

The Immunologic Basis of Non-IgE-Mediated Reactions

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KEY CONCEPTS

- Genetic, environmental, and developmental factors as well as antigenic properties of food proteins influence the development of non-IgE-mediated food allergy.
- The mucosal barrier in concert with the innate and adaptive arms of the immune system comprises the primary defense of the gastrointestinal tract against luminal antigens.
- The uptake and transport of luminal antigens is facilitated by intestinal epithelial cells, microfold cells, and dendritic cells.
- Food antigens are presented to T-cells in association with MHC class II molecules.
- Oral tolerance is a physiologic, active non-response to an encountered antigen and a failure of its induction leads to immunologic reactions to foods.

Introduction

The first authentic report of food hypersensitivity, over 2300 years ago, is attributed to Hippocrates for his findings that there exist individual differences in reactions to milk ingestion [1,2]. Quantitatively, food proteins account for one of the largest antigenic challenges confronting the human immune system [3]. Nonetheless, only a small number of foods instigate the majority of abnormal immune responses.

Abnormal responses to foods may be classified as toxic, such as to food contaminants, which are not dependent on individual susceptibility; and non-toxic, which are dependent on individual susceptibility. Non-toxic responses may be separated into non-immune mediated, such as food intolerance to lactose, and immune mediated, such as food allergy. The mechanisms of immune-mediated adverse reactions may be further divided into IgE-mediated or immediate-in-time and non-IgE-mediated or delayed-in-time responses [4,5]. Consistent with the global increase in the prevalence of atopic diseases, there has been a significant rise in both IgE and non-IgE-mediated food allergies [6,7].

This chapter provides a comprehensive overview of the immunologic basis of non-IgE-mediated, as well as mixed IgE-mediated mechanisms of food allergy. We elaborate

on factors affecting the development of food allergy; the immunologic anatomy and defense mechanisms of the gut which avert the development of food allergy; the processing of enteral food antigens and their presentation to immune competent cells of the gastrointestinal tract; and the effector cells and inflammatory mediators critical to the propagation and consequences of abnormal reactions to foods.

Development of food allergy

Genetic, environmental, and developmental factors, as well as a number of antigenic characteristics of food proteins, appear to influence the onset of food allergy. Genetic factors, such as a family history of atopic disease [8] and genetic polymorphisms [9], have been implicated. Early infectious exposure [10,11], rural upbringing with exposure to animals [12], and commensal [13] and pathogenic microorganisms within the GI tract are environmental factors that have been correlated with reduced atopic sensitization. Developmental factors, which include immaturity of the gut mucosa and the gut immune system, as seen among infants and children, may contribute to the development of food allergy. In addition, a number of antigenic characteristics of food proteins also impact the occurrence of food allergy.

The genetic basis of IgE-mediated food allergies have been more thoroughly characterized [14–19] than for non-IgE-mediated food allergies [20,21]. Studies of familial clustering, twin studies, and isolation of genetic polymorphisms

illustrate the genetic influences on non-IgE-mediated diseases. For example, celiac disease (CD), a prototypical non-IgE-mediated food allergy, shows familial clustering [22,23] and a high concordance rate of approximately 75% among monozygotic twins [24,25]. This disease has also been shown to be associated with two conventional DQ molecules, HLA-DQ2 and HLA-DQ8 [20,26,27]. Moreover, studies showing the genetic contribution of the HLA region on the familial clustering of CD suggest that HLA haplotypes are an important genetic background to the development of CD. However, additional susceptibility factors need to be identified [27]. Genetic associations with disease have also been made in eosinophilic esophagitis where familial clustering occurs, and observation of a single nucleotide polymorphism in the human eotaxin-3-gene has been made [28]. The genetic basis of food allergy, however, remains unclear [29].

A number of environmental factors appear to influence the development of allergy [30]. Studies show that improved social conditions may lead to a more “sanitary” living environment which may increase the risk for developing allergies, including food allergy [31]. A dominant role of early environmental exposures in the development of immune tolerance has also been reported. For instance, a farm upbringing, particularly with exposure to animals and livestock, has been shown to be relatively protective against the development of allergies [32]. This concept is often referred to as the “hygiene hypothesis” and is supported by a recent study which documented a decreased prevalence of allergic sensitization in children growing up on farms compared to their counterparts residing in the same geographic regions [33]. The inheritance of primary eosinophilic disorders appears to be multi-factorial, with an interplay between genetic and environmental factors where a majority of individuals are atopic [9]. Moreover, individuals demonstrate symptomatic improvement when the offending food allergen is eliminated from their diet [34].

