Immunological Tolerance: Therapeutic Induction

Secondary article

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Article Contents

Protection from the development of autoimmune diseases and acceptance of transplanted organs are due to effective tolerance to self-antigens and alloantigens, respectively. Various immunological strategies have been developed to induce therapeutic tolerance to control autoimmune disease or prevent graft rejection in antigen specific or non-specific manner.

Introduction

Immunological tolerance can be defined as an unresponsiveness of T and/or B cells to self antigens. Low responsiveness of T cells and/or B cells to self antigens could be termed partial tolerance. Protection from the development of autoimmune diseases and acceptance of transplanted organs or tissue are due to effective tolerance to self antigens and alloantigens, respectively. The breakdown of tolerance to self antigens, due to mechanisms that are still obscure, culminates in the development of autoimmune diseases. It has been suggested that tolerance to self antigens occurs by clonal deletion, anergy, ignorance and/or suppression by so-called regulator cells and the cytokine(s) they secrete (e.g. transforming growth factor β, TGFβ). Also, deviation from pathogenic to nonpathogenic T- and/or B-cell responses to self antigens could induce tolerance to pathogenic T- and/or B-cell responses. The therapeutic induction of tolerance is indicated in the treatment or prevention of autoimmune diseases and in the prevention of transplanted organ or tissue rejection. The ultimate goal is to induce self antigen-specific or alloantigen-specific tolerance to cure autoimmune diseases or prevent graft rejection, respectively. However, nonself antigen-specific or nonalloantigen-specific tolerance with minimal global immunosuppression and toxicity could also be effective in ameliorating autoimmune diseases or preventing graft rejection. This review will discuss the various strategies for therapeutic induction of tolerance and the possible mechanisms involved in animal models of autoimmune diseases and transplantation.

Situations in which Tolerance Induction is Desirable

Primarily, therapeutic tolerance induction is desirable in two situations: (1) in cases of autoimmune disease that could be either prevented or ameliorated; and (2) when graft rejection could be prevented by therapeutic induction of tolerance. **Table 1** lists autoimmune diseases that are amenable to therapeutic tolerance induction. The animal models of autoimmune diseases, and the autoantigens suggested as being involved in these diseases, are also given; however, this is not an exclusive list of autoimmune diseases, and the list is still growing. In diseases like insulin-dependent (type I) diabetes mellitus (IDDM), a significant portion of the islet β cells are damaged at the time of diagnosis, and because islet β cells cannot be regenerated, the disease has to be controlled by insulin therapy or islet β-cell transplantation. Therefore, tolerance to autoantigens involved in IDDM should be achieved before the development of type I diabetes in order to prevent the disease. In myasthenia gravis (MG), the neuromuscular junctions or end plates are destroyed by antibodies and complement. Fortunately, neuromuscular junctions can be regenerated, and therefore MG could possibly be ameliorated and remission induced by preventing further destruction by antibodies and complement. This could be achieved by therapeutic tolerance induction to acetylcholine receptor (AChR)-specific T and B cells after the establishment of disease. Similar therapeutic tolerance induction could be attempted in other autoimmune diseases in which target tissue can regenerate, or at the initial onset of disease, when the damage is minimal (e.g. rheumatoid arthritis (RA)). Ulcerative colitis, autoimmune atrophic gastritis, sympathetic ophthalmia and discoid lupus erythematosus also are considered to be autoimmune diseases. The autoantigens for these diseases are obscure and animal models for the diseases are not available.

Strategy for Inducing Tolerance

Table 2 illustrates the most promising strategies of inducing antigen-specific or nonspecific tolerance. If one wishes to induce therapeutic tolerance to a given autoimmune disease, the immunopathogenic mechanisms of that autoimmune disease should be understood for therapy to

Table 1 Autoimmune diseases in humans, the mouse counterpart, and the autoantigen(s)

