

The Immunological Basis of IgE-Mediated Reactions

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KEY CONCEPTS

- Sensitization to food allergens can occur via the gastrointestinal tract (true food allergens) or via the pulmonary route (cross-reactive aeroallergens).
- Food allergens enter the mucosal barrier and can be transported throughout the body in an immunologically intact form.
- A dysregulated immune response to food allergens, consisting of a strong Th2 and IgE response and a low regulatory T-cell and IgG/IgA response, leads to allergic disease.
- Genetic and environmental (hygiene hypothesis) factors influence the individual immune reactions to food allergens.
- Cross-linking of IgE on tissue mast cells triggers the release of pro-inflammatory mediators and initiates the acute-phase reaction and the recruitment of eosinophils, basophils, and lymphocytes.

Introduction

Food allergy defined as immune-mediated food intolerance can be divided into “IgE-mediated” disorders (immediate-type gastrointestinal hypersensitivity, oral allergy syndrome, acute urticaria and angioedema, allergic rhinitis, acute bronchospasm, anaphylaxis) and “non-IgE-mediated” (dietary protein-induced enterocolitis and proctitis, celiac disease, and dermatitis herpetiformis). This classification has been extended by supposing a third subgroup of “mixed IgE- and non-IgE-mediated” disorders such as allergic eosinophilic esophagitis and gastroenteritis, atopic dermatitis, and allergic asthma [1,2].

In this chapter, the underlying immune mechanism of IgE-mediated allergic reactions with a particular focus on food allergy will be discussed. The development of food allergy is a multi-step process, requiring repetitive challenges with a particular food antigen, in contrast to non-immune-mediated reactions which can cause symptoms even after a single food exposure. The disease is preceded by a sensitization phase without symptoms, in which allergen-specific T- and B-cells are primed and IgE is produced. Recurrent

allergen challenge of sensitized individuals results in IgE cross-linking bound on tissue mast cells that subsequently release their pro-inflammatory mediators.

Route of sensitization

Food allergy might result from sensitization to ingested food proteins or to aeroallergens through the respiratory route. Several pollen allergens can confer cross-reactivity to homologous proteins in plant foods. It has been suggested that oral sensitization only occurs when allergens are highly resistant to digestion in the gastrointestinal tract, while pollen food cross-reactive proteins are labile [2]. The route of sensitization might therefore influence the allergenic pattern on a molecular level and influence the clinical manifestation after challenge. This relationship has been confirmed in a recent multi-center study across Europe [3]. In the Netherlands, Austria, and northern Italy apple allergy is mild (>90% present exclusively oral symptoms) and precedes birch pollen allergy. The apple allergy arises as a result of the cross-reactivity between the birch pollen allergen Bet v 1 and the apple allergen Mal d 1. In Spain, exposure to birch pollen is virtually absent and the main apple allergen is Mal d 3. The authors suggested that apple allergy in Spain is a result of a primary sensitization to peach and its major allergen Pru p 3, which is cross-reactive to Mal d 3. Both proteins belong to

the non-specific lipid transfer proteins, which are resistant to proteolysis. Consequently, about 35% of the Spanish patients have systemic reactions after double-blind, placebo-controlled food challenges with apple [3].

Allergen uptake in the intestine

The intestinal mucosa is constantly challenged with food and the commensal flora, which may be harmful after uncontrolled uptake. Therefore, innate and adoptive mechanisms have been developed to control the immune balance to food and commensals and to fend off pathogens [1,4,5]. Gastric acid, mucus, an intact epithelial layer, digestive enzymes, and the intestinal peristaltic are unspecific factors forming the “non-immunological” barrier [6]. The immunological defense mechanisms include innate (antimicrobial peptides, immune cells expressing pattern recognition molecules, etc.) and adaptive mechanisms (lymphocytes, IgA) [4]. Despite this tight mucosal barrier, macromolecules and intact bacteria can pass through or can be even actively taken up by the intestinal epithelium. Macromolecular uptake can be beneficial in delivering essential growth factors and in sampling the antigenic milieu of the gastrointestinal tract in order to enable the induction of immune tolerance to environmental antigens [6,7].

Breakdown of the intestinal barrier is associated with the development of food allergy. Neutralization of gastric acid results in increased mucosal transport of ingested proteins and sensitization to allergens [8]. Intestinal permeability is increased in patients suffering from food allergy [9]. Interestingly, one study showed that intestinal permeability is increased in patients with bronchial asthma, supporting the hypothesis that a general defect of the mucosal system may facilitate the development of allergic diseases [10]. Further evidence that a barrier dysfunction is a risk factor for developing food allergy comes from the notion that early introduction of solid food in babies (immature barrier) [11] and IgA deficiency or retarded IgA development in infants [12] is associated with a higher risk of atopy.

Many food allergens are fairly stable to heat, acid, and proteases making them resistant to digestion, a critical role allowing them to get in contact with the intestinal immune system. It has been demonstrated that ingested food proteins can be transported throughout the body in an immunologically intact form [2,13]. This might explain why symptoms of food allergies are not restricted to the gastrointestinal tract, but very often cause additionally or even exclusively extra-intestinal symptoms. Considering that the intestine is an immunologically privileged site, it is not surprising that hyperresponsive reactions to food allergens occur in some patients only outside the gastrointestinal system, independent from the site of initial antigen uptake.

