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REVIEW

Indication of Immune Activation in Patients with Perceived Food Hypersensitivity

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Abstract Majority of the patients with perceived food hypersensitivity have irritable bowel syndrome (IBS), and a significant proportion of IBS patients also attribute their gastrointestinal complaints to food items. Different factors such as disturbed intestinal fermentation, enteric dysmotility, post-infectious changes and altered microbial flora in the colon as well as psychological disturbances likely play a role in the pathophysiology and symptoms generation in patients with food hypersensitivity. In addition, a number of studies in these patient groups indicate that local, systemic and mucosal immune systems are activated. The question now is no longer intestinal immune activation, but how the immune system is activated in these patients. In the following review, the potential pathogenetic role of the immune system and evidence of immune activation are reported in patients with perceived food hypersensitivity.

Keywords Food hypersensitivity · Food allergy · Irritable bowel syndrome · Immune activation · Immune system · Intestinal permeability

Introduction

Food hypersensitivity is an overarching term which covers all kinds of adverse reactions to food. Almost one-third of the general population report hypersensitivity reactions to food, but the prevalence of food allergy in adults by showing IgE sensitization is very low, around 1–3 % [1]. Food hypersensitivity reactions consist of any abnormal reaction after ingestion of a food, and include food allergy (immunological mechanism, IgE or non-IgE-mediated), food intolerance (non-immunological mechanisms) and food aversions. The diagnosis is not always easy, and it can create a major frustration for both the patients and health care professionals.

There is an increasing appreciation of the role of the food hypersensitivity to the development of functional gastrointestinal disorders, especially irritable bowel syndrome (IBS). A significant proportion of IBS patients (up to 70 %) attribute their gastrointestinal symptoms to food that activates an abnormal response [2]. Although the symptoms are provoked by meals, the relationship to particular food items is sometimes difficult to show. In IBS patients, the number of positive responses by skin prick test (19.4 %) was significantly higher than in the controls (4 %, $p < 0.01$), suggesting that some patients with IBS have indeed immune-mediated problems with food [3].

Our interdisciplinary research group dedicated to such patients has examined more than 400 patients between 2001 and 2011, and they concluded that the majority (around 90 %) of the patients with self-reported food hypersensitivity had IBS [4]. They had also several other systemic symptoms, psychological disturbances and intestinal fermentation problems as generally seen in patients with IBS. Administration of low-digestible carbohydrates (LDC) often replicated their habitual gastrointestinal symptoms. In addition, a considerable number of patients showed evidence of immune activation as also seen in patients with IBS. Then, the question is no longer immune activation, but how the immune system is activated in these patients (Fig. 1).

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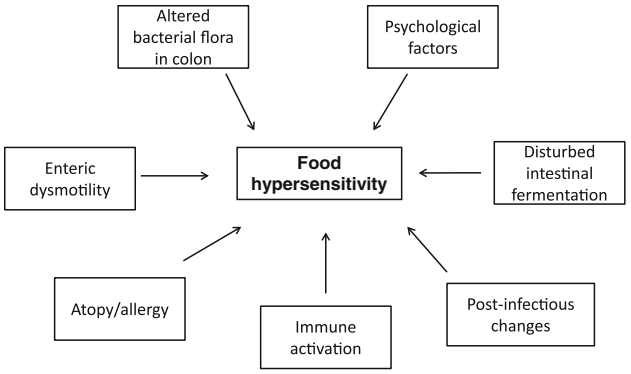

Fig. 1 Schematic representation of biological factors involved in the pathophysiology of food hypersensitivity

This review is focused on recent literature and the results of our own studies on the topic of the potential pathogenetic role of the immune system and indication of immune activation in patients with perceived food hypersensitivity.

