

Allergy and the Gut

Stephan C. Bischoff Julika H. Mayer Michael P. Manns

Department of Gastroenterology and Hepatology, Medical School of Hannover, Hannover, Germany

Key Words

Food allergy · Intestinal allergy · Inflammatory bowel disease · Irritable bowel syndrome · Mast cells · Eosinophils · Interleukin-4

Abstract

There have frequently been doubts as to the relevance of food allergy, in particular as far as the involvement of the intestinal tract is concerned. Several studies, however, have confirmed the existence of allergic reactions in the gut, with an estimated prevalence of about 1–2% in adults. Clinical symptoms are unspecific and include nausea, vomiting, abdominal pain, cramping and diarrhea. Intestinal mast cells, as well as intestinal eosinophils, have been shown to be involved in the pathogenesis of food-allergy-related enteropathy. In addition to classical IgE-dependent degranulation, further agonists have been demonstrated for mast cell activation, for example IL-4. The methods used to confirm the diagnosis of intestinal allergy are still insufficient. Until now, blinded oral challenge procedures with food antigens have been accepted as the 'gold standard' in diagnosing food allergy, although these tests have practical problems. Therefore, new test systems have been developed, such as endoscopic provocation tests, that may improve diagnostic procedures. Elimination diet still presents the main basis of therapy. Aspects to be focused on in the future are the role of IgE-independent allergic mecha-

nisms in intestinal allergy, the impact of cross-reactivity with other allergens and the relationship to other inflammatory bowel diseases such as Crohn's disease, ulcerative colitis, celiac disease and irritable bowel syndrome.

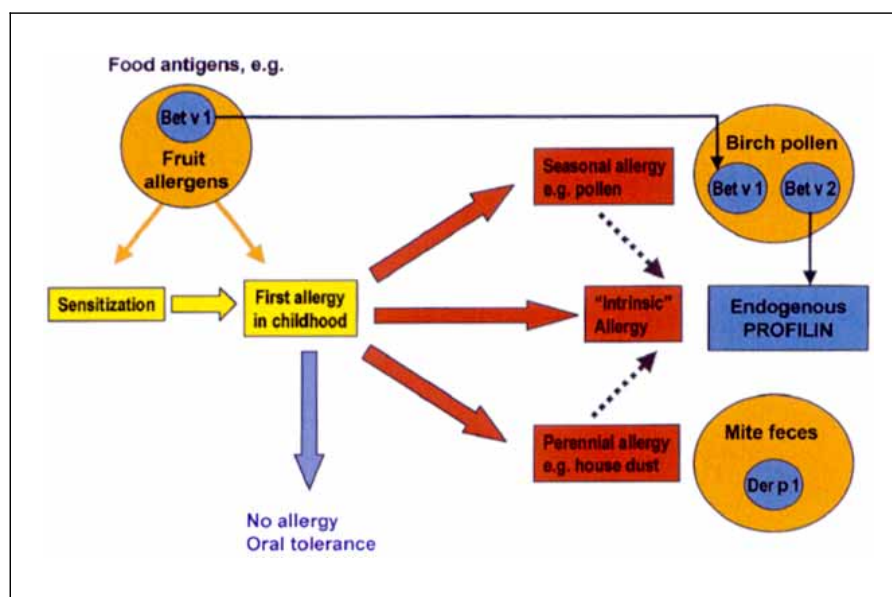
Copyright © 2000 S. Karger AG, Basel

Introduction

Enormous progress has been made in our understanding of allergic diseases and their management during the last 3 decades since the discovery of IgE, in particular in the field of asthma and rhinitis. However, some other fields of allergy have remained rather obscure, e.g. hypersensitivity reactions that are not based on the classical type I anaphylactic reaction, often occurring rather in a delayed fashion. Furthermore, there have frequently been doubts as to the clinical relevance of food allergy, in particular as far as the involvement of the intestinal tract is concerned. Especially in adults, the significance of gastrointestinal (GI) allergy is still questioned. There is, however, indeed substantial evidence in the literature as well as from our own experience that allergic reactions exist in the GI tract, and that this food-allergy-related enteropathy is of relevance not only in children, but in certain adult patients as well [1–3].

One of the major problems with GI allergy is the fact that its manifestations can hardly be visualized – as is pos-

Fig. 1. The natural course of allergy (current hypotheses). Onset of allergic disease accompanied by sensitization to food proteins (e.g. fruits etc.) is frequently observed in early childhood, possibly because of an immature immune system or incomplete gastrointestinal barrier. In most cases, children lose food intolerance, but a few fail to develop oral tolerance during childhood. The latter may develop seasonal allergy, e.g. against pollens that cross-react with food allergens. Others develop unrelated allergies which may occur also in patients who never suffered from allergic diseases before.



sible in skin reactions – or measured – as by bronchial obstruction or hyperresponsiveness in allergic asthma. Our knowledge of epidemiology, clinical manifestation, and specific therapy is poor, mainly due to a lack of standardized diagnostic procedures and due to the inaccessibility of the target organ. The role of GI hypersensitivity reactions in the natural history of disorders such as inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis) and irritable bowel syndrome also remains controversial [4–6]. Furthermore, the understanding of the pathomechanisms involved is limited.

Epidemiology

A recent population study by Young et al. [7] used double-blind placebo-controlled food challenges to confirm reported reactions to food on an objective basis. The results of this survey suggest that the prevalence of food allergy in adults (general population) is about 1.4%. In this study, 28% of the individuals with a diagnosed food allergy had intestinal symptoms. Due to the limitations of the diagnostic means used to identify afflicted patients, the prevalence may have been underestimated in this study. Using a broad array of diagnostic means, including mucosal food challenge procedures, we could confirm intestinal food allergy in 3.2% of adult patients suffering from inflammatory bowel disease or irritable bowel syndrome [2]. Compared to adults, a higher prevalence of

food allergy can be found in children of less than 3 years. According to the literature, 2–8% [8, 9] of children are afflicted. The most relevant allergen in childhood seems to be cow's milk. Most children tend to 'outgrow' their allergy by the 3rd [10] or, at the latest, 6th [11] year of life. A small group, however, undergoes a so-called change of level that is a change from food allergy involving the skin and gut to allergic diseases of the respiratory tract triggered by inhalative allergens (fig. 1). The factors determining the natural course of allergy during life are unknown.

Clinical Presentation

Clinical Symptoms of GI Allergy

Allergic reactions of the GI tract may occur from within minutes to several hours after ingestion of the offending food allergen, and present as a variety of clinical findings. These include nausea, vomiting, abdominal pain, cramping and distension, flatulence, constipation or diarrhea [1, 12, 13]. Occult fecal blood loss and malabsorption have also been reported in association with allergic responses of the GI tract.

The existence of a delayed-type or chronic form of allergic enteropathy with persistent inflammatory alterations of the intestinal mucosa has not yet been proven.

Table 1. Clinical aspects of allergic reactions in the gut

Clinical symptoms	Relevant allergens	Differential diagnoses
Nausea	milk	irritable bowel syndrome
Vomiting	hazelnut	Crohn's disease
Abdominal pains	wheat	ulcerative colitis
Cramping	apple	eosinophilic gastroenteritis
Distension	pork	celiac disease
Flatulence	egg	lactase deficiency
Constipation	celery	infectious diseases
Diarrhea	carrots	tumor diseases
Intestinal edema	inhalants?	pseudoallergic reactions

According to our own experience, the most relevant allergens for intestinal food allergy are milk proteins, hazelnut, wheat, apple, pork and egg [14, 15].

Table 1 shows a synopsis of the clinical aspects of allergy in the gut.

Association with Other GI Diseases

The clinical features of GI allergy are variable and unspecific and also typify other GI disorders, such as irritable bowel syndrome, Crohn's disease and ulcerative colitis [16, 17]. Thus, a differential diagnosis often proves to be difficult. A crucial aspect that has to be focused on in the near future is whether this similarity of symptoms is purely coincidental, or whether it is part of a causal relationship between GI hypersensitivity reactions and other GI disorders. There are, for instance, a few studies suggesting an association between food and irritable bowel syndrome [6]. Furthermore, the relationship between food-allergy-related enteropathy and inflammatory bowel diseases (Crohn's disease, ulcerative colitis) has to be elucidated. Although there are hints from the literature that there might be a link between these disorders [2, 4, 5], a distinct causality has not been proven so far, and clear epidemiological data do not yet exist. The same applies to celiac disease, where a food as responsible agent (wheat protein gliadin) is well known; the mechanism by which the disease is caused, however, is not fully understood [18]. Many patients with celiac disease are sensitized towards multiple other food proteins, which may reflect either a hypersensitive immune status or an increase in sensitization because of increased gut permeability caused by celiac disease.