Both commensal and pathogenic microorganisms in the gut appear to stimulate local B-cells and T-cells to induce normal development of the GI mucosal immune system. Animals reared in a germ-free environment have an underdeveloped GI mucosal immune system [13,35]. Toll-like receptors (TLRs) and secretion of chemokines, such as CCL20, IL-8, and MIP3 α , which are involved in luminal bacterial recognition, appear to be critical for the development of the innate as well as the adaptive arm of the mucosal immune system [36,37]. It has been proposed that disturbances in the gut flora, along with disruption of the gut barrier and breakdown of innate mucosal immunity caused by enteral pathogenic microorganisms, may contribute to the development of allergies. Further, microorganisms play a role in the pathogenesis of reflux esophagitis [38], gastritis and gastric ulcers [39], and infectious diarrheas [40], which are characterized by disruptions of the gut barrier resulting in irregularities in permeability and antigen transport across

the GI epithelium, perhaps fostering the development of food allergy.

The prevalence of food allergy is higher among infants and in children who are less than 3 years of age [41]. In infants, the relative immaturity of the GI mucosal immune system and mucosal barrier functions may be responsible for the increased prevalence [1]. The mucosal immune system immaturity is characterized by low basal acid output in the stomach, relative low levels of proteolytic activity, and immaturity of the barrier function. These conditions result in decreased luminal breakdown of antigen which leads to increased antigen absorption, as well as absorption of large antigenic molecules that interact with the mucosal immune system and predispose to food allergy [30].

Antigenic characteristics of food proteins may contribute to specific food allergies [42]. Physical characteristics of food proteins, such as size of the antigen, relative abundance [43] and resistance to acidic and enzymatic denaturation and digestion [44], their immunogenicity and the method by which they are presented to T-cells, are key determinants of their antigenic potential [45]. Food proteins that are allergenic also tend to be resistant to processing and heating and to acidic degradation and digestion within the GI tract [46].

Gut anatomy

The primary anatomical constituents which relate to immunologic responses in the GI tract include mucus, the glycocalyx, microvilli, the epithelial layer, the lamina propria, the muscularis mucosa, and gut-associated lymphoid tissue (GALT) (Fig. 3.1). The mucus layer is composed of mucin, free protein, dialyzable salts and is 95% water. It forms an adherent mucus gel layer over epithelial cells and creates a near-neutral pH at the epithelial surface, which is resistant to acidic and proteolytic digestion, thereby protecting the underlying mucosa [47]. Internal to the mucus layer is the cell surface coat, the glycocalyx, which is composed primarily of carbohydrates and contains various enzymes such as enteropeptidases, dipeptidases, and disaccharidases; and non-enzymatic proteins that are essential for terminal digestion of food and absorption of nutrients [48,49]. Luminal to the epithelial cells and beneath the glycocalyx are dense microvilli, which increase the absorptive surface area [50]. The epithelial layer is singular and composed of columnar cells, and together with the luminal mucus, functions as the primary separation between the mucosal immune system and the microbiota and enteral food antigens [51]. The epithelium also contains mucus goblet cells, undifferentiated crypt epithelial cells, and intra-epithelial lymphocytes, each of which performs a unique and integrated function (Fig. 3.1(a)). The intestinal epithelial cells (IECs) are bound by tight junctions at their apical surfaces, which function as a selective barrier to prevent the absorption of harmful

