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Determinants of allergenicity

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The question "What makes an allergen an allergen?" has puzzled generations of researchers, and we still do not have a conclusive answer. Despite increasing knowledge about the molecular and functional characteristics of allergens that have been identified, we still do not fully understand why some proteins are clinically relevant allergens and most are not. Different approaches have been taken to identify the structural and functional features of allergens, aiming at developing methods to predict allergenicity and thus to identify allergens. However, none of these methods has allowed a reliable discrimination between allergenic and nonallergenic compounds on its own. This review sums up diverse determinants that contribute to the phenomenon of allergenicity and outlines that in addition to the structure and function of the allergen, factors derived from allergen carriers, the environment, and the susceptible individual are of importance.

Key words: *Allergen, structure, intrinsic function, pollen-associated lipid mediators, adjuvant*

Abbreviations used

DC: Dendritic cell
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
DEP: Diesel exhaust particle
PALM: Pollen-associated lipid mediator
TCR: T-cell receptor
TLR: Toll-like receptor
Treg: Regulatory T

Allergies are immune-mediated hypersensitivity reactions that can affect various organs, most commonly the skin, airways, and gut (ie, the interface between organism and environment). The prevalence rates of allergic conditions have increased at an alarming rate throughout the world in the past 50 years.[1] According to current concepts, T_H2 responses constitute a prerequisite for the development of type I hypersensitivity reactions, which lead to IgE production and arming of mast cells and basophils with specific IgE. Subsequent allergen encounter leads to IgE-mediated mast cell/basophil degranulation and release of proinflammatory mediators, cytokines, and chemokines that ultimately are responsible for the inflammatory response seen in type I hypersensitivity. Although the biology of T_H2 cells and their influence on B cells and immunoglobulin class-switch recombination are well understood, little is known about the mechanisms that control the initial T_H2 polarization in response to allergens. Some studies put forward allergen-dependent mechanisms determined at the dendritic cell (DC) level because of particular attributes of the specific protein.[2-4] Others suggest T cell–dependent[5,6] or individual[7] factors leading to a predominance of the T_H2 response. In principle, all these factors together can add to the allergenicity of a given substance. The complexity of allergen exposure conditions, however, hampers efforts to understand why a given protein in an individual at a certain time point induces an "allergic" immune response and not a "healthy" or tolerogenic immune response. Here we review the multidimensional determinants contributing to the allergenicity of a protein that induces type I allergy, focusing on the structure and function of the allergen and on the biogenic and anthropogenic adjuvants promoting proallergic immune responses.

Type I hypersensitivity reactions, such as rhinitis allergica and allergic asthma, are the most common allergic diseases, with current prevalence rates ranging from 5% to 30% in industrialized countries.[8] Type I allergies are mediated by the production of IgE specific for otherwise harmless environmental substances, most of which are proteins. According to the World Health Organization/International Union of Immunological Societies definition, a protein is listed as an allergen when it causes a specific IgE antibody response in at least 5 individuals.[9] Most allergenic molecules that elicit IgE-mediated immune responses are derived from plants, animals, and fungi. Allergen nomenclature has been developed and is maintained by the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Subcommittee (www.allergen.org).[10] Allergens are named by using the first 3 letters of a genus, a single letter for the species, and a number according to priority of allergen discovery and purification; for example, Phl p 1 is the group 1 major allergen from grass pollen of *Phleum pratense*. Currently, more than 1000 protein allergens have been sequenced, and the numbers of known allergens are steadily increasing. However, only a very small percentage of the total number of proteins from environmental sources that our immune system encounters elicits an allergic reaction.

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FROM T-CELL RESPONSE TO IgE PRODUCTION AND ALLERGIC DISEASE

Type I reactions and symptoms can be explained by molecular-molecular interactions between the antigen and its corresponding IgE antibody. This aspect is situated at the end of a cascade of events leading to allergy. A precondition for this cascade to start is specific recognition of the allergen on antigen-presenting cells, which, in the case of T-cell responses, is accomplished by the antigen-specific T-cell receptor (TCR). However, the TCR-transmitted signal alone is insufficient to induce T-cell responses; it requires additional modulation by costimulatory molecules with positive or negative regulatory function. A third signal derives from antigen-presenting cell–originated cytokines that codecide the ensuing T-cell subtype (T_H1, T_H2, T_H17, and regulatory T [Treg] cells). IL-12, for example, is the key cytokine to induce T_H1 responses. A necessary and sufficient DC-derived T_H2-skewing signal is not described to date, perhaps because it does not exist. This gap was filled with the default T_H2 hypothesis, claiming that the T_H2 pathway represents a default mechanism that can be in certain cases disturbed by other polarizing cytokines, such as IL-12 (skewing to T_H1), IL-6, IL-23, IL-1β (skewing to T_H17), and IL-10 (skewing to a regulatory type).

