Antigen absorption: food, fire or fuel?

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The epithelium of the gastrointestinal tract is constantly exposed to varieties of antigens. In healthy individuals, only small amounts of ingested dietary antigens are absorbed. The normal immune response to absorbed food antigens is one of tolerance, which enables food to play its nutritive ("food") role without causing disease. Breakdown in tolerance may result in a spectrum of clinical problems, including food allergy, food sensitive enteropathy and food intolerance ("fire"). When food-sensitive enteropathy is subclinical, continued ingestion of the offending food antigen sometimes results in development of tolerance and resolution of the enteropathy. The development of tolerance to a specific food antigen under these circumstances may be prevented by briefly excluding the antigen from the diet, substituting it with a different antigen and then reintroducing the first antigen. In this situation, the second food antigen not only prevents the mucosal recovery expected if the infant had been continuously fed food containing the first antigen alone, but frequently seems to worsen the damage when the first antigen is reintroduced ("fuel"). While genetic constitution seems to be the major player in the heightened IgE responsiveness in atopic subjects, the pathophysiology of food-sensitive enteropathy in non-atopic children is less well understood. Complex interplay between environmental factors such as breast feeding, and host factors such as the integrity of the absorptive gut epithelium and its immunological responsiveness at the time of introduction of various food antigens seems to be important in its genesis as well as in its tendency to be a transient disorder of infancy.

Introduction

The gastrointestinal epithelium is repeatedly exposed to a variety of dietary, microbial and other antigens from the immediate post-natal period. Although adverse reactions to food antigens are uncommon, there is now a good deal of both *in vivo* and *in vitro* evidence which indicates that small amounts of antigens gain access to the tissues after penetrating the gastrointestinal epithelium in children as well as in adults. In the normal host the entry of such antigens into the intestinal mucosa is usually of no clinical consequence and results in a state of immunological tolerance. Antigen entry into the intestinal mucosa may, however, have a significant pathogenic role in a variety of human disorders, including food allergy, coeliac disease and transient food sensitive enteropathies (FSE) in children. This review will address the physiology and developmental aspects of antigen absorption and the immunological response of the normal host to the absorbed antigen, and discuss the complex interplay between food antigens, antigen-absorption and abnormal immune response to the antigen in the pathogenesis of transient FSE in children.

Antigen absorption in the normal host

The intestinal lumen of the exclusively breast-fed infant contains only trivial amounts of foreign dietary antigens, presumably from foods consumed by the mother. In contrast, the gut lumen of the formula-fed infant is exposed to varieties of food proteins which are potential sources of numerous foreign antigens. The normal gastrointestinal tract is endowed with a highly efficient machinery to minimise entry of food antigens into the mucosa and body tissues (Tables 1 and 2). Furthermore, the small amounts of food antigens which normally gain access to tissues across the absorptive epithelium, usually fail to evoke adverse local or systemic effects, thanks to an ingenious and efficient immune system.

Table 1. Intestinal barrier for antigen absorption: non-specific mechanisms

A. Minimise Intake	
	Anorexia, food aversions, vomiting
B. Digestive	
	Peristalsis, secretions, mucus, Proteolysis
C. Structural	
	Epithelial integrity

Non-specific mechanisms which minimise antigen absorption

The normal gastrointestinal tract has a very efficient digestive system which ensures an almost complete proteolytic breakdown of ingested proteins into non-antigenic peptides and amino acids. The extent to which different food proteins are degraded and rendered either non-antigenic or immunogenic is a function of intestinal maturity. Studies in rats have shown conclusively that some dietary proteins are degraded incompletely in immature animals, compared with mature animals, resulting in increased binding of antigens to enterocyte

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microvillous membranes[1,2]. In the human, macromolecular absorption by histologically normal intestinal mucosa in childhood has been shown to be a normal phenomenon in an *in vitro* study[3]. In keeping with experimental work in immature animals, increased macromolecular absorption has also been demonstrated in premature infants[4].

A critical determinant of macromolecular absorption is the structural integrity of intestinal epithelium. A study using intestinal organ culture showed increased number of enterocytes permeable to macromolecules in histologically abnormal mucosae compared with normal mucosae[3]. Increased mucosal permeability to large molecular weight (4000) polyethylene glycol has been found in patients with food allergy and atopic dermatitis, or atopic dermatitis alone[5]. Whether these patients had mucosal histological abnormalities is not known as morphological studies had not been carried out. Finally, increased macromolecular absorption has also been demonstrated in severely malnourished children[6]. From these aforementioned studies, it is clear that antigen absorption is a normal physiological phenomenon and that increased antigen absorption is a consequence of either intestinal immaturity or increased passive permeability of damaged enterocytes.