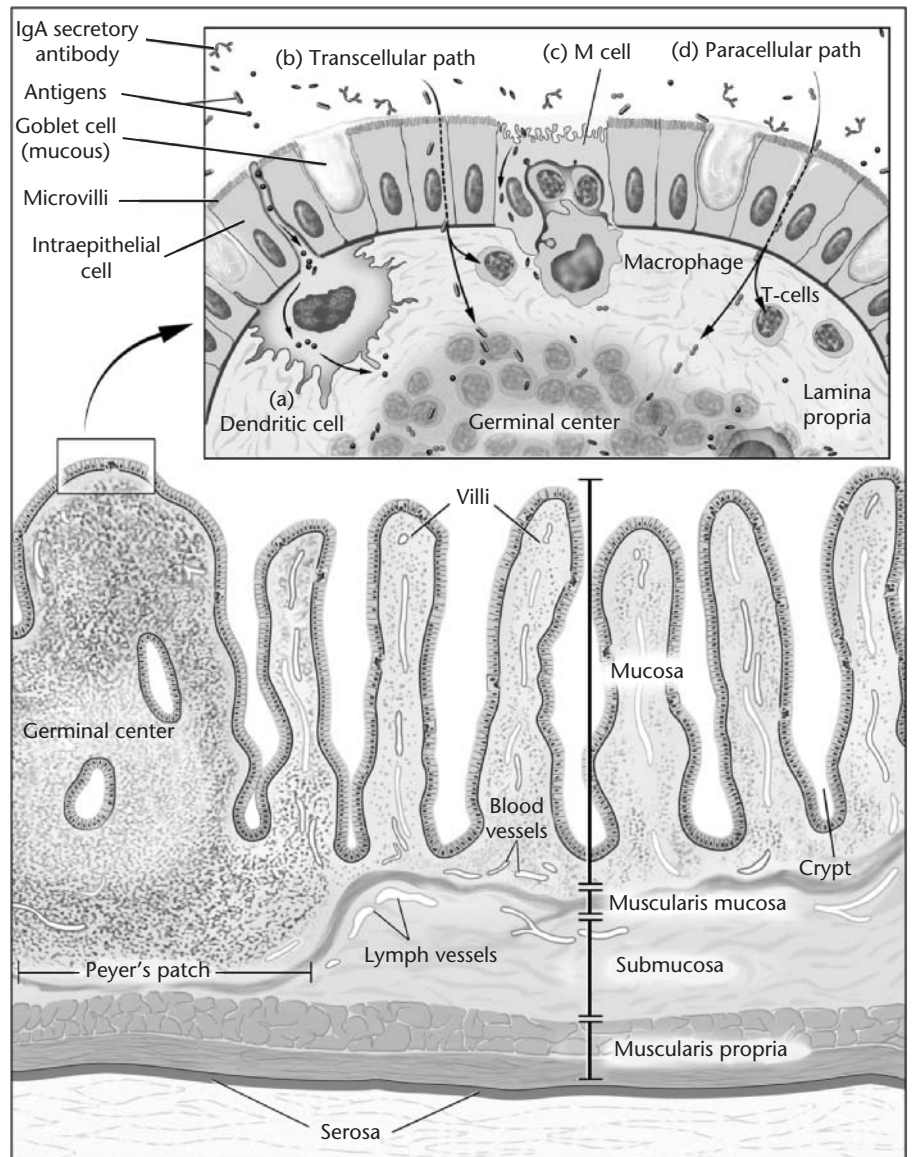


Figure 3.1 Ultrastructure of the GI wall illustrating some of its physical characteristics which comprises the intrinsic factors of the defense mechanisms. (Inset) Pathways of antigen uptake in the gut: (a) dendritic cells extend foot processes to sample luminal antigens; (b) antigen uptake across the apical surface of the IECs; (c) uptake of antigens by M cells, which subsequently deliver them to the germinal centers; and (d) uptake of antigen via the tight junctions of the IECs.

viruses, bacteria, and antigens, while allowing the transport of essential nutrients [52]. The lamina propria is a connective and supportive tissue layer between the basement membrane upon which the epithelium rests and the underlying muscularis mucosa. The lamina propria contains significant numbers of adaptive and innate immunocompetent cells: dendritic cells, T-cells (predominantly CD4+ and TCR $\alpha\beta$ + cells), plasma cells (mostly IgA producing), eosinophils, macrophages, and mast cells [53].

The mucosal immune system of the GI tract which resides within the mucosal layer is considered to be the largest immunologic organ in the body [51]. Humans have a well-developed gut immune system by 19 weeks' gestation, similar to most lymphoid tissues. The GALT is larger in children than in adults, and consists of lymphoid follicles and lymphoid cells. The lymphoid follicles are distributed

within the wall of the GI tract as Peyer's patches and also as solitary lymphoid follicles in the small intestine [54,55]. Lymphoid cells are present diffusely within the epithelium as intra-epithelial lymphocytes, within the lamina propria, and in the Peyer's patches [56]. Peyer's patches serve as antigen sampling sites for the gut immune system [57]. Each Peyer's patch consists of many follicles, and each follicle is made up of a central germinal center. The germinal center develops after antigenic exposure at birth [58] and is composed of B-lymphocytes and surrounded by a number of T-lymphocytes [59]. Overlying the follicle is a dome region consisting of the follicle-associated epithelium (Fig. 3.1) which contains the specialized microfold cells (M cells) [60,61]. M cells are derived from IECs and have microfolds on their luminal surface unlike epithelial cells which possess microvilli [62]. The M cells facilitate the uptake

of antigens from the gut lumen and present them to dendritic cells and macrophages which are contained beneath the follicle-associated epithelium. In the small intestine, other lymphoid tissues aggregates have also been described, which include isolated mucosal lymphoid follicles and submucosal lymphoid aggregations that are thought to be solitary Peyer's patch follicles [55]. The appendix is a prominent constituent of the GALT. It is organized into a large number of repeating lymphoid follicles which are morphologically similar to those present in the Peyer's patches [63].

Within the colon, lymphoid follicles may be present as lymphoglandular complexes, which are organized lymphoid structures [64] and appear to be intimately associated with the luminal epithelium. The complexes are located at points of defects in the muscularis mucosa and consist of compact spherical aggregates of lymphocytes situated below the muscularis mucosa. These complexes are also in continuity with a less clearly circumscribed collection of lymphocytes located within the colonic lamina propria. Individual lymphoid follicles may be found within the colonic submucosa and lamina propria, and are most common in the rectum [56]. The cell types present in these lymphoglandular complexes, such as dendritic cells, T- and B-lymphocytes, macrophages, and epithelial cells, have ultrastructural characteristics similar to M cells and appear to be similar to cells that exist in the lymphoepithelial complexes of the small intestine [64].

Defense mechanisms

The GI epithelium functions, in essence, as a gatekeeper, allowing the passage of essential nutrients necessary for growth and development, while maintaining an effective barrier against antigenic food proteins, and commensal and pathogenic microorganisms. The mucosal barrier in conjunction with the innate and adaptive immune systems comprises the primary host defense of the GI tract. The innate immune system provides protection by barrier mechanisms, while the adaptive immune system prevents indiscriminate immune responses to innocuous antigens [65].

