Digestion and Uptake of Dietary Antigens– A Physiological Overview

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The digestive process is likely to be important in the development of sensitisation to dietary antigens. While immediate reactions can be triggered by minute amounts of native antigen at mucosal surfaces in already sensitised persons, the initial sensitisation requires antigen presentation to the adaptive immune system by antigen presenting cells, usually within the gastrointestinal tract. The normal processes of digestion may thus modulate potential sensitisation events by determining the characteristics of dietary constituents, prior to their presentation to the mucosal immune system. For example, reduction of gastric acid production by use of proton pump inhibitors significantly promotes allergic sensitisation in mice and humans by allowing absorption of larger and more immunogenic fragments [1,2].

Digestion and absorption of nutrients

While nutrients may sometimes be ingested as a relatively pure intake of carbohydrate, protein and fat, most meals will consist of some mixture of these major constituents. However, there are distinct absorption mechanisms.

Carbohydrate absorption

Dietary carbohydrates consist mainly of starches, sucrose and lactose. Dietary starches such as amylopectin are digested by amylases, which break down α 1,4 bonds between monosaccharides. Amylases encountered are initially of salivary origin (before inactivation by gastric acid) and subsequently pancreatic [3,4]. They do not break down α 1,6 bonds, so complex carbohydrates remain intact at this stage. Amylase digestion releases simpler sugar structures, including maltose, maltotriose and polymerised oligosaccharides of up to 9 sugars. The subsequent hydrolysis of disaccharides, oligosaccharides and complex oligosaccharides occurs at the enterocyte brush border mediated by α-dextrinase and glucoamylase, which liberate glucose from polymers, and the disaccharidases lactase and sucrase-isomaltase.

Subsequent absorption of monosaccharides is mediated by specific enterocyte carrier proteins, notably the sodium glucose linked transporter (SGLT-1), which actively transports glucose into the enterocyte, and the glucose transporter-5 molecule (GLUT5), which transports fructose in a non sodium dependent fashion [3,4]. The monosaccharides are transported out of the enterocyte by facilitated transport mediated by another glucose transporter GLUT2.

Although dietary carbohydrates are not usually considered as having potential for sensitisation, they may have indirect effects. Glucose absorption via SGLT-1 has effects upon epithelial barrier function, including induced rearrangement of tight junction molecules and enhancement of paracellular permeability [4,5]. Classic experiments by Attisook and Madara demonstrated paracellular permeation of an 11 amino acid oligopeptide during glucose absorption [5]. While this chain length is just too short for optimal activation of T cells following antigen presentation, modest further increase in paracellular permeability due to enteropathy of whatever kind may allow penetration of sufficient intact antigen to allow T cell responses [3,4].

Effects of small intestinal enteropathy on carbohydrate absorption

Enteropathy may allow carbohydrate-induced immune effects due to malabsorption within the small intestine, as expression of lactase or sucrase-isomaltase are downregulated upon mucosal T cell activation [4]. Malabsorbed carbohydrates pass down the gut, and are used as metabolic fuel by the gut flora. Different bacteria have different abilities to use malabsorbed carbohydrate, variably upregulating metabolic genes in the presence of mucosal lactose (the classic *lac*-operon discovered by Jacob and Monod in *E coli* includes β-galactosidase, lactose permease and thiogalactoside transacetylase). Specific bacteria, such as *bacteroides thetaiotamicron* function as scavengers of unabsorbed dietary complex carbohydrates, and may in fact induce gene expression within the enterocytes to facilitate this process, to mutual benefit [6]. Intriguingly, amongst the genes induced within the host by this organism [7], small proline rich protein 2a (sprr2a) has been implicated in mucosal allergic responses [8].

In addition to bacteria, luminal fungi may also use dietary carbohydrates for energy substrates. While the role of gastrointestinal fungal colonisation in allergy has been controversial, recent studies of antibiotic-induced fungal dysbiosis have demonstrated that these organisms may induce T_H2 cell recruitment to the lung, and support pulmonary allergic

responses [9]. Carbohydrate absorption mechanisms may thus have indirect effects upon mucosal tolerance events.

Protein absorption

Clearly the most important direct effects of dietary components upon allergies relate to proteins. Protein absorption starts within the lumen of stomach, where gastric acid denatures and activates pepsinogens I and II into pepsins [3,4]. These have a broad specificity, preferentially splitting peptide bonds at phenlylalanine, tyrosine and leucine residues. Subsequent proteolysis is efficiently mediated by pancreatric enzymes released in response to cholecystokinin, and polypeptides are digested by endopeptidases of different specificity. Trypsin, released from trypsinogen by brush border enterokinase, splits newly-formed peptides only at basic amino acids at the amino end, chymotrypsin splits at aromatic amino acids and elastase uncharged small amino acids at the carboxy end. There are several active transporters of amino acids and dipeptides located on the apical enterocyte surface, while peptides are broken down into amino acids within the enterocyte.