The fourth signal derives from the microenvironment. Here so-called adjuvant factors of extrinsic and intrinsic nature are the decision makers. Classic T_H1 adjuvants are bacterially derived molecules, such as LPS, and were identified to act through pattern-recognition receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain containing proteins (NODs), Dectin, and dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN/CD209).[11,12] Concerning exogenous T_H2 adjuvants,[13] first evidence was given from helminth-derived molecules to skew the immune system toward T_H2.[14] However, recent data suggest that also allergen carriers can release T_H2-promoting factors (see below).[15,16] Together these mechanisms suggest that fine-tuning T-cell functions is not only accomplished by the orchestrated interaction of TCRs, costimulatory molecules, and cytokines but also by adjuvant factors present in the local microenvironment.[17]

Depending on the susceptibility of the individual, either a healthy or an allergic immune response to the allergen arises. In the case of a healthy immune response, the production of IgG4 or IgG1 allergen-specific antibodies prevails. On the T-cell side, the healthy response is characterized by the presence of allergen-specific Treg cells,[18] whereas in allergic individuals reduced frequencies of allergen-specific Treg cells are reported[19] and successful immunotherapy is accompanied by a normalization of the Treg cell compartment.[20] In susceptible individuals T_H2-dominated immune responses lead to production of allergen-specific IgE. T_H2-derived IL-4 adds to the development of IgE-switched B cells, which in turn undergo transformation to IgE-producing plasma cells, some of which are long lived and thus responsible for a long-lasting sensitization status.[21] Aalberse[22] suggested 2 routes that lead to IgE production: (1) the atopic route (relevant for allergens from pollen and mites) in which a direct switch from μ to ϵ is common and (2) the "modified T_H2" route (used by allergens from pets) in which the class switch to IgE is often preceded by a switch to IgG4. According to Aalberse and Platts-Mills,[23] the choice between these 2 routes is determined at the level of the germinal center activity .

With the formation of allergen-specific IgE, the final step of the allergy cascade is attained. Every subsequent encounter with theallergen leads to cross-linking of FcϵRI-bound IgE on effector cells, such as mast cells and basophils, by using multivalent allergen, resulting in cellular activation and release of proinflammatory mediators responsible for the symptoms of allergic diseases. Recent observations suggest that this response is not only dependent on the presence of 2 epitopes within the allergenic protein but is also profoundly influenced by other factors that reduce the threshold mast cell activation. In this context it has been shown that cell-surface receptors, such as specific G protein–coupled receptors and KIT (stem cell receptor, c-KIT/CD117), synergistically enhance FcϵRI-mediated mast cell degranulation. Activating mutations in critical signaling molecules might also contribute to such responses, underlying the importance of individual susceptibility to allergic responses.[24]

Loci and susceptibility genes for allergic sensitization and disease have been described.[25,26] Among others, these include genes involved in IgE regulation (eg, gene encoding signal transducer and activator of transcription 6, IL-4 receptor α, IL-4, and IL-13),[27,28] regulation of alveolar macrophage function (eg, the neuropeptide S receptor gene *GPR154*),[29] or genes that code for microbial pattern-recognition receptors (eg, CD14 and TLRs).[30] Polymorphisms in pattern-recognition receptors are discussed as a possible molecular mechanism of the hygiene hypothesis,[31-33] which postulates that exposure to microbial components early in childhood protects against the development of allergy and asthma.[34,35] In addition, common loss-of-function variants within the gene encoding filaggrin, a protein that is essential for epidermal barrier function, have been identified as predisposing factors for atopic eczema[36] and allergic sensitization in patients with atopic eczema.[37-39] Therefore interactions between genetic factors (eg, MHC class II haplotype and polymorphisms of genes controlling epithelial integrity or pattern-recognition receptors) and environmental factors (eg, allergen dose and exposure to adjuvants or microbial stimuli) can influence the outcome of the immune response and with that the susceptibility to allergic sensitization and disease (Fig 1).[25,40]

ALLERGEN SOURCE AND INDIVIDUAL EXPOSURE

The most important sources of allergens are wind-dispersed pollen grains from trees, grasses, and weeds, followed by excretions of house dust mites and cockroaches, fungal spores, and animal dander and insect venoms.

