

Review

Food Allergy, Oral Tolerance and Immunomodulation—Their Molecular and Cellular Mechanisms

Shuichi KAMINOGAWA

Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

This paper describes the molecular and cellular mechanisms of food allergy and oral tolerance including immunomodulation. Food allergy is triggered by an aberrant immune response elicited by oral administration of dietary antigens. Oral tolerance is a state of immunological unresponsiveness induced by oral administration of dietary antigens and is thought to serve to suppress food allergy. This review first describes the characteristic properties of T and B cells relating to milk allergy and also the location of binding sites to T and B cells on allergen molecules. The immunogenicity of allergens is shown to be reduced by the modulations of the T cell binding site, using sophisticated methods such as site-specific mutagenesis. Furthermore, this review focuses on oral tolerance with special reference to the identification of lymphocyte compartment subsets and the immunological mechanism relating to oral tolerance. Finally, the application of oral tolerance for the treatment of allergy is speculated on.

Key words: food allergy; immunomodulation; milk allergen; oral tolerance; immunotherapy

For many years it was thought that macromolecules such as protein antigens did not enter the circulation *via* the intestinal ingestion route. However, it is now established that intact and partially digested antigens can pass through the mucosal barrier of the intestinal tract. Such antigens encounter the gut-associated lymphoid tissue (GALT),^{1–4)} a very well-developed immune network that has evolved not only to protect the host from ingested pathogens but also to prevent host adverse immune reactions to ingested dietary protein.

The interaction of orally administered antigens with GALT induces characteristic immunological responses such as production of immunoglobulin A (IgA) and induction of oral tolerance (Fig. 1). IgA can prevent the initial adhesion of pathogens to epithelial cells that precedes infection by the transepithelial route. On the other hand, oral tolerance is a state of systemic immunological unresponsiveness induced by oral administration of protein antigens that serves to avoid aberrant immune reactions.

Food allergy is triggered by an aberrant immune response elicited by oral administration of dietary antigens such as milk and egg proteins. It is generally accepted that food allergies are generated only when immunological barriers such as oral tolerance are breached. Thus, oral tolerance is thought to be an immune mechanism which serves to suppress food allergy.¹⁾

The number of patients with allergic disease is increasing world-wide and, consequently, effective new treatments for this common disease are required. Although many drug therapies are available for intervention in allergic reactions, most of these treatments cannot be applied without side-effects. Therefore, new immunotherapies, such as those described below, are being investigated with the aim to provide more safe and effective means of treatment. One approach is the modification of allergens to achieve a non-pathogenic molecular state or to provide allergens with an immunosuppressive function by partial alteration of their primary structure. A second approach is to induce oral tolerance to pathogenic allergens.

In this review, the molecular and cellular mechanisms of

food allergy and oral tolerance including immunomodulation will be described.

Food Allergy

Molecular and cellular mechanism of food allergy

The word “allergy” comes from a Greek term that means altered reactivity, and thus includes all mechanisms of immune hypersensitivity.

Allergic disease affects 30% of the population of Japan and includes food allergy, dermatitis, asthma, and anaphylaxis. Food allergy is often the first manifestation of allergic disease in young allergic patients. The clinically important allergen sources in food are eggs, milk, legumes, seafood, cereals, meat, and occasionally others.

Food allergy is an aberration of the immune system manifested by skin, gastrointestinal, and respiratory syndromes. Allergic diseases are classified depending on the basis of the mechanism at onset as follows: type I, immediate hypersensitivity; type II, hypersensitivity; type III, immune complex-mediated hypersensitivity; and type IV,

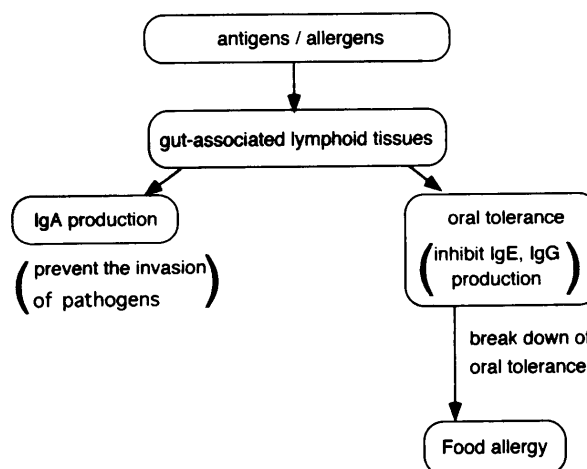


Fig. 1. Illustration of Immunological Response in Intestinal Immune System.

delayed-type hypersensitivity. In the case of food allergies, type I reactions are most common among these four types.^{5,6)}

The mechanism of food allergy (*i.e.*, type I allergy) is shown in Fig. 2. The type I allergic reaction is characterized by the production of allergen-specific IgE and the activation of mast cells, and is summarized below.