In the context of intestinal allergy, eosinophilic gastroenteritis must also be mentioned. It is a rare disease of unknown etiology characterized by pronounced eosino-

phil accumulation and activation in the GI tract. The eosinophil infiltration can involve all parts of the GI tract, from the esophagus to the rectum. In most cases, the infiltration is limited to the mucosa, but sometimes the muscle layer or the subserosa are predominantly affected. This disease presents clinically with abdominal pain, cramps, nausea, diarrhea, malabsorption and, if the subserosa is infiltrated, eosinophilic ascites. It is interesting that 25–50% of these patients are reported to suffer from food allergy [19, 20].

Cross-Allergy

It is well known that pollen allergy can be associated with food allergy to fruits, nuts and vegetables. This complex is best established for the oral allergy syndrome, but probably also applies to food-allergy-related reactions of the gut. Cross-reactive IgE antibodies were found to be responsible for this clinical phenomenon in patients with inhalant allergy. The first relevant cross-reactive allergens identified almost a decade ago were the major birch pollen allergen Bet v 1 and birch profilin (Bet v 2). Allergens homologous to Bet v 1 have been detected in apple, pear, celery, carrot and potato [21]. Furthermore, common IgE epitopes of Bet v 1 and Mal d 1, the major apple allergen, leading to allergic cross-sensitization have been proven [22]. Profilin is an actin-binding protein in higher plants, and it represents the cross-reactive allergen for almost 20% of all pollen-allergic patients [23]. We found that administration of recombinant Bet v 1 to the colonic mucosa in selected patients is effective in inducing a mucosal wheal-and-flare reaction, suggesting that this allergen is of significance also in the gut. It is not yet clear if the inhalant sensitization subsequently leads to food hypersensitivity, or if the sensitization process is initially determined by Bet v 1-related food allergens. It is possible that food allergens prime T cells, which consequently also react with pollen allergens. The earliest foreign antigens in infant nutrition are usually apples, carrots and other vegetables containing Bet v 1 homologues. The immaturity of the mucosal immune system in childhood can lead to allergic sensitization to Bet v 1, which may account for a subsequent sensitization to pollen allergens [22]. This might be the reason for the observation that most children with food hypersensitivity are likely to 'outgrow' their allergic enteropathy, but are predisposed to develop pollen allergy later on (fig. 1). Genetic engineering of recombinant hypoallergenic Bet v 1 derivatives may produce candidates for specific immunotherapy [24].

Pathogenesis

In contrast to allergic disorders of human skin and respiratory mucosa, little is known about the pathophysiology of intestinal hypersensitivity reactions, and most of the available information comes from animal studies [25]. For mast cells in particular, our knowledge is to a large degree based on studies in rodents, the results of which cannot be easily transferred to the human system because of substantial functional differences between rodent and human mast cells. In order to most closely reflect the clinical situation, we performed in vitro studies using exclusively purified human cell preparations (eosinophils isolated from peripheral blood, mast cells isolated from surgery specimens of intestinal tissue) or immunohistochemical studies in colon biopsies taken from food-allergic human individuals after allergen provocation.

Intestinal Mast Cells

Regulation. Mast cells are the well-recognized key cells of type I hypersensitivity reactions. Almost no IgE-independent triggering agents have been found for human mucosal mast cells [26], in contrast to human basophils and eosinophils, but also in contrast to rat peritoneal mast cells and human skin mast cells. One of the most important findings with regard to mast cell biology was the discovery of stem cell factor (SCF), the c-kit ligand, in the early nineties. SCF is known to regulate the development of human mast cells from progenitor cells [27]. In addition, we found that SCF profoundly regulates mature human mast cell functions. We were able to demonstrate that SCF is a unique cytokine enhancing histamine release and leukotriene production in human lung and GI mast cells [26, 28, 29]. Further studies have revealed that SCF selectively promotes GI mast cell survival and proliferation [30] and enhances the adhesion of human mast cells to extracellular matrix proteins [paper submitted]. SCF is produced by stroma cells, fibroblasts and endothelial cells, but not by lymphocytes [27]. Thus, SCF cannot be claimed responsible for the link between mast cells and the specific immune system, as was presumed for many years. In rodents, IL-3 was found to be a major mast cell regulator, which is obviously different in humans, since Valent et al. [31] showed that human mast cells do not express the IL-3 receptor. Very recently, we were able to demonstrate that IL-4 seems to be a decisive regulator of human intestinal mast cells [32]. IL-4 by itself has no visible effect on mast cells. In the presence of SCF, however, IL-4 strongly enhances mast cell proliferation and survival, and increases IgE-dependent mediator release. IL-4

acts on mast cells directly through the IL-4 receptor expressed on human mast cells. However, in contrast to previously published papers, we found no significant IL-4 production in human intestinal mast cells, whereas human basophils are a potent source of IL-4 after stimulation [32, 33]. In conclusion, constitutively produced SCF seems to be required for the presence of mast cells, whereas IL-4 regulates the functional status of mast cells, which is probably different in allergic individuals after provocation. Figure 2 illustrates the different mechanisms involved in mast cell regulation.

Distribution and Effects on the Intestinal Tract. Mast cells are found at multiple body sites, including the GI tract. The mast cell density in the intestinal mucosa is even higher than, for example, in the skin. Between 2 and 3% of lamina propria cells in the gut are mast cells, which are located in the neighborhood of blood vessels, nerves and lymphatic tissue. Mast cells manifest a considerable heterogeneity with regard to phenotype, protease content and functional properties. As early as 20 years ago, mast cells were shown to be involved in the pathomechanisms of intestinal disorders such as Crohn's disease, ulcerative colitis and celiac disease. For example, degranulating mast cells were found in the gut mucosa of patients with Crohn's disease [34], and elevated mast cell numbers in the jejunal mucosa were detected in patients with celiac disease [35]. Mast cell activation also seems to be of importance in GI food allergy. It has been shown that intestinal mucosal mast cells degranulate and release histamine upon allergen stimulation in rats that had been sensitized to cow's milk or egg albumin [36]. Also, some human studies have demonstrated the involvement of mast cells in hypersensitivity reactions of the GI tract. In endoscopic intragastral provocation tests performed by Reimann and Lewin [37] and Bagnato et al. [38], mast cell and histamine changes in the mucosa were reported. Consistent with these findings, we also found mast cell activation in cecal biopsies after colonoscopic allergen provocation [14]. By virtue of the mediators and proteases they release, mast cells have a significant potential to modulate gut function. The cross-linking of IgE receptors has been shown to stimulate epithelial ion transport, increase vascular permeability, diminish barrier integrity, and cause mucosal edema and contraction of intestinal smooth muscle [36, 39]. These alterations may account for the clinical findings associated with food-allergy-related enteropathy. The effects of mast cells on the GI tract are illustrated in figure 3.

Further Mediators. Mast cells not only release their granule contents, but also synthesize several mediators de

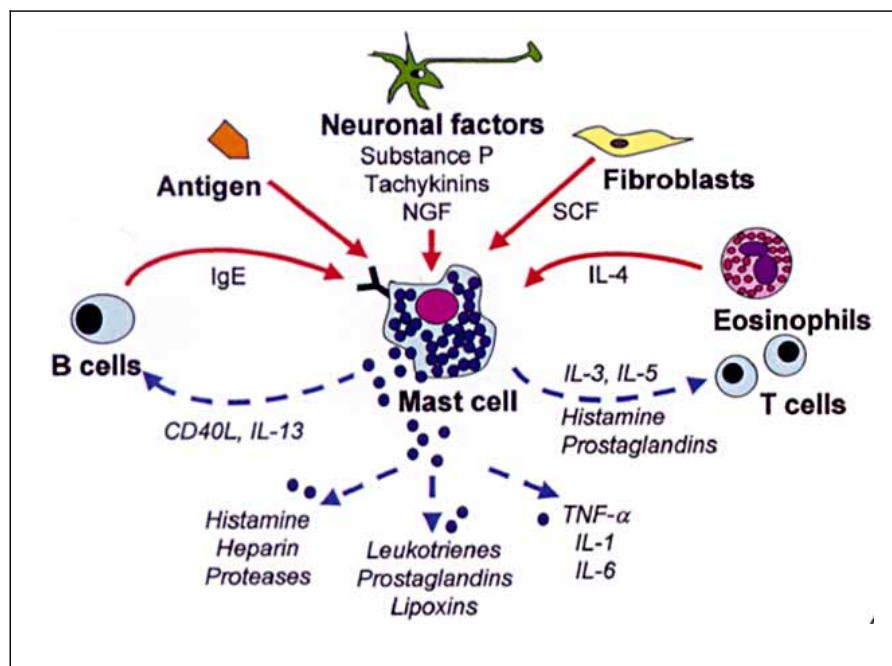


Fig. 2. Regulation of human mucosal mast cells.