An abnormal immune response may be observed when there are perturbations in these defense mechanisms, which in certain disease processes may be triggered by small amounts of residual, non-degraded dietary enteral proteins [66]. The GI defense system may also be divided into extrinsic factors, which are features that restrict the quantity of antigen that is able to reach the epithelial surface, and intrinsic factors, which are characteristics of the physical barriers of the GI wall. The extrinsic and intrinsic factors appear to act synergistically to limit the absorption of food antigens.

The extrinsic factors consist of proteolysis of food proteins, GI acidity, mucin production, peristalsis, and secretory IgA. The stomach produces proteolytic enzymes, such as pepsin

and papain, while the small intestine harbors trypsin, chymotrypsin, and the pancreatic proteases that lead to protein denaturation and degradation which alters the epitopes necessary for immunologic recognition [42,44]. A number of diseases and the effects of some medications lead to reduced gastric acidity, which may promote increased antigen absorption [67]. Patients with cystic fibrosis are deficient in pancreatic enzymes, which may lead to increased antigen absorption [68]. Mucus produced by goblet cells coats the epithelial surface and acts as a physical barrier, while its viscous nature in unison with the peristaltic activity of the gut impedes the access of food antigens. Peristaltic waves results in mixing of mucus with food antigens, which limits the interaction with the absorptive epithelial surface and subsequent uptake [69]. Large intestine peristaltic waves are fewer and less vigorous compared to the small intestine, which promotes the absorption of food antigens [70]. Secretory antibodies provide the immunologic barrier within the gut lumen. Breast milk, especially colostrum, appears to provide, as well as enhance, secretory IgA production, a majority of which remains within the gut lumen [71]. Luminal IgA binds to food antigens which hastens their transport within the GI tract [72]. IgA may also act as a cell surface receptor and attach to food antigens, which facilitates their transport into epithelial cells where such antigens are digested within the phagolysosomes [73,74].

The intrinsic barrier consists of the microvillus and IECs with their tight junctions, intracellular organelles, and proteolytic enzymes. Food antigen must maneuver across the components of the extrinsic barrier prior to coming in contact with the intrinsic barrier. The abundant microvilli, which cover the epithelial cell surface, constitute a significant barrier due to their size, close apposition to each other [75], and their negative charge [76]. The IECs appear to be more than just passive barriers to the luminal contents, given the presence of a selectively permeable membrane at the base of the microvilli [77]. The IECs are hyperpolarized and joined together by tight junctions which further augment barrier function [78]. Food proteins are ultimately endocytosed into vesicles where they are acidified and degraded by proteases and delivered to lysosomes where most antigens are eventually destroyed.

Oral tolerance

Immune responses against foods may lead to decreased absorption of food constituents and essential nutrients. Abnormal responses to foods may result in intestinal pathology, as exemplified by CD and other food-sensitive enteropathies. Under physiologic conditions, when a novel antigen is ingested, IgA antibodies are secreted in the mucosa, which is followed by a systemic humoral and/or a cell-mediated immune response. Subsequently, a systemic and local immune hyporesponsiveness may develop which

prevents a deleterious immune response with subsequent encounter of the specific antigen. This is referred to as oral tolerance [79].

Although the mechanisms of oral tolerance have been primarily elucidated in animal models [80,81], there does exist clinical and experimental evidence of oral tolerance in humans [82]. Studies have suggested that at least two mechanisms are responsible for the development of oral tolerance: induction of clonal anergy (or deletion) of antigen-specific T-cells, and stimulation of regulatory T-cells (Treg) which mediate active suppression of the immune response to food antigens. Clonal anergy results from a lack of co-stimulatory molecules on the antigen-presenting cells, namely CD80 and CD86, or interaction with inhibitory co-stimulatory molecules, such as CD152 or cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) and PD-1 [83–87]. Clonal anergy is an outcome of the activation of apoptotic pathways which permanently remove antigen-specific T-cells [88]. Further, a single high dose of food antigen is more likely to induce tolerance by clonal anergy whereas repeated intake of low doses may stimulate Treg cell activity [89–91]. Stimulation of Treg cells is the other mechanism by which oral tolerance may be induced. However, recent studies show that clonal anergy and active regulation by Treg cells are not necessarily distinct aspects of T-cell function [86]. Current research suggests that Treg cells are more likely to be CD4+ cells than the earlier believed CD8+ T-cells. Several subsets of CD4+ Treg cells have been identified, which include TGF- β producing Th3 cells, IL-10 producing Tr1 and CD4+CD25+ Treg cells [86]. Th3 cells are formed in the GALT and appear to be pivotal in the mediation of tolerance to dietary antigens which are ingested in low doses, and inhibit the activation of all lymphocytes in close proximity. This is often referred to as bystander tolerance. Th3 cells then migrate to lymphoid organs where they suppress immune responses by hindering the generation of effector cells, and to target organs where they suppress disease by releasing antigen non-specific cytokines [92]. Studies have shown that compared to normal individuals, children suffering from food allergies, immediate as well as delayed, may have reduced numbers of Th3 cells in their duodenal mucosa, which supports the finding that TGF- β is an important regulator of intestinal immunity in humans [93]. Antigen and naïve T-cell interactions lead to a preferential induction of Treg cells which secrete downregulatory cytokines such as IL-10, TGF- β , and IL-4. IL-10 appears to downregulate inflammatory cytokines such as IL-1 α , IL-6, and TNF- α , which are secreted by gut wall macrophages upon interacting with luminal bacteria [94]. Thymus dependent CD4+CD25+ Treg cells have been shown in experimental studies to prevent colitis, possibly relating to increased levels of TGF- β [95]. Oral tolerance once induced suppresses T-cell allergic responses, the basis of most non-IgE-mediated food-allergic