After allergens have entered the body through the gastrointestinal tract, which comprises the first line of defense against foreign materials, the allergens interact with the following four kinds of cell groups in the body: 1) antigen presenting cells including macrophages and dendritic cells; 2) T cells; 3) B cells; 4) mast cells. The allergens are firstly internalized and processed by antigen presenting cells, and are subsequently presented to T cells.

T cells are classified into $CD4^+$ T cells (consisting of types Th1 and Th2) and $CD8^+$ T cells. Helper T cells (Th2-type $CD4^+$ T cells) play an essential role in activation of B cells to secrete antibodies, while inflammatory T cells (Th1-type $CD4^+$ T cells) activate macrophages to kill intracellular parasites. $CD8^+$ T cells kill target cells infected with cytosolic pathogens or downregulate the immune response.

Most helper T cells recognize the processed antigens in association with major histocompatibility antigen complex (MHC) class II molecules on the surface of antigen presenting cells *via* their T cell antigen receptors (TCR). Complex formation by three different molecules is a key reaction in the allergic response. The processed regions of the allergen which participate in formation of this complex are designated as T cell determinants. Each T cell determinant is composed of an agretope and an epitope: they are the interacting site of peptides derived from the antigen in complex with the MHC molecule and the T cell antigen receptor, respectively. Such interaction leads to the T cell activated state. Simultaneously, allergens stimulate B cells by binding immunoglobulin E (IgE) on the cell surface.

The activated T cells provide help to the allergen-stimulated B cells through direct cell-cell interaction and secretion of cytokines which promote their proliferation and differentiation into IgE antibody-producing cells. This IgE antibody binds to a receptor on mast cells. After

binding of allergen to cell-associated IgE, the stimulated mast cells degranulate to release chemical mediators such as leukotrienes and histamine, which cause the allergic symptoms.

The binding sites on allergens recognized by IgE are defined as B cell epitopes (or determinants) and the regions on the IgE molecule which are directly involved in contact with the allergen molecule are designated as paratopes. The same designations are used in the case of other classes of antibodies such as IgG, IgM, IgA, and IgD.

It is generally accepted that the onset of allergy is principally governed by the level of allergen specific IgE in patients. However, the immune functions of GALT have an influence on the onset of symptoms in food allergy. The production of IgE is suppressed through oral tolerance and a breakdown of tolerance leads to the onset of food allergy.

T cells and antibodies in allergic patients

Since T and B cells play important roles in allergic reactions, the characteristic properties of T cells and antibodies from milk-allergic patients have been examined in an effort to discover the cellular mechanisms of food allergy. In these studies, the reactivity of peripheral blood mononuclear cells from cow's milk-allergic patients to α_{s1} -casein, the major allergen in cow's milk,⁷⁾ was analyzed.⁸⁾ α_{s1} -Casein specific T cell lines from cow's milk allergic patients were established and these cell lines could be classified into three groups: those containing predominantly $CD4^+CD8^-$ T cells, those containing both $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells, and those containing predominantly $CD4^-CD8^+$ T cells. $CD8^+$ T cells were obtained at an unexpectedly high frequency from these patients.⁸⁾ These T cell lines produced interferon- γ (IFN- γ) as well as interleukin (IL)-4. These results suggest that $CD8^+$ T cells specific for α_{s1} -casein as well as $CD4^+$ T cells are primed by the stimulation with α_{s1} -casein in milk-allergic patients, and that both $CD8^+$ and $CD4^+$ T cells may play a key role in the onset, progression of, or recovery from cow's milk allergy.

