

Peptide-based therapeutic vaccines for allergic and autoimmune diseases

Mark Larché¹ & David C Wraith²

Allergic and autoimmune diseases are forms of immune hypersensitivity that increasingly cause chronic ill health. Most current therapies treat symptoms rather than addressing underlying immunological mechanisms. The ability to modify antigen-specific pathogenic responses by therapeutic vaccination offers the prospect of targeted therapy resulting in long-term clinical improvement without nonspecific immune suppression. Examples of specific immune modulation can be found in nature and in established forms of immune desensitization. Understanding and exploiting common mechanisms such as the ability to induce antigen-specific regulatory cells should allow the development of effective therapeutic strategies for both forms of immunopathology. Targeting pathogenic T cells using vaccines consisting of synthetic peptides representing T cell epitopes is one such strategy that is currently being evaluated with encouraging results. Future challenges in the development of therapeutic vaccines include selection of appropriate antigens and peptides, optimization of peptide dose and route of administration and identifying strategies to induce bystander suppression.

Allergic and autoimmune diseases are manifestations of immunological hypersensitivity. They arise when mechanisms controlling responses to innocuous environmental antigens (such as allergens), or to endogenous host ('self') proteins, break down. Why certain individuals suffer from particular hypersensitivities is unclear, but there is evidence that both genetic and environmental factors influence susceptibility.

Analysis of genes contributing to allergic¹ and autoimmune disorders² has shown that susceptibility arises from complex multigenic interactions. Many genetic elements controlling immune responses are polymorphic. Notably, genes encoding major histocompatibility complex (MHC; also known as human leukocyte antigens or HLA) class I and II are strongly associated with certain autoimmune diseases^{3,4}. Such associations support a prominent role for T cells in the recognition of a restricted number of epitopes derived from autologous or cross-reactive exogenous proteins (such as viral proteins). Interestingly, despite the well-described role of T cells in the pathogenesis of allergic inflammation, few HLA-allergic disease associations exist. Explanations for the difference between allergy and autoimmunity may include the fact

that responses to self-epitopes are tightly regulated through central and peripheral tolerance mechanisms. In contrast, responses to exogenous proteins, containing many HLA-binding epitopes, are not regulated to the same extent, particularly through central tolerance.

The dramatic recent increase in the prevalence of allergic sensitization and autoimmune diseases, particularly in industrialized countries, provides evidence for the additional role of environmental factors in the pathogenesis of immune hypersensitivity. Improved sanitation, mass vaccination programs and widespread use of antibiotics have been associated not only with reduced burden of infectious disease, but also with declining immunological interaction with microorganisms⁵. Reduced exposure to predominantly T helper type 1 (T_H1) response-inducing environmental stimuli was initially proposed to explain the increased incidence of allergic sensitization ('hygiene hypothesis'). But parallel increases in the prevalence of T_H1-mediated autoimmune diseases such as type 1 diabetes⁶ implies immunological dysfunction common to both T_H1- and T_H2-mediated diseases. Clinical studies have shown the coexistence of both T_H1- and T_H2-mediated mechanisms in asthma⁷. Thus, it seems unlikely that deficiency in T_H1 or T_H2 responses drives pathology associated with the other. Nor is it likely that simple, mutual antagonism of T_H1 and T_H2 responses in autoimmunity and allergy represent viable therapeutic approaches. Indeed, experimental models have shown the potential for exacerbation of disease through this strategy^{8,9}.

The hygiene hypothesis, in its original form, did not incorporate mechanisms of immune regulation common to T_H1 and T_H2 processes. Thus, alternative explanations for the increased occurrence of disease may include the simple concept of clonal competition, in which hypersensitivity may be passively controlled by homeostatic regulation arising through expansion of immune cells specific for prevalent infectious agents, a simple competition for scarce resources precluding hyper-reactivity to innocuous antigens¹⁰. In addition, it seems likely that regulatory mechanisms are established during early life in response to childhood infections and exposure to environmental organisms and their products¹¹ (Fig. 1). Data, derived predominantly from experimental animal models, show that distinct populations of immunoregulatory T cells limit, or protect organisms from, immune pathology¹². A number of subsets have been described, including 'natural' CD4⁺ CD25⁺ cells and those expressing the transcription factor FoxP3, an apparent marker of regulatory potential. T_H3 cells synthesizing transforming growth factor (TGF)- β and Tr1 cells secreting interleukin (IL)-10 have been described in both murine and human models. Functional deficits in CD25⁺ and Tr1 subsets of regulatory cells have been reported in both allergic^{13–16} and autoimmune diseases^{12,17–20}. Whether these observations reflect an intrinsic defect in the regulatory cells themselves (a concept difficult to reconcile with the antigen-selective nature of these diseases), or their inability to control over-exuberant effector responses, remains to be established.

¹Department of Allergy & Clinical Immunology, Imperial College London, Faculty of Medicine, Dovehouse Street, London, SW3 6LY, UK. ²Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK. Correspondence should be addressed to M.L. (m.larche@imperial.ac.uk).

Published online 5 April 2005; doi:10.1038/nm1226

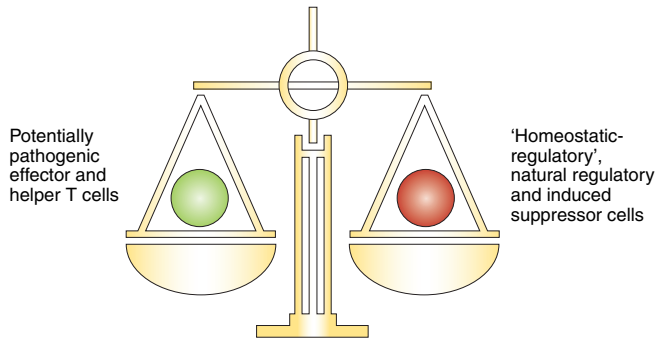


Figure 1 Balancing effector and regulatory T cell responses. The immune system has evolved to protect the individual from death by infection, while avoiding recognition of the body's own antigens. Furthermore, the response to foreign agents must be both appropriate and moderate, to avoid more damage than the agent itself. It now appears that aberrant responses to self-antigens and hypersensitivity towards foreign antigens are both limited by regulatory or suppressor cells. As such, the immune system should be seen as a fine balance between potentially pathogenic effector cells and various different types of regulatory cells.

Current treatment: the unmet need

Current pharmacologic treatments for autoimmune and allergic disorders (such as glucocorticosteroids, cyclophosphamide, methotrexate and antihistamines) are largely palliative (rather than curative) and some may result in nonspecific immunosuppression. This may be associated with a range of complications including infections, the development of tumors and disruption of natural regulatory mechanisms. Long-term use of palliative drugs is associated with significant compliance and economic issues. For these reasons, there is renewed enthusiasm for strategies aiming to reinstate homeostasis toward environmental and self-antigens. Here we discuss how peptide-based 'therapeutic vaccines' may recover immunological tolerance through the induction of immunoregulatory T cells and consider how vaccines of the future may be designed.

