

Oral Tolerance: Mechanisms and Therapy of Autoimmune Diseases

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Abstract

Oral tolerance is a state of immune hyporesponsiveness induced by the oral or mucosal exposure to antigens. This state is dependent on the dose of the oral antigen administered, with a low dose stimulating regulatory T cell development leading to an active immune suppression that is transferable via T cells. The active mechanism appears to be a cytokine mediated immune deviation with a predominant Th2 and Th3 response (TGF- β). In contrast, high dose oral antigens lead to clonal deletion and anergy. The active suppression of low dose oral tolerance can also suppress an unrelated immune response (bystander suppression) paving the way for therapy of autoimmune diseases like rheumatoid arthritis. Oral tolerance has been effective in the treatment of autoimmune diseases like experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA) and insulin-dependent diabetes in animals. However, recent studies in human autoimmune diseases have not been as effective but the results are encouraging and more work is required to understand the mechanisms involved and other factors that may modulate the response.

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Introduction

Immunologic tolerance is a hallmark of the immune system whereby immune cells are tolerant of self antigen. Historically, this tolerance was thought to be secondary to the removal of self-reactive T cells during development and maturation in the thymus of the immune cells - clonal deletion.^{1,2} However, the true mechanisms are complex and comprise many components. Recent studies have shown that the mechanisms of tolerance include not only a deletion of self-reactive cells in the thymus and the periphery but also "silencing" of self-reactive cells. This silencing may be due to an anergising signal whereby immune T and B cells are rendered incapable of responding in a functional way to nominal antigens.^{1,2} It can also be due to an active suppression via other immune cells via suppressive cytokines or mediators. Additionally, it has also been established that self-reactive T cells can be found in normal individuals. Why these T cells do not cause pathology in most individuals but in some lead to autoimmune disease is currently the subject of many studies. The foundations for therapy of autoimmune diseases thus lie in the understanding of how these autoreactive T cells can be controlled and tolerised.

Oral tolerance has been recognised as a method of inducing tolerance to a variety of antigens via exposure of the mucosal surface of the gut to antigens. The exact mechanisms have remained obscure for many years. However, it has come into intense scrutiny in recent years as the mechanisms become more understood and the potential for application immense. Oral tolerance was first described by Wells³ in 1911 when it was observed that guinea pigs were resistant to egg protein anaphylaxis when the animals were fed the same protein orally prior to antigen challenging. Subsequently, in 1946 Chase⁴ described diminished contact sensitisation to a hapten (DNCB - dichloronitrobenzene) if animals were fed DNCB prior to immune stimulation. As a consequence of this, other workers were able to confirm oral tolerisation by a variety of other antigens like ovalbumin and sheep RBCs in other animals.⁵ The first description of this in humans was to the antigen, keyhole limpet haemocyanin (KLH).⁶ These observations established oral tolerance as an effective means of modulating the immune response via oral antigen administration. This state of oral tolerance is dependent on the dose of the antigen fed (Fig. 1). At high antigen doses, T cells were rendered anergic or deleted and with low doses, development of regulatory

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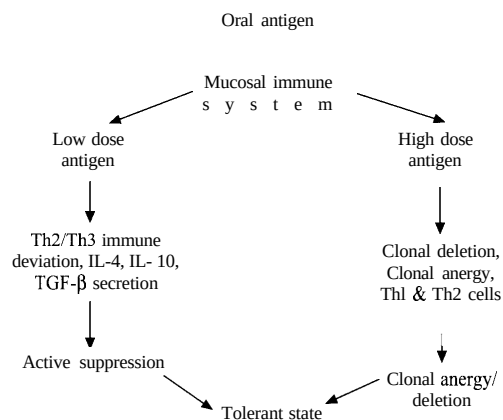


Fig. 1. Oral tolerance and antigen dose.

T cells secreting suppressive cytokines like TGF- β and IL-4 were enhanced.⁷

Mucosal Immune System

The gastrointestinal tract has in recent years been found to be more than just an organ for digestion and absorption of food and nutrients. Scattered within the intestinal epithelium and lamina propria are intraepithelial lymphocytes (IELs) and lamina propria lymphocytes (LPLs) respectively.⁸ These IELs have special characteristics and have an extrathymic mode of development. In addition, there are also nodules of lymphoid tissue within the small intestines (Peyer's patches) that may be important in the mucosal immune response and lymphocyte trafficking to and from the gut.⁸ Thus, the gastrointestinal tract can also be viewed as an important immune organ that protects the host from ingested organisms as well as suppressing immune reactions to the ingested food. This gut associated lymphoid tissue (GALT) is thus directly exposed to antigens within the gut and regulate immune responses to these antigens (Fig. 2). Furthermore, specialised antigen presenting cells, M cells, are present in the gut and these may play an important role in processing and presenting antigen to the GALT. Thus, the selective immune responses generated in the GALT may be responsible for the systemic hyporesponsiveness to the ingested antigen.

