

REVIEW ARTICLE

The contribution of biotechnology toward progress in diagnosis, management, and treatment of allergic diseases

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Abstract

‘Biotechnology’ has been intuitively used by humans since thousands of years for the production of foods, beverages, and drugs based on the experience without any scientific background. However, the golden era of this discipline emerged only during the second half of the last century. Incredible progresses have been achieved on all fields starting from the industrialization of the production of foods to the discovery of antibiotics, the decipherment of the genetic code, and rational approaches to understand and define the status we now call ‘healthy’. The extremely complex interactions between genetic background, life style, and environmental factors influencing our continuously increasing life span have become more and more evident and steadily generate new questions which are only partly answered. Here, we try to summarize the contribution of biotechnology to our understanding, control, and cure of IgE-mediated allergic diseases. We are aware that a review of such a vast topic can never cover all aspects of the progress achieved in the different fields.

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Although considered the pandemics of the 21st century (1) with an increasing impact on healthcare burdens for therapy and prevention (2–6) and on politics (7–9), allergy is not a novel disease (10). The first description of the symptomatic ‘Catarrhus aestivus’ (hay fever or hay asthma) termed by Blackley (11) occurred almost 140 years ago, and the first description of allergen-specific immunotherapy (ASIT) by Noon in the reputed journal ‘Lancet’ (12) is more than 100 years old (Table 1). Allergic diseases can be roughly subdivided into four groups according to the organs affected: allergic rhinitis and rhinosinusitis affecting the upper respiratory tract (13), allergic asthma affecting the lower respiratory tract (14), atopic eczema affecting the skin (15), and food allergy affecting the gut (16). The common hallmark of these

diseases is their association with the production of allergen-specific IgE which, compared with other immunoglobulin isotypes, is present only in minute amounts in the serum of sensitized individuals (17). The pivotal discovery of IgE by Ishizaka et al. (18) more than 40 years ago opened a new era in medical science: the allergology (Table 1). For a long time, allergic diseases have not represented a major health problem as shown by their prevalence among the general population (Fig. 1). However, since the second half of the last century, the prevalence of allergic diseases has, for largely unknown reasons (19–21), dramatically increased. The continuous improvement of diagnostic tests made possible by improved biochemical preparation of standardized extracts (22) and the biotechnological production of recombinant allergens (23, 24)

Table 1 Milestones in the development of molecular allergology

Year	Topics	Reference
1873	First description of allergy	11
1911	First immunisation against pollens	12
1967	Discovery of IgE	18
1988	First allergen cloned	35
1992	First skin tests with a recombinant allergen	213
1996	First description of a recombinant allergen on ImmunoCaps	214
2002	First description of an allergen-microarray	30
2004	First use of a recombinant allergen for immunotherapy	215

might partly explain the increase in sensitization to allergens around the world (25). However, in spite of the substantial improvement achieved, not all problems related to the *in vitro* and *in vivo* diagnosis of allergic diseases have been solved (26, 27). The spectacular progress in genetic engineering, biochemistry, molecular and cellular biology, and 'omics' technologies including proteomics, genomics, and next-generation sequencing during the past three decades substantially contributed to an improved understanding of the mechanisms involved in the development of IgE-mediated diseases. New concepts such as component-resolved diagnosis and patient-tailored treatment of allergic diseases (28), microarray-based diagnosis (29, 30), anti-IgE treatment (31), and novel application routes for immunotherapy emerged (32) and entered clinical trials (33) (Table 1). The aim of this work was to summarize the contribution of biotechnology to the exciting progress achieved in understanding mechanisms, diagnosis, management, and treatment of IgE-mediated diseases experienced over the past few years.

Allergens, allergenicity, and cross-reactive structures

Molecular allergology lagged behind the progress in genetic engineering and molecular biology for almost two decades (Table 1) (34). However, with the molecular cloning of the first allergen in 1988 (35), a spectacular development of this young scientific discipline started. To date, the most important allergens from mites, animal dander, pollens, insects, fungi, and foods have been cloned and characterized (www.allergen.org), the allergen nomenclature has been recently updated (36), and more than 40 three-dimensional allergen structures are in the Protein Database (www.uniprot.org). However, the reason why few proteins are allergenic whereas the majority are not remains unsolved perhaps because all efforts to understand allergenicity have been focused on studies of structural features of allergens and B- and T-cell epitopes (37), largely ignoring external and patient's intrinsic factors influencing the deregulated immune response against allergens that are normally innocuous environmental substances for healthy individuals. Environmental factors such as oxidative stress, for example, might provoke unfolding of protein-inducing responses promoting inflammation (38) and therefore favouring a switch toward the production of allergen-specific IgE (39). Although the elucidation of the primary, secondary, and three-dimensional structures of allergens has not significantly contributed to understand allergenicity, the accumulated knowledge strongly contributed to understand the roles played by cross-reactivity (1, 33, 40, 41), polysensitization (42), and cross-reactive carbohydrate determinants (43) in allergic diseases. Recombinant allergens producible as perfectly standardized substances strongly contributed to improve the sensitivity and specificity for the detection of soluble allergen-specific serum IgE. In this context, it is important to understand that allergen-specific serum IgE, necessary for the development of IgE-mediated

