New Treatment Avenues: Oral Tolerance — Mechanisms and Applicability to Human Diseases

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Immunological tolerance is a fundamental property of the immune system that provides a mechanism for self-nonself discrimination. The concept of oral tolerance, first described in 1911 by Wells [1], refers to a form of peripheral tolerance where mature lymphocytes in the peripheral lymphoid tissues are rendered nonfunctional or hyporesponsive by prior oral administration of an antigen [2]. Other mucosal routes, such as the nasal and bronchial mucosa, have also been effective in modulating systemic immune responses. Orally administered antigens encounter the gut-associated lymphoid tissue, which is a well-developed immune network that not only protects the host from ingested pathogens but also prevents the host from reacting to ingested proteins. Among the primary areas in the GALT[1], where specific immune responses are generated, are Peyer's patches, which are interspersed among the villi. Once in the patches, the antigens encounter a dense web of cells that process and present them to T cells, which then differentiate into various kinds of effector T cells that mediate oral tolerance and/or oral immunization. Effector T cells subsequently migrate out of the patches and traffic to systemic lymphoid tissue, thus ensuring that the consequences of mucosal immunization and tolarization are effective throughout the immune system [3].

Mechanisms of Oral Tolerance

The mechanisms by which oral tolerance is induced have recently been extensively reviewed [2–4]. The primary mechanisms mediating oral tolerance include deletion, anergy of antigen-specific T cells, and active cellular suppression [5–7], the primary determining factor being the dose of fed antigen [6,8]. Low doses favor active suppression, whereas high doses favor deletion and anergy [Figure 1].

Active cellular suppression

This event is mediated by the induction of regulatory T cells in the GALT and their migration to the systemic immune system. One of the primary mechanisms of active cellular suppression is via secretion of suppressive cytokines such as transforming growth factor-beta and interleukins-4 and -10 following antigen-specific triggering [7,9]. TGF-β[2] is produced both by CD4+ and CD8+ GALT-derived T cells [10] and is an important mediator of the active suppression component of oral tolerance. CD4+ cells that primarily produce TGF-β appear to be a unique T cell subset. Termed Th3 cells, they are different from Th1 and Th2 cells, provide help for immunoglobin A production, and primarily secrete TGF-β [2,7]. Although CD4+ T cells appear to be the most important suppressor T cells mediating oral tolerance, a number of reports have pointed to CD8+ suppressor γδ-T cells as also mediating oral tolerance [3].

Bystander suppression

This concept refers to a state in which tolerized regulatory cells secrete suppressive cytokines (e.g., TGF-β) in an antigen-specific fashion, but their release into a local microenvironment may then also suppress ongoing immune responses to an unrelated, but anatomically colocalized antigen. The outcome may be complete abrogation of an autoimmune response or attenuation of the inflammatory process. The idea of bystander suppression may solve a major conceptual problem in designing antigen or T cell-specific therapy for inflammatory autoimmune diseases in which the autoantigen is unknown, or where there are reactivities to multiple autoantigens at the target tissue. Because regulatory cells induced by oral antigen secrete antigen-nonspecific cytokines after being

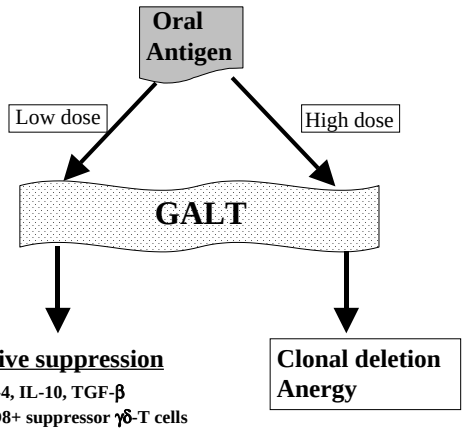

Figure 1. Mechanisms of oral tolerance induction.

[1]GALT = gut-associated lymphoid tissue
[2]TGF-β = transforming growth factor-β

triggered by the fed antigen, they suppress inflammation in the microenvironment where the fed antigen is localized. Thus, for a human organ-specific inflammatory disease, one need not know the specific antigen that is the target of an autoimmune response; all that is necessary is feeding an antigen capable of inducing regulatory cells which then migrate to the target tissue and suppress inflammation. Examples include the suppression of proteolipid protein-induced experimental autoimmune encephalomyelitis by feeding with myelin basic protein [11], suppression of adjuvant and antigen-induced arthritis by feeding with type II collagen [12,13], or the suppression of viral-induced diabetes by oral insulin [14].