The route of exposure, dose, and function of the allergen are crucial to mount an allergic sensitization. Sensitization occurs at the site of allergen exposure, such as the airways and skin, but can also occur through the gastrointestinal tract. In general terms, exposure to low allergen concentrations induces IgE production and allergy, whereas exposure to high allergen doses induces tolerance through Treg cells, a modified T_H2 cell response with the production of high levels of allergen-specific IgG4 antibodies, or both that can block the binding of IgE-allergen complexes to effector cells of the allergic immune response.[41-44]

But what is considered "high" and "low" dose when it comes to allergen exposure? Individual allergen exposure is still difficult to measure and can only be estimated. The inhalation of indoor allergens from cats and dogs has been calculated to be in the range of less than 1 ng to 300 ng per day depending on the presence of the animal in the house.[45] Concentrations of house dust mite allergens

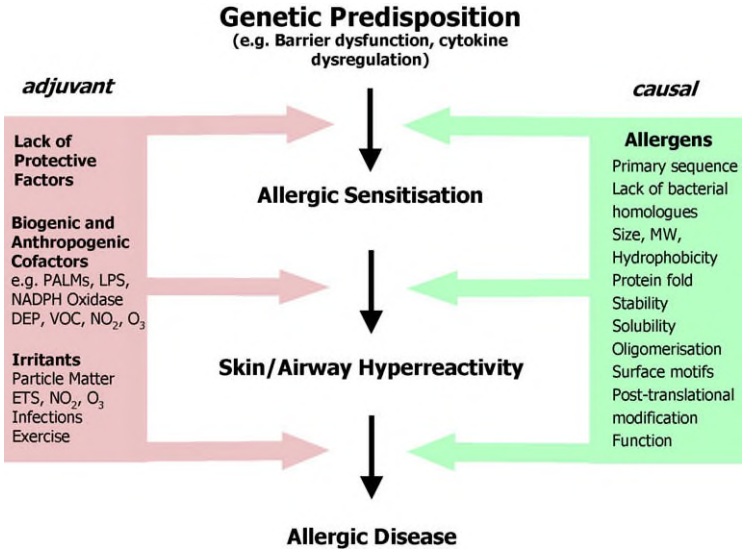

FIG 1. Determinants of allergic diseases. Allergy is the result of an orchestrated interplay of genetic predisposition and exposure to allergens and adjuvant environmental cofactors that leads to allergic sensitization, skin and airway hyperreactivity, and allergic disease. *NADPH*, Reduced nicotinamide adenine dinucleotide phosphate; *VOC*, volatile organic compound; *ETS*, environmental tobacco smoke; *MW*, molecular weight.

in indoor air are too low to be quantified reliably. Concentrations in dust samples are high enough to be detected and show that the major allergen Der p 1 is up to 400-fold lower than concentrations of major cat and dog allergens.[46] Exposure to outdoor allergens, such as the birch pollen major allergen Bet v 1, during the pollen season can be approximated based on pollen flight and allergen release data obtained *in vitro*[47,48] to be on the order of 0.03 to 1 ng/d. Therefore nanogram quantities or less of indoor or outdoor allergens seem be sufficient for the induction of allergic sensitization and disease.

However, not everybody who is exposed will become sensitized and have allergies. Aside from the individual exposure conditions, there is a high variability in the individual responsiveness to a given allergen dose. The development of an allergic (T_H2 cell and IgE-dominated) immune response crucially depends not only on the concentration of available antigen but also on the strength of the T cell–activating signal. Here MHC haplotype variability between individuals determines the binding affinity of antigenic peptides to individual MHC class II molecules and their presentation to TCRs.[42,49] In other words, what might be a high allergen dose for some individuals could be a low dose for others if the allergenic peptide has a lower affinity for that individual's MHC molecules.