Background to the approach of therapeutic vaccination

There is evidence that both allergic and autoimmune pathology, resulting from deficient immune regulation, is reversible. Allergic children can 'grow out' of food allergies¹⁵, autoimmune diseases such as rheumatoid arthritis can transiently resolve without intervention during pregnancy²¹ and 'specific' allergen immunotherapy (SIT) (or in some cases, natural exposure to allergen²²) can modulate the antigen-specific immune response, resulting in long-term disease modification²³. Identifying common elements of these processes should provide the basis for development of therapeutic interventions for inflammatory diseases including allergy and autoimmunity.

Treatment of allergic rhinitis and asthma with SIT is of proven efficacy^{23,24} and has been practiced for almost a century²⁵. Subcutaneous or sublingual administration of the sensitizing protein(s) modifies immunity and reduces allergen sensitivity. Mechanistic studies show downregulation of T_H2 responses in peripheral blood^{26,27} or increased T_H1 responses in the tissue^{28,29}, or both. Recently, increased numbers of cells containing mRNA encoding IL-10 (and in some cases TGF- β) or the proteins themselves have been reported in blood^{30,31} and tissues^{32,33} of treated individuals. Moreover, IL-10-secreting regulatory T cells are known to suppress pathology in experimental models of autoimmune diseases^{34–37}. Further evidence that the resolution of allergic disease may involve factors other than simple translation of T_H2 responses to T_H1 has been obtained from several models. Beekeepers exposed to multiple

stings are protected from severe allergic reactions through a mechanism involving IL-10-secreting T cells^{38,39}. IL-10 has immunosuppressive functions on both T cells and mast cells. As with SIT for venom and aeroallergen sensitivity, protection of beekeepers is associated with induction of IgG isotypes, particularly IgG4, an isotype selectively promoted by IL-10 (ref. 40).

Cross-linking of allergen-specific IgE on the surface of mast cells and basophils leads to activation and degranulation, with the release of mediators such as histamine and leukotrienes. Furthermore, IgE on the surface of antigen-presenting cells (APC) and B cells enhances uptake of allergen (allergen focusing) for presentation to T cells. IgG may downregulate the allergic response by competition with IgE for allergen binding (classical 'blocking antibody' theory), prevention of aggregation of receptor-bound IgE through steric hindrance, or by interference with antigen focusing by IgE bound to APC. Interestingly, a major role for mast cells has recently been described in the pathogenesis of autoimmune diseases^{41,42}, providing further evidence to support common mechanisms in allergic and autoimmune pathology. Related mechanisms in pathogenesis are likely to be amenable to common approaches in therapy.

Historically, increased $T_H1:T_H2$ cytokine ratios and induction of IgG following SIT focused therapeutic strategies in allergy on exploitation of the mutual antagonism of T_H1 and T_H2 responses, a concept adopted in reverse, with respect to autoimmune disease⁴³. For example, glatiramer acetate (also known as GA, Cop-1, Copaxone), one of the most widely prescribed drugs for multiple sclerosis, is a random amino acid copolymer (poly (YEAK)_n) known to activate T cells specific for myelin basic protein (MBP) and induce T_H2 responses⁴⁴. These responses may relate to the occurrence of adverse events of an allergic nature, such as flushing, urticaria, pruritis and chest tightness⁴⁵. Long-term follow up of subjects enrolled in the pivotal phase 3 evaluation of this compound showed gradual reduction in annual relapse rates (relapse was defined as the appearance of neurologic abnormalities persisting for at least 48 hours and preceded by a relatively stable or improving neurologic state of at least 30 days)^{46,47}. But these studies were conducted in an open-label fashion and comparisons were made with baseline data rather than placebo-treated groups. Interestingly, a recent meta-analysis of all randomized controlled trials of glatiramer acetate concluded that active treatment showed no significant advantage over placebo and that current data did not support the continued use of glatiramer acetate in multiple sclerosis⁴⁵. However, further studies will be required to resolve this issue.

Glatiramer acetate binds to several MHC molecules including HLA-DRB1*1501 (encoded by a disease-associated allele in multiple sclerosis) *in vitro*. It has been suggested that glatiramer acetate competes with MBP epitopes for presentation to T cells, reducing activation of MBP-specific clones. Recently, modified copolymers based on similar sequences (VWAK and FYAK), blocked native epitope binding to MHC class II in a mouse model of multiple sclerosis, polarized T-cell cytokine responses toward a ' T_H2 ' profile and generated regulatory populations of T cells secreting IL-4 and IL-10 that were able to transfer protection from disease⁴⁸. Furthermore, defined synthetic 15-mers (peptides of 15 amino acids in length), have been designed based on the HLA-binding characteristics of glatiramer acetate and the immunodominant native epitope MBP_{83–99}. Peptides with high affinity for HLA-DRB1*1501 suppressed experimental autoimmune encephalomyelitis in a humanized SJL/J mouse model. Protection was associated with production of IL-4 and IL-10 by splenocytes and lymph node cells⁴⁹. Clinical evaluation of such compounds, which are of defined lengths and structures different from those of glatiramer acetate, is eagerly awaited.

In murine models, IL-10 has generally been considered a T_H2 cytokine. But in human studies and increasingly in experimental models, IL-10 is being regarded as an immunosuppressive or regulatory cytokine.

Thus, autoimmune processes may be ameliorated by the induction of a combination of T_H2 (IL-4) and regulatory responses (IL-10, TGF- β), just as allergic disease can be addressed by the induction of both T_H1 (interferon (IFN)- γ) and immunoregulation (IL-10, TGF- β). The search is now on for strategies to safely and effectively induce regulatory responses, in an antigen-specific fashion, for the treatment of both allergic and autoimmune conditions.

Mucosal tolerance

Mucosal administration of antigen frequently results in downregulation of the immune response and is associated with the induction of regulatory cells⁵⁰. In 1829, Dakin observed a means by which the cutaneous hypersensitivity to poisonous vegetables such as poison oak and poison ivy could be avoided⁵¹; “mystical, marvellous physicians, or favoured ladies with knowledge inherent, say the bane will prove the best antidote, and hence advice the forbidden leaves to be eaten, both as preventive and cure to the external disease.” This was one of the earliest recorded examples of mucosal tolerance, a phenomenon that waited more than 150 years for the discovery of its mechanism.

Mucosal tolerance induction has been evaluated in numerous experimental models of allergy and autoimmune disease⁵⁰, but clinical data from trials in humans have been generally disappointing. Trials of oral tolerance involved extracts of myelin in multiple sclerosis, retinal extract for autoimmune uveitis, bovine collagen in rheumatoid arthritis and insulin in type 1 diabetes⁵². Relatively low doses of antigen were administered but did not provide significant clinical benefit. In fact, administration of a complex mixture of antigens in uveitis was associated with worsening of disease⁵³. Additional trials of mucosal tolerance are required to investigate dosing effects, the nature of the autoantigen (*i.e.*, purified soluble antigens versus complex mixtures of antigens) and the use of mucosal adjuvants designed to enhance tolerance induction^{54,55}. Furthermore, a systematic comparison of mucosal versus systemic (intravenous, subcutaneous, intradermal) antigen administration is required in humans.