T-cell response in IgE-mediated allergy

A hallmark of IgE-mediated allergic disorders is the generation of allergen-specific CD4⁺ Th2 lymphocytes. These cells produce a characteristic Th2-cytokine profile consisting of IL-4, IL-5, IL-9, and IL-13. IL-4 and IL-13 induce IgE class-switching in B-cells, IL-4 and IL-9 are important growth and activation factors for mast cells, and IL-5 promotes eosinophil development and recruitment. IL-13 additionally triggers mucus secretion in the lung and provokes airway hypersensitivity [14,15]. In the 1990s, allergic sensitization to harmless environmental proteins (allergens) was attributed to a dysregulation of the Th1/Th2 balance. However, the simple dichotomy of the Th1/Th2 system has been challenged by the discovery of a plethora of new T-cell subsets, including Th17 cells [16], non-classical T-cells such as NKT [17] and $\gamma\delta$ T-cells, different subsets of CD8⁺ T-cells (Tc1 and Tc2), and, most importantly, regulatory T (Treg) cells [14,18,19]. The actual concept states that allergies and also autoimmune diseases result from a dysbalance between a protective Treg response and a disease inducing effector Th2 (in the case of allergy) or Th1/Th17 response (in the case of autoimmune diseases; recent observations suggest that Th17 cells are the main effectors) [16]. However, it is clear that different effector T-cell subsets have counter-regulatory functions, which also play a role in the immune-regulatory network [14,18,19].

Several subtypes of Treg cells have been described, which also have some overlapping phenotypes. Naturally occurring CD4⁺CD25⁺FoxP3⁺ Treg cells are distinguished from antigen-driven IL-10 (Tr1) and TGF- β (Th3)-secreting CD4⁺ Treg cells. The former subset originates from the thymus and acts by cell–cell contact in an antigen-independent manner. Tr1 and Th3 cells originate in the periphery and operate by the production of the anti-inflammatory cytokines IL-10 and TGF- β via an antigen-driven mechanism [14,18]. However, inducible and naturally occurring Treg cells share a functional relationship. The modulatory functions of Treg cells have also been attributed to the production of IL-10 and TGF- β and can eventually be induced in the periphery. Antigen-specific Treg cells may also function through cell–cell contact independently of IL-10 and TGF- β . It has been suggested that IL-10 producing T cells might not be exclusively generated from naïve CD4⁺, but also from Th1 or Th2 cells that have undergone chronic stimulation, resulting in the disappearance of effector cytokines [18].

Evidence that both subtypes of Treg cells play a major role in the prevention of allergen sensitization and modulation of disease activity comes from human and animal studies [18]. The function of Treg cells are impaired or dysregulated in allergic patients [20]. It is noteworthy to mention that a large number of healthy individuals are sensitized to allergens. However, these persons likely mount a balanced immune response, consisting of allergen-specific

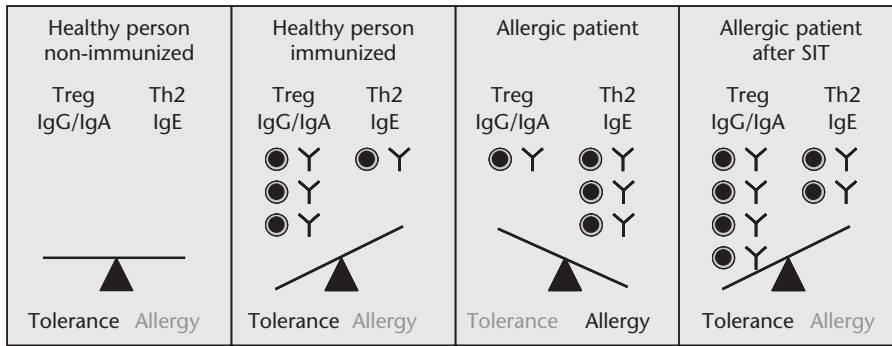


Figure 2.1 Immune response to allergens in healthy and allergic individuals.

Tr1 cells and high levels of protective antigen-specific IgG4 and IgA (Fig. 2.1) [19,20]. Treg cells modulate different cells and effector functions, which are associated with allergic diseases. They downregulate Th2 cell activation, stimulate IgG4 (indirectly induced by IL-10) and IgA (induced by TGF- β) class-switch in B-cells [18], and might inhibit mast cell and eosinophil functions through the production of IL-10 and TGF- β [21,22]. Further evidence for the importance of Treg cells in the prevention of allergy arises from the finding that successful specific immunotherapy (SIT) is associated with a decrease in allergen-specific Th2 cell responses and the induction of allergen-induced Tr1 and TH3 cells (Fig. 2.1) [23,24].

B-cell response in IgE-mediated allergy

Antigen-specific IgE produced by B-cells is essential for type I allergic reactions. Apart from its pathological function in allergies, antigen-specific IgE is an important component of protective immunity against helminths [25].

IgE class-switch recombination (CSR) is strongly dependent on antigen-specific Th2 cells [26], although some evidence exists that other cells, including mast cells, basophils, and eosinophils, can provide the required signals [27,28]. Naïve B-cells capture their specific antigen (allergen) by the B-cell receptor (BCR), process it, and present it in the context of MCH class II to Th2 cells. This interaction illustrates why B- and T-cell epitopes are frequently found in the same protein. Th2 cells provide the major signals responsible for the IgE CSR; IL-4 and IL-13, as well as CD40L on T-cells, which binds CD40 on B-cells. The activation of the transcription factors STAT6 (induced by IL-4 and IL-13) and NF- κ B (induced by CD40) in the B-cells promotes the CSR to IgE [26]. Given the potent, and potentially life-threatening effects of allergen-specific IgE, the class-switch is tightly controlled by several antagonizing signals, such as cytokines (IFN- γ , IL-21, TGF- β), B-cell surface receptors (CTLA4, CD45, BCR, CD23), and transcription factors (BCL6, ID2) [26]. Activated B-cells subsequently expand and are subjected to affinity maturation by somatic hyper mutation

(SHM). However, there is less evidence for mutational maturation of antigen selection by IgE compared to IgG, although the existence of high-affinity IgE antibodies has convincingly been demonstrated [29]. CSR and SHM take place in secondary lymphoid organs, from which the cells migrate to mucosal effectors sites and undergo terminal differentiation to plasma cells [4]. However, it is becoming increasingly apparent that CSR and SHM may also occur at mucosal sites of allergic patients [30,31].

