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REVIEW

Gut permeability and food allergies

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Summary

Intestinal permeability is a critical feature of the gastrointestinal epithelium as it must allow an efficient passage of nutrients and restrict the entry of larger molecules, such as protein antigen, in order to facilitate appropriate immune responses towards food antigens. The proper regulation of the epithelial barrier relies on multiple, intricate physiological and immunologic mechanisms, in terms of which recent progresses regarding the cellular and molecular components have been unravelled. In genetically predisposed individuals, breakdown of oral tolerance can occur, leading to the inadequate production of allergen-specific IgE and the recruitment of mast cells in the gastrointestinal mucosa. Under such conditions, the intestinal permeability towards allergen is altered via different mechanisms, with IgE–CD23-mediated transport across the mucosa playing an important amplification role. Additionally, during the effector phase of the allergic reaction, when mast cells degranulate, a series of inflammatory mediators, such as proteases and cytokines, are released and further affects intestinal permeability. This leads to an increase in the passage of allergens and hence contributes to perpetuate the inflammatory reaction. In this review, we describe the importance of properly balanced intestinal permeability in oral tolerance induction and address the processes involved in damaging the intestinal barrier in the sensitized epithelium and during allergic reactions. We conclude by speculating on the effect of increased intestinal permeability on the onset of sensitization towards dietary antigens.

Introduction

The gastrointestinal tract is a very large surface, whose main function is to digest and absorb food. A single layer of epithelial cells forms a very selective barrier between the outside environment and the host, allowing the transport of nutrients while keeping larger molecules and bacteria within the lumen. This thin physical barrier is backed up by the gastrointestinal mucosal immune system, which is able to raise discriminating immune responses as a function of the antigen nature. The immune system has the difficult task of maintaining gastrointestinal homeostasis by keeping up a state of non-responsiveness towards dietary antigens and a symbiotic relationship with commensal bacteria, while initiating proper protection against potential pathogenic intruders to prevent the host's infection. The default immune response in the gut is oral tolerance, a state of active inhibition of immune responses to antigens first given orally. However, in some genetically predisposed individuals, food allergy can develop and is thought to be the consequence of the failure to establish or maintain tolerance towards normally harmless antigens [1]. In this review, we first describe the various pathways by which an antigen can cross the intestinal mucosa in a healthy individual and how this is linked to the induction of oral tolerance. We next develop on what is known regarding the modification of intestinal permeability in a sensitized mucosa of atopic individuals and the consequences of allergic reactions on intestinal permeability. Finally, the influence of increased intestinal permeability on the development of allergies in predisposed subjects is discussed.

Intestinal barrier and induction of tolerance in a healthy gut

Antigen degradation in the lumen

Before any contact between the epithelium and a food protein antigen is established, the latter is modified by gastric acids and stomach, pancreatic and small intestinal

brush-border proteases. Degradation into tripeptides, dipeptides and single amino acids then allows efficient absorption by enterocytes [2]. Destruction of immunogenic conformational epitopes by the gastric acidic environment and intestinal proteases appears to be crucial to promote tolerance via immune ignorance [3]. Besides degradation by enzymatic activities, the size-dependent filter properties of the mucus layer covering the epithelium [4] and peristalsis have been postulated to affect the availability of proteins at the surface of the epithelium. A proportion of partially or even undigested proteins can still reach the surface of the epithelium and enter the mucosa. The ability of molecules to cross the epithelium is dependent on its size, shape, polarity, three-dimensional structure and aggregation status [5, 6].

Transport of antigens from the lumen to the mucosa

Several routes allow the passage of molecules and include transport via the specialized microfold (M) cells of Peyer's patches (PP) and isolated lymphoid follicles (ILF) or across the epithelium, either through the cells (transcellular pathway) or between the cells (paracellular pathways) (Fig. 1).

PP and ILF. The mucosa-associated lymphoid tissue, comprising PP and ILF, is covered by the follicle-associated epithelium containing microfold (M) cells. These cells have the capacity to engulf particulate antigens, and transport them to underlying dendritic cells in the subepithelial dome region, which, upon contact with naïve T cells, will contribute towards mounting an immune response of various intensities and orientations. It is noteworthy that PP are dispensable for induction of tolerance against soluble antigens; oral administration of a soluble antigen in PP-deficient mice results in the same frequency of dendritic cells and macrophages in mesenteric lymph nodes (MLN), peripheral lymph nodes and spleen [7]. In support of this, knock-out mice devoid of PP, but with fully developed MLN, are competent in establishing systemic tolerance against an antigen given by the oral route [8]. This suggests that antigen-presenting cells loaded with antigens emanating from ILF or from the mucosa are sufficient to induce efficient oral tolerance.