Regulatory T cells have been shown to have an important role in murine mucosal tolerance, with effector phenotype depending upon the nature of the antigen. Thus, protein antigens tend to induce TGF- β -secreting T_H3 cells⁵⁶, whereas short peptide antigens elicit IL-10-secreting Tr1 cells^{34,35,57}. Importantly, regulatory cells induced through mucosal tolerance have been shown to mediate bystander (or ‘intermolecular’) suppression⁵⁸, a process through which regulatory cells specific for one protein suppress the response of nearby effector cells to another protein. The latter seems to require copresentation of the two antigens by the same APC^{59,60}.

Bystander suppression is an important feature of mucosal and other forms of antigen-induced suppression because, as shown in mouse models, it may override the phenomenon of ‘epitope spreading.’ Epitope spreading was first described as a complication of autoimmune disease whereby the initiating immune response expands with time to include responses to other antigens⁶¹. Similar mechanisms have not been reported in allergic diseases (atopic dermatitis may prove to be the exception), although few studies have had the opportunity to address this issue. The issue of epitope spreading has been raised as a potential barrier to the development of effective immunological strategies to combat autoimmune diseases. Therapeutic vaccines must have the potential to modulate pathogenic responses to several antigens, as responses to individual proteins may wax and wane within an individual. Ideally, effective vaccines should generate a network of intramolecular (‘linked’) and intermolecular (‘bystander’) suppression, such that tolerance induced to one protein (MBP, for example) can be exploited to regulate responses to others (proteolipid protein, for example). Although such processes have

been shown to operate in rat models⁵⁸, there is currently little evidence of such activity in human studies. This issue remains a high priority for future vaccine design and evaluation.

Nature of the antigen for an effective therapeutic vaccine

The aim of a therapeutic vaccine is to achieve effective modulation of immune responses. Administration of the intact antigen would avoid having to select specific epitopes to suit MHC-disparate individuals. But intact antigen can activate mast cells and basophils, by cross-linking IgE in allergic individuals, as well as activating pathogenic B and T cells in both allergic and autoimmune diseases. For example, marmosets given a ‘tolerogenic’ treatment with myelin oligodendrocyte glycoprotein were protected against induced autoimmune encephalomyelitis but suffered a later lethal demyelinating disorder⁶². Furthermore, mucosal administration of whole proteins may induce pathogenic cytotoxic T lymphocytes (CTL)^{63,64}. Hanninen and colleagues compared different mucosal routes of administration in the mouse and found that oral or nasal delivery of ovalbumin (OVA) primed for OVA-specific CTL⁶⁴. Protein delivered by either route induced diabetes in mice expressing antigen in the pancreas. For these reasons, strategies to modify the structure of antigens have been investigated, including chemical modification⁶⁵ or disruption of tertiary structure of the protein⁶⁶, identification of hypoallergenic isoforms of allergens⁶⁷ and the use of synthetic peptides representing MHC class II-restricted T cell epitopes.

Peptide-based therapeutic vaccines

As short linear peptide sequences generally lack the ability to cross link adjacent IgE molecules on mast cells and basophils, a particular advantage of synthetic T cell epitopes in allergic disease is the avoidance of IgE-mediated activation. Early promise with peptide therapy in experimental models of allergy^{68,69} led to evaluation of synthetic peptides for immunotherapy in clinical trials^{70–78} (for a summary of clinical studies see Table 1). Studies using longer peptides (greater than 20 amino acids in length) were often associated with adverse events, some of which may have been related to residual IgE cross-linking capability. In general, peptide therapy was shown to reduce sensitivity to allergen and downregulate allergen-specific proliferative and cytokine responses in the blood. In studies evaluating treatment with cat allergen peptides, reduced skin reactivity to allergen was accompanied by decreased T_H1 and T_H2 responses in the blood and increases in IL-10 (ref. 77). Furthermore, airway hyperreactivity was reduced in asthmatic subjects⁷⁹ and nasal symptom scores improved in subjects with allergic rhinitis⁷⁸. Studies using peptides from a bee venom allergen (phospholipase A_2) showed improved tolerance of injected allergen challenge and partial protection from sting challenge in bee venom-allergic subjects⁷⁴.

Despite evidence from animal models that autoimmune conditions can also be prevented and treated with peptide therapy, the approach has been slower to translate to the clinic^{80–92}. Two recent studies evaluating peptides derived from heat-shock proteins for the treatment of diabetes and rheumatoid arthritis have provided encouraging results. Individuals with newly diagnosed type 1 diabetes were treated by subcutaneous injection at three time points (at study entry, 1 month and 6 months) with 1 mg of a peptide (p277) derived (and slightly modified) from the heat-shock protein hsp60 (ref. 93). After 10 months, islet cell function had been maintained in the treated group but had declined in placebo controls. In the treated group, peripheral blood T cells produced more IL-10 and IL-13, indicating modulation toward a T_H2 -regulatory phenotype⁹³. In a phase 1 study of individuals with rheumatoid arthritis, a peptide derived from the bacterial heat-shock protein dnaJ was administered by mucosal (oral) delivery. Analysis of peripheral blood responses to antigen showed that although there was no overall

Table 1 Clinical studies of therapeutic peptide vaccination

Indication	Peptide (antigen source)	Route of delivery	Cumulative dose	Outcomes	References
Allergy: Cat	Fel d1	Subcutaneous/intradermal	5,000–6,000 mcg	Sensitivity to allergen challenge in skin and lung. Peripheral immune responses.	70–73, 76–79
Allergy: Bee venom	Api m1 (phospholipase A2)	Subcutaneous/intradermal	~400 mcg	Sensitivity to allergen challenge in skin. Wild bee sting. Peripheral immune responses.	74, 75
Autoimmunity: Multiple sclerosis	Myelin basic protein (MBP)	Subcutaneous/intradermal	Up to ~1,800 mg	Clinical scores, magnetic resonance imaging, peripheral immune responses.	100, 101
Autoimmunity: Type 1 diabetes	Hsp60 (heat shock protein)	Subcutaneous	3 mg	Pancreatic islet beta cell mass. Insulin requirements. Hemoglobin concentration. Peripheral immune responses.	93
Autoimmunity: Rheumatoid arthritis	dnaJ (bacterial heat shock protein)	Subcutaneous	Up to ~9,000 mg	Clinical scores, laboratory measurements, peripheral immune responses.	94

change in the numbers of antigen-specific cells after treatment, there were significant increases in the percentage of T cells producing IL-4 and IL-10 and concomitant reductions in IFN- γ and tumor necrosis factor (TNF)- α ⁹⁴. These results may reflect the induction of a regulatory T cell population after therapy. But the study was not placebo controlled and did not report clinical outcomes. As a result, no conclusions can be drawn in relation to clinical efficacy.