Secreted antibodies provide humoral immunity against pathogens, but persistent production of specific IgE is also a hallmark of type I allergies. IgE plasma level is the lowest (<100 μ g/l in normal adults) and the biological half-life is the shortest (~12 hours in the serum [32] and ~14 days in the skin [33]) of all immunoglobulin classes. Elevated titers of antigen-specific IgE are found in allergic patients and after helminth infections, even in the absence of antigen for several years [25,34]. This conclusion is consistent with clinical experience, since patients can develop recurrent allergic symptoms despite long-term allergen avoidance. Stable maintenance of B-cell memory can be divided into two broad categories: long-lived plasma cells and memory B-cells. Three competing concepts, which are not mutually exclusive, might explain humoral memory. First, short-lived plasma cells (which do not divide) are constantly generated from memory B-cells, a process that might be driven by persisting antigen. Second, long-lived plasma cells with a defined half-life of several weeks develop from cytokine-receptor or Toll-like receptor (TLR) activated memory B-cells. Third, memory arises from long-lived plasma cells that survive in appropriate survival niches, which are located in the bone marrow and possibly in secondary lymphoid organs and inflamed tissue [35]. Whether IgE is predominantly produced within or outside mucosal sites is a matter of debate [35,36]. The fact that allergen-specific IgE production can be transferred by bone marrow transplantation argues that bone marrow-derived IgE might contribute to the sensitization of effector cells at mucosal surfaces [37]. The persistence of allergen-specific IgE even under immunosuppressive therapy suggests that long-lived plasma cells

(possibly located in the bone marrow) contribute substantially to IgE-mediated allergy, because long-lived plasma cells are resistant to immunosuppression [35].

Allergen-specific IgG and IgA

Allergen-specific IgG1, IgG4, and IgA are frequently detectable in allergic and non-allergic individuals. It has been proposed that these immunoglobulin subclasses might prevent allergic reactions and that the ratio of antigen-specific IgE and antigen-specific IgG determines the severity of an allergic reaction following allergen exposure (Fig. 2.1) [14,19,38]. Evidence for the protective effect of allergen-specific IgG arises from SIT studies. Although specific IgE levels not always decrease after successful SIT [39], several studies show that allergen-specific IgG1 and, in particular, IgG4 levels increase [40–42], most likely as a result of the change in the T cell profile (Fig. 2.1).

Allergen-specific IgG may compete with IgE for the binding of allergens and inhibit, therefore, high-affinity Fc receptor (FcεRI) cross-linking [14,43–45]. Furthermore, allergen-specific IgG (in particular IgG1) may co-aggregate FcγRIIB with FcεRI. FcγRIIB contains an intra-cytoplasmatic immunoreceptor tyrosine-based inhibitory motif (ITIM) and has been reported to inhibit IgE-induced mast cell and basophil activation [43,46]. Its inhibitory function in allergy has

been demonstrated by studies using knock-out mice [47]. Co-cross-linking of FcγRIIB and FcεRI might also be exploited for the engineering of safe therapeutic agents for SIT that maintain all B- and T-cell epitopes. A human IgG1 Fc fragment fused to the cat allergen Fel d 1 was reported to inhibit Fel d 1-induced activation of human mast cells and basophils sensitized with serum from patients allergic to Fel d 1; and also Fel d 1-induced anaphylaxis in human FcεRIα transgenic mice [48]. However, the relationship between the efficacy of SIT and the induction of allergen-specific IgG has also been questioned, because some studies failed to observe a correlation [45,49]. These conflicting data might be explained by the fact that allergen-specific IgG can also have immune-enhancing effects. For example, IgG can stimulate immune and inflammatory cells such as mast cells and eosinophils through binding to Fcγ receptors (Table 2.1) [50,51]. Allergen-specific IgG may enhance allergic reactions by the formation of larger allergen aggregates (super-cross-linking) [52] or by activating complement (IgG1-3, not IgG4). Furthermore, the binding of certain IgG to allergens can enhance IgE affinity, which may be due to changes of the three-dimensional allergen structure [53]. However, IgG4 seems to be of particular importance to prevent allergic reactions. It binds only with low affinity to Fcγ receptors and does not activate complement. Furthermore, it has been shown very recently that IgG4 antibodies are dynamic molecules

Table 2.1 Fc receptors on mast cells, eosinophils, and basophils

Receptor (CD)	Chains	Binding affinity	Ligands	Expression MC, E, B	Other cells
FcγRI (CD64)	α, γ	Ig1: 10^8 M^{-1}	(1) IgG1 = IgG3, (2) IgG4, (3) IgG2	MC, E ¹	M, N ¹ , DC
FcγRII-A (CD32)	α	Ig1: $2 \times 10^6 \text{ M}^{-1}$	(1) IgG1, (2) IgG2 ² = IgG3, (3) IgG4	MC, E, B ³	M, N, LC, P
FcγRII-B (CD32)	α ⁴	Ig1: $2 \times 10^6 \text{ M}^{-1}$	(1) IgG1 = IgG3, (2) IgG4, (3) IgG2	MC, E, B ³	M, N, B
FcγRIII (CD16)	α, β, γ	Ig1: $5 \times 10^5 \text{ M}^{-1}$	(1) IgG1 = IgG3, (2) IgG4, (3) IgG2	E	M, N, B
FcαRI (CD89)	α, γ	IgA1, IgA2: 10^7 M^{-1}	IgA1 = IgA2	E	M, N
FcεRI	α, β, γ	IgE: 10^{10} M^{-1}	IgE	MC, B, E ^{1,5}	M ⁵ , DC ⁵ , LC ⁵
FcεRII (CD23)	Single	IgE: 10^8 M^{-1}	IgE, others ⁶	E	B, T, M, LC, P

MC, mast cells; E, eosinophils; B, basophils; M, monocytes; N, neutrophils; B, B-cells; T, T-cells; DC, dendritic cells; LC, Langerhans' cells; P, platelets.