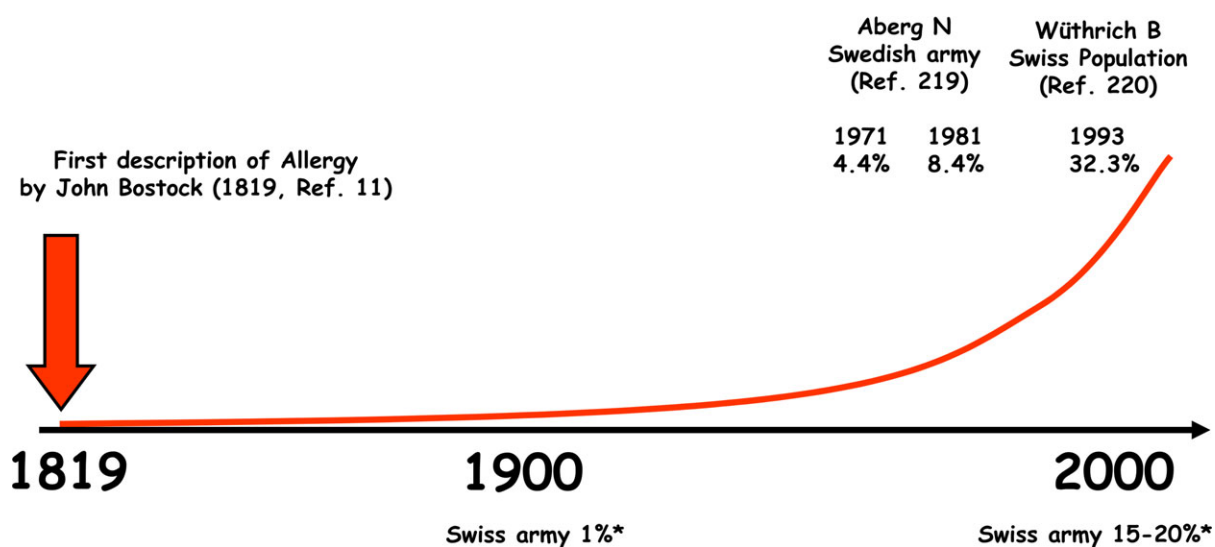


Figure 1 Increase in the prevalence of allergic diseases during the past two centuries (*Data obtained from Dr. Th. Kündig, personal communication).

hypersensitivity reactions, is an excellent marker to diagnose the atopic status of allergic individuals (44) which, however, is not necessarily correlated with symptoms (44, 45). In contrast to complex allergen extracts that are hard to produce in a standardized form, recombinant allergens can be produced as perfectly standardized diagnostic and therapeutic preparations satisfying the increasing safety profiles required for medical applications in humans.

Component-resolved diagnosis of allergy and allergen microarrays

Complex natural allergenic sources such as foods, mites, and fungi contain many species-specific and cross-reactive allergens. The composition of the extracts depends on many factors including raw material used for the preparation, extraction procedure, batch-to-batch variation, protease content, and storage conditions. These factors strongly influence the final composition potency and stability of allergen preparations and, as a consequence thereof, the outcome of diagnostic tests. Most allergen preparations derived from fungi, molds, foods, and some complex pollens are still not yet available as standardized products. The diagnostic sensitivity depends basically on two variables: the concentration of the single allergens of the test preparation that can be influenced by standardization and the pattern of sensitization of the subject to single allergens that can vary from individual to individual. Therefore, it is of paramount importance, especially in view of a possible therapeutic intervention, to know the exact sensitization profile of the individual to allow a patient-tailored, component-resolved treatment. The availability of highly pure recombinant allergens allows today to analyze the sensitization profiles of allergic individuals in detail (46, 47), to reduce the number of allergens required for a reliable diagnosis (48), to identify the most appropriate diagnostic markers (49–51), and to distinguish multiple sensitization from cross-reactivity (52–54). Except their use in novel therapeutic approaches and their contribution to the elucidation of basic mechanisms involved in the development of allergy and asthma discussed later, the availability of purified natural and recombinant allergens further contributed to the development of fast and sensitive component-resolved diagnostic procedures based on microarrays (30, 42, 55–57). Although the allergen microarray technology is not yet fully automated as it is the case for other microarray-based systems such as DNA microarrays (58, 59), this novel diagnostic approach will replace older technologies in the near future.

