

Antigen-specific tolerance strategies for the prevention and treatment of autoimmune disease

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Abstract | The development of safe and effective antigen-specific therapies is needed to treat patients with autoimmune diseases. These therapies must allow for the specific tolerization of self-reactive immune cells without altering host immunity to infectious insults. Experimental models and clinical trials for the treatment of autoimmune disease have identified putative mechanisms by which antigen-specific therapies induce tolerance. Although advances have been made in the development of efficient antigen-specific therapies, translating these therapies from bench to bedside has remained difficult. Here, we discuss the recent advances in our understanding of antigen-specific therapies for the treatment of autoimmune diseases.

Adjuvant

An agent mixed with an antigen that increases the immune response to that antigen after immunization.

To date, the majority of therapies approved by the US Food and Drug Administration (FDA) for autoimmune disease have focused on the global inhibition of immune inflammatory activity. Although nonspecific immune suppression is partially effective in inhibiting autoreactive immune-cell function, the drugs used to suppress the immune response have numerous side effects and continuous therapy is not conducive to long-term host survival. The goal of ongoing research in immune tolerance is the development of autoantigen-specific treatments that allow for the specific blockade of the deleterious effects of self-reactive immune-cell function, while maintaining the ability of the immune system to clear non-self antigens. Although CD4⁺ T cells can discriminate between specific peptide antigens, the T-cell receptor (TCR) cannot intrinsically distinguish self from non-self peptides. During thymic selection of CD4⁺ T cells, the majority of T-cell clones with high-affinity TCRs that recognize self are deleted as a consequence of self-antigen presentation by thymic epithelial cells¹. However, the thymic negative-selection process is not perfect, and therefore self-reactive CD4⁺ T cells are present in the peripheral T-cell repertoire of healthy individuals. For example, myelin basic protein (MBP)-specific CD4⁺ T cells can be found in the peripheral blood of both healthy individuals and individuals with multiple sclerosis (MS)². The difference between the MBP-specific CD4⁺ T cells in these two groups is

the activation status of the cells. Self-reactive CD4⁺ T cells that escape negative selection in the thymus must be held in check by additional peripheral tolerance mechanisms, and it is thought that the ability to tightly control and avoid the activation of peripheral self-reactive T cells is crucial for avoiding autoimmunity.

The advantage of peptide-specific therapy over other forms of therapy is that it lacks potential metabolic activity and can limit the range of the response to the desired pathogenic peptide epitopes without increasing the possibility for hyperactivation of self-reactive T cells. Likewise, the use of different routes of administration (such as the intravenous administration of antigen-coupled cells or the mucosal or intravenous administration of soluble peptides), the dose schedule and the adjuvants used in combination with the therapy may provide a more efficient means to specifically induce tolerance and/or induce the deviation of the response towards an anti-inflammatory cytokine profile. This Review focuses on research in experimental mouse models that uses various autoantigen- or peptide-specific strategies aimed at the induction of self-tolerance for the potential treatment of human autoimmune diseases. The benefits and limitations of each of the various methods of antigen-specific therapy are discussed, along with their putative mechanisms of action and potential use in clinical trials.

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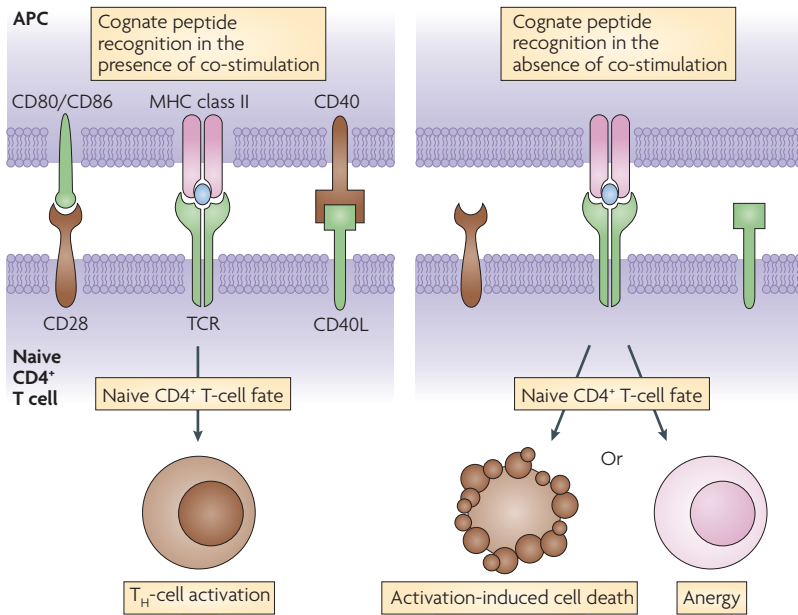


Figure 1 | CD4⁺ T-cell activation and tolerogenic strategies. A naive CD4⁺ T cell receives antigen-specific signals (signal 1) through its clonally derived T-cell receptor (TCR), which interacts with antigenic peptides presented by MHC class II molecules expressed on the surface of antigen-presenting cells (APCs). A second set of signals exists, which includes signals delivered by secreted cytokines that are produced by either the APC or the activated CD4⁺ T cell itself, and signals delivered by co-stimulatory molecules expressed by activated APCs, such as CD80 and/or CD86, which interact with the CD28 co-receptor that is constitutively expressed by CD4⁺ T cells. The overall effect of CD28 ligation is to increase the level of T-cell proliferation and cytokine production, promote cell survival, and enhance the expression of CD40 ligand (CD40L) and adhesion molecules necessary for trafficking, such as $\alpha_4\beta_1$ -integrin (also known as VLA4). The co-stimulatory molecule pairs CD28–CD80/CD86 and CD40–CD40L, and cellular adhesion molecules represent putative therapeutic targets for blockade of autoreactive CD4⁺ T-cell activation and trafficking to inflammatory sites. In addition to CD28 co-stimulation, various cytokines produced by activated dendritic cells, macrophages, or B cells are required to direct the differentiation of naive T cells to a T_H1-cell phenotype (induced by interleukin-12 (IL-12) and interferon- γ), a T_H2-cell phenotype (induced by IL-4) or a T_H17-cell phenotype (induced by IL-6 and transforming growth factor- β (TGF β), and maintained by IL-23). Various methodologies used to induce CD4⁺ T-cell unresponsiveness rely on the alteration of signal 1 and/or inhibition of co-stimulatory molecule stimulation (signal 2) resulting in CD4⁺ T-cell anergy. The difficulty in the development of these therapies lies in maintaining their antigen specificity to allow the host to still recognize and react to non-self antigens.

Models of peptide-induced tolerance

T-cell-mediated autoimmune diseases, such as MS and type 1 diabetes, are initiated and maintained by the presentation of self antigen by activated antigen-presenting cells (APCs) to self-reactive T cells. According to the two-signal hypothesis for the activation of CD4⁺ T cells, a naive antigen-specific CD4⁺ T cell requires both the stimulation of the TCR (signal 1) and of co-stimulatory molecules (signal 2), such as CD28, that provide both proliferative and survival signals to the T cell to become fully activated (FIG. 1). One proposed mechanism for the maintenance of peripheral self tolerance is dependent on the presentation of self antigen by APCs that express low levels of surface co-stimulatory molecules. The level of co-stimulatory-molecule expression and cytokine production by APCs is regulated by the presence or absence of inflammation, infectious agents and other pathological

Altered-peptide ligands (APLs). APLs are peptide analogues that are derived from the original antigenic peptide. They commonly have amino-acid substitutions at T-cell receptor (TCR)-contact residues. TCR engagement by these APLs usually leads to partial or incomplete T-cell activation. Antagonistic APLs can specifically antagonize and inhibit T-cell activation that is induced by the wild-type antigenic peptide.

conditions that are sensed by pattern-recognition receptors, including Toll-like receptors (TLRs). Therefore, it is speculated that each of the different routes used for the induction of peptide-specific tolerance allows for the presentation of specific peptides to autoreactive CD4⁺ T cells in a non-inflammatory manner. In this way, the APC would process and present a peptide in the absence of activating stimuli. There are currently four different protocols employed for inducing peptide-specific immune tolerance — soluble-peptide-induced and DNA-vaccination-induced tolerance, mucosal (oral or nasal)-induced tolerance, coupled-cell-induced tolerance, and altered-peptide ligand (APL)-induced tolerance. Each of these methods is discussed in the following sections, as well as their putative mechanisms of action.