Altered peptide ligands

Various groups have investigated the use of altered peptide ligands (APL) or T cell antagonists as potential vaccines for autoimmune disease^{95–99}. Such peptides share MHC binding characteristics with the native peptide sequence but, as a result of amino acid substitutions (particularly in residues that contact the T cell receptor), deliver antagonist or partial agonist signals, modifying T cell activation and cytokine production. Two trials of APL therapy in multiple sclerosis evaluated modified forms of the immunodominant epitope MBP_{83–99}. The clinical response to treatment was heterogeneous with no clear improvement in clinical outcomes. Both studies were suspended as a result of adverse events^{100,101}. In one study, hypersensitivity reactions were linked to T_{H2} skewing of T cells during treatment. Similar reactions in a related study were associated with expansion of MBP_{83–99}-specific T_{H1} cells that had high antigen avidity. In two of three subjects who developed atypical disease exacerbations, worsening of clinical parameters correlated with expansion of antigen-specific T_{H1} cells during treatment. In both studies, subjects on low-dose protocols seemed to tolerate APL therapy better. Indeed, in a secondary analysis of outcomes, subjects treated with 5 mg of APL weekly showed a reduction in the total number and volume of contrast (gadolinium)-enhancing lesions after 4 months of treatment compared to baseline ($P = 0.09$ following Bonferroni correction for multiple comparisons)¹⁰⁰. These preliminary observations are currently being evaluated in further trials. Outcomes in APL treatment may reflect the broad specificity of the T cell repertoire. Thus, as shown in an experimental model of autoimmunity¹⁰², an APL can function as an antagonist for a T cell clone expressing receptor A but as an agonist for a clone expressing receptor B. Therefore, there may be no advantage in using T cell antagonists or APL because of the difficulties of designing APL that antagonize all clonotypes specific for a particular epitope.

The role of peptide dose in tolerance induction

Despite demonstrated efficacy in animal models, little is known about the most effective dose for induction of tolerance by peptide therapy. In mice, dose ranges extend from a few micrograms¹⁰³ to milligrams⁹⁷,

and fundamental differences may exist in the mechanisms underlying low- versus high-dose tolerance. For example, in a number of experimental animal models using higher-dose regimens, substantial deletion of antigen-specific cells occurred^{97,104,105}. Anergy was also a feature of some high-dose models¹⁰⁶. Of the smaller number of studies of low-dose tolerance, none have evaluated cell death as a contributing factor. Despite potential differences, both high- and low-dose models are characterized by the induction of populations of T cells with regulatory activity^{12,94,107,108}.

In human studies, even ultra-low doses of peptide have been shown to modulate T cell responses. Intradermal injection of microgram quantities of synthetic allergen peptides induced transient activation of antigen-specific T cells in the airways. In asthmatic individuals, this was manifested as bronchoconstriction measurable a few hours after injection^{76,109}. In these individuals, transient T cell activation was followed by months of marked and enduring hyporesponsiveness, which has recently been linked to the induction of regulatory CD4⁺ T cells¹⁰⁸. Similar transient activation before tolerance was described in a number of mouse experimental models^{34,104,110–112}. The occurrence of contrast-enhancing lesions during peptide therapy in multiple sclerosis may also be related to transient effector T cell activation. With allergen peptides, induction of bronchoconstriction was not required for the ensuing peptide-induced hyporesponsiveness^{77,79}. Although this does not preclude low-level activation of antigen-specific T cells before systemic tolerance induction, it implies that activation of effector T cells is not an absolute requirement for tolerance. Thus, the magnitude of the initial effector T cell response may be controlled (for example, by reducing dose) to avoid T cell-dependent disease exacerbations such as bronchoconstriction in asthma and brain lesions in multiple sclerosis.

Differences in protein or peptide dose may not translate into a substantially different pharmacologically active dose. The route of administration, physical and biological half-life, and solubility of the tolerogen probably have roles in determining the active dose (and the immunological context of that dose) reaching the blood and lymphatics. For example, microgram doses of allergen peptides administered intradermally resulted in systemic manifestations of tolerance^{76,77}, whereas the same preparation delivered by inhalation (nebulized in saline and inhaled orally) did not¹¹³. This observation suggests that the route of administration can determine tolerogenicity and that transient activation of effector T cells may be dissociated from ensuing tolerance because it was possible to transiently activate effector T cells in the airways through both routes. It may be that a threshold plasma dose of tolerogen must be achieved to establish systemic tolerance, even in low-dose regimens.

Although intradermal or intravenous administration may require the lowest doses to achieve this, the inconvenience of injection may favor higher-dose regimens using intranasal delivery, which has been shown to lead to rapid systemic delivery in experimental models¹¹⁴.

Peptide selection

Appropriate selection of epitopes and corresponding peptides for therapeutic vaccines is crucial for success. Translation of findings in murine models is complicated by the use of inbred strains of mice. Polymorphism of the genes encoding human MHC class I and II presents a challenge for peptide vaccine design. In autoimmune diseases, HLA associations with disease provide a natural platform for vaccine design once target antigens have been identified^{115,116}. Whereas few diseases (such as ankylosing spondylitis) are overwhelmingly linked to the genes encoding HLA, a small number of epitopes (capable of binding to the disease-associated HLA molecule) selected from the most important target proteins may form the basis of an effective peptide vaccine (key features for peptide selection are shown in Box 1).

The lack of HLA association in allergic diseases, coupled in some cases with prolific polymorphism in target allergens, initially presents a more complex scenario. But the few detailed studies of the MHC-binding characteristics of primary sequence from both allergens and parasite antigens^{109,117,118} (Larché, M. & Mailleré, B., unpublished observations) indicates that relatively short regions of sequence contain multiple overlapping MHC-binding motifs. On this basis it has been possible to design peptide vaccines based on a small number of MHC-binding peptides that theoretically provide coverage of the majority of the population (assuming that a minimum of one epitope must be recognized to achieve a biological response)¹¹⁸.

The role of APCs

Further important features of an effective therapeutic peptide vaccine are the ability to bind to class II MHC proteins in a conformation that mimics the naturally processed epitope of the self-antigen or allergen¹¹⁹, together with solubility of peptide components and the lack of an associated innate immune response. Peptides that are insoluble can induce local inflammation¹²⁰. Soluble peptides can bind directly to dendritic cells (DCs) in lymphoid tissues^{114,121} and may activate regulatory T cells. Alloreactive human T cells stimulated with mature DCs produce IL-2 and IFN- γ and proliferate strongly¹²². In contrast, a number of studies in mice and humans have shown that antigens carried by immature DCs induce IL-10-secreting T cells. Repetitive *in vitro* stimulation of alloreactive human T cells with immature DCs generated anergic regulatory T cells secreting IL-10 (ref. 122). Moreover, Tr1-like T cells were induced after culture with bone marrow-derived DCs and IL-10 (ref. 123). A natural population of DCs with the same phenotype also exists *in vivo*. A single injection of immature DCs loaded

with an influenza peptide led to the generation of cells that produced IL-10 (refs. 124,125). A similar study showed that repeated injections of self-peptide-loaded immature bone marrow-derived DCs protected against subsequent induction of autoimmune disease, through the generation of IL-10-producing CD4⁺ T cells¹²⁵. Taken together, these studies imply that DCs are the most likely APCs for tolerance induction after soluble peptide administration (Fig. 2).