T- and B-cell epitopes and basophil activation tests

T-cell epitopes play a crucial role in immune responses for the induction of cytotoxic T-cell responses and in providing help to B cells for the development of antibody responses. They represent short linear fragments of protein antigens processed through the proteasome and bind to the MHC I or MHC II on antigen-presenting cells. The peptide/MHCs are recognized by the T-cell receptor and initiate a cascade of

events leading to T-cell activation and differentiation. T-cell epitopes are easy to define experimentally (60) and to produce in large quantities under GMP conditions by classical chemical synthesis (61). It is therefore not astonishing that defined T-cell epitopes have been largely used in immunology for basic research and for the development of vaccines (62, 63).

The characterization of B-cell epitopes is much more complicated because the majority of them represent three-dimensional structures (37) difficult to define without co-crystallization of antigens and antigen-specific FAB fragments (64, 65). The identification of B-cell epitopes, however, strongly contributed to improve our understanding of structural aspects of allergens (37) and of the pivotal role they play in the induction of hypersensitivity reactions against small molecules (66–68) and allergens (68, 69) through cross-linking of allergen-specific IgE bound to high-affinity FcεRI receptors on effector cells (70).

The development of the basophil activation test (BAT) allowed investigating IgE-mediated allergies without *in vivo* provocations that are associated with a potential risk of generating systemic allergic reactions (71).

Development of novel vaccines

The development of vaccines is one of the biggest stories of success in human and veterinary medicine (72). Since Edward Jenner's first introduction of a therapeutic approach using a smallpox virus derived from cow (Latin *vacca*) in 1796, the use of vaccines has mainly contributed to the eradication of infectious diseases. The cow-derived 'drug' was called vaccine, and the therapy was termed vaccination, which still has an important impact on the prevention of infectious diseases. Here, we want to point out the increasing incidence of allergies and the economical impact of a subtype of vaccination, ASIT, can have. In contrast to vaccination for the prevention of infectious diseases, ASIT aims at generating an allergen-specific peripheral tolerance to allergens in established deregulated immune responses.

A major caveat in the application and development of novel vaccines to be used for treating allergic patients is the fact that the agents used for immunization (or desensibilization) can evoke severe side-effects (7, 73).

This is due to the fact that patients undergoing ASIT have by definition specific IgE against the allergen and thus face hypersensitivity reactions upon re-exposure to the offending allergen. Therefore, intensive research has been carried out with the aim to overcome these problems. Investigations about successful ASIT start with a careful elucidation of the underlying mechanisms. This description includes the documentation of clinically observed phenomena such as the interference of antibodies (71), adverse side-effects (73), the role of B and T cells, as well as the involvement of APCs and effector cells during the progress of the therapy (74–78). Biotechnology strongly contributed to the improvement of allergy vaccines by chemical modification of natural allergens (79, 80) and even more by genetic engineering of recombinant allergens starting from the natural sequences, the generation

of hypoallergenic variants including isoforms and folding variants (81–84), and the *de novo* engineering of vaccines combining B- or T-cell epitopes (84, 85). Some of these experimental vaccines have been successfully tested in clinical trials (86, 87) and demonstrated to have an increased safety profile and reduced side-effects compared with allergen extracts.

The large variation in size of naturally occurring antigens ranging from ~10 nm for viruses to more than 100 000 nm for fungi and protozoa and the long evolutionary optimization of the immune system to deal with these dangers open a plethora of possibilities for design and biotechnological production of synthetic vaccines with different properties as amply documented (28, 33, 72).

The steadily increasing understanding of the interactions between innate and adaptive immunity, the discovery of pattern recognition receptors such as Toll-like receptors (TLRs), dectins, and C-type lectin receptors promoted a comeback of research on adjuvants. In turn, new agonistic or antagonistic immune potentiators such as monophosphoryl lipid A (88), or CpGs as TLR agonists (89), attenuated or living microbes (e.g., *Lactobacillus*, *Lactococcus*, *Bifidobacterium*) (21, 90), or small molecular compounds such as imidazoquinolines or 1,25-dihydroxyvitamin D3 have been identified and are currently under intensive investigation.