Soluble-peptide-induced and DNA-vaccination-induced tolerance.

Injection of high doses of soluble peptides (or the production of peptides following DNA vaccination) leads to a state of T-cell unresponsiveness (referred to as anergy) owing to a block in T-cell proliferation and/or interleukin-2 (IL-2) production, or results in activation-induced cell death (AICD) after T-cell re-stimulation with the cognate peptide (FIG. 2a)^{3–6}. It is thought that tolerance induced by soluble peptides may be useful for antigen-specific immunotherapy for the treatment of human autoimmune diseases. Non-obese diabetic (NOD) mice spontaneously develop type 1 diabetes, which is characterized by T-cell-mediated inflammation of the pancreatic islets (insulinitis) and the eventual destruction of the insulin-producing β -cells⁷. Prevention of type 1 diabetes in NOD mice can be achieved by inducing specific T-cell tolerance to pancreatic β -cell autoantigens prior to the total destruction of all the islet β -cells. BDC2.5 TCR-transgenic NOD mice express the rearranged α - and β -chain TCR genes of a diabetogenic T-cell clone that is reactive to an unknown β -cell auto-antigen, and immunization of these mice with a panel of β -cell-derived peptides does not induce active disease. Interestingly, direct immunization of BDC2.5 mice with β -cell-derived peptides causes a large percentage of the BDC2.5 mouse T cells to undergo AICD. Strikingly, the administration of soluble peptides to recipients of activated BDC2.5 mouse T cells results in protection from disease⁸, suggesting that high-affinity peptide analogues of autoimmune epitopes might be useful as therapeutic modulators in active autoimmune disease. These data demonstrate that intravenous administration of soluble peptides can result in long-lasting tolerance to an auto-antigen in experimental mouse models of disease and perhaps in humans.

In addition, the administration of insulin or various insulin peptides through different routes has protected NOD mice from developing type 1 diabetes^{9,10}. NOD mice immunized by intramuscular injection of a plasmid encoding the B-chain of insulin had a delayed onset of disease and a lower disease incidence than non-immunized mice¹¹. However, in a separate study using young NOD mice, administration of recombinant DNA encoding the mouse insulin A-chain or B-chain fused with the Fc portion of IgG to stabilize

the insulin chains led to an earlier onset and accelerated progression of type 1 diabetes¹². In another study, a DNA vaccine vector encoding a membrane-bound form of pre-pro-insulin did not efficiently induce tolerance by itself, but was tolerogenic when

co-administered with a vaccine that enhanced the expression of inhibitory co-stimulatory molecules¹³. Therefore, it is critical to choose the correct target antigen and correct form of antigen to successfully induce tolerance with DNA vaccines.

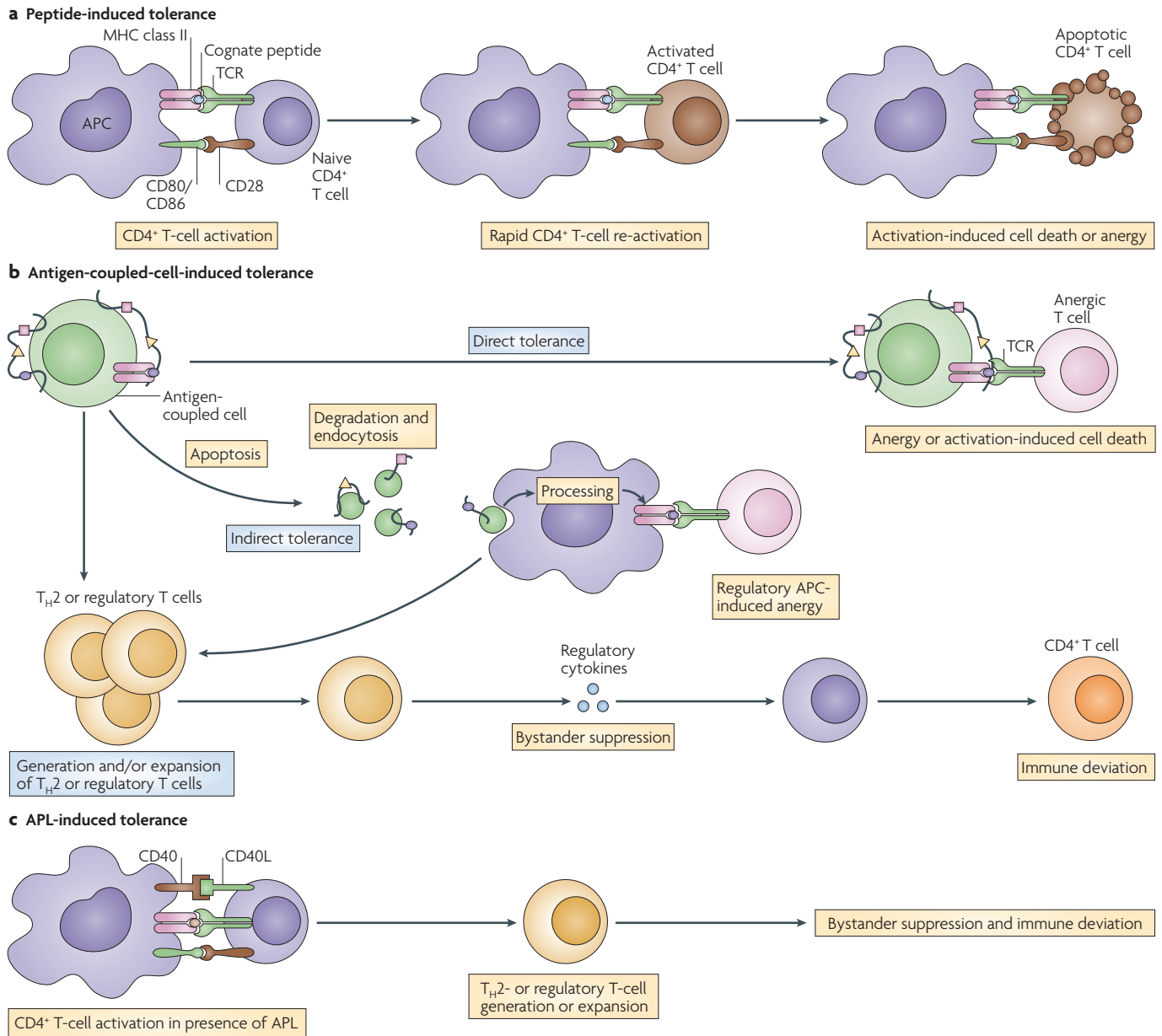


Figure 2 | Proposed mechanisms of peptide-induced tolerance. a | In tolerance induced by soluble peptide and/or DNA vaccination, the presence of excess antigenic peptide is thought to induce an initial proliferative burst of the autoreactive CD4⁺ T cells, but the subsequent re-activation of the proliferating CD4⁺ T cells is inhibited due to activation-induced cell death (AICD) or anergy. **b** | In antigen-coupled-cell-induced tolerance, first, antigen-coupled cells induce direct tolerance following interaction of the T-cell receptor (TCR) on the host, antigen-specific CD4⁺ T cells with the antigen-coupled cells, leading to the induction of anergy through TCR stimulation in the absence of co-stimulation. Second, antigen-coupled cells induce indirect or cross-tolerance through the re-presentation of the apoptotic antigen-coupled cells by ‘tolerogenic’ host antigen-presenting cells (APCs) leading to T-cell anergy. Third, antigen-coupled cells directly or indirectly activate regulatory T cells and/or T helper 2 (T_H2) cells, and this results in bystander suppression of autoreactive CD4⁺ T cells through the action of regulatory cytokines, such as transforming growth factor-β (TGFβ), interleukin-10 (IL-10) and/or IL-4. **c** | In tolerance induced by altered-peptide ligands (APLs), which uses a mechanism similar to indirect tolerance induced by antigen-coupled cells, T-cell unresponsiveness results from the induction of anergy and/or the activation of regulatory T cells or T_H2-cell responses, or both.