Duration of tolerance

The duration of tolerance induced by peptide therapy has not been studied extensively in humans, but preliminary data suggest that a single administration modifies the immune response to allergen for several months⁷⁶. Experimental models have yielded similar results, although in disease prevention rather than treatment protocols. The dominant epitope for autoimmune encephalomyelitis in the H-2^u mouse is the N-terminal nonamer of MBP¹²⁶. Mucosal (intranasal)⁸⁰ or systemic (intraperitoneal)¹²⁷ administration of a soluble form of this peptide was effective in preventing autoimmune disease. Specific suppression of the response to the N-terminal peptide was virtually complete for 1–6 weeks after administration of a single dose¹²⁸. From 8 weeks onward, respon-

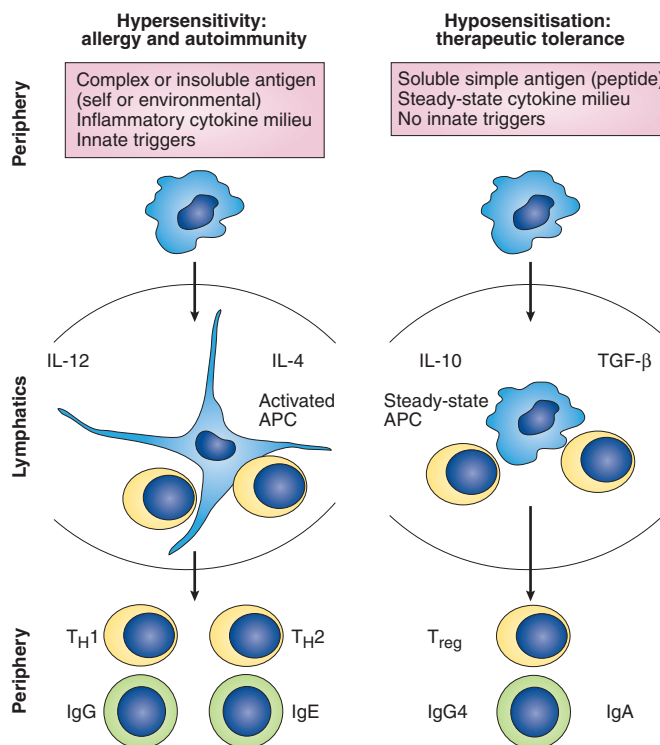


Figure 2 Antigen-presenting cells direct T cell differentiation. Hypersensitivity to self-proteins or to environmental antigens (such as pollen, animal and food proteins) may arise as a result of antigen encounter in a proinflammatory environment. Antigen-presenting cells (APC) encounter antigen in the presence of proinflammatory cytokines (T_H1 or T_H2) and innate triggers such as ligands of Toll-like receptors. APC differentiate and migrate to draining lymph nodes where they stimulate proinflammatory T_H1 and/or T_H2 responses, which may ultimately manifest themselves in the periphery as autoimmune or allergic responses. Delivery of antigen in a simple, soluble form (e.g., peptides) in the absence of proinflammatory cytokines or innate triggers, results in the maintenance of APC in a steady state as they migrate to draining lymphatics. Subsequent encounter with T cells results in the generation of regulatory responses. Manipulation of such mechanisms may provide the basis for intervention with therapeutic vaccines.

BOX 1 DESIRABLE VACCINE CHARACTERISTICS

- Soluble in aqueous solution
- Short linear sequences (avoiding tertiary structure)
- Native protein sequence (APL may behave unpredictably)
- Systemic or mucosal administration
- Failure to trigger innate immune mechanisms (i.e., TLRs)
- Appropriate MHC-binding characteristics
 - Promiscuous MHC binding may be advantageous in allergic diseases where strong HLA-disease associations are lacking.
 - The peptide must mimic the naturally processed epitope in order to induce tolerance among relevant cells.

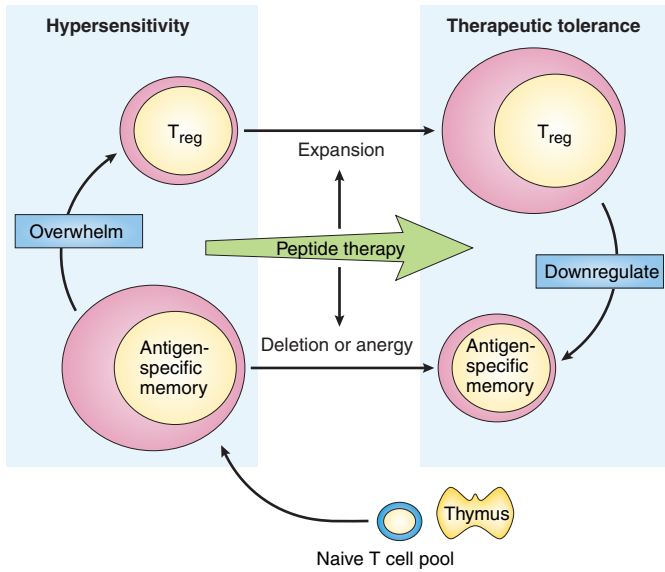


Figure 3 Peptide therapy expands the regulatory T cell pool. Under certain conditions, antigen exposure leads to a pool of antigen-specific effector memory cells that is capable of overwhelming the available regulation. This leads to T cell hypersensitivity and manifestations of disease. Peptide therapy expands the regulatory pool, allowing it to downregulate the aberrant response, while reducing the size of the effector pool. In the case of high-dose peptide therapy, induction of regulatory cells may be associated with deletion of effector cells, whereas at low peptide doses, some effectors may be rendered anergic. With time, the effector memory T cell pool may be replenished by recent thymic emigrants or antigen-specific cells differentiated from the naive T cell pool.

siveness slowly recovered in euthymic, but not adult, thymectomized mice. Such treatment substantially reduced mean maximal grades of disease, when disease was induced 1 week after treatment. But mice were barely protected from disease induction 16 weeks after peptide treatment. This observation suggests that T cell tolerance following peptide therapy is a thymus-independent, peripheral phenomenon, the reversal of which is dependent on new T cells being exported from the thymus (Fig. 3). In practice, sustained suppression by peptide therapy will probably require repeated doses of suitably selected peptide antigens.

In conclusion, we believe the uncertainty surrounding 'specific' immunotherapy is diminishing as our understanding of immune regulation progresses. Investigators in the field are entering an era in which many of the questions surrounding this area of immunology will be addressed. These include clarification of the appropriate dose of antigen, the selection of peptide epitopes for induction of bystander suppression, the optimal route of administration for each specific disease and perhaps, the selection of adjuvants to enhance the therapeutic effect of soluble antigens.

ACKNOWLEDGMENT

The authors would like to thank A. B. Kay for critical review of the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Medicine* website for details).