However, also the usage of modified vaccinia virus Ankara (MVA) has a long history in safe vaccinations for smallpox, and MVA has been used as an expression vector to produce vaccines to fight against other infectious diseases and, recently, also against cancer (72). Regarding allergies, genetically modified VA was used to prevent the onset of intestinal allergy to ovalbumin (OVA) in a murine model of food allergy by vaccination of the mice with MVA expressing OVA on the surface. This vaccination induced a strong OVA-specific Th1 immune response mediated by the induction of IFN- γ , and protective OVA-specific IgG2a antibody responses, highlighting the potential of older vaccination concepts and the need for further research of these type of vaccines in allergy (91, 92).

More complex synthetic multicomponent vaccines are, for example, virus-like particles (VLP) that represent a way to mimic a virus infection for the immune system. They represent self-assembling entities, usually made from a major capsid protein of a virus that can be filled or decorated with cargo molecules such as nucleic acids, adjuvants, polypeptides, or proteins, either by active engineering or chemical coupling or by simply providing of the agents in the medium where the assembly is performed (93–95). Although VLP vaccines have made it to commercially available vaccines for, for example, HPV (GardasilTM, CervarixTM) (95), the usage of VLP in allergology and ASIT remains quite limited to date (96, 97).

In addition to the main stream developments described above, many other concepts for the development of allergy vaccines have been proposed (98), but only a few of them entered clinical studies.

However, not only the biochemical properties of a vaccine and the adjuvant used drive the immune response but also

the route of application has a decisive influence on the therapeutic outcome as summarized in the next section.

Novel routes of application: SCIT, SLIT, ILIT, and clinical trials with recombinant allergens

Beyond optimizing the immunological properties of allergy vaccines produced by biotechnological manipulations, the convenience of the therapeutic treatment for the patient and for the treating allergologist is still largely an unmet goal (99). A successful vaccine should induce long-term and specific tolerance to the allergen, should be modular (100) allowing fusion of different allergens (101), and should act on a short time with a few injections (102). In clinical routine, the most commonly used form of allergy vaccination is still the subcutaneous route (SCIT) performed by injection of increasing amounts of allergen. Although the SCIT for insect venom and pollen allergy is highly effective in curing the diseases (103, 104), the number of injections needed to achieve protection is high (105), local reactions at the side of injection are frequent, and the risk of anaphylactic and systemic reactions is low, however, not negligible (106). In SCIT, the allergen preparation is injected subcutaneously, but the immunological response is generated in the draining lymph nodes. Because only <1% of the injected protein reaches the draining lymph nodes, SCIT requires higher doses of allergen compared with other routes of application (107).

Adverse effects, together with the wish to deliver the vaccine more directly to the target organs or to the lymph nodes where the immune response is taking place, stimulated the search for novel routes of application. Sublingual immunotherapy (SLIT), as a model system to target mucosal tissues, was first described in 1986 (108). Numerous studies have been published since then, proving the efficacy and safety of SLIT (109–113). SLIT is generally considered to have an improved safety profile over SCIT, as most reactions are local and transient and are thus seldom leading to an interruption of the treatment. The good safety profile and the convenient route of application for the patients lead to a very good compliance rate of around 97% for SLIT compared with 75% for SCIT (106). Studies on the distribution of SLIT vaccines in humans and mice show that SLIT vaccines bind to the epithelial cells within the time frame of a few minutes, cross the mucosa in 15–30 min, and are thereafter captured and processed by antigen-presenting cells (114–116). The complete elucidation of the uptake mechanisms and targeting of APCs in mucosal tissues could open new ways to optimize SLIT vaccines by biotechnology.

One approach to increase the amount of vaccine finally reaching the side of immunological action, the lymph node, is the direct injection into subcutaneous lymph nodes. This concept was first introduced in classical vaccinology (116), but has been adapted for usage in ASIT in 2001/2005 (117–119). A direct comparison of different routes of immunization, SCIT, intraperitoneal, or intralymphatic immunotherapy (ILIT), showed a 1000-fold less amount of vaccine necessary

to elicit the same specific antibody titers in mouse (118). ILIT has also been combined with modular antigen-translocating vaccines which directly target the MHC class II antigen presentation pathway (120) and showed excellent results in a phase I/IIa clinical study after only three injections of low amounts of vaccine (101). There is of course an increasing number of clinical trial reports involving many kinds of different biotechnologically engineered allergen vaccines (86, 87, 121–124) which, in combination with different routes of application, indicate a great therapeutic (104) and economic potential for the future treatment and prevention of allergic and asthmatic diseases (125).