Intestinal Barrier and Permeability

The gastrointestinal barrier is a complex structure that allows transfer of fluids, electrolytes and nutrients from gut lumen to the systemic circulation, but at the same time, it protects against invading pathogens and several allergens including foods. There are several components which contribute to this process to provide an active barrier against foreign antigens. They are mainly non-specific defence systems, innate or adaptive immune systems, the mucus layer, epithelial cells and intercellular tight junctions [5–7].

Briefly, the non-specific defence system in the body consists of gastric acid, digestive enzymes, mucus, an intact epithelial layer forming tight junctions and peristaltic movement [7]. The innate immune responses include phagocytic cells in blood such as neutrophils, eosinophils, and monocytes, and tissue phagocytic cells in the tissue such as macrophages. They also include alternative complement pathways, natural killer cells, toll-like receptors (TLRs) and antimicrobial peptides (defensins and cathelicidins), whereas adaptive immune responses mainly consist of lymphocytes (T or B cells), Peyer's patches (PPs), secretory IgA and cytokines. The innate immune system provides a first line of defence against antigens by neutrophils and macrophages, but they could not always eliminate the allergens. Then, the lymphocytes of the adaptive immune system are involved in this process to provide a more lasting defence [8]. Tight junctions are positioned between epithelial cells in intestinal mucosa, and they are key molecules to provide control of paracellular permeability (pathway). Tight junctions have a complex protein system which are made of membrane proteins (occludin, caludin), scaffold proteins (zonula occludens (ZO-1 and -2) and regulatory kinases (myosin light chain kinase) affecting the integrity of the intestinal barrier, and they regulate transition of electrolytes and small molecules from gut lumen to systemic circulation [9, 10]. In addition, several mediators and cytokines such as proteases, histamine, Th1 (TNF-α, IL-1β) and Th2 cytokines (IL-4, -5 and -13) also have an effect on epithelial barrier function [6].

Changes in intestinal barrier structure, intestinal permeability, can be measured by several permeability marker probes, namely, mono- or oligo-saccharides, ^{51}Cr-labelled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) and related substances, and polyethylene glycol (PEG) [11]. Monosaccharides such as mannitol and L-rhamnose mainly use small transcellular aqueous pores (transcellular pathway), whereas the absorption of lactulose, cellobiose, raffinose (oligosaccharides), and ^{51}Cr-EDTA go through the larger pores located at the tight junction between cells (paracellular pathway). PEG molecules have both hydrophilic and lipophilic properties and may pass through both transcellular lipophilic brush border membranes and tight junctions, depending on its lipid solubility, geometric shape, and molecular size [12].

Most of the existing data on intestinal permeability and food allergies concern children, and the results are contradictory. Children with a cow's milk allergy and asymptomatic children who have been fed cow's milk formula versus those who were breast-fed, have increased intestinal permeability [13]. However, Falth-Magnusson et al. [14] found normal permeability even after challenge with cow's milk. Another study by Jalonen [15] shed light on these findings. Urinary lactulose/mannitol (L/M) ratios that were measured before cow milk's challenge were normal in control subjects and allergic children, whereas after 3 days of continuous challenge, there was a significant increase in permeability [15]. In a similar study, not only was intestinal permeability increased (L/M ratio) compared with healthy controls but it also positively correlated with their severity of symptoms in patients with food allergy [16]. We recently showed indications of "atopic bowel" in patients with food hypersensitivity, and when they were divided into two groups as atopic and non-atopic, atopic patients had significantly increased intestinal permeability of ^{51}Cr-EDTA than non-atopic patients [17]. All findings regarding permeability in patients with food allergy and hypersensitivity suggest that intestinal permeability may not be a consequence of primary (reduced mucosal barrier) but rather a secondary (allergen-induced inflammation) event.

Increased intestinal permeability and changes in tight junctions' proteins have also been shown in patients with

IBS patients. Piche et al. [18] demonstrated elevated permeability on colonic mucosal biopsies from both patients with diarrhoea-dominant IBS (IBS-D) and constipation-dominant IBS (IBS-C). Furthermore, decreased protein levels of both occludin and ZO-1 expression in biopsies were found in patients with IBS-D [19, 20]. Further studies are warranted to explore the possible role of immune activation on intestinal barrier dysfunction.