¹ Inducible.

² Only some allotypes of FcγRII-A bind IgG2.

³ CD32 expression has been shown, but to date it is not clear whether FcγRII-A or FcγRII-B is expressed.

⁴ Contains an ITIM motif (inhibitory).

⁵ β chain is not expressed.

⁶ See text (IgE receptors). Modified from Janeway CA *et al.* [55].

that exchange Fab arms by swapping a heavy chain and attached light chain (half-molecule) with a heavy-light chain pair from another molecule, which results in bispecific antibodies. IgG4 molecules thereby lose their ability to cross-link antigen and to form immune complexes under most conditions. This mechanism might provide the basis for the anti-inflammatory activity attributed to IgG4 [53b].

Obviously, the clinical consequences of an allergen exposure are influenced by several factors: (i) allergen structure, (ii) dose and duration of exposure, (iii) epitope specificity and affinity of the antibodies, (iv) absolute and relative amounts of immunoglobulin subclasses, (v) expression profiles of Fc receptors on effector cells, (vi) composition and activation status of immune cells in the exposed tissue, and (vii) profile of allergen-specific effector T cells [14,54]. This demonstrates that monitoring of SIT by measurement of antigen-specific immunoglobulins can be sometimes misleading and that complex biological systems are required to analyze the immunological effects of SIT.

Genes and environment

It is generally acknowledged that risk factors for the development of allergic diseases include genetic and environmental factors, but certainly also individual factors such as psychological conditions.

Sibling and family studies have revealed that the genetic background affects the risk of developing allergy. These observations can be related to several gene polymorphisms. Not surprisingly, many of these genes encode for key factors of Th2-type and IgE-related immune reactions, such as FcεRI β chain, IL-4R, IL-13, and STAT6 [56,57]. Interestingly, also genes for innate immune receptors, such as CD14 and NOD1, might be associated with allergy [58,59]. These analyses suggest that the threshold of the immune system to environmental stimuli is controlled by natural genetic variation and gene–environment interaction.

Prevalence of allergic disorders is considerably lower in developing countries and in rural area in comparison to urban areas within one country. Furthermore, the number of allergic patients has strongly increased within the last decades, further arguing that environmental factors are substantially responsible for atopy [60,61]. However, there is little consistent evidence to suggest that obvious risk factors, such as increased exposure to indoor allergens, pollution, or changes in diet and breast-feeding, could account for the rise in allergic diseases. Another category of environmental factors that show overwhelming inverse association with atopy are infections, vaccinations, absence of antibiotic treatment, traditional farming environments, older siblings, day care attendance, and pet ownership [60–62]. These findings lead to the “hygiene hypothesis” which proposes that reduced exposure to particular microbiological stimuli [63], which decrease with improved living standards and higher

personal hygiene, might result in an increased risk of developing allergy. Indeed, perinatal treatment (mothers pre-natal and infants 6-month postnatal) with the probiotic *Lactobacillus GG* strain significantly reduced the development of allergies up to the age of 4 years [64]. Although the “hygiene hypothesis” is widely accepted, the underlying mechanisms are controversial. In particular, the molecular link between environmental stimuli and immune hyper-responsiveness is far from being understood. It has been suggested that a chronic stimulation of the immune system by microbes creates a kind of immunotolerant environment. This concept is attractive, since it would also explain the known relation between increased hygiene standards, decline in infection rates, and increase in autoimmune diseases [65]. Tolerance induction might involve both the innate and the adaptive immune systems. Animal studies show protective effects of certain TLR ligands in allergen-induced inflammation [60]. Furthermore, microbial components such as CpG-containing immunostimulatory DNA, a TLR9 agonist, or the bacterial cell wall component monophosphoryl lipid A have been used successfully as adjuvants in clinical studies for SIT. These compounds considerably improve immunological surrogate markers and clinical outcome [66,67]. Chronic infections might induce Treg cells which provide non-specific bystander suppression [61]. Moreover, it has been suggested that cross-reactive IgE and IgG-binding structures exist in allergens and parasite antigens [61]. Interestingly, parasite-specific IgG4 antibodies can inhibit IgE-mediated degranulation of effector cells isolated from allergic patients, suggesting that chronic parasite infections induce allergen-cross-reactive “blocking antibodies” [68].

Innate immune recognition of allergens

The concept that the driving force for the induction of an adaptive immune response is the innate immune recognition system in dendritic cells and other immune cells has been generally accepted [69]. In light of this model, it is interesting to note that certain allergens contain immune stimulatory properties that target the innate immune system. For example, pollens contain intrinsic NADPH oxidase activity, generating reactive oxygen species (ROS) [70] and/or bioactive lipids (phytoprostanes) [71]. These pollen intrinsic bioactivities have been shown to instruct a Th2 cell polarization. Furthermore, the pollen NADPH-induced ROS vigorously augments specific IgE production and allergic airway inflammation induced by the major pollen antigens in mice [70]. Several allergens have enzymatic functions, frequently protease activities that facilitate transepithelial allergen delivery and spreading, but also activation of epithelial cells via protease-activated receptors, resulting in the production of pro-inflammatory cytokines [15]. Most interestingly, a recent study reports that the major peanut allergen Ara h 1 is

recognized by DC-SIGN, a C-type lectin acting as a pathogen recognition molecule. Ara h 1 challenged dendritic cells primed a Th2-skewed T-cell response [72]. Ara h 1 is an N-glycan that shares structural similarities to N-glycans from *Schistosoma mansoni* egg antigens, that are well studied Th2 PAMPs (pathogen-associated molecular pattern). These data suggest that at least some allergens provoke allergies by a two-signal strategy, in which signal 1 is the innate response and signal 2 the adaptive response.