Some children with food induced disorders such as cow's milk allergy (CMA) and coeliac disease have been known to show marked aversion to the offending foods and would either spit out or vomit the food if forced to ingest them. Although such a "protective" behaviour might help reduce the antigen intake, with consequent decreased antigen absorption, it might also contribute to serious nutritional consequences such as failure to thrive, impaired growth and malnutrition.

Table 2. Intestinal barrier for antigen absorption: specific mechanisms

A.	Secretory immunoglobulin A (sIgA)
B.	Human milk IgA (Breast-fed infants)

Specific mechanisms which minimise antigen absorption

In addition to the non-specific intestinal mechanisms which militate against excessive entry of antigens into the epithelium and lamina propria discussed above, the gastrointestinal tract mounts a significant local immune response to dietary antigens through the gut associated lymphoid system. (GALT) T lymphocytes have been shown to migrate into human foetal intestine at about 12-14 weeks gestation, and their numbers increase gradually thereafter.[7] By about 20 weeks of gestation these T-cells are capable of responding to luminal stimuli by the production of cytokines such as interleukin-2 (IL-2) and interferon gamma (IFN-γ)[8]. Further, GALT is capable to responding to food antigens by production and secretion of secretory immunoglobulin A (sIgA) to the epithelial luminal surface. This response aids in diminishing antigen absorption by immune exclusion or by increasing the ability of brush border peptidases to completely hydrolyse antigenic peptides into non-antigenic molecules[9]. The sIgA response, however, is not developed in premature infants until about 35 weeks gestation[10]. Maturation of the sIgA response occurs throughout infancy and early childhood. A recent study which measured salivary IgA in infants showed that sIgA increased more rapidly in the first six months after birth in infants who were exclusively breast-fed than in those who were exclusively bottle-fed[11]. Human milk contains soluble factors mitogenic to B-cells[12]. These factors include, among others, epidermal growth factor and interleukin-6 (IL-6)[13,14]. IL-6 has been shown to stimulate IgA synthesis by human appendix B-cells[15]. Further, recent study by Ramsay *et al* in which targeted disruption of the gene that encodes IL-6 in mice resulted in greatly reduced numbers of IgA-producing cells at mucosae, the mucosal defect in IgA secretion as well as antigen-specific IgA antibody production could be restored by local application of IL-6 expressed by a recombinant vaccinia virus[16]. These interesting observations suggest a convincing physio-logical role for human milk in regulating antigen absorption by the infant gut when the gastrointestinal tract is most vulnerable to immunopathologic insults due to enhanced absorption of luminal antigens.

Normal response to absorbed dietary antigens

The small quantities of food antigens that are normally absorbed evoke local as well as systemic immune responses which not only minimise antigen entry, but also result in the development of tolerance rather than food-induced disease. The exact immunological mechanisms which result in tolerance are very complex and poorly understood. Suffice it to say that genetic constitution, maturity and immunological reactivity of the gastro-intestinal tract at the time of introduction of food and environmental factors such as breast feeding, are some of the major critical factors determining normal development of tolerance.

Limited studies in infants and children suggest that tolerance is associated with a local immune response consisting of formation and secretion of sIgA to the mucosa and a cellular immune response characterised by absence of abnormal T-cell activation. Antigen absorption is therefore not only a normal physiological event, but perhaps an essential necessity for the phenomenon of tolerance to food proteins. Teleologically, therefore, antigen absorption has ensured survival of the host by allowing food proteins to play their essential nutritive role ("food") without causing food-induced disease.

Adverse gastrointestinal reactions to food antigens ("Fire")

Normal antigen absorption followed by the development of tolerance to food proteins is a developmental, maturational process which is susceptible to complex modulatory effects of genetic and environmental influences. Failure of development of tolerance or the loss of tolerance after it has been well-established is a potential threat, albeit small, associated with repeated food consumption. Considering the fact that all ingredients for a potentially serious and harmful immunopathological adverse reaction to food are present in close proximity within the gut micro-environment, ie, food antigens in the

lumen, antigen processing cells in the mucosa and T-lymphocytes in the epithelium and lamina propria, it is indeed surprising that food sensitive gastrointestinal disease does not occur in all individuals. Fortunately, such reactions occur in less than 10% of the population. Not unexpectedly, they are more common in young infants in their first few weeks and months of life than in older children and adults. The clinicopathological spectrum of food-induced immunological disorder is indeed quite wide, and at least one half of affected children show predominantly gastrointestinal symptoms. With the exception of IgE mediated reactions, convincing evidence for causal relation between any immune reaction to food antigens and adverse clinicopathological disorders has not been established. Further discussion of various immuno-pathological and non-immunological mechanisms thought to be important in the genesis of adverse reactions to food is beyond the scope of this review. An excellent recent review of food allergy in children is provided by Stern[16].