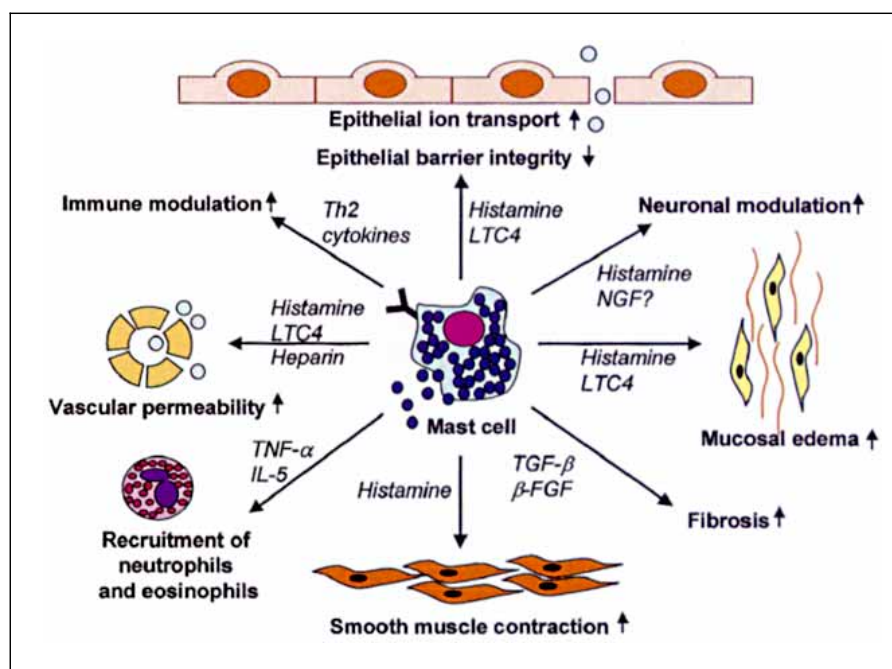


Fig. 3. Effects of mast cells on the GI tract. LTC4 = Leukotriene C4; FGF = fibroblast growth factor.

novo upon activation, e.g. leukotriene C4 and prostaglandin D2. More recently, it was found that in rodents, mast cells are capable of producing TNF- α and many other cytokines [40]. These findings suggest that mast cells, apart from their role as proinflammatory cells, may have multiple physiological functions with regard to immunoregulation and host defense, especially against parasites

and bacteria [41, 42]. As we were able to show recently, human intestinal mast cells also produce low levels of TNF- α constitutively, and IgE cross-linking substantially enhances TNF- α production [43]. Apart from proinflammatory cytokines such as TNF- α , which attracts neutrophils, human intestinal mast cells produce Th2-type cytokines such as IL-3, IL-5 and IL-13, which attract eosino-

phils and other cell types, a finding that may be of particular relevance for allergic diseases [44, 45]. In contrast to proinflammatory cytokines, Th2 cytokines are only produced in mast cells stimulated by IgE cross-linking. The addition of IL-4 to the cells strongly upregulates Th2-type cytokines. In the presence of SCF and IL-4, low levels of Th2 cytokines can be detected even without further stimulation with IgE cross-linking [34, 45]. These data show that human intestinal mast cells stimulated by IgE cross-linking contribute to the production of selected Th2 cytokines, particularly in the presence of IL-4, which may be a mechanism of amplification of allergic inflammation.

IgE-Independent Mast Cell Activation. Furthermore, IgE-independent mechanisms of mast cell activation have to be taken into account. Functional receptors other than the high-affinity IgE receptor (FcεRI) have been implicated in the immediate hypersensitivity response of IgE-deficient mice, suggesting that, for example, IgG1 receptors (FcγRI/II/III) expressed by mast cells may be involved in anaphylactic reactions [46]. In addition, nitric oxide has been described as a further regulator of mast cell degranulation [47].

The interaction between mast cells and nerves is gaining increasing attention. In airway mucosa, tachykinins [substance P and neurokinins (NKs)] released from nerves have been shown to influence vascular permeability, stimulate secretion and enhance the recruitment and activation of inflammatory cells. These effects are mediated by NK-1 receptors. In rodents, substance P binds to NK receptors on mast cells, which subsequently release serotonin. Our own unpublished results indicate that human intestinal mast cells also express NK-1 upon stimulation. Thus, substance P may have an indirect effect on smooth muscle cells which prompts the smooth muscle cells to contract [48, 49]. Circulating levels of nerve growth factor (NGF) are increased in humans with allergic disease [50]. The high-affinity NGF receptor, TrkA, is expressed on rodent mast cells, and NGF has been shown to increase the number of mast cells as well as to prime histamine release [51, 52]. Potential cell sources of NGF in allergic inflammation are mast cells, eosinophils, T and B cells, dendritic and epithelial cells and fibroblasts. Although the role of tachykinins and NGF in allergy in the human gut has not been studied so far, it is indeed possible that these concepts can be applied to the intestinal mucosa, too, as some data suggest [53]. There might be therapeutic options offered by knowledge of the role of neuropeptides in allergic inflammation, e.g. by NK-1 receptor antagonists. The proof of a role for NK receptor antagonists, however, has yet to be delivered.

Intestinal Eosinophils

Role in Inflammatory Bowel Diseases. Several recent studies and reviews have focused on the function of eosinophilic granulocytes in GI disease [54, 55]. There is increasing evidence that eosinophils are involved in the pathogenesis of noninfectious inflammatory bowel diseases such as Crohn's disease, ulcerative colitis and celiac disease, mainly by force of the cytotoxic proteins they release upon activation. Electron microscopic as well as immunohistochemistry studies have revealed the activation of eosinophils in both Crohn's disease and ulcerative colitis. Moreover, in patients with these diseases, increased levels of eosinophil granule proteins, such as eosinophil cationic protein (ECP) and eosinophil protein X (EPX), have been found in feces, gut perfusates and gut lavages [56–61].

Role in Allergic Enteropathy. Less is known about the role of eosinophils in the pathophysiology of GI hypersensitivity reactions, although the importance of eosinophils in allergic conditions of other organs is well established [62]. In recent years, however, some findings have been reported that imply an involvement of eosinophils in food-allergy-related reactions of the gut. For example, we and others have found that the levels of eosinophil granule proteins such as ECP or EPX in feces is elevated in patients with intestinal food allergy [59, 63]. Local stimulation of the gut mucosa of food-allergic patients with the relevant antigens leads to an activation of mucosal eosinophils as detected by electron microscopy [14] and immunohistochemistry [unpubl. data], as well as to intestinal secretion of ECP [64]. In both studies, eosinophil activation took place within 20 min of allergen challenge of the mucosa, whereas until now, eosinophil activation has been associated in particular with allergic late-phase reactions occurring 2–4 h after allergen challenge in some individuals, mainly in the respiratory system and the skin [62]. These data suggest that eosinophils may be involved in the immediate phase of food-allergy-related enteropathy.