Allergic inflammation

Once an individual is sensitized and allergen-specific IgE has been formed, recurrent antigen exposure readily induces the manifestations of atopic disease. The response has been categorized into three phases: (i) acute or immediate-phase reaction, (ii) late-phase reaction, and (iii) chronic allergic inflammation (Fig. 2.2).

An acute reaction occurs when the allergen, after crossing the mucosa, binds to antigen-specific IgE on the surface of mast cells and eventually basophils. This induces cross-linking of FcεRI resulting in the release of pro-inflammatory mediators, such as histamine, eicosanoids, and cytokines. Clinical signs of the acute response (weal and flare) develop within seconds to minutes. A particular characteristic of intestinal food allergy might be a delayed “acute reaction” because of the passage time of dietary antigens through the esophagus and the stomach. The immediate reaction may be followed by a late-phase reaction starting after 4–48 hours. Mast cell-derived mediators induce expression of adhesion molecules on endothelial cells, which bind its ligand on the surface of eosinophils, basophils, Th2 cells, and NKT cells [17,73]. This leads to the preferential extravasations of these cells through vessel walls into sites of inflammation. Their recruitment to the target organ depends on the production of a number of chemokines [74]. Within the tissue, infiltrating cells are further activated by the inflammatory environment and allergens via antigen-specific recognition (IgE on basophils and MHC class II-dependent antigen presentation to Th2 cells). Late-phase reactions may be developed independent of IgE. It has been reported that birch pollen-related food allergen that lost capacity to bind to IgE because of cooking, but retained their T-cell stimulatory potency, does not induce an acute-phase reaction such as the oral allergy syndrome; but can still induce a late-phase response like atopic eczema [73]. Repeated allergen exposure may lead to a chronic inflammatory response causing persistent infiltration of mast cells, eosinophils, basophils, and lymphocytes and subsequent chronic structural changes of the tissue, such as mucus cell and smooth muscle hypertrophy, fibrosis, and organ dysfunction. The specific clinical features of each of the different phases vary according to the anatomical site affected (Fig. 2.2).

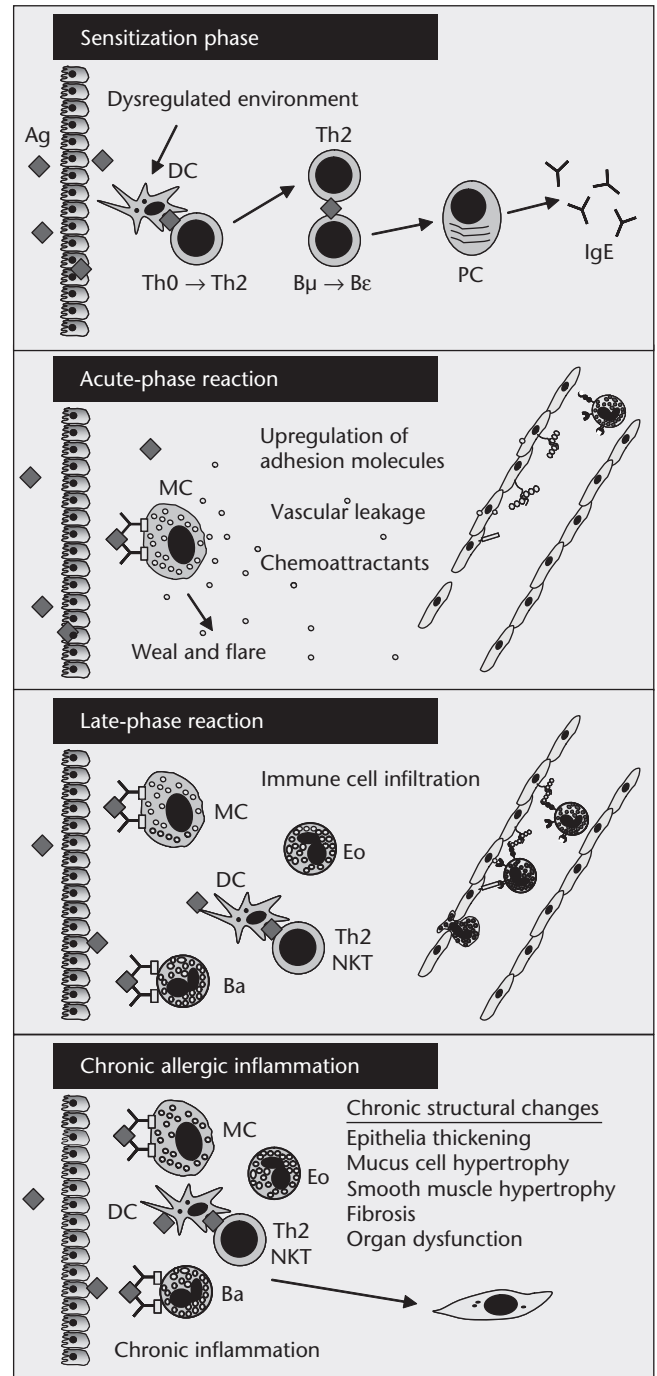


Figure 2.2 Phases of allergic disease. For details see text. Ag, antigen; DC, dendritic cell; Th0, naïve CD4⁺ lymphocyte. Th2, Th2 lymphocyte; Bμ, naïve B-cell; Bε, primed B-cell after IgE class-switch; PC, plasma cell; MC, mast cell; Eo, eosinophil; Ba, basophil; NKT, NKT cell.

