

2 Definition of An Allergen (Immunobiology)

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INTRODUCTION

A variety of terms are used to define the substance that stimulates an atopic reaction. Which words are used depends on the terms chosen to denote the sensitivity (1). In the context of a general immunological reaction, the triggering substance is called an antigen. In modern usage, an antigen is any substance that induces a state of sensitivity and/or resistance as a result of coming into contact with appropriate tissues of an animal body (2). The observed sequence—exposure to substance, latency period, manifestation of substance-specific sensitization upon reexposure to substance—is characteristic of immunological memory, and indicates that B cells and/or T cells are involved.

In 1923, Coca and Cooke coined the word “atopy” to describe a type of sensitized state (3); through the intervening years, the term subsequently has been defined as an adverse immune reaction involving immunoglobulin E (IgE). The term “allergen” has been used to define the substance that induces specific IgE antibodies; exposure can be by inhalation, ingestion, tactile contact, or by injection. Thus, allergens are defined in terms of the body's response to them. Interestingly, not all individuals have a demonstrable IgE response to “known” allergens. That there is a genetic basis for atopic predisposition has been recognized for nearly a century (4). Ultimately, the response to an allergen is complex and influenced by the interplay of multiple factors: characteristics of the host (including genetic susceptibility), the environment, and physical properties of the allergen itself (5). Although we choose to define an allergen as an antigen that will induce and interact specifically with IgE, the differences between allergens and antigens are blurred. The question therefore arises as to whether all antigens can be allergens under the proper conditions.

PROPERTIES OF AN ALLERGEN/ANTIGEN/IMMUNOGEN

An operationally defined antigen (*i*) shows immunogenicity (i.e., a capacity to establish a state of sensitivity and/or stimulate the formation of corresponding antibody) and (*ii*) reacts specifically with the responding tissue and/or those antibodies (2). The two properties are not always associated. If immunogenicity is observed, the molecule responsible is more broadly defined as an immunogen. Immunogenicity is not an inherent property of a molecule as is molecular weight. Haptens (low molecular weight compounds, such as drugs) are not immunogenic in and of themselves, but do possess/contain antigenic epitopes (6). In the proper context, such as may exist after a hapten has covalently bound to a larger protein, the hapten-protein complex taken up and processed by an antigen-presenting cell, and the hapten/antigenic epitope presented in the context of MHC (cell surface antigens of the major histocompatibility complex)—they produce and react specifically with the corresponding antibodies that were formed against hapten-protein complexes. A molecule acts as an antigen whenever an organism's immune system recognizes it; if that recognition results in identification as “foreign,” the immune system responds to it. Thus, a molecule might function as an antigen in one organism but not in another. This chapter is primarily concerned with molecules recognized as antigens by the humoral system of humans.

Normally, antigen recognition by both T and B cells is required for elicitation of humoral immunity. B-cell recognition, and eventual specific antibody response, is directed toward a unique surface region of the antigen. B-cell epitopes are conformational and generally have a surface area of 500 to 1000 Å² (7,8). The antibody's antigen-binding region, composed jointly by

variable regions of the light and heavy chains, is called the "paratope" and forms a tightly fitting complementary surface with the antigen's B-cell epitope. The juxtapositioning of charges and hydrophobic mountains or valleys within epitope and paratope produces the free energy for the binding reaction. The precise fit of the two surfaces excludes most of the hydration water, tightening the complex (9). Because the antibody is directed toward a specific epitope, it will recognize another antigen if it carries the same or a very similar epitope. This is the basis for observed cross-reactivity between antigens (10,11). The surface of an antigen represents a quilt of putative epitopes (12). How many of those putative epitopes dominate the antibody response varies from case to case (8,11). The structure and position of dominating epitopes are being described for an increasing number of protein antigens (13). To date, the prediction of allergen cross-reactivity has mainly been based on protein homology (i.e., linear sequence data).

Next, consideration must be given to the function of antibodies in general and those specifically involved in interaction with allergens (14). The most important feature for an antibody is its ability to recognize an antigen and to form a complex with its target epitope. The function of an antibody to allergen is thought by many to arm an antibody receptor situated on effector cells, such as mast cells, and wait for the antigen/allergen to come and cross-link the receptors. It is the cross-linking reaction that an allergen, in general, must accomplish. For that purpose an allergen must carry at least two suitably separated epitopes, allowing the molecule to form a bridge. One epitope might be enough for the functioning of an antigen, but it is probably not for an allergen. For some purified allergens, the antibody response is predominantly toward three to four dominant epitopes (15). Both the IgG and IgE responses in the same sensitized individual recognize the same epitopes (16). From a receptor aggregation point of view, a favorable topography of epitopes would probably contribute to the potency of an allergen. One might argue that the necessary high affinity of antibody would limit the inherited libraries capable of producing such antibodies and thus restricting some antigens to become allergens. Although IgE affinities toward allergens are exceptionally high, nonallergic individuals are able to mount an equally high-affinity response of IgG to the same allergens acting in this case as antigens (15). Thus, high affinity by itself is not a necessary step toward atopy. In addition, in skin test negative clinically allergic people, lower-affinity antibodies can act in allergic reactions (17,18). Affinity is correlated to the ability to cross-link receptors (19). Thus, high affinity is correlated with the strength of atopic reactions, but achieving that affinity seems not to be the limiting factor in characterizing allergens among antigens.

The necessity of link formation of two separated epitopes might also induce a lower limit in molecular weight where crowding on small surfaces could limit a cross-linking activity. Studies of Amb a 5 reveal that at least three epitopes are present on that 2500 MW protein (16). How much smaller it can go without the necessity of dimerization or polymerization of the putative allergen is not known; it is likely, however, that the probability of finding an antigen with allergenic properties is lessened at the lower molecular weight.