diseases. A breakdown of oral tolerance may lead to CD and cow's milk-protein allergy in which aberrant CD4+ T-cell responses to gliadin and milk-protein antigens, respectively, lead to mucosal injury [96].

Antigen transport

Nutrients and antigens from enteral food are primarily absorbed by IECs across their apical surface or through their tight junctions or by M cells. IECs absorb antigens in a fluid phase as well as soluble antigens, whereas M cells primarily deliver samples of large particulate antigens to the lymphoid tissues via an active vesicular transport [97,98]. Studies have suggested that this membrane traffic is charge dependent, as is seen in polarized epithelial cells [99]. The M cells have a limited number of cytoplasmic lysosomes, which makes intracellular processing of antigenic foods unlikely. The large particulate antigens which are absorbed across M cells are more likely to be of bacterial and viral origin [100].

Antigens are transported at the apical surface via the transcellular pathway or through the tight junctions by means of the paracellular pathway. Across the apical surface, antigens are transported in membrane-bound vesicles by pinocytosis (Fig. 3.1) [76]. The tight junctions, under physiologic conditions, make paracellular transport of antigens and other macromolecules almost unachievable. The tight junctions appear to be dynamic structures. Activation of certain transport systems embedded within the apical membrane of the IEC may lead to transient and reversible increases in permeability. For example, activation of the Na+ coupled transport of glucose and amino acids dilates tight junctions and allows for increased absorption of food antigens [101,102]. Similarly, TNF- α , IFN- γ , IL-4, and IL-13 increase epithelial permeability, but TGF- β appears to enhance barrier functions [103–106].

Antigen processing and presentation

The uptake, processing, and presentation of food antigens to naïve T-cells are necessary for the mounting of an immune response by immune competent cells of the GI tract. The uptake of antigens peaks during the neonatal period and decreases as the gut matures. In the adult GI tract, minute quantities of ingested food antigens may be absorbed and transported to the portal venous and systemic circulations in immunologically intact forms [107]. Subsequent to uptake, the processing of antigenic food proteins by IECs is achieved by proteolysis within endosomes. The antigens may then be presented to T-cells by eosinophils and mast cells, the non-professional antigen-presenting cells, which express class II MHC molecules on their cell surface when activated; and dendritic cells, macrophages, and B-cells, the professional antigen-presenting cells that constitutively express class II MHC molecules. However, IECs appear to be the only non-professional antigen-presenting cells that constitutively

express MHC class II molecules on their surface [108]. The antigen may be taken up by the antigen-presenting cells by endocytosis, which is non-specific and less efficient than by receptor-mediated methods which appear to be more efficient [109]. Only professional antigen-presenting cells with the following three key characteristics are able to activate normally naïve T-cells to become memory and effector cells [110]. First, there must be expression of surface glycoproteins, which are products of class II MHC molecules. Second, absorption of antigens must occur by either receptor-mediated or fluid-phase endocytosis. Finally, there must be processing of the absorbed antigens within intracytoplasmic organelles, forming a complex with products of class II MHC molecules and presenting them to T-cells.

In addition to their role in barrier function and as non-professional antigen-presenting cells, IECs may also play a role in the regulation of regional immunologic function. These cells absorb and process antigens and may present them directly to T-cells in an MHC-dependent manner [111]. The IECs express class II MHC molecules, mostly on their basolateral surface, where they interact with lymphocytes in the intra-epithelial spaces and in the lamina propria. The expression of MHC molecules appears to be enhanced during gut inflammation [112]. Absorbed luminal antigens may be processed by dissimilar proteolytic enzymes contained in different phagolysosomes that generate a diversity of antigenic epitopes which ultimately interact with T-cells [113]. An antigen absorbed at the apical surface usually may not elicit an immune response, but may if it is absorbed at the basolateral surface [114]. In contrast to professional antigen-presenting cells, IECs may selectively activate CD8⁺ suppressor T-cells which enhance the suppression of the gut immune response [115]. This process appears to be regulated by the non-classical MHC class I molecule CD1d and an IEC membrane glycoprotein, the CD8 ligand gp180 [116–118]. The precise mechanisms implicated and the roles played in downregulating the mucosal immune responses, however, remain to be characterized.