A list of common and some uncommon gastrointestinal manifestations of food protein-induced adverse reactions in children is shown in Table 3. Among the various features listed, bloody diarrhoea due to colitis in infants less than three months of age deserves special mention.[17] It is particularly, though not exclusively, seen in entirely breast-fed infants and is a transient disorder with complete recovery in subsequent months. Careful clinical observations suggest an aetiological role for food antigens from maternal diet which have been secreted into her milk after systemic absorption in the intestine.

The European Society for paediatric gastroenterology and nutrition has established a working group for the diagnostic criteria for food allergy. Recommendations of this working group should be of further interest to those interested in food allergy.[18]

Table 3. Gastrointestinal manifestations of adverse reactions to food antigens

A. Acute
Vomiting
Diarrhoea
Abdominal Pain/Colic
Haematemesis (rare)
B. Chronic
Diarrhoea
Vomiting
Malabsorption
Anorexia, food aversion
Impaired growth
Bloody diarrhoea due to colitis

Food sensitive enteropathy (FSE)

Small intestinal mucosal damage (enteropathy) is now a well-recognised histopathological feature of gastrointestinal adverse reactions to food in children. While cow's milk protein (CMP) and soy protein (SP) have been the most common antigens involved, FSE had been documented in relation to egg protein, rice, fish, gluten and others. With the exception of gluten-sensitive enteropathy (coeliac disease) FSE caused by other dietary proteins is usually a transient disorder affecting children in the first 2-3 years of life. The enteropathy resolves completely when offending antigens are excluded from the child's diet but it recurs if the antigen is reintroduced into the diet within a few days or weeks. After prolonged periods of exclusion, however, usually 12 months or more, the enteropathy does not recur and the child remains tolerant to the food protein(s) thereafter. In contrast, mucosal sensitivity to gluten in coeliac disease is a permanent and lifelong phenomenon. The immunological mechanisms that result in failure of development of tolerance or, less commonly, breakdown in established tolerance to food protein and FSE are poorly understood. Immunogenicity of the antigens, structural integrity of the gut epithelium, luminal factors such as microbial flora and breast milk, as well as genetic constitution and its variable effects on gastrointestinal immunoreactivity during critical, particularly vulnerable periods of life, such as infancy and during recovery from acute viral gastroenteritis have all been considered to be important. The intestine of young infants seems to be particularly vulnerable to disturbances of regulatory mechanisms which normally prevent immunopathologic damage due to antigen entry into the mucosa. The histological and immunopathological lesions of the mucosa in FSE have all the hallmarks of damage caused by abnormal activation of T-lymphocytes. The remarkable similarities between the immunopathological lesions seen in the mucosae of human foetal intestinal explants which have been exposed *in vitro* to various luminal mitogens to activate T-lymphocytes and the pathology of the mucosa in FSE lend strong support to this hypothesis[8]. Recent studies which employed sophisticated molecular genetic techniques to selectively inactivate IL-10 gene and create a mouse mutant phenotype defective in IL-10 production and secretion have thrown further light on immunological mechanisms which may be relevant to FSE[19]. In this IL-10 "knock-out" mouse model, the small bowel mucosal lesions resulting in impaired growth and anaemia consequent to malabsorption bear remarkable similarity to FSE in infants. A role for luminal antigenic stimulus in the immunopathology of the small intestinal lesions in this mouse model became apparent when it was observed that the lesions attenuated when the mutants were cared for in a specific-pathogen-free environment. Uncontrolled macrophage activation in IL-10-deficient mice may have been responsible for enhanced stimulation and activation of T-helper cell subset 1 (Th-1). IL-10 is known to suppress the macrophage-dependent activation of Th 1 cells and natural killer cells (NK). Enhanced production of cytokines by Th-1 cells and NK, and the specific effects of these cytokines on enterocytes such as aberrant expression of MHC class II molecules on their surface, in the phase of normal development of B-lymphocytes into antibody producing plasma cells under the influence of Th-2 cells and luminal antigens entering the mucosa, may account for the enteropathy in this mouse model. The chain of events starting with antigen entry into the mucosa, followed by dysregulated and enhanced activation of T-cells which induce immunopathology rather than tolerance, and culminating in a clinical syndrome of FSE with chronic diarrhoea, malabsorption, failure to thrive,

with growth and nutritional deficit can therefore be justifiably described as "fire" caused by antigen absorption.

Causative effect of antigenically unrelated food proteins in potentiating mucosal lesions in FSE

In common with many other forms of intestinal mucosal insults, the mucosal damage in FSE is not always accompanied by clinical manifestations. After exclusion of the offending antigen from the diet, however, both the mucosal damage and symptoms resolve. This suggests that the mucosal damage is initiated and maintained by continued antigen entry in the phase of perturbed and heightened immunoreactivity to the antigens. Some infants whose FSE is caused by CMP are often found to be intolerant to other antigenically unrelated proteins such as SP[20]. This phenomenon is particularly common if SP is introduced into the diet when the mucosal damage caused by CMP has not yet resolved. In this scenario, there is progressive damage to the small bowel mucosa, suggesting the possibility that the mucosal damage induced by one protein increases permeability of the mucosa to other antigens, consequently leading to more severe mucosal response to the second food protein antigens.

