

Gut barrier dysfunction in food allergy

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Intestinal permeability tests used in the diagnosis of allergic diseases in response to oral food challenge have led to the conclusion that constitutive defects of the intestinal barrier are not the primary cause of allergic diseases. However, perturbation of environmental factors (infection, stress), by increasing intestinal permeability and enhancing danger signals, may favour food allergy in susceptible individuals. The mechanisms of enhanced permeability to specific and bystander antigens have been delineated as well as the molecular events involved in the sequential phases of allergic reactions. Intestinal absorption of food antigens and immune responses are mutually dependent, and luminal (environmental) and serosal (intrinsic) factors synergize to maintain a self-perpetuating

cycle in which antigens penetrate the mucosa and induce allergic inflammation. *Eur J Gastroenterol Hepatol* 17:1279–1285 © 2005 Lippincott Williams & Wilkins.

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Introduction

Every day the digestive system handles a considerable amount of dietary proteins. These are mainly digested into small peptides or amino acids, by secreted enzymes or peptidases inserted in the enterocyte microvillus membrane and absorbed by sodium-coupled transport systems. A small amount of residual, non-degraded, dietary proteins can, however, potentially trigger the immune system because a significant amount of protein fragments reaches the duodenum and lower parts of the digestive tract [1]. The initiation of an immune response to food antigens requires interactions between non-degraded proteins and gut-associated lymphoid tissue. The mucosal environment, the nature of antigens and the pathways by which they access the mucosal immune system, are important factors involved in the outcome of the immune response. The capacity of a given food antigen to resist enzymatic hydrolysis is highly variable and often determines its allergenicity [2]. Cow's milk proteins present different susceptibility to the sequential hydrolysis by pepsin and trypsin. In a study of healthy volunteers, half of the β -lactoglobulin ingested was recovered intact at the jejunal level [1] and therefore represents a potential danger in susceptible, atopic, individuals. It is also important to note that mucosal and systemic immune responses are preferentially stimulated by particulate antigens, taken up through M cells of the follicle-associated epithelium, while soluble dietary antigens transported by enterocytes are likely to induce tolerance [3].

Antigen sampling by the intestinal epithelium

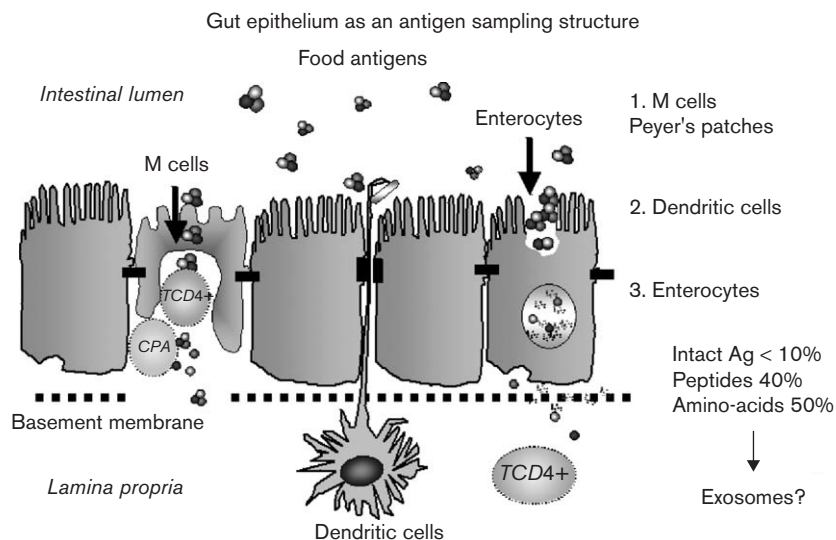
The intestinal epithelium normally acts as a barrier to restrict the diffusion or uptake of luminal antigens (Fig. 1). This barrier is maintained by a continuous layer

of polarized epithelial cells, joined at their apical side by junctional complexes which prevent the passage of peptides and proteins with antigenic properties [4], and of macromolecular compounds greater than 600 Da, between cells [5,6]. In addition, mucus and entrapped secretory immunoglobulin A (IgA) also restrict the absorption of dietary antigens. Although tight junctions between epithelial cells avoid free diffusion of luminal macromolecules toward the internal milieu along the paracellular route, enterocytes have the capacity to transcytose tiny amounts of antigenic material present in the intestinal lumen to the underlying immune system.

Role of Peyer's patches in soluble antigen sampling

Although particulate antigens can cross intestinal epithelium overlying Peyer's patches more readily than soluble antigens, some evidence suggests that food antigens may cross via this route. The rapid uptake and transport of horseradish peroxidase (HRP) by M cells, with subsequent uptake by lymphocytes, was initially described as a specific route for antigen uptake into the intestinal lymphoid system [7]. The role of Peyer's patches in the development of oral tolerance to food antigens was suggested from mice studies, where antigen-specific CD4⁺CD25⁺ T(reg) cells, which have characteristics of anergic T cells and actively suppress antibody production, were induced in Peyer's patches upon oral administration of protein antigen [8,9]. However, in various animal models of allergy, Peyer's patches are also described as a portal of entry for food allergens. For example, in mice, material derived from digestion of peanuts was almost exclusively delivered, in a large amount and highly immunogenic form, to the inductive sites of the intestinal immune system via M cells [10]. In

Fig. 1



Gut epithelium represents an efficient barrier against the massive entry of potentially noxious molecules or infectious particles. However, it is also able to sample luminal content, along different transport pathways. These include M cells in the follicle-associated epithelium (Peyer's patches), direct sampling of dendritic cells in the intestinal lumen through extension of dendrites and constitutive transcytosis across columnar enterocytes, leading to partial degradation of antigens and probably to the release of antigen presenting structures called exosomes. These are vesicles bearing major histocompatibility complexes (MHC) Class II/antigen-derived peptide complexes.

a murine model of cow's milk allergy, a specific role for lymphocytes in Peyer's patches, in tolerance to β -lactoglobulin, was also suggested, and the low IL-10 production in Peyer's patches was suspected to favour symptoms of food allergy [11]. Altogether, it is not really known whether Peyer's patches are the predominant transport pathway for allergen delivery to the mucosal immune system, because columnar epithelial cells are also actively involved.

Columnar epithelium

Food antigen absorption is not restricted to M cells overlying Peyer's patches but can also take place across columnar absorptive epithelium. For a long time it has been proposed that antigen processing by epithelial cells might be involved in the development of oral tolerance [12], although the precise role of the intestinal epithelium in this phenomenon remains elusive. The existence of a macromolecular transport pathway by transcytosis across the enterocytes was initially described by Walker *et al.* [13]. This transport pathway was further characterized using human intestinal epithelial cell lines and animal and human intestinal biopsies, studied *in vitro*, in Ussing chambers [14,15]. Radiolabelled-proteins such as HRP allowed quantification and characterization of protein transport and processing across the gut. The efficiency of the gut barrier was demonstrated in these studies as they found that less than 1% of proteins present in the intestinal lumen could cross the intestinal mucosa, and, of the small amount internalized by enterocytes and

conveyed to the serosal side of the epithelium, 90% was hydrolysed in the endosome-lysosomal system and only a minute amount was delivered intact to the serosal pole of the epithelium. This gut processing did not lead to total breakdown of antigen, since 40% of the processed material consisted of small but still immuno-reactive peptides, with a molecular mass (~ 1100 Da) compatible with a binding to MHC restriction molecules [15]. Diffusion of antigens between enterocytes (paracellular leakage) is not considered a physiological transport pathway for macromolecules, due to the small molecular cut-off of tight junctions (< 600 Da) [5] and as the integrity of tight junctions is maintained even at sites of desquamation at the top of the villi [16]. The transcytosis of a limited amount of luminal antigens has been described at different levels of the digestive tract (stomach, small intestine, colon), with an increasing gradient of efficiency, from the proximal to the distal part of the intestine. Besides these two classical macromolecular transport pathways, a new possibility for antigen delivery to the mucosal immune system has recently been described. This new possible mechanism implies that dendritic cell protrusions extend through the tight junctions of epithelial cells into the intestinal lumen, to catch luminal material without disrupting epithelial integrity. This phenomenon, however, is mainly aimed at collecting luminal bacteria by picking them up directly within the intestinal lumen, especially in inflammatory conditions [17]. It is less clear whether food antigens can also be captured directly in the lumen by this process.

Intestinal epithelial cells can take up and process luminal antigens and have the capacity to present peptidic epitopes to CD4⁺ T cells, at least *in vitro* [18,19]. They are often described as non-professional antigen presenting cells expressing MHC class II molecules, although their molecular conformation may differ from that of professional antigen presenting cells [20]. In physiological conditions, MHC class II molecule expression remains low but is upregulated by pro-inflammatory cytokines, enhancing the capacity of antigen presentation. Indeed, IFN- γ has been shown not only to upregulate the expression of MHC class II molecules on the intestinal epithelial cell line HT29-19A, but also to increase the paracellular and transcellular transport of potentially antigenic material [15]. Although enterocytes can take up and present antigens, their ability to interact directly with CD4⁺ T cells *in vivo* is probably restricted due to the presence of a basement membrane, which is not easily permeable to immune cells, separating enterocytes and CD4⁺ T cells. Recently, the capacity of enterocytes to secrete membrane vesicles (exosomes), enriched in MHC class II/peptide complexes [21] and presenting high immunogenic capacity [22], has been demonstrated. These vesicles could play a role as messengers, transmitting peptidic information from the intestinal lumen to the underlying immune system, via enterocytes, by diffusing through pores in the basal membrane, in order to interact with non adjacent immune cells. The role of epithelial exosomes in the induction of oral tolerance [23] or sensitization [22] is currently under investigation.

Gut barrier dysfunction in food allergy: cause or consequence?

Abnormalities in intestinal permeability are not the hallmarks of food allergy, since they are reported in most inflammatory digestive diseases. Although allergic inflammation led to epithelial dysfunction with altered antigen handling, most studies have concluded that constitutive modification of epithelial permeability is not involved in the development of food allergy. Indeed, increased epithelial permeability was more the consequence, rather than the cause, of food sensitization, although it participates in a self-perpetuating cycle that maintains allergic inflammation. Although constitutive abnormalities in intestinal permeability are not recorded in atopic patients, environmental events such as infection or stress may have consequences on the susceptibility to allergic diseases.

Environmental events modulate intestinal permeability and the development of food allergy

Enteric infections

There is substantial evidence that enteric infections and inflammation tend to increase the amount of antigen penetrating the intestinal mucosa, influencing the outcome of antigen presentation to T cells (sensitization instead of tolerance) and directing the immune response

towards allergy, in susceptible individuals. Indeed, in addition to the epithelial damage and increased permeability caused by infectious agents, pro-inflammatory cytokines are known to switch dendritic cells, the major professional antigen presenting cells, from a tolerogenic to an immunogenic state [24]. Results obtained using animal models of food allergy to test this hypothesis are nevertheless contradictory. Some results are consistent with this hypothesis. For example, the gastric pathogen *Helicobacter pylori* decreases food antigen breakdown by epithelial cells during transcytosis, increasing the passage of intact antigens [25]. This property may explain the frequent persistence of inflammation after eradication of the bacteria, suggesting that sensitization to bystander food antigens may have occurred during infection. The observation that *H. pylori* gastric infection is associated, in susceptible subjects, with the development of allergic disorders, further supports this hypothesis [26]. In a model of sulphonic acid-induced colitis in guinea pigs, the rate and intensity of sensitization to cow's milk proteins was increased relative to control animals [27]. In the same way, in guinea pigs, the increased intestinal permeability to milk proteins observed in malnourished animals was associated with higher β -lactoglobulin-specific IgE titres, and with an enhanced intestinal anaphylactic response to β -lactoglobulin, compared to well-nourished animals [28]. In contrast, another study indicated that increased intestinal permeability was not associated with higher incidence or severity of food allergy in weanling rabbits. Indeed, entero-adherent *Escherichia coli* infection increased the rate of transcytosis and delayed gut closure to food proteins [29], without increasing allergic sensitization [30].

In children, rotavirus infection, the most frequent cause of diarrhoea, has been associated with altered intestinal permeability [31,32]. The frequency of persistent diarrhoea following viral enteritis suggests that sensitization to food antigens may occur during the infection [33,34]. Despite increasing evidence from epidemiological studies that support the hygiene hypothesis [35], i.e., that growing incidence of food allergy is linked to aseptic environmental conditions, some results still suggest that frequent infections in infancy are associated with an increased prevalence of allergic symptoms later in life [36], leaving the question open.

Stress

Mechanisms linking psychological stress to atopic disease continue to be elucidated and have been reviewed recently [37]. Various hormones or neuromediators that regulate immune and inflammatory processes are produced during stressful periods. Interestingly, animal models of maternal deprivation have shown that neonatal stress has a strong impact on intestinal permeability later in life, in basal conditions [38], and after acute stress in

adult animals [39]. Increased intestinal absorption of intact proteins due to stress has also been demonstrated in rats [40]. It is possible, although not proven, that altered epithelial permeability observed in stressed individuals may play a role in the maturation of the immune system. There is evidence that parental stress is associated with subsequent onset of wheezing in children between birth and 1 year [41]. It has been speculated that stress triggers hormones in the neonatal period which may influence Th2 cell predominance. Interestingly, relationships between caregiver stress and markers of early childhood immune response were noted. High levels of caregiver stress have been associated with higher total IgE levels, greater allergen-specific proliferative response, increased production of TNF- α and reduced IFN- γ in infants predisposed to atopy [42]. Thus, stress may influence atopy through the enhancement of neuroimmune responses to environmental factors.

Effect of food allergy on intestinal permeability and barrier function

Intestinal permeability in allergic patients

Gut barrier dysfunction is a common feature of digestive and cutaneous manifestations of food allergy. Although the first site at which food allergens encounter the immune system in allergic patients is the digestive system, defects in intestinal barrier capacity are observed in patients presenting either intestinal or cutaneous symptoms [43], due to the spreading of cells of mucosa-associated lymphoid tissue.

Permeability is a general feature of the intestine, reflecting the capacity of the epithelial layer to allow or restrict the passage of molecules of various size, from the intestinal lumen to the internal compartment. One of the most widely used intestinal permeability tests *in vivo* is the differential sugar absorption test lactulose/mannitol (L/M), in which the ratio of urinary excretion of a relatively large molecule, lactulose, is compared with that of a relatively small molecule, mannitol, after oral ingestion [44]. In this test, mannitol is considered an inert small molecule diffusing through a large number of small pores in the cell membrane that exclude lactulose [45,46], and lactulose is used as a marker of paracellular permeability. This test has been used successfully in the diagnosis of food allergy in adults [47] and cow's milk allergy in children [48], where, after milk challenge, the L/M ratio increased relative to the pre-challenge level. For a long time, a considerable number of clinical studies have reported that food allergy in the active phase, i.e., before food allergen eviction from the diet, is associated with increased luminal to serosal passage of substances of various size. It is noticeable that this increase in intestinal permeability is not specific to food allergy since it is observed in many other pathological conditions (prematurity, coeliac disease, cystic fibrosis, Crohn's disease, acute and chronic diarrhoea) but can become more

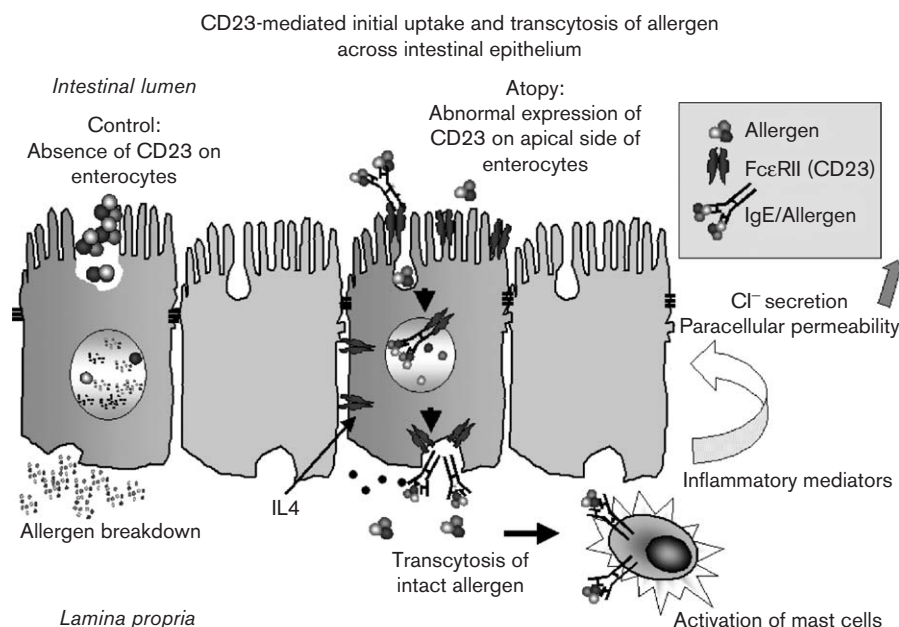
specific when associated with the offending food challenge.

Although the sugar permeability tests offer a convenient non-invasive method to measure changes in permeability in response to offending allergens, they do not reflect absorption of larger food antigens responsible for the abnormal immune response, as shown in guinea pigs [49], rats [50] or human infants [51]. Later on, it was possible to use additional methods which, although more invasive, gave precise information on the antigen handling by the intestinal mucosa of patients with food allergy. Large molecules, like the protein marker HRP or milk proteins (α -lactalbumin, β -lactoglobulin), were used to test gut barrier defects in response to offending macromolecules. Paracellular and transcellular routes of epithelial transport have been shown to be involved in these barrier defects. By using duodenal biopsies mounted in Ussing-type chambers, measurement of polarized transport of radiolabelled protein markers (HRP, β -lactoglobulin) was made possible. Indeed, in duodenal biopsies from infants with active cow's milk allergy, a significant increase in HRP transport was observed [14] that, after treatment of the infants with a diet that did not contain cow's milk, returned to normal values. These studies supported the idea that a constitutive increase in intestinal handling of food antigens is not the primary cause for the development of food allergy, but rather a secondary phenomenon.

Gut barrier dysfunction in food allergy: mechanisms involved

Animal studies have allowed us to gain insight into the mechanisms of intestinal barrier defects during food allergy. Studies conducted by M. Perdue's research group have shown disturbances in intestinal function in response to sensitizing antigens, in animal models of food allergy. The major involvement of mast cells in immediate allergic responses was explored using fragments of small intestine mounted in Ussing chambers. It was shown that mucosal or serosal challenge with sensitizing antigens led to the degranulation of mast cells and to the release of mediators (histamine, serotonin, prostaglandins, RMCP II, leukotrienes, cytokines) acting synergistically with neuromediators [52,53] to activate Cl⁻ and water secretion. In addition, epithelial permeability to small and large molecules also increased, due to selected mast cell mediators in conjunction with neural stimulation [54]. More recently, two stages of antigen transport were described following sensitization: phase I consisted of the extremely rapid transmucosal transfer of specific allergen, independent of mast cell degranulation, whereas phase II occurred later, implied mast cell activation, and resulted in a general increase in permeability [55]. It was subsequently shown that, in rats, the specificity of the enhanced allergen transport in phase I was due to the expression of CD23

Fig. 2



In healthy individuals, food antigens present in the intestinal lumen can be taken up by enterocytes but are readily degraded within the lysosomal system leading to the passage of a few peptides and a tiny amount of intact antigenic material. In contrast, in atopic individuals, the overexpression of CD23, the low affinity receptor for IgE, allows the protected transfer of IgE/allergen complexes and the passage of intact allergens to the internal compartments.

(FcεRII), the low affinity IgE receptor, on the apical membrane of epithelial cells following sensitization [56]. These results shed light on the previous unexplained observation that CD23 expression was increased at the epithelial level, in intestinal biopsies of patients with food allergy [57,58]. The binding of HRP to the IgE-CD23 complex at the apical pole of intestinal cells from HRP-sensitized rats appeared to reduce HRP degradation during transepithelial transport, since the quantity of intact HRP appearing in the serosal buffer was much greater in the sensitized rats than in controls [56]. Such protection of CD23-mediated allergen transport from degradation was also confirmed directly by analysing the molecular form of radiolabelled HRP or β-lactoglobulin after transepithelial transport across jejunal mucosa of sensitized or control mice (Fig. 2). The presence of intact proteins was demonstrated by radio-reverse phase high performance liquid chromatography in sensitized mice but was not observed in control mice [59]. The role of CD23 in the early events leading to rapid transepithelial passage of allergens was further supported by the inhibition by anti-CD23 antibodies of, not only enhanced antigen transport, but also of reduced mast cell mediated short-circuit current (*I*_{sc}), which is a direct measure of net ion transport, and of permeability responses following hypersensitization reactions [56]. It is interesting to note that, recently, different splice forms of CD23, including CD23b and CD23bΔ5, have been shown, in mice, to

display different transport properties, CD23b mediating the transfer of IgE/allergen complexes across epithelial cells and the delivery of native allergen to the serosal immune system and CD23bΔ5 mediating the transport of free IgE across polarized epithelial cells [60]. Finally, a cytokine known to be predominantly secreted upon T-cell stimulation in allergic patients, namely IL-4, was shown to significantly decrease intestinal epithelial cell (T84) monolayer resistance and to increase apical-to-basal transepithelial HRP transport. Electron microscopic analysis demonstrated that both transcellular and paracellular pathways were affected. The enhanced production of IL-4 in allergic conditions has also been shown to upregulate the expression of CD23 on epithelial cells, a property that may explain, at least in part, the role of IL-4 in enhancing permeability [61].

Conclusion

Food allergy is a common problem of increasing incidence and, besides well-known genetic factors predisposing to atopic immune responses to exogenous antigens, environmental factors have been shown to have a considerable impact on the development of allergic disease. Intestinal permeability has been the target of multiple studies, initially aimed at diagnosing allergic reaction in response to oral food challenge, and later providing evidence that constitutive defects of the

intestinal barrier to antigenic-sized molecules were not the primary cause of allergic manifestations. Later, animal models allowed a better understanding of the permeability defects associated with the ingestion of allergens in sensitized rodents, and delineation of the molecular events involved in the sequential phases of anaphylactic reactions. In fact, both permeability and immune responses are mutually dependent, and both luminal (environmental) and serosal (intrinsic) factors can synergize to maintain a self-perpetuation cycle in which antigens penetrate the mucosa and induce allergic inflammation.

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