Dendritic cells are derived from circulating monocytes, which originate from bone-marrow-derived myeloid precursors [119,120]. Dendritic cells are specialized for the uptake, processing, transport, and presentation of antigens, as well as the priming of naïve T-cells. During differentiation, dendritic cells upregulate expression of MHC class II molecules which increases their antigen-presenting efficiency. They also alter their expression of chemokine receptors and production of cytokines which are vital to T-cell differentiation [121,122]. Microbes within the intestinal lumen also appear to stimulate dendritic cells to secrete immunostimulatory cytokines, including IL-12, which upregulate the expression of MHC class II molecules, as well as produce co-stimulatory molecules [121]. Within gut lymphoid follicles, dendritic cells may be classified as follicular and non-follicular cells. Follicular dendritic cells express antigens,

which are vital for the maintenance of memory B-cells. The non-follicular dendritic cells are preferentially localized within the dome regions of Peyer's patches, the lamina propria, in T-cell zones and in certain other parts of the GALT.

The uptake of antigens by dendritic cells may be achieved by macropinocytosis or by receptor-dependent mechanisms [123,124]. Dendritic cells may also send foot processes between IECs [125] or they may become lodged between adjacent IECs and endocytose luminal antigens before migrating to the lamina propria [126]. Subsequently, they reach secondary lymphoid organs, such as mesenteric lymph nodes, where they may interact with and activate naïve T-cells [127]. Occasionally, antigens may reach dendritic cells within secondary lymphoid organs by direct dissemination through draining gastrointestinal lymphatics or bloodstream.

T-cells

The production of food-antigen-specific IgE antibodies is facilitated by cytokines produced by T-cells, such as IL-4 and IL-13. Non-IgE-mediated food allergies result in part from an imbalance between inflammatory cytokines secreted by T-cells such as, IFN- γ , TNF- α , and IL-15 and regulatory cytokines such as IL-10 [128,129].

Antigenic stimulation of naïve T-cells leads to priming, followed by proliferation into memory T-cells and subsequent entrance into the circulation. From the vasculature, memory T-cells may return to the GI tract to function as effector cells in disease pathogenesis. Increased expression of $\alpha 4\beta 7$ on memory T-cell subsets correlates with enhanced recruitment into Peyer's patches. Naïve T-cells which are $\alpha 4\beta 7$ low and the subset of memory T-cells which are $\alpha 4\beta 7$ high are equally well recruited to Peyer's patches. However, the subset of memory T-cells which are $\alpha 4\beta 7$ low are excluded [130]. The specific ligand for $\alpha 4\beta 7$ on vascular endothelial cells within the high endothelial venules is mucosal addressin-cell adhesion molecule-1 (MAdCAM-1), which facilitates the migration of T-cells. Stimulation of peripheral T-cells by β lactoglobulin present in cow's milk results in the selective increased expression of $\alpha 4\beta 7$, which suggests that allergen exposure enhances the migration of memory T-cells [131]. Patients with subclinical CD have increased numbers of T-cells within the gut mucosa which proliferate further when the individual becomes symptomatic [132,133].

CD is characterized by the presence of gluten-specific CD4⁺ T-cells in the lamina propria and increased numbers of intra-epithelial lymphocytes of the TCR $\alpha\beta$ ⁺ CD8⁺CD4[−] and TCR $\gamma\delta$ ⁺ CD8[−]CD4[−] lineage [134]. Cytokines secreted by CD4⁺ cells, intra-epithelial lymphocytes, and IECs are the primary effectors of mucosal injury in CD. Gluten-activated mucosal CD4⁺ cells secrete IFN- γ , which together with TNF- α secreted by macrophages, leads to increased

permeability and direct cytotoxic effect on the small IECs [135,136]. Activated stromal cells within the lamina propria are induced by TNF- α to produce keratinocyte growth factor (KGF), an epithelial mitogen, which stimulates small intestinal epithelial cell proliferation and results in crypt cell hyperplasia [137]. IECs, dendritic cells, and macrophages produce IL-15, which is upregulated within the lamina propria and the intestinal epithelium during active disease and plays a critical role in the pathogenesis of CD [138]. Gliadin may act independently or in consort with IL-15 on IECs to activate them and induce the expression of the non-conventional HLA I molecule, MHC class I chain A related molecule (MICA). Expression of MICA leads to direct cytotoxicity of IECs in an antigen non-specific manner [139]. IL-15 may also upregulate the expression of NKG2D, the natural killer receptor which is normally expressed on most NK cells as well as on CD8⁺ TCR $\alpha\beta$ ⁺ and TCR $\gamma\delta$ ⁺ cells [140–142]. MICA serves as a ligand for the activating natural killer cell receptor, NKG2D, which may result in lymphocyte-mediated cytotoxicity of IECs and in villus atrophy and small intestinal mucosal injury [143]. However, T-cell mechanisms which mediate injury and exert their damaging effects on the mucosa are yet to be completely understood.