A cautionary note for the use of intravenous injection of soluble myelin peptide monomers or oligomers in mice with pre-established, adjuvant-induced experimental autoimmune encephalomyelitis (EAE; a mouse model of MS) is that this treatment regimen was found to induce a fatal anaphylactic response in various mouse strains¹⁴. Furthermore, intravenous administration of soluble myelin oligodendrocyte glycoprotein (MOG) in a primate model of EAE was shown to exacerbate disease¹⁵. Owing to the highly variable outcome of this type of treatment, there is currently a significant level of uncertainty regarding the safety of soluble-peptide-induced tolerance. Anaphylactic responses to intravenous peptide administration appear to occur in an antigen-specific manner, meaning that the same antigen must be administered during both the initial sensitization phase and the re-challenge phase to induce the effect. In light of the fact that recurrent tolerogenic treatments may be required to ameliorate disease progression, soluble-peptide-induced anaphylaxis is a significant safety concern.

One of the contributing factors to the induction of anaphylaxis may be the route of peptide injection and the primary site of peptide deposition that is associated with each of the various routes. For example, following intravenous injection, peptide quickly traverses the body enabling wide-spread activation of tissue-resident mast cells. This is in contrast to what occurs with intradermal and subcutaneous injections, in which the peptide is phagocytosed by local APCs that then migrate to the local draining lymph node. In addition, the method used to induce disease in these models may also influence the incidence of anaphylaxis. For example, priming a mouse with a peptide in complete Freund's adjuvant has been shown to induce antigen-specific IgE production¹⁶, which triggers anaphylaxis, whereas in a patient with ongoing MS the autoreactive antibodies would be mainly of the IgG isotype¹⁷. Also, proteolipid protein (PLP) peptide PLP_{178–191}, which is a B-cell epitope and induces IgG production, failed to induce anaphylactic shock during autoimmune disease treatment¹⁴. Another contributing factor to the potential mechanisms of intravenously administered soluble-peptide-induced anaphylaxis is thought to correlate with the thymic expression of each antigen, and presumably the extent of central tolerance¹⁸. This hypothesis is, however, not supported by findings from our laboratory showing that equivalent levels of anaphylaxis were induced in mice with pre-established EAE regardless of whether the peptide was expressed in the thymus. For example, MBP_{Ac1–11} and MBP_{84–104} are expressed in the thymus, whereas PLP_{139–151} and MOG_{35–55} are not, but all of these peptides equally induced anaphylaxis following administration to mice with actively induced EAE¹⁴.

Peptide-induced mucosal tolerance. The biological basis for the induction of tolerance by the mucosal (oral or nasal) route is that when food is ingested, the individual foreign antigens that are component parts of the food are usually tolerated by the host, except in the case of food allergy. T cells found within the gastrointestinal tract are exposed daily to large doses of ingested foreign antigen

and yet they remain largely unresponsive to these non-self antigens. For this reason, peptide-induced tolerance using the mucosal route is an alluring treatment for autoimmune disease, as it is antigen-specific, it uses an easy route of administration, and it carries decreased risk of toxicity when compared with the injection of soluble antigen or with DNA vaccination.

The efficiency of oral tolerance is dependent on various factors, including the animal model, the type of antigen and the dose of treatment used¹⁹. Depending on the treatment dose (either high or low dose), two different effector mechanisms of tolerance are apparently induced^{20–22}. High-dose oral treatment results in the induction of T-cell anergy or the deletion of peripheral antigen-specific T cells^{23,24}. At high doses, antigen can rapidly diffuse through the gastrointestinal wall and into the systemic circulation, where it can induce T-cell unresponsiveness. By contrast, low doses of oral antigen act by bystander suppression or by inducing regulatory-cell-driven tolerance within the target organ^{25,26}. Low-dose antigen is taken up by mucosa-associated APCs that activate regulatory T cells to secrete suppressive cytokines, such as transforming growth factor- β (TGF β), IL-4 and IL-10 (REF. 27).

Oral administration of antigen has been shown to suppress the initiation of autoimmune disease in multiple animal models, including EAE and models of uveitis, colitis and asthma^{28,29}. Multiple studies have shown that the oral or nasal pre-administration of soluble myelin peptides protects against the induction of EAE, but attempts to treat EAE after the onset of clinical symptoms with oral peptide-induced tolerance have been less successful^{30–35}. However, suppression of ongoing EAE can be achieved through combinational treatments using myelin antigens and soluble IL-10 delivered either orally or nasally³⁶. Besides the administration of native peptides, oral administration of APLs, such as glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd.), have been shown to suppress the severity of EAE in mice and result in decreased T-cell proliferation, decreased IL-2, IL-6 and interferon- γ (IFN γ) production and increased secretion of IL-10 and TGF β ³⁷. APL therapy will be more thoroughly discussed in a following section. Similarly, administration of MBP peptides via the nasal route has been shown to inhibit the induction of EAE³⁸. Although these experimental models appear to show the potential efficacy of mucosal peptide-induced tolerance, clinical trials of patients with MS using orally administered whole bovine myelin did not show any differences between the placebo and treated groups^{29,39}. Mucosal peptide-induced tolerance, thus, appears to be effective at preventing the induction of EAE but it is significantly less effective in treating pre-established autoimmunity in the central nervous system (CNS). So, although the use of mucosal peptide-induced tolerance remains an attractive possibility for preventing the onset of autoimmune disease, this therapy is currently limited in its ability to induce tolerance in ongoing disease, potentially limiting its usefulness for treating human autoimmune disease.

Anergy

A state of unresponsiveness to antigen. Anergic T cells or B cells cannot respond to their cognate antigens under optimal conditions of stimulation.

Activation-induced cell death

(AICD). A form of regulated cell death, which is induced during lymphocyte activation. During a normal immune response, most antigen-specific lymphocytes undergo AICD.

Anaphylaxis

Severe and rapid allergic reaction triggered by the activation of high-affinity Fc receptors for IgE in sensitized individuals. An anaphylactic shock is the most severe type of anaphylaxis and will usually lead to an individual's death in minutes if left untreated.

Central tolerance

The lack of self-responsiveness that occurs as lymphocytes develop. It is associated with the deletion of autoreactive clones. For T cells, this occurs in the thymus.

Bystander suppression

The extension of tolerogen-induced suppression of immune responses that are directed against antigens not structurally related to the tolerogen but expressed by the same target cell or organ.