Systemic Immune Activation

Mediators

Several mediators which are released from mast cells [histamine, tryptase, prostaglandins (PG) and leukotriens (LT)], eosinophils [eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophil peroxidase (EPO), major basic protein (MBP), PG and LT], basophils (histamine and LT) and neutrophils [myeloperoxidase (MPO), human neutrophil lipocalin (HNL), lactoferrin, calprotectin and lysozyme] have been measured in serum, urine and stool in patients with food hypersensitivity [21–25]. The notion that mast cells, eosinophils and basophils become activated after food allergen exposure (provocation test) to skin, lung or intestine, is further emphasized by histological studies showing degranulation and cytokine production in these cell types and by the measurement of enhanced levels of proinflammatory mediators after allergen provocation tests. Increased levels of histamine, ECP and EPX have been reported by several investigators in patients with intestinal food allergy [22, 25, 26]. In addition, methylhistamine which is a metabolite of histamine, can be determined in urine in patients with food allergy [27, 28]. LT and PG have also been used to monitor inflammation in allergic disorders, mostly in patients with airway allergy, asthma and drug allergy. Both LTE_4 and PGD_2 metabolite 9α, 11β-PGF_2 can be determined in the urine, although there is rather limited information on this in relation to food allergic reactions [29]. Regarding cytokines, some studies have reported an imbalance of interleukin (IL)-4 and interferon-γ (INF-γ) in children and adults patients with food hypersensitivity [30, 31]. Furthermore, INF-γ and IL-2 have been reported to be elevated in food allergic reactions [32].

Recent studies on IBS patients have demonstrated increased levels of proinflammatory cytokine such as IL-6 and IL-8 in serum, and as well as elevated levels of IL-1β, TNF-α and IL-6 in unstimulated and lipopolysaccaride stimulated blood mononuclear cell cultures [33]. In addition, an abnormal IL-10/IL-12 ratio, which is an indication for inflammatory response, has also been shown in IBS patients [34]. Although mast cells have mainly been involved during allergic reactions as a key to effector cells, they are also important in host defence, homeostasis, recruitment and activation of eosinophils, B and T cells. Mediators released by activated mast cells evoke enteric nervous system responses, visceral hypersensitivity and increased mucosal permeability, as well as symptom generation, including bowel habit changes and abdominal pain [35].

Dendritic Cells and B Cell-Activating Factor

The intestinal mucosa is constantly in contact with food proteins and foreign microorganisms. Dietary protein antigens interact with specific antigen presenting cells (APCs), which help to activate regulatory T cells, usually resulting in a suppression of immune responses [36]. Dendritic cells (DCs) are recognised as the most potent APCs with a unique ability to initiate and maintain primary immune responses [37, 38]. There are different pathways of antigen sampling in the intestinal epithelium. The particulate (insoluble) antigens are taken up by either DCs which extend their dendrites into the lumen by crossing the tight epithelium or M cells which overlie PPs and can take up antigens and deliver them to subepithelial DCs. However, soluble antigens cross the epithelium through transcellular or paracellular routes, via brush border membrane and tight junctions, respectively [39, 40]. After sampling of antigen by DCs, they present them as peptide-MHC complexes to effector T-cells in lymphoid organs.

Up to 70 % of the IBS patients report adverse reactions to one or more food items, and almost 90 % of IBS patients show evidence of immune activation [2, 41]. Previous immunological studies indicate important functions of T and B cells in patients with IBS [42]. Thus, if intestinal allergens are involved in food hypersensitivity, intestinal DCs may play a role. Therefore, various DC populations in patients with self-reported food hypersensitivity were analysed in one of our studies. Major groups of DCs and cell surface markers were not significantly different between patients and healthy controls, but monocyte-derived DCs (moDCs) from atopic patients expressed significantly more CD38 compared to moDCS from non-atopic patients. Moreover, LPS stimulated moDCs from atopic patients produced significantly more IL-10 compared to non-atopic patients. CD38 expression was also correlated with total serum IgE levels [43].