In a subset of individuals, the underlying mechanism of cow's milk allergy may be non-IgE mediated. Persons with cow's milk allergy, in contrast to individuals with CD, usually do not develop villous atrophy nor an increased mononuclear cell infiltrate within the lamina propria. However, TCR $\gamma\delta$ ⁺ CD8⁺CD4[−] cells may occur as the majority of intra-epithelial lymphocytes, which suggests that a cytokine imbalance leads to the disease phenotype [144]. CD is primarily Th1 biased, whereas cow's milk-protein allergy in individuals with an atopic predisposition appears to be predominantly Th2 biased. This is evidenced by a high production of IL-4, IL-5, and IL-13 and a low production of IFN- γ [145,146]. However, non-atopic individuals may exhibit a Th0-like cytokine phenotype [147]. A recent study has demonstrated differences in an immune activation profile between individuals with non-IgE-mediated cow's milk allergy and CD. The group with cow's milk allergy demonstrated an upregulation of CCR4 and IL-6mRNA and downregulation of IL-18 and IL-2mRNA within the gut mucosal tissue, suggesting a Th2-biased immune response. In contrast, individuals with CD showed upregulation of IFN- γ and downregulation of IL-12p35, IL-12p40, and IL-18-specific mRNA [148].

Eosinophils

Eosinophils play an important role in the pathogenesis of allergic disorders in the lung, skin, and gut [149]. Mixed IgE and non-IgE food-allergic reactions are the basis of eosinophil-associated gastrointestinal disorders (EGID) [1,9]. Individuals with EGID are often allergic to multiple foods,

have an eosinophil-rich infiltrate within the wall of the esophagus, stomach and small and large intestines [150], an elevated serum IgE in a majority and peripheral eosinophilia in a few [151,152].

Numerous inflammatory mediators have been implicated in the recruitment of eosinophils to tissues. Of these, eotaxin, which is constitutively expressed by the GI epithelium, and IL-5 appear to be relatively specific eosinophil chemoattractants [153]. Eotaxin appears to modulate the recruitment of eosinophils by selectively binding and signaling through the chemokine receptor, CCR3, found primarily on eosinophils [154]. Eotaxin also appears to facilitate the movement of eosinophils from blood vessels to the gut tissue, which is dependent on the interaction of $\alpha 4\beta 7$ present on eosinophils with MAdCAM-1 [155]. In a physiologic state, eosinophils exist in small numbers within the GI wall and the presence of relatively large numbers denotes an underlying disease process. However, the esophageal wall lacks eosinophils and their presence indicates a pathologic course [156]. IL-5 has also been shown to be involved in eosinophil differentiation, proliferation, survival, recruitment, and trafficking within the GI tract and has been implicated as the principal modulator of gut inflammation in EGID [157].

A step-wise interaction between endothelial cells of the blood vessels and eosinophils promotes their migration to the mucosal tissues. The rolling of an eosinophil over the endothelial cell surface is assisted primarily by P-selectin, the adhesion molecule present on endothelial cells [158]. Rolling is followed by adherence facilitated by adhesion molecules of the integrin family [159]. Within the mucosal tissues, eosinophil survival is cytokine dependent, where GM-CSF increases the survival of tissue eosinophils and IL-12 appears to increase apoptosis [160].

Studies have demonstrated that in addition to eosinophils, the cellular component of the inflammatory infiltrate in EGID also consists of increased numbers of activated CD4⁺ T-cells and mast cells [161]. Monocytes and neutrophils are other cell types that are associated with the disease [162]. Eosinophils propagate disease pathogenesis and may instigate mucosal injury by release of inflammatory mediators, eosinophilic cytotoxic granule proteins, cytokines, and reactive oxygen intermediates. Cytokines, immunoglobulins, and complement components may activate eosinophils to generate numerous inflammatory mediators, such as IL-1, IL-3, IL-4, IL-5, IL-13, GM-CSF, TNF- α , MIP 1 α , and vascular endothelial cell growth factor. This suggests that eosinophils may modulate the many features of the immune response [163]. Furthermore, epithelial growth, fibrosis, and tissue remodeling may be influenced by eosinophil-derived TGF- β and the eosinophilic cytotoxic granule proteins including eosinophilic cationic protein [164], major basic protein [165], and eosinophil peroxidase [166]. The eosinophilic cationic protein may insert pores into the IEC membrane, which leads to the entry of other toxic

Table 3.1 An overview of immune mechanisms and symptoms of non-IgE-mediated and mixed food allergies

Disease	Immune mechanism	Symptoms
Food-protein-induced enterocolitis	Cell mediated	Profuse vomiting, diarrhea (±microscopic blood), severe symptoms may lead to lethargy, dehydration, and shock
Food-protein-induced proctocolitis	Cell mediated	Gradual onset bleeding progressing to streaks of blood, infant typically thriving and usually well
Food-protein-induced enteropathy (gluten-sensitive enteropathy)	Cell mediated	Dyspepsia, reflux, diarrhea, abdominal distension, flatulence, failure to thrive, other symptoms depend on extraintestinal manifestations
Allergic eosinophilic esophagitis	Cell mediated and/or IgE mediated	Difficulty in feeding, failure to thrive, gastroesophageal reflux, vomiting, epigastric pain, dysphagia and food impaction
Allergic eosinophilic gastroenteritis	Cell mediated and/or IgE mediated	Recurrent abdominal pain and vomiting, failure to thrive, peripheral blood eosinophilia (50%).

molecules [167]. Major basic protein induces smooth muscle reactivity and may initiate degranulation of mast cells and basophils [168].