Published online at <http://www.nature.com/naturemedicine/>

1. Cookson, W. Genetics and genomics of asthma and allergic diseases. *Immunol. Rev.* **190**, 195–206 (2002).
2. Merriman, T.R. & Todd, J.A. Genetics of autoimmune disease. *Curr. Opin. Immunol.* **7**, 786–792 (1995).
3. Sawcer, S. *et al.* A genome screen in multiple sclerosis reveals susceptibility loci on

- chromosome 6p21 and 17q22. *Nat. Genet.* **13**, 464–468 (1996).
4. Bali, D. *et al.* Genetic analysis of multiplex rheumatoid arthritis families. *Genes Immun.* **1**, 28–36 (1999).
5. Strachan, D.P. Hay fever, hygiene, and household size. *Br. Med. J.* **299**, 1259–1260 (1989).
6. Gale, E.A. A missing link in the hygiene hypothesis? *Diabetologia* **45**, 588–594 (2002).
7. Heaton, T. *et al.* An immunoepidemiological approach to asthma: identification of *in vitro* T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* **365**, 142–149 (2005).
8. Hansen, G., Berry, G., DeKruyff, R.H. & Umetsu, D. T. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J. Clin. Invest.* **103**, 175–183 (1999).
9. Lafaille, J.J. *et al.* 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 (1997).
10. Barthlott, T., Kassiotis, G. & Stockinger, B. T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med.* **197**, 451–460 (2003).
11. Bach, J.F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* **347**, 911–920 (2002).
12. Wraith, D.C., Nicolson, K.S. & Whitley, N.T. Regulatory CD4(+) T cells and the control of autoimmune disease. *Curr. Opin. Immunol.* **16**, 695–701 (2004).
13. Ling, E.M. *et al.* Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* **363**, 608–615 (2004).
14. Cavani, A. *et al.* Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. *J. Immunol.* **171**, 5760–5768 (2003).
15. Karlsson, M.R., Rugtveit, J. & Brandtzaeg, P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J. Exp. Med.* **199**, 1679–1688 (2004).
16. Ou, L.S., Goleva, E., Hall, C. & Leung, D.Y. T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. *J. Allergy Clin. Immunol.* **113**, 756–763 (2004).
17. Balandina, A., Lecart, S., Dartevielle, P., Saoudi, A. & Berrih-Aknin, S. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* **105**, 735–741 (2005).
18. de Kleer, I.M. *et al.* CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J. Immunol.* **172**, 6435–6443 (2004).
19. Arif, S. *et al.* Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest.* **113**, 451–463 (2004).
20. Viglietta, V., Baecher-Allan, C., Weiner, H.L. & Hafler, D.A. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* **199**, 971–979 (2004).
21. Barrett, J.H., Brennan, P., Fiddler, M. & Silman, A.J. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. *Arthritis Rheum.* **42**, 1219–1227 (1999).
22. Platts-Mills, T., Vaughan, J., Squillace, S., Woodfolk, J. & Sporik, R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* **357**, 752–756 (2001).
23. Durham, S.R. *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* **341**, 468–475 (1999).
24. Bousquet, J. *et al.* Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. *Ann. Allergy Asthma Immunol.* **81**, 401–405 (1998).
25. Noon, L. Prophylactic inoculation against hay fever. *Lancet* **i**, 1572–1573 (1911).
26. Ebner, C. *et al.* Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* **27**, 1007–1015 (1997).
27. Secrist, H., Chelen, C.J., Wen, Y., Marshall, J.D. & Umetsu, D.T. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J. Exp. Med.* **178**, 2123–2130 (1993).
28. Varney, V.A. *et al.* Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J. Clin. Invest.* **92**, 644–651 (1993).
29. Wachholz, P.A. *et al.* Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. *Immunology* **105**, 56–62 (2002).
30. Francis, J.N., Till, S.J. & Durham, S.R. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J. Allergy Clin. Immunol.* **111**, 1255–1261 (2003).
31. Jutel, M. *et al.* IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* **33**, 1205–1214 (2003).
32. Nouri-Aria, K.T. *et al.* Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J. Immunol.* **172**, 3252–3259 (2004).
33. Nasser, S.M., Ying, S., Meng, Q., Kay, A.B. & Ewan, P.W. Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur. J. Immunol.* **31**, 3704–3713 (2001).
34. Burkhart, C., Liu, G.Y., Anderton, S.M., Metzler, B. & Wraith, D.C. Peptide-induced T cell regulation of experimental autoimmune encephalomyelitis: a role for IL-10.