This leads also to the issue of thresholds for the effects of allergen exposure on sensitization and development of disease. The relationships between allergen exposure in childhood and atopic sensitization or asthma are both complex and controversial. Most intensively discussed in this field are the correlation of exposure, sensitization to house dust mite and cat,[50] and the possible development of asthma. Sporik et al[51] suggested in 1990 that in addition to genetic factors, exposure in early childhood to house dust mite allergens is an important determinant of the subsequent development of asthma and opened a nonended discussion. Of note, although exposure to high levels of Der p 1 antigen in infancy did influence asthma at 11 years in these subjects,[51] this effect was not statistically significant at 22 years.[52] In addition, stringent environmental control, leading to a drastic reduction of house dust mites, resulted in an increased rather than decreased risk of atopy; however, there were surprisingly better results for some measurements of lung function in high-risk children at the age of 3 years.[53] The dose-related immune response to allergens derived from cat exposure appears to be even less straightforward. In countries in which the prevalence of cat ownership is low or rare (<5%), positive associations between either pet allergen in house dust[54] or pet ownership and sensitization have been reported.[55,56] However, studies in areas with a greater frequency of pet ownership demonstrated that despite a positive association with sensitization, cat ownership was inversely associated with wheeze, potentially suggesting an IgE-independent protective mechanism in this community.[57]

MOLECULAR FEATURES OF ALLERGENS

The allergens that elicit type I allergies are mostly proteins or glycoproteins and cluster in less than 2% of all (9318) known protein families (AllFam database: www.meduniwien.ac.at/allergens/allfam).[58,59] This would seem to imply that structural and biochemical similarities between allergenic proteins and the comparison of allergenic and nonallergenic members of the same protein family could explain what determines allergenicity. The primary structure (amino acid sequence) of a protein allows its physicochemical properties, such as molecular weight, isoelectric point (charge), hydrophobicity, and stability, to be predicted. Computational analysis of the major allergens has shown that most are relatively small (<70 kd) negatively charged proteins with low hydrophobicity and high stability.[10] In addition, posttranslational modifications, such as glycosylation or the presence of disulfide bonds, can increase the stability and bioavailabilty of allergens.[60,61] Although this approach allows us to define common molecular features of allergens, none of the above parameters or combinations thereof allows a reliable discrimination of allergens and nonallergens.

It has been proposed recently that a common denominator for allergens could be the lack of protein sequences found in bacterial proteins.[62] Homology searches showed that most common allergens have no bacterial homologues, whereas nonallergenic proteins from the same species have large numbers thereof. However, there were several exceptions to this rule, and bacterial proteins themselves can act as type I allergens inducing IgE responses.[63]

Progress in structural biology and bioinformatics has provided detailed information about the secondary and tertiary structures of more than 200 allergens (see the RCSB protein Data Bank: www.rcsb.org/pdb/).[10] Most allergens can be grouped into 4 structural families when classified according to their protein folds: (1) antiparallel β-strands; (2) antiparallel β-strands closely associated with one or more α-helices; (3) α- and β-structures not closely associated; and (4) α-helical structure. Again, though, none of the structural features allows a reliable discrimination between allergenic and nonallergenic proteins.[64] However, determination of the 3-dimensional structures of allergens allowed explanation (and possibly prediction) of cross-reactivity between homologous molecules from different sources. Allergenicity related to the ability of a protein to induce symptoms based on cross-reactivity is the cause of pollen-food syndromes, such as birch and apple. Cross-reactivity of environmental allergens to human antigen based on molecular mimicry is discussed to play an important pathogenic role (eg, in atopic dermatitis).[65]

A more recent approach indicates that allergen-specific motifs can be identified by means of *in silico* mapping of molecular surface features. The analysis of conservation patterns of surface residues in 4 allergen families and their nonallergen homologues showed the presence of allergen-specific patches with a high proportion of surface-exposed hydrophobic residues.[66] Whether this holds true for other allergen families and the nature of the functional relevance of allergen-specific surface motifs remain to be determined.