For antigens to act as allergens, they must elicit T-cell-dependent responses and be able to form at least two, preferably three or four, spatially separated epitopes. This establishes some lower molecular weight limit and raises the question of whether the majority of T-cell-dependent antigens become allergens. They certainly have the ability, but whether they become an antigen depends on circumstances (20). A series of investigations of allergy-prone families found that although the tendency to be sensitive to allergens was inherited, the choice of allergens among antigens seemed to be totally random. Clinically, there was no correlation between the type of specific allergen sensitivity seen in the mother or father versus the children. Thus, all antigens encountered fulfilling the two criteria above may become allergens by a purely random process (21).

The sea of molecules acting as allergens is organized and named according to a schema proposed by World Health Organization/International Union of Immunologic Societies (WHO/IUIS). The molecules are labeled by the first three letters of the genus and the first letter of the species they are isolated from, and then by an Arabic numeral indicating the sequence of isolation. Der p 1 is the first isolate from *Dermatophagoides pteronyssinus*, a house-dust mite. The distinction between major and minor allergens is a functional classification; in current usage, a major antigen is one to which >50% of allergic patients react.

Antigens/allergens are generally proteins, glycoproteins, or lipoproteins of plant or animal origin. Many of the major allergens (including those from mites, animal danders,

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proteins of plant or tes, animal danders,

pollens, insects, and foods) have been cloned and sequenced (22). For many of these, the three-dimensional structure is also now known; either because it has been directly visualized via crystallography or has been modeled, that is, the structure is inferred on the basis of sequence homology with other, solved, antigens. It does not appear that these molecule types can be divided into allergic/atopic or nonallergic/non-atopic on an a priori structural reason (23). Antigens are derived from proteins with a variety of biological functions, including proteases, pathogenesis-related proteins, seed storage proteins, ligand-binding proteins, lipid-transfer proteins, calcium-binding proteins, and other structural proteins; in toto, they have been identified as coming from more than 120 distinct protein families (24–26). However, the majority of the plant and animal allergens are clustered within just a few of these families (24). Classification of allergens into groups with structural similarity may help predict cross-reactivity or may provide other useful information (10). For example, biological function, such as the proteolytic enzyme allergens of dust mites, may directly influence the development of IgE responses (i.e., via direct cleavage of CD23 from the B-cell surface, thus inhibiting negative feedback regulation) (27). This same antigen might directly initiate inflammatory responses in the lung, such as those associated with asthma (28,29). An antigen's intrinsic structural or biological properties may also influence the extent to which it persists in the indoor and outdoor environments or retains its allergenicity while within the digestive tract (30).

In the future, structural biology and proteomics may continue to enable the identification of motifs, patterns, and structures of clinical and immunological significance.

ALLERGEN: ROUTE AND AMOUNT OF EXPOSURE

Exposure to allergen is typically necessary to develop an allergic IgE immune response. However, the presence of cross-reacting antibodies as well as autoantibodies may complicate the picture. The skin and mucosal surfaces, present in upper and lower respiratory tract, GI tract, genital tract, and mammary glands, are the body's barriers to encounters with allergens and other environmental factors. The presence of these barriers safeguards the internal milieu by keeping foreign items out. First, they act as physical barriers and thus prevent penetration of high molecular antigens; Schneeberger reported that the molecular weight cutoff above which nasal and alveolar membranes are impermeable is between 40,000 and 60,000 Da (31). Next, the skin and mucosal barriers are the first sites of contact with the innate and adaptive immune systems, both of which are involved in the defense of the body.

The innate immune system is the host defense mechanism that is encoded in the germline genes of the host (32). It involves barrier mechanisms such as the epithelial cells layers, secreted mucus layers, and epithelial cilia; soluble proteins and bioactive small molecules in biological fluids, that is, complement and defensin, released from cells (cytokines, chemokines and bioactive amines and enzymes); as well as cell surface receptors that identify by binding molecular patterns expressed on the surfaces of invading microbes and other foreign substances. Innate mucosal defense consists of many soluble and cellular elements, including complement, secretory leukocytes, protease inhibitors, surfactant protein, defensin, mucins, slatherin, lactoferrin, cystatins, lysozyme, mannose-binding lectin, thrombospondins, and collectin, as well as secretory agglutins.

The adaptive system exhibits specificity for its target antigens. It is based primarily on the antigen-specific receptors on the surfaces of the T and B lymphocytes (33). The antigen-specific receptors of the adaptive response are assembled by somatic rearrangement of germline gene elements to form both intact T-cell receptors (TCRs) and B-cell antigen-specific receptors (Ig). The adaptive mucosal immune system involves two main tissue systems: (i) the tonsils, Peyer's patches, and isolated lymphoid follicles and (ii) the diffuse mucosal immune system consisting of intra-epithelial lymphocytes and the lamina propria. IgA is the main mucosal antibody (34).

The organized mucosal tissues play an important role in the inductive stage of an immune response (35). The mucosal surfaces present in the upper and lower respiratory tract and in the GI tract are important routes of entry for development of allergic sensitization (36); they also represent key sites for initiation of non-IgE responses (37,38). Immune responses, whether healthy or aberrant, are induced via other routes of exposure as well. IgE sensitization has been found following injection of allergens, such as penicillin metabolite acting as a hapten, or enzymes delivered by stinging insects (14).

Once encountered, the amount/dose of exposure, duration, as well as other modulating pollutants are a few of the many environmental factors that influence the type of response to an antigen/allergen. Allergens appear to induce IgE production at relatively low doses (39). Marsh has estimated that the mean adult annual dosage of individual allergenic components is probably in the nanogram range (14). The ambient level of mite allergen that a normal individual is exposed to has been measured to fluctuate at around 100 pg/m³. House dust with mite content >2 µg mite/g dust is associated with sensitization in children (expert consensus) (40). Clinical studies suggest that the duration of exposure needed for IgE sensitization varies; "days" for parasitic allergens, "months" for some constant/perennial allergen exposure, to "years" of exposure for seasonal allergens such as pollens are needed to develop IgE antibodies.