- Int. Immunol.* **11**, 1625–1634 (1999).
35. Sundstedt, A., O'Neill, E. J., Nicolson, K. S. & Wraith, D. C. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells *in vivo*. *J. Immunol.* **170**, 1240–1248 (2003).
 36. Barrat, F.J. *et al.* In vitro generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* **195**, 603–616 (2002).
 37. Mathisen, P.M., Yu, M., Johnson, J.M., Drazba, J.A. & Tuohy, V.K. Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells. *J. Exp. Med.* **186**, 159–164 (1997).
 38. Akdis, C.A. *et al.* Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. *J. Clin. Invest.* **98**, 1676–1683 (1996).
 39. Akdis, C.A., Blesken, T., Akdis, M., Wuthrich, B. & Blaser, K. Role of interleukin 10 in specific immunotherapy. *J. Clin. Invest.* **102**, 98–106 (1998).
 40. Jeannin, P., Lecoanet, S., Delneste, Y., Gauchat, J.F. & Bonnefoy, J.Y. IgE versus IgG4 production can be differentially regulated by IL-10. *J. Immunol.* **160**, 3555–3561 (1998).
 41. Galli, S.J., Nakae, S. & Tsai, M. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* **6**, 135–142 (2005).
 42. Pedotti, R., De Voss, J.J., Steinman, L. & Galli, S.J. Involvement of both 'allergic' and 'autoimmune' mechanisms in EAE, MS and other autoimmune diseases. *Trends Immunol.* **24**, 479–484 (2003).
 43. Liblau, R.S., Singer, S.M. & McDevitt, H.O. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today* **16**, 34–38 (1995).
 44. Duda, P.W., Schmied, M.C., Cook, S.L., Krieger, J.I. & Hafler, D.A. Glatiramer acetate (Copaxone) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. *J. Clin. Invest.* **105**, 967–976 (2000).
 45. Munari, L., Lovati, R. & Boiko, A. Therapy with glatiramer acetate for multiple sclerosis. *Cochrane Database Syst. Rev.* CD004678 (2004).
 46. Johnson, K.P. *et al.* Glatiramer acetate (Copaxone): comparison of continuous versus delayed therapy in a six-year organized multiple sclerosis trial. *Mult. Scler.* **9**, 585–591 (2003).
 47. Johnson, K.P., Ford, C.C., Lisak, R.P. & Wolinsky, J.S. Neurologic consequence of delaying glatiramer acetate therapy for multiple sclerosis: 8-year data. *Acta Neurol. Scand.* **111**, 42–47 (2005).
 48. Stern, J.N. *et al.* Amelioration of proteolipid protein 139-151-induced encephalomyelitis in SJL mice by modified amino acid copolymers and their mechanisms. *Proc. Natl. Acad. Sci. USA* **101**, 11743–11748 (2004).
 49. Stern, J.N. *et al.* Peptide 15-mers of defined sequence that substitute for random amino acid copolymers in amelioration of experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* **102**, 1620–1625 (2005).
 50. Faria, A.M. & Weiner, H.L. Oral tolerance: mechanisms and therapeutic applications. *Adv. Immunol.* **73**, 153–264 (1999).
 51. Dakin, R. Remarks on a cutaneous affection produced by certain poisonous vegetables. *Am. J. Med. Sci.* **4**, 98–100 (1829).
 52. Weiner, H.L. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. *Immunol. Today* **18**, 335–343 (1997).
 53. Nussenblatt, R.B. *et al.* Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. *Am. J. Ophthalmol.* **123**, 583–592 (1997).
 54. Sun, J.B., Rask, C., Olsson, T., Holmgren, J. & Czerkinsky, C. Treatment of experimental autoimmune encephalomyelitis by feeding myelin basic protein conjugated to cholera toxin B subunit. *Proc. Natl. Acad. Sci. USA* **93**, 7196–7201 (1996).
 55. Williams, N.A., Hirst, T.R., & Nashar, T.O. Immune modulation by the cholera-like enterotoxins: from adjuvant to therapeutic. *Immunol. Today* **20**, 95–101 (1999).
 56. Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., & Weiner, H.L. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* **265**, 1237–1240 (1994).
 57. Maron, R., Melican, N.S., & Weiner, H.L. Regulatory Th2-type T cell lines against insulin and GAD peptides derived from orally- and nasally-treated NOD mice suppress diabetes. *J. Autoimmun.* **12**, 251–258 (1999).
 58. al Sabbagh, A., Nelson, P.A., Akselband, Y., Sobel, R.A., & Weiner, H.L. Antigen-driven peripheral immune tolerance: suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis by aerosol administration of myelin basic protein or type II collagen. *Cell. Immunol.* **171**, 111–119 (1996).
 59. Anderton, S.M., Burkhart, C., Liu, G.Y., Metzler, B. & Wraith, D. C. Antigen-specific tolerance induction and the immunotherapy of experimental autoimmune disease. *Novartis Found. Symp.* **215**, 120–131 (1998).
 60. Alpan, O., Bachelder, E., Isil, E., Arnheiter, H. & Matzinger, P. 'Educated' dendritic cells act as messengers from memory to naive T helper cells. *Nat. Immunol.* **5**, 615–622 (2004).
 61. Lehmann, P.V., Forsthuber, T., Miller, A. & Sercarz, E.E. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature* **358**, 155–157 (1992).
 62. Genain, C. P. *et al.* Late complications of immune deviation therapy in a nonhuman primate. *Science* **274**, 2054–2057 (1996).
 63. Blanas, E., Carbone, F.R., Allison, J., Miller, J.F. & Heath, W.R. Induction of autoimmune diabetes by oral administration of autoantigen. *Science* **274**, 1707–1709 (1996).
 64. Hanninen, A., Braakhuis, A., Heath, W. R., & Harrison, L. C. Mucosal antigen primes diabetogenic cytotoxic T-lymphocytes regardless of dose or delivery route. *Diabetes* **50**, 771–775 (2001).
 65. Norman, P.S., Lichtenstein, L.M., & Marsh, D.G. Studies on allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever. *J. Allergy Clin. Immunol.* **68**, 460–470 (1981).
 66. Niederberger, V. *et al.* Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc. Natl. Acad. Sci. USA* **101** Suppl 2, 14677–14682 (2004).
 67. Valenta, R. *et al.* Genetically engineered and synthetic allergen derivatives: candidates for vaccination against type I allergy. *Biol. Chem.* **380**, 815–824 (1999).
 68. Hoyme, G.F., O'Hehir, R.E., Wraith, D.C., Thomas, W.R., & Lamb, J.R. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J. Exp. Med.* **178**, 1783–1788 (1993).
 69. Briner, T. J., Kuo, M. C., Keating, K. M., Rogers, B. L. & Greenstein, J. L. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc. Natl. Acad. Sci. USA* **90**, 7608–7612 (1993).
 70. Norman, P.S. *et al.* Treatment of cat allergy with T-cell reactive peptides. *Am. J. Respir. Crit. Care Med.* **154**, 1623–1628 (1996).
 71. Maguire, P., Nicodemus, C., Robinson, D., Aaronson, D. & Umetsu, D.T. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin. Immunol.* **93**, 222–231 (1999).
 72. Simons, F.E., Imada, M., Li, Y., Watson, W.T., & HayGlass, K.T. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int. Immunol.* **8**, 1937–1945 (1996).
 73. Pene, J. *et al.* Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. *J. Allergy Clin. Immunol.* **102**, 571–578 (1998).
 74. Muller, U. *et al.* Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J. Allergy Clin. Immunol.* **101**, 747–754 (1998).
 75. Fellrath, J.M. *et al.* Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J. Allergy Clin. Immunol.* **111**, 854–861 (2003).
 76. Oldfield, W.L., Kay, A.B. & Larche, M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J. Immunol.* **167**, 1734–1739 (2001).
 77. Oldfield, W.L., Larche, M. & Kay, A.B. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* **360**, 47–53 (2002).
 78. Alexander, C., Tarzi, M., Larche, M. & Kay, A. B. The effect of Fel d 1-derived T cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. *Allergy*, in the press.
 79. Alexander, C., Ying, S., Kay, B. & Larche, M. Fel d 1-derived T cell peptide therapy induces recruitment of CD4CD25; CD4 interferon-gamma T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. *Clin. Exp. Allergy* **35**, 52–58 (2005).
 80. Metzler, B. & Wraith, D.C. 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 (1993).
 81. Anderton, S.M. & Wraith, D.C. Hierarchy in the ability of T cell epitopes to induce peripheral tolerance to antigens from myelin. *Eur. J. Immunol.* **28**, 1251–1261 (1998).
 82. Liu, J.Q. *et al.* 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 (1998).
 83. Staines, N.A. *et al.* Mucosal tolerance and suppression of collagen-induced arthritis (CIA) induced by nasal inhalation of synthetic peptide 184-198 of bovine type II collagen (CII) expressing a dominant T cell epitope. *Clin. Exp. Immunol.* **103**, 368–375 (1996).
 84. Chu, C.Q. & Londei, M. Differential activities of immunogenic collagen type II peptides in the induction of nasal tolerance to collagen-induced arthritis. *J. Autoimmun.* **12**, 35–42 (1999).
 85. Prakken, B.J. *et al.* Peptide-induced nasal tolerance for a mycobacterial heat shock protein 60 T cell epitope in rats suppresses both adjuvant arthritis and nonmicrobially induced experimental arthritis. *Proc. Natl. Acad. Sci. USA* **94**, 3284–3289 (1997).
 86. Tian, J. *et al.* Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J. Exp. Med.* **183**, 1561–1567 (1996).
 87. Daniel, D. & Wegmann, D. R. 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 (1996).
 88. Elias, D. *et al.* Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine burst and downregulates autoimmunity to various beta-cell antigens. *Diabetes* **46**, 758–764 (1997).
 89. Thurau, S.R., Chan, C.C., Suh, E. & Nussenblatt, R.B. Induction of oral tolerance to S-antigen induced experimental autoimmune uveitis by a uveitogenic 20mer peptide. *J. Autoimmun.* **4**, 507–516 (1991).
 90. Karachunski, P.I., Ostlie, N.S., Okita, D.K. & Conti-Fine, B.M. Prevention of experimental myasthenia gravis by nasal administration of synthetic acetylcholine receptor T epitope sequences. *J. Clin. Invest.* **100**, 3027–3035 (1997).
 91. Paas-Rozner, M. *et al.* Oral administration of a dual analog of two myasthenogenic T cell epitopes down-regulates experimental autoimmune myasthenia gravis in mice.