Mechanism of Activation. The mechanism of the observed rapid eosinophil activation is unclear. Both a directly stimulating effect of the allergen on eosinophils, e.g. by cross-linking surface-bound IgE or IgA, or an indirect effect, e.g. via mast cell mediators which can induce eosinophil degranulation [65], may be hypothesized. Eosinophil mediator release caused by IgE cross-linking has not been shown consistently – although eosinophils express components of the high-affinity IgE receptor in the cytoplasm – probably because the components are not assembled to a functional surface receptor [66, 67]. In

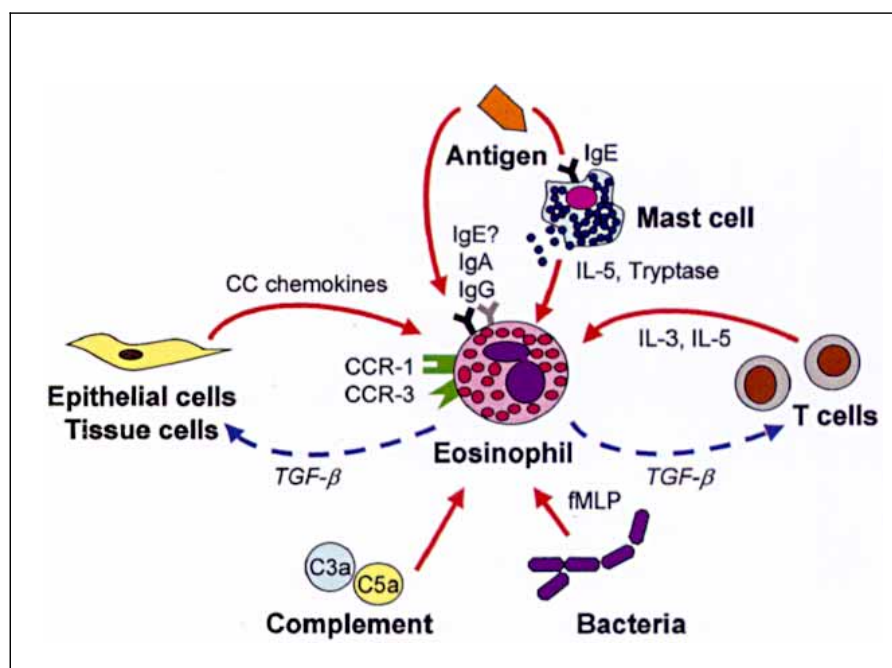


Fig. 4. Regulation of human eosinophils.

addition, several IgE-independent triggering agents have been found for basophils and eosinophils, e.g. the bacterial product fMLP and the anaphylatoxins C3a and C5a [68–71]. More recently, it has been shown that several chemokines, which are a family of small polypeptides working as chemotactic cytokines, are potent basophil and eosinophil triggers and chemoattractants. The chemokines MCP-2, MCP-3, RANTES, MIP-1 α , and eotaxin 1 and 2 are potent eosinophil agonists, and are produced by various cells (T cells, monocytes, macrophages, endothelial cells, fibroblasts). Among these, eotaxin 1 and eotaxin 2 lead to exclusive recruitment of eosinophils. Most of these chemokines bind to the chemokine receptor CCR-3, which is expressed on eosinophils [62, 72, 73]. Interestingly, some chemokines preferentially induce mediator release, whereas others act mainly as chemoattractants. The reason for these differential and redundant effects became clear when the chemokine receptors that are expressed on human eosinophils were discovered [74]. It has been repeatedly suggested that chemokines are significantly involved in the regulation of inflammatory effector cells and in the determination of the type of inflammation [75–77]. We have been able to describe particular sets of cytokines modulating the effector functions of eosinophils. For example, IL-3, IL-5 and GM-CSF enhance IgE-independent mediator release in human eosinophils [70, 71]. It has been shown by several groups that this set of cytokines is upregulated at sites of allergic

inflammation, confirming that IL-3, IL-5 and GM-CSF are important regulators of allergic inflammation, in particular of the late-phase reaction [78]. The release of these cytokines and chemokines by T cells, eosinophils, mast cells and other cells is presumably a most relevant step in the pathogenesis of the late-phase reaction, characterized not only by increased vascular permeability and edema but by the infiltration of the tissue with inflammatory cells. Figure 4 shows the mechanisms of eosinophil activation and the interaction between eosinophils and other cell types.

Diagnostic Procedures

General Remarks

A suspicion of GI food allergy is frequently expressed, but unfortunately, it can be confirmed objectively in very few cases. Thus, a substantial skepticism exists in relation to this kind of disease in the medical profession, particularly among gastroenterologists. The major reason for this is that the methods available to confirm the diagnosis of GI allergy are insufficient at present, and therefore it remains difficult to identify afflicted patients on an objective basis. The classical allergologic tests [skin test, radioallergosorbent test (RAST)] often fail to deliver reliable results in situations where the GI tract is exclusively involved. Thus, new laboratory methods were established

to measure IgE or eosinophil-derived mediators in stool samples [57–59, 79]. In addition, a few attempts have been made to develop local provocation tests. Most of them, however, have not exceeded experimental status yet.

A selection of diagnostic procedures for intestinal allergy is listed below. It is not possible to give the exact specificity and sensitivity of these methods, as there is no gold standard of diagnostics for gut allergy with which these methods can be compared. In addition to allergologic tests, it is important to perform careful gastroenterological diagnostics. Since the symptoms of intestinal allergy are unspecific and variable, the diagnosis is to a large part a diagnosis by exclusion. The lactose tolerance test, malabsorption tests, microbiological examinations, sonography and endoscopy can serve to exclude nonallergic causes of GI disorders [13].

The main principles of the diagnosis of GI allergy are summarized in table 2.

In vitro Tests

Radioallergosorbent Test. The RAST or appropriate enzymatic tests allow the quantification of allergen-specific IgE in serum. Elevated total IgE or high levels of IgE antibodies to specific foods may suggest the presence of an IgE-mediated allergic disorder. They cannot, however, establish the diagnosis of a clinical food allergy. The sensitivity in particular is low compared to oral food challenge tests, and normal values of such parameters do not exclude intestinal allergy [15, 80, 81]. A possible explanation of this discrepancy may be that hypersensitivity reactions of the gut are mediated by *local* IgE that is not necessarily reflected by serum levels. Older studies showed that the IgE concentration in feces and intestinal juice does not correlate with IgE concentration in the blood [79, 82]. Huggins and Brostoff [83] described a group of patients with dust-mite-allergic rhinitis who had a negative RAST and skin test, whereas in nasal fluid, specific IgE was detected that did not correlate with serum levels. A similar phenomenon might be present in the GI mucosa. Alternatively, one can speculate that in patients who present with GI allergy, but have negative RAST results, IgE-independent mechanisms such as IgG-mediated reactions, immune complex disease or lymphocyte-triggered reactions are involved in the pathogenesis of GI allergy.

Measurement of Inflammatory Markers in Feces. In patients with allergic enteropathy, elevated levels of eosinophilic granule proteins, such as ECP and EPX, can be measured in feces [59, 63]. However, increased fecal concentrations of ECP and EPX are also found in patients

Table 2. Diagnostic means proposed to confirm GI (food) allergy

<i>I. Patient's history (3/6)</i>
– history of food intolerance
– improvement of symptoms after exclusion diet
– improvement of symptoms after treatment with oral DNCG for >1 month
– negative GI diagnostics
– history of allergy (extrinsic asthma, seasonal rhinitis, atopic dermatitis)
– history of atopy (eczema in childhood) or afflicted first-degree relatives
<i>II. Laboratory tests (3/6)</i>
– elevated total IgE in serum
– specific IgE against food antigen in serum
– eosinophilia in serum/intestinal mucosa
– elevated levels of ECP or EPX in serum/feces
– positive SPTs using food allergens
– facultative: BHRT, CAST, TOP (?)
<i>III. Provocation test (after oral elimination diet) (1/2)</i>
– DBPCFC
– COLAP test

DNCG = Disodium cromoglycate; BHRT = basophil histamine release test; CAST = cellular allergen stimulation test; TOP = tissue oxygenation provocation. The numbers in parentheses indicate how many items should be fulfilled to confirm the diagnosis of food allergy.

with Crohn's disease and ulcerative colitis [57–61]. Single attempts have also been made to measure IgE levels in feces from allergic individuals [79, 84].

'Ex vivo' Stimulation Tests

Basophil Histamine Release Test. It has been shown that isolated peripheral blood-derived basophils from patients with food allergy have an increased rate of spontaneous histamine release compared to controls [85]. Dispersed mast cells and basophils from the jejunal mucosa of patients with GI allergy have been found to release histamine when stimulated with the relevant food extracts [86]. These test procedures, however, are really of experimental character and are not established in routine diagnostics yet.

Cellular Allergen Stimulation Test. This is an alternative in vitro approach similar to the basophil histamine release test [87]. There has been only limited experience with this test procedure so far, but sensitivity and specificity seem to be at least comparable to those of the RAST [88].