ALLERGIC SENSITIZATION

The innate and adaptive immune systems work together to protect the organism from foreign substances that may possess a diverse collection of pathogenic mechanisms. Though the innate and adaptive immune responses are different in their mechanisms of action, synergy between them is essential for a fully effective immune response (41,42). The innate system is the first line of host defense; it sets the stage for the development of an adaptive response to the antigen/allergen.

To explore the possible positioning of allergens within the antigen family, features of antigen in its function as an initiator of humoral response have to be considered. First, antigens (regardless of their allergenic properties) can be broadly divided based on whether or not they require T-cell help when eliciting a humoral response. The thymus-independent pathway allows direct activation of antigen-specific B-cell clones, thus eliminating the need for a T-cell epitope. Most bacterial sugar-based antigens belong to this class (43). Protein antigens, including allergens, are thymus dependent; this means that to act as antigen and trigger a humoral, antibody-based response, the molecule has to be able to first interact and activate antigen-specific T cells.

T cells are unable to recognize antigen in the absence of antigen presentation (with the exception of the superantigens) (44). The TCR is restricted to recognizing antigenic peptides displayed in the context of molecules of the major histocompatibility complex (MHC) (45). In humans, human leukocyte antigens (HLA) are encoded by genes of the MHC complex, located on chromosome 6. With the exception of some cell types, all cells are capable of presenting antigen and activating the adaptive response via MHC class I. Dendritic cells, macrophages, and B cells play a major role in the innate response, and also act as professional antigen-presenting cells (APCs). APCs phagocytize exogenous foreign substances, such as allergens, bacteria, parasites, or toxins in the tissues, and then migrate, via chemotactic signals, to T-cell-enriched draining lymph nodes (46). During migration, dendritic cells undergo a maturation process in which they lose phagocytic capacity and develop an increased ability to communicate with T cells. This maturation process is dependent on signaling through pattern recognition receptors, such as the members of the Toll-like receptor family, which occurs following binding of pathogen-associated molecules (41,47). Lysosome-associated enzymes digest phagocytized proteins into smaller peptides. These peptides are loaded into the antigen-binding clefts of MHC class II molecules for display (i.e., as "T-cell epitopes"); MHC class II molecules bind peptides that are 10 to 30 amino acids long with a core region of 13 amino acids containing primary and secondary anchor residues. On the APC surface the MHC-peptide antigen complexes are available for recognition by any naïve CD4⁺ T cell passing through the lymph node. CD4⁺ helper T lymphocytes are immune response mediators and play an important role in establishing and maximizing the capabilities of the adaptive immune response. Several different subtypes of CD4⁺ T cell can be activated by professional APCs, with each type of T cell being specially equipped to deal with different foreign substance, whether it be allergenic, bacterial, viral, or a toxin (48). The type of T cell activated, and therefore the type of response generated, depends, in part, on the context in which the antigen was first encountered by the APC.

When a naïve T_H0 cell contacts an antigen and is stimulated through its antigen receptor, it begins to polarize along a lineage-determining developmental pathway (49,50). T_H1, T_H2, T_H17, and T regulatory (Treg) (Tr1) cells all develop from the same naïve T_H0 cell, under the

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influence of genetic and environmental factors acting at the level of antigen presentation. The predominance of a given cytokine in the microenvironment of the responding T_H0 cell is an important modulatory factor in this process (51). Signals through contact molecules, as well as through cytokine receptors, elicit a complex series of molecular interactions that culminate in the binding of lineage-specific transcription factors to multiple regulatory elements in the promoters, and subsequent activation of a differentiation pathway (52). Following activation, naïve T_H0 cells differentiate toward T_H1 in the presence of IL-12, which upregulates IFN- γ via signal transducer and activator of transcription 4 (STAT-4), leading to IFN- γ -mediated activation and induction of the T_H1 lineage-determining transcription factor T-bet, subsequently leading to production of T_H1 cytokines, including IFN- γ , IL-2, and TNF- β . T_H2 cell differentiation occurs in response to IL-4, which activates STAT6, resulting in induction of the T_H2 lineage-determining transcription factor GATA-3, and leading to production of the T_H2 cytokines IL-4, IL-5, and IL-13 (53–55). The T_H17 subset develops in response to TGF- β plus IL-6; STAT-3 is activated and induces the T_H17 lineage-specific transcription factor ROR γ t, which is an orphan nuclear receptor, (56). Treg cells can also be generated from naïve T_H0 cells; TGF- β induces the transcription factor FOXP3, leading to production of the suppressive cytokines, TGF- β and IL-10 (57). The molecular mechanisms of these pathways involve cross talk on the level of the transcription factors: GATA-3 not only increases transactivation of the IL-4 locus promoter but also inhibits production of IFN- γ ; T-bet interferes with T_H17 cells and directly blocks GATA-3 while binding to its own targets. For the T_H1 , T_H2 , and T_H17 lineages, the differentiation process includes a cascade of events that results in genetic imprinting—reorganization of the histone/chromatin structure such that the determined T-cell polarization is subsequently maintained (58–61). A sketch of the various T helper subsets and the cytokines thought to be key for induction and maintenance of their polarization is presented in Figure 1A.

As mentioned above, early IL-4 production favors T_H2 polarization, whereas IFN- γ and IL-12 in the absence of IL-4 promote T_H1 polarization. While the source of IL-4 produced in the beginning of the immune response is not fully understood (the naïve T_H0 cells themselves, mast cells, and/or basophils, NKT cells), both IL-12 and the IFNs responsible for T_H1 polarization are produced during innate immune responses (62). For example, many bacteria and viruses contain one or more components able to interact with the Toll-like receptors present on dendritic cells and NK cells; IL-12 and IFN are among the cytokines released as a consequence of that activation. It is likely that T_H2 priming can occur either as a default pathway in the absence of Toll-like receptor signaling (it prevents rejection of the developing fetus in utero) or through currently unidentified T_H2 -activating receptors. Although T_H2 cells are characterized, defining the properties of allergen-specific T cells is difficult in human beings because of their low frequency within the T-cell repertoire.