Human	Mouse	Autoantigens
Myasthenia gravis	Experimental autoimmune myasthenia gravis	Acetylcholine receptor
Hashimoto thyroiditis	Experimental autoimmune thyroiditis	Thyroglobulin
Uveitis	Experimental autoimmune uveitis	Interphotoreceptor retinoid binding protein
Rheumatoid arthritis	Collagen type II-induced arthritis	Type II collagen
Type I diabetes (IDDM)	NOD (IDDM)	Islet β-cell antigens, glutamic acid decarboxylase and insulin
Graves disease (thyrotoxicosis)	–	Thyroid stimulating hormone receptor
Primary myxoedema	–	–
Goodpasture syndrome	–	Type IV collagen
Pemphigus vulgaris	–	Desmoglein 3
Pemphigoid	–	Epidermal basement membrane protein
Pernicious anaemia	–	Intrinsic factor, gastric parietal cell antigen
Addison disease	–	Adrenal gland antigen
Autoimmune haemolytic anaemia	–	Erythrocyte membrane protein
Idiopathic thrombocytopenic purpura	–	Platelet membrane protein
Multiple sclerosis	Experimental autoimmune encephalomyelitis	Myelin basic protein, Myelin oligodendrocyte glycoprotein, proteolipid protein
Systemic lupus erythematosus	NZB/NZW, MRl/lpr	DNA, RNA, Smith (SM) antigen
Primary biliary cirrhosis	–	Pyruvate dehydrogenase
Coeliac disease	–	Gluten (gliadin)
Vasculitis	–	Neutrophil cytoplasmic antigen
Sjögren syndrome	–	Ribonucleoprotein antigens (RO/SSA and La/SSB)
Scleroderma	Tight-skin mice	–

be effective. For predominantly cell-mediated (cellular infiltration and/or local cytokine release causing lesions) diseases like experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS), one must target the pathogenic cells and their secreted products (cytokines) that cause the lesion. The ideal approach is therefore either to eliminate the pathogenic populations of T lymphocytes or to suppress the pathogenic cytokines by antigen-specific or nonspecific therapy. Antibody and complement-mediated diseases like experimental autoimmune myasthenia gravis (EAMG) and MG could be effectively treated by eliminating or suppressing the pathogenic B-cell population and/or the T and natural killer (NK) cell population and cytokines (e.g. tumour necrosis factor, (TNF)) involved in the disease in an antigen-specific or nonspecific manner. Therapeutic induction of tolerance in systemic autoimmune disease like systemic lupus erythematosus (SLE) is more complicated because of pathological lesions on numerous organs and tissues. Therefore, at present, nonantigen-specific tolerance would be the ideal choice to treat SLE.

Antigen-specific

Numerous strategies have been adopted to eliminate or suppress the pathogenic T-cell population in EAE,

Table 2 Promising therapeutic tolerance induction strategy

Antigen-specific	Nonantigen-specific
Systemic, oral or nasal administration of high or low doses of autoantigen or its T cell epitope(s)	Administration of soluble cytokine receptor (TNFR:Fc) or antibodies to cytokine (anti-TNFα)
Antibody to TCR BV chain or TCR BV chain peptide vaccination in diseases with highly restricted TCR gene usage	Gene therapy by delivering regulatory protein (i.e. IL-4, TGFβ) to the site of inflammation
TCR antagonist peptide vaccination	
DNA-encoding self-peptide or TCR peptide vaccination	

IL-4, interleukin 4; TCR, T-cell receptor; TGFβ, transforming growth factor β; TNFR, tumour necrosis factor receptor.

collagen type II-induced arthritis (CIA), EAMG and experimental autoimmune uveitis (EAU) in an antigen-specific manner. Among the approaches, systemic or mucosal tolerance to high or low doses of self antigen or its immunodominant T-cell epitope(s) has been very successful. Apart from a few studies, most of this tolerance induction was initiated before the established autoimmune diseases in animal models. Multiple doses of large amounts of antigens are usually necessary once disease (e.g. EAE, EAU) is established (Whitacre and Campbell, 2000). The immunosuppressive effects are greater when the antigen is administered before challenge. Encouraging results from animal models have led to clinical trials with oral tolerance to specific antigens in MS, RA, uveitis, thyroiditis and type I diabetes (Weiner, 1997). The purity of the antigen preparation appears to play an important role in the success of oral tolerance induction in the clinical setting.