Tissue Oxygenation Provocation. Intestinal biopsies taken endoscopically undergo oxygenation in a so-called 'Tabbox' system and are incubated with food antigens. The authors found that relevant allergens lead to significantly increased rates of mediator secretion (tryptase, ECP, TNF) from intestinal tissue. These functional release experiments are used for allergen identification [89]. An appreciable advantage of this method is the possibility of challenging the involved organ in an 'ex vivo' manner without the risk of inducing an anaphylactic reaction in the patient. However, controlled studies to validate these preliminary data are still lacking.

In vivo Provocation Tests

Skin Prick Test. Like the RAST, the skin prick test (SPT) indicates the presence of systemic allergen-specific IgE, and thus, it has similar drawbacks to the RAST; a patient who experiences hypersensitivity reactions in the GI tract does not necessarily have positive skin reactions after prick test provocation, maybe due to local intestinal IgE production. Accordingly, the usefulness of the SPT for the diagnosis of intestinal hypersensitivity is limited [14, 80].

Elimination and Rechallenge Diet. Elimination diet and rechallenge can be a valid, objective method for confirming a diagnosis of food-allergy-related enteropathy [1]. In practice, however, this approach has limitations because of difficulties in eliminating the food allergens suspected to be of relevance, which depends on a correct choice of the food to be avoided, the compliance of the patient and the type of food. In addition, a properly carried out elimination and reintroduction diet is a protracted procedure which takes days or even weeks. To avoid fatal reactions, patients have to be carefully selected, and challenges should be performed in hospital settings.

Double-Blind Placebo-Controlled Food Challenge. Oral challenge procedures with food substances are acknowledged as the 'gold standard' for the confirmation of intestinal food allergy [13, 80]. In this test, food antigen as well as placebo is given in a blinded form (e.g. in capsules), and the patients' symptoms after ingestion are registered. At present, a positive double-blind placebo-controlled food challenge (DBPCFC) is considered the only conclusive evidence of food allergy, provided it is performed properly [13]. However, there are several practical problems connected with the DBPFC, e.g. the read-out system. The test procedure was originally developed for patients with food-induced urticaria or atopic dermatitis. In these cases, the alterations after ingestion of a food can easily be demonstrated and registered. The most significant draw-

back of the DBPCFC in GI allergy, however, is that most patients present only subjective symptoms which cannot be objectively verified (e.g. abdominal pain, cramps, nausea), so the interpretation of the test results is often unsatisfactory [13] and depends on the patients' subjective reports of their experienced symptoms. An additional disadvantage is the variable time interval between ingestion and the onset of symptoms, which may occur hours or days after ingestion if, for example, the colonic mucosa is the target organ. Finally, the DBPCFC does not discriminate between food allergy and food intolerance. Our own experiences with the DBPCFC in patients with allergic enteropathy were to a great extent disappointing [14].

Jejunal Perfusion. An alternative approach with a more objective read-out system has been proposed by Bengtsson et al. [64]. During endoscopy of the upper intestinal tract, these authors 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. Increased concentrations of ECP and histamine in the perfusate were measured in food-allergic patients after allergen challenge. This test has only been performed in patients with cow's milk allergy so far. The rather large amount of allergen solution required for the challenge (10 ml of cow's milk) increases the risk of an anaphylactic reaction of the patient. A minor disadvantage of this technically complicated procedure is that the mucosa changes are not visible, and biopsies cannot be taken. So far, this technique is really reserved to research settings.

Gastroscopic Provocation Tests. In previous studies, attempts were made to challenge gastric mucosa with food allergens during gastroscopy. Local mucosa changes with edema, reddening and bleeding were reported in cases of challenge with a food allergen. However, these tests could not be developed for clinical practice [37, 38].

Colonoscopic Allergen Provocation Test. We resumed the idea of local challenge in the GI tract and developed the colonoscopic allergen provocation (COLAP) test [14, 15]. During colonoscopy, liquid (food) antigen extracts, a buffer control and a positive control containing histamine are injected into the mucosa of the cecum with a fine needle. The antigen extracts are selected according to the patient's history and/or RAST results. Three or four different antigens are tested in each patient. After 20 min, the wheal-and-flare reaction of the provoked mucosa is registered semiquantitatively using a score from 0 to 4, and biopsies are taken from the provocation areas as well as from unprovoked cecal mucosa. To date, more than

150 patients with suspected GI food allergy and 10 healthy volunteers have undergone the COLAP test. No systemic anaphylactic reactions occurred in response to intestinal challenge, except one case of generalized urticaria. In the case of a positive mucosa reaction, wheal-and-flare began approximately 2 min after antigen challenge and was maximally pronounced after 10–15 min. Whereas no positive mucosa reactions to antigen challenge were seen in the control group, about 50% of all antigens tested positive in the patient group. Histamine injection led to a mucosal wheal-and-flare reaction in 70% of the patients and 80% of the control individuals, while buffer injection was always negative. Antigen-induced wheal-and-flare reactions correlated with the patients' history of adverse reactions to food, but not with serum levels of total or specific IgE or skin test results.

The clinical relevance of the COLAP test results has to be evaluated. A preliminary follow-up showed that an individual diet with strict elimination of each food that tested positive in the COLAP, in a few cases combined with additional antiallergic medication, seems to achieve an improvement of symptoms in the majority of patients [14]. A disadvantage of this technique is that late-phase reactions escape the diagnosis, unless a second colonoscopy with reevaluation of the provoked areas is performed the next day. Besides its application as a diagnostic technique, the COLAP provides a model of intestinal allergy in humans and thus additionally serves as a tool to study the underlying mechanisms leading to mucosal wheal-and-flare reaction. Immunohistochemical surveys of biopsies can deliver new information concerning the cells and mechanisms involved in allergic reactions of the gut. For example, a rapid activation of eosinophils after allergen application could thus be demonstrated.

Therapy

In the situation where relevant food allergens as a cause of GI complaints can be identified, a diet with elimination of the responsible food is a causal therapy. The practicability of such an elimination diet is, however, limited by the number of allergens and quite often also by the frequency of the allergen within normal nutrition. Food (extracts) that serve as ingredients for common products, e.g. egg, wheat, soy and spice, can hardly be eliminated.

If an elimination diet cannot be performed properly due to the above reasons, or because the responsible foods could not be identified, antiallergic medication is required. A preparation of disodium cromoglycate that is

effective locally in the GI tract is available. Due to a low side-effect rate, oral cromoglycate can be used in children as well. In more severe cases, therapy with steroids might be inevitable. To what extent locally acting steroids, e.g. budesonide, are helpful in GI food allergy, has not been studied yet. In conclusion, the therapeutic options for hypersensitivity reactions in the gut are at present poorly evaluated.

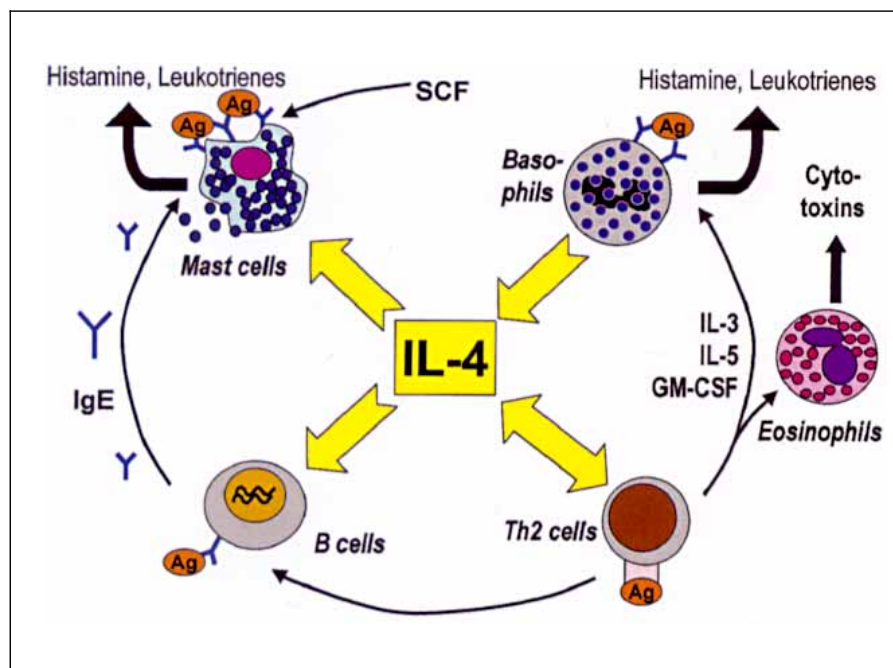
Several innovative antiallergic drugs are under development at present, e.g. tolerogenic peptides, anti-CD4, anti-IgE, anti-IL-5 or anti-IL-4 strategies. The first clinical studies with recombinant epitopes, anti-IgE antibodies, or IL-4 receptor antagonists were performed in asthmatic individuals. The results suggest that these strategies might present a benefit for allergic patients [90–95]. In particular, recent results from clinical studies on the use of anti-IgE antibodies are promising. It is unclear whether these therapeutic approaches are effective in the GI tract as well.