Allergen-responsive B lymphocytes develop from bone marrow precursors before antigenic stimulation; they then migrate to and populate peripheral lymphoid tissues, where they complete their maturation and are available to interact with foreign antigen (63,64). Humoral responses are initiated by the recognition of antigens by B cells specific for each antigen (65). Antigen binds to the IgM and IgD receptors on naïve B cells and activates them; a clonal proliferation of antigen-specific cells results, and these cells continue to differentiate. Some of these become effector cells that actively secrete IgM antibody, others become memory B cells, and still others undergo heavy chain isotype class switch so that IgG, IgA, and IgE can be produced. Isotype switch is stimulated by CD4 $^+$ helper T-cell signals, which include the various cytokines and CD40-CD40 ligand interaction. Affinity maturation can also occur at this point.

Primary sensitization may occur in predisposed naïve individuals on their initial encounter with the allergen. The cellular and molecular pathways that lead to sensitization are quite similar to those that lead to a future recognition reaction in sensitized people; however, the cellular participants are probably different. The cells recruited for sensitization response cannot come from the memory cell compartment but only from the naïve cell population. Furthermore, as heavy chain isotype switching and affinity maturation increase with repeated exposures to protein antigens/allergens, the absence of traces of high-affinity antibody favors cells that do not use the Ig as a receptor in antigen-presenting function. This may push the concentration limits for recognition higher than those that develop in sensitized individuals. For some purified allergens, the antibody response is predominantly toward three to four dominant epitopes (15). Both the IgG and IgE responses in the same sensitized individual recognize the same epitopes (16). The rough outline of this essential process leading to

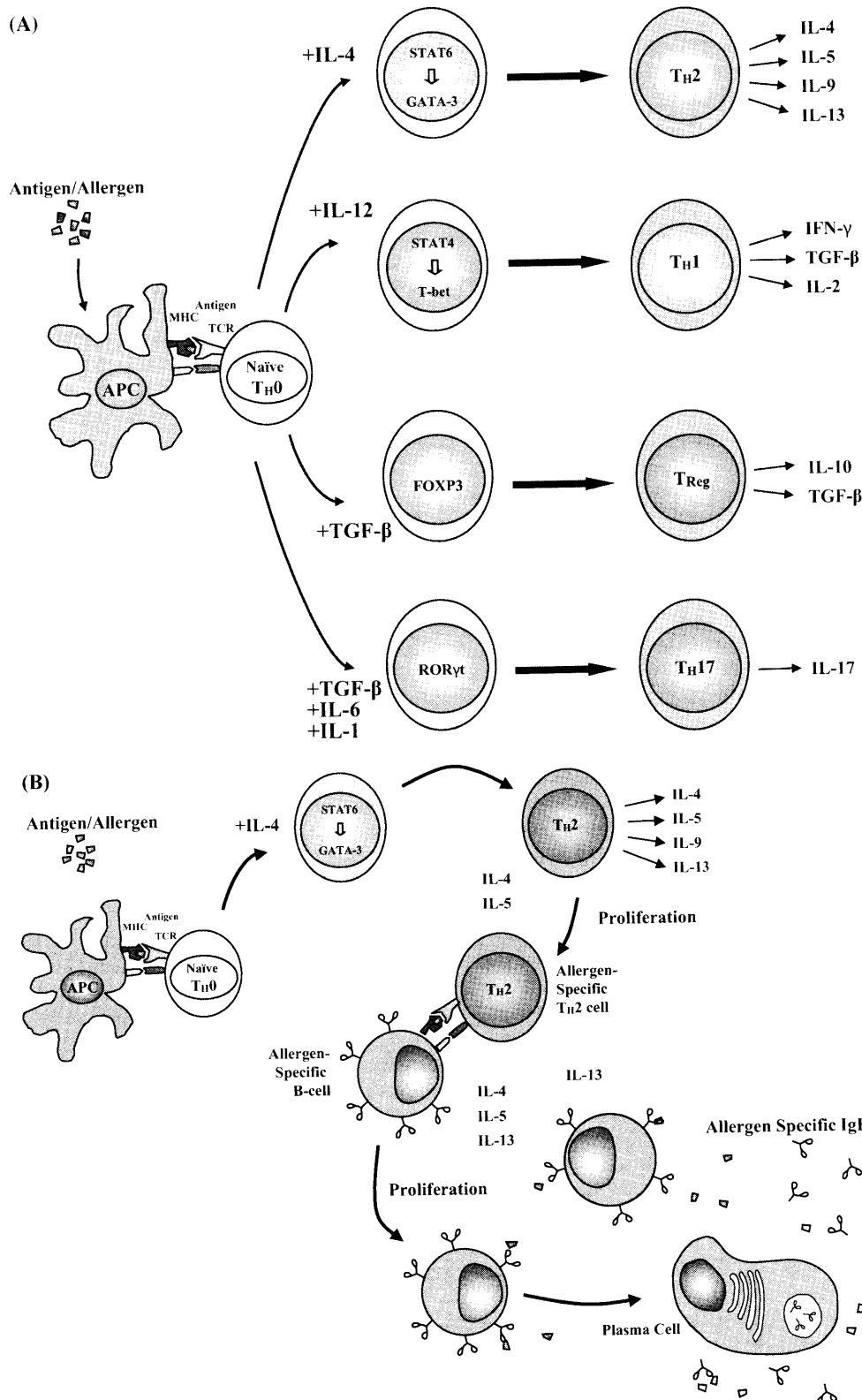


Figure 1 (A) T helper cells of various subsets are thought to develop from the same naïve T_H0 cell under the influence of both genetic and environmental factors acting at the level of antigen presentation. (B) Early exposure to and production of IL-4 results in polarization toward a T_H2 phenotype and is associated with production of allergen-specific IgE.

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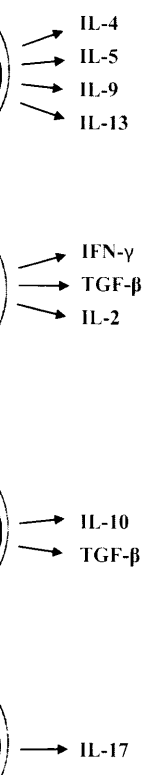
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production of IgE antibody is sketched in Figure 1B. The process is similar for other subclasses of antibody production as well, except that different cytokines are involved.