Epithelial cells. Because of the vast surface area they constitute, epithelial cells are important players in the selective permeability of the intestinal barrier. The barrier

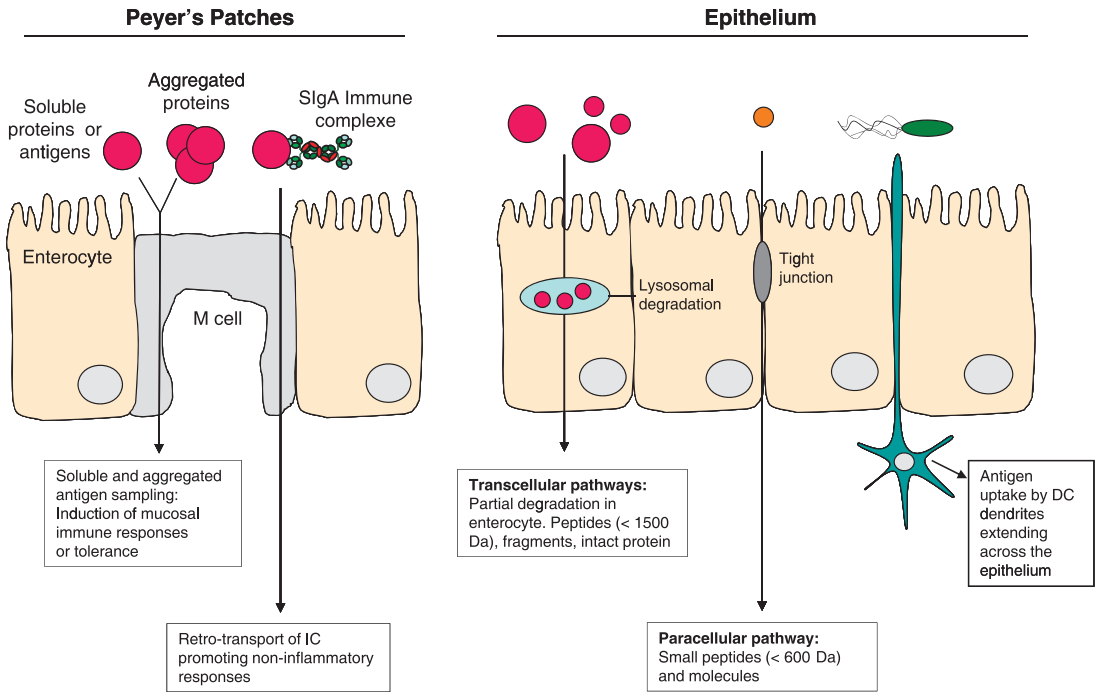

Fig. 1. Differential pathways of antigen sampling in the healthy epithelium. Organization of the gut epithelium makes it an efficient tight barrier with filtering properties against the entry of pathogenic agents and possibly harmful molecules such as toxins and allergens. Microfold (M) cells present on the surface of the follicle-associated epithelium in intestinal Peyer's patches transport particulate antigens and aggregated proteins for presentation by local dendritic cells, resulting in the onset of a tolerogenic type of immune responses under steady-state conditions. Secretory IgA-based immune complexes are similarly taken up by M cells and promote the induction of non-inflammatory cytokines (TGF-β and IL-10), ensuring low reactivity against the transported antigen. Partially degraded proteins and a small proportion of intact proteins are taken up by enterocytes. Degradation along the phago-lysosomal pathway occurs, thus resulting in the loss of potentially allergenic properties. Paracellular selective leakage provides access to ions, amino acids and carbohydrates, which are important in ensuring liquid fluxes and maintenance of transepithelial gradients. Direct intestinal sampling of bacterial antigens by dendritic cells extending their dendrites across the tight epithelium and release of exosome vesicles (not drawn) represent other plausible pathways.

function is guaranteed upon sealing of the paracellular pathway between enterocytes, a process relying on the assembly of different transmembrane and intracellular proteins forming tight junctions, adherens junctions and desmosomes [9]. In particular, tight junctions are multi-protein complexes made of membrane proteins (claudin, occludin), scaffold proteins (zonula occludens I and II) and regulatory kinases (myosin light chain kinase) affecting the integrity of the actomyosin ring and therefore permeability [10]. Paracellular transport across the epithelial barrier is highly regulated and is able to leave access to small molecules ($\leqslant 4$ Å), but also to larger molecules including small peptides and bacterial lipopolysaccharides (500–1500 Da) through the 'leak pathway' [11–13]. Enterocytes can also take up and process macromolecules transcellularly by endocytosis [5]. Most of the absorbed macromolecules will be degraded by the enzymes present in the endocytic vesicles and lysosomes. However, a small proportion of intact antigens can reach the lamina propria, the intestinal lymph and circulation [14]. These antigens are mostly taken up by antigen-presenting cells present in the lamina propria, processed and presented to the surrounding T cells or alternatively to T cells in the MLN, after dendritic cell migration. Interestingly, enterocytes also express MHC class II molecules and can present antigens to intra-epithelial lymphocytes [15]. Exosomes, defined as MHC class II molecules-bearing membrane vesicles released from enterocytes, might also convey antigen-derived peptides across the basal membrane and activate local T cells to induce an immune response [16]. It is uncertain whether this takes place *in vivo* and the relative importance of this mechanism in the induction or the maintenance of oral tolerance compared with the other antigen-presenting cells remains difficult to evaluate.

A particular subset of dendritic cells ($CD103^-$, $CX3CR1^+$) is known to extend their dendrites in the lumen to collect bacteria under steady-state conditions, a process further accentuated upon inflammation [17]. It is not yet known whether this process can also take place for food antigens and whether this could contribute to the selective passage of antigens across the intestinal barrier and further presentation to the mucosal immune system.

