effects of MBP 68-86 and 87-99. Int. Immunol. **10:** 1139–1148

114 Zhang G. X., Shi F. D., Zhu J., Xiao B. G., Levi M., Wahren B. et al. (1998) Synthetic peptides fail to induce nasal tolerance to experimental autoimmune myasthenia gravis. J. Neuroimmunol. **85:** 96–101

115 Zou L. P., Zhu J., Deng G. M., Levi M., Wahren B., Diab A. et al. (1998) Treatment with P2 protein peptide 57-81 by nasal route is effective in Lewis rat experimental autoimmune neuritis. J. Neuroimmunol. **85:** 137–145

116 Wildner G., Hunig T. and Thurau S. R. (1996) Orally induced peptide-specific γ/δ TCR + cell suppress experimental autoimmune uveitis. Eur. J. Immuonl. **26:** 2140–2148

117 Staines N. A., Narper N., Ward F. J., Malmstrom V., Holmdahl R. and Bansal S. (1996) Mucosal tolerance and suppression of collagen-induced arthritis (CIA) induced by nasal inhalation of synthetic peptide 184–198 of bovine type II collagen (C II) expressing a dominant T cell epitope. Clin. Exp. Immunol. **103:** 368–375

118 Khare S. D., Krco C. J., Pawelski J. R., Griffiths M. M., Luthra H. S. and David C. S. (1994) Oral administration of human collagen peptide 250-270 suppresses collagen-induced arthritis in DBA/1 mice by inhibiting a Th1 response. Arthritis Rheum. **37:** S398

119 Gaur A., Wiers B., Liu A., Rothbard J. and Fathman C. G. (1992) Amelioration of autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. Science **258:** 1491–1494

120 Sasamoto Y., Kawano Y. I., Bouligny R., Wiggert B., Chader G. J. and Gery I. (1992) Immunomodulation of experimental autoimmune uveoretinitis by intravenous injection of uveitogenic peptides. Invest. Ophthalmol. Vis. Sci. **33:** 2641–2649

121 Fuller B. E., Okayasu I., Simon L. L., Giraldo A. A. and Kong Y. M. (1993) Characterization of resistance to murine experimental autoimmune thyroiditis: duration and afferent action of thyroglobulin- and TSH-induced suppression. Clin. Immunol. Immunopathol. **69:** 60–68

122 Tian J., Clare-Salzler M., Herschenfeld A., Middleton B., Newman D., Mueller R. et al. (1996) Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. Nat. Med. **2:** 1348–1353

123 Nussenblatt R. B., Caspi R. R., Mahdi R., Chan C. C., Roberge F., Lider O. et al. (1990) Inhibition of S-Ag induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-Ag. J. Immunol. **144:** 1689–1695

124 Dick A. D., Cheng Y. F., McKinnon A., Liversidge J. and Forrester J. V. (1993) Nasal administration of retinal antigens suppresses the inflammatory response in experimental allergic uveoretinitis. Br. J. Ophthalmol. **77:** 171–175

125 Peterson K. E. and Braley-Mullen H. (1995) Suppression of murine experimental autoimmune thyroiditis by oral administration of porcine thyroglobulin. Cell. Immunol. **166:** 123–130

126 Daniel D. and Wegmann D. R. (1996) Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9-23). Proc. Natl. Acad. Sci. USA **93:** 956–960

127 Matsumoto Y., Tsuchida M., Hanawa H. and Abo T. (1994) T cell receptor peptide therapy for autoimmune encephalomyelitis: stronger immunization is necessary for effective vaccination. Cell. Immunol. **153:** 468–478

128 Rosloniec E. F., Brand D. D., Whittington K. B., Stuart J. M., Ciubotaru M. and Ward E. S. (1995) Vaccination with a recombinant V alpha domain of a TCR prevents the development of collagen-induced arthritis. J. Immunol. **155:** 4504–4511

129 Formby B. and Shao T. (1993) T cell vaccination against autoimmune diabetes in nonobese diabetic mice. Ann. Clin. Lab. Sci. **23:** 137–147

130 Beraud E., Kotake S., Caspi R. R., Oddo S. M., Chan C. C., Gery I. et al. (1992) Control of experimental autoimmune uveoretinitis by low dose T cell vaccination. Cell. Immunol. **140:** 112–122

131 Warren K. G. and Catz I. (1995) Administration of myelin basic protein synthetic peptides to multiple sclerosis patients. J. Neurol. Sci. **133:** 85–94

132 Gold D. P., Smith R. A., Golding A. B., Morgan E. E., Dafashy T., Nelson J. et al. (1997) Results of a phase I clinical trial of a T-cell receptor vaccine in patients with multiple sclerosis. II. Comparative analysis of TCR utilization in CSF T-cell populations before and after vaccination with a TCR beta 6 CDR2 peptide. J. Neuroimmunol. **76:** 29–38

133 Moreland L. W., Morgan E. E., Adamson T. C., Fronek Z., Calabrese L. H., Cash J. M. et al. (1998) T cell receptor peptide vaccination in rheumatoid arthritis: a placebo-controlled trial using a combination of Vbeta3, Vbeta14 and Vbeta17 peptides. Arthritis Rheum. **41:** 1919–1929

134 Laar J. M. van, Vries R. R. de and Breedveld F. C. (1993) T cell vaccination in humans: the experience in rheumatoid arthritis. Clin. Exp. Rheumatol. **8:** S59–S62