In addition to the thymus-dependent and thymus-independent classes of antigens, a third antigen class exists: a "superantigen" is an antigen capable of triggering nonspecific activation of many T cells, leading to wide antibody response. These are proteins produced by many pathogens (including bacteria, mycoplasma, and viruses), which can bind to the variable region of the beta chain (V-beta) of the T-cell receptor and cross-link it to MHC class II molecules on the surface of APCs. Superantigens function as intact molecules; they are not processed and presented by APCs. The extent of T-cell stimulation is a function of the frequency of T cells bearing V-betas that can bind a specific superantigen. There has been some speculation about the superantigenic nature of some allergic responses (66).

Most people, both atopic and nonallergic, mount a vigorous response to antigens, utilizing all subclasses of immunoglobulins except IgE (67,68). The atopic people mount the same response, but in addition they have an IgE response (69). The major difference in immune antibody response to antigen and allergen is consequently quite narrowly localized. The additional production of high-affinity IgE is directed to the dominant epitopes of the antigen. The epitopes seem to be the same recognized by other antibody classes. Unusual patterns of response by other subclasses of antibodies has been frequently mentioned, especially that of appearance of enhanced IgG₄ response. This may appear in individual circumstances, but studies of large populations of immune-response profiles to allergens have not revealed any systematic differences.

There is intrinsically very little in the structure of antigens that determines whether they will become allergens or not. Allergens are created by the selective response to them as they are presented as normal antigens (70); consequently, whether a molecule is an "antigen" or an "allergen" probably ultimately rests in the circumstances under which the presentation takes place.

GENETIC FACTORS MODULATING THE IMMUNE RESPONSE TO ALLERGENS

The atopic immune response is a complex condition involving genetic as well as environmental factors (Fig. 2). The evidence for genetic factors being involved in the different phenotypes of atopy has consisted of their aggregation in families, increased prevalence in first-degree relatives, and increased concordance in monozygotic twins compared with dizygotic twins (71,72). Genetic investigations to determine where the genes are located have used many approaches including forward genetics, candidate genes, genome screens, fine mapping, and functional genomics using statistical linkage and association analysis (73). These

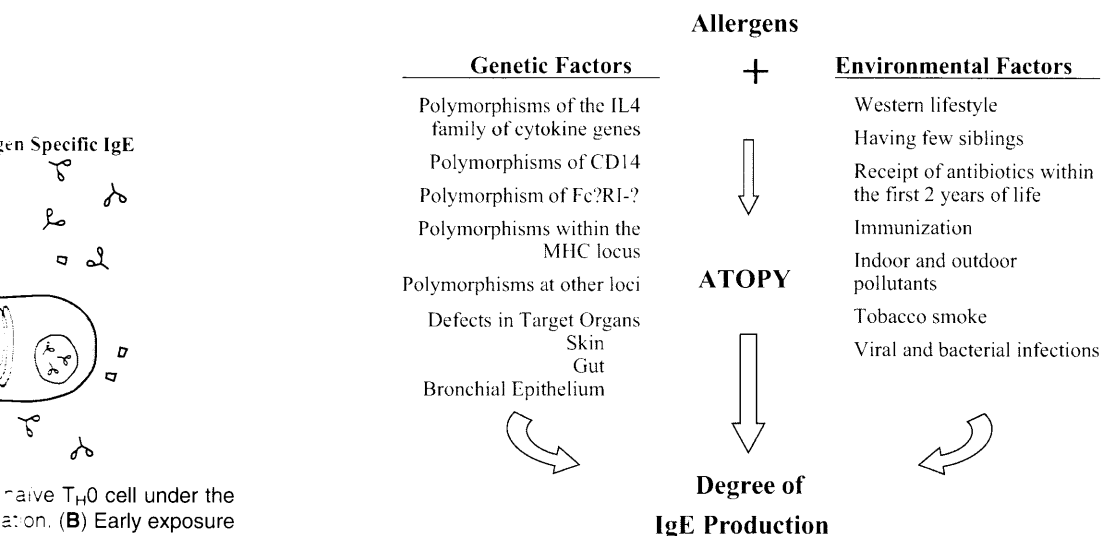


Figure 2 Examples of the environmental and genetic factors implicated in IgE response to allergen.

methods considered genetic heterogeneity, gene-gene interaction, and gene-environmental interaction. Atopy, defined as an adverse specific IgE immune reaction, has been studied using a variety of phenotypes including serum IgE levels, skin test reactivity, specific skin test reactivity, and specific serum IgE levels (74).

Jackola et al. demonstrated that all individuals usually respond with an immune response to a foreign allergen, but in atopy there is an increased production of a high-affinity IgE antibody while non-atopic individuals produce an equally high-affinity IgG₁ antibody (75). The clinical conditions resulting from an atopic mechanism may consist of asthma, some forms of urticaria, and allergic rhinitis. It is evident that the ultimate clinical picture of asthma will involve a variety of genetic factors that interact with each other and with environment exposures such as allergens, smoke, weight, infectious agents, diet, endotoxins, drugs, and diesel particles, all of which may change with time. There is no consensus regarding the mode of inheritance and/or the genes that are involved. Even less is known about the clinical course of these phenotypes (74). The main studies that have been used are the positional cloning or the genome screen and the candidate gene or association approaches (73).

Genome Wide Linkage Analysis for Asthma and Atopy and Related Phenotypes

The number of regions having linkage with atopy and related phenotypes such as asthma is at least 20. The replicated regions reported for the atopy and asthma phenotype have been on 1p, 2q, 4q, 5q, 6p, 12q, 13q, 14q, 19q, and 21q; for total serum IgE sites on 2q, 3q, 5q, 6p, 7q, and 12q; atopy on chromosomes 3q, 4q, 6p, 11q, 17q, 20p; and blood eosinophil counts on 15q (73,76-78). Blumenthal et al. reported on a genome-wide search for an atopy gene in three ethnic groups from the Collaborative Study on the Genetics of Asthma (CSGA). They found linkage on chromosomes 11q and 20p (79). The same group investigated mite sensitivity and noted linkage for the combined group on chromosomes 19q and 20q (80). These studies indicate the complexity of these conditions.