Although most oral antigens are degraded by digestive processes, some of the antigens may enter the systemic circulation unaltered or as partially degraded antigen.^{9,10} How the systemic entry of these antigens lead to tolerance is currently still unclear.¹¹ Work in our laboratory indicates these systemically absorbed antigens may also have systemic effects such as thymic deletion (manuscript in preparation).

Mechanisms

Recent studies have indicated that oral tolerance is

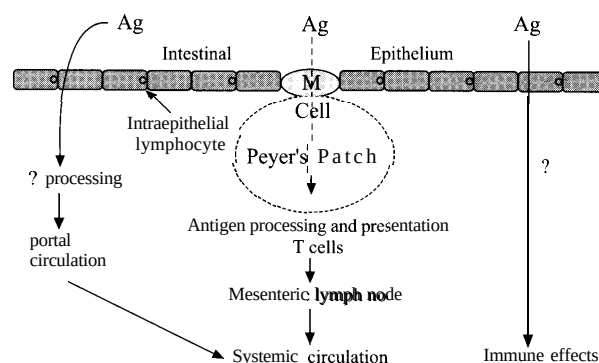


Fig. 2. Mucosal immune system.

mediated via several mechanisms-clonal deletion, clonal anergy and active suppression¹²⁻¹⁶ (Fig. 1). The dominant mechanism used is determined by the amount of antigen fed orally. With high doses of antigen, the frequent finding is that of clonal deletion in the Peyer's patches and clonal anergy of the remaining antigen-specific T cells. Under such conditions, the tolerant state could not be transferred from one animal to another via the transfer of T cells. The experimental animals were tolerant as the antigen-specific T cells were removed or rendered inactive.

When animals were fed low doses of antigen, the primary mechanism contributing to oral tolerance is active suppression.¹⁷ Here, the antigen induces the production of regulatory T cells in the GALT, primarily in the Peyer's patches, such that suppressive cytokines like TGF- β , IL-4 and IL-10 are produced by the cells.¹⁸ These cytokines in turn downregulate the antigen-specific systemic response for the antigen. These T cells have been shown to be both CD4+ as well as CD8+ T cells. Older studies showed that CD8+ T cells¹⁹ were the main cells transferring tolerance during adoptive transfer to naive animals but recent work suggested that both CD4 and CD8 T cells were equally capable of mediating the oral tolerant state.^{18,20-25} In addition, oral tolerance could still be elicited in CD8 depleted mice using antibody and in mice lacking CD8+ T cells.²⁴⁻²⁶ Thus, T cells activated via the mucosal route have a predominant Th2 immune response with enhancement of IL-4 secretion and lower IFN- γ production with some cells producing predominantly TGF- β also known as Th3 cells,²⁷ while T cells stimulated at non-mucosal sites tend to produce more IL-2.²⁸ Furthermore, in mice lacking TGF- β widespread inflammation was found showing that TGF- β has an anti-inflammatory and immune suppressive effect.²⁹

CD4+ regulatory T cells have also been cloned from mice fed with myelin basic protein (MBP) and these cells have been shown to transfer protection against experimental autoimmune encephalomyelitis (EAE) in naive mice.²¹ These T cells have been shown to produce IL-4

and TGF- β and help in mucosal IgA production.^{21,27} These MBP specific Th2 and Th3 T cell clones have also been found in human multiple sclerosis patients fed with MBP.³⁰ In a model of contact hypersensitivity, lack of MHC class II and CD4+ T cells abrogated oral tolerance and also primed the mice for contact sensitivity.³¹ This indicated that in this system, the CD4+ T cells were crucial to the oral tolerisation process.

The activation of regulatory T cells after low dose oral antigens has been shown to suppress an immune response towards other antigens.³² This bystander suppression is the result of the release of suppressive cytokines that downmodulate the immune response to another antigen provided the original oral antigen is also present. Thus, in animals fed with ovalbumin, a subsequent immune response to another antigen like MBP could be dampened if the animals are immunised with both MBP and ovalbumin but not with MBP alone. Furthermore, tissue-specific bystander suppression could be observed in proteolipid protein (PLP) induced EAE in SJL mice fed with MBP or MBP peptides.³³ This has allowed investigators to move beyond a conceptual problem in the therapy of autoimmune diseases-lack of knowledge on the inciting autoantigen. In most instances, the autoantigens triggering autoimmune are unknown and during an autoimmune response there are both intra and interantigenic epitope spreading so that many epitopes may be involved. For example, in human diabetes, there is evidence to show that glutamic acid dehydrogenase (GAD), insulin, heat shock protein (HSP) and other antigens are recognised by self-reactive T cells.³⁴ Thus, the knowledge of the true target antigen of the autoimmune response becomes immaterial and oral feeding of any antigen capable of inducing regulatory T cells to the target tissue is all that is required for suppression of the autoimmune response.