The response of $CD8^+$ T cells, discovered in this experiment, is characteristic of cow's milk allergic patients since

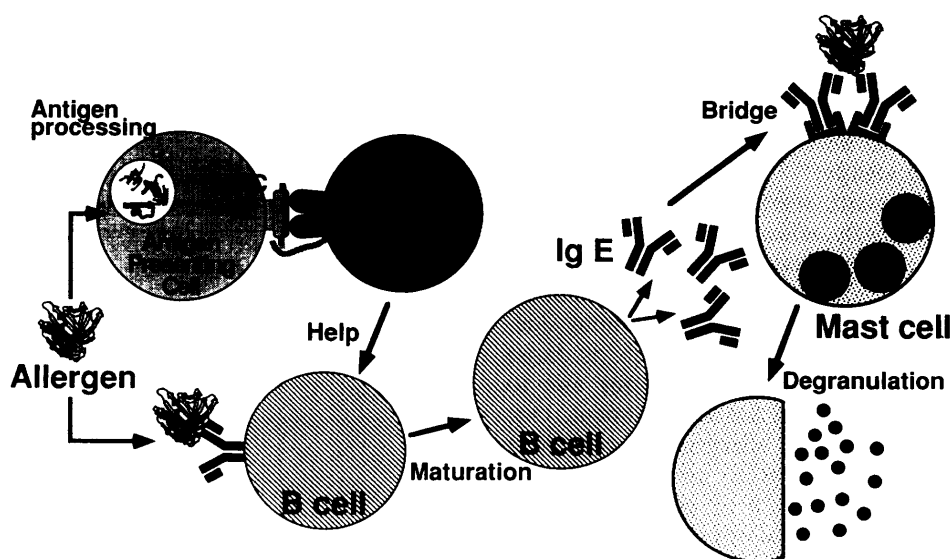


Fig. 2. Mechanism for Type I Allergy.

only CD4⁺ T cells are reactive to allergen were found in the case of mite, pollen, and egg allergic patients. Furthermore, we discovered that the antigenic determinants of α_{s1} -casein recognized by T cells from a milk allergic patient had a common alignment, Glu(X)₆-Lys. In addition, the epitopic site recognized by IgE was determined in the studies using synthetic peptides. These experiments showed that only a limited region of α_{s1} -casein (181–199) was recognized by specific IgE antibodies from allergic patients. In contrast, the determinants recognized by immunoglobulin G₁ (IgG₁) and IgG₄ were spread more extensively over the whole molecule of α_{s1} -casein. I speculate that the immunological mechanism of the production of IgE having a particular specificity is strongly related to induction of allergy.

T and B cell determinants of milk allergens

Allergens interact with four kinds of cell groups in the body, as mentioned above. Characterization of the key structural features of allergen molecules, in particular the sites involved in these interactions, is expected to provide information essential for understanding the cellular and molecular mechanisms of the allergic reaction. Thus, we have analyzed the T cell determinants of α_{s1} -casein^{9,10} and β -lactoglobulin, the major milk allergens, in the studies of mice.

The locations of the T cell determinants of α_{s1} -casein were found by a T cell proliferation assay, in which 13 synthetic overlapping peptides (19–20 amino acid residues) encompassing the entire sequence of the protein were tested for lymph node cells from three different strains of mice [BALB/c, C3H/He, and C57BL/6]. As shown in Fig. 3, it is clear that only limited regions of α_{s1} -casein were recognized by the T cells and the profiles of the T cell determinants differed among the three murine strains, each possessing a distinct type of MHC (H-2^d, H-2^k, and H-2^b). The number and the location of T cell determinants are various among these mice. Response to some determinants were strong (dominant) and those to others were weak (subdominant). The immunodominance of a T cell

determinant is mainly dependent upon the MHC haplotype of the mouse and may be explained by the reactivity of the peptide with MHC molecules, the availability of a specific T cell repertoire, and the antigen presentation pattern.

Also, the location of the T cell determinants of β -lactoglobulin have been analyzed in mice^{11,12} and this study is outlined later in this review.

B cell epitopes (determinants) are restricted areas of the antigen molecule which are recognized by the combining sites or paratopes of immunoglobulin molecules. It is generally accepted that B cell epitopes on protein molecules are composed of 15–22 amino acid residues. Analysis of protein antigens and antibody interactions has led to the identification of only a few residues as critical elements for binding to an antibody, while the entire surface of native globular protein molecules possibly contributes to induce antibody production. It has been postulated that molecular regions having large segmental mobility tend to be B cell epitopes.¹⁴

The locations of B cell epitopes on the α_{s1} -casein molecule were examined in studies of mice.^{14,15} Three different strains of mice (BALB/c, C3H/He, and C57BL/6) were immunized intraperitoneally with α_{s1} -casein, and the extent of binding of each peptide to IgG from these mice was measured by ELISA. We found that only a few regions of the protein molecule were recognized and that the response varied according to the strain of mice tested.