Candidate Genes for Asthma and Atopy and Related Phenotypes

Genome-wide linkage analysis and/or biological factors are the basis used for the selection of candidate genes studied for association. A variety of genetic association investigations for atopy and asthma phenotypes have been performed in different populations, many times yielding some reproducible but also many nonreplicable results (73,76-78). The candidate genes for atopy and asthma include allelic variants of genes of known immunological significance; variants in over 100 genes have been described (73,78). Some of the most common candidate gene variants are mentioned in the following paragraphs.

Chromosome 2q Region

This site identified in genome screens to be linked with the atopy and asthma phenotype contains the interleukin 1 receptor antagonist (IL-1RN) and the cytotoxic T lymphocyte antigen-4 (CTLA4) genes. Single nucleotide polymorphisms (SNPs) in CTLA4 are associated with asthma, serum IgE, asthma severity, airway responsiveness, and asthma (73,81-86). P-selectin has been reported as an atopy susceptibility locus (87).

Chromosome 5q and 16q Regions

Activation of the interleukin 4 receptor (IL-4RA) stimulates the production of total serum IgE. Interleukin 4 (IL-4) is located on chromosome 5q31 and IL-4RA on chromosome 16p12, both of which are found on genomic regions linked to the asthma phenotype. Many studies demonstrate that functional SNPs in the promoters of IL-4 and IL-4RA are associated with the atopy and asthma phenotypes (73,76,78). An article by Basehore et al. suggests that the 3017G/T variant or the haplotypes it identifies influence IL-4's ability to modulate total serum IgE levels (73,77,88). They suggest that inconsistencies with previously reported IL-4 associations might be due to population differences in allele frequencies, linkage disequilibrium with this SNP or haplotype, or both. Vercelli further discusses the discrepancies reported regarding CD14 polymorphisms, atopy, and endotoxin switch (89). Other genes on chromosome 5, including interleukin 13 (IL-13), monocyte differentiation antigen CD14 (CD14), serine protease inhibitor kazal type 5 (SPINK5), and leukotriene C4 synthase (LTC4S) have been

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of asthma will
with environment
endotoxins, drugs, and
regarding the mode
the clinical course
positional cloning or

Phenotypes

such as asthma is at
type have been on 1p,
3q, 5q, 6p, 7q, and
eosinophil counts on 15q
atopy gene in three
CSGA). They found
mite sensitivity and
80). These studies

for the selection of
investigations for
relations, many times
78). The candidate
immunological
of the most common

asthma phenotype
toxic T lymphocyte
TLA4 are associated
asthma (73,81-86).

of total serum IgE.
osome 16p12, both of
type. Many studies
associated with the
suggests that the 3017G/
late total serum IgE
rted IL-4 associations
equilibrium with this
reported regarding
on chromosome 5,
CD14 (CD14), serine
LTC4S) have been

studied. SNPs in the promoter and coding regions of these genes are associated with immune response and asthma phenotypes (73,76-78,90).

Chromosome 6p21 Region

Human leukocyte antigen DRB1 (HLA-DRB1), tumor necrotic factor alpha (TNF- α), and lymphotoxin alpha (LTA) are associated with asthma phenotypes (73). HLA class II molecules are involved in controlling the immune response to allergens (73). Early association studies demonstrate that several purified allergens, such as ragweed Amb a 5 and 6, olive Ole e 1, and *Lolium perenne* 1, 2, and 3, are associated with HLA. Functional SNPs and/or haplotypes in HLA-DRB1 are associated with allergic specific IgE responses. Moffatt et al. noted a relationship of atopy, respiratory function, and HLA-DR in Aboriginal Australians (91).

Chromosome 11q13 Region

The gene for the beta chain of the high-affinity receptor for IgE on chromosome 11q13, a genome region linked to atopy, has been reported. SNPs and/or haplotypes in FCER1B are associated with atopy, asthma, and their associated phenotypes (73,78).

Chromosome 12q Region

SNPs and haplotypes for the STAT-6 and nitric oxide synthase 1 (NOS1) are described to be associated with asthma and atopy (73,76,78).

Chromosome 17q Region

The predominant eosinophil chemoattractant on chromosome 17q is the gene for eotaxin (SCYA11), which is involved in allergic inflammation (73,76,78).

These are a few of the many atopy and asthma susceptibility genes suggested in the literature. Even though the loci and variants are identified, their function needs to be established. This is done through functional studies that involve SNPs in coding sequences of genes (promoter, enhancers, and chromatin structure). Over 55 genes with functional information have been reported; a selected few of these are noted in Table 1. Polymorphisms that may have function effects include IL-10, CD14, CCL5, Filaggrin, IL-17, IL-13, and the cysteinyl leukotriene receptors (73,78,92).

Asthma Susceptibility Loci Identified by the Positional Cloning Approach

Five potential asthma susceptibility genes or complexes have been identified using a positional approach (93). These are ADAM33, DPP10, PHF11 and SETDB2, GPRA, and SPINK5 (94-98). The replication studies of ADAM33 and asthma have resulted in conflicting results (99-102). To date, there is no definite replication of the association between GPRA, PHF11 and DPP10, and asthma and/or atopy phenotypes by groups independent from those publishing the original reports (73,76,78,93).

Gene-Environmental Interactions

The influence of environmental factors, including pets, endotoxin, viruses, smoke, and pharmacological agents, on the expression of genes and the ultimate clinical phenotype is being investigated and will be further summarized in a following section "Environmental Factors Modulating the Immune Response to Allergens." Investigation of gene-environmental interactions will be important in understanding the genetic basis of asthma and atopy. Even though there is genetic predisposition, environmental factors probably modulate the effect, causing either tolerance or susceptibility (73,74).