Future Directions

Many questions remain to be answered in the field of GI food allergy, including the most appropriate means of diagnosis and the basic pathomechanisms involved in intestinal allergic reactions. By now, blinded oral challenge procedures with food antigens are accepted as the 'gold standard' in diagnosing food allergy, although these tests have several practical problems, especially in patients with intestinal manifestations of food hypersensitivity. However, new test systems have been developed, such as the measurement of IgE and eosinophil parameters in stool samples or endoscopic provocation tests performed locally in the intestinal mucosa. These tests may improve the possibility of identifying patients with food-allergy-related enteropathy, and in addition, they can provide new insights into the pathophysiology of intestinal hypersensitivity reactions. Nevertheless, the basic mechanisms of allergic enteropathy still remain largely unclear. Further investigations are necessary for an understanding of the pathophysiology of GI allergy, because such knowledge is important for the development of reliable diagnostic tests and pharmacological agents for treatment of this disease.

A particular aim of future research is to elucidate the mechanisms of IgE-independent allergic reactions. For example, the role of IgG in atopy is still controversial. The relationship between IgG levels in serum and clinical symptoms has been found to be rather weak, which may

Fig. 5. Proposed role of IL-4 in the pathogenesis of allergic inflammation. In humans, IL-4 is produced predominantly by T lymphocytes of the Th2 phenotype and by basophils, but not by mast cells. Because of the multiple crucial effects of IL-4 on the priming of T lymphocytes to the Th2 phenotype, on IgE production in B lymphocytes, and the proliferation and mediator release of tissue mast cells, IL-4 has been suggested as an important therapeutic target for the treatment of allergic diseases.



be due to a variable antigen/antibody balance in serum or tissue. Some data suggest that IgG4 is a protective rather than a sensitizing antibody. On the other hand, atopic symptoms are reported to be associated with high levels of IgG4 antibodies. The significance of the measurement of specific IgG in serum is still doubtful [96, 97].

The central role of mast cells and eosinophils in allergic inflammation is well established. Using our newly developed COLAP test, as well as immunohistochemical and electron microscopy techniques, we were able to confirm this knowledge also for GI allergy caused by food proteins. Our *in vitro* data show that particular cytokines and growth factors (IL-3, IL-5, GM-CSF for eosinophils, SCF and IL-4 for mast cells) are of major importance not only for the development and recruitment of inflammatory effector cells, but also for the enhanced functional activity and increased mediator release in response to classical triggering agents. A local overproduction of these cytokines, in particular Th2-type cytokines, may cause an enhanced inflammatory reaction leading to allergic inflammation. What causes enhanced expression of these cytokines? It is generally believed that they are mainly produced by lymphocytes of the Th2 phenotype. Increased numbers of Th2 cells may be generated either because of genetic alterations or because of allergen-dependent T cell activation via antigen-presenting cells. The observation that basophils, eosinophils and mast cells also produce dif-

ferent cytokines upon appropriate stimulation renders the situation even more complex. Inflammatory cells, besides T cells, may contribute to the local production of Th2 cytokines in an allergen-specific manner. These findings provide new diagnostic and therapeutic targets, such as IL-4, which is a key molecule of allergic inflammation regulating T, B and mast cells (fig. 5).

Currently, several genetic studies are under way to evaluate potential genetic factors that confer an atopic disposition. Allergic disorders are not determined by a single major gene and do not show a simple pattern of inheritance. They belong to 'complex' diseases in which environmental and multiple genetic factors interact in a complex fashion to produce susceptibility and expression. New techniques for scanning the human genome promise advances in tracking the origins of these disorders. Two common genetic approaches have been used to localize and evaluate heritable components of allergy: genome-wide screens and candidate gene studies. The candidate gene approach has been applied to the promoter region of a number of cytokine genes and chromosomal regions related to allergic pathology, such as chromosomes 5, 6, 11, 12, 14 and 16. These chromosomes contain genes, e.g. for IL-4, IL-5, IL-4 receptor, SCF, the β -chain of the high-affinity IgE receptor, which are accepted as causing susceptibility to atopy. It is hoped that knowledge from gene studies will have a practical clini-

cal value. Determining the inheritance of a set of polymorphisms in a subject may allow the prediction of those at risk of developing allergy and may allow more accurate disease prevention strategies. There are several questions which will hopefully be answered by gene studies, such as the cause of the increase in the incidence of allergy, and the reason why certain environmental fac-

tors trigger allergy in some, but not all, exposed subjects. In the future, genetics may result in the development of novel, more specific therapeutic strategies in allergy [98–100]. Nevertheless, we are still far from understanding the genetic basis of asthma and atopy and their interaction with the environment.