Respiratory burst enzyme pathways in eosinophils generate superoxide that may cause mucosal damage [169]. Eosinophil peroxidase generates toxic hydrogen peroxide and halic acids which may trigger further injury [170]. Neutrophils also generate lipid mediators, such as LTC4, LTD4, and LTE4 which lead to increased vascular permeability, mucin secretion, and smooth muscle contraction [171]. Moreover, the extent of GI wall eosinophil infiltration and the quantity of eosinophilic cytotoxic proteins correlate with disease severity [172,173].

Table 3.1 lists diseases with non-IgE and mixed immune-mediated mechanisms and a summary of their clinical features [174–178].

Food-protein-induced enterocolitis and proctocolitis

Food-protein-induced enterocolitis and proctocolitis occur predominantly in infants with food allergies and are characterized by severe small and large intestine mucosal injury. The common dietary culprits implicated in the pathogenesis of food-allergic reactions in infants are cow’s milk and soybean. Cereal grains (rice, oat, barley), fish, poultry, and vegetables are infrequent offenders [179]. The diagnosis is chiefly made by clinical symptoms and challenge testing, but may also be supported by resolution of symptoms after dietary elimination of the perpetrator protein. Typical symptoms consist of vomiting and diarrhea with presence of blood, leukocytes, eosinophils, and increased carbohydrate content in the stool [180]. Histologic studies of endoscopic biopsy specimens in symptomatic patients reveal non-specific markers of inflammation, which include prominent eosinophilia and plasma cells, crypt abscesses, and mild villous injury. A few infants may also show evidence of gastritis and esophagitis [181].

Celiac disease

CD is often categorized as an autoimmune disorder affecting the small intestines, induced by the intake of gluten in wheat and analogous proteins present in barley and rye. CD is closely associated with genes that code HLA-II antigens, mainly of DQ2 and DQ8 classes [182,183]. CD may manifest early in life following the introduction of gluten in the diet or may develop later in life. The clinical manifestations include abdominal pain with distension, dyspepsia, presence of gastroesophageal reflux disease (GERD), recurrent episodes of altered bowel habits (diarrhea and/or constipation), weight loss, bone disease, anemia, and weakness. Symptoms tend to remit upon strict compliance to a gluten-free diet. The demonstration of circulatory IgA antibodies to transglutaminase (tTG-IgA) is supportive of the diagnosis. Histologic examination of endoscopic samples is confirmatory [184]. However, patchy involvement of the mucosa may lead to a false-negative diagnosis. Histologic changes within the mucosa include villus atrophy/flatten-ing, crypt hyperplasia, thickening of the epithelial basement membrane, and reduced numbers of goblet cells. Evidence of mucosal inflammation is manifested by an increase in intra-epithelial lymphocytes and an influx of immune cells within the small intestinal lamina propria, and loss of basal nuclear orientation as well as change of the IECs to a cuboidal morphology.

Allergic eosinophilic esophagitis and gastroenteritis

Primary eosinophilic gastrointestinal disorders include eosinophilic esophagitis, gastritis, gastroenteritis, enteritis, and eosinophilic colitis. These diseases are occurring with increasing frequency and are mediated by mixed immune mechanisms [185]. They are characterized by an eosinophil-rich infiltrate within the gut wall in the absence of other causes of gut wall eosinophilia such as drug reactions, parasitic infections, and malignancy. The constellation of

symptoms includes abdominal pain, dysphagia, vomiting, diarrhea, gastric dysmotility, irritability, and failure to thrive [186]. The diagnosis of primary eosinophilic gastrointestinal disorders is contingent upon the histologic assessment of endoscopic biopsy samples with vigilant consideration of the quantity, location, and characteristics of the eosinophilic infiltration [9].

Conclusions

We have reviewed non-IgE-mediated mechanisms of food allergy. We have detailed the barrier functions of the gut; the processing, absorption, and presentation of antigens to the immune competent cells; the mounting of a response; and the inflammatory changes and mucosal damage as propagated by infiltrating cells within the gut mucosa. It is apparent that non-IgE-mediated gastrointestinal allergic diseases may be associated with gastrointestinal epithelial barrier dysfunction. However, it is not clear if barrier dysfunction is an outcome, or a contributing factor to development of food allergies. Intertwined in the disease pathogenesis are roles of T-cells which are pivotal to the induction of oral tolerance as well as the propagation of disease, and eosinophils which are central in the pathogenesis and modulation of eosinophilic gastrointestinal disorders. An appreciation of the immune mechanisms involved in food hypersensitivities and its associated diseases, and the counseling of genetically susceptible individuals will facilitate the development of new and novel approaches to treating patients with these diseases.

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