Nonspecific

Nonantigen-specific tolerance with minimal global immunosuppression and toxicity could play an important role in treating autoimmune diseases. TNF has been suggested as being involved in the inflammatory process of autoimmune disease (i.e. RA) pathogenesis. Therefore, soluble TNF receptor (which could bind TNF and neutralize its activity) could be used in the treatment of RA. The p75 TNFR:Fc fusion protein (etanercept, enbrel) as monotherapy for RA has met with great success. A multicentre, randomized, placebo-controlled trial enrolled 180 patients who received placebo or etanercept and 75% achieved benefits, compared to 14% of the patients who received placebo. Further, etanercept lacks immunogenicity.

Anti-CD4 antibody, which depletes or suppresses the function of CD4 cells involved in the disease, has not only prevented, but also reversed, animal models of autoimmune diseases (Christadoss and Dauphinee, 1986). Anti-CD4 antibody therapy has been performed in RA and MG. The therapeutic efficacy is not very striking. Blocking costimulatory molecules B7/CD28 or CTLA4 immunoglobulin (Ig) prevented animal models of autoimmune diseases. But, blocking costimulatory molecules *in vivo* might induce global immunosuppression, and, accordingly, this form of therapy should be discouraged.

Gene therapy

Deoxyribonucleic acid (DNA) vaccination with T-cell receptor (TCR) BV8.2 (TCR BV gene used by encephalotogenic T cells) or encephalotogenic peptides has protected mice from developing EAE (Waisman *et al*., 1996; Moreland *et al*., 1997). The local expression of regulatory proteins (i.e. interleukin (IL)-4, TGFβ, and IL-10) could be directed to the sites of autoimmune inflammation and deliver the regulatory protein by gene therapy. This form of gene therapy has been attempted in animal models of autoimmunity and allografting. Localized central nervous system production of IL-4 ameliorated the clinical signs of EAE in myelin basic protein (MBP)-immunized mice (Shaw *et al*., 1997). In a recent clinical trial, synoviocytes were infected *ex vivo* with a retrovirus gene encoding the IL-1 receptor antagonist (IL-1Ra), and injected intra-articularly (reviewed in Whitacre and Campbell, 2000). In CIA adenovirus transfer of the viral IL-10 gene periarticularly to mouse paws suppressed the development of CIA in both injected and uninjected paws (Whitacre and Campbell, 2000). Immunoglobulins can serve as tolerogenic carriers for antigens, and B cells can function as tolerogenic antigen-presenting cells (APCs). A retroviral vector was constructed containing a major uveitogenic interphotoreceptor retinoid-binding protein (IRBP) epitope in frame with the mouse IgG1 heavy chain. Peripheral B cells were transduced with this construct and infused into syngeneic recipients. A single infusion of tranduced cells before IRBP challenge protected mice from developing uveitis(Agarwal *et al*., 2000).

Transplantation tolerance

1. Graft survival of hetertopic cardiac allografts was prolonged by oral tolerance to immunodominant major histocompatibility complex (MHC) donor-

derived synthetic class I peptide (Zavazava *et al*., 2000).
2. Transient antibody therapy directed against the adhesion molecule LFA-1 (CD11a) was sufficient to induce donor-specific tolerance to pancreatic islet allografts in multiple donor–recipient strain combinations (Nicolls *et al*., 2000).
3. Blockage of the CD28/B7 T-cell costimulatory pathway prolonged allograft survival. CTLA4 Ig gene transfer into the myocardium allowed indefinite graft survival (Guillot *et al*., 2000).
4. It is possible that nonimmunogenic retroviral vectors that allow permanent transgene expression in dendritic cells and promote localized delivery of the immunosuppressive transgene product, could promote immune deviation and alloantigen-specific T-cell hyporesponsiveness (Takayama *et al*., 2000).