Oral tolerance and clonal deletion

Feeding with high doses of antigen can lead to oral tolerance mediated by mechanisms other than active suppression, namely clonal deletion or anergy. Evidence for T cell deletion has been reported primarily after oral administration of ovalbumin. Apoptosis of antigen-specific lymphocytes was demonstrated *in vivo* both locally (within Peyer's patches) and systemically (in the thymus and spleen) [5,10]. Both Th1 and Th2 cells were deleted following their initial activation, manifested by decreased IL-2[3], interferon-gamma and IL-4 production, whereas cells that secrete TGF-β were resistant to deletion. The apoptotic mechanisms involved in clonal deletion in response to fed protein are largely unknown. It was found that the degree of peripheral tolerance is enhanced when antigen feeding is combined with systemic administration of anti-IL-12 antibodies [10], implying that IL-12 negatively regulates high dose antigen feeding-induced apoptosis. The loss of peripheral cells was Fas-mediated, since more than 90% of antigen-specific Fas+ T cells were lost. In addition, antagonizing Fas reversed the phenomenon when cells were stimulated *in vitro*. Nonetheless, the deletion mechanisms responsible for oral tolerance after administration of high doses of antigen are yet to be determined.

Oral tolerance and clonal anergy

High doses of orally administered antigen may result in inactivation of antigen-specific cells, i.e., anergy. Anergy is defined as an unresponsive state of T cells, such that they are incapable of proliferating or secreting IL-2. Although functionally inert, anergic T cells remain intact; and in many experimental systems anergy can be reversed through exposure of T cells to IL-2. There are several experimental models of oral tolerance that showed no evidence of active suppression or presence of antigen-reactive lymphocytes [8,15]. Suppression of Th1 lymphocyte function after oral administration of ovalbumin has been demonstrated [15]. The impaired ability of cells to respond to antigen *in vitro* could be restored by a period of culture with exogenous IL-2, indicating the continued presence of antigen-reactive T cells. Interestingly, Th2 responses were not diminished after ovalbumin feeding, suggesting that anergy is more readily induced in the Th1 lymphocyte population.

[3]IL-2 = interleukin-2

Oral Tolerance in Experimental Autoimmune Diseases

Autoimmune diseases are regarded as a failure of the body's immune system to maintain tolerance to selected self-antigens. Keeping autoreactive cells under control is an ongoing and dynamic process, and a shift in this balance can lead to the emergence of self-reactivity and autoimmune disease. A milestone in the area of mucosal tolerance was the recognition that self-antigens could be administered to prevent and treat autoimmune diseases. Early studies showed that animals receiving self-antigens orally, *prior to* immunologic challenge, were protected from subsequent disease induction [16,17]. These early studies were expanded into a variety of model systems [Figure 2] representing a wide spectrum of human diseases with autoimmune components [4].

Experimental autoimmune enchephalomyelitis

EAE[4] is an autoimmune inflammatory disease of the central nervous system, mediated by CD4+ Th1 cells and accepted as a model for multiple sclerosis. EAE can be induced by MBP[5] or by other myelin proteins such as proteolipid protein, myelin oligodendrocyte glycoprotein, or purified peptides derived from these proteins. Feeding MBP or its fragments prevented animals from developing EAE following challenge with MBP [16]. Studies in the chronic–relapsing EAE models indicated that oral administration of myelin antigens or proteolipid protein could protect diseased animals from further relapses [18]. A novel method for oral tolerance induction in EAE was recently introduced [19] using copolymer-1, a synthetic amino acid copolymer that simulates MBP immunologically. Feeding mice or rats with Cop-1[6] resulted in inhibition of EAE induction, associated with down-regulation of T cell immune responses to MBP. Oral Cop-1 was even more effective than oral MBP in suppressing EAE in rats.

Experimental arthritis

Oral administration of type II collagen suppresses several models of arthritis, including collagen-induced arthritis [17], adjuvant arthritis [12], pristane-induced arthritis, and antigen-induced arthritis [13]. These diseases are induced in rats and susceptible strains of mice, and exhibit clinical signs and histopathologic changes similar to those seen in rheumatoid arthritis. Successful peripheral tolerance was also accomplished via the nasal and aerosol routes.