B cell-activating factor (BAFF) is a member of the tumour necrosis factor (TNF) superfamily and an important regulator of peripheral B cell survival, maturation and immunoglobulin class-switch recombination [44]. Increased concentrations of BAFF in different body fluids were previously shown in autoimmune and allergic diseases, infections and malignancies [45]. Recently,

increased concentrations of BAFF in blood were shown in patients with self-reported food hypersensitivity, indicating immune activation [46].

Local Immune Activation

Luminal Provocation

Local immediate reactions may be implicated in gastrointestinal food hypersensitivity. In previous studies, the investigators isolated an approximately 10-cm long segment in the proximal part of the jejunum by a two-balloon, six-channel tube and perfused it with an allergen solution. Samples of the perfusate were collected after 20 min. Bengtsson et al. [21] indicated increased intestinal secretion of ECP and histamine after intestinal challenge in patients with cow's milk intolerance. Intraluminal administration of food antigens may induce intestinal release of mast cells tryptase, histamine, prostaglandin D_2 and eosinophils peroxidase activity already within 30 min after challenge [47]. In our studies, we were able to visualise local intestinal reactions in response to direct intraduodenal administration of suspected allergens (luminal provocation) using both endoscopic and transabdominal ultrasonography, as well as magnetic resonance imaging [48–50]. A rapid intestinal response to direct food challenge, characterized by thickening of the intestinal wall, increased peristalsis and influx of large amounts of fluid into the intestinal lumen was seen. Notably, influx of fluids into the intestinal lumen is typical of immediate IgE-mediated food allergy responses [47] following degranulation and release of mediators from mucosal mast cells. This suggests that despite lack of systemic food-specific IgE, the reactions may have been mediated by local IgE [51]. Considering that many of the patients had known non-gastrointestinal atopic disease and thus were likely to have a high density of IgE-armed mast cells in their duodenal mucosa, a local IgE-mediated reaction seems an even more reasonable explanation of the visualised changes [17]. However, further validation studies are necessary to establish the clinical utility of these new provocation tests.

Mediators and B Cell-Activating Factor

Immediate hypersensitivity reactions are consequences of reaginic antibody production (usually IgE) and their attachment to mast cells. Subsequent exposure to antigen induces mast cell degranulation with release of stored and newly formed mediators like histamine and tryptase [52]. Released mast cell products recruit other immunocompetent and inflammatory cells, which in turn contribute to the allergic reactions, particularly late-phase responses [47]. Such markers of inflammation can also be measured in gut lavage fluid. In one study we analysed gut lavage fluid samples for histamine, ECP, calprotectin and tryptase, and found that histamine and ECP concentrations were high in patients with food hypersensitivity confirmed by DBPCFC, but more research is needed to clarify this association [53].

In addition to serum, increased BAFF levels in gut lavage fluids in patients with food hypersensitivity was also reported, suggesting local immune activation [46]. Local BAFF production may contribute to intestinal accumulation of B lymphocytes and intestinal production of immunoglobulins in patients with self-reported food hypersensitivity.