IgE receptors

Most of the IgE is bound to its FcεRI expressed on mast cells, basophils, monocytes, dendritic cells, Langerhans cells, eosinophils, and platelets (Table 2.1). Monomeric IgE binds

to FcεRI with high affinity and has a very slow dissociation rate (half-life of about 20 hours). The FcεRI is composed of an IgE-binding α chain, a tertaspanning transmembrane β chain, and a homodimeric disulfide-linked γ chain. The β chain, which is only expressed in mast cells and basophils, functions as an amplifier and in its absence the receptor initiates only weak signals. Cross-linking of FcεRI initiates signaling mediated through the immunoreceptor tyrosine-based activation motif (ITAM) encoded in the cytoplasmic tails of the β and γ chains. For a detailed description of the signaling events via FcεRI, the reader is referred to some recent and comprehensive reviews [46,75–77]. In brief, downstream signaling results in intracellular Ca⁺⁺ release and activation of PKC (protein kinase C), MAPK (mitogen-activated protein kinase) pathways, NF-κB (nuclear factor-kappaB), PI3K (phosphoinositide-3 kinase), and PLA₂ (phospholipase A₂). In mast cells and basophils these events result in degranulation, generation of arachidonic acid metabolites, and enhanced expression of genes encoding for pro-inflammatory cytokines and chemokines. IgE binding in the absence of antigen increases receptor expression, induces anti-apoptotic signals, and triggers low-level cytokine production in mast cells [78,79]. The downstream signaling induced by monomeric IgE binding remains elusive. In antigen-presenting cells, FcεRI has been shown to facilitate antigen presentation by IgE-dependent capture of antigens [36,80].

The low-affinity IgE receptor (FcεRII/CD23) is not a member of the Ig superfamily. CD23 is a type II integral membrane protein with a C-lectin domain at the distal C-terminal end of the extracellular sequence. The lectin domain contains the binding sites for all known ligands of CD23 including IgE, complement receptors CR2, CR3, and CR4 (also termed CD21, CD18-CD11b, CD18-CD11c, respectively), and vitronectin. CD23 facilitates antigen presentation of B-cells and acts as a negative feedback regulator of the IgE class-switch. In enterocytes, CD23 facilitates the bidirectional transport of IgE–antigen complexes and thus may participate in antigen sampling from the intestinal lumen [36].

Mast cells

Mast cells are widely distributed throughout the body, frequently found around blood vessels, attached to nerves and at mucosal surfaces. Bone marrow-derived mast cell progenitors migrate via the peripheral blood into the tissue, where they undergo final maturation under the influence of local microenvironmental factors. Stem cell factor (SCF), produced either in a soluble or membrane-bound form by fibroblasts, endothelial cells, and stromal cells, is the essential factor for both mast cell maturation and survival of mature mast cells [81]. The importance of SCF and its receptor KIT is stressed by the fact that KIT-deficient mice basically lack mast cells. Mature mast cells are long-living cells that maintain the capability to grow. In particular IL-4, but also IL-3

and IL-9, induces proliferation of tissue mast cells in an SCF-dependent manner [82–84].

Human mast cells are commonly classified according to their protease content and related ultrastructural signatures of their granules. Mast cells containing only tryptase (MC_T) predominate in the lung and intestinal mucosa. Tryptase and chymase positive mast cells (MC_{TC}) are mainly located in the skin and the intestinal submucosa. It has been suggested that these subtypes can be further classified according to their responsiveness to certain IgE-independent agonists. This heterogeneity might reflect that mast cells exhibit differences in biochemical and functional properties, depending on the anatomical site in which the cells reside; and/or the biological process, in which they participate [76].

Mast cells exert their biological functions mainly by the release of humoral mediators. Mast cell, as well as eosinophil and basophil, mediators can be categorized into three groups: (i) preformed secretory granule-associated mediators, (ii) *de novo* synthesized eicosanoid metabolites, and (iii) cytokines and chemokines which are mainly *de novo* synthesized but are also sometimes found to be stored within secretory granules (Table 2.1). Secretory granule-associated mediators of mast cells are mainly histamine, proteases, and proteoglycans. Histamine exerts its wide-ranging biological activities via binding to four histamine receptors (H1–4). With regard to allergic inflammation, the H1-receptor seems to be of particular importance. Its activation affects the function of blood vessels (dilation and increased permeability), smooth muscles (contraction), epithelial cells (mucus production), and Th2 lymphocytes (recruitment) [85]. Mast cell proteases can cleave several host proteins, including protease activating receptors, and have been linked to immune as well as non-immune functions of mast cells, such as tissue repair and fibrinolysis [86]. Very recently, two exiting studies showed that mast cell-derived proteases cleave endogenous (endothelin-1, induced by bacterial infection) and exogenous (snake and honeybee venoms) toxins and subsequently limit pathology [87,88]. Moreover, mast cell proteases mediate cleavage of allergens. This might be an important negative feedback loop terminating or weakening allergic inflammation [89]. The main mast cell-produced metabolites of arachidonic acid (and their prominent function) are PGD₂ (smooth muscle contraction, chemoattractant for eosinophils, and Th2 cells), LTC₄ (increase of vascular permeability, mucus production, and smooth muscle contraction), and LTB₄ (chemoattractant for neutrophils, eosinophils, and CD8⁺ T-cells) [90]. A significant amount of cytokines and chemokines are produced by mast cells. Among them, IL-3, IL-5, and IL-13 seem to be specifically important in allergic reactions [91]. They mediate basophil (IL-3), and eosinophil recruitment (IL-5), IgE class-switching, mucus production, and airway hyperreactivity (all IL-13). Although detectable in rodent mast cells [92], human mast cells produce no or only small amounts of IL-4 [27,91].