Antigen- or peptide-coupled-cell-induced tolerance. One of the more promising methods to induce tolerance for the prevention and treatment of autoimmune diseases, and the prevention of transplant rejection, is intravenous treatment with antigen-coupled, ethylene carbodiimide (ECDI)-fixed splenocytes (referred to here as antigen-coupled cells) (FIG. 2b). Treatment with antigen-coupled

cells can induce anergy *in vitro* and peripheral tolerance *in vivo*^{40–42}. For example, ECDI-fixed peripheral-blood lymphocytes (PBLs) coupled with MBP peptides selectively induce anergy *in vitro* in human T helper 1 (T_H1)-cell but not T_H2-cell clones⁴³. *In vivo*, intravenous injection with myelin-antigen-coupled cells induces rapid and long-lived antigen-specific tolerance in mice with EAE.

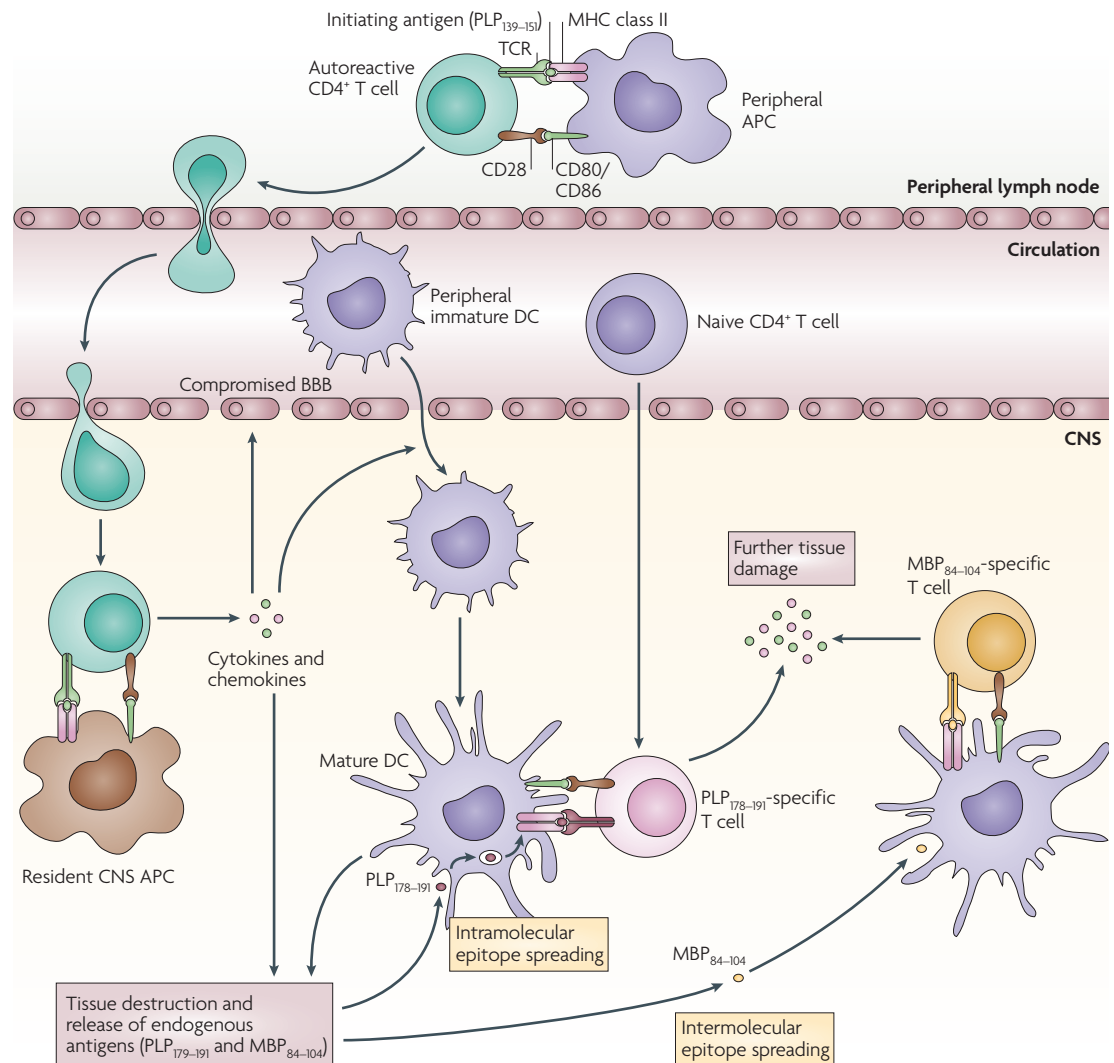


Figure 3 | Epitope spreading. Animal models of multiple sclerosis (MS) have helped to identify putative mechanisms by which epitope spreading occurs. In relapsing–remitting experimental autoimmune encephalomyelitis (EAE), the activation of the autoreactive CD4⁺ T cells that are specific for the initiating antigen epitope (CD4⁺ T cell depicted in green) occurs in the draining lymph node. Following activation, the effector CD4⁺ T cells enter the circulation and extravasate into the CNS. Once in the CNS, the autoreactive CD4⁺ T cells initiate myelin destruction through the activation of resident and infiltrating antigen-presenting cells (APCs). The activated infiltrating immune cells secrete cytokines and chemokines that not only recruit immune cells into the central nervous system (CNS), but also help to open the blood–brain barrier (BBB). Besides the re-activation of the CD4⁺ T cells that are specific for the initiating antigen, myelin antigens are released, phagocytosed, processed, and presented principally within the CNS by peripherally derived myeloid dendritic cells (DCs) to naive CD4⁺ T cells, both of which can enter through the compromised BBB. For example, in proteolipid protein (PLP) peptide PLP_{139–151}-induced relapsing–remitting EAE in SJL/J mice the initiating epitope is PLP_{139–151}, and the population of CD4⁺ T cells that recognize this peptide is responsible for the initial acute phase of disease. During the acute phase of disease the destruction of myelin allows for the release of both PLP and myelin basic protein (MBP). Due to antigen availability and CD4⁺ T-cell precursor frequency, the activation of the secondary population of CD4⁺ T cells specific for PLP_{178–191} occurs during the primary relapse (known as intramolecular epitope spreading). During the secondary relapse, CD4⁺ T cells specific for MBP_{84–104} are activated (known as intermolecular epitope spreading). TCR, T-cell receptor.

The fixation of donor cells with ECDI in the presence of antigen results in the formation of peptide bonds between free amino and carboxyl groups, which bind the peptide to the cells generating a tolerogenic carrier system. Another interesting aspect of antigen-coupled-cell-induced tolerance is the finding that multiple peptides, as well as intact proteins, can be coupled to a single donor cell allowing for the simultaneous targeting of multiple T-cell specificities. This may be crucial for antigen-specific tolerance therapy for chronic autoimmune diseases, in which tolerance to multiple T-cell epitopes may be necessary to treat these diseases due to the phenomenon of epitope spreading^{44–46} (FIG. 3). In contrast to soluble-peptide-induced tolerance, the use of peptide- or protein-coupled cells reduces the risk of anaphylaxis, as the protein has been chemically crosslinked to the surface of the carrier cell.