Induction of oral tolerance

Dendritic cells derived from the gut (either from PP, ILF or lamina propria) display a special tolerogenic phenotype compared with systemic dendritic cells [18, 19]. This confers to the majority of gut-associated dendritic cell subtypes the ability to preferentially generate a tolerogenic immune response, mostly by generating regulatory T cells [20]. It remains unclear whether induction of the immune response can take place within the tissue exclusively or whether antigens must reach the MLN to be presented to T cells. Oral tolerance to ovalbumin is partially abrogated when migration of dendritic cells to MLN is abolished [21], suggesting that at least a portion of dendritic cells needs to reach the MLN to initiate efficient oral tolerance. It is generally accepted that feeding of a high dose of protein antigen leads to tolerance acquisition mediated by T cell clonal anergy and apoptosis [22]. Conversely, active suppression resulting in the onset of populations of regulatory T cells with various phenotypes (Th3, Tr1, $CD4^+CD25^+$) takes place after the oral delivery of a low dose of antigen [23]. This suggests that the amount of antigen encountered, and thus its degree of passage through the intestinal barrier, influences the type of response subsequently generated. The outcome of the immune response can vary from no detectable response (ignorance) to the induction of antigen-specific antibodies such as IgGs and secretory IgAs (SIgA) in large amounts. The difference in the intensity of the response depends on many factors such as the genetic background of the individual, the nature of the antigen and its resistance to protease.

In genetically susceptible individuals, however, the process of oral tolerance is abrogated and an inappropriate immune response towards an antigen is mounted. The generation of allergen-specific Th2 cells secreting IL-4, IL-13 and IL-9 is observed and will favour the production of allergen-specific IgE by plasma cells. Mast cells – the key effector cells in food allergy – are recruited in the lamina propria and bind allergen-specific IgE on the membrane high-affinity FcεRI. Upon second exposure to the allergen, during the effector phase, mast cell degranulation occurs as a consequence of bound IgE cross-linking by allergens. The impact of allergic reactions on gut permeability during the effector phase is described in more detail in the next sections.

Intestinal permeability in patients with food allergies

Early studies of subjects with food allergies demonstrated that intestinal permeability towards sugars, assessed using the lactulose/mannitol (L/M) test, was increased in patients after an allergen challenge [24] or in patients before an eviction diet [25]. The values of L/M ratio returned to normal when patients followed a strict eviction diet, demonstrating that increased intestinal permeability is a specific consequence of allergic reactions. A similar study, however, identified augmented L/M ratio in food allergy patients on an eviction diet since 6 months. Interestingly, not only was intestinal permeability increased compared with healthy controls but it also positively correlated to their severity of symptoms [26]. This suggests that increased intestinal permeability might also constitute a cause of allergic reactions, or alternatively, that the allergic status of the intestinal mucosa remains present even long after the last allergen exposure and prevents intestinal permeability from returning to normal values.

Although the sugar permeability test is a convenient non-invasive method to measure changes of intestinal

permeability, it reflects the passage of small molecules through the mucosa and does not necessarily imply a high permeability to larger antigens such as protein allergens. *Ex vivo* or *in vitro* studies offer an alternative to evaluate intestinal permeability more specifically. Measurement of transepithelial electrical resistance, either of tissues mounted in Ussing chambers or of epithelial cell monolayers grown on filters, reflects the passage of ions through the paracellular pathway. In addition, the passage of larger molecules, such as high-molecular-weight dextrans, horseradish peroxidase or specific allergens can also be quantified, which is largely indicative of epithelial crossing along the transcellular route. Using these techniques, it was demonstrated that the transcellular passage of horseradish peroxidase was increased in jejunal biopsies of allergic children and returned back to normal after an eviction diet [27].

Altogether, these data indicate that intestinal permeability towards small molecules, sugars and allergens is increased during the effector phase of allergic reactions, a process relying on several different pathways. To shed light on the underlying molecular mechanisms leading to the disturbance of intestinal permeability during an allergic reaction, studies with rodent models of sensitization turned out to be highly instructive.

Molecular mechanisms leading to increased intestinal permeability during allergic reactions

Impact of sensitization and mast cell degranulation on intestinal permeability

A series of elegant studies using intestinal tissues from sensitized rats mounted in Ussing chambers demonstrated that intestinal permeability alterations occur in a biphasic manner. Within 2 min following challenge of the sensitized epithelium by the allergen (phase I; Fig. 2), the latter was taken up by enterocytes transcellularly, rapidly distributed in endosomal compartments and recovered in the lamina propria. This phenomenon was enhanced by