In a substantial proportion of infants with FSE caused by CMP, it has been observed that if the mucosal damage caused by CMP is subclinical, then complete resolution of the lesion may yet occur despite continued ingestion of CMP, suggesting that tolerance develops despite increased permeability of the epithelium to antigens in some infants[21]. In some similar cases of FSE due to SP, we have observed worsening of mucosal damage and overt clinical symptoms due to SP if the infant was taken off SP, placed on CMP for 12 to 24 hours and was then re-fed SP[20]. It is probable that interposition of CMP had injured the mucosa further and made it vulnerable to SP rechallenge in these infants. This phenomenon, where a second food protein taken later prevents the development of tolerance to the first protein taken earlier underscores the complex and changing inter-relationship between abnormal antigen entry, mucosal reactivity and food antigens. Perhaps some antigens "fuel" the "fire" caused by other antigens.

The transient nature of FSE in infants is now well-known. The exact immunohistological mechanisms resulting in the development of tolerance to food antigens, however, remain poorly understood. Limited observations in Finnish children showed that the development of tolerance was associated with altered intestinal local immune response to food antigens[22]. The intestine of infants who developed tolerance showed significant rise in food antigen-specific antibody-secreting cells of IgA isotype. Clinical tolerance seems to coincide with the ability of the mucosa to mount a local immune response to food antigens, particularly of the IgA isotype. As cytokines such as IL-4 and IL-6 and growth factors such as transforming growth factor beta 1 (TGFβ1) influence the development of mucosal IgA production, it is conceivable that tolerance is primarily associated with maturation of T cell subsets capable of influencing gut mucosal B-cells with these cytokines and growth factors.

Alternatively, the possibility that the primary event associated with clinical tolerance is restoration of epithelial integrity due to a combination of maturational events and prolonged period of antigen exclusions, and that the normal local humoral immune response coinciding with clinical tolerance is a secondary event cannot be excluded. Indeed, these two alternative mechanisms are not mutually exclusive, especially in the light of recent observations that enterocytes and intraepithelial lymphocytes (IEL) influence each other's function in a complementary, physiologically meaningful fashion[23,24]. In their recent elegant studies, Cepak *et al*[23] showed that E-cadherins expressed on the basolateral plasma membranes of enterocytes were the counter-receptors for $\alpha^E\beta^7$ integrins expressed on the plasma membrane of IEL. The unique heterophilic interaction between these two classes of counter-receptors would therefore explain the tissue-specific compartmentalisation of IEL. Equally important is the observation by Boismenu and Havran that IEL of the $\gamma\delta$ subset produce keratinocyte growth factor which promotes growth of cultured epithelial cells[24]. This suggests that $\gamma\delta$ IEL have a physiological role in surveillance and repair of damaged enterocytes. In this context the observation that T cells bearing the $\gamma\delta$ TcR represent less than 1% of CD3 + cells in the lamina propria while they constitute about 10% of IEL is highly relevant[8]. Further clinical and experimental studies to improve our understanding of the phenomenon of tolerance and sensitivity of the intestine to food antigens should be of immense value in the appropriate management and prevention of food-protein-sensitive disorders.

Acknowledgement: Miss CA Frazer for preparation of the manuscript.

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Asia Pacific Journal of Clinical Nutrition (1995) Volume 4, Number 4: 371-375

抗原吸收：作爲食物、供應養料或引起不適？

摘要

胃腸道上皮經常接觸多種多樣的抗原，在健康個體，僅少量攝取的抗原被吸收。人體對食物抗原吸收後的正常免疫反應是耐受，這樣可使食物發揮其營養作用而不產生疾病。耐受消滅將引起一系列的臨床問題，包括食物過敏、食物過敏性腸病和食物耐受不良。在輕度食物過敏性腸病時，如繼續攝取該種食物抗原（第一種食物抗原），有時可建立耐受性而消除腸病。但是如果用不同食物抗原（第二種食物抗原）暫時代替，然後再次用第一種食物抗原，反而防礙了這種耐受性的建立。在這種情況下，第二種食物抗原不但防礙了腸黏膜的康復，而且當再次用第一種抗原時，常常會引起更壞的損害。在特應性個體遺傳體質在加強 IgE 反應中似是主要的，在非特應性兒童其食物過敏性腸病的病理生理仍了解不多。在採用各種食物抗原時，環境因素如母乳喂養和宿主因素如吸收腸黏膜的完整和它的免疫反應的相互作用，對嬰兒的發病似是重要的。

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