Table 2.2 Mediators of mast cells, eosinophils, and basophils

Mast cells	
Granule-associated	Histamine, tryptase, chymase, carboxypeptidase A, heparine, chondroitinsulfate E, many acid hydrolases, catepsin G
<i>De novo</i> synthesized	LTC ₄ , LTB ₄ , PGD ₂ , PAF
Cytokines/chemokines	IL-1β, IL-3, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-13, IL-16, IL-18, IL-25, TNF-α, TGF-β, GM-CSF, MIP-1α, bFGF, VPF/VEGF, and others
Eosinophils	
Granule-associated	ECP, EDN (formerly called EPX), MBP, EPO, CLC
<i>De novo</i> synthesized	LTC ₄ , LTB ₄ , PAF, PGE ₁ /E ₂ , thromboxane B ₂ , oxygen metabolites (H ₂ O ₂ , O ₂ ⁻)
Cytokines/chemokines	IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, TNF-α, TGF-α, TGF-β, GM-CSF, RANTES, MIP-1α, MCP-1, eotaxin-1, VPF/VEGF, PDGF-B
Basophils	
Granule-associated	Histamine, chondroitin sulfate A, neutral protease with bradykinin-generating activity, β-glucuronidase, elastase, cathepsin G-like enzyme, MBP, CLC, granzyme B (induced by IL-3)
<i>De novo</i> synthesized	LTC ₄
Cytokines/chemokines	IL-4, IL-13, MIP-1α

Cross-linking of the FcεRI is the most potent trigger to activate mast cells for the release of all three classes of mediators. Degranulation and eicosanoid production occur within minutes. Cytokines, if not stored within the granules, are mainly transcriptionally regulated and produced within 2–6 hours [91]. IL-3, IL-4, and IL-5 enhance FcεRI-mediated reactions [83,91,93], ensuring an autocrine and paracrine positive feedback loop, because the main producers of these cytokines are mast cells (IL-3, IL-5) and, after recruitment, basophils (IL-4), eosinophils (IL-3, IL-5), and Th2 cells (IL-4, IL-5) (Table 2.2). IL-4 induces IL-5 production by mast cells even in the absence of IgE-cross-linking [93]. On the other hand, the central modulatory cytokines of Treg cells, IL-10 and TGF-β, have been shown to induce apoptosis in mast cells (TGF-β) and decrease FcεRI-induced mediator production (IL-10 and TGF-β) [21,22]. Several IgE-independent mast cell triggers have been described. SCF, complement factors (C5a), neuropeptides (substance P), adenosine, IgG-cross-linking of FcγRI, and several TLR ligands stimulate mast cell effector functions, but are substantially less effective than FcεRI-mediated signals [50,94].

Considering the multiple biological effects of mast cell mediators, one can propose several possible functions of mast cells *in vivo*. A mouse mast cell “knock-in” model has considerably contributed to the understanding of the role of mast cells in several pathologies. Apart from allergies, recent studies point out that mast cells are important effector cells in immune complex-induced autoimmune models (mediated via Fcγ on mast cells) [95] and confer protection against acute bacterial infections [94,96,97]. Using this mast cell knock-in model, it has been shown that mast cells contribute to all stages of immunopathology in allergic asthma, which are the immediate-phase, late-phase, and chronic allergic

reactions. Mast cell effector functions were mainly, but not exclusively, dependent on FcεRI/FcγRIII expression [98].

An exiting new field of mast cell research concerns the questions whether and how mast cells contribute to the instruction of the adaptive immune response? Several lines of evidence now indicate that mast cells deliver signals important for dendritic cell activation and T-/B-cell priming, such as cytokines (e.g. TNF-α) and co-stimulatory molecules (CD40L, OX40 ligand). *In vivo*, delayed-type hypersensitivity responses may be decreased in the absence of mast cells, although data are conflicting [99]. However, recent studies suggest that mast cells mediate regulatory functions [100,101]. In an allograft model, mast cells were absolutely essential in Treg cell-dependent peripheral tolerance. IL-9 represented the functional link through which Treg recruited and activated mast cells to mediate regional immune suppression [101]. However, it remains elusive as to whether mast cells play a role (either activating or tolerizing) during the sensitization phase of allergies.

Despite the new enthusiasm about the multiple functions of mast cells in immune diseases and immune regulation, one should consider that most of the data derived from the murine system, in particular with regard to mast cell functions, may not always reflect the human situation [102].

Basophils

Basophils have often been considered as the circulating progenitor of tissue mast cells, because of their similar morphology and staining characteristics due to the basophilic granule contents; and their overlapping functional properties. It is now generally accepted that mast cells and basophils originate from separate lineages [103,104]. Basophils fully

mature within the bone marrow and are subsequently released to the peripheral blood, where they form the smallest population of leukocytes (0.5–1% of total leukocytes, considerably increased in allergic patients). IL-3 is the most important basophil growth factor, but other growth factors such as IL-5, GM-CSF, NGF, and TGF- β have been identified [103]. Basophils enter the tissue at sites of inflammation, being directed by adhesion molecules and chemoattractants [74,105]. This array of growth factors and chemoattractants largely overlap with the factors promoting eosinophil development and recruitment. This may explain the combined involvement of both cell types in many diseases. In contrast to mast cells, both basophils and eosinophils are short-living cells, surviving in the tissue only for several days [103,106]. Basophils have been detected particularly in allergic late-phase reactions within the skin and the lung [107]; whereas their involvement in gastrointestinal pathologies is largely unknown.

Similar to mast cells, basophils release large amounts of histamine and LTC₄, but no PGD₂, which is specifically mast cell-derived. Basophils are a major source of IL-4 and IL-13 [108,109] that can be released upon IgE-dependent and IgE-independent stimulation. On a per cell basis, activated basophils produce more IL-4 and IL-13 than any other cell type. Basophils produced a much more limited cytokine profile than mast cells and eosinophils (Table 2.1). However, the specific expression of IL-4, which is hardly produced by mast cells and eosinophils, suggests a particular role for basophils in the antigen-specific priming of Th2 cells [110].

