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Review Article

Food allergens and mucosal immune systems with special reference to recognition of food allergens by gut-associated lymphoid tissue

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Abstract

Food allergy, triggered by an aberrant immune response elicited by orally ingested food allergens, is generated through a complicated mechanism because the allergen interacts with the mucosal immune system (the gut-associated lymphoid tissue, GALT) and the resulting immune response affects the generation of allergy. This review will describe the process by which antigens or allergens are recognized by the GALT and the characteristic immune responses induced thereafter. Orally administered antigens induce distinct immune responses in the Peyer's patches, lamina propria and the intestinal epithelium. In addition to these local immune responses in the gut, ingested antigens are known to affect systemic immunity. These may induce a suppressed state of systemic immune responsiveness, which is called oral tolerance, or in some cases they may elicit a systemic IgE antibody response which may lead to allergic reactions. Information on the regions on food allergens recognized by T cells and IgE antibodies is important in understanding the fates of food allergens after being recognized by the GALT. The structure of T and B cell epitopes on food allergens and the possibility of modulation of allergic reactions by amino-acid substituted analogs of allergen-derived peptides will also be discussed.

Key words: altered peptide ligands, epitope, food allergy, gut-associated lymphoid tissue (GALT), IgE, oral tolerance.

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Introduction

Food allergy, triggered by an aberrant immune response elicited by orally ingested food allergens, is generated through a complicated mechanism because the allergen interacts with the mucosal immune system and the resulting immune response affects the generation of allergy. The oral administration of antigen or allergen is known to induce a suppressed state of immune responsiveness, which is called oral tolerance. Thus, the same allergen can elicit both the generation and the suppression of allergy. It is unknown how these interesting immune reactions which function in opposing directions are controlled by the mucosal immune system. To understand these complicated immune responses, the process of recognition of food allergens and the resulting response by the mucosal immune system must be elucidated. Initially, this review will describe the process by which allergens are recognized by the mucosal immune system and thereafter, characteristic immune responses and oral tolerance will be described. The allergic reaction to food, usually a type I reaction, consists of plural processes such as immunoglobulin (Ig) E production, IgE binding to mast cells, and inflammation. However, many problems remain unresolved. For example, the regions on allergen molecules which are bound by IgE and T cells should be analyzed. In this review, the structure of the T and B cell epitopes on food allergens and the modulation of allergic reactions by amino-acid substituted analogs of allergen-derived peptides will be described.

Food allergens and the gut-associated lymphoid tissue

Many of the food allergens that commonly induce hypersensitivity to food in infants or adults have been identified. Egg white, milk, and soybean proteins are major food

allergens in the case of infants. In Japan, cereal proteins (i.e. proteins in rice, buckwheat and other grains), especially, are recognized as allergens as infants grow older. The components of these foods responsible for the disease are allergenic proteins. After being ingested in the gut, such allergenic proteins interact with the mucosal immune system of the gastrointestinal tract, namely the gut-associated lymphoid tissue (GALT). The GALT is composed of organized lymphoid tissues termed Peyer's patches (PP), the epithelium, lamina propria (LP) and the mesenteric lymph nodes (Fig. 1).[1] Peyer's patches contain T and B lymphocytes, macrophages and dendritic cells. The lamina propria also contains dendritic cells and scattered lymphocytes throughout including T cells and abundant plasma cells. The epithelium contains epithelial cells and T lymphocytes termed intraepithelial lymphocytes (IEL).

T cells play a central role in regulating immune responses. We will focus on the response of T cells to ingested antigen and the regulation of the immune response by them.

Response in Peyer's patches and lamina propria

T cells recognize antigenic peptide/major histocompatibility complex (MHC) complexes on the surface of antigen-presenting cells (APC). Thus, the first step in induction of the T cell response is antigen uptake and antigen presentation. It has been shown that microorganisms in the intestinal lumen are taken up by M cells which cover the PP and these are transferred to underlying macrophages and dendritic cells.[2] Although it is not clear whether soluble dietary proteins are taken up in a similar manner, it has been demonstrated that PP-derived dendritic cells can present antigens which they have acquired *in vivo* to stimulate T cells *in vitro*.[3] It has also been demonstrated that intestinal dendritic cells acquire orally administered antigen, the origin of which may be either the PP or LP.[4]

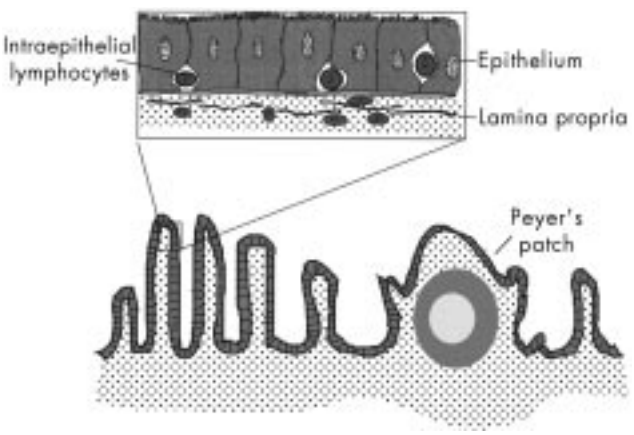

Fig. 1 A schematic illustration of the gut-associated lymphoid tissue.