Finally, the quaternary structures of allergen oligomers influence allergenicity by facilitating IgE receptor cross-linking, increasing allergen stability (protection from proteolytic degradation) and resulting in the formation of additional IgE-binding epitopes localized in regions of monomer-monomer contact.[67]

Therefore even though we can describe common features of allergens, structural biology has failed thus far to provide reliable discriminators that allow the identification of allergens.[64,68,69] Still, the fact that an allergen induces a switch to monoclonal, oligoclonal, or polyclonal responses of the IgE isotype to this specific structure (which might well be cross-reactive with homologous structures) strongly indicates that structural elements related to the specific allergens must be involved in the process. However, we have to expand the picture of factors inducing switch (T_H2 and IgE) processes with a variety of patient-specific (genetic background) issues and intrinsic function of the allergen but also with a large variety of environmental factors, both anthropogenic and biogenic, occurring with allergen exposure.

INTRINSIC FUNCTION OF ALLERGENS

A large number of allergens have intrinsic biologic functions. They act as proteases, pectate lyases, trypsin inhibitors, calcium-binding proteins, lipid transfer proteins, actin-binding proteins, and others. Some of these biologic activities can contribute to allergenicity by increasing the tissue distribution of the allergen through digestion of extracellular matrix (eg, hyaluronidase), degradation of cellular adhesion molecules (eg, house dust mite major allergen Der p 1 or the *Penicillium* allergen Pen ch 13[70,71]), or direct toxic effects (eg, melitin and phospholipases) on cells of the microenvironment.[72] Others, such as the major birch pollen allergen Bet v 1, act as membrane-binding proteins by binding to membrane phospholipids, which might help them to cross the mucosal barrier and facilitate access of the allergen to antigen-presenting cells.[73]

The most extensively studied allergens with intrinsic enzymatic activity are the house dust mite group 1 allergens Der p 1 from *Dermatophagoides pteronyssinus* and Der f 1 from *Dermatophagoides farinae*, which belong to the papain-like cysteine protease family.[74,75] These provide a clear example of how the biologic functions of allergens can influence allergenicity: Der p 1 can cleave several cell-surface molecules, such as CD23 from B cells.[76] On B cells, membrane-bound CD23 and its soluble fragments have been shown to be involved in IgE regulation. Under physiologic conditions, the binding of IgE to CD23 delivers a negative IgE regulatory signal to B cells. When CD23 is not occupied by IgE, it undergoes proteolytic cleavage, releasing soluble CD23. Depending on the size, these fragments either upregulate (<25-kd fragment) or downregulate (16-kd fragment) IgE synthesis.[77] Schulz et al[78] demonstrate that Der p 1 cleaves a 25-kd fragment of CD23, suggesting that Der p 1 might upregulate IgE synthesis by virtue of its ability to cleave CD23.[79,80] Der p 1 can also cleave CD25, CD40, DC-SIGN (CD209), and DC-SIGN receptor, which can bias the capacity to induce T_H2 cell responses.[3,81] Der p 1–mediated cleavage of CD25 on T cells interrupts an autocrine positive feedback of IL-2 expression required for T_H1 differentiation.[82] In addition, cleavage of CD25 on Treg cells is likely to interfere with their capacity to suppress T-effector cells through cytokine consumption.[83] A lack of regulation can in turn facilitate the development of T_H2-dominated allergic immune responses.

Immunization experiments in mice using active and inactive Der p 1 demonstrate that proteolytic activity is crucial for allergic sensitization and can even facilitate sensitization to bystander antigens.[84,85] In addition, Zhang et al[86] proposed that Der p 1 differs from other C1 cysteine peptidases because the presumably regulatory prodomain ends in competitive proteolysis and degradation of the propiece by Der p 1 itself. This contrasts with the well-established behavior of other C1 family cysteine peptidases in which untethered propieces control unchecked proteolysis by the mature enzyme because they are persistent nondegradable inhibitors.[86]

The enzymatic activity of allergens can also act by changing the microenvironment in which allergens are encountered. Tissue homeostasis and epithelial barrier function depend on the presence of inhibitors that counterbalance endogenous protease activities. This delicate balance can be disrupted by the group 1 allergens, which degrade antiproteases, such as α1-proteinase inhibitor, elafin, or secretory leukocyte protease inhibitor.[87] This results in enhanced endogenous protease activity that can weaken the epithelial barrier and facilitate allergen access to DCs in subepithelial tissue. In addition, Der p 1 and Der f 1 can inactivate lung surfactant proteins A and D, which are known to inhibit the binding of inhaled allergens to cell-sequestered IgE[88] and can degrade tight-junction proteins in airway epithelium, which results in increased availability of allergens to DCs beneath the epithelial barrier.[89] Immunization experiments in mice using active and inactive Der p 1 show that the proteolytic activity of Der p