References

- 1 Finn R: Food allergy – fact or fiction: A review. *J R Soc Med* 1992;85:560–564.
- 2 Bischoff SC, Herrmann A, Manns MP: Prevalence of adverse reactions to food in patients with gastrointestinal disease. *Allergy* 1996;51:811–818.
- 3 Sampson HA: Food allergy. Part 1: Immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717–728.
- 4 Jones VA, Dickinson RJ, Workman E, Wilson AJ, Freeman AH, Hunter JO: Crohn's disease: Maintenance of remission by diet. *Lancet* 1985;ii:177–180.
- 5 Ballegaard M, Bjergstrom A, Bronndum S, Hylander E, Jensen L: Self-reported food intolerance in chronic inflammatory bowel disease. *Scand J Gastroenterol* 1997;32:569–571.
- 6 Zwetschenbaum JF, Burakoff R: Food allergy and the irritable bowel syndrome. *Am J Gastroenterol* 1988;83:901–904.
- 7 Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R: A population study of food intolerance. *Lancet* 1994;343:1127–1130.
- 8 Kristjansson I, Ardal B, Jonsson JS, Sirudsson JA, Foldevi M, Björkstén B: Adverse reaction to food and food allergy in young children in Iceland and Sweden. *Scand J Prim Health Care* 1999;17:30–34.
- 9 Bock SA: Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics* 1987;79:683–688.
- 10 Host A, Halken S: A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990;45:587–596.
- 11 Bishop JM, Hill DJ, Hosking CS: Natural history of cow milk allergy: Clinical outcome. *J Pediatr* 1990;116:862–867.
- 12 Bischoff SC: Mucosal allergy: Role of mast cells and eosinophil granulocytes in the gut. *Baillière's Clin Gastroenterol* 1996;10:443–459.
- 13 Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Björkstén B, Moneret-Vautrin D, Wüthrich B: Adverse reactions to food. *Allergy* 1995;50:623–635.
- 14 Bischoff SC, Mayer J, Wedemeyer J, Meier PN, Zeck-Kapp G, Wedi B, Kapp A, Cetin Y, Gebel M, Manns MP: Colonoscopic allergen provocation (COLAP): A new diagnostic approach for gastrointestinal food allergy. *Gut* 1997;40:745–753.
- 15 Bischoff SC, Mayer J, Meier PN, Zeck-Kapp G, Manns MP: Clinical significance of the colonoscopic allergen provocation test. *Int Arch Allergy Immunol* 1997;113:348–351.
- 16 Podolsky D: Inflammatory bowel disease. Part 1. *N Engl J Med* 1991;325:928–937.
- 17 Podolsky D: Inflammatory bowel disease. Part 2. *N Engl J Med* 1991;325:1008–1018.
- 18 Hed J: Coeliac disease: A food allergy? *Monogr Allergy*. Basel, Karger, 1996, vol 32, pp 204–210.
- 19 Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR: Eosinophilic gastroenteritis: A clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. *Gut* 1990;31:54–58.
- 20 Naylor AR: Eosinophilic gastroenteritis. *Scott Med J* 1990;35:163–165.
- 21 Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O: Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962–969.
- 22 Fritsch R, Bohle B, Vollmann U, Wiedermann U, Jahn-Schmid B, Krebitz M, Breiteneder H, Kraft D, Ebner C: Bet v 1, the major birch pollen allergen, cross-react at the level of allergen-specific T helper cells. *J Allergy Clin Immunol* 1998;102:679–686.
- 23 Valenta R, Duchene M, Ebner C, Valent P, Silaber C, Deviller P, et al: Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
- 24 Vrtala S, Hirtenlehner K, Susani M, Hufnagl P, Binder BR, Vangelista L, Pastore A, Sperr WR, Valent P, Ebner C, Kraft D, Valenta R: Genetic engineering of recombinant hypoallergenic oligomers of the major birch pollen allergen, Bet v 1: Candidates for specific immunotherapy. *Int Arch Allergy Immunol* 1999;118:218–219.
- 25 Crowe SE, Perdue MH: Gastrointestinal food hypersensitivity: Basic mechanisms of pathophysiology. *Gastroenterology* 1992;103:1075–1095.
- 26 Bischoff SC, Dahinden CA: c-kit ligand: A unique potentiator of mediator release by human lung mast cells. *J Exp Med* 1992;175:237–244.
- 27 Galli SJ, Tsai M, Wershil BK: The c-kit receptor, stem cell factor, and mast cells. What each is teaching us about the others. *Am J Pathol* 1993;142:965–974.
- 28 Bischoff SC, Dahinden CA: Effect of c-kit ligand on mediator release by human lung mast cells. *Int Arch Allergy Immunol* 1992;99:319–322.
- 29 Bischoff SC, Schwengberg S, Wordelmann K, Weimann A, Raab R, Manns MP: Effect of c-kit ligand, stem cell factor, on mediator release by human intestinal mast cells isolated from patients with inflammatory bowel disease and controls. *Gut* 1996;38:104–114.
- 30 Bischoff SC, Schwengberg S, Raab R, Manns MP: Functional properties of human intestinal mast cells cultured in a new culture system. Enhancement of IgE receptor-dependent mediator release and response to stem cell factor. *J Immunol* 1997;159:5560–5567.
- 31 Valent P, Besmer J, Sillaber CH, Butterfield JH, Eher R, Majdic O, Kishi K, Kleptko W, Eckersberger F, Kaltenrunner R, Lechner K, Bettelheim P: Failure to detect IL-3 binding sites on human mast cells. *J Immunol* 1990;145:3432–3437.
- 32 Bischoff SC, Sellge A, Lorentz A, Sebald W, Raab R, Manns MP: Interleukin-4 enhances proliferation and mediator release in mature human mast cells. *Proc Natl Acad Sci USA* 1999;96:8080–8085.
- 33 Brunner T, Heusser CH, Dahinden CA: Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J Exp Med* 1993;177:605–611.
- 34 Dvorak AM, Monahan RA, Osage JE, Dickerson GR: Mast-cell degranulation in Crohn's disease. *Lancet* 1978;i:498.
- 35 Strobel S, Busuttill A, Ferguson A: Human intestinal mucosal mast cells: Expanded population in untreated coeliac disease. *Gut* 1983;24:222–227.
- 36 Patrick MK, Dunn IJ, Buret A, Miller HRP, Huntley JF, Gibson S, Gall DG: Mast cell protease release and mucosal ultrastructure during intestinal anaphylaxis in the rat. *Gastroenterology* 1988;94:1–9.
- 37 Reimann HJ, Lewin J: Gastric mucosal reactions in patients with food allergy. *Am J Gastroenterol* 1988;11:1212–1219.

- 38 Bagnato GF, Di Cesare E, Caruso RA, Gulli S, Cugliari A, Morabito Lo Prete A, Previti M, Muscara M, Bottari M: Gastric mucosal mast cells in atopic subjects. *Allergy* 1995;50:322–327.
- 39 D'Inca R, Ramage JK, Hunt RH, Perdue MH: Antigen-induced mucosal damage and restitution in the small intestine of the immunized rat. *Int Arch Allergy Appl Immunol* 1990;91:270–277.
- 40 Gordon JR, Burd PR, Galli SJ: Mast cells as a source of multifunctional cytokines. *Immunol Today* 1990;11:458–464.
- 41 Echtenacher B, Männel DN, Hultner L: Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* 1996;381:75–77.
- 42 Malaviya R, Ikeda T, Ross E, Abraham SN: Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF- α . *Nature* 1996;381:77–80.
- 43 Bischoff SC, Lorentz A, Schwengberg S, Weier G, Raab R, Manns MP: Mast cells are an important cellular source of tumour necrosis factor α in human intestinal tissue. *Gut* 1999;44:643–652.
- 44 Lorentz A, Schwengberg S, Mierke C, Manns MP, Bischoff SC: Human intestinal mast cells produce IL-5 in vitro upon IgE receptor cross-linking and in vivo in the course of intestinal inflammatory disease. *Eur J Immunol* 1999;29:1496–1503.
- 45 Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC: Human intestinal mast cells are capable of producing different cytokine profiles: Role of IgE receptor cross-linking and IL-4. *J Immunol* 2000;164:43–48.
- 46 Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ: Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc γ RIII. *J Clin Invest* 1997;99:901–914.
- 47 Iikura M, Takaishi T, Hirai K, Yamada H, Iida M, Koshino T, Morita Y: Exogenous nitric oxide regulates the degranulation of human basophils and rat peritoneal mast cells. *Int Arch Allergy Immunol* 1998;115:129–136.
- 48 Joos GF, Lefebvre RA, Bullock GR, Pauwels RA: Role of 5-hydroxytryptamine and mast cells in the tachykinin-induced contraction of rat trachea in vitro. *Eur J Pharmacol* 1997;338:259–268.
- 49 Suzuki R, Furuno T, McKay DM, Wolvers D, Teshima R, Nakanishi M, Bienenstock J: Direct neurite-mast cell communication in vitro occurs via the neuropeptide substance P. *J Immunol* 1999;163:2410–2415.
- 50 Bonini S, Lambiase A, Bonini S, Angelucci F, Magrini L, Manni L, Aloe L: Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA* 1996;93:10955–10960.
- 51 Marshall JS, Gomi K, Blennerhassett MG, Bienenstock J: Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. *J Immunol* 1999;167:4271–4276.
- 52 Nilsson G, Forsberg-Nilsson K, Xiang Z, Hallbook F, Nilsson K, Metcalfe DD: Human mast cells express functional TrkA and are a source of nerve growth factor. *Eur J Immunol* 1997;27:2295–2301.
- 53 Castagliuolo I, Wang CC, Valenick L, Pasha A, Nikulasson S, Carraway RE, Pothoulakis C: Neutensin is a proinflammatory neuropeptide in colonic inflammation. *J Clin Invest* 1999;103:843–849.
- 54 Furuta GT, Ackerman SJ, Wershil BK: The role of the eosinophil in gastrointestinal diseases. *Curr Opin Gastroenterol* 1995;11:541–547.
- 55 Levy AM, Kita K: The eosinophil in gut inflammation: Effector or director? *Gastroenterology* 1996;110:952–954.
- 56 Dvorak AM: Ultrastructural evidence for release of major basic protein-containing crystalline cores of eosinophil granules in vivo: Cytotoxic potential in Crohn's disease. *J Immunol* 1980;125:460–462.
- 57 Raab Y, Fredens K, Gerdin B, Hällgren R: Eosinophil activation in ulcerative colitis. *Dig Dis Sci* 1998;43:1061–1070.
- 58 Levy AM, Gleich GJ, Sandborn WJ, Tremaine WJ, Steiner ML, Phillips SF: Increased eosinophil granule proteins in gut lavage fluid from patients with inflammatory bowel disease. *Mayo Clin Proc* 1997;72:117–123.
- 59 Bischoff SC, Grabowsky J, Manns MP: Quantification of inflammatory mediators in stool samples of patients with inflammatory bowel disorders and controls. *Dig Dis Sci* 1997;42:394–403.
- 60 Bischoff SC, Mayer JH, Nguyen Q-T, Stolte M, Manns MP: Immunohistochemical assessment of intestinal eosinophil activation in patients with eosinophilic gastroenteritis and inflammatory bowel disease. *Am J Gastroenterol* 1999;94:3521–3529.
- 61 Berstad A, Borkje B, Riedel B, Elsayed S, Berstad A: Increased fecal eosinophil cationic protein in inflammatory bowel disease. *Hepato-gastroenterology* 1993;40:276–278.
- 62 Rothenberg ME: Eosinophilia. *N Engl J Med* 1998;338:1592–1600.
- 63 Kosa L, Kereki E, Borzsonyi L: Copro-eosinophil cationic protein (ECP) in food allergy. *Allergy* 1996;51:964–966.
- 64 Bengtsson U, Knutson TW, Knutson L, Danaeus A, Hällgren R, Ahlstedt S: Eosinophil cationic protein and histamine after intestinal challenge in patients with cow's milk intolerance. *J Allergy Clin Immunol* 1997;100:216–221.
- 65 Raible DG, Schulman ES, DiMuzio J, Cardillo R, Post TJ: Mast cell mediators prostaglandin D2 and histamine activate human eosinophils. *J Immunol* 1992;148:3536–3542.
- 66 Gounni AS, Lamkhioed B, Delaporte E, Dubost A, Kinet JP, Capron A, Capron M: The high-affinity IgE receptor on eosinophils: From allergy to parasites or from parasites to allergy? *J Allergy Clin Immunol* 1994;94:1214–1216.
- 67 Capron M, Gounni SA, Morita M, Truong MJ, Prin L, Kinet JP, Capron A: Eosinophils: From low- to high-affinity immunoglobulin E receptors. *Allergy* 1995;50(suppl 25):20–23.
- 68 Bischoff SC, Brunner T, de Weck AL, Dahinden CA: Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J Exp Med* 1990;172:1577–1582.
- 69 Bischoff SC, de Weck AL, Dahinden CA: Interleukin 3 and granulocyte/macrophage-colony-stimulating factor render human basophils responsive to low concentrations of complement component C3a. *Proc Natl Acad Sci USA* 1990;87:6813–6817.
- 70 Takafuji S, Bischoff SC, de Weck AL, Dahinden CA: IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C4 in response to soluble agonists. *J Immunol* 1991;147:3855–3861.
- 71 Takafuji S, Bischoff SC, de Weck AL, Dahinden CA: Opposing effects of tumor necrosis factor- α and nerve growth factor upon leukotriene C4 production by human eosinophils triggered with N-formyl-methionyl-leucyl-phenylalanine. *Eur J Immunol* 1992;22:969–974.
- 72 Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA: RANTES and macrophage inflammatory protein 1 α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489–1495.
- 73 Bischoff SC, Krieger M, Brunner T, Rot A, von Tscherner V, Baggiolini M, Dahinden CA: RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. *Eur J Immunol* 1993;23:761–767.
- 74 Baggiolini M, Dahinden CA: CC chemokines in allergic inflammation. *Immunol Today* 1994;15:127–133.
- 75 Alam R, Grant JA: The chemokines and the histamine-releasing factors: Modulation of function of basophils, mast cells and eosinophils. *Chem Immunol* 1995;61:148–160.
- 76 Rollins BJ: Chemokines. *Blood* 1997;90:909–928.
- 77 Sallusto F, Lanzavecchia A, Mackay CR: Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol Today* 1998;19:568–574.
- 78 Kay AB, Ying S, Varney V, Gaga M, Durham SR, Moqbel R, Wardlaw AJ, Hamind Q: Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991;173:775–778.
- 79 Kolmannskog S, Haneberg B: Immunoglobulin E in feces from children with allergy. *Int Arch Allergy Appl Immunol* 1985;76:133–137.
- 80 Sampson HA: Food allergy. Part 2: Diagnosis and management. *J Allergy Clin Immunol* 1999;103:981–989.
- 81 Bengtsson U, Nilsson-Balknäs U, Hanson LÅ, Ahlstedt S: Double blind, placebo controlled food reactions do not correlate to IgE allergy in the diagnosis of staple food related gastrointestinal symptoms. *Gut* 1996;39:130–135.
- 82 Belut D, Moneret-Vautrin DA, Nicolas JP, Grilliat JP: IgE levels in intestinal juice. *Dig Dis Sci* 1980;25:323–332.