Mechanisms Involved

Systemic high-dose T-cell epitope tolerance

Acetylcholine receptor dominant T-cell epitope (α146–162) peptide-induced systemic tolerance can prevent the development of clinical EAMG in C57BL6 mice (Wu *et al*., 1997). A high dose of α146–162 peptide suppressed AChR-specific T-cell responses to AChR and its dominant α146–162 and subdominant α182–198 peptides by suppressing IL-2, interferon γ (IFNγ), and IL-10 production of α146–162 peptide-specific lymph node cells. Tolerance induced by α146–162 peptide injection is antigen-specific, as a keyhole limpet haemocyanin (KLH)-specific immune response is not suppressed by α146–162 peptide injection. AChR-activated CD4 cells in CD8-deficient mice could be effectively tolerized (Deng *et al*., 2001). Suppression of lymphocyte proliferative response to AChR and α146–162 peptide after injection with α146–162 peptide in incomplete freund adjuvant (IFA), was preceded by the augmented expression of CD69 and Fas molecules (initial activation molecules) and B7.2 molecules on AChR-immune lymph node cells Deng *et al*., 2001). Production of AChR and α146–162 peptide-specific T-cell proliferative response and IL-2 production was suppressed by high-dose α146–162 injection in B6 mice, but not in B6.lpr mice deficient in Fas. Further, high-dose α146–162 peptide injection failed to suppress clinical EAMG and anti-AChR antibody response in B6.lpr mice. Therefore, Fas-mediated apoptosis could play an important role in systemic high-dose α146–162 peptide-induced tolerance. Evidence for augmented apoptosis of BV6 cells within the AChR-immune lymph node cells 24 h after high-dose α146–162 peptide injection was also demonstrated. No T_H1 to T_H2 immune deviation after tolerance induction was observed; however, exogenous IL-2 addition *in vitro* recovered the proliferative response to AChR and α146–162 peptide in tolerized cells, suggesting clonal anergy also play a role in systemic high-dose α146–162 peptide tolerance (Deng *et al*., 2001).

A scenario of cellular events that could take place after systemic injection of a high dose of α146–162 peptide in IFA into AChR-immunized B6 mice to induce tolerance might be as follows: (1) first, there is activation of CD4 cells (B cells?) specific for α146–162 peptide, and augmented expression of CD69, Fas and B7.2 molecules; (2) next, Fas-FasL-mediated apoptosis of a portion of α146–162 peptide-specific (e.g. BV6) cells occurs; followed by (3) reduction of IL-2 production due to deletion of IL-2 producing α146–162 peptide-reactive (e.g. BV6) cells; leading to (4) clonal anergy of part of α146–162 peptide-specific cells which has escaped apoptosis; and (5) clonal anergy of other AChR (α182–198 peptide)-specific cells due to local IL-2 deficiency (infectious tolerance or determinant spread (Wu *et al*., 1997)). Therefore, antigen-specific therapy of EAMG and MG could be achieved by Fas-mediated apoptosis and anergy of AChR-dominant, peptide-reactive T cells by high-dose dominant T-cell epitope tolerance by activating Fas on the AChR pathogenic epitope-reactive CD4 cells.

Oral tolerance

Following oral administration of antigens, proteins enter the gastrointestinal tract where they interact with the gut-associated lymphoid tissue (GALT). The dendritic cell has recently emerged as the most likely candidate APC in oral tolerance. Within 6 h of oral antigen administration, there is an upregulation of CD69 on the relevant antigen-specific T-cell population in the GALT, as well as in the spleen and lymph nodes. It is decreased by 48 h after feedings. The expression of CD69 is accompanied by an increase in the size of the activated T cells in the spleen, mesenteric lymph nodes and Peyer patches. This activation is accompanied by augmented T-cell proliferation, and secretion of IL-2, IFNγ, and IL-4. Similar activation events are observed whether low or high doses of antigen are fed (Weiner, 1997; Whitacre and Campbell, 2000). Antigen administered repetitively in low doses induced the appearance of IL-4-, IL-10- and TGFβ-producing T cells specific for the fed antigen. The production of TGFβ secretion is thought to be a pivotal event in low-dose tolerance, with TGFβ secretion causing a nonspecific reduction in the activity of nearby cell types (including autoreactive T cells) (Weiner, 1997). Following oral administration of high doses of antigen, antigen-reactive T cells have been shown to ultimately die by apoptosis. IL-12 has been identified as a pivotal regulator for both low- and high-dose oral tolerance.