So far thymic derived $\alpha\beta$ T cells appear to be responsible for the process of oral tolerisation. The mucosal immune system is also substantially populated with $\gamma\delta$ T cells, many of which are extrathymic in origin and are either CD4-CD8- or CD8 $\alpha\alpha$ + (in contrast to thymic CD8 $\alpha\beta$ + cells). Recent evidence suggest that depending on conditions, these cells may abrogate oral tolerance³⁵ or enhance oral tolerance.³⁶ They are also important in the proliferation of the lining epithelial cells³⁷ and provide T cell help for IgA production.“”

Modulators of Oral Tolerance

As oral tolerance shows good potential as a means of immune regulation, many investigators have studied other factors that may enhance or abrogate oral tolerance. As one of the major mechanisms of oral tolerance is that of a balance between Th1 and Th2 cytokines, use of cytokines and anti-cytokine antibodies has been studied to modulate oral tolerance. Parenteral IFN- γ (Th1)

has been shown to abrogate oral tolerance³⁹ while IL-4 (Th2) enhances oral tolerance (unpublished data). Interestingly, mice lacking IL-4 have been found to have impaired mucosal responses but intact oral tolerance to high dose antigens.^{40,41} IL-12 is a cytokine secreted by macrophages and mononuclear cells and has been shown to bias an immune response towards the Th1 and away from a Th2 response. Appropriately, anti-IL-12 enhanced oral tolerance leading to an increase in TGF- β production and T cell apoptosis.⁴² Orally administered IL-4 and IL-10 have also been found to be effective in enhancing oral tolerance (unpublished data). In another recent study, low dose methotrexate (non-immune suppressive) has been shown to enhance oral tolerisation to MBP⁴³ and may have potential use in the treatment of rheumatoid arthritis. Cholera toxin has been found to be an immune adjuvant and primes for an immune response rather than cause tolerance and has been used as an adjuvant for mucosal immunisation. However, the B subunit of the toxin coupled to antigen has been found to be effective in inducing tolerisation but the exact mechanism is still unclear.^{44,45} Surprisingly, another potent adjuvant lipopolysaccharide (LPS) could enhance oral tolerisation to MBP.⁴⁶ An anti-chemokine antibody has been shown to block oral tolerance showing that factors involved in lymphocyte trafficking may be important in the process.⁴⁷ Recently, a study revealed that caution should be applied in oral tolerisation as under certain circumstances, immune priming may occur instead of tolerisation. Here, in a MHC class I restricted T cell transgenic system, oral antigen led to the autoimmune destruction of β islet cells and diabetes in the animals.⁴⁸ Thus, these various modalities have good potential to modulate the oral tolerisation to antigens but care is required in further understanding the mechanisms behind the process before application in the therapy of autoimmune diseases.

Treatment of Autoimmune Diseases

The initial work performed in mice and rats showed that oral administration of MBP could prevent or reduce the incidence of EAE. These were studies which showed for the first time that oral tolerisation could be effective as means of therapy.^{49,51} This provided the impetus for more studies which showed that the mechanisms of oral tolerisation differ depending on the dose of the administered antigen. As discussed above, high dose antigen leads to deletion and anergy while low doses cause active suppression that could be transferred via T cells.^{14,15} In addition, analysis of the brains and spinal cords of animals revealed that treated animals had higher levels of TGF- β and lower levels of TNF- α and IFN- γ in the diseased tissue.⁵² Long-term administration of oral MBP could modulate chronic EAE in animals and decreases the number of relapses in these animals.⁵³ In a TCR transgenic system specific for MBP, mice develop EAE

but disease could be suppressed via oral MBP.⁵⁴

With the success of oral tolerisation against EAE, other animal models of autoimmune disease were used to test therapy (Table I). Oral collagen type II could suppress arthritis induced by collagen,^{55,56} adjuvant⁵⁷ and direct intra-articular antigen,⁵⁸ while oral mycobacterial HSP65 had been found to suppress adjuvant arthritis.⁵⁹ Studies in experimental autoimmune uveitis (EAU) were also equally positive showing protection with oral retinal S protein.^{60,61} Non-obese diabetic (NOD) mice developed spontaneous diabetes mellitus that is immune mediated. Oral insulin delay and in some prevent diabetes in NOD mice.^{62,63} Like in other models, the main mechanism appears to be secondary to immune deviation to a Th2 cytokine response with decrease in IFN- γ production and reduced insulinitis.⁶⁴ Additionally, nasal and bronchial exposure of antigen could also protect against disease.^{65,66} Furthermore, in a lymphocytic choriomeningitis virus (LCMV) system, oral insulin could suppress diabetes due to an immune response to a viral antigen⁶⁷ and a Th2 cytokine shift was also apparent. With the good success in the animal models, it became imperative to study oral tolerisation therapy in human autoimmune disease.