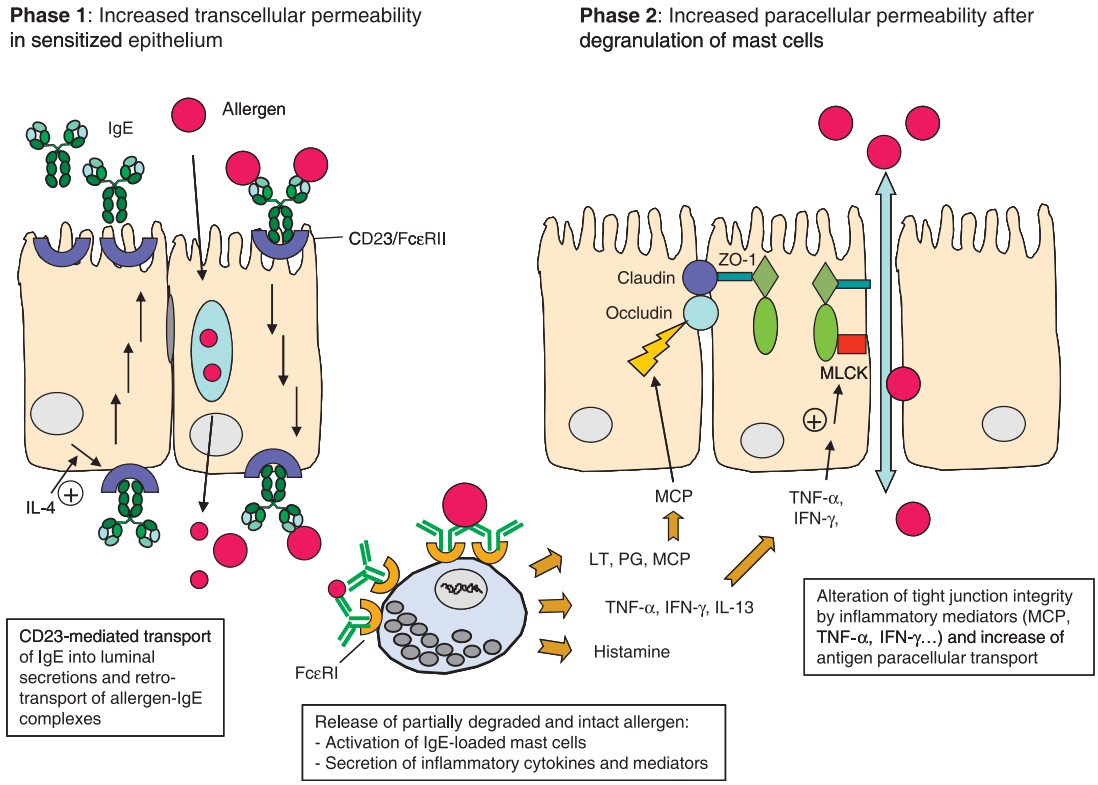

Fig. 2. Mechanism of increased intestinal permeability in the sensitized epithelium. In allergic subjects, mast cells loaded with allergen-specific IgE are present in the lamina propria. Small amounts of intact protein can pass transcellularly and trigger mast cell activation via cross-linking of bound IgE. In addition, elevated IL-4 present in individuals with an atopic background contributes towards up-regulation of the low-affinity IgE receptor (CD23, FcεRII) on the basolateral and apical poles of intestinal epithelial cells. This triggers the secretion of luminal IgE produced by IL-4-induced plasma cells; upon binding of dietary antigens, transepithelial transport back to the lamina propria is initiated, leading to the passage of intact antigen capable of binding and activating mast cells (phase I). Upon degranulation of mast cells, mediators such as cytokines, histamine, leukotrienes (LT), prostaglandins (PG) and proteases (MCP) are released and influence ion secretion and modify paracellular permeability (phase 2). Alteration of the epithelial permeability occurs upon disorganization of the actomyosin ring through activation of myosin light-chain kinase (MLCK) and changes in the architecture of tight junctions resulting from clipping of occludin by MCP. This leads to the entry of greater amounts of undigested allergen through the paracelluar pathway, which further strengthen the intensity of the allergic reaction.

sensitization of the animal, but was independent of the presence of [28], or activation [29], of mast cells. In the second phase, starting a few minutes after allergen exposure, massive allergen translocation occurred through the paracellular pathway (Fig. 2). This increase of paracellular permeability was dependent on both sensitization and the presence of activated mast cells. Secretion of chymases by activated mast cells affected tight junctions within 20–30 min and opened the otherwise sealed paracellular route, and eventually allowed intact antigen to cross the disturbed barrier [30].

In addition, mouse mast cell protease-1 was shown to increase mucosal leakiness via the degradation of the tight junction protein occludin [31]. Besides chymases, mast cells also release histamine, which promotes the secretion of both mucus and electrolytes, thus impacting on the physiology of the mucosa [32]. Mast cells secrete a wide range of cytokines such as TNF-α, IL-13 and IL-8, which are all implicated in the regulation of epithelial cells' permeability. TNF-α is known to influence both paracellular and transcellular permeability *in vitro* and *in vivo* [33, 34]. IL-13, a Th2 cytokine involved in an allergic reaction, also influences the paracellular permeability (measured with dextran passage and TER) either through PI3K activation and apoptosis induction [35], or by activation of STAT6, leading to increased ion selectivity and diminished TER [36]. IL-4, another Th2 cytokine secreted by activated Th2 cells, also causes a reduction of TER and enhances transepithelial fluxes of Dextran 4000 in T84 cells [37] and *in vivo* [38]. The synergizing combination of these events promotes an increase of paracellular permeability post-degranulation, triggering a massive passage of allergen molecules, thus resulting in more severe local and possibly systemic reactions.

A prerequisite for the degranulation of mast cells was the transcellular transport of the allergen, which was increased in sensitized epithelium even before degranulation of mast cells can be measured [28, 29]. It was then demonstrated that allergen–IgE complexes were transported from the lumen to the serosa via the low-affinity IgE receptor FcεRII (CD23) expressed on enterocytes [39]. Enhanced transepithelial antigen uptake was abolished in sensitized CD23 knock-out mice, as well as in IL-4 invalidated mice, in support of the regulatory function of the cytokine in CD23 expression [40]. This is consistent with the overexpression of CD23 in intestinal epithelial cells of patients with food allergy [41] and elevated levels of allergen-specific IgE in intestinal secretions of allergic patients [42]. Two major isoforms of CD23 exist (CD23a and CD23b localized at the apical pole only), resulting in the bidirectional transport of IgE mediated by CD23a, and different kinetics of apical to basolateral transport of allergen–IgE complexes [43]. In addition, different splice forms of CD23b were identified, which selectively transcytose allergen–IgE complexes or free IgE [44]. Together, this confers to the various forms of CD23 the capacity to bring IgE in luminal secretions, and transport allergen-bound IgE back into the serosa rich in mast cells. Notably, the uptake of IgE–allergen complexes from the luminal side protects from lysosomal degradation [45], thus ensuring that intact, allergenic forms reach the serosal environment. How cellular exocytosis of free allergen takes place following CD23/IgE-mediated epithelial transport remains to be established.