In studies of β -lactoglobulin, we have identified four regions containing B cell epitopes: 21–40, 41–60, 102–124, and 149–162.¹⁶ Three of these four regions overlap with the T cell determinants of β -lactoglobulin, and these sites are thought to play an important role in antibody production. Considering the three-dimensional structure of β -lactoglobulin, three of the four regions correspond to the structure of a turn or random coil between strands of the β -sheets, and these regions are known to be exposed on the outside surface of the folded protein molecule. These results are consistent with the antigenic structure of other globular proteins.^{17,18}

Determinant position and the immune response

The antigens (or allergens) processed in antigen presenting cells are presented to CD4⁺ T cells. These T cells, activated by the antigen-presenting cells, stimulate B cells to differentiate into antibody-producing cells. Lymphokines such as IL-2, IL-4, and IL-5, and adhesion molecules have been postulated to be functional molecules relevant to T cell help. Various relationships occur among the determinants recognized by interacting T and B cells such as: commonality of the T and B cell epitopes, negative and positive selection, intermolecular/intrastructural T cell help, and directional help.⁷

α_{s1} -Casein is a suitable protein antigen for use in analysis of the interaction between T and B cells, since the specificity of both the T cell response and the antibody response can be studied. An analysis of T and B cell responses to α_{s1} -casein in C3H/He mice has shown particular relationships between the T and B cell determinants.

We have investigated the ability of synthetic overlapping peptides of α_{s1} -casein to elicit a specific antibody response without the involvement of any carrier proteins. Synthetic peptides which contained both the T and B cell deter-

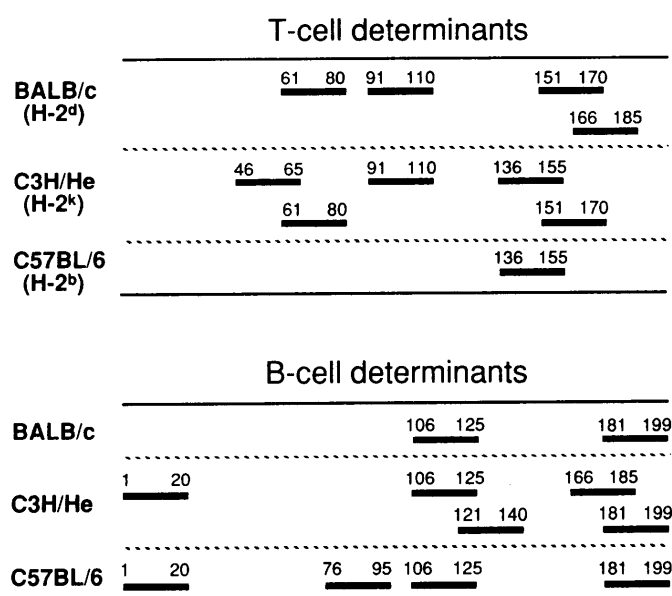


Fig. 3. T-Cell and B-Cell Epitopes of α_{s1} -Casein in Three Strains of Mice.

minants of α_s -casein gave rise to many peptide-native protein cross-reactive antibodies, while most of the peptides which failed to elicit an antibody response did not contain T cell determinants.^{9,10)} These results suggest that the contiguous presence of T and B cell determinants on an antigen may be beneficial or essential to induce an antibody response as has been observed in other antigen systems. Conversely, these observations imply that enzymatic digestion may lead to a reduction of allergenicity. Furthermore, in these studies of antigenic peptides derived from β -casein and β -lactoglobulin, we demonstrated that the distance between T and B cell determinants is important in elicitation of antibody production.^{19,20)}

Modulation of allergic reactions by manipulation of T cell determinants

One of the possible methods of reducing the allergenicity of food components is to digest antigenic protein with protease, which result in degraded antigenic determinant regions. Infant formulas²¹⁾ and rice²²⁾ manufactured by such methods are commercially available for allergic patients. However, these products may not always be able to prevent an allergic reaction and the removal of all high molecular weight proteins, including both allergenic and nonallergenic molecules, often leads to additional problems in food processing, taste, or nutritional efficiency. Therefore, alternative strategies are required to develop foods that can prevent both the onset of allergy and further hypersensitive responses.

Significant progress in our understanding of a cellular and molecular basis of T cell antigen recognition and activation has opened up research into T cell regulation. It is known that TCR on CD4⁺ T cells bind peptides generated from protein antigens or analog of these peptides in complexes with class II MHC molecules on antigen-presenting cells, and there are many different outcomes following TCR triggering. These include activation, clonal deletion, partial activation or functional inactivation (anergy). It is of potential therapeutic value to establish analog peptides and site-directed protein modifications that are effective to elicit the allergen specific T cell unresponsive state.

Focusing on these objectives, we have tried to develop a hypoallergenic protein by site-directed mutagenesis. At first, we analyzed the molecular details of the T cell determinant of β -lactoglobulin. The agretope was analyzed by a binding assay, using purified MHC ClassII (I-A^b) molecules and peptides of p119–133 and analog peptides in B6 mice. The results indicated that ¹²⁶Pro and ¹²⁸Val were the residues important for binding to this class II molecules.

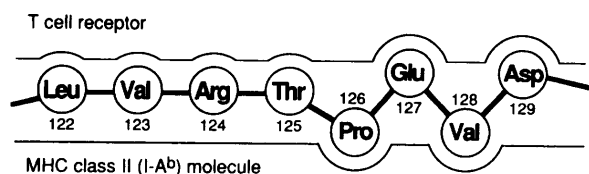


Fig. 4. T Cell Determinant and I-A^b Binding to p119–133 in β -Lactoglobulin.

The importance of the side-chains of each residue for binding to TCR or A^b molecules is indicated by the size of the arc.

The interactions among the peptide from β -lactoglobulin, the MHC class II molecule, and TCR are illustrated in Fig. 4.

Presumably, a T cell determinant can be destroyed by some alteration affecting an amino acid residue within the agretope, and protein engineering techniques such as site-directed mutagenesis should be useful to obtain detailed information about the relationship between the structure of β -lactoglobulin and its immunological function. Hence, using a yeast expression system, we used site-directed mutagenesis to further investigate its antigenic features. β -Lactoglobulin was directed to be expressed in *Saccharomyces cerevisiae* and secreted into the growth medium.²³⁾ By site-directed mutagenesis we obtained a mutant form of β -lactoglobulin altered at the anchor residue (¹²⁶Pro → ¹²⁶Ala). This mutant form of β -lactoglobulin showed much less immunogenicity than the wild-type protein, suggesting a new strategy for obtaining proteins with low immunogenicity.

It is generally accepted that T cell responses are inhibited by analogs of antigenic peptides with a single substitution at TCR contact residues.^{24–27)} These peptides are designated as TCR antagonists and are expected to provide a powerful tool to approach antigen-specific immunointervention in allergies and autoimmune diseases.

In term of the sites of interaction between region 119–133 of β -lactoglobulin and TCR (Fig. 4), we designed analog peptides and finally could identify TCR antagonist peptides for a panel of murine helper T cell clones specific for the 119–133 region. D129A, which is an analog of p119–133 with a single substitution of Ala for a TCR contact residue ¹²⁹Asp, showed an inhibitory effect on the proliferative responses of T cell clones. Therefore, D129A was identified as a TCR antagonist peptide. D 129A was also inhibitory for the immune responses to p119–133 *in vivo*. When T cell clones or lines are established from allergic patients, peptides potentially useful for therapeutic treatment of allergic reactions can be identified.

Application of a monoclonal antibody as a probe to analyze the conformational change in allergens

We have applied monoclonal antibodies directed against β -lactoglobulin to distinguish between different structural forms of this protein such as denatured, renatured, and recombinant forms, and mutant forms generated by site-directed mutagenesis.^{28–31)} The gene structure of the monoclonal antibodies was also analyzed.³²⁾

Furthermore, we have studied the interaction between monoclonal antibodies and β -casein.^{33,34)} These studies have successfully led to new information contributing to the overall view of antigen–antibody interactions.

Oral Tolerance

The mechanism of oral tolerance

The human immune system receives most of its external stimuli at mucosal surfaces, mainly from ingested food and bacteria, while ingested food rarely sensitizes lymphocytes. Antigens administered pergastrically typically induce a state of systemic immunological unresponsiveness called oral tolerance, whereas antigens administered parenterally induce an obvious immune response. As such, the oral

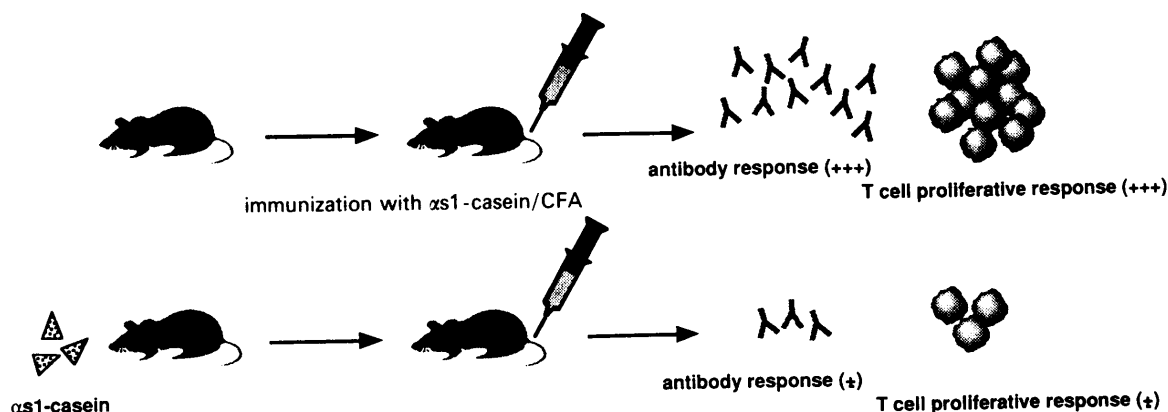


Fig. 5. The State of Oral Tolerance Can be Confirmed as Shown in This Figure.

tolerance is a form of antigen-driven peripheral immune tolerance.³⁵⁻³⁷⁾

The state of oral tolerance can be confirmed in experiments as shown in Fig. 5. Mice fed a control diet or a diet containing α_{s1} -casein, were immunized with α_{s1} -casein, together with adjuvant. Markedly reduced proliferation of T cells and lower levels of antibody production were observed in the case of mice fed the α_{s1} -casein containing diet, compared with control mice.

The remaining sections of this review focus on oral tolerance with special reference to i) identification of the lymphoid organs, tissues, and cells which participate in the induction of oral tolerance, ii) the cellular mechanism of immunological unresponsiveness in oral tolerance, iii) application of oral tolerance for the treatment of allergy and autoimmune disease.

Organs and tissues

GALT, which consist of lymphoid nodules termed Peyer's patches, intestinal epithelial cells, intraepithelial lymphocytes (IEL), and lymphocytes scattered throughout the lamina propria may play an important role in induction of oral tolerance. Whether other organs and tissues contribute in some manner to the induction of oral tolerance remains to be seen.

Cells

It is controversial which kinds of lymphocytes contribute to the induction of oral tolerance. In our studies for an attempt to identify the lymphocytes involved in oral tolerance,³⁸⁾ we have transferred lymphocyte subpopulations from orally tolerant mice to SCID and nude mice, which are fine recipients for lymphocyte transfer because these mice lack both T and B cells, and only T cells, respectively. The state of oral tolerance was successfully transferred principally with T cells. Furthermore, we have clearly demonstrated that the state of oral tolerance can be maintained principally by CD4⁺ T cells.

CD4⁺ T cells can be classified as subpopulations of two types (Th1 and Th2) based on their different patterns of secretion of cytokines. Th1 cells secrete IL-2 and IFN- γ , whereas Th2 cells produce IL-4, IL-5, IL-6, and IL-10. Th1 cells provide efficient help for the production of IgG2a, while Th2 cells provide help for production of IgG1 and IgE.

The selective inhibition of Th1 type responses in systemic

sites has been observed following the oral administration of antigen in C3H/He mice. Therefore, the inhibition of T cell proliferation and cytokine production by orally administered antigen was examined, focusing on the relevance of the Th1/Th2 response. Spleen cells from mice fed low doses (10 μ g–100 μ g/feeding) of α_{s1} -casein secreted IFN- γ and IL-2 but not IL-4, IL-10, or TGF- β . In the case of mice fed high doses (> 1 mg) of antigen, IFN- γ and IL-2 production was minimal. Stronger inhibition was observed for antigen-specific T cell proliferative responses and IgG2a antibody production but not for IgG1 responses, indicating that Th1 responses were preferentially inhibited.

Transgenic mice, expressing the T cell antigen receptor gene for ovalbumin, were orally administered ovalbumin and the T cell responses were examined.³⁹⁾ We also studied oral tolerance using transgenic mice and demonstrated that the production of IFN- γ and IL-2 was suppressed but an increase in production of IL-4 production was observed, suggesting that Th1 cells were preferentially tolerized.

Also, we have tried to discover the role of CD8⁺ T cells in the induction of oral tolerance using B6 mice. Most CD8⁺ T cells recognize endogenous antigens in the context of MHC class I molecules. These cells are involved in protection of the host against virus infections and play a role in graft-versus-host reactions. Recent findings indicate that some CD8⁺ T cells perform an important role in downregulation of the immune system.^{40,41)} Furthermore, it has been reported that the CD8⁺ T cell population contributes to oral tolerance⁴²⁻⁴⁴⁾; transfer of CD8⁺ T cells from orally tolerized mice induces immunological tolerance, and it has been shown that TGF- β produced by CD8⁺ T cells is responsible for oral tolerance in the rat system.

We have examined the effects of oral administration of α_{s1} -casein on the response of CD8⁺ T cells in mice. Administration of α_{s1} -casein abolished the proliferative responsiveness of purified CD4⁺ T cells, while the response of CD8⁺ T cells was similar to that observed in the case of control mice. However, CD8⁺ T cell clones from the tolerant mice produced considerable amounts of TGF- β , while those from control mice produced only small amounts. These results indicate the possibility that CD8⁺ T cells contribute to the induction of oral tolerance through active suppression as described below. It remains controversial, however, whether CD8⁺ T cells are necessary for the induction of oral tolerance, since oral tolerance can be

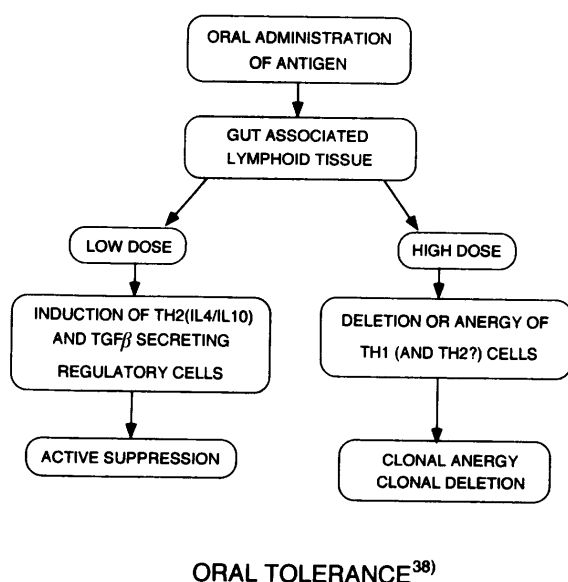


Fig. 6. Mechanisms of Oral Tolerance.

induced in mice depleted of CD8⁺ T cells.⁴⁵⁾ The other properties of CD8⁺ T cell clones related to this experiment were reported by our group.^{46–49)}

Cellular mechanisms

Three basic mechanisms are involved in antigen-driven tolerance, and these are clonal anergy, active suppression, and clonal deletion (Fig. 6). Anergy is defined as a state of T lymphocyte unresponsiveness characterized by the absence of proliferation, IL-2 production, and diminished expression of the IL-2 receptor.⁵⁰⁾ Active suppression is defined as a state of T lymphocyte unresponsiveness induced by the direct action of regulatory-cells secreting inhibitory factors such as TGF- β and IL-10, following antigen specific triggering.^{36,44)} Clonal deletion is a process in which cells are selectively destroyed *via* a mechanism such as apoptosis.⁵¹⁾

It has been postulated recently that oral tolerance is due to anergy-driven tolerance or active suppression (Fig. 6). An important factor determining a form of peripheral tolerance following oral administration of antigen is the dose of antigen fed (Fig. 6). Low doses of antigen favor the generation of active suppression, whereas high doses of antigen favor anergy-driven tolerance. However, these forms of oral tolerance are not mutually exclusive and may occur simultaneously.³⁶⁾

Recently, we demonstrated that the *ad libitum* uptake of casein diet generates oral tolerance due to anergy in mice.³⁸⁾ It was shown that CD4⁺ T cells from the tolerant mice inhibit the helper activity of intact CD4⁺ T cells. These results imply that anergy and active suppression are both principal mechanisms of oral tolerance under our experimental conditions.

Many studies of oral tolerance in murine autoimmune models have shown that active suppression is a primary mechanism.³⁶⁾ Such studies have led to the identification of regulatory cells generated in tolerant mice. The regulatory cells are CD8⁺ or CD4⁺ T cells and these act *via* the secretion of antigen non specific downregulatory cytokines of TGF- β following antigen specific triggering.

Structure and quantity of antigens

The relationships between the structure of antigens and the induction of oral tolerance have been investigated. We have examined the immune response to orally administered antigens in mice, comparing the effects of native and heat-denatured β -lactoglobulin.⁵²⁾ Milk whey protein contains β -lactoglobulin, which elicited a systemic humoral response when fed to mice as a constituent of the diet. The serum antibodies were of the IgG class, and these recognized mainly β -lactoglobulin. Feeding a native whey protein diet induced oral tolerance. Furthermore, when the animals were fed heat-denatured whey protein, the degree of oral tolerance induced was similar to that observed in the case of animals fed the native whey protein diet. In contrast, mice fed heat-denatured whey protein displayed lower levels of antibody production compared with those fed native whey protein without immunization. Thus, oral administration of denatured whey protein induced tolerance to native protein, but did not elicit antibody production.

The induction of oral tolerance by peptides was also investigated.⁵³⁾ Taking into consideration that orally administered protein antigens are subject to enzymatic degradation in the gastrointestinal tract, we examined whether an enzymatic digest of milk proteins could induce oral tolerance. A tryptic digest of casein, containing mainly peptide fragments smaller than 6000 kDa, was fed to mice as a constituent of their diet. Mice fed the casein-digest diet responded poorly to subsequent immunization with casein, indicating that oral tolerance to casein was induced in these animals. The results suggest the presence of immunosuppressive peptide fragments in the casein digest, which may be of use for preventing milk allergy.

As described previously, we have located the T cell determinants of α_{s1} -casein recognized in C3H/He mice.^{9,10)} We found that the intradermal administration of α_{s1} -casein induced profound immunological tolerance in the T cell and antibody responses.⁵⁴⁾ Therefore, we examined whether intradermal administration of a dominant peptide could induce immunological tolerance in the antibody response against the native whole protein.⁵⁴⁾

C3H/He mice were intradermally administered with the peptide corresponding to amino acid residues 91–110 of α_{s1} -casein, which includes the dominant T cell determinant, and subsequently immunized with α_{s1} -casein. Immunological tolerance in T cell response against α_{s1} -casein was successfully induced in these mice.

In terms of the above results we tried to induce oral tolerance by administration of peptides. Oral administration of a peptide comprising the dominant T cell determinant (91–110) to C3H/He mice successfully induced immunological tolerance. Miller *et al.* have reported that oral administration of a peptide containing the T cell determinant of myelin basic protein suppresses experimental autoimmune encephalomyelitis.⁵⁵⁾ Collagen-induced arthritis is also known to be suppressed by oral administration of a peptide derived from type II collagen.⁵⁶⁾

T and B cell determinants escaping from oral tolerance

We have shown that feeding bovine casein, a major milk allergen, as a constituent of the diet induces oral tolerance

in mice. However, there remains a question as to whether there are differences among T or B cell determinants in the degree of tolerance induced in this system. It seems important to identify determinants which elicit only weak oral tolerance because such determinants could potentially induce harmful food allergy. In a murine model of autoimmune disease, for example, there is evidence that T cells specific for certain determinants on self antigens are less susceptible to tolerance, suggesting that such T cells may play a role in autoimmunity.^{57,58)}

We have attempted to ascertain whether there are differences among T and B cell determinants of α_s1 -casein in the degree of tolerance induced in mice orally administered this antigen.⁵⁹⁾ Our experiments show that the degree of tolerance induced in response to different determinants in α_s1 -casein varied and that cellular and humoral responses to certain determinants were not inhibited in mice orally tolerant to α_s1 -casein. These results suggest that the activation of T and B cells recognizing certain determinants on the allergen molecule that are ineffective or weakly effective to elicit oral tolerance may be involved in the pathogenesis of food allergy.⁶⁰⁾

Oral tolerance as immunotherapy

It is believed that induction of oral tolerance can be applied to the treatment of allergic reactions as means of immunotherapy. Although a number of such studies have been reported, the efficacy of this immunotherapy is equivocal. Recently, promising results demonstrating a reduction of allergic symptoms by oral immunotherapy have been obtained in the case of house dust mite allergy.⁶¹⁾ It is reported that oral administration of a body extract of the mite in a tight capsule led to an improvement of clinical symptoms, and serum levels of IL-5 in these patients decreased significantly.⁶²⁾ Treatment by oral immunotherapy should be attempted in other cases of allergic disease.

Concluding Remarks

In this review, it has been described that food allergy is triggered by T cell and B cell response, and that the modification of allergen downregulate T cell and B cell response. We have also demonstrated that oral tolerance was due to an antigen-driven immunological unresponsive state induced by cellular functions and that the different states of oral tolerance can be induced by changing dose and molecular size of orally administered antigens. These experimental results lead us to the conclusion that the modification of allergens is a promising treatment for food allergies. Furthermore, it has been shown that oral tolerance is a potential immunotherapy for food allergy, other allergies, and other hypersensitivities such as autoimmune diseases.

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