Experimentally, the use of antigen-coupled cells to induce tolerance not only prevents the onset of EAE in mice, but is also an effective treatment for ameliorating the progression of established disease in both active and adoptive-transfer models of EAE^{45,47–50}. The induction of peripheral tolerance with antigen-coupled cells is also useful for defining immunodominant myelin proteins, as progression of disease can be inhibited by cells coupled with the spread epitope (FIG. 3). For example, MBP_{84–104}-specific tolerance significantly inhibits relapsing–remitting EAE initiated by MBP-primed lymph-node-derived T cells, and PLP_{139–151}-specific tolerance significantly inhibits active relapsing–remitting EAE induced by either mouse spinal-cord homogenate (MSCH) or intact PLP. This indicates that the MBP_{84–104} and PLP_{139–151} peptides are immunodominant in their respective proteins, but also that other epitopes can contribute to disease induced by the intact proteins, as complete inhibition of the disease was not achieved by treatment with these peptides^{42,51–55}. Furthermore, peripheral tolerance induced by antigen-coupled cells is useful for defining the specificity and pathological contribution of epitope spreading (FIG. 3) to endogenous myelin epitopes in clinical relapses. Tolerance studies showed a major pathological contribution of PLP_{139–151}-specific T cells to the relapses in MBP_{84–104}-induced relapsing–remitting EAE and of PLP_{178–191}-specific T cells to the relapses in PLP_{139–151}-induced EAE, and that responses to the initiating epitope do not have a major role in the chronic disease phase^{42,45,54,56,57}. Epitope spreading has been shown to initiate in the CNS⁵⁸ and is primarily initiated by peripherally derived CD11b⁺CD11c⁺ myeloid dendritic cells that drive the differentiation of T_H17-cell responses to endogenous myelin peptides⁵⁹. The induction of tolerance by antigen-coupled-cell treatment has also been shown to be an effective therapy in other disease models, including experimental autoimmune thyroiditis⁶⁰, uveitis⁶¹, and neuritis⁶² and in the NOD mouse model of diabetes⁶³. Unlike soluble-peptide therapy, in which (depending on the disease-inducing autoantigen) the tolerizing antigen can induce an anaphylactic response that results in the death of treated mice, antigen-coupled-cell therapy

does not induce an allergic response, regardless of the antigen used, and appears to be well tolerated at all stages of disease^{14,18}.

The mechanism of action of antigen-coupled-cell-induced tolerance has yet to be fully elucidated; however, the route of administration, the dosage used, the levels of co-stimulatory molecules expressed (the two-signal hypothesis) and the extent of T_H1-cell polarization and regulatory T-cell induction are all probable factors that contribute to the efficacy of the treatment. One potential mechanism for antigen-coupled-cell-induced tolerance is by suboptimal T-cell activation through the engagement of the TCR in the absence of co-stimulation (FIG. 2b). *In vitro* studies revealed that ECDI-treated splenocytes pulsed with soluble peptide antigen were unable to deliver crucial co-stimulatory signals for the activation of T_H1-cell clones and resulted in anergy⁶⁴. A second potential mechanism is the indirect induction of tolerance, wherein ECDI crosslinking induces apoptosis of the antigen-coupled cells thereby leading to phagocytosis and re-presentation of the antigen by tolerogenic host APCs in a non-inflammatory manner (FIG. 2b); a mechanism we have termed 'cross tolerance'.

Data from our laboratory demonstrate that the effectiveness of antigen-coupled-cell-induced tolerance in PLP_{139–151}-induced EAE in SJL/J mice is dependent on having a low level of CD80 and CD86 expression on the fixed APCs and that cytotoxic T-lymphocyte antigen-4 (CTLA4) signalling is crucial for the maintenance of tolerance⁶⁵. Programmed cell death 1 ligand (PDL1)–PD1 engagement has also been shown to be important for the maintenance of insulin-coupled-cell-induced tolerance in the NOD mouse model of type 1 diabetes⁶³. The efficiency of this therapy is also critically dependent on the route of administration; intravenous administration of antigen-coupled cells induces tolerance, whereas neither the intraperitoneal or subcutaneous routes induce tolerance, with subcutaneous administration actually enhancing immune responses to the target antigen⁵¹. However, *de novo* antigen processing by the antigen-coupled cells is not a contributing factor to the induction of tolerance, as ECDI induces apoptosis in these cells and therefore they cannot process antigen⁶⁶. Furthermore, ECDI fixation is absolutely necessary for the induction of tolerance, and inclusion of antigen-processing inhibitors in the coupling reaction does not reverse the tolerance phenotype⁶⁷.

Induction of tolerance by antigen-coupled cells occurs even if the donor cells are coupled with intact proteins rather than defined peptide epitopes or if they are derived from allogeneic donors or from donors that are deficient in MHC class I and/or MHC class II molecules⁶⁶. On the basis of these findings, along with the finding that antigen-coupled-cell-induced tolerance is obviated in splenectomized recipients (S.D.M., unpublished observations), it is proposed that the ability of ECDI to nonspecifically crosslink antigen or peptide to the cell surface while inducing apoptosis in the cell, allows the donor cells to be perceived by the host in a non-inflammatory (non-immunogenic) fashion in the spleen. This, in turn, would allow immature

Epitope spreading

The *de novo* activation of autoreactive T cells by self-antigens that have been released after T-cell or B-cell-mediated bystander tissue damage.

(tolerogenic) host splenic APCs — possibly plasmacytoid dendritic cells, which have been previously implicated in the induction of self tolerance^{68,69} — to reprocess and re-present the coupled antigen in a tolerance-inducing manner.

T cells that have been tolerized by antigen-coupled cells and recovered from the CNS of mice with ongoing EAE produce higher amounts of the anti-inflammatory cytokines IL-10 and TGF β , compared with control mice. This pattern of increased regulatory cytokine production is suggestive of an increase in natural regulatory T-cell function in ongoing EAE⁴⁶. Furthermore, regulatory T cells appear to have a role in antigen-coupled-cell-induced tolerance, and are required for the long-term maintenance of tolerance, but are not necessary for tolerance induction (S.D.M., unpublished observations). Collectively, the induction of tolerance with antigen-coupled cells appears to work by several distinct synergistic mechanisms, thereby increasing their therapeutic efficacy.

Altered-peptide-ligand-induced tolerance. In order to induce peptide-specific tolerance for the effective prevention and treatment of disease, it is speculated that either the peptide(s) responsible for disease induction or the dominant peptide(s) driving ongoing autoimmunity must be identified. However, the development of APLs both support and refute this hypothesis. APLs can compete with the naive ligand for TCR binding, thereby altering the cascade of signalling events necessary for full T-cell activation. Functionally, since APLs contain one or more amino-acid substitutions, they typically bind with lower affinity to the TCR than the native peptide and may function as either antagonists or partial agonists (FIG. 2c). Antagonistic APLs induce T-cell anergy, whereas partial-agonist APLs induce incomplete activation of T cells. This partial activation can induce cytokine production in the absence of proliferation, thereby inducing immune deviation from T_H1-cell- and T_H17-cell-dependent responses to T_H2-cell- and T_H3-cell-dependent responses, or induce bystander suppression through the induction of regulatory T cells^{70,71}. Although both antagonistic and partial-agonistic APLs are intended to induce tolerance, caution must be taken to ensure that the desired outcome of tolerance induction is obtained.

A number of groups have created APLs of various myelin epitopes to test the therapeutic efficacy of these reagents in treating established autoimmune disease in the CNS. *In vivo* administration of these myelin APLs appear to prevent or reverse clinical disease progression in EAE, and APL administration seems effective regardless of the administration route — subcutaneous, intraperitoneal (in incomplete Freund's adjuvant), intranasal or intravenous⁷²⁻⁷⁴. The ability of APL treatment to modulate MBP₈₇₋₉₉-induced disease in rats was first tested by immunizing rats with native MBP₈₇₋₉₉ peptide alone or in combination with a TCR-antagonistic peptide. The level of tumour-necrosis factor (TNF) and IFN γ produced by the lymph nodes from mice immunized with MBP₈₇₋₉₉ combined with a TCR antagonist was decreased

compared with mice immunized with MBP₈₇₋₉₉ alone⁷⁵. Likewise, treatment of SJL/J mice with APLs of PLP₁₃₉₋₁₅₁ is associated with the induction of a T_H2-cell-skewed immune response. As evidence for immune deviation, therapeutic clones responsive to PLP₁₃₉₋₁₅₁-derived APLs produce IL-4, IL-10, IL-13 and TGF β , and antibodies directed against each of these cytokines attenuate the protective influence of APL-mediated treatment⁷⁶. An example of a clinically relevant APL is glatiramer acetate, which is a random mixture of glutamic acid, lysine, alanine and tyrosine peptides of various lengths. Glatiramer acetate has been reported to induce immune deviation from a mixed T_H1- and T_H17-cell-type response to a T_H2-cell-type response, but not to induce anergy or the deletion of the autoreactive T cells⁷⁷. This finding would suggest that a putative mechanism of action for APLs is through the induction of immune deviation from a T_H1-cell response to a T_H2-cell response.