1 is crucial for allergic sensitization and can even facilitate sensitization to bystander antigens.[85,90]

Among the allergens currently listed in public-domain databases (eg, Allergome or the Structural Database of Allergenic Proteins), more than 80 different allergens are listed as proteases. For many of these, we are just beginning to understand how the biologic activity can influence allergenicity. An excellent example is the recent observation[91] that the proteolytic activity of papain is crucial for the development of allergic immune responses by inducing the recruitment of basophils to T-cell areas of draining lymph nodes during the priming phase of the response. The recruited basophils produce T_H2 cell–promoting cytokines (IL-4 and thymic stromal lymphopoietin) in response to the (protease) allergen and are required for the subsequent T_H2 cell differentiation *in vivo*. The results indicate a novel model for the innate initiation of T_H2 cell responses by basophils based on sensing the protease activity of allergens, a mechanism that might also be effective in sensing proteases during helminth infections.

Despite good evidence for a role of protease function in allergenicity, many other allergens, such as Fel d 1, Der p 2, and Der p 5, have no protease activity,[92,93] and some even function as cysteine protease inhibitors (eg, Fel d 3).[94] Either we still have to discover the relevant activity of these allergens, or more likely, the allergenicity of these proteins is independent of their biologic function. Furthermore, we have to take into account that not all proteases are allergenic. Taken together, specific intrinsic activities can contribute to the allergenicity of certain proteins, but they are not an obligatory prerequisite for an allergen. In addition, thus far no single biologic function has been identified as a reliable discriminator of allergenic proteins.

BIOGENIC COFACTORS CONTRIBUTING TO ALLERGENICITY

Allergen research has mainly focused on identifying single allergens and characterizing their biochemical, structural, and functional properties. Little attention has been paid to the natural context under which these allergens are encountered by the organism. Except for testing in clinical settings, individuals will never be exposed to isolated allergens but are usually exposed to their carriers. This implies that other factors being liberated from the allergen carrier or exposed together with the allergen might influence the host's response to the allergen.

For example, microbial molecules are ubiquitous in ambient air and dust. Consequently, individuals are exposed to the allergen together with these microbial molecules that directly activate receptors expressed on cellular constituents of the innate immune system.[95] These pathogen-associated molecular patterns include TLR ligands, which can dramatically influence antigen-specific immunity. For example, mice and human subjects immunized with antigen and immunostimulatory sequence oligodeoxynucleotide (TLR9), have robust T_H1-biased adaptive responses and are protected from T_H2-biased airway hypersensitivities.[96] However, the nature,[97] dose,[98,99] and exposure frequency[17] of TLR ligand seem to be of central importance for the outcome of the ensuing T-cell response, which perhaps is one reason for conflicting results concerning the immunologic basis of the hygiene hypothesis.[100,101]

Chitin, a biopolymer of N-acetyl-β-D-glucosamine that is present in insects, mites, fungal spores, helminths, and crustaceans, has previously shown to induce T_H1 responses or even the prevent allergy.[102,103] A recent report of Reese et al,[104] however, demonstrates that chitin, even though not recognized as an allergen in itself, can act as a potent inducer of IL-4–producing eosinophils and basophils, which might then facilitate the development of IgE-dominated immune responses. Chitin-induced eosinophil and basophil recruitment is independent of TLRs and involves BLT1 receptor–dependent accumulation of alternatively activated macrophages.[104]

Along the same lines, pollen grains not only release allergens but also nonallergenic, bioactive, pollen-associated lipid mediators (PALMs),[105] which have proinflammatory and immunomodulatory effects on the cells of the allergic immune response. Proinflammatory PALMs (eg, oxylipins) attract and activate eosinophils and neutrophils independently of the sensitization status of the donor (ie, from allergic and nonallergic donors),[106,107] suggesting that they act primarily as an adjuvant that can enhance inflammatory processes, such as during the elicitation phase of the allergic response. Immunomodulatory PALMs, such as E1 phytoprostanes, inhibit DC production of IL-12 and T_H1-type chemokines and increase the capacity of DCs to induce T_H2 cell differentiation and recruitment, which indicates that they support the generation of a T_H2 cell–dominated allergic immune response.[16,108,109] Finally, pollens release nonallergenic reduced nicotinamide adenine dinucleotide phosphate oxidases, which have detrimental effects on airway epithelial cells through the generation of reactive oxygen species.[110,111]