Fat absorption

Bile production is important in fat absorption, as their detergent-like effects allow emulsification of fats into small globules within the lumen [3,4]. Digestion of dietary fats is then promoted by pancreatic lipase, a water-soluble enzyme that acts on the surface of globules to produce free fatty acids and monoglycerides from dietary lipids. Fat absorption then requires initial formation of micelles within the glycocalyx, a process promoted by bile salts but also requiring surfactants and alkaline phosphatase [10,11]. The lipophilic components of digested fats are then taken up directly by the enterocytes [12]. Putative transporter molecules include CD36 (fatty acid translocase) and fatty acid transporter protein 4 (FATP4), while intracellular transport of ingested fats is mediated by intestinal fatty acid binding protein (I-FABP) [12]. The potential relevance of fat intake in allergic sensitisation has been poorly recognised, but recent data have identified a mechanism in which lipids may induce T cell and cell activation following presentation by the non-classical MHC molecule CD1d [13,14].

Bulk antigen absorption

In addition to breakdown and absorption of the major components of the diet, there exists also pathways for uptake of larger molecules, potentially including whole proteins [15,16]. Thus molecules such as horseradish peroxidase (HRP) may be transported through the cell and

presented to the mucosal immune system, either intact or undergoing partial digestion [17]. A shuttling mechanism, involving clathrin-coated pits, facilitates this sampling of luminal contents [16]. The molecules absorbed in this way are then directed to specific intracellular compartments, and may then be presented to the mucosal immune system after loading onto both classical and non-classical Major Histocompatability Complex (MHC) molecules [18,19]. Enterocytes may thus function as non-professional antigen presenting cells (APC's) [19]. They do not express co-stimulatory molecules such as CD80 or CD86, and will thus tend to induce anergy in potentially reactive T cells [19]. This may make a significant contribution to the mechanisms of oral tolerance.

While class II MHC molecules are not normally expressed by small bowel epithelial cells, they are upregulated by T_H1 mediated immunopathology, in response to increased interferon-γ, and are thus strongly expressed in conditions such as coeliac disease and tropical enteropathy. Antigen transport from the lumen is also enhanced in the presence of IFN-γ [17,20]. The combination of enhanced transport and induced expression of Class II MHC may allow presentation of antigens to CD4 T helper cells in circumstances of T_H1-mediated mucosal inflammation.

Intraepithelial cells are distinct from lamina propria lymphocytes, being dominated by CD8 and γδ T cells, as well as Natural Killer (NK) cells and rare populations unusual in other sites. While classic CD8 cells respond to endogenous antigen after presentation by class I MHC molecules, the other intraepithelial lymphocytes may respond to a variety of exogenous antigens, presented by non-classical MHC molecules such as CD1d [21]. The potential importance of this pathway is that antigen presentation is not limited to peptides alone, and indeed glycolipids and lipid molecules themselves may modulate T cell reactivity [21]. While this phenomenon has been best studied from the viewpoint of host defence, recent evidence suggests that this may play a role in allergic responses to lipid antigen. A T_H2 dominated response to inhaled plant lipid antigens, inducing IgE-mediated allergy, was induced by CD1d-mediated presentation in mice [13,14]. Similar responses were seen to ingested ovalbumin [13]. These emerging data suggest that the lipid composition of food antigens may also play a role in sensitisation, supporting experimental data of enhanced IgE sensitisation to β-lactoglobulin in rats by using milk (which contains only 10% β-lactoglobulin) in comparison to purified β–lactoglobulin alone [22].

Penetration of intact antigens from the lumen

Paracellular permeability

Overall permeability of the intestinal barrier is greatly increased during foetal life. In some species, such as the pig, this enhanced permeability continues after birth prior to a rapid reduction of paracellular permeability, known as "gut closure". Evidence in preterm infants, based on penetration of β-lactoglobulin from ingested cows' milk-based feeds, suggests that gut closure occurs in humans before birth, and probably before 33 weeks gestation [23]. Factors subsequently increasing paracellular permeability may promote sensitisation to dietary antigen by allowing ingress of antigen fragments sufficiently large to allow presentation by subepithelial dendritic cells and macrophages in a pro-inflammatory context. Thus sensitisation to cow's milk protein in infants classically followed infectious gastroenteritis [4]. Within the developing world, the prognosis of tropical enteropathy may directly correlate with measurements of paracellular permeability [24]. Cytokines inducing paracellular leakiness include IFN-γ and TNF-α, while those having the opposite effect of promoting tight junction integrity (IL-10 and TGF-β) also play a central role in mucosal immune tolerance [25,26,27]. Molecules regulating tight junction integrity include zonulin (ZO-1) and claudins, which may be modulated by cytokines and pathogens [28]. A further contribution to paracellular barier function comes from charged proteoglycans on the basolateral epithelial surface, and their loss or degradation allows penetration of macromolecules such as albumin in either direction across the epithelium [29].