It has been reported that APLs can suppress effector T cells through various other mechanisms, in addition to their antagonistic or partial-agonistic activity. The crystal structure of HLA-DR2 complexed with MBP₈₄₋₁₀₂ confirmed that lysine (E) at position 91 serves as a major TCR contact site, and phenylalanine (Y) at position 90 acts as an anchor that interacts with the MHC class II molecule. APLs were generated that contain repetitive four-amino-acid sequences and are designed to interfere with T-cell activation, while maintaining the ability to bind to MHC class II molecules. The peptide sequence EYYKEYYYKEYYK was found to ameliorate MBP₈₄₋₁₀₂-induced EAE in Lewis rats⁷⁸.

Two different APL mimics of MBP — the amino acid copolymers poly(FYAK)_n and poly(VWAK)_n — inhibited disease progression in both MBP₈₅₋₉₉ (REF. 79) and PLP₁₃₉₋₁₅₁-induced EAE⁸⁰. Although both of these copolymers are similar in sequence and therapeutic outcome, they appear to mediate their protective influence through distinct mechanisms. Poly(VWAK)_n induced anergy in effector T cells directly, whereas poly(FYAK)_n promoted T-cell proliferation and induced the production of anti-inflammatory cytokines in MBP₈₅₋₉₉-induced EAE. This example demonstrates how a two-amino-acid modification in an APL can elicit distinct mechanisms of immunosuppression and tolerance. This is in contrast to the PLP₁₃₉₋₁₅₁-induced EAE model, in which poly(FYAK)_n and poly(VWAK)_n treatment both significantly inhibited clinical disease progression by competing for peptide binding with MHC class II molecules and inducing the production of regulatory cytokines, such as IL-4 and IL-10.

Clinical trials using peptide-specific tolerance

Clinical trials have been carried out using various antigen-specific tolerance strategies in multiple autoimmune diseases including MS, rheumatoid arthritis and type 1 diabetes (TABLE 1). Clinical trials are often designed to parallel experiences in animal models of the disease, however, the transition between outcomes in experimental animal models and human trials is often not straight forward. To date, the clinical efficacy of antigen- or peptide-specific immunotherapies for the treatment of

T_H3 cells

A CD4⁺ helper T-cell subset that is characterized phenotypically by the secretion of TGF β .

pre-existing autoimmune disease is still uncertain. Multiple factors need to be considered when moving from animal models to clinical trials, including, but not limited to: the route of antigen administration; the dosage and frequency of antigen administration; the development of robust immunological assays to measure the outcome and monitor follow-up; and the time of administration (prevention versus intervention). For example, the induction of tolerance to MBP was examined in a Phase I clinical trial in patients with chronic-progressive MS using a peptide that is immunodominant for MBP-specific T cells and B cells. In this study the induction of tolerance was monitored by quantification of MBP-specific autoantibodies in cerebrospinal fluid (CSF). Although this study showed that intravenous injection of MBP₈₅₋₉₆ peptide decreased the level of autoantibodies present in the CSF for 3–4 months post treatment and showed a correlation between the decreased levels of MBP₈₅₋₉₆-specific autoantibodies and a decrease in disease severity, the direct induction of tolerance was not shown. Therefore, it is possible that the decreased level of autoantibody in the CSF was due to antigen blockade¹⁷.

As mentioned above, mucosal tolerance is a natural immunological event and therefore the use of the mucosal route to induce antigen-specific tolerance in the clinical setting is particularly attractive due to the ease of delivery. Multiple clinical trials have recently been conducted to examine the clinical benefit of the oral administration of insulin for the prevention and treatment of type 1 diabetes⁸¹⁻⁸⁵. These studies have found no (or minimal) change or improvement in β -cell function. The induction of oral tolerance has also been tested in patients with MS without any clear indication of clinical efficacy⁸⁶. Mixed results have been reported for the use of oral tolerance in the treatment of rheumatoid arthritis, with either no effect on clinical disease⁸⁷, or increased production of IL-4 and IL-10 and decreased production of IL-2, IFN γ and TNF by T cells following treatment⁸⁸. Therefore, although oral administration of antigen appears to be a safe method of tolerance induction and is effective for inhibiting the induction of autoimmune diseases in animal models, to date there is no robust indication of the efficacy of this therapy for the treatment of autoimmune disease in humans.

APLs (with at least one amino-acid substitution at positions necessary for TCR engagement) that were shown to compete with the natural ligand and interfere with T-cell activation have also been tested in clinical trials. As discussed previously, APL therapy successfully inhibits EAE in mice, however, two separate Phase II clinical trials testing an APL of MBP₈₃₋₉₉ (the immunodominant HLA-DR2-restricted T-cell MBP epitope) in patients with MS were halted due to safety concerns^{89,90}. Magnetic resonance imaging (MRI) was used to monitor the number of CNS lesions in patients undergoing therapy, and the incidence of clinical relapse and hypersensitivity reactions were monitored. In the first clinical trial, which included only eight patients, the APL was tested at a dose of 50 mg. Following treatment, participants had a higher incidence of MS exacerbations,

as determined by both MRI and clinical criteria and the APL cross-stimulated self-antigen-reactive T_H1 cells⁸⁹. A second double-blind placebo-controlled clinical trial included 142 patients receiving various APL doses. In contrast to the previous study, there was some evidence of improvement in the patients that received the 5 mg dose, based on decreased volume and number of gadolinium-enhancing lesions, as determined by gadolinium-enhanced MRI⁹⁰. This study was halted because 9% of patients developed hypersensitivity reactions, and most of these patients were in the group that received the highest dose. A potential problem with this approach is that a particular APL may be antagonistic for certain T-cell clones, but at the same time be an agonist or super-agonist for peptide-specific clones expressing different TCRs. These studies demonstrate the difficulty in moving from animal models to human patients.

In contrast to these trials, which used the MBP APL, glatiramer acetate is the only approved APL therapy for the treatment of MS. Therapy with glatiramer acetate typically consists of daily subcutaneous dosage of 20 mg, and clinically the treatment has proven beneficial for a subset of patients with relapsing–remitting MS⁹¹. A recent trial was conducted to test the safety, tolerability and efficacy of a 40 mg per day subcutaneous dosage in relapsing–remitting MS and the higher dosage was found to be well tolerated and had increased efficacy at inhibiting relapses than the currently approved dosage⁹². Parenteral administration of an APL of the amino acids 9–23 of the insulin B-chain has also been tested in type 1 diabetes⁹³. Data from this Phase I clinical trial suggested that the treatment switched the T_H1-cell-dominant pathogenic response to a T_H2-cell regulatory phenotype and a Phase II multi-dose study is underway to validate these initial findings.