- 83 Huggins KG, Brostoff J: Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* 1975;ii: 148–150.
- 84 Andre F, Andre C, Colin L, Cavagna S: IgE in stools as indicator of food sensitization. *Allergy* 1995;50:328–333.
- 85 Sampson HA, Broadbent KR, Bernhisel-Broadbent J: Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N Engl J Med* 1989;321:228–232.
- 86 Nolte H, Stahl-Skov P, Kruse A, Schiøtz PO: Histamine release from dispersed human intestinal mast cells. A method using biopsies from children and adults. *Allergy* 1989;44:543–553.
- 87 De Weck AL, Stadler BM, Urwyler A, Wehner HU, Bühlmann RP: Cellular allergen stimulation test (CAST) – a new dimension in allergy diagnostics. *ACI News* 1993;5:9–14.
- 88 Rossi RE, Monasterolo G, Operti D: A comparative study of the tryptase release test and the cellular allergen stimulation test (CAST) in mite sensitive patients. *Clin Exp Allergy* 1998; 28:752–757.
- 89 Raithel M, Matek M, Baenkler HW, Jorde W, Hahn EG: Mucosal histamine content and histamine secretion in Crohn's disease, ulcerative colitis and allergic enteropathy. *Int Arch Allergy Immunol* 1995;108:127–133.
- 90 van Neerven RJJ, Ebner C, Yssel H, Kapsenberg ML, Lamb JR: T-cell responses to allergens: Epitope-specificity and clinical relevance. *Immunol Today* 1996;17:526–532.
- 91 Nicodemus C, Philip G, Jones N, Hirani S, Norman P: Integrated clinical experience with tolerogenic peptides. *Int Arch Allergy Immunol* 1997;113:326–328.
- 92 Kon OM, Sihra BS, Barkans J, Compton CH, Barnes NC, Kay AB: In vivo effects of a PRIMATEZED® anti-CD4 monoclonal antibody, SB210396 (IDEC CE9.1), on CD4+ T lymphocytes in chronic severe asthma. *J Allergy Clin Immunol* 1998;101:S9.
- 93 Corne J, Djukanovic R, Thomas L, Warner J, Botta L, Grandordy B, Gygax D, Heusser C, Patalano F, Richardson W, Kilchherr E, Staehelin T, Davis F, Gordon W, Sun L, Liou R, Wang G, Chang TW, Holgate S: The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: Efficacy, safety, and pharmacokinetics. *J Clin Invest* 1997;99:879–887.
- 94 Garlisi CG, Kung TT, Wang P, Minnicozzi M, Umland SP, Chapman RW, Stelts D, Crawley Y, Falcone A, Myers JG, Jones H, Billah MM, Kreutner W, Egan RW: Effects of chronic anti-interleukin-5 monoclonal antibody treatment in a murine model of pulmonary inflammation. *Am J Respir Cell Mol Biol* 1999;20:248–255.
- 95 Borish LC, Nelson HS, Lanz M, Claussen LR, Martin DW, Garrison L: Phase I/II study of interleukin-4 receptor (IL-4R) in moderate asthma. *J Allergy Clin Immunol* 1998;101: S8.
- 96 Vercelli D, De Monte L, Monticelli S, Di Bartolo C, Agresti A: To E or not to E? Can an IL-4-induced B cell choose between IgE and IgG4? *Int Arch Allergy Immunol* 1998;116:1–4.
- 97 Jenmalm MC, Björkstén B, Macaubas C, Holt BJ, Smallacombe TB, Holt PG: Allergen-induced cytokine secretion in relation to atopic symptoms and immunoglobulin E and immunoglobulin G subclass antibody responses. *Pediatr Allergy Immunol* 1999;10: 168–177.
- 98 Barnes KC, Marsh DG: The genetics and complexity of allergy and asthma. *Immunol Today* 1998;19:325–332.
- 99 Anderson GG, Morrison JFJ: Molecular biology and genetics of allergy and asthma. *Arch Dis Child* 1998;78:488–494.
- 100 Thomas NS, Wilkinson J, Holgate ST: The candidate region approach to the genetics of asthma and allergy. *Am J Respir Crit Care Med* 1997;156:S144–151.

Copyright: S. Karger AG, Basel 2000. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.