Although T cell responses induced by oral administration of antigen in the PP or LP have been examined in many studies, most have focused on the response induced by oral administration of an antigen together with an adjuvant such as cholera toxin. In general, dominant T-helper (Th) 2 responses (secretion of interleukin (IL)-4, IL-5 and IL-6) have been observed.[5–7] Nevertheless, the secretion of Th1 cytokines such as IL-2 and interferon (IFN)-γ was observed in some cases.[6,7] In the case of administration without an adjuvant, Weiner and his colleagues have consistently shown that TGF-β-secreting cells are induced in the PP and in other sites in the GALT.[1,8,9] However, another study has shown that cells secreting IFN-γ were induced by oral administration of antigen.[10] In our own studies, we have examined T cell responses induced by oral administration of antigen using ovalbumin (OVA)-specific T cell receptor transgenic (TCR-Tg) mice.[11] Most T cells in these mice recognize OVA, enabling us to amplify and detect the T cell response to orally administered antigen. We found that cells which are capable of secreting high levels of IL-5 are induced in the PP of the OVA-fed TCR-Tg mice (S Kaminogawa *et al*., unpubl. data, 1998).

Although slightly different results have been obtained in different systems with regard to the responses in the PP or LP, it is likely that distinct antigen presentation or a distinct population of T cells is responsible for the distinct responses in these organs. With respect to this, Everson *et al*. have shown that dendritic cells in the PP preferentially induce Th2-type responses.[12] In contrast, we have found that APC populations from the PP preferentially induce a T cell population that secretes IL-2, and IFN-γ but not IL-4 or IL-10 (S Kaminogawa *et al*., unpubl. data, 1998). There are also reports which suggest that GALT-derived T cells have a distinct ability to produce cytokines. Saparov *et al*. have shown that LP T cells express IFN-γ or IL-10 in response to antigenic stimulation, while spleen and PP cells express predominantly IL-2 as determined by the *in situ* hybridization technique.[13] We have found that PP T cells from unsensitized TCR-Tg mice secrete higher levels of IL-5 compared to splenic T cells in response to identical antigenic stimulation (S Kaminogawa *et al*., unpubl. data, 1998). However, the molecular bases of the induction of the distinct responses in PP or LP remain unclear.

RESPONSE OF INTRAEPITHELIAL LYMPHOCYTES

Intraepithelial lymphocytes are a large population of T lymphocytes present in the epithelial layer of villi in the intestinal lumen. Intraepithelial lymphocytes have various unique characteristics in that firstly, many cells express the γδ-T cell receptor and secondly, many cells express the CD8αα homodimer.[14] These cells are also distinct in that they are of extrathymic origin. It has been unclear whether these cells recognize antigens present in the gut lumen. As for αβ-IEL, this population has been implicated in recognition of antigens present in intestinal microorganisms (or their products). This is based on previous reports which have shown that the number of αβ-IEL in germ-free mice is fewer than that in mice housed under conventional conditions.[15] However, it is likely that the expansion of αβ-IEL in response to the intestinal flora results from a mechanism of stimulation not involving MHC/peptide/TCR complexes, such as superantigenic stimulation targeted to the TCR or cytokines secreted from epithelial cells. Although the response of IEL to orally administered antigen has been demonstrated in only a few studies, there is evidence that this population does actually recognize luminal antigen. A relationship between the number of IEL and the oral administration of antigen has been shown for celiac disease[16] and for a food-sensitive enteropathy model established by cyclophosphamide treatment.[17] $CD4^+$ IEL in orally immunized animals have been shown to prevent parasitic infection.[18] $CD4^+$ IEL in mice orally primed with sheep red blood cells (SRBC), secreting Th2 cytokines, were found to be capable of providing a helper function for antigen-specific B cell responses.[19] We have found that feeding OVA to the above mentioned TCR-Tg mice resulted in an increased frequency and an increased proliferative capacity of $CD4^+$ αβ-IEL (M Goto *et al*., unpubl. data, 1998). These IEL secreted primary IFN-γ in response to antigenic stimulation. Intestinal epithelial cells (IEC) express class II molecules, and can present antigens to T cells *in vitro*.[20] Thus, orally administered antigen may be presented by IEC in our system.

REGULATION OF LOCAL RESPONSES IN THE GUT-ASSOCIATED LYMPHOID TISSUE

The above mentioned GALT-derived T cells regulate immune responses to ingested antigen. The most important of these is the induction of a secretory IgA response. IgA not only prevents the invasion of pathogens but also may prevent uptake of allergens.[21] IgA production is enhanced by cytokines such as TGF-β, IL-5 and IL-6.[22] As discussed, these cytokines have been shown to be secreted by GALT-derived T cells. Much less is known about the regulation of other reactions by T cells in response to ingested antigen. IL-5 is known to activate eosinophils,[23] and IFN-γ may mediate inflammatory responses or act on IEC to upregulate their class II molecules[24] or open tight junctions.[25] Such reactions may possibly be involved in food allergy.

ORAL TOLERANCE

In addition to inducing immune responses in the gut, the oral administration of antigen may affect systemic immune responses. It has long been recognized in experimental animals that oral administration of antigen induces hyporesponsiveness of systemic immunity.[1,26] Recently, this has also been described in human studies.[27]

We, as well as others, have clearly demonstrated that the state of oral tolerance is maintained principally by $CD4^+$ T cells.[28,29] In some systems it has been reported that the $CD8^+$ T cell population also contributes to oral tolerance.[30] In such cases, $CD8^+$ T cells which recognize exogenous antigen[31] are involved.

Three basic mechanisms are involved in oral tolerance: clonal anergy, active suppression, and clonal deletion (Fig. 2). Anergy is defined as a state of T lymphocyte unresponsiveness characterized by the absence of proliferation or IL-2 production.[32] Recently, it has been shown that the induction of anergy is mediated by signals through CTLA-4.[33] Active suppression is defined as a state of inhibition of immune responses by regulatory T cells secreting

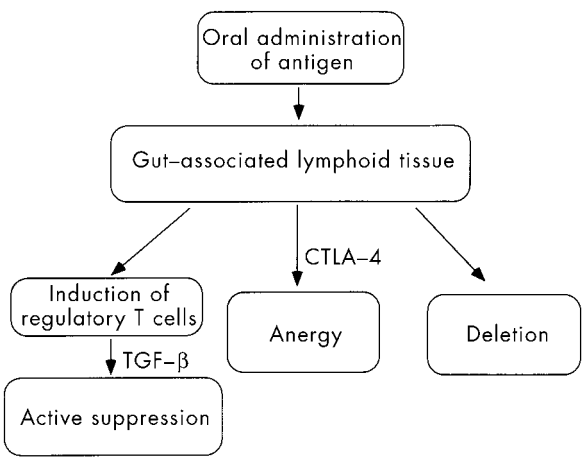

Fig. 2 The mechanisms of oral tolerance.

inhibitory factors such as TGF-β, following antigen specific triggering.[1,9] Clonal deletion is a process in which cells are selectively destroyed via apoptosis.[34] These forms of oral tolerance are not mutually exclusive and may occur simultaneously. All three mechanisms have been demonstrated clearly using TCR-transgenic mice.[34–36] However, much remains to be elucidated concerning the cellular and molecular processes involved in these phenomena. One important issue is the site of induction of oral tolerance. The regulatory T cells mediating active suppression have been shown to be induced in the GALT[8,9] and deletion of antigen-reactive T cells after oral administration of antigen has been demonstrated in the PP.[34] However, these observations do not exclude the possibility that these events may occur in other sites. It is known that orally administered antigen enters the circulation and a recent study has clearly demonstrated systemic presentation of orally administered antigen,[37] suggesting a role for organs other than the GALT in the induction of oral tolerance.

There are several other important features of oral tolerance to note. First, the dose of antigen seems to be an important factor in determining which form of tolerance is induced. Low doses of antigen favor the generation of active suppression, whereas high doses of antigen favor anergy or deletion.[38] Second, it has been demonstrated that Th1 responses are more susceptible to tolerance induction than Th2 responses.[39,40] Nevertheless, Th2 responses, including IgE antibody responses are inhibited upon induction of oral tolerance under appropriate conditions.[40–42]

IgE response induced by oral administration of antigen

Oral administration of antigen induces oral tolerance in systemic immunity and thus, administration by the oral route is usually inefficient in inducing a systemic immune response. However, it has been demonstrated that, in some cases, a systemic antibody response is elicited in response to ingested antigen. Administration of antigen with adjuvant, or administration of components of micro-organisms, efficiently induces such a response. In addition, feeding a protein antigen without any adjuvant may also induce a systemic response, albeit a weak response. For example, feeding a large amount of antigen as a constituent of the diet constantly for long periods induces a systemic IgG response[43,44] and in some cases even an IgE response.[45] Many patients allergic to food display circulating IgE antibodies. Thus, clarifying the mechanism underlying the induction of IgE antibodies to ingested antigen is important in order to understand the etiology of food allergy. However, at present this process is poorly understood. This has mostly been due to a lack of experimental models in which IgE antibodies are elicited by ingested antigen. Recently, Ito *et al*. have shown that oral administration of milk casein as a constituent of the diet to DBA/2 mice elicited a serum IgE response with a slightly enhanced Th2 response in the liver, mesenteric lymph nodes, and spleen.[45] We found that feeding OVA as a constituent of the diet to OVA-specific TCR-Tg mice elicited a strong serum IgE response. OVA-specific IgE antibodies were detected 2 weeks after feeding, and the IgE titer became elevated to levels comparable to those induced by immunization with OVA + alum after 4 weeks of feeding (S Kaminogawa *et al*., unpubl. data, 1998).

It has been hypothesized that oral tolerance is a mechanism preventing food allergy in healthy individuals.[46] Thus, induction of a systemic antibody response by ingested antigen may be considered to be due to a breakdown of tolerance or insufficient induction of tolerance. However, the relationship between induction of oral tolerance and the development of an antibody response to orally administered antigen remains unclear. In our system using Tg mice, splenic T cells from ovalbumin (OVA)-fed mice with a high IgE titer showed a diminished cytokine response to antigenic stimulation, which is indicative of T cell tolerance. However, we found that 1 week after the start of feeding, T cells capable of secreting large amounts of IL-4 were induced in the spleen (S Kaminogawa *et al*., unpubl. data, 1998). The results suggested that an aberrant response in the induction phase of T cell tolerance resulted in the development of an antibody response. Further studies are required to clarify whether the IgE response to an ingested allergen and food allergy are related to a breakdown of oral tolerance.

The T- and B-cell epitopes recognized on food allergens

The review will now deal with the structure of T- and B-cell epitopes on food allergens. Such information will contribute to the understanding of the fates of food allergens after being recognized by the GALT. It is useful to know whether the GALT has a characteristic antigen recognition system.

The primary structures of several allergenic proteins have been determined and these allergens are named according to the recommendations of the International Union of Immunological Societies (Table 1). It is generally

Table 1. Major allergens in foods

Food allergen	Allergen name*	Mass (kDa)**
Chicken egg	*Gal d*1 (ovalbumin)	45
	*Gal d*2 (ovomucoid)	28
Cow's milk	α_{S1}-casein	23
	β-lactoglobulin	18.4
Soybean	*Gly m*1A, *Gly m*1B	8
	*Gly m*Bd (7S globulin)	30
Peanut	*Ara h*1	63.5
	*Ara h*2	17.5
Brazil nut	*Ber e*1 (2S albumin)	16–16.4
Yellow mustard	*Sin a*1 (2S albumin)	15
Oriental mustard	*Bra j*1 (2S albumin)	16
Codfish	*Gad c*1	13
Shrimp	*Pen a*1 (tropomyosin)	36
	*Met e*1 (tropomyosin)	34
	*Par f*1	39
Apple	*Mal d*1	17.7

*According to the recommendations of the IUIS Subcommittee for Allergen Nomenclature. **Molecular mass of the allergen.

considered that there are common structural characteristics among the allergenic proteins in food (i.e. firstly, the molecular mass is between 10 and 100 kDa and secondly, these proteins show high stability to heat or enzyme treatment). The structural features of OVA and α_{S1}-casein have been fully clarified;[47–50] there have been several reports on the determinant regions within their amino-acid sequences recognized by T cells or B cells (IgE or IgG) and a few reports on the functions of specific T cells isolated.[51–60] With regard to the peanut allergenic protein *Ara h*2, the allergenic structure recognized by specific IgE has been investigated[61] and T cell clones specific for crude peanut extract have been established.[62] As for almost all of the other allergenic proteins, only the binding abilities of specific-IgE to the proteins have been analyzed. In addition, there have been several reports of analysis performed demonstrating the cross-reactivity between allergenic proteins in various foods, and between food allergens and allergens in items other than food.[63–65] However, there have been only a few reports on the amino acid sequences functioning as binding regions recognized by specific-IgE and on the isolation and characterization of specific-T cell lines.[66] Thus, although many food allergens have been identified to date, the intention of this review is mainly to introduce findings concerning OVA and α_{S1}-casein and the determinants on these two proteins recognized by IgE and T cells in detail.

Ovalbumin is the major component (54%) of egg white protein and is considered to be one of the most important allergens in egg white. This protein is a water-soluble glycoprotein composed of 386 amino acid residues with a molecular mass of 45 kDa.[48,49] The molecular structure of OVA has been defined by X-ray crystallography.[50] Based on this structural information, it has been determined that amino acid residues 1–11 of OVA (OVA1–11),[52] OVA323–339,[53] OVA34–46, OVA47–55,[54] and OVA41–172, OVA301–385[55] are the regions recognized by OVA-specific IgE from allergic patients. Moreover, Honma *et al*. demonstrated that OVA peptides have the ability to induce histamine release from basophils in patients with egg allergy.[56] There have been a few reports concerning the establishment of OVA-specific T cell lines from such patients.[57,58] Katsuki *et al*. studied the determinants in detail using a panel of 187 overlapping 13 mer peptides and determined their dominant restriction elements.[58] These T cell lines were all Th2 type cells and such cells may play a crucial role in the process of allergic inflammation including IgE production.[58]

α_{S1}-Casein is known to be one of the most significant allergens in cow's milk. It consists of 199 amino acid residues and it has a flexible and linear conformation rather than a rigid and compact one.[47] These characteristic structural features of α_{S1}-casein are advantageous for epitope analysis using overlapping peptides. We have analyzed the determinants recognized by IgE, IgG_4 and T cell lines specific for α_{S1}-casein, using synthetic overlapping peptides and natural fragments of this protein.[60] In these analyses, a commonality of epitopes in the C-terminal region of α_{S1}-casein recognized by the IgE from each of 9 patients with cow's milk allergy was found. The determinants recognized by IgE were included among those recognized by IgG4, widely distributed on α_{S1}-casein. In addition, amino acid sequences common to the determinants on α_{S1}-casein recognized by Th2 type T cell lines from each of 3 patients were found. Spuergin *et al*. detected three IgE and IgG binding regions within the amino acid sequence of α_{S1}-casein.[59]

From these results of determinant analysis and studies concerning cross-reactivity among food allergens, it seems possible that IgE which interacts with only a limited region on allergen proteins may be synthesized in cases of food allergy and that this region may contain common epitopes for IgE from various individual food allergic patients. If so, studies clarifying the structure of allergens may allow us to understand the etiology of allergen-specific manifestations of food allergies. Concerning this, it is necessary to analyze whether the unique T and B cell epitope patterns in the food allergic patients

already described are influenced by the digestion in the gut.

MODULATION OF ALLERGIC REACTIONS BY AMINO ACID-SUBSTITUTED ANALOGS OF FOOD ALLERGEN-DERIVED PEPTIDES

Recent studies have revealed that T cells show altered responses to amino acid-substituted analogs of a specific peptide antigen.[67,68] Some analog peptides called partial agonists induce partial activation of T cells: for example, cytokine production without proliferation,[69] alteration of the cytokine production pattern,[70,71] and induction of anergy,[72,73] due to partial signaling. Another group of analog peptides can inhibit T cell responses to the parent peptide in an antigen-specific manner and these are designated TCR antagonists.[74] These analog peptides capable of altering T cell responses are collectively called altered peptide ligands. These are expected to be effective tools for achieving antigen-specific modulation of immune responses in treatment of allergies, including food allergy, and autoimmune diseases.[26,75,76]

In allergic patients, Th2-type responses which promote production of IgE and activation of eosinophils seem to be dominant. This is because most of the T cell clones specific for an allergen established from several allergic patients show high levels of IL-4 and IL-5 production.[77–80] Therefore, administration of analog peptides preferentially inducing production of IFN-γ,[81] which is a potent inhibitor of Th2-type responses, would be effective for inhibiting the onset of allergy or remitting the symptoms. We have identified an analog of a peptide derived from α_{s1}-casein, which significantly enhances IFN-γ production from specific $CD8^+$ T cells *in vivo* in a mouse model.[82] From patients allergic to α_{s1}-casein, $CD8^+$ T cell lines specific for α_{s1}-casein have been established in high frequency.[83] Given that these $CD8^+$ T cells play a key role in suppressing the allergic reactions, an analog peptide with enhanced ability to induce IFN-γ production from $CD8^+$ T cells may be useful for the treatment of allergic patients.

Several reports have shown inhibitory effects of TCR antagonist peptides *in vivo* on the onset of T cell-mediated experimental autoimmune diseases, such as experimental allergic encephalomyelitis[84–87] and experimental allergic uveitis.[88] In antibody-mediated immune diseases, such as type I allergy, inhibition of pathogenic antibody production as well as inhibition of T cell responses is crucial to ameliorate the disease. We have examined the effect of a TCR antagonist peptide on specific-antibody production *in vivo* in a mouse model. A TCR antagonist peptide, an analog of a peptide derived from β-lactoglobulin, which is a major cow's milk allergen, efficiently inhibited the production of antibodies, including IgE, specific for the β-lactoglobulin-peptide, when the TCR antagonist was co-administered with the parent peptide at the time of the first immunization (S Kaminogawa, unpubl. data, 1998). Our results suggest that TCR antagonists may be highly effective as preventive inhibitors of pathogenic-antibody production in food allergic patients.

CONCLUSION

It was our purpose to describe how ingested food allergens up-regulate IgA and IgE production and down-regulate peripheral immune responses (oral tolerance) through the mucosal immune system. How food allergens are bound by IgE and T cell receptors and the site of IgE production are other important issues which have yet to be clearly described. Thus, we believe that the elucidation of these aspects will lead to a fuller understanding of the mechanism of generation of food allergy and other immunologically mediated diseases such as Crohn's disease. However, there are many important problems not mentioned in this review. For example, how immunologic interactions between the GALT and intestinal bacteria affect the mucosal and systemic peripheral immune responses. We hope to have the opportunity to understand and describe these issues in the future.

REFERENCES

1 Weiner HL, Friedman A, Miller A *et al.* Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol.* 1994; 12: 809–37.
2 Neutra MR, Pringault E, Kraehenbuhl J-P. Antigen sampling across epithelial barriers and induction of mucosal immune responses. *Annu. Rev. Immunol.* 1996; 14: 275–300.
3 Kelsall BL, Strober W. Distinct populations of dendritic cells are present in the subepithelial dome and T cell regions of the murine Peyer's patch. *J. Exp. Med.* 1996; 183: 237–47.
4 Liu LM, MacPherson GG. Antigen acquisition by dendritic cells: Intestinal dendritic cells acquire antigen administered orally and can prime naive T cells in vivo. *J. Exp. Med.* 1993; 177: 1299–307.
5 Xu-Amano J, Kiyono H, Jackson RJ *et al.* Helper T cell subsets for immunoglobulin A responses: Oral immunization with tetanus toxoid and cholera toxin as adjuvant

selectively induces Th2 cells in mucosa associated tissues. *J. Exp. Med.* 1993; 178: 1309–20.
6 Wilson AD, Bailey M, Williams NA, Stokes CR. The *in vitro* production of cytokines by mucosal lymphocytes immunized by oral administration of keyhole limpet hemocyanin using cholera toxin as an adjuvant. *Eur. J. Immunol.* 1991; 21: 2333–9.
7 Clarke CJ, Wilson AD, Williams NA, Stokes CR. Mucosal priming of T-lymphocyte responses to fed protein antigens using cholera toxin as an adjuvant. *Immunology* 1991; 72: 323–8.
8 Santos LM, al SA, Londono A, Weiner HL. Oral tolerance to myelin basic protein induces regulatory TGF-β-secreting T cells in Peyer's patches of SJL mice. *Cell. Immunol.* 1994; 157: 439–47.
9 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994; 265: 1237–40.
10 Hoyne GF, Callow MG, Kuhlman J, Thomas WR. T-cell lymphokine response to orally administered proteins during priming and unresponsiveness. *Immunology* 1993; 78: 534–40.
11 Sato T, Sasahara T, Nakamura Y *et al.* Naive T cells can mediate delayed-type hypersensitivity response in T cell receptor transgenic mice. *Eur. J. Immunol.* 1994; 24: 1512–6.
12 Everson MP, Lemak DG, McDuffie DS, Koopman WJ, McGhee JR, Beagley KW. Dendritic cells from Peyer's patch and spleen induce different T helper cell responses. *J. Interferon Cytokine Res.* 1998; 18: 103–15.
13 Saparov A, Elson CO, Devore-Carter D, Bucy RP, Weaver CT. Single-cell analyses of $CD4^+$ T cells from αβ T cell receptor-transgenic mice: A distinct mucosal cytokine phenotype in the absence of transgene-specific antigen. *Eur. J. Immunol.* 1997; 27: 1774–81.
14 Guy-Grand D, Vassalli P. Gut intraepithelial T lymphocytes. *Curr. Opin. Immunol.* 1993; 5: 247–52.
15 Bandeira A, Mota ST, Itohara S *et al.* Localization of γδ T cells to the intestinal epithelium is independent of normal microbial colonization. *J. Exp. Med.* 1990; 172: 239–44.
16 Halstensen TS, Brandtzaeg P. Activated T lymphocytes in the celiac lesion: Non-proliferative activation (CD25) of $CD4^+$ α/β cells in the lamina propria but proliferation (Ki-67) of α/β and γ/δ cells in the epithelium. *Eur. J. Immunol.* 1993; 23: 505–10.
17 Ohtsuka Y, Yamashiro Y, Maeda M *et al.* Food antigen activates intraepithelial and lamina propria lymphocytes in food-sensitive enteropathy in mice. *Pediatr. Res.* 1996; 39: 862–6.
18 McDonald V, Robinson HA, Kelly JP, Bancroft GJ. Immunity to *Cryptosporidium muris* infection in mice is expressed through gut $CD4^+$ intraepithelial lymphocytes. *Infect. Immun.* 1996; 64: 2556–62.
19 Fujihashi K, Yamamoto M, McGhee JR, Kiyono H. αβ T cell receptor-positive intraepithelial lymphocytes with $CD4^+$, $CD8^-$ and $CD4^+$, $CD8^+$ phenotypes from orally immunized mice provide Th2-like function for B cell responses. *J. Immunol.* 1993; 151: 6681–91.
20 Bland PW, Warren LG. Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. *Immunology* 1986; 58: 1–7.
21 Swarbrick ET, Stokes CR, Soothill JF. Absorption of antigens after oral immunisation and the simultaneous induction of specific systemic tolerance. *Gut* 1979; 20: 121–5.
22 Ramsay AJ, Bao S, Beagley KW *et al.* Cytokine gene knockout mice – lessons for mucosal B-cell development. In: Kagnoff MF, Kiyono H (eds). *Essentials of Mucosal Immunology*. San Diego: Academic Press, 1996; 247–61.
23 Takatsu K, Tominaga A, Harada N *et al.* T cell-replacing factor (TRF) /interleukin 5 (IL-5): Molecular and functional properties. *Immunol. Rev.* 1988; 102: 107–35.
24 Kohyama M, Hachimura S, Nanno M, Ishikawa H, Kaminogawa S. Analysis of cytokine producing activity of intestinal intraepithelial T cells from TCR β-chain and δ-chain mutant mice. *Microbiol. Immunol.* 1997; 41: 353–9.
25 Adams RB, Planchon SM, Roche JK. IFN-γ modulation of epithelial barrier function. Time course, reversibility, and site of cytokine binding. *J. Immunol.* 1993; 150: 2356–63.
26 Kaminogawa S. Food allergy, oral tolerance and immunomodulation – their molecular and cellular mechanisms. *Biosci. Biotech. Biochem.* 1996; 60: 1749–56.
27 Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J. Immunol.* 1994; 152: 4663–70.
28 Hirahara K, Hisatsune T, Nishijima K, Kato H, Shiho O, Kaminogawa S. $CD4^+$ T cells anergized by high dose feeding establish oral tolerance to antibody responses when transferred in SCID and nude mice. *J. Immunol.* 1995; 154: 6238–45.
29 Garside P, Steel M, Liew FY, Mowat AM. $CD4^+$ but not $CD8^+$ T cells are required for the induction of oral tolerance. *Int. Immunol.* 1995; 7: 501–4.
30 Chen Y, Inobe J, Weiner HL. Induction of oral tolerance to myelin basic protein in CD8-depleted mice: Both $CD4^+$ and $CD8^+$ cells mediate active suppression. *J. Immunol.* 1995; 155: 910–6.
31 Hisatsune T, Nishijima K, Kohyama M, Kato H, Kaminogawa S. $CD8^+$ T cells specific to the exogenous antigen. Mode of antigen recognition and possible implication in immunosuppression. *J. Immunol.* 1995; 154: 88–96.
32 Schwartz RH. Costimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 1992; 71: 1065–8.
33 Samoilova EB, Horton JL, Zhang HD, Khoury SJ, Weiner HL, Chen YH. CTLA-4 is required for the induction of high dose oral tolerance. *Int. Immunol.* 1998; 10: 491–8.
34 Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995; 376: 177–80.
35 Chen Y, Inobe J, Kuchroo VK, Baron JL, Janeway CJ, Weiner HL. Oral tolerance in myelin basic protein T-cell

receptor transgenic mice: Suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. *Proc. Natl Acad. Sci. USA* 1996; 93: 388–91.
36 Van Houten N, Blake SF. Direct measurement of anergy of antigen-specific T cells following oral tolerance induction. *J. Immunol.* 1996; 157: 1337–41.
37 Gutgemann I, Fahrer AM, Altman JD, Davis MM, Chien YH. Induction of rapid T cell activation and tolerance by systemic presentation of an orally administered antigen. *Immunity* 1998; 8: 667–73.
38 Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc. Natl Acad. Sci. USA* 1994; 91: 6688–92.
39 Melamed D, Friedman A. *In vivo* tolerization of Th1 lymphocytes following a single feeding with ovalbumin: Anergy in the absence of suppression. *Eur. J. Immunol.* 1994; 24: 1974–81.
40 Yoshida T, Hachimura S, Kaminogawa S. The oral administration of low-dose antigen induces activation followed by tolerization, while high-dose antigen induces tolerance without activation. *Clin. Immunol. Immunopathol.* 1997; 82: 207–15.
41 Garside P, Steel M, Worthey EA *et al.* T helper 2 cells are subject to high dose oral tolerance and are not essential for its induction. *J. Immunol.* 1995; 154: 5649–55.
42 Van Halteren AG, van der Cammen MJ, Cooper D, Savelkoul HF, Kraal G, Holt PG. Regulation of antigen-specific IgE, IgG1, and mast cell responses to ingested allergen by mucosal tolerance induction. *J. Immunol.* 1997; 159: 3009–15.
43 Kim SM, Enomoto A, Hachimura S, Yamauchi K, Kaminogawa S. Serum antibody response elicited by a casein diet is directed to only limited determinants of α_{S1}-casein. *Int. Arch. Allergy Immunol.* 1993; 101: 260–5.
44 Enomoto A, Konishi M, Hachimura S, Kaminogawa S. Milk whey protein fed as a constituent of the diet induced both oral tolerance and a systemic humoral response, while heat-denatured whey protein induced only oral tolerance. *Clin. Immunol. Immunopathol.* 1993; 66: 136–42.
45 Ito K, Inagaki-Ohara K, Murosaki S *et al.* Murine model of IgE production with a predominant Th2-response by feeding protein antigen without adjuvants. *Eur. J. Immunol.* 1997; 27: 3427–37.
46 Mowat AM. The regulation of immune responses against dietary protein antigens. *Immunol. Today* 1987; 8: 93–8.
47 Kumosinski TF, Brown EM, Farrel HM Jr Three-dimensional molecular modeling of bovine caseins: α_{S1}-casein. *J. Dairy Sci.* 1991; 74: 2889–95.
48 McReynolds L, O'Malley BW, Nisbet AD *et al.* Sequence of chicken ovalbumin mRNA. *Nature* 1978; 273: 723–8.
49 Nisbet A, Saundry RH, Moir AJG, Fothergill LA, Fothergill JE. The complete amino-acid sequence of hen ovalbumin. *Eur J. Biochem.* 1981; 115: 335–45.
50 Stein PE, Leslie AGW, Finch JT, Turnell WG, McLaughlin PJ, Carrell RW. Crystal structure of ovalbumin as a model for the reactive centre of serpins. *Nature* 1990; 347: 99–102.
51 Elsayed S, Hammer AS, Kalvenes MB, Florvaag E, Apold J, Vik H. Antigenic and allergenic determinant of ovalbumin. I. Peptide mapping, cleavage at the methionyl peptide bonds and enzymic hydrolysis of native and carboxymethyl OA. *Int. Arch. Allergy Appl. Immunol.* 1986; 79: 101–7.
52 Elsayed S, Holen E, Haugstad MB. Antigenic and allergenic determinant of ovalbumin. II. The reactivity of the NH2 terminal decapeptide. *Scand. J. Immunol.* 1988; 27: 87–91.
53 Johnsen G, Elsayed S. Antigenic and allergenic determinant of ovalbumin. III. MHC Ia-binding peptide (OA323–339) interacts with human and rabbit specific antibodies. *Mol. Immunol.* 1990; 27: 821–7.
54 Elsayed S, Stavseng L. Epitope mapping of region 11–70 of ovalbumin (*Gal d*1) using five synthetic peptides. *Int. Arch. Allergy Immunol.* 1994; 104: 65–71.
55 Kahlert H, Petersen A, Becker WM, Schlaak M. Epitope analysis of the allergen ovalbumin (*Gal d*II) with monoclonal antibodies and patient's IgE. *Mol. Immunol.* 1992; 29: 1191–201.
56 Honma K, Kohno Y, Saito K *et al*. Allergenic epitopes of ovalbumin (OVA) in patients with hen's egg allergy: Inhibition of basophil histamine release by haptenic ovalbumin peptide. *Clin. Exp. Immunol.* 1996; 103: 446–53.
57 Holen E, Elsayed S. Specific T cell lines for ovalbumin, ovomucoid, lysozyme and two OA synthetic epitopes, generated from egg allergic patients' PBMC. *Clin. Exp. Allergy* 1996; 26: 1080–8.
58 Katsuki T, Shimojo N, Honma K, Tsunoo H, Kohno Y, Niimi H. Establishment and characterization of ovalbumin-specific T cell lines from patients with egg allergy. *Int. Arch. Allergy Immunol.* 1996; 109: 344–51.
59 Suergin P, Mueller H, Walter M, Schiltz E, Forster J. Allergenic epitopes of bovine α_{S1}-casein recognized by human IgE and IgG. *Allergy* 1996; 51: 306–12.
60 Nakajima-Adachi H, Hachimura S, Ise W *et al.* Determinant analysis of IgE and IgG4 antibodies and T cells specific for bovine α_{S1}-casein from the same patients allergic to cow's milk: Existence of α_{S1}-casein-specific B cells and T cells characteristic in cow's milk allergy. *J. Allergy Clin. Immunol.* 1998; 101: 660–71.
61 Stanley JS, King N, Burks AW *et al.* Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen *Ara h*2. *Arch. Biochem. Biophys.* 1997; 342: 244–53.
62 De Jong EC, Spanhaak S, Martens BP, Kapsenberg ML, Penninks AH, Wierenga EA. Food allergen (peanut)-specific TH2 clones generated from the peripheral blood of a patient with peanut allergy. *J. Allergy Clin. Immunol.* 1996; 98: 73–81.
63 Ebner C, Hirschwehr R, Bauer L *et al.* Identification of allergens in apple, pear, celery, carrot and potato: Cross-reactivity with pollen allergens. *Monographs Allergy* 1996; 32: 73–7.
64 Reese G, Jeoung BJ, Daul CB, Lehrer SB. Characterization of recombinant shrimp allergen Pen a1 (tropomyosin). *Int. Arch. Allergy Immunol.* 1997; 113: 240–2.

65 Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M *et al.* Cloning and sequencing of *Mal d* 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to *Bet v* 1, the major birch pollen allergen. *Biochem. Biophys. Res. Commun.* 1995; 214: 538–51.
66 Ball G, Shelton MJ, Walsh BJ, Hill DJ, Hosking CS, Howden MEH. A major continuous allergenic epitope of bovine β-lactoglobulin recognized by human IgE binding. *Clin. Exp. Allergy* 1994; 24: 758–64.
67 Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: Molecular mechanisms and role in T cell biology. *Annu. Rev. Immunol.* 1996; 14: 1–27.
68 Sette A, Alexander J, Ruppert J *et al.* Antigen analogs/MHC complexes as specific T cell receptor antagonists. *Annu. Rev. Immunol.* 1994; 12: 413–31.
69 Evavold BD, Allen PM. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science* 1991; 252: 1308–10.
70 Windhagen A, Scholz C, Höllsberg P, Fukaura H, Sette A, Hafler DA. Modulation of cytokine patterns of human autoreactive T cell clones by a single amino acid substitution of their peptide ligand. *Immunity* 1995; 2: 373–80.
71 Kohyama M, Kakehi M, Totsuka M, Hachimura S, Hisatsune T, Kaminogawa S. Selective induction of $CD8^+$ T cell functions by single substituted analogs of an antigenic peptide: Distinct signals for IL-10 production. *FEBS Lett.* 1998; 423: 138–42.
72 Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature* 1993; 363: 156–9.
73 Sloan-Lancaster J, Evavold BD, Allen PM. Th2 cell clonal anergy as a consequence of partial activation. *J. Exp. Med.* 1994; 180: 1195–205.
74 De Magistris M, Alexander J, Coggeshall M *et al.* Antigen analog-major histocompatibility complexes act as antagonists of the T cell receptor. *Cell* 1992; 68: 625–34.
75 Fairchild PJ, Thorpe CJ, Travers PJ, Wraith DC. Modulation of the immune response with T-cell epitopes: The ultimate goal for specific immunotherapy of autoimmune disease. *Immunology* 1994; 81: 487–96.
76 Hoyne GH, Kristensen NM, Yssel H, Lamb JR. Peptide modulation of allergen-specific immune responses. *Curr. Opin. Immunol.* 1995; 7: 757–61.
77 Yssel H, Johnson KE, Schneider PV *et al.* T cell activation-inducing epitopes of the house dust mite allergen Der p I. Proliferation and lymphokine production patterns by Der p I-specific $CD4^+$ T cell clones. *J. Immunol.* 1992; 148: 738–45.
78 Spiegelberg HL, Beck L, Stevenson DD, Ishioka GY. Recognition of T cell epitopes and lymphokine secretion by rye grass allergen Lolium perenne I-specific human T cell clones. *J. Immunol.* 1994; 152: 4706–11.
79 Van Neerven RJ, van de Pol MM, van Milligen FJ, Jansen HM, Aalberse RC, Kapsenberg ML. Characterization of cat dander-specific T lymphocytes from atopic patients. *J. Immunol.* 1994; 152: 4203–10.
80 Carballido JM, Carballido PN, Terres G, Heusser CH, Blaser K. Bee venom phospholipase A2-specific T cell clones from human allergic and non-allergic individuals: Cytokine patterns change in response to the antigen concentration. *Eur. J. Immunol.* 1992; 22: 1357–63.
81 Matsuoka T, Kohrogi H, Ando M, Nishimura Y, Matsushita SRA. Altered TCR ligands affect antigen presenting cell responses: Up regulation of IL-12 by an analogue peptide. *J. Immunol.* 1996; 157: 4837–43.
82 Totsuka M, Kakehi M, Kohyama M, Hachimura S, Hisatsune T, Kaminogawa S. Enhancement of antigen specific IFN-γ production from $CD8^+$ T cells by a single amino acid substituted peptide derived from bovine α_{S1}-casein. *Clin. Immunol. Immunopathol.* 1998; 88: 277–86.
83 Nakajima H, Hachimura S, Nishiwaki S *et al.* Establishment and characterization of α_{S1}-casein-specific T-cell lines from patients allergic to cow's milk: Unexpected higher frequency of $CD8^+$ T-cell lines. *J. Allergy Clin. Immunol.* 1996; 97: 1342–9.
84 Franco A, Southwood S, Arrhenius T *et al.* T cell receptor antagonist peptides are highly effective inhibitors of experimental allergic encephalomyelitis. *Eur. J. Immunol.* 1994; 24: 940–6.
85 Karin N, Mitchell DJ, Brocke S, Ling N, Steinman L. Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor antagonism and reduction of interferon γ and tumor necrosis factor α production. *J. Exp. Med.* 1994; 180: 2227–37.
86 Kuchroo VK, Greer JM, Kaul D *et al.* A single TCR antagonist peptide inhibits experimental allergic encephalomyelitis mediated by a diverse T cell repertoire. *J. Immunol.* 1994; 153: 3326–36.
87 Nicholson LB, Murtaza A, Hafler BP, Sette A, Kuchroo VK. A T cell receptor antagonist peptide induces T cells that mediate bystander suppression and prevent autoimmune encephalomyelitis induced with multiple myelin antigens. *Proc. Natl Acad. Sci. USA* 1997; 94: 9279–84.
88 Singh DP, Kikuchi T, Singh VK, Shinohara T. A single amino acid substitution in core residues of S-antigen prevents experimental autoimmune uveitis. *J. Immunol.* 1994; 152: 4699–705.