Peptide-coupled-cell tolerance is a tolerogenic therapy yet to be tested in the clinic. As discussed previously, it has been demonstrated to be an efficient, safe process to induce antigen-specific tolerance in multiple animal autoimmune models, including models of MS and type 1 diabetes, with the advantage of having efficacy for the treatment of established disease. A Phase I and II clinical trial has been awarded provisional support by the [Immune Tolerance Network](#) pending FDA approval, to test safety and efficacy of peptide-coupled cells in patients with early relapsing–remitting MS. In this clinical trial, it is proposed that isologous peripheral-blood leukocytes (PBLs) — collected from patients with MS using plasmapheresis — that are ECDI-coupled with a cocktail of 5–7 previously identified immunodominant myelin peptides (including MBP₁₁₁₋₁₂₉, MBP₈₃₋₉₉, MBP₁₄₆₋₁₇₀, MOG₃₅₋₅₅ and PLP₁₃₉₋₁₅₄, which cause the expansion of peripheral blood T cells) will be intravenously re-infused into these patients⁹⁴. It is hoped that this peptide-cocktail approach will induce long-term tolerance in effector autoreactive T cells, as well as prevent future relapses by tolerizing naive T cells specific for potential endogenously released myelin epitopes, without compromising immune responses to foreign pathogens. A second clinical trial, also supported by the Immune Tolerance Network and that is currently under development,

Gadolinium-enhanced magnetic resonance imaging

An imaging technique in which gadolinium is introduced as a contrast agent, allowing short data-acquisition times, large anatomical coverage and improved image quality.

Table 1 | Human antigen-specific tolerance-based clinical trials for autoimmune disease

Antigen-specific therapy	Autoimmune disease	Treatment	Clinical trial outcome	References
Oral tolerance	Type 1 diabetes	A double-blind French study; insulin or placebo fed daily at 2.5 mg or 7.5 mg for 1 year	No effect on deterioration of β -cell function	82
		A multi-centre double-blind US study; insulin or placebo fed to adults and children with new-onset disease	Preliminary analysis suggested preserved β -cell function*	83
		A multi-centre double-blind Italian study; 5 mg daily insulin treatment of patients with new-onset type 1 diabetes for 12 months	No clinical effect on β -cell function; increased production of TGF β by PBMCs	84,99
		Large efficacy study (DPT1) of 7.5 mg daily insulin treatment of at-risk non-diabetic subjects	No prevention of type 1 diabetes onset; however, a subgroup with high insulin autoantibody levels had delayed disease onset. A repeat study planned focusing on this subgroup.	81
	MS	Treatment with bovine myelin	Decreased MRI lesions in HLA-DR2 ⁺ males; no effect on clinical relapse; large placebo effect	86, [‡]
Rheumatoid arthritis		Multi-centre double-blind Phase II clinical trial; collagen II treatment in doses from 0.02 to 2.5 mg per day for 6 months	Positive effects in the group that received the lowest dose	87
		Phase II clinical trial; 15 patients were treated with a 15mer peptide derived from <i>Escherichia coli</i> HSP dnaJ (dnaJ _{P1}) given at 0.25, 2.5 or 25 mg per day for 6 months	Treatments were well tolerated; immune deviation observed from a pro-inflammatory to tolerogenic T-cell response (decrease in TNF and increase in IL-10 production)	88
Oral APL	MS	Phase III clinical trial; copaxone (Teva Pharmaceutical Industries Ltd) or placebo given daily at 5 mg or 50 mg	No clinical, MRI or immunological effects; a Phase II clinical trial with copaxone given at 300 mg or 600 mg doses in progress	86, [‡]
Parenteral APL	MS	An MBP APL given weekly at 50 mg subcutaneously for up to 9 months.	Two out of eight patients showed increased brain lesions; one showed hypersensitivity; three others had non-specific side effects; one patient dose was lowered to 5 mg and still developed increased MS lesions; study suspended	89
		Randomized, double-blind clinical trial in 142 patients; placebo or a 5 mg, 20 mg or 50 mg dose of MBP APL given weekly subcutaneously for 4 months; then decreased to 5 mg weekly	No significant difference in relapse rate of treated and placebo groups but volume of new brain lesions was reduced in some of the patients that received 5 mg throughout; 9% developed hypersensitivity to the therapy and the trial was stopped	90
		Randomized, double-blind clinical trial; dose-comparison of 20 mg and 40 mg of copaxone given subcutaneously in relapsing–remitting MS	Higher dose was well tolerated and a decrease in relapse rate observed	92
	Type 1 diabetes	Phase I clinical trial of the insulin APL NBI-6024 consisting of five biweekly injections subcutaneously at doses of 0.1 mg, 1 mg or 5 mg	Shift from T _H 1-cell responses to T _H 2-cell regulatory phenotype; Phase II multi-dose study underway	93
		Phase II double-blind clinical trial of DiaPep277, a HSP60-derived peptide, 1 mg given subcutaneously in 4–8 dosages during the two-stage clinical trial; male participants only	Treatment was well tolerated; preserved β -cell function as measured by C-peptide concentrations in the treated group compared to placebo group; lower need for exogenous insulin in the treated group	117,118
TCR vaccination	MS	Phase I and II clinical trial of trivalent TCR peptide vaccine IR903 (Neurovax; Orchestra Therapeutics)	Induced specific TCR-reactive T-cell responses; increased expression of FOXP3; currently in Phase II clinical trial	119
Peptide-coupled PBLs	MS	Intravenous administration of ECDI-fixed, autologous PBLs coupled with five immunodominant myelin peptides	Phase I and II clinical trial to test safety and efficacy pending	[§]

*Only in patients over 20 years of age when diagnosed and fed 1 mg insulin. [‡]AutoImmune Inc. [§]Immune Tolerance Network. APL, altered-peptide ligand; DPT1, diabetes prevention trial-1; ECDI, ethylene carbodiimide; FOXP3, forkhead box P3; HSP, heat shock protein; IL, interleukin; MBP, myelin basic protein; MRI, magnetic resonance imaging; MS, multiple sclerosis; PBL, peripheral-blood lymphocyte; PBMC, peripheral-blood mononuclear cells; TCR, T-cell receptor; TGF β , transforming growth factor- β ; T_H, T helper; TNF, tumour-necrosis factor.