Five phase II studies of oral collagen type II (CII) in RA have been performed thus far, with over 1100 patients. In

patients receiving 100–500 μg chicken CII, improvement was noted in some, as indicated by a decrease in the number of swollen and tender joints. Doses in the range of 20–60 μg collagen per day were optimal, whereas doses in the 1–10 mg range were not (reviewed in Whitacre and Campbell, 2000).

The overall results from numerous trials on oral tolerance in the treatment of human chronic autoimmune diseases (RA, MS) are disappointing because they fail to demonstrate clinical efficacy.

Nasal tolerance

Intranasal administration of the human AChR extracellular domain of the α subunit prevented the induction of EAMG in rats, and immunosuppressed ongoing disease. This prevention was accompanied by a marked reduction in the proliferative T-cell response and IL-2 production in response to AChR, reduced antiself AChR antibody titre, and an isotype switch of IgG2 to IgG1 (Barchan *et al*., 1999). In addition, nasal tolerance to an α-chain recombinant fragment of AChR or its peptide could downregulate T_H1 and upregulate T_H2 cytokine and TGFβ (reviewed in Deng *et al*. (2001)).

Antibody to TCR BV chain or TCR BV chain peptide vaccination

Antibody to TCR BV chain primarily deletes *in vivo* pathogenic TCR BV-expressing cells in EAE when there is a highly restricted usage of TCR BV genes. Analysis of T-cell receptor BV gene usage in cells isolated from the arthritic joints of BUB mice showed that TCR BV chain gene usage was limited to TCR BV3 and BV10 gene families. Immunization with TCR BV3 and BV10 peptides completely blocked the development of clinical and subclinical inflammation, formation of pannus and synovial hyperplasia, and the erosion of cartilage and bone (Haqqi *et al*., 1996). Immunization with TCR BV peptide failed to suppress the humoral response to chicken CII, but elicited significant levels of anti-BV3 and BV10 peptide antibodies. The deletion of TCR BV3 and BV10 cells by anti-BV3 and BV1 peptide antibodies elicited by BV3 and BV10 peptide immunization appear to be the main mechanism of prevention of CIA.

TNF receptor fusion protein in the treatment of RA

Marked suppression of acute phase proteins such as C-reactive protein (CRP) was observed after TNF blockade and the changes in CRP were associated with changes in IL-6. A reduction in soluble E-selectin and intercellular adhesion molecule 1 (ICAM-1) was observed after anti-TNFα antibody treatment. Reduction in inflammation was attributed to reduced vascular permeability as a consequence of downregulation of vasculoendothelial growth factor.

Maintenance of Tolerance

In most of the studies, tolerance was induced either before or during the priming of autoantigens. Very few studies have been performed to study the maintenance of therapeutic antigen-specific or nonspecific tolerance, and we do not know how long therapeutic tolerance could last. We studied the kinetics of the maintenance of tolerance after a single tolerizing dose of AChR in AChR-primed mice. C57BL6 mice were immunized with 20 μg AChR in complete Freund adjuvant (CFA). A week later, they were injected intraperitoneally with either 500 μg α146–162 peptide or phosphate buffered saline (PBS) in IFA. Three, 7 and 28 days after the induction of tolerance, AChR and α146–162 peptide-specific T-cell proliferation, and IFNγ and IL-2 production were measured. Suppression of AChR and the α146–162 peptide-specific proliferative response could be observed from day 3 to day 28 after a single high dose of α146–162 in IFA. Suppression of α146–162 peptide- and AChR-specific IFNγ and IL-2 production was also observed until day 28. Therefore, a single intraperitoneal injection of 500 μg α146–162 peptide in IFA, could induce T-cell low response to AChR and α146–162 peptide for at least 28 days, as measured by proliferation, and by production of IFNγ, and IL-2 (Deng *et al*., 2001). The recovery of AChR-specific lymphocyte response on day 28 after α146–162 peptide-induced tolerance indicates that for long-term T-cell tolerance, α146–162 peptide injection should be repeated. A combination therapy in which T-cell epitope tolerance could be followed by specific anticytokine therapy might maintain tolerance for an extended period. The ultimate goal is the induction of permanent tolerance to the autoantigens in a given autoimmune disease, with minimal nonspecific immunosuppression and side effects.

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