Summary

The current understanding of the immune system suggests that the upregulation of IgE synthesis in atopy is due to the induction of IgE isotype utilization at the DNA level in B cells. The start of IgE synthesis appears to involve a number of signals followed by direct T- and B-cell interaction. They require prior engagement of the TCR with antigenic fragments (peptides)

Table 1 Candidate Genes of Atopy and Allergy

Type	Examples
Cytokines influencing atopic phenotype	
Eosinophil growth-, activation-, and apoptosis-inhibiting factors	IL-5, IL-3, GM-CSF, CCL11, CCL5
Mast-cell growth factors	IL-3, IL-9, IL-10, SCF, TGF- β
Histamine-releasing factors	CCL2 (MCP-1), CCL7 (MCP-3), CCL5
IgE isotype switch factors	IL-4, IL-13
Inhibition of IgE isotype switch	IFN- γ , IL-12, IL-18, IL-23
Lipoxygenase pathway metabolism	5-LO, 5-LO-activating peptide, leukotriene C4 synthase
Proinflammatory cytokines	IL-1 α , IL-1 β , TNF- α , IL-6
Anti-inflammatory cytokines	TNF- β , IL-10, IL-1R α
Receptors	
Antigen receptors	T-cell receptors (α/β , γ/δ), B-cell receptor (IG, κ/λ light chains)
IgE receptor	Fc ϵ RI β chain, Fc ϵ RII (CD23)
Cytokine gene receptors	IFN- γ R β chain IL-1R, IL-4R, TNF receptors, common γ -chain
Adhesion molecules	VLA-4, VCAM-1, ICAM-1, LFA-1
Corticosteroid receptor	Grl-hsp90
Neurogenic receptors	β 2-Adrenergic, cholinergic receptors
Nuclear transcription factors	GATA-3, T-bet, NF- κ B, I κ B, NFAT, STAT-1/2, STAT-4, STAT-6
Other molecules of importance	
MHC and antigen processing	HLA class I and II molecules, TAP-1 and TAP-2, LMP
Cell signaling	CTLA-4, CD28, JAK1
Barriers and other defense	SPINK5, Clara cell protein 16, endothelin 2

Abbreviations: CCL, chemokine ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen; hsp, heat-shock protein; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LFA, lymphocyte function-associated antigen; LMP, large multicatalytic proteasome; LO, lipoxygenase; MHC, major histocompatibility complex; SCF, stem cell factor; TAP, transporter associated with antigen processing; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

that are recognized on MHC class II molecules on antigen-presentation cells. INF- α appears to be a major downregulator of IgE synthesis. There are at least two major genetic controls of atopy. One, which is non-epitope specific, is noted using the phenotypes of total serum IgE levels and skin test reactivity in general. The genes may reside on a variety of different loci and chromosomes, that is, IL-4 on chromosome 5q, IgE receptor on chromosome 11q, and IFN- γ on chromosome 12q. Another is epitope specific and appears to be associated with the HLA system. Therefore, there appears to be several levels involved in selectivity: (i) epitope-specific level that is related to the HLA system, (ii) purified allergen level (molecular selection) that is only partially HLA associated and dependent on size, and (iii) complex or natural allergen level involving many epitopes selective for organisms. There are probably too many surface epitopes, and too many HLA polymorphic types to demonstrate any specific HLA association.

In view of the complexity of atopy, it is not completely clear how many genes and environmental factors are involved. The genome screens suggest that there may be many genes with modulatory effects. It is likely that many of the genes that influence immunity will prove to be polymorphic. As a result, almost any immune response gene may be found to have an effect on any immune-mediated disease. To determine the clinical relevance of these polymorphisms, they should be tested in case-control studies involving patients with different manifestations and severity of the disease. The samples should be obtained from representative populations with environmental factors being defined. A good understanding of these relationships is needed for the proper management of atopy and its related conditions.

ENVIRONMENTAL FACTORS MODULATING THE IMMUNE RESPONSE TO ALLERGENS

A substantial increase in the prevalence of atopic diseases has occurred in the western world over the last few decades (103). These increased numbers cannot be explained simply on the basis of heightened awareness (leading to increased diagnosis of existent disease). Nor would

we expect to find this time period. The interplay between allergens, air impact the immune system (104,105). The animals, microbial family size, and early/increased exposure (106). It was believed that there may be that children sensitization, communities.

A possible during the period production; the influence the genetically susceptible such as dogs and animals can be from an atopic a strong cell-positive role in atopy (117).

The results There are many asthma and observations. Other environmental exposure have picture. It appears (118,119).

ALLERGIC ATOPY (INCLUDING)

The resulting discharge, and shortness of asthmalgia and conditions a histological reaction by a physical

Once initiated. Upon more inflammatory allergic reactions ranging from specific immune cells, eosinophil importance allergen involved cells' contact histamine functions of

we expect to detect change/drift in the genetic composition of the affected populations during this time period. Since expression of the atopic phenotype occurs as a result of complex interplay between genetic and environmental factors, the environment must be of major importance in the development and increased prevalence of atopy. Western living conditions, allergens, air pollution such as smoke and diesel fumes, and microbial exposure all may impact the immune system, and together influence an individual's risk for being atopic (104,105). The hygiene hypothesis postulates that reduced early childhood exposure to farm animals, microbes, and endotoxin or other microbial products, due to a combination of smaller family size, improved living standards, higher personal hygiene, changes in infant diet, and early/increased use of antibiotics, might result in increased risk for developing atopic disease (106). It was based on the observations, many of which have not been consistently confirmed, that there may be an inverse relationship between some infections and atopic parameters and that children who grow up in a farming environment may have less asthma (atopic sensitization, asthma, "hay fever") than children of the same age and living in the same communities, but not growing up on a farm (107-110).