Also, house dust mites were shown to harbor adjuvant activity, first of all because of the above-described proteolytic activity of the major house mite allergen Der p 1. In addition, Krishnamoorthy et al[112] demonstrate that house dust mite whole-body extracts promote cell-surface expression of KIT and its ligand, stem cell factor, on mouse DCs, resulting in sustained signaling downstream of KIT, upregulation of the Notch ligand Jagged-2, and finally IL-6 secretion. The authors hypothesize that IL-6 upregulation could limit the T_H1 response while promoting the T_H2 and T_H17 pathways.

Collectively, allergen carriers, such as pollen grains and house dust mites, represent packages of danger signals that can influence the outcomes of immune responses in many ways (Fig 2). For a better understanding of allergenicity, we will have to address and integrate in more detail the role of nonallergenic cofactors present during allergen exposure that favor the development of allergic sensitization and disease.

ENVIRONMENTAL POLLUTANTS AS CONTRIBUTING FACTORS

The same holds true for environmental pollutants, which can facilitate the development of type I allergies through several different mechanisms. The best-studied particulate pollutants are diesel exhaust particles (DEPs), which were shown to turn a harmless neoantigen into an allergen capable of inducing high levels of allergen-specific IgE.[44,113] Susceptibility genes for the adjuvant effect of DEPs have been identified (eg, glutathione-S-transferase and Nrf2) and suggest that DEP-induced oxidative stress play a central role in this process.[114-117] In addition, particulate matter can act directly on local antigen-presenting cells, such as mucosal DCs, and modulate their function by changing their surface phenotype and cytokine profile (reduced IL-12 production), resulting in a proallergic pattern of innate immune activation.[118] Because allergic sensitization and the elicitation of

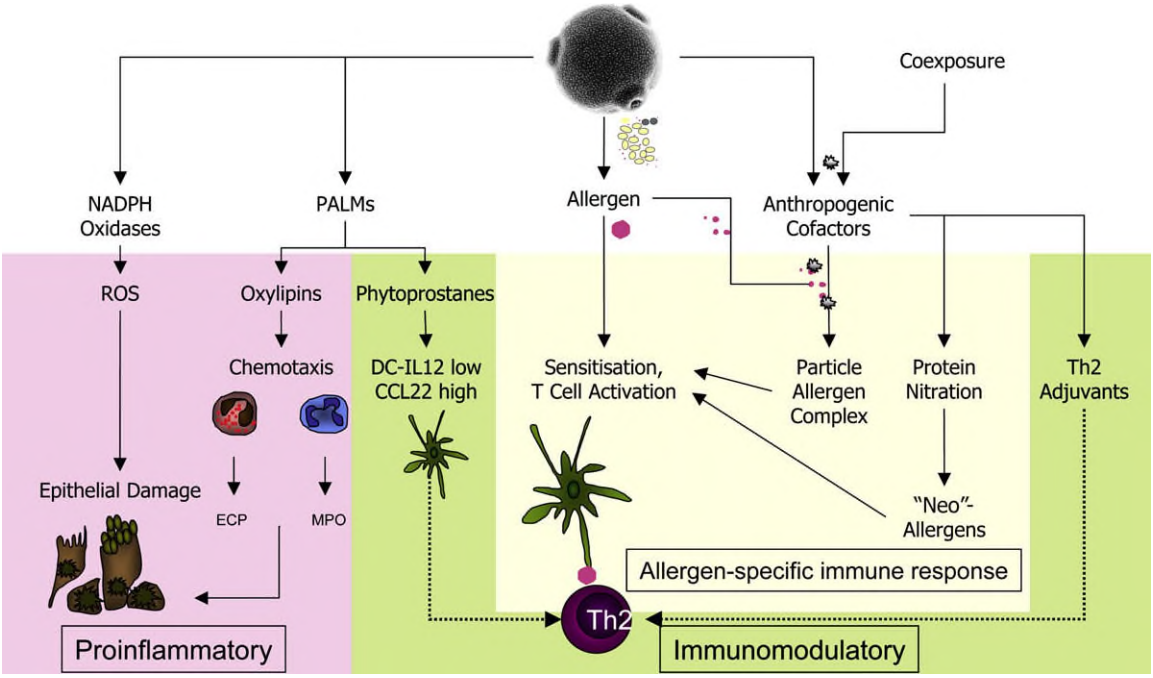

FIG 2. Interplay between the allergen source, environmental cofactors, and cells of the allergic immune response during exposure to pollen grains. In the context of type I allergy, pollens have generally been regarded as allergen carriers. Aside from allergens, pollens release many other substances with proinflammatory and immunomodulatory effects on cells of the allergic immune response. In addition, pollens can carry biogenic and anthropogenic factors that influence allergen release, generate novel allergenic epitopes, and modulate the epithelial microenvironment of an allergen encounter. *NADPH*, Reduced nicotinamide adenine dinucleotide phosphate; *ROS*, reactive oxygen species; *ECP*, eosinophil cationic protein; *MPO*, myeloperoxidase.

symptoms are dose-dependent phenomena, factors that modulate the bioavailability of allergens can influence allergenicity.[119] This can occur at the site of allergen exposure in the body, where pollutants (eg, O_3, NO_2, and volatile organic compounds) act as irritants and induce pulmonary inflammation and disruption of epithelial barrier homeostasis, which facilitates the access of allergens to effector cells of the allergic immune response. It can also occur at the level of the allergen carriers, such as pollen grains, where exposure to gaseous pollutants can alter the release of allergens.[119] In addition, traffic-related pollutants, such as NO_2 and O_3, facilitate the release of allergen-rich cytoplasmic granules from pollen and therefore increase the quantity of allergens in the respirable submicronic fraction.[120] Finally, pollutants such as NO_2 and O_3 at relevant atmospheric concentrations can lead to the nitration of airborne allergens, such as Bet v 1.[121] The detection of IgE specific for nitrated Bet v 1a, which does not bind unmodified Bet v 1 or nitrated unrelated proteins, implies that nitration generates novel allergenic epitopes.[122] Interestingly, functional IgE specific for nitrated Bet v 1a is detected in serum samples of patients who are allergic to birch pollen,[122] which indicates that allergen nitration is relevant *in vivo* and can contribute to allergenicity in polluted environments.

SUMMARY AND FUTURE PERSPECTIVES

Rapid progress in molecular and clinical allergy research has advanced our understanding of the structural and functional nature of allergens and has led to improved classifications according to taxonomy and protein families. This knowledge has resulted in better characterization and standardization of allergen extracts, the design of novel hypoallergenic mutants for safer allergen-specific immunotherapy, and the development of novel strategies for advanced diagnostics and patient-tailored therapies in the management of allergic diseases.[123]

Despite this advancement, we still struggle when it comes to the following questions: "What makes an allergen an allergen?" or, the other way round, "Why are all the other proteins derived from allergen carriers nonallergenic?" Clearly, allergenicity cannot be determined based on structural features alone, be they sequential or conformational. A list of additional determinants needs to be integrated to understand the complex phenomenon of allergenicity. Several important questions remain unanswered: What determines the "major" or "minor" nature of an allergen? Which factors are responsible for turning immunologic sensitization at the IgE level into clinically relevant allergic immune responses/inflammation? What are the relevant gene–environment interactions that influence the outcome of allergic responses? The genetic makeup of an individual (specifically polymorphisms in genes involved in immune and barrier function) is an important predisposing determinant for the development of T_H2 cell–dominated allergic immune responses. Biogenic and anthropogenic environmental cofactors released from or associated with allergen carriers might be equally important as contributors to allergic sensitization and disease manifestation. Even though this has been conclusively shown for various single components, the questions that remain concern which of the many components in what combination in which patients with what kind of genetic background act as decisive elements leading to allergic disease.

Future research should not only try to elucidate the structural basis of allergenicity but should focus with equal intensity on the many additional factors from the environment and the host that determine the outcome of the response to allergens. This might finally result in new concepts of diagnosis, therapy, and prevention and might enable interventions at a regulatory level in public health.

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