- Proc. Natl. Acad. Sci. USA* **97**, 2168–2173 (2000).
92. Zou, L.P. *et al.* Antigen-specific immunosuppression: nasal tolerance to PO protein peptides for the prevention and treatment of experimental autoimmune neuritis in Lewis rats. *J. Neuroimmunol.* **94**, 109–121 (1999).
 93. Raz, I. *et al.* Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* **358**, 1749–1753 (2001).
 94. Prakken, B.J. *et al.* Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **101**, 4228–4233 (2004).
 95. Sloan-Lancaster, J. & Allen, P. M. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu. Rev. Immunol.* **14**, 1–27 (1996).
 96. Franco, A. *et al.* T cell receptor antagonist peptides are highly effective inhibitors of experimental allergic encephalomyelitis. *Eur. J. Immunol.* **24**, 940–946 (1994).
 97. Karin, N., Mitchell, D.J., Brocke, S., Ling, N. & Steinman, L. Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor antagonism and reduction of interferon gamma and tumor necrosis factor alpha production. *J. Exp. Med.* **180**, 2227–2237 (1994).
 98. Kuchroo, V.K. *et al.* A single TCR antagonist peptide inhibits experimental allergic encephalomyelitis mediated by a diverse T cell repertoire. *J. Immunol.* **153**, 3326–3336 (1994).
 99. Nicholson, L.B., Murtaza, A., Hafler, B.P., Sette, A. & Kuchroo, V.K. A T cell receptor antagonist peptide induces T cells that mediate bystander suppression and prevent autoimmune encephalomyelitis induced with multiple myelin antigens. *Proc. Natl. Acad. Sci. USA* **94**, 9279–9284 (1997).
 100. Kappos, L. *et al.* Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in relapsing MS Study Group. *Nat. Med.* **6**, 1176–1182 (2000).
 101. Bielekova, B. *et al.* Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med.* **6**, 1167–1175 (2000).
 102. Anderton, S.M., Manickasingham, S.P. & Wraith, D.C. Fine specificity of myelin basic protein reactive T-cells: implications for T-cell receptor antagonism. *Biochem. Soc. Trans.* **25**, 659–661 (1997).
 103. Chai, J.G., James, E., Dewchand, H., Simpson, E. & Scott, D. Transplantation tolerance induced by intranasal administration of HY peptides. *Blood* **103**, 3951–3959 (2004).
 104. Kearney, E.R., Pape, K.A., Loh, D.Y. & Jenkins, M.K. Visualization of peptide-specific T cell immunity and peripheral tolerance induction *in vivo*. *Immunity* **1**, 327–339 (1994).
 105. Critchfield, J. M. *et al.* T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* **263**, 1139–1143 (1994).
 106. Paas-Rozner, M., Sela, M., & Mozes, E. The nature of the active suppression of responses associated with experimental autoimmune myasthenia gravis by a dual altered peptide ligand administered by different routes. *Proc. Natl. Acad. Sci. USA* **98**, 12642–12647 (2001).
 107. Apostolou, I. & Von Boehmer, H. *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med.* **199**, 1401–1408 (2004).
 108. Verhoef, A., Alexander, C., Kay, A.B. & Larche, M. T Cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. *PLoS Med.*, in the press.
 109. Haselden, B.M., Kay, A.B., & Larche, M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J. Exp. Med.* **189**, 1885–1894 (1999).
 110. Vidard, L., Colarusso, L.J., & Benacerraf, B. Specific T-cell tolerance may be preceded by a primary response. *Proc. Natl. Acad. Sci. USA* **91**, 5627–5631 (1994).
 111. Webb, S., Morris, C. & Sprent, J. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* **63**, 1249–1256 (1990).
 112. Hoyne, G.F., Askonas, B.A., Hetzel, C., Thomas, W.R., & Lamb, J.R. Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4+ T cells precedes the development of tolerance *in vivo*. *Int. Immunol.* **8**, 335–342 (1996).
 113. Ali, F.R., Oldfield, W.L., Higashi, N., Larche, M. & Kay, A. B. Late asthmatic reactions induced by inhalation of allergen-derived T cell peptides. *Am. J. Respir. Crit. Care Med.* **169**, 20–26 (2004).
 114. Metzler, B., Anderton, S.M., Manickasingham, S.P., & Wraith, D.C. Kinetics of peptide uptake and tissue distribution following a single intranasal dose of peptide. *Immunol. Invest.* **29**, 61–70 (2000).
 115. Arentz-Hansen, H. *et al.* The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med.* **191**, 603–612 (2000).
 116. Martin, R., McFarland, H.F. & McFarlin, D.E. Immunological aspects of demyelinating diseases. *Annu. Rev. Immunol.* **10**, 153–187 (1992).
 117. Southwood, S. *et al.* Several common HLA-DR types share largely overlapping peptide binding repertoires. *J. Immunol.* **160**, 3363–3373 (1998).
 118. Texier, C. *et al.* HLA-DR restricted peptide candidates for bee venom immunotherapy. *J. Immunol.* **164**, 3177–3184 (2000).
 119. Anderton, S.M., Viner, N.J., Matharu, P., Lowrey, P.A. & Wraith, D.C. Influence of a dominant cryptic epitope on autoimmune T cell tolerance. *Nat. Immunol.* **3**, 175–181 (2002).
 120. Shen, C.R. *et al.* Peptides containing a dominant T-cell epitope from red cell band 3 have *in vivo* immunomodulatory properties in NZB mice with autoimmune hemolytic anemia. *Blood* **102**, 3800–3806 (2003).
 121. Santambrogio, L., Sato, A.K., Fischer, F.R., Dorf, M.E. & Stern, L.J. Abundant empty class II MHC molecules on the surface of immature dendritic cells. *Proc. Natl. Acad. Sci. USA* **96**, 15050–15055 (1999).
 122. Jonuleit, H., Schmitt, E., Schuler, G., Knop, J. & Enk, A. H. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* **192**, 1213–1222 (2000).
 123. Wakkach, A. *et al.* Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation *in vivo*. *Immunity* **18**, 605–617 (2003).
 124. Dhodapkar, M.V., Steinman, R.M., Krasovsky, J., Munz, C. & Bhardwaj, N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. Med.* **193**, 233–238 (2001).
 125. Menges, M. *et al.* Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. *J. Exp. Med.* **195**, 15–21 (2002).
 126. Zamvil, S.S. *et al.* T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature* **324**, 258–260 (1986).
 127. Liu, G.Y. & Wraith, D.C. Affinity for class II MHC determines the extent to which soluble peptides tolerize autoreactive T cells in naive and primed adult mice—implications for autoimmunity. *Int. Immunol.* **7**, 1255–1263 (1995).
 128. Metzler, B. & Wraith, D.C. Inhibition of T-cell responsiveness by nasal peptide administration: influence of the thymus and differential recovery of T-cell-dependent functions. *Immunology* **97**, 257–263 (1999).