A possible explanation for the protective effects of exposure to bacteria or their products during the period when sensitization occurs in early life is via increased stimulation to IFN- γ production; the lack of microbial stimulation of sufficient intensity may, paradoxically, influence the maturation of the immune system, causing a predominance of T_H2 cell subtype in genetically susceptible individuals (111-114). Early exposure to allergens from domestic pets, such as dogs and cats, is related to atopic sensitization (115,116). Like farm animals, domestic animals can be a source of endotoxin and may thus bias the overall immune response away from an atopic or T_H2 response. It is also postulated that only those infections that stimulate a strong cell-mediated immune response and long living memory immunity may play a positive role resulting in a shift toward the T_H1 type response and prevention of asthma and atopy (117).

The results seen in some studies may be a result of selection and environmental bias. There are many problems in accepting the hygiene hypothesis. These include the finding that asthma and atopy are more prevalent in the core city compared with the suburbs and the observations that autoimmune processes, which are thought to involve T_H1, are increasing. Other environmental factors such as diesel fumes, occupational inhalants, and allergen exposure have been noted to affect the immune response to allergens and the resulting clinical picture. It appears that environmental factors may enhance either sensitization or normalization (118,119).

ALLERGIC ATOPIC REACTIONS AND INFLAMMATION (INCLUDING PATHOLOGY)

The resulting clinical allergic reactions may vary from having symptoms of sneezing, nasal discharge, and nasal congestion associated with allergic rhinitis; coughing, wheezing, and shortness of breath with evidence of reversible airway obstruction; as well as certain forms of urticaria and angioedema, and anaphylaxis. Inflammation is an important feature of these conditions as summarized above; it is a dynamic process that consists of cytological and histological reactions that occur in tissues in response to injury or abnormal stimulation caused by a physical, chemical, or biological agent.

Once the individual begins to develop sensitization to an allergen, inflammation is initiated. Upon reexposure to the allergen, the immune system is further activated, resulting in more inflammation. This ultimately determines the clinical picture of allergy/atopy. The allergic reaction results from the involvement and interactions of a variety of cell types, ranging from monocytes and macrophages to T cells involved in the development of the specific immune reaction, as well as the granulocytic cells of the myeloid series (i.e., mast cells, eosinophils, neutrophils, and platelets). The interactions of all these cells are of importance in the inflammatory response. One of the steps following reexposure to the allergen involves the interaction with its specific IgE, attached by way of Fc ϵ RI and Fc ϵ RII to cells containing mediating substances. Important mediators are thought to include histamine, cytokines, and leukotrienes. Mediators released by some cells regulate the functions of others. The acute symptoms of allergies, such as sneezing, wheezing, and

urticaria, may be due to the release of mediators from the mast cells, such as histamine, whereas the chronic symptoms such as bronchial hyperreactivity may be explained on the basis of eosinophil-mediated tissue damage. T cells are of the T_H2 type and produce IL-4 and IL-5, which potentiate the terminal differentiation and activation of eosinophils. Basic proteins, together with the platelet-activating factor and leukotrienes secreted by eosinophils, probably also contribute to these chronic symptoms. Cell-adhesion molecules are also important in inflammation. A series of cell-adhesion molecules mediate interaction between vascular endothelium and leukocyte cell surfaces. The three major families of adhesion molecules, which contribute to this process are integrins, selectins, and immunoglobulin-like receptors. Other mediators of the inflammatory response that may be important are the complement system and heat-shock proteins. Therefore, as a result of the interaction of the allergen in a sensitized individual, a variety of cells and humoral components are activated, which results in inflammation and determines the clinical picture. The end result for exposure to an allergen is transient and/or chronic inflammation. The molecular and tissue changes found are common to all inflammatory processes. The difference between atopic allergy and all other inflammatory processes lies in causation. In the case of atopic allergy, it is linked to aberrant humoral response to foreign molecules whether these responses are IgE, IgG, or direct cellular reactions, as in the case of some late-phase reactions (120,121).

The nature of the immune reaction to an allergen and the resulting clinical picture is dependent on many steps influenced by host and environmental factors such as properties of the allergen, route of exposure, as well as genetic controls.

SALIENT POINTS

- Allergens/antigens have two properties: (i) immunogenicity (i.e., the capacity to stimulate the formation of the corresponding antibody and/or a state of sensitivity) and (ii) the ability to react specifically with those antibodies and/or the responding tissue. The two properties are not always associated.
- Allergens are antigens that induce the production of an IgE-specific antibody that will interact with the inducing antigen.
- From the chemical standpoint, there seems to be little to differentiate allergens from other antigens.
- There appear to be four restrictions for a molecule to become an allergen: (i) it must possess a surface to which the antibody can form a complementary surface, (ii) it must have an amino acid sequence in its backbone able to bind the MHC II alleles of the responding individual, (iii) the free energy of interaction of the allergen with the antibody should be adequate to ensure binding at low concentrations, and (iv) it must form at least two epitopes able to act as a bridge.
- The nature of the immune reaction to an allergen is dependent on many steps influenced by host and environmental factors.
- Genetic factors include multiple genes regulating non-epitope-specific factors, such as those on chromosome 5q, as well as those that are allergen epitope-specific, including genes in the MHC on chromosome 6.
- The duration, route, and amount of exposure, as well as other modulating pollutants, are a few of the environmental factors that influence the type of response seen to an allergen.
- Atopy clinically defined is an inflammatory condition resulting from an allergen producing an adverse immune reaction.

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DEFINITIONS

Antigens are substances which the animal immune system recognizes. *Allergens* are a subset of antigens that induce an IgE subclass antibody response. *Haptens* are substances that induce a humoral response but do not induce antibody on their own. *Immunogens* are substances that induce the production of an antibody. *B-cell epitopes* are regions of an antigen to which an antibody is directed. *T-cell epitopes* are regions of an antigen that are displayed by MHC molecules. *T helper cell 2 (TH2)* cells are a subset of T cells that are dominant in allergic diseases. *T helper cell 1 (TH1)* cells are a subset of T cells that are dominant in autoimmune diseases.

REFERENCES

1. Blