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Immunological response to food

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The gastrointestinal tract has a remarkable capacity for adaptation to the enormous variety of foods which are consumed. The many digestive functions of the gut are coordinated by complex neural and humoral networks. However, in addition to these primarily-nutritional properties, the gastrointestinal tract has several different immunological functions. These are influenced, in their induction and expression, by digestion and motility, and also by primarily-immunological regulatory mechanisms. There are a number of reasons why the mucosal immune system has properties quite separate and distinct from the systemic immune apparatus. Against a background of continuous low-grade immune responses to food antigens and commensal bacteria, it is necessary that the gut allows absorption of nutritious substances and tolerates the presence of many useful micro-organisms. However, there remains the need to mount, rapidly and efficiently, a range of protective immune responses to enteric pathogens in order to eliminate infections and prevent reinfection. Thus, immune responses to enteric antigens, including those of food, are in general under continuous suppression (oral tolerance). Interruption of this important homeostatic property of the gut can be considered the primary pathogenesis of a number of important food allergic diseases.

Gastrointestinal immune responses and their regulation

Antigens encountered within the gut will include those of ingested food and food additives, commensal micro-organisms and a great variety of viral, bacterial, protozoal and helminth pathogens and their secretions. These antigens are confronted with gut-associated lymphoid tissues which comprise nodular lymphoid tissues within the wall of the gut (the Peyer's patches and appendix), the mesenteric lymph nodes, the phagocytic system of the liver, many single lymphoid cells scattered throughout the mucosa, immunoglobulins both locally secreted and derived from the serum, and various immunologically non-specific humoral and cellular agents such as lysozyme, mucus and macrophages. In addition to these mucosal-associated cells and secretions, components of the systemic immune apparatus may be recruited into the tissues in disease states. For example, IgE plasma cells, IgG and IgM antibodies, and peripheral T and B cells, have all been identified in diseased gut mucosa, either in experimental animals or in human disease.

Many types of lymphocyte are continuously moving around the body, and the

gut-associated lymphocytes have distinct traffic routes. 'Gut-associated' small T and B lymphocytes move into the Peyer's patches and mesenteric lymph nodes from the bloodstream via post-capillary venules, and exit from these organs into the lymph. These cells play vital roles in regulation of the induction phase in immune responses to antigens encountered within the Peyer's patches. On the other hand, large immunoblasts of both T and B types are induced in Peyer's patches and mesenteric nodes as a result of immune stimulation. These cells have distinctive morphological characteristics and a tendency to 'home' back to the gastrointestinal tract mucosae. This continuous traffic allows for widespread distribution of antibody-producing cells and T blasts, and the capacity for specific immune reactions to antigen is spread throughout the length of the gut.

Traffic of lymphoid cells has been described in several mammalian species, and there is no reason to suspect that there is not also such a traffic in man. This means that the scattering of lymphoid cells in a biopsy of small or large intestine will reflect, fairly accurately, the antigen exposure of the intestine within the previous 1 or 2 weeks.

When antigen is injected parenterally (as in the use of protective vaccines) the usual responses are induction of active systemic immunity in the form of serum antibodies of IgM and IgG classes and, with some antigens, active cell-mediated immunity (CMI) (e.g. the tuberculin reaction). When an antigen is administered via the gut, several types of immune response may be evoked (Fig. 1) and these are

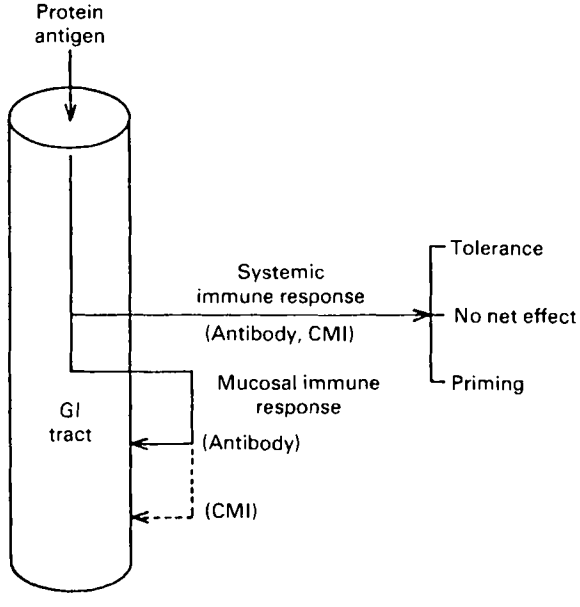

Fig. 1. Patterns of systemic and mucosal immune responses which may be elicited by the ingestion of a protein antigen. In health, the usual pattern is of induction of tolerance of the systemic immune system, and of a mucosal secretory antibody response. CMI, cell-mediated immunity; GI, gastrointestinal tract.

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not mutually exclusive. There may be induction or suppression of antibody or CMI, at gut level or systemically. It is worth re-emphasizing the differences between active immunity in which antigen-reactive cells or specific antibody develop, and immunological tolerance which is a specific immune response leading to suppression of reactions if the same antigen is subsequently given systemically. Active immune responses can readily be detected and measured in man as well as in animals, whereas the phenomenon of immunological tolerance to ingested protein has been studied mainly in small laboratory rodents.

We have postulated that the various types of immune response which develop after antigen is administered enterically can be explained on the basis of induction of different types of immunoregulatory T cells in the gut-associated lymphoid tissues (Ferguson & Strobel, 1983). Within the Peyer's patches, conditions are optimal for T cell interaction with antigen. Peyer's patches contain precursors of lamina propria IgA cells and antigen; the antigen, presented to T cells by macrophages within the organized lymphoid tissues of the gut, leads to the induction of antigen-specific T helper cells for IgA and activation of T suppressor cells for other immunoglobulin classes. Experiments with mouse Peyer's patches and mesenteric lymph node cells have shown dual activation of these different helper and suppressor T cells (Richman *et al.* 1981). We have examined the modulation of mucosal CMI by manipulating the immune status of animals at the time of first encounter with antigen via the gut. For example, when cyclophosphamide is used to inhibit suppressor cells there may be stimulation of mucosal CMI (Mowat & Ferguson, 1981) and recent experiments with the adjuvant muramyl dipeptide are leading to similar results. Results of these animal experiments are so consistent that they should provide an impetus for studies of immunoregulatory T cells in patients with intestinal inflammatory and allergic disease.

It is to be emphasized that, even in the relatively well-defined sphere of experimental animal research, a whole battery of investigations are necessary to define patterns of immune responses, induced by feeding in a single situation. This is illustrated in Table 1 which highlights the difficulties in this work, particularly because some of the investigations will require refeeding or further injections of antigen, which radically change the immune status of the animal. Analogous experiments have not been done in the human species or in large animals such as the bovine, pig, sheep, dog and cat.

Oral tolerance and systemic priming in humans

Although there have been no definitive studies of oral tolerance, three published studies (Korenblat *et al.* 1968; Lowney, 1968; Dastur *et al.* 1981) suggest that this probably does exist in man. Lowney (1968) used male prisoners whom he skin tested for sensitization to dinitrochlorobenzene (DNCB). He used two groups of subjects; those in one (experimental) group received two to three buccal applications of DNCB whereas those in the other (control) group did not. Following subsequent skin challenge with DNCB, less than half (seven out of

Table 1. *Typical protocol for study of the immunological effects of a first encounter with antigen via the gut*

Response	Methods	Comment
Active systemic immunity:		
Antibody	Serum-antibody titres	Study time-course
Cell-mediated immunity (CMI)*	Skin test	Best method of in vivo lymphocyte function
Systemic tolerance*	Follow antibody and CMI responses to systemic immunization	Positive and negative control groups essential
Mucosal antibodies	Antibody-containing or secreting cells in lymph or mucosa (immunoblasts, plasma cells). Antibodies in secretions, bile	Study time-course; optimal techniques still uncertain. In some species very little antibody is transported into bile
Mucosal CMI*	Refeed antigen and study effects on villi, crypts and intraepithelial lymphocytes	Indirect methods include in vitro tests on mucosal cells or mesenteric lymph node cells. Significance of results uncertain.

*Immune status of the animal is altered when these measurements are made, since further doses of antigen must be administered in order to perform the test.

seventeen) of the experimental group showed evidence of delayed hypersensitivity, whereas nearly all (twenty-five out of twenty-six) of the control group were sensitized. This is good evidence for development of oral tolerance to a contact-sensitizing agent.

Korenblat *et al.* (1968) measured serum-antibody titre to bovine serum albumin (BSA) in a group of American volunteers. They were then fed daily for 3 weeks with large doses of BSA (27·3 mg/kg per d (12 mg/lb per d)). Those in whom antibody to BSA was absent from the serum before the experiment showed little or no rise in serum-antibody titre after the large feeds of BSA: they were tolerant to fed BSA. Those who already had circulating anti-BSA, however, showed a rise in anti-BSA titre after the feeds: they were not tolerant to fed BSA. When seven of the volunteers were systemically immunized with 1 mg BSA, a rise in serum anti-BSA was found in those who were not tolerant (by the previously-mentioned definition) but no rise was found in those who were tolerant. This kind of experiment may be useful for examining the properties and control of immune-regulatory T cells. It would be useful to extend such studies (with appropriate ethical precautions) to other food antigens.

The third relevant paper, by Dastur *et al.* (1981), indicates that most of the adult population of India have circulating antibodies to tetanus toxin and that in such populations it appears to be much more difficult to induce a good systemic immune response with an ordinary parenteral tetanus vaccination. This suggests that Indians are tolerant to tetanus toxin (probably because they are colonized by *Clostridium tetani* and normally have the toxin in the gut).

These three papers seem to constitute the entire literature on oral tolerance in man. This is an important subject, however, since if oral tolerance can be reimposed in a food-allergic individual, it has implications for immunotherapy by specifically reversing a food hypersensitivity state.

Another approach to investigation of oral tolerance in man is to define circumstances in which this is certainly absent, i.e. in which there is active systemic immunity to antigens which are normally encountered only by the gut. The natural group of antigens to study is those of foods, and indeed there is plenty of evidence that normal human infants, patients with diseases of the small intestine, and patients with a number of other diseases, have serum antibodies to a range of foods. These are not of the IgE class (IgE antibodies and food allergic disease are discussed later in the context of atopic eczema). The antibodies concerned may be IgM, IgG or IgA and, because of technical aspects when different immunoglobulin classes are examined, it is not possible to draw any overall conclusions with regard to the importance or otherwise of a preponderance of any particular immunoglobulin.

We have recently embarked on a study of immune responses to foods in healthy blood donors (as inferred by the presence in plasma of relatively high titres of antibodies). We have selected a range of antigens derived from very disparate types of food, and covering a range of chemistry. To date, results are available for antibodies to β-lactoglobulin (cow's-milk protein), hen's-egg ovalbumin, gliadin (a plant storage protein and gum arabic (a tree exudate widely used in food processing). Results for a group of fifty blood donors are summarized in Table 2 and it is clear that, when an ELISA technique is used, substantial numbers of healthy individuals have detectable antibodies to foods and in some the titres are as high as those observed in severe small bowel disease such as untreated coeliac disease. There appears to be no particular grouping of the antigens we have studied although, if a patient has high titre of antibody to one antigen, usually significantly high titres are present to several others also. This simple technique of screening for active systemic immunity will allow us to establish whether there is a genetic predisposition to this state in healthy adults, whether there is any association with HLA status, with IgA deficiency or blood group. This type of work emphasizes the need for very critical appraisal of studies in which very sensitive techniques (such as immunofluorescence) are used to demonstrate antibodies to foods in patients with chronic diseases such as schizophrenia, or in patients who are convinced that they have food allergy although they do not conform to recognized clinical descriptions of these states. Creation of immune complexes when antigen is absorbed and combines with antibody, is considered by Dr Levinsky in another paper of this symposium (pp. 81–86).

IgE class antibodies to inhaled antigens are the hallmark of the atopic state and of atopic diseases such as hay fever, asthma and eczema. Atopic children may have accompanying IgE responses to foods such as cow's-milk antigens, and food allergic disease which often (although by no means always) relates to the same foods. On the other hand, it is unusual for IgE food antibodies, and food allergic

Table 2. *Antibodies to food proteins detected by ELISA technique in plasma from fifty blood donors*

(ELISA technique and cut-off 'positive' optical density reading differ for each antigen)

Antigen	Negative or borderline result	Positive result	
		Intermediate	High
β-Lactoglobulin	39	10	1
Ovalbumin	40	8	2
Gliadin	47	2	1
Gum arabic	43	5	2

reactions, to occur in atopic adults and this is almost unknown in non-atopic individuals. Reactions which mimic allergic states do occur and have been discussed at length in a recently published report (Royal College of Physicians and British Nutrition Foundation (Joint Report), 1984). The atopic state, and specific IgE antibodies, can be recognized by using prick tests (for example with Bencard allergens) and by in vitro tests such as the radio-allergo-sorbent technique (RAST) for circulating IgE class antibodies. It must be emphasized, however, that the characterization, purification and standardization of food antigens is much less advanced than is the case for common inhalant antigens. Thus laboratory diagnosis of food allergic disease or food allergic reactions is still in its infancy and, to date, the only method for diagnosis of a state of food allergy is to prove food intolerance by clinical means, and back this up with laboratory evidence that the food intolerance is accompanied by abnormal immune responses which would be consistent with the hypersensitivity mechanism postulated.

Immunological responses to foods in coeliac disease and atopic eczema

Interesting and useful information on immunity to foods can be accrued by simple clinical documentation in patients with clearly-defined food allergic disease. We are currently studying cellular and humoral immunity in adult patients with coeliac disease and with severe atopic eczema, before initiation of any elimination diet treatment.

Immediate hypersensitivity and serum IgE antibodies. Patients were asked directly if there was a history of angio-oedema associated with ingestion of foods. Twelve of thirty-two atopic eczema patients had had angio-oedema, precipitated either by fish or eggs, and one of the twenty-eight atopic asthma–rhinitis controls also had had a previous allergic reaction to fish (Barnetson, 1980). No such history was elicited in twenty-eight healthy controls and in twenty adult coeliac patients.

The Phadebas RAST technique was used to detect IgE antibodies to foods in serum. Only in patients with atopic eczema were there high RAST scores with food allergens. In general, those patients who had high RAST scores with foods also had high titres of antibodies to inhalant allergens, and also had high levels of total circulating IgE (Barnetson *et al.* 1981).

Other serum antibodies. In untreated coeliac disease, the majority of patients

have serum antibodies to many foods, not only gluten but others such as milk or eggs, which they can tolerate without ill effects. We are currently using precipitin and ELISA techniques to study serum antibodies to foods in these patients, and preliminary results indicate that about 40% of patients with atopic eczema have serum precipitins reacting with a variety of food antigens (Barnetson *et al.* 1983).

In vitro studies of peripheral blood lymphocytes. There have been many reports of peripheral blood lymphocyte reactivity to gluten or gliadin in coeliac disease. In general, using either lymphocyte transformation tests or leucocyte migration inhibition tests, positive results are frequently obtained in lymphocytes from patients with coeliac disease and infrequently with lymphocytes from non-coeliac patients (Holmes *et al.* 1976; Bullen & Losowsky, 1978). We have performed lymphocyte transformation tests in seven patients with severe atopic eczema all of whom had a history of food-precipitated angio-oedema. No transformation was obtained when lymphocytes from six egg-allergic patients were cultured with ovalbumin, and no transformation was obtained when lymphocytes from the single milk-allergic patient were cultured with α-lactalbumin or with β-lactoglobulin.

Jejunal biopsy pathology. Our experimental work has implied that features of jejunal biopsy pathology, villus atrophy, crypt hyperplasia and intraepithelial lymphocyte infiltrate, are indirect markers of mucosal CMI reactions (Mowat & Ferguson, 1982). In order to make the diagnosis of coeliac disease, it is of course necessary to demonstrate severe partial villus atrophy or subtotal villus atrophy with crypt hyperplasia in a jejunal biopsy. These typical features were present in all coeliac patients studied. In contrast, of twenty-five patients with atopic eczema, twenty-four have entirely normal jejunal biopsy pathology. One patient with severe atopic eczema had villus atrophy and crypt hyperplasia, and subsequent investigations have shown that he has coeliac disease. It is of interest that treatment of this patient with a gluten-free diet produced considerable clinical improvement in his eczema.

The absence of intestinal mucosal villus atrophy and crypt hyperplasia and the negative lymphocyte transformation tests with foods tend to suggest that patients with severe atopic eczema do not have CMI to food antigens, although they clearly have immediate hypersensitivity and IgE antibodies to the foods concerned. We have found no evidence as yet of any primary intestinal mucosal abnormality in atopic eczema and suggest that the defect in immunoregulation is related to IgE responses in general. However, the secondary effects of type 1 hypersensitivity reactions in the intestinal mucosa may increase intestinal permeability so that substantial amounts of many foods are absorbed across the intestinal mucosa and this may lead to the induction of the serum-precipitating antibodies, pathogenetic effects of which remain to be defined. On the other hand, it is clear that the immunoregulatory abnormality in coeliac disease does not encompass IgE-antibody responses, although coeliac patients frequently have enhanced serum and secretory humoral responses to many foodstuffs. In coeliac disease, there is also circumstantial evidence that intestinal CMI to foods and other antigens contributes to the enteropathy.

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