Human trials using oral tolerance for multiple sclerosis (MS), rheumatoid arthritis, uveitis and insulin dependent diabetes (IDDM) are currently on-going (Table I). Commercial companies like Autoimmune Inc. have been set up to use novel immune tolerisation in the therapy of autoimmune disease. Initial studies on oral tolerance were performed on patients with multiple sclerosis and rheumatoid arthritis.^{68,69} The results while not as impressive as that in animals were positive although long-term consistent results were lacking. How-

ever, the trials did reveal that there were no significant systemic toxicity or exacerbation of disease during the trial. In vitro studies also supported the positive trend showing that in patients treated with bovine myelin, MBP and PLP specific T cells secreting TGF- β could be found.⁷⁰

In a more recent phase III double-blind placebo-controlled trial in relapsing remitting MS (515 patients), a single dose of bovine myelin was given.⁷¹ Here, there were no clinical differences between the treated and control groups although MRI data suggested a positive change in the myelin-treated groups. A high placebo effect was seen in this study which could have obscured a true clinical effect.

A recent study of 280 patients with rheumatoid arthritis given oral chicken type II collagen in doses ranging from 20 to 2500 μ g showed no differences in the high dose treatment groups while there were significant responses in those on the lowest dose (20 μ g) of treatment (unpublished data). In general, a higher prevalence of responders were seen in the treated group compared with the placebo. In another study where oral collagen was given to 360 patients, significant differences from baseline were found in those treated with 60 μ g of collagen per day. However, no statistical differences were found between the treated and placebo groups. Here again, a significant placebo response was obtained.⁷²

Studies in other immune mediated diseases like uveitis, contact allergy and IDDM have also been performed and showed positive trends in oral tolerisation.⁷³⁻⁷⁵ A study is currently underway to study the effects of oral human insulin in the prevention of new onset IDDM in at risk individuals in a Diabetes Prevention Trial (DPT). This will be the first human study attempting to delay or prevent an autoimmune disease by oral tolerisation, the benefits of which have been definitively established in animal models. Transgenic plants have recently been developed to express autoantigens and early studies on NOD mice fed with plant-derived GAD antigen showed protection against diabetes mellitus.⁷⁶

Thus, these early studies indicate that there are positive trends in the use of oral tolerance in human disease.

The Future

Despite improved understanding in the mechanisms of oral tolerance, much of the immune response to antigens on mucosal surfaces still remain unclear. In fact, exposure to antigens in the nasal and bronchial mucosa also leads to tolerance induction.^{65,77} This has important implications for the treatment of allergic and hypersensitivity reactions as well. It is also unclear how antigen is processed in the intestinal mucosa and presented to immune cells and what roles the resident immune cells play in modulating the immune response.⁷⁸

TABLE I: ORAL TOLERANCE THERAPY

Animals	Self-Antigens
EAE	MBP, PLP
Arthritis	Type II collagen, HSP
Uveitis	S-antigen
Myasthenia gravis	Acetylcholine receptor
Diabetes (NOD)	Insulin, GAD
Transplantation	MHC peptide, alloantigen
Thyroiditis	Thyroglobulin
Humans	
Multiple sclerosis	Bovine myelin
Rheumatoid arthritis	Chicken type II collagen
Uveitis	Bovine S-antigen
IDDM	Recombinant human insulin

EAE: experimental autoimmune encephalomyelitis; GAD: glutamic acid decarboxylase; HSP: heat shock protein; IDDM: insulin-dependent diabetes mellitus; MBP: myelin basic protein; MHC: major histocompatibility complex; NOD: non-obese diabetic mice; PLP: proteolipid protein;

Many different antigen presenting cells are resident in the mucosa and perhaps many different molecules regulating lymphocyte trafficking and activation are involved. Furthermore, under certain circumstances, oral antigens may prime for an immune response.^{49,79-81} More studies need to be done in order to determine the optimal dosing and conditions to obtain a good clinical response. In addition, more trials are being designed using recombinant human proteins that are purer and incorporating adjuvants and other drugs in combination. The appeal of oral tolerisation still remains strong as it is simple to administer and virtually free of adverse effects. Thus, more work and understanding are required before one can fully harness the power of this mode of immune regulation and use it safely for the treatment of various autoimmune conditions.

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