Immunological mechanisms underlying allergic diseases

The advances experienced in biotechnology over the last decades have significantly contributed to improve the knowledge of the immunological mechanisms underlying allergic diseases (76, 126–130). Allergy consists of two well-defined steps: the sensitization and effector phases (131). The sensitization phase occurs during the first contact with the allergen and is characterized by the differentiation and clonal expansion of allergen-specific CD4⁺ Th2 cells that produce IL-4 and IL-13 and contribute to the induction of class switch to the ϵ immunoglobulin heavy chain in B cells (74, 132). Allergen-specific IgE antibodies (Abs) bind to the high-affinity Fc ϵ RI on the surface of mast cells and basophils, and consequently, the patient becomes sensitized. The effector phase occurs after a new encounter with the offending allergen, which induces the cross-linking of the IgE–Fc ϵ RI complexes on sensitized effector cells, and triggers the release of anaphylactogenic substances that lead to the clinical symptoms associated with immediate reactions (133, 134). If the contact with the allergen persists, late-phase reactions leading to tissue inflammation and injury might occur. Late-phase reactions initiate through IgE-mediated presentation of allergens by DCs and consequent activation of allergen-specific memory Th2 cells which, in turn, produce high levels of IL-4, IL-5, IL-9, and IL-13. These cytokines together with the participation of other cell subsets such as the ILC2 contribute to maintain inflammation, high levels of allergen-specific IgE Abs, mucus production, eosinophilia, and contraction of smooth muscle cells, thus leading to the most severe clinical manifestations of allergy such as chronic persistent asthma, allergic rhinitis, atopic dermatitis, or anaphylaxis. Other T-cell subsets such as Th1 and Th17, or the recently discovered Th9 and Th22, can also contribute to the development of allergic diseases (124, 134). On the other hand, the generation and maintenance of allergen-specific regulatory T (T_{reg}) cells is a key event for healthy immune responses to allergens (76, 134, 135). T_{reg} cells are a heterogeneous population of T cells with suppressive capacity that can be broadly classified into 2 main groups: the thymus-derived naturally occurring CD4⁺ CD25⁺ forkhead box protein 3 (FOXP3)⁺ T_{reg} cells, termed natural T_{reg} cells, and the inducible T_{reg} cells, which are generated in the periphery after antigenic stimulation.

The role of the innate immune system in allergic diseases

Recent findings have also shown that the innate immune system plays an important role in the context of allergy (136–139). Dendritic cells (DCs) with the capacity of linking innate and adaptive immune responses are essential for the initiation and maintenance of allergic diseases (139–142). Human circulating DCs can be broadly divided into two different functional groups, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) (7, 134). mDCs can be further divided into type 1 mDCs expressing BDCA1 and type 2 mDCs expressing BDCA3 (142). For a long time, it was believed that only immature or partially mature DCs under noninflammatory conditions were able to generate functional T_{reg} cells (143) and that mature DCs induced specific effector Th cells after encountering different pathogens in specific environments (144, 145). However, recent findings indicated that fully mature DCs, even under inflammatory conditions, are also able to induce functional T_{reg} cells using a large battery of soluble molecules (TGF- β , IL-10, IDO, or RALDH), as well as different costimulatory molecules (ICOS-L, PD-L1, ILT-3, ILT-4, 4-1BB, CD80, CD86, or MHC II) (146, 147). The normal response to innocuous allergens should be the generation of functional T_{reg} cells; however, under certain circumstances, DCs can promote the generation of allergen-specific Th2 responses (148–150). Although the detailed mechanisms are not fully understood yet, several studies demonstrated that specific biochemical features of allergenic proteins might well act as Th2-skewed adjuvants. An axis involving epithelial cell-derived thymic stromal lymphopoietin (TSLP) and OX40-L expression on DCs was linked to the generation of Th2 immune responses in the absence of IL-12 (150, 151). The epithelial barrier not only acts as a simple physical barrier but also responds to environmental insults such as pathogens, pollution, cigarette smoke, or allergens with the production of antimicrobial peptides and inflammatory cytokines or chemokines (139, 152, 153). After allergen encounter, the epithelium releases Th2-promoting cytokines such as TSLP, IL-25, or IL-33, which activate different cell types including DCs and the recently discovered type 2 innate lymphoid cells (ILC2) (154–157). Altered barrier function promotes the generation of DCs with the capacity to generate allergen-specific Th2 cells and/or induce activation of ILC2 that produce large amounts of IL-13 and IL-5, thus contributing to perpetuation of the allergic reactions. Although the cross talk among epithelial cells, DCs, and ILC2 in the context of allergic diseases needs to be explored in further detail, there is compelling evidence showing that the role of Th2 cytokines in the development of allergy might well have innate and adaptive cellular sources (157).