After cross-linking of Fc ϵ RI, the release of histamine and eicosanoid is nearly complete by 20 minutes, whereas IL-4 and IL-13 production follows a time course with a maximal response after 4 and 20 hours, respectively. Small amounts of IL-4 (<10 pg/10⁶ basophils) become detectable within 5–10 minutes after stimulation, suggesting that preformed IL-4 is released [106]. IgE-independent secretagogues are the anaphylatoxins C3a and C5a, platelet-activating factor (PAF), eosinophil-derived major basic protein (MBP), cytokines (IL-3, IL-5, GM-CSF), and chemokines (MCP-1, -3, eotaxin, RANTES, MIP-1 α , IL-8). Of particular interest is the observation that IL-3, IL-5, and GM-CSF only induce small amounts of mediator release, but substantially enhance the effects of almost all IgE-dependent and IgE-independent agonists. The latter seems to be of greater importance; particularly in the allergic late-phase reaction characterized by enhanced cytokine production, and has been named “basophil priming.” Similar observations could be made for other inflammatory cells such as eosinophils, suggesting a rather general way of inflammatory cell regulation [103,106,111].

A growing body of evidence suggests that basophils are involved in the defense against helminth infections [112]. However, basophil *in vivo* studies are limited, because a basophil-deficient mouse strain does not exist. Mukai *et al.*

recently demonstrated a series of elegant transfer studies, that basophils, in the absence of T-cells and reacting mast cells, induce an IgE-dependent delayed-onset allergic inflammation, whereas mast cells were necessary for the immediate-phase response [113].

Eosinophils

Eosinophils fully mature within the bone marrow, from which they enter the bloodstream. IL-3, IL-5, and GM-CSF are particularly effective in regulating eosinophil growth and maturation. Of these three, IL-5 is the most specific and potent. Eosinophils normally account for only 1–3% of peripheral blood leukocytes and, under physiological conditions, their presence in tissue is primarily limited to the gastrointestinal mucosa which forms the largest eosinophil reservoir of the body. In the course of several diseases, including allergy, eosinophilic gastroenteritis, and helminth infection, eosinophils can selectively accumulate in the peripheral blood or any tissue [28,114]. Recruitment of eosinophils depends on the production of a number of chemokines (e.g. RANTES and the eotaxins). Only IL-5 and the eotaxins selectively regulate eosinophil trafficking [28,74]. Anti-IL-5 treatment reduced tissue eosinophilia in asthma patients by 55%, however, without affecting symptoms [115].

Eosinophils secrete an array of cytotoxic granule cationic proteins that are present in large quantities in the cells: eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), major basic protein (MBP), and eosinophilic peroxidase (EPO). The enzymatic activities and several functions of these proteins have been defined and have been recently reviewed in detail [28,116]. Apart from their toxic effects and antiviral activity, these proteins activate mast cells (EPO, MBP) and suppress T-cell proliferation (ECP) and immunoglobulin synthesis (ECP). Eosinophils produce several eicosanoid metabolites, oxygen radicals, and multiple cytokines/chemokines [28] (Table 2.1).

C5a, C3a, and PAF cause degranulation in eosinophils, whereas other stimuli, such as the cytokines IL-3, IL-5, and GM-CSF, have a weak or no direct effect. However, this set of cytokines “prime” eosinophils for enhanced mediator release to other stimuli, including otherwise ineffective agonists [117]. Interestingly, PAF produced by eosinophils has been considered as an autocrine secretagogue [118]. Furthermore, chemokines, such as the CCR3 ligands MCP-3, MCP-4, RANTES, and eotaxin, induce degranulation in eosinophils [119]. Eosinophils express Fc α RI, Fc γ RII, and Fc γ RI (inducible, Table 2.2) and secretory IgA and IgG are strong signals for degranulation [51,120,121] mediating for example antibody (or complement)-dependent cellular toxicity against helminthes. The role of the Fc ϵ RI on the eosinophil is still a matter of controversy [122]. Eosinophil activation by cytokines and immunoglobulins is critically

dependent on $\beta 2$ -integrins, especially on Mac-1 binding to ICAM-1 [123], suggesting that their activity is silenced in the bloodstream.

There is strong evidence that eosinophils play a considerable role in the defense against helminthes. This is supported by findings in both humans and animal models [28,124]. Recently, two eosinophil-deficient mouse models have been developed. In both strains, eosinophils substantially impact on experimental allergic asthma, but apart from this common finding, they give divergent results. While the *PHIL* mice are completely protected from developing airway hyperresponsiveness and show partial protection from airway mucous metaplasia, the Δ dbl GATA mice lack improvement of these parameters, and rather show attenuation of airway remodeling. However, airway remodeling has not been investigated in the *PHIL* mice [125,126].

Conclusion

The allergen-specific Th2 and B-cell priming, the function of IgE and Fc ϵ RI, and the biology of allergic effector cells have been intensively studied during the last few decades. The current advances in understanding these fundamental immunological mechanisms lead to the design of new treatment approaches, of which some have reached the level of clinical trials or approval [14,115,127]. We are only beginning to understand the regulatory network of the immune system, in particular the function of Treg cells and “blocking” allergen-specific antibodies. Therefore, we still need to learn how we can direct the immune system toward a tolerizing response to introduce more rational and safer vaccine strategies [14]. The link between environmental stimuli and allergy remains poorly understood and requires further investigation.

A specific problem concerning food allergy lies in the fact that the general pathophysiological concepts of allergy have mainly been developed in model systems of non-food-related atopy. This applies also to this chapter, which reviews in large part data generated in studies of non-food allergy. This is further reflected by the fact that new drugs are often designed for the treatment of asthma, rhinoconjunctivitis, atopic dermatitis, or insect allergy, but rarely for food allergy. The pathophysiology and the clinical management of food allergy might be considered more complex in comparison to other allergic disorders, in particular, as the gastrointestinal tract is difficult to access for investigation and the symptoms are often variable and unspecific. Therefore, a better understanding of the specific immunopathology of food allergy and an improvement in the diagnostic approach are necessary to provide improved treatment options.

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