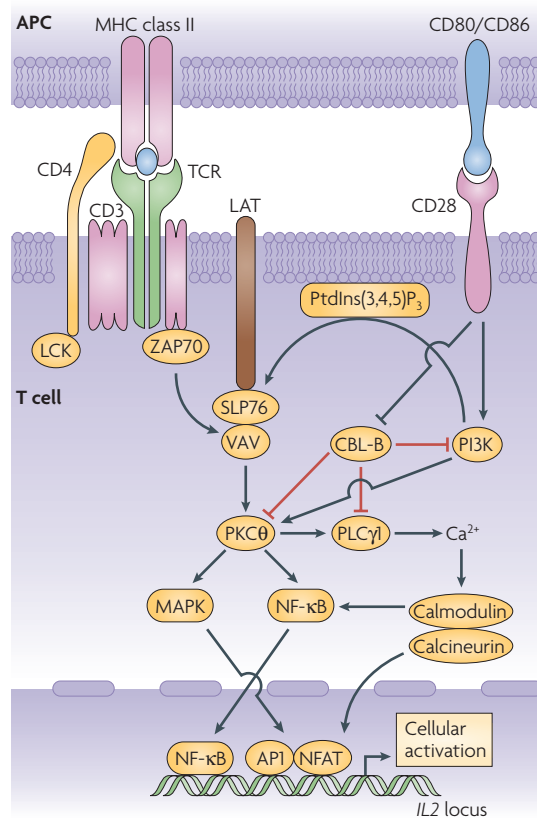


Figure 4 | Signal transduction pathways involved in T-cell anergy. Signals delivered by the engagement of the T-cell receptor (TCR; signal 1) and co-stimulatory molecules (such as CD28; signal 2) induce different signalling pathways that result in the activation of multiple transcription factors. Ligation of the TCR by peptide–MHC complexes triggers the recruitment of signalling molecules, such as phospholipase Cγ1 (PLCγ1), which induces the Ca²⁺ influx, and nuclear factor of activated T cells (NFAT) and protein kinase Cθ (PKCθ), which regulate the nuclear factor-κB (NF-κB) and activator protein 1 (AP1) pathways, respectively. These pathways control nuclear transcriptional and gene activation. In the nucleus, NFAT cooperates with AP1 and other transcription factors to induce a programme of gene expression that leads to interleukin-2 (IL-2) production. TCR engagement in the absence of co-stimulation results in the induction of NFAT proteins without concomitant AP1 activation. In the absence of cooperative binding to AP1 (FOS and JUN), NFAT regulates the transcription of a distinct set of anergy-inducing genes, such as Casitas B-lineage lymphoma B (CBL-B). Anergy-associated factors inhibit T-cell function at different levels leading to T-cell unresponsiveness. LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; PtdIns(3,4,5)P₃, phosphatidylinositol-3,4,5-triphosphate; ZAP70, ζ-chain-associated protein kinase of 70kDa.

E3 ubiquitin ligase
An enzyme that is required to attach the molecular tag ubiquitin to proteins. Depending on the position and number of ubiquitin molecules that are attached, the ubiquitin tag can target proteins for degradation in the proteasomal complex, sort them to specific subcellular compartments or modify their biological activity.

proposes to treat patients with new-onset type 1 diabetes with isologous PBLs ECDI-coupled with intact insulin. These therapeutic antigen-specific tolerance strategies all involve the common goal of restoring self tolerance in autoimmune diseases while also avoiding overall global immunosuppression. Although each of these strategies

has shown positive effects of variable efficacy in multiple animal models of autoimmune disease, translating these peptide-specific therapies to the clinical setting will undoubtedly require overcoming many obstacles.

Future directions

One probable contributing factor to the variable results gained from tolerogenic approaches for the treatment of experimental autoimmune diseases in the clinical setting is the diversity of human MHC polymorphisms. Continued research to better understand the underlying molecular mechanisms of tolerance and to enhance the specificity and efficacy of each of these treatment strategies, perhaps using combinatorial approaches, is thus necessary to deal with the complexity of the human immune system. Since short-term blockade of CD28–CD80/CD86 interactions has been proposed to induce long-term unresponsiveness of T cells undergoing activation at the time of treatment, the use of CD28–CD80/CD86 inhibitory reagents, such as CTLA4–immunoglobulin fusion protein, are currently being tested in Phase I and II clinical trials. Recent attempts to further test the therapeutic efficacy of bypassing co-stimulatory signals have shown the significant therapeutic potential of non-mitogenic CD3-specific monoclonal antibody treatment in animal models of both type 1 diabetes and EAE^{95–97}. More recently a humanized form of the OKT3 (Orthoclone; Ortho Biotech) CD3-specific monoclonal antibody mutated to prevent binding to Fc receptors (OKT3 Ala-Ala) demonstrated some success in Phase I and II clinical trials for delaying the onset of type 1 diabetes and for treating psoriatic arthritis^{98–101}. This non-mitogenic CD3-specific monoclonal antibody induces a suboptimal level of TCR-mediated signalling¹⁰² and the success of therapy is thought to be due to multiple mechanisms, including the induction of anergy in pathogenic T cells, immune deviation and activation of regulatory T cells^{97,103}. However, therapy with OKT3 Ala-Ala was associated with some side effects, including a moderate cytokine-release syndrome and symptoms of Epstein–Barr viral mononucleosis. Thus, broad-based TCR-directed therapies may have other long-term effects on the immune system.

Studies in animal models of autoimmune disease also showed efficacy of monoclonal antibody therapies directed against molecules involved in the recruitment and activation of lymphocytes and monocytes, including antibodies specific for α₄β₁-integrin (also known as VLA4), such as natalizumab (Tysabri; Biogen Idec, Inc. and Élan Corporation Plc)^{104,105}, and antibodies specific for chemokines, such as CC-chemokine ligand 3 (CCL3)¹⁰⁶, or pro-inflammatory cytokines, such as lymphotoxin¹⁰⁷. These and other immunosuppressive strategies necessitate the physical deletion or inactivation of entire subsets of T cells or the nonspecific inhibition of antigen presentation, pro-inflammatory cytokine production or T-cell trafficking, which could potentially compromise the ability of the host to combat opportunistic pathogens and/or result in an increased risk of neoplasia. The inherent dangers in these approaches are illustrated by the deaths of several participants in

a clinical trial of MS following treatment with Tysabri from progressive multifocal leukoencephalopathy (PML), an infection of the CNS by JC virus that destroys myelin-producing oligodendrocytes¹⁰⁸. Thus, owing to the side effects with these forms of antibody therapy, antigen-specific tolerance strategies have the best therapeutic potential, although more precise knowledge of the antigen(s) and epitope(s) involved in the ongoing pathogenic process in a particular autoimmune disease will be required.

Another emerging area of study is the determination of the mechanisms underlying the molecular regulation of tolerance induction. T-cell activation is regulated by nuclear factor of activated T cells (NFAT) through its interaction with the activator protein 1 (AP1) signalling complex¹⁰⁹. Besides the positive regulation of transcription when dimerized with AP1, NFAT forms complexes with other transcription factors, and can also directly regulate transcription¹¹⁰. One of the common mechanisms of tolerance induction presented in this Review is the induction of autoreactive CD4⁺ T-cell tolerance through TCR stimulation in the absence of co-stimulation. Current molecular studies show that in the absence of co-stimulation-induced AP1 activation, NFAT regulates the transcription of a specific programme of genes involved in the negative regulation of TCR signalling (FIG. 4)^{109,111}. Among the proteins that are expressed by anergic T cells are E3 ubiquitin ligases, such as ITCH (itchy homolog E3 ubiquitin protein ligase), CBL-B (Casitas B-lineage

lymphoma B) and GRAIL (gene related to anergy in lymphocytes)^{110,112–114}. For example, a characteristic feature of T cells from CBL-B-deficient mice is a lower T-cell activation threshold, which results in hypersensitivity following TCR engagement and activation of downstream signalling pathways without the normal requirement for co-receptor stimulation¹¹⁵. Furthermore, the dysregulation of TCR-induced signalling cascades associated with T-cell survival, such as the phosphoinositide 3-kinase (PI3K) pathway, is associated with the loss of self tolerance and the development of autoimmune disease¹¹⁶. These two signalling pathway intermediates (CBL-B and PI3K pathways) are putative candidate mechanisms by which peptide-specific tolerance is induced in CD4⁺ T cells. Development and/or identification of therapeutics that either inhibit signalling intermediates of T-cell activation or promote anergy-associated signalling intermediates, when used in combination with peptide-specific tolerance therapies presents a possible combinatorial strategy that may increase therapeutic efficacy while maintaining antigen specificity. Therefore, continued research to enhance specificity and efficacy of treatment in all these strategies is necessary in both animal models as well as in the clinic with advanced patient screening using modern genomic and pharmacogenomic techniques. The use of these tolerogenic approaches in combination with non-antigen-specific therapies also has the potential to eventually provide 'tailored therapy' to deal with the complexity of the human immune system.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Gene:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
CD28 | CD80 | CD86 | MBP | MOG | PLP

OMIM:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
multiple sclerosis | type 1 diabetes

FURTHER INFORMATION

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Immune Tolerance Network: www.immunetolerance.org

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