Mechanisms by which allergens disrupt the barrier

The improvements of molecular biology and protein chemistry tools allowed the generation of a large number of purified natural allergens and their recombinant and mutated forms, which has significantly contributed to better understand the

structural nature of the allergenic proteins as well as to identify allergens able to induce primary sensitization (158–163). Although what makes a protein an allergen is still an open question as there is no single structural feature able to unequivocally predict the allergenicity of a protein, there are some properties that are common to many allergens. For example, in general, allergens are relatively small (<70 kDa) proteins or glycoproteins that belong to <2% of all known protein families. Allergenic proteins are frequently highly stable to digestive and thermal treatments, and they usually display low hydrophobicity, which increases their solubility in the mucosa. Despite some exceptions, there are two properties that should be common to all true allergens: (i) their ability to trigger the production of IgE antibodies and (ii) their capacity to elicit allergic symptoms via an IgE-mediated mechanism. Therefore, at certain stage, allergenic proteins must be recognized by the immune system in an immunologically active form (164). Mucosal surfaces cover around 400 m² of the human body and represent the first contact point of allergens with the immune system. Depending on the way of entrance to the body, the allergens encounter the immune system at different places (165). For food allergens, this occurs in the oral and gastrointestinal mucosa, but for aeroallergens from pollen, house dust mite, fungi, or animal dander, the encounter takes place in the nasal and airway mucosa. Food and aeroallergens can also directly contact with the tonsils as they constitute a first line of mucosa-associated lymphoid tissue at the entrance of the respiratory and digestive tracts (166). For contact allergens, the first encounter

with the immune system occurs at the skin (167). In any case, allergenic proteins need to be transported through the epithelial barrier to reach the immune system. This transport can be para-cellular, for example, due to disruption of the intercellular junctions [tight junctions (TJ), adherent junctions, or desmosomes] as well as trans-cellular through different mechanisms including diffusion, transcytosis, endocytosis, or active transport (168). In addition, DCs can directly capture allergens due to their capacity to insert their dendrites between epithelial cells to scan the lumen (164).

Allergenic proteins can be classified as type I allergens (proteases able to promote Th2 responses) or type II allergens (nonproteases able to disrupt the barrier by different mechanisms and also promote Th2 responses). Type I allergenic proteases alter the integrity of the barrier by degrading TJ proteins and increasing the para-cellular transport, which favors the exposure of any protein from the environment to the mucosal immune system. The cysteine protease Der p 1, the major allergen from house dust mite *Dermatophagoides pteronyssinus*, was the first allergen described to show proteolytic activity (169). High-molecular-weight allergenic proteases with serine and/or aminopeptidase activity able to disrupt the barrier through degradation of TJ proteins such as occluding, claudin-1, or E-cadherin are also contained in different pollens including *Olea*, *Dactylis*, *Cupressus*, and *Pinus* (170). Several studies demonstrated the presence of allergenic proteases in *Alternaria alternata*, other fungi, and foods (171–173). Allergenic proteases can also alter the capacity of epithelial cells to mount innate immune responses. Der p 1

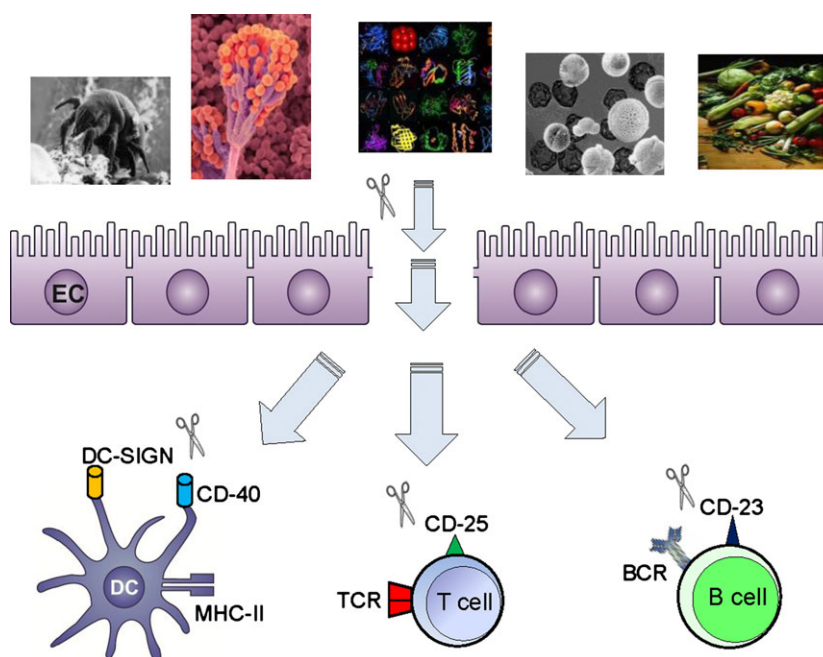


Figure 2 Type I allergens with proteolytic activity act on multiple targets contributing to promote Th2 immune responses. They disrupt epithelial barriers by degrading TJ proteins to cross the epithelium and reach subepithelial areas where they come in contact

with different cells of the immune system. Type I allergen might also cleave different additional targets such as DC_SIGN or CD40 on DCs, CD25 on T cells, and CD23 on B cells favouring the generation and maintenance of Th2 immune responses.

and the serine protease Der p 9 are able to induce cytokine release from airway epithelium favoring pro-inflammatory adaptive immune responses in a process mediated by PAR-2 but not PAR-1 (174). Similar effects were reported for cockroach allergens and other pollen and fungal proteases (164). Interestingly, a large number of studies also showed that allergenic proteases target other relevant immune system cells promoting the generation of Th2 immune responses. Der p 1 cleaves CD23 on B cells, thus disrupting the negative feedback loop for IgE production (Fig. 2) (164). Type II allergens can also disturb the barrier and promote the induction of Th2 responses by different mechanisms (Fig. 3). Der p 2 possesses a MD-2-related lipid recognition domain that allows a direct interaction of the allergen with the TLR4 complex and with LPS, thus directly activating TLR4 signaling even in the absence of MD-2 (175). Different studies showed alternative ways of crossing the barrier including direct uptake of allergens by epithelial cells (176–178). Bet v 1, the major birch pollen allergen, binds to conjunctival epithelium, and it is transported through only in allergic patients (176). Combination of transcriptomic and proteomic tools demonstrated that 22 putative receptors are expressed at higher levels in allergic epithelium and that Bet v 1 is transported through

the epithelium within lipid rafts/caveolae in an antibody-dependent process. Similarly, the major soybean allergen P34, which is resistant to proteolysis *in vitro*, is transported through intestinal epithelial cells by a caveolae-mediated mechanism (177). It was also demonstrated that Pru p 3, the major peach allergen, crosses the epithelial cell barrier without disturbing the integrity of TJs via lipid rafts inducing the production of the Th2-promoting cytokines TSLP, IL-33, and IL-25 (178). A recent study using site-directed mutagenesis and comparative analysis of Bet v 1 and related food allergens revealed intrinsic structural features of Bet v 1 that account for its high intrinsic allergenicity, suggesting that structural changes might well alter the sensitization capacity of allergens (179).

Mechanisms of immunotherapy

The understanding of the mechanisms operating in ASIT has also significantly improved in parallel with the recent advances in biotechnology (180–182). A very rapid desensitization of effector cells occurs early during ASIT, which impairs mast cell and basophil degranulation and consequently systemic anaphylaxis (182–185). The immunological changes associated with successful ASIT include the generation of allergen-specific T_{reg} and/or Th1 cells, inhibition of Th2 responses, decreases in allergen-specific IgE, and increases in allergen-specific IgG and IgA, as well as a reduction in infiltrating inflammatory cells (134). Several studies demonstrated that the generation and maintenance of functional allergen-specific T_{reg} cells able to inhibit ongoing allergic responses represents a critical step for the induction of permanent T-cell tolerance (183, 186, 187). The use of novel biotechnological tools including allergen-specific tetramer molecules, purified natural and recombinant allergens alone or chemically coupled to specific fluorophores, proteomic and genomic tools, as well as novel next-generation sequencing has significantly contributed to the better understanding of the mechanisms operating during the restoration of healthy immune responses to allergens during ASIT. The use of major histocompatibility complex class II tetramer molecules, coupled to specific peptides of Bet v 1, demonstrated that the generation of functional Bet v 1-specific CD4⁺ FOXP3⁺ T_{reg} cells in human tonsils occurs by mechanisms partially depending on pDCs (187). It was also shown that in human tonsils and peripheral blood, allergen-specific T-cell tolerance is broken by triggering of TLR4 or TLR8 and proinflammatory cytokines (188). Interestingly, a recent study confirmed tonsils as organs where immunoregulation takes place (189). In this study, quantification of mRNA expression levels of different genes by qPCR demonstrated that tonsillar cytokine expression is closely related to viral infections and age. Also, the analysis of epigenetic modifications at DNA level has gained increasing interest in the field of allergy (190–192). A recent study demonstrated that successful peanut SLIT resulted in an increased induction of allergen-specific T_{reg} cell linked to the hypomethylation of the FOXP3 promoter (192). These data suggest that epigenetic modifications in allergen-specific T_{reg} cells might be predictive of a state of clinical

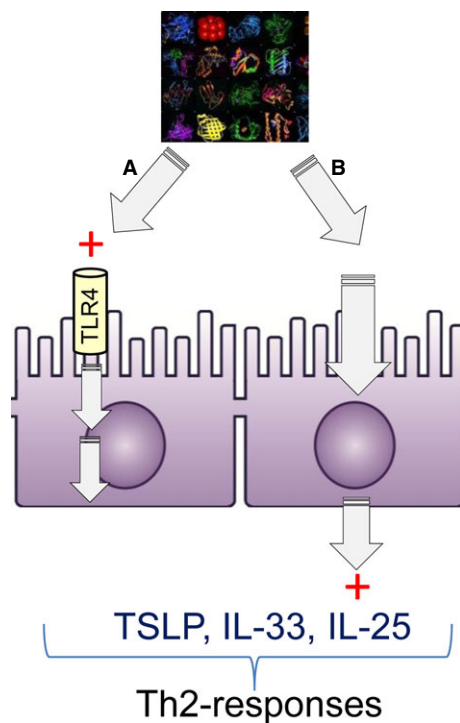


Figure 3 Type II allergens, without proteolytic activity, can induce Th2 responses by different mechanisms of action. For example, Der p 2 mimics components of the TLR4 signaling complex even in the absence of LPS (A). Pollen (e.g., Bet v 1) or food allergens (e.g., P34 from soybean or Pru p 3) can interact with lipid rafts to become transported across the epithelium in a trans-cellular manner where they induce the production of Th2-promoting cytokines such as TSLP, IL-33, or IL-25 (B).

tolerance during oral immunotherapy. In humans, tolerogenic IL-10⁺ PLA-specific regulatory B cells were also recently identified as new key players in the restoration of tolerance in allergic patients receiving ASIT (193). Supporting these data, recent findings in mice also demonstrate that tolerogenic CX3CR1⁺ B cells suppress food allergy-induced intestinal inflammation (194).

In conclusion, the use of novel biotechnological tools has significantly contributed to delineate the most important mechanisms involved in the pathogenesis of allergy and ASIT, as well as to the identification of new cell subsets and molecules implicated in these processes. This progress will contribute not only to improve diagnosis and management, but also to the development of more efficient and safer immunotherapeutic treatments for allergic and asthmatic diseases.

Future perspectives and conclusions

Biotechnology will also in future strongly contribute to the progress in the field of allergic and asthmatic diseases providing tools and biomarkers allowing a better understanding of the mechanisms leading to these diseases. Although many aspects of the progress achieved have not been discussed in detail due to space constraints such as, for example, the field of proteomics and biomarkers (129, 195–197), miRNA (128, 198, 199), the many effects of cytokine antibodies and other small molecules (184, 200, 201), or the potential of IgE-based therapeutic approaches to cure cancer (202), we hope we have covered the major aspects. Biotechnology has and will further serve as an innovation motor in all fields of life science including allergy. However, innovation alone without

practical implementation never had an impact on our society. It is therefore of utmost importance that the progress achieved in basic and clinical science becomes translated in political actions and in practical applications. The outstanding progress obtained during the few past years and the limited financial resources ask for a large discussion and definition of the future needs in appropriate forums (7, 203). A tremendous work has been carried out by the EAACI during the past years to define needs and guidelines for prevention (204–206), management (3, 4, 204, 207, 208), and treatment of allergic disease (209–212). In conclusion, we are convinced that the scientific and political efforts with engagement of all stakeholders, including basic science and applied biotechnology, will allow to further speed up the progress aiming at preventing, controlling, and reducing the social and economic burdens caused by IgE-mediated diseases.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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