Experimental antiphospholipid syndrome

APS[7] is characterized by high titers of anticardiolipin and anti-β2-glycoprotein-I autoantibodies and/or lupus anticoagulants associated with thromboembolic phenomena, thrombocytopenia, recurrent fetal loss, as well as other multisystemic involvement. An experimental model for APS was induced in naive mice by immunization with pathogenic autoantibodies — aCL[8] or β2GPI[9] [20–23].

[4]EAE = experimental autoimmune encephalomyelitis
[5]MBP = myelin basic protein
[6]Cop-1 = copolymer-1
[7]APS = antiphospholipid syndrome
[8]aCL = anticardiolipin

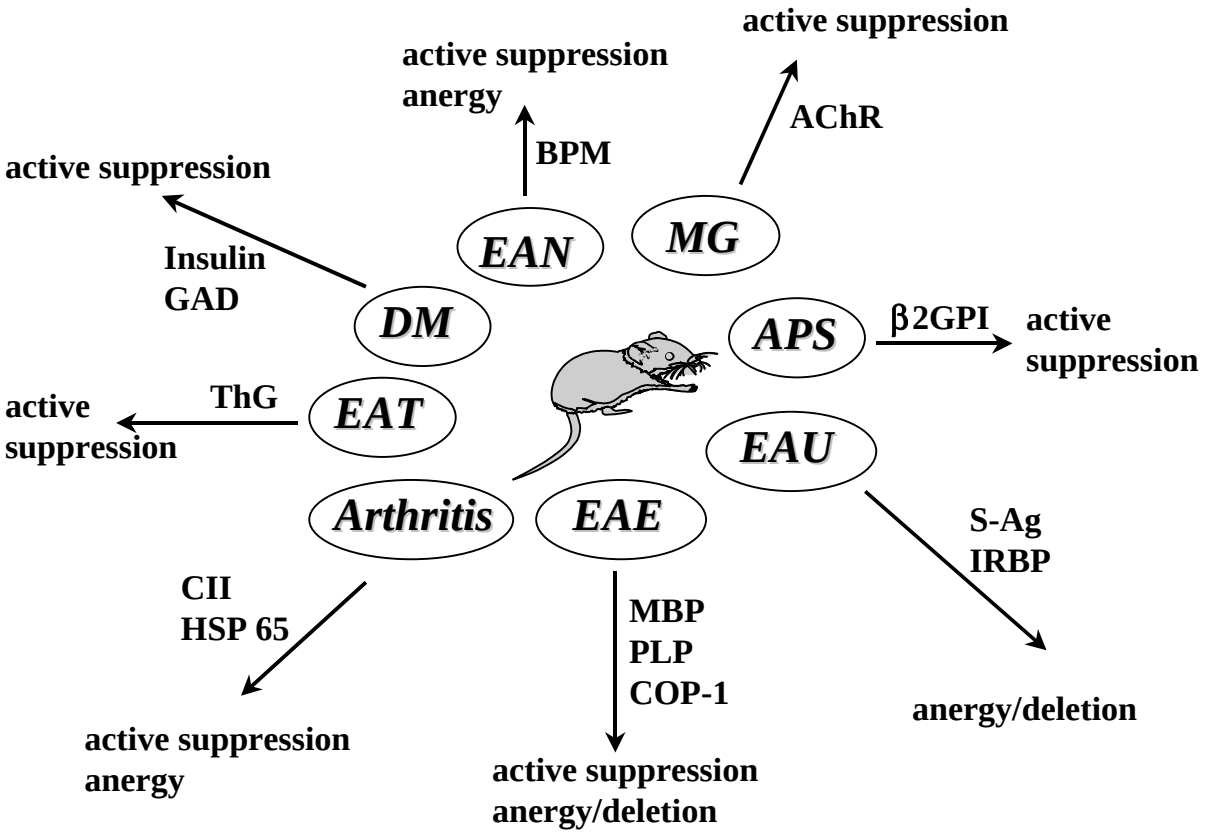

Figure 2. Induction of mucosal tolerance in animal models for autoimmune diseases.

APS = antiphospholipid syndrome, DM = diabetes mellitus, EAE = experimental autoimmune encephalomyelitis, EAN = experimental autoimmune neuritis, EAT = experimental autoimmune thydoiditis, EAU = experimental autoimmune uveoretinitis, MG = myasthenia gravis. , MBP = myelin basic protein, PLP = proteolipid protein, CII = collagen type II, HSP65 = mycobacterial heat-shock protein, ThG = thyroglobulin, GAD = glutamic acid decarboxylase, BPM = bovine peripheral myelin, AchR = acetylcholine receptor, β2GPI = β2-glycoprotein I, IRBP = interphotoreceptor retinoid binding protein.

Employing this model, Blank et al. [24] recently induced oral tolerance in BALB/c mice by feeding them with low dose β2GPI. The treated group had low titers of anti-β2GPI and aCL antibodies in the serum, lack of fetal resorptions, low incidence of thrombocytopenia, and normal values of activated partial thromboplastin time. β2GPI given orally before priming with β2GPI resulted in the complete prevention of experimental APS development, while β2GPI given at an early stage of the disease reduced clinical manifestations.

Experimental autoimmune uveoretinitis

Immunization of Lewis rats or B10.A mice with retinal antigen produces the histopathological features of EAU[10], a T cell-mediated autoimmune disease that simulates uveoretinitis in humans. Studies in both acute and chronic–relapsing EAU have indicated that oral administration of S-antigen, S-Ag[11]-derived peptides, interphotoreceptor retinoid binding protein, and even recombinant *Escherichia coli* (expressing retinal S-Ag) before immunization can protect animals from EAU [8,25,26]. Successful peripheral tolerance has also been obtained via the nasal route.

Experimental insulin-dependent diabetes mellitus

An experimental autoimmune model of human IDDM[12] is the non-obese diabetic mice, characterized by a Th1 cytokine pattern. Oral insulin can prevent the development of IDDM in these mice considerably, the effect being mediated through a T cell-dependent mechanism with a shift of balance from a Th1 to a Th2 and Th3 pattern of cytokine expression [27]. Investigations of other models of diabetes for their applicability to oral tolerance have also been successful [14].

Experimental autoimmune myasthenia gravis

Myasthenia gravis and its experimental model EAMG[13] are immune disorders characterized by circulating antibodies and lymphocyte autoreactivity to nicotinic acetylcholine receptor. EAMG can be induced by immunizing animals with purified AChR[14]. Oral administration of AChR to Lewis rats prior to immunization with AChR resulted in prevention or delay in the onset of EAMG [28]. Prevention of EAMG has also been established following intranasal AChR administration. Recombinant fragments

[9]β2GP1 = β2-glycoprotein-1
[10]EAU = experimental autoimmune uveoretinitis
[11]S-Ag = S-antigen
[12]IDDM = insulin-dependent diabetes mellitus
[13]EAMG = experimental autoimmune myasthenia gravis
[14]AChR = acetylcholine receptor

corresponding to the entire extracellular domain of the human AChR α-subunit (Hα1-210), and to portions of it that encompass the main immunogenic region or the ligand-binding site of AchR, were cloned and shown to modulate the *in vivo* course of EAMG [29]. The tolerizing and suppressive potential of mucosal administration of these recombinant peptides was recently studied [30]. Intranasal administration of these recombinants prevented the induction of EAMG in rats and suppressed an ongoing disease. Similarly, oral administration of Hα1-210 fragment prior to EAMG induction had a clear beneficial effect on the disease's clinical symptoms, manifested by increased weight and normal content of AChR.

Other experimental models

- Experimental autoimmune neuritis is a disease that mimics the clinical and histopathological features of Guillian-Barre syndrome as well as other chronic–relapsing demyelinating neuropathies. EAN[15] is induced in animals by immunization with peripheral nerve myelin (bovine peripheral protein). Oral administration of BPM[16] strongly suppressed clinical and histological signs of EAN subsequently induced by BPM [4]. Oral therapy with BPM after onset of myelin-induced EAN ameliorated the further course of disease only slightly, but reduced lethality of the disease significantly.
- Experimental autoimmune thyroiditis is an inflammatory autoimmune disease-induced immunization with thyroglobulin or by passive transfer of activated CD4+ T cells. Oral administration of ThG[17] prior to immunization suppressed the severity of passively transferred experimental autoimmune thyroiditis [4]. Also, feeding with human ThG effectively reduced the immune responses in mice immunized with ThG [31].
- Experimental autoimmune cholangitis is a mouse model for primary biliary cirrhosis, characterized by pathological Th1 CD4+ cell responses to immunization with pyruvate dehydrogenase complex. Feeding SJL mice with high or low doses of PDC[18] prior to immunization significantly down-regulated the *in vitro* T cell proliferation and/or skewed the response to PDC toward Th2 phenotype, with elevated IL-4 and IL-10 levels [32].

Studies of Oral Tolerance in Patients

The findings obtained in experimental animal models have prompted several clinical trials of oral tolerance in patients with myasthenia gravis, rheumatoid arthritis, uveitis, and IDDM [2,4]. The results of the trials are conflicting, and although some improvement has been noted in some of the patients, broad-ranging clinical improvement has not yet been observed. A more accurate choice of antigens, as well as more precise dosing and timing of antigen administration, might lead to better results in the future.

In a one-year double-blind study, 30 individuals with relapsing–remitting myasthenia received daily capsules of bovine myelin or a control protein to determine the effect of oral tolerization to myelin antigens on the disease [33]. Six of 15 individuals in the myelin-treated group had at least one major exacerbation, compared to 12 of 15 in the control group who had an attack. T cells reactive with MBP were reduced in the myelin-treated group. No toxicity or side effects were noted. Males fed myelin were observed to have fewer exacerbations than placebo-treated patients. This trend was not observed in female patients. However, in a placebo-controlled double-blind phase III trial of single-dose bovine myelin in 515 patients with relapsing-remitting myasthenia, no improvement was found in the treated group of patients compared with the controls [2].

In an open-label study testing the oral administration of CII[19] in 10 children with juvenile rheumatoid arthritis, 8 patients showed reductions in both swollen and tender joint counts after 3 months of treatment. There were no adverse events that were considered to be treatment related [34]. In a randomized, double-blind trial involving 60 patients with severe RA[20], a decrease in the number of swollen joints and tender joints occurred in patients fed chicken CII for 3 months but not in those who received a placebo. Four patients in the collagen group had complete remission of the disease; no side effects were evident [35]. In contrast, in a double-blind, placebo-controlled trial of 90 patients with RA treated for 12 weeks with bovine CII at 1 or 10 mg/day or with placebo, there were no significant differences among the three groups in terms of response to treatment [36]. In another recent multicenter, double-blind, placebo-controlled trial of oral CII in patients with RA [37], 274 patients with active RA were enrolled to receive placebo or 1 of 4 dosages of oral CII for 24 weeks. An improvement was detected in the group of patients receiving the lowest dosage (20 μg). The presence of serum antibodies to CII at baseline was significantly associated with an increased likelihood of responding to treatment.

In a phase I/II randomized masked trial, patients with endogenous uveitis who were dependent on immunosuppressive agents were randomly assigned to receive either retinal S-Ag or placebo [38]. Although not statistically significant, patients given S-Ag were more likely to be tapered off their chronically administered systemic immunosuppressive therapy. Recently, a synthetic peptide mimic of disease-associated HLA-B haplotype sequence was administered orally to patients with autoimmune uveitis [39]. This peptide was shown to cross-react with S-Ag and to induce EAU in rats, while protecting the animals from EAU if fed prior to challenge with S-Ag or IRBP[21]. Five patients received oral peptide therapy 3 times weekly for 12 weeks. There was no observed toxicity and several patients were able to discontinue their steroid treatment due to amelioration of the ocular inflammation.

Finally, some preliminary data have suggested that oral tolerance could be obtained in humans by administering small doses of subcutaneous insulin to prediabetic patients

[15] EAN = experimental autoimmune neuritis
[16] BPM = bovine peripheral myelin
[17] ThG = thyroglobulin
[18] PDC = pyruvate dehydrogenase complex
[19] CII = type II collagen
[20] RA = rheumatoid arthritis
[21] IRBP = interphotoreceptor retinoid binding protein

[40]. Several trials are currently under way in children and adults at high risk, and only at completion of those trials will it be clearer whether oral tolarization strategies can be used in IDDM.

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