The sum of these studies unravelled the multi-partner processes implicated in the increase of intestinal permeability observed after allergen-induced degranulation of mast cells, and shed light on one mechanism of entry of the allergen after sensitization. Besides the entry of allergen complexed to IgE, free allergens can cross the epithelium and trigger allergic reactions; the importance of the molecular form and cellular processing for its allergenic properties is discussed in the next section.

Importance of the three-dimensional structure of the allergen for the induction of allergic reactions

As mentioned earlier, antigens are largely modified and degraded by enterocytes within the endocytic vesicles during their passage along the transcellular pathway. Likewise, such modifications have important implications during the effector phase of allergic reactions. Transport of a model antigen across polarized Caco2 cell monolayers was increased by structural changes resulting from chemical modifications, digestion or exposure to a lactic acid bacterium [46]. Further, passage through the epithelial cell induced conformational changes in the native antigen, resulting in reduced immunogenicity. Incubation of modified antigen with rat basophil leukaemia cells loaded with specific IgE led to different degrees of activation, indicating that variations in immunogenicity did not correlate strictly with allergenicity. The importance of the three-dimensional nature of epitopes has been studied in allergic humans: individuals who possess IgE specific for linear epitopes were reactive to any cooked or partially digested forms of the antigen, whereas those with IgE recognizing predominantly conformational epitopes turned out to exhibit partial clinical tolerance [47].

Moreover, the degree of aggregation of an allergen influenced its ability to cross the epithelium and thus to induce degranulation of mast cells. A study conducted by Roth-Walter et al. [6] demonstrated that particulated antigens such as caseins (naturally found as micelles) or aggregated α-lactalbumin (ALA) and β-lactoglobulin (BLG) (induced after pasteurization) could not be taken up by epithelial cells, but instead were directed to M cells of PP. This contributed to reduce allergic responses of sensitized animals, because aggregated allergens could not reach mast cells located under the enterocytes. By comparison, soluble ALA and BLG were transcytosed by

enterocytes and were thus more prone to induce allergic reactions in the mucosa. This indicated that structural changes in processed food somehow protect against allergic reactions in sensitized animals by impairing uptake through epithelial cells. However, directing allergens to PP by aggregation significantly increased the Th2-associated antibody and cytokine response, and thus potentially enhanced sensitization of the animal to this allergen. Intriguingly, as for intestinal IgE, the level of SIgA was higher in mice sensitized with aggregated proteins than with soluble ALA or BLG. One can speculate that tetravalent SIgA neutralizes allergen, leading to its aggregation, therefore adding to competition for binding to IgE the capacity to direct complexes in the mucus and PP [48], ultimately limiting allergic responses.

Implication of allergen neutralization by SIgA for allergic reactions

Neutralization of allergen occurs with the abundant SIgA found in gastrointestinal secretions and contributes towards limiting the access of allergen to the lamina propria and thus the inflammatory responses [49]. In addition, neutralization by IgA inhibits FcεRI-dependent activation of mast cells [50]. SIgA immune complexes are retrotransported in PP and interact with dendritic cells [51], resulting in attenuated antigen-specific antibody production, T cell response and DC maturation. In combination with an antigen carrying danger signals, SIgA are likely to favour a reduction of cytokine expression including IL-6, IFN-γ and TNF-α, with no effect on IL-10 involved in the maintenance of tolerance [52]. It is thus tempting to speculate that the retrotransport of antigen–SIgA complexes is important for the maintenance of tolerance towards innocuous proteins. The importance of IgA is further illustrated by the finding that mice sensitized with BLG have much lower IgA-producing cells in PPs, as well as reduced faecal SIgA when compared with mice actively tolerized with the same protein [53]. However, the presence of allergen-specific SIgA is not always increased in successfully tolerized animals, and can even be present in large amounts in sensitized ones without conferring protection [54]. Further, the importance of SIgA against allergic diseases remains unclear with respect to recent clinical studies; patients with IgA deficiency display an increased risk of food hypersensitivity at the age of 4 years solely [55], whereas in another cohort, IgA deficiency does not show any correlation with food allergy [56]. Furthermore, pIgR KO mice, which are unable to produce secretory IgA and have increased intestinal permeability, display a greater immune response towards bacteria, but not towards food antigens [57]. This suggests that SIgA production is more critical for homeostasis towards commensal bacteria than food antigens. Further studies are required to clarify the importance of SIgA in the maintenance of oral tolerance, and hence the integrity of the intestinal barrier.

Overall, although it is clearly established that intestinal permeability is increased as a consequence of sensitization and during the effector phase of an allergic reaction, a question that remains is whether increased intestinal permeability can constitute a cause of sensitization and subsequent allergy.

Could increased intestinal permeability predispose to food allergies?

Clinical studies evaluating intestinal permeability by L/M tests revealed contradictory results in patients on an eviction diet [25, 26], making it difficult to evaluate whether increased intestinal permeability is a constitutive defect. Furthermore, because intestinal permeability values of sensitized mucosa (even on an eviction diet) are most likely not representative of the values obtained before allergy develops, these parameters do not allow to draw definitive conclusions.

Hence, does increased intestinal permeability lead to food antigen sensitization? Many barrier defects have been associated with autoimmune bowel inflammatory diseases [58], but to date, none of them has been found to be specific to food allergy/hypersensitivity. This suggests that intestinal barrier abnormalities alone are not sufficient to trigger dietary allergies. Likewise, pathological conditions resulting in increased permeability such as helminth infection appear to be protective against food allergy [59]. In contrast, *Helicobacter pylori*-associated gastritis in humans has been shown to correlate positively with food allergy [60]. Increased passage of intact or improperly processed antigen across the epithelium has been identified as a possible mechanism [61]. Many confounding factors such as the induction of regulatory T cells following infections, or the dilution of allerqen-specific IgE in parasite-specific IgE upon parasite infections prevent to end up with solide causative evidence that increased intestinal permeability is linked to the development of food allergies.

A piece of evidence might come from an interesting study demonstrating that overexpression of IL-9 in the gut predisposes mice to oral antigen sensitization through a combined increase of intestinal recruitment of mast cells and intestinal permeability [62]. Remarkably, such a mechanism did not require that mice be initially primed with a mixture of antigen and adjuvant. IL-9-deficient mice failed to develop anaphylaxis, consistent with a central role of the cytokine in the process. Interestingly, IL-9 signalling contributed strictly to oral antigen-induced anaphylaxis, but not parenteral administration, and was mediated by IgE exclusively [63]. Furthermore, when a normal intestinal permeability was restored by the action of the mast cell stabilizer cromolyn, intestinal

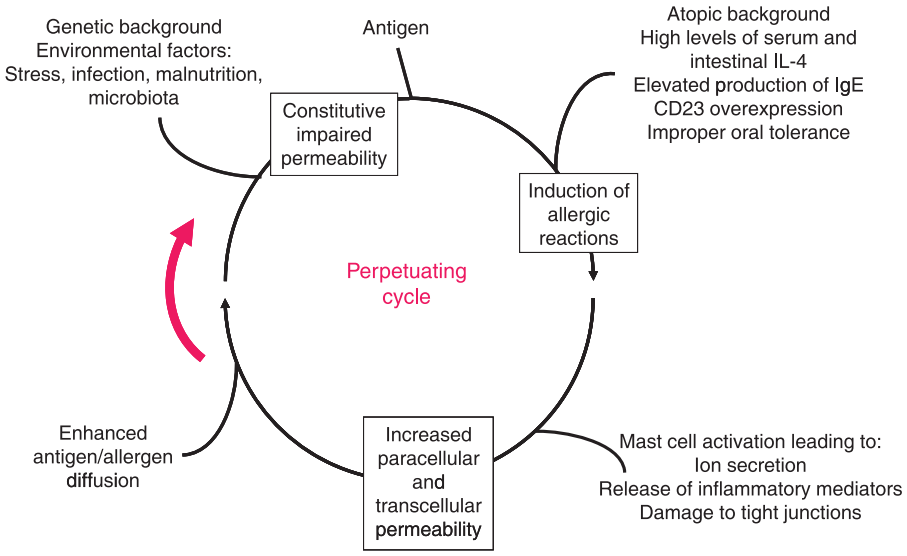

Fig. 3. Intestinal permeability and allergy: cause and/or consequence. Poorly defined factors associated with the genetic background or the environment may serve as priming causes to favour the entry of antigen and elicit the production of antigen-specific IgE in predisposed individuals. This constitutive passage will in turn promote the onset and amplification of allergy-related reactions culminating in the activation of mast cells and release of mast cell mediators with pro-inflammatory properties capable of negatively impacting the epithelial barrier function. Further damages will trigger the uncontrolled passage of more antigen, now considered as an allergen, leading to the perpetuation of the cycle allergen entry–IgE binding–mast cell degranulation–increased epithelial permeability.

anaphylaxis was abrogated, confirming the crucial importance of intestinal permeability for the effector phase of intestinal anaphylaxis. In addition, the total IgE production remained low in cromolyn-treated animals, suggesting that intrinsic sensitization is favoured by increased intestinal permeability. The amounts of allergen-specific IgE were, however, not measured, leaving the question of increased sensitization to allergens vs. increased permeability open.

Conclusion

Although we now know in great detail the mechanisms by which the mucosal immune system is regulated and discriminates between dangerous and innocuous antigens, we do not have much of an understanding as to the basic reasons why a dietary protein will lead to intestinal sensitization. In particular, it remains unclear how the initial priming by an antigen will improperly instruct the intestinal immune system to mount a response that results in the production of IgE, and in conjunction with the recruitment of mast cells, will convert the antigen into an allergen. Partial and somehow frustrating explanations can be found in environmental factors and/or the genetic background, but one has to concede that this is not fully satisfactory to justify the constitutive trigger(s) that dysregulate(s) homeostatic, steady-state processes in atopic individuals. In an attempt to look at the situation from a more optimistic point of view, the accumulation of mechanistic knowledge on what happens in sensitized epithelia can already serve to design more targeted and efficacious treatments to help preserve the integrity of the epithelial barrier. The egg and hen paradigm of who is first makes it difficult, however, to tackle novel avenues of research to eventually answer the question of the cause or the consequence that remains open in the complex relationship between dietary allergy and epithelial permeability (Fig. 3).

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References

1 Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004; **113**:805–19.
2 Erickson RH, Kim YS. Digestion and absorption of dietary protein. *Annu Rev Med* 1990; **41**:133–9.
3 Untersmayr E, Scholl I, Swoboda I *et al.* Antacid medication inhibits digestion of dietary proteins and causes food allergy: a fish allergy model in BALB/c mice. *J Allergy Clin Immunol* 2003; **112**:616–23.
4 Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. *Nat Rev Immunol* 2006; **6**:148–5.
5 Snoeck V, Goddeeris B, Cox E. The role of enterocytes in the intestinal barrier function and antigen uptake. *Microbes Infect* 2005; **7**:997–1004.
6 Roth-Walter F, Berin MC, Arnaboldi P *et al.* Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. *Allergy* 2008; **63**:882–90.

7 Kunkel D, Kirchhoff D, Nishikawa S, Radbruch A, Scheffold A. Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. *Eur J Immunol* 2003; **33**:1292–301.

8 Spahn TW, Weiner HL, Rennert PD *et al*. Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. *Eur J Immunol* 2002; **32**:1109–13.

9 Turner JR Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009; **9**:799–809.

10 Su L, Shen L, Clayburgh DR *et al*. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. *Gastroenterology* 2009; **136**:551–63.

11 Van Itallie CM, Holmes J, Bridges A *et al*. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* 2008; **121**:298–305.

12 Watson CJ, Hoare CJ, Garrod DR, Carlson GL, Warhurst G. Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J Cell Sci* 2005; **118**:5221–30.

13 Ménard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. *Mucosal Immunol* 2010; **3**:247–59.

14 O'Hagan DT, Palin KJ, Davis SS. Intestinal absorption of proteins and macromolecules and the immunological response. *Crit Rev Ther Drug Carrier Syst* 1988; **4**:197–220.

15 Hershberg RM, Cho DH, Youakim A *et al*. Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. *J Clin Invest* 1998; **102**:792–803.

16 Van Niel G, Mallegol J, Bevilacqua C *et al*. Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. *Gut* 2003; **52**:1690–7.

17 Niess JH, Brand S, Gu X *et al*. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* 2005; **307**:254–8.

18 Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol* 2008; **8**:435–46.

19 Manicassamy S, Reizis B, Ravindran R *et al*. Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. *Science* 2010; **329**:849–53.

20 Kang SG, Lim HW, Andrisani OM, Broxmeyer HE, Kim CH. Vitamin A metabolites induce gut-homing FoxP3+regulatory T cells. *J Immunol* 2007; **179**:3724–33.

21 Worbs T, Bode U, Yan S *et al*. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006; **203**:519–27.

22 Melamed D, Friedman A. Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. *Eur J Immunol* 1993; **23**:935–42.

23 Tsuji NM, Mizumachi K, Kurisaki J. Interleukin-10-secreting Peyer's patch cells are responsible for active suppression in low-dose oral tolerance. *Immunology* 2001; **103**:458–64.

24 Dupont C, Barau E, Molkhou P, Raynaud F, Barbet JP, Dehennin L. Food-induced alterations of intestinal permeability in children with cow's milk-sensitive enteropathy and atopic dermatitis. *J Pediatr Gastroenterol Nutr* 1989; **8**:459–65.

25 André C, André F, Colin L, Cavagna S. Measurement of intestinal permeability to mannitol and lactulose as a means of diagnosing food allergy and evaluating therapeutic effectiveness of disodium cromoglycate. *Ann Allergy* 1987; **59**:127–30.

26 Ventura MT, Polimeno L, Amoruso AC *et al*. Intestinal permeability in patients with adverse reactions to food. *Dig Liver Dis* 2006; **38**:732–6.

27 Heyman M, Grasset E, Ducroc R, Desjeux JF. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. *Pediatr Res* 1988; **24**:197–202.

28 Berin MC, Kiliaan AJ, Yang PC, Groot JA, Kitamura Y, Perdue MH. The influence of mast cells on pathways of transepithelial antigen transport in rat intestine. *J Immunol* 1998; **161**:2561–6.

29 Berin MC, Kiliaan AJ, Yang PC, Groot JA, Taminiau JA, Perdue MH. Rapid transepithelial antigen transport in rat jejunum: impact of sensitization and the hypersensitivity reaction. *Gastroenterology* 1997; **113**:856–64.

30 Scudamore CL, Thornton EM, McMillan L, Newlands GF, Miller HR. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995; **182**:1871–8.

31 McDermott JR, Bartram RE, Knight PA, Miller HR, Garrod DR, Grencis RK. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc Natl Acad Sci USA* 2003; **100**:7761–6.

32 Bischoff SC, Kramer S. Human mast cells, bacteria, and intestinal immunity. *Immunol Rev* 2007; **217**:329–37.

33 Wang F, Graham WV, Wang Y, Witkowski ED, Schwarz BT, Turner JR. Interferon-gamma and tumor necrosis factor-alpha synergize to induce intestinal epithelial barrier dysfunction by up-regulating myosin light chain kinase expression. *Am J Pathol* 2005; **166**:409–1.

34 Clayburgh DR, Musch MW, Leitges M, Fu YX, Turner JR. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea in vivo. *J Clin Invest* 2006; **116**:2682–94.

35 Prasad S, Mingrino R, Kaukinen K, Hayes KL, Powell RM, MacDonald TT, Collins JE. Inflammatory processes have differential effects on claudins 2, 3 and 4 in colonic epithelial cells. *Lab Invest* 2005; **85**:1139–62.

36 Weber CR, Raleigh DR, Su L *et al*. Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *J Biol Chem* 2010; **285**: 12037–48.

37 Wisner DM, Harris LR III, Green CL, Poritz LS. Opposing regulation of the tight junction protein claudin-2 by interferon-gamma and interleukin-4. *J Surg Res* 2008; **144**:1–7.

38 Madden KB, Whitman L, Sullivan C *et al*. Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. *J Immunol* 2002; **169**:4417–22.

39 Yang PC, Berin MC, Yu LC, Conrad DH, Perdue MH. Enhanced intestinal transepithelial antigen transport in allergic rats is mediated by IgE and CD23 (FcepsilonRII). *J Clin Invest* 2000; **106**:879–86.

40 Yu LC, Yang PC, Berin MC *et al*. Enhanced transepithelial antigen transport in intestine of allergic mice is mediated by IgE/CD23 and regulated by interleukin-4. *Gastroenterology* 2001; **121**:370–81.

41 Kaiserlian D, Lachaux A, Grosjean I, Graber P, Bonnefoy JY. Intestinal epithelial cells express the CD23/Fc epsilon RII molecule: enhanced expression in enteropathies. *Immunology* 1993; **80**:90–5.

42 Belut D, Moneret-Vautrin DA, Nicolas JP, Grilliat JP. IgE levels in intestinal juice. *Dig Dis Sci* 1980; **25**:323–32.
43 Li H, Nowak-Wegrzyn A, Charlop-Powers Z *et al*. Transcytosis of IgE-antigen complexes by CD23a in human intestinal epithelial cells and its role in food allergy. *Gastroenterology* 2006; **131**:47–58.
44 Montagnac G, Yu LC, Bevilacqua C *et al*. Differential role for CD23 splice forms in apical to basolateral transcytosis of IgE/allergen complexes. *Traffic* 2005; **6**:230–42.
45 Bevilacqua C, Montagnac G, Benmerah A *et al*. Food allergens are protected from degradation during CD23-mediated transepithelial transport. *Int Arch Allergy Immunol* 2004; **135**:108–16.
46 Bernasconi E, Fritsche R, Corthésy B. Specific effects of denaturation, hydrolysis and exposure to *Lactococcus lactis* on bovine beta-lactoglobulin transepithelial transport, antigenicity and allergenicity. *Clin Exp Allergy* 2006; **36**:803–14.
47 Vila L, Beyer K, Jarvinen KM, Chatchatee P, Bardina L, Sampson HA. Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. *Clin Exp Allergy* 2001; **31**:1599–606.
48 Kadaoui KA, Corthésy B. Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. *J Immunol* 2007; **179**:7751–7.
49 Smits HH, Gloudemans AK, van Nimwegen M *et al*. Cholera toxin B suppresses allergic inflammation through induction of secretory IgA. *Mucosal Immunol* 2009; **2**:331–9.
50 Pasquier B, Launay P, Kanamaru Y *et al*. Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. *Immunity* 2005; **22**:31–42.
51 Favre L, Spertini F, Corthésy B. Secretory IgA possesses intrinsic modulatory properties stimulating mucosal and systemic immune responses. *J Immunol* 2005; **175**:2793–800.
52 Boullier S, Tanguy M, Kadaoui KA *et al*. Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. *J Immunol* 2009; **183**:5879–85.
53 Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *J Allergy Clin Immunol* 2004; **114**:377–82.
54 Perrier C, Thierry AC, Mercenier A, Corthésy B. Allergen-specific antibody and cytokine responses, mast cell reactivity and intestinal permeability upon oral challenge of sensitized and tolerized mice. *Clin Exp Allergy* 2009; **40**:153–62.
55 Janzi M, Kull I, Sjoberg R *et al*. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol* 2009; **133**:78–85.
56 Aghamohammadi A, Cheraghi T, Gharagozlou M *et al*. IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol* 2009; **29**:130–6.
57 Sait LC, Galic M, Price JD *et al*. Secretory antibodies reduce systemic antibody responses against the gastrointestinal commensal flora. *Int Immunol* 2007; **19**:257–65.
58 Marchiando AM, Graham WV, Turner JR. Epithelial barriers in homeostasis and disease. *Annu Rev Pathol* 2010; **5**:119–44.
59 Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; **296**:490–4.
60 Corrado G, Luzzi I, Lucarelli S *et al*. Positive association between helicobacter pylori infection and food allergy in children. *Scand J Gastroenterol* 1998; **33**:1135–9.
61 Matysiak-Budnik T, Terpend K, Alain S *et al*. *Helicobacter pylori* alters exogenous antigen absorption and processing in a digestive tract epithelial cell line model. *Infect Immun* 1998; **66**:5785–91.
62 Forbes EE, Groschwitz K, Abonia JP *et al*. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med* 2008; **205**:897–913.
63 Osterfeld H, Ahrens R, Strait R, Finkelman FD, Renauld JC, Hogan SP. Differential roles for the IL-9/IL-9 receptor alpha-chain pathway in systemic and oral antigen-induced anaphylaxis. *J Allergy Clin Immunol* 2010; **125**:469–76.