Mucosal Immune Activation

Mast cells are found in all layers of the gastrointestinal tract but predominantly in the lamina propria and submucosa, preferentially around blood vessels and nerves. Two mast cell subtypes have been described in rodents, the connective tissue mast cells, the dominant subtype in the skin and peritoneal cavity, and the mucosal mast cells located predominantly in intestinal lamina propria and in lung mucosa [54]. However, human mast cells are commonly classified according to their protease content. Mast cells containing only tryptase predominate in the lung and intestinal mucosa. Tryptase- and chymase-positive mast cells are located mainly in the skin and the intestinal submucosa [54–57]. In the intestinal submucosa, mast cells and primary sensory neurons have close interactions [58]. Food antigens might have contact with intestinal epithelial cells, resulting in the release of cytokines from these cells. These cytokines diffuse into the lamina propria and activate primary sensory afferent neurons whose cell bodies are present in the dorsal root ganglia. Activation of primary sensory neurons causes early release of substance P and calcitonin gene-related peptide (CGRP), which stimulate mucosal mast cells and other resident immune cells, such as macrophages [59]. Substance P and CGRP are the principal neuropeptides released upon activation of sensory neurons, the former being a powerful vasodilatory peptide and considered of major importance for vasodilation [60]. In addition, this close interaction between mast cells and primary afferent neurons may contribute to neuronal hyperexcitability. Tryptase signals to cells through proteinase activated receptors (PARs). Mast cell tryptase induces activation of PAR-2 located on enteric nerves and visceral afferents causing long-lasting neuronal hyperexcitability, resulting in visceral hypersensitivity and abnormal gut motor function [61]. Hence, like in IBS, mast cells and mediator release interaction with mucosal innervation might also contribute to abdominal symptom generation in patients with intestinal food hypersensitivity.

Mast cells influence both innate and adaptive immune systems including intestinal barrier function, and thus, they may work as a "gate keeper" [62, 63]. DCs, macrophages and mast cells express TLRs and PARs, which are important for host defense against bacteria in the lumen. In addition, mast cells express receptors for several cytokines, and release of TNF-α, IL-1β and granulocyte–macrophage colony-stimulated factor (GM-CSF) promotes DC migration, maturation and activation. Several studies regarding IL-9, -29 and -33 indicate the important role of these cytokine in mast cell-mediated responses in food allergies [62]. Mast cells can release IL-29 in response to antigenic stimulation or lipopolysaccharide (LPS). IL-33 is responsible for activating mast cells which is resulted with stimulating production of IL-5, -4 and -13, whereas IL-29 rather contributes to mast cell recruitment in response to different stimuli [64–66]. IL-9 contributes to allergen sensitization. In an experimental study, IL-9 deficient mice did not develop intestinal allergies in response to oral antigen, whereas overexpression of IL-9 in the intestine led to an intestinal mastocytosis, intravascular leakage and increased intestinal permeability, supporting the contribution of IL-9 to mast cell responses during food allergy [67].

In both IBS and food hypersensitivity, inflammatory cells, especially mast cells, are involved in the pathogenesis [52, 61, 68]. Barbara et al. [61] indicated that 77 % of the IBS patients showed an increased area of mucosa occupied by mast cells in colon as compared with controls, and there was a 150 % increase in the number of degranulating mast cells. In addition, mast cell products like histamine and tryptase were increased in the mucosa of these patients. The latest studies indicate that mast cell activity not only increased in colon, but also other areas of the gut in IBS patients. In addition to elevated numbers of rectal mast cells in patients with IBS-D, significantly increased numbers of mast cells in the jejunum with corresponding higher levels of tryptase were also shown in another study [69, 70]. Furthermore, this phenomenon was extended to the first part of the small bowel by showing elevated mast cell numbers in the duodenum in both IBS-D and IBS-C patients, indicating that mucosal inflammation in IBS is not restricted to the lower gut [71]. Recent studies have also shown the importance of TLRs in IBS. TLRs are pattern recognition molecules regulating innate immunity, and they are activated by various bacterial and viral cell components. Binding between TLRs and these cell components results in production of inflammatory cytokines. TLRs are found in high numbers on colonic epithelial cells and monocytic cells. Increased express of TLRs, especially TLR-2 and 4, have been demonstrated in both colonic mucosa and periphery (blood) in IBS patients, indicating immune dysregulation in this group of patients [72–74].

Also in patients with food hypersensitivity, the increased numbers of mast cells in duodenum was shown [17]. Compared to non-atopic patients, the atopic patients had significantly higher numbers of IgE-positive cells in the duodenal mucosa, which in the majority of cases were mast cells "armed" with IgE. Whether atopic disease plays a role in the actual development of food-related gastrointestinal symptoms is indeed an interesting topic for future research. However, the findings regarding increased numbers of "IgE-armed" mast cells, BAFF and DCs support the notion of immune activation in patients with food hypersensitivity.

Gut Microbial Dysbiosis

Human gut microbiota play a key role in the maintenance of host health by providing nutrients and energy, and contributing to the maturation and development of the immune system. Microbe–host relationships are tightly interrelated, such that host factors can induce functional changes in the microflora that, in return, affect host biology [75].

Intestinal dysbiosis is a condition with microbial imbalances in gut. Altered profiles of gut microbiota and its relation with intestinal and extra-intestinal symptom (impaired quality of life and fatigue) generation have been shown in patients with IBS. In humans, conditions that are known to predispose to IBS, such as enteric infection, antibiotic use, and stress, may all change the intestinal microbiota [76], which in turn may trigger local and systemic immune activation, low-grade mucosal inflammation, increased intestinal permeability, and disturbances of intestinal motility and fermentation.

Patient's habitual gastrointestinal symptoms are often reproduced after administrating LDC. The fermentation process in gut after carbohydrate intake is complex in that it involves several functional groups of bacteria, and a functional intestinal dysbiosis in gut might induce an alteration in carbohydrate metabolism. Intestinal fermentation of LDC yields both gases (e.g., hydrogen, methane and carbon dioxide) and short-chain fatty acids (SCFA, e.g., acetic, propionic and butyric acids), and should normally take place mainly in the proximal colon. Excessive, impaired or altered fermentation, as well as fermentation in other parts of the gastrointestinal tract (i.e., small intestine, rectum), may cause meal-related functional gastrointestinal symptoms. Accordingly, patients with self-reported food hypersensitivity have a different profile of fecal SCFA than healthy controls, characterized by increased proportions of butyric acid [4]. This finding may have clinical implications, especially since butyrate has been shown to induce visceral hypersensitivity in rodents [77]. Intestinal

fermentation of LDC by a microflora skewed to produce butyric acid may thus be a potential cause of LDC intolerance.

Changes in the composition of the luminal and mucosal gut flora may lead to mucosal inflammation, and several changes such as increase in mucosal cellularity (enterochromaffin cells, lamina propria T lymphocytes and mast cells), modified pro-inflammatory/anti-inflammatory cytokine balance and disordered neurotransmission have been observed in IBS [78]. Furthermore, gut microbiota also contribute to neurobehavioral changes that are commonly reported as extra-intestinal symptoms. A link between gastrointestinal dysfunction including alterations in gut microbiota composition and cognitive problems was shown in chronic fatigue syndrome (CFS) [79]. The comorbidity of CFS and IBS is associated with increased plasma levels of proinflammatory cytokines such as IL-6, -8, -1β and TNFα, a systemic inflammatory situation which may in part be due to the translocation of commensal bacteria resulting from an increased intestinal permeability. Gut inflammation and loss of mucosal integrity may therefore contribute to the pathogenesis of the disease.

Conclusions

Several factors such as intestinal dysbiosis, increased gut permeability, stress, psychological (anxiety and depression), immunological and local factors may be associated with symptom generation in patients with perceived food hypersensitivity. Mast cells are "gate keepers," and they are not only involved in allergic reactions, but also in host defence including recruitment and activation of other immunity cells which may evoke the symptom generation. Their importance and involvement during both the systemic and intestinal immune activation was shown in both patients with food hypersensitivity and IBS. DCs and gut microbes via binding to TLRs may also contribute to this process. Further studies should be focused both on how the immune system is activated and which trigger factors are involved in the link between gut microbiota and intestinal mucosal response in patients with perceived food hypersensitivity.

Conflict of interest None.

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