Antigen sampling from the lumen

In addition to bulk antigen ingestion by the intestinal epithelium, and presentation to the immune system by absorptive enterocytes, there are specific mechanisms by which dietary antigen may be sampled from the gut lumen and presented to the immune system [30]. Specialised epithelial cells, named microfold (M) cells after their ultrastructural appearance, allow easy ingress of both bacterial and dietary antigens because of their relative porosity [30]. These are situated abundantly within the dome epithelium of organised lymphoid tissues within the gut, notably the Peyer's patches of the terminal ileum. Permeation of antigen is substantially enhanced through follicle-associated epithelium, occurring through enhanced transcellular permeation via endosomal compartments [31]. Thus epithelium appears functionally distinct in this region, rather than simply leakier.

M cells were previously thought to be located exclusively within the dome epithelium of organised lymphoid tissue. Recent findings point to a more widespread location, and they may be identified within the epithelium of villi (villous M cells) in mice, where they appear to have similar function of antigen sampling [32]. While dendritic cells are plentiful within organised lymphoid tissue beneath M cells, and may thus recognise antigen that has trafficked through this specialised epithelium, there is also evidence that they may sample luminal antigen more diffusely, away from organised follicle-associated epithelium. *In vitro* data suggested that they might directly affect tight junction integrity, allowing themselves to extend processes through into the lumen [33]. This process is mediated by their expression of molecules such as ZO-1, occludin and claudin and dependent upon the chemokine fractalkine (CXC3L) by the epithelium and its receptor on dendritic cells [33,34,35,36].

M cells may be induced *in vitro* by co-culture of epithelial cell monolayers with B cells [37,38]. This implies that ongoing mucosal immune reactivity might potentially further upregulate antigen uptake by increasing the proportion of M cells to absorptive enterocytes. One other mechanism by which the M cell phenotype may be induced is by ingestion of non-steroidal anti-inflammatory drugs (NSAIDS) such as indomethacin [39]. Increased M cell formation was induced *in vivo* in rats within 12 hours of a single injection of indomethacin, in association with local infiltration of lymphocytes.. Although this regimen is sufficiently potent to induce significant bowel inflammation in rats, the potential consequences of NSAID ingestion may theoretically promote sensitisation by both increase in paracellular permeability and by direct antigen uptake and presentation through M cell hyperplasia.

Bacterial exposures represent an important modulatory factor for antigen delivery and responses, not least by regulating dendritic cell function. Importantly, blockade of innate immune responses to the gut flora induced dietary sensitisation in transgenic mice [40]. Bacterial exposure also has a trophic effect on M cell numbers, although localised at specific sites within follicle-associated epithelium [41]. Expression of the microbial pattern recognition molecules toll-like receptors (TLRs) differs between M cells and absorptive enterocytes, with increased apical expression of TLR4 [42]. Transport of bacteria across M cells was found to be dependent on expression of TLRs and integrins [43]. Uptake mechanisms for dietary antigens are so far less clear within this specialised epithelium.

Regulation of antigen absorption by IgE and IgA

Recent evidence points to a direct role of IgE in antigen uptake that appears to be antigen-specific. Sensitisation of mice to HRP was followed by induced mast cell activation within resected jejunal specimens [44]. Even prior to mast cell activation, transport of HRP (but not a control dietary antigen) was increased from the mucosal surface, mediated by endocytic uptake [44]. HRP was extensively distributed within the enterocyte, and penetrated through to the subjacent lamina propria. Permeation was strikingly less in unsensitised controls, and was restricted to the apical compartment. The effect of mast cell activation was to promote additional paracellular transport of HRP through enhanced tight junction leakiness. Thus the physiological sequence, if applicable to humans, would be of enhanced antigen-specific transcellular uptake of sensitising antigens, followed by a wider antigen non-specific increase in paracellular uptake if subepithelial mast cells were activated by the initially penetrating antigen.

The low affinity IgE receptor CD23 (FcεRII) is expressed by enterocytes following allergic stimulation [45]. This may have physiological consequences for ingestion of allergens. Another study of uptake of HRP after sensitisation also demonstrated significantly increased uptake compared to unsensitised mice, and intact HRP was visualised within the serosal compartment within 10 minutes of mucosal exposure [46]. This increased flux was significantly decreased by anti-CD23 antibody, suggesting that it is involved in what appears to be a protected antigen transport pathway. Similar results were obtained for the milk antigen β-lactoglobulin in sensitised mice [46]. These pathways of enhanced allergen uptake may be inhibited by medications. Experimental data suggests that the enhanced uptake of "bystander" proteins by intestinal epithelium, following specific antigen sensitisation, may be reduced by necrodomil or corticosteroids, but only corticosteroids reduced paracellular sugar permeability [47].

In contrast to the facilitatory role played by IgE in allergen absorption, IgA may have a contrary effect of limiting penetration and sensitisation [48]. Childhood IgA deficiency may therefore promote early sensitisation [49]. IgA regulation may occur at three sites, within the lumen and within and beneath the epithelium. The transport of IgA from the lamina propria across the epithelium is mediated by binding to the polymeric immunoglobulin receptor. Secretory IgA is thus densely expressed within the luminal glycocalyx, and presents an antigen-specific barrier for ingress of luminal antigen following sensitisation. During its

intracellular transit to the lumen, IgA is capable of binding antigen and thus to block apical to basal transcytosis, as has been demonstrated for HIV virus [50]. Finally, subepithelial binding was demonstrated in a transgenic mouse model, in which animals carrying ovalbumin specific T cells were sensitised by oral antigen - subsequent parenteral antigen administration was followed by its excretion across the gut lumen [51]. The intensity of antigen expression within the epithelium mirrored that of the polymeric immunoglobulin receptor, and was strongest in the crypts. Further study showed a unidirectional transfer from basal to apical surfaces, only when the antigen was bound to IgA as an immune complex [51]. Thus the long recognised secretion of IgA, from the mucosal to luminal surface of the small intestine, may function in part as an antigen-excretory system.

Antigen absorption in inflammation

Enhanced transcellular antigen transport has been reported in inflammatory bowel disease, in addition to previously recognised increases in paracellular permeability [52]. A distinct change in enterocyte phenotype has been reported in these circumstances, whereby HRP and ovalbumin were transported unusually rapidly to late endosomes and trans-Golgi vesicles within freshly resected human intestine, in comparison to tissue from patients without inflammatory bowel disease [51]. Cells showing this phenomenon were called Rapid Antigen uptake into the Cytosol Enterocyte (RACE) cells [53]. Whether these are a truly distinct lineage or simply reflect sublethal stress events is unknown, and their presence in other enteropathies has yet to be reported. RACE cells show overlap features with M cells, although differing in some features of intracellular antigen location. It is thus possible that these cells may represent the human analogue of the villous M cells recently reported in rodents [32]. As these cells are shed preferentially into the lumen, leaving a gap within the epithelial monolayer, it is also possible that these represent cells in early apoptosis, although expression of caspase-3 is not increased above normal epithelium [53]. Such inflammation-induced enhancement of transcellular permeability may have implications in other dietary sensitising events. Certainly there is evidence of enhanced gliadin antigen delivery to Golgi complexes in coeliac disease, and its association within this compartment of what appeared to be shed αβ and γδ T cell receptors [54].

Other modulators of sensitisation

Many other factors may alter epithelial permeability, and thus promote sensitisation. Heat stressed epithelium shows increased transcellular transport, as seen in inflamed epithelium [55].

There is experimental evidence suggesting that chronic psychological stress may also promote transepithelial penetration of antigen through follicle associated epithelium [56]. Subsequent experiments have confirmed in rats that chronic stress can indeed provoke sensitisation to luminal contents [57]. Stressed rats developed evidence of both intestinal inflammation and IgE antibodies against ingested HRP, abrogated by antagonist to corticotrophin-releasing hormone (CRH) [57].

Intestinal surfactants play a role in fat absorption, in particular promoting micelle formation [10,11], while exogenous surfactants may be found in a variety of commercially available foodstuffs. Study of intestinal epithelial monolayers gives *in vitro* evidence that transepithelial resistance is lowered by exogenous surfactants, and found evidence of cytoskeletal rearrangements within the enterocyte that promoted paracellular leakiness, in this case permitting excess permeation of ovomucoid [58]. Exogenous surfactants may induce mast cell degranulation [59] while endogenous surfactants such as surfactant protein-D may have a contrasting effect of inhibiting activation [60]. In addition, ingestion of ethanol may induce excess penetration of larger antigen fragments, due to the effects of ethanol of increasing paracellular permeability - this effect was most marked following chronic ethanol exposure [61].

These data collectively suggest that the processes of antigen presentation to the mucosal immune system may be modulated by a variety of factors, including stress, common medications such as NSAIDS, ingested surfactants and alcohol consumption. This might in part account for variability in threshold sensitivity for severe intestinal allergic responses, particularly as mast cell reactivity may also be upregulated by many of the same physical and chemical stressors, likely mediated via transient receptor potential (vanilloid) (TRPV) cation channels [57,59, 62-64]. There is potential for establishment of a vicious circle favouring exaggerated immune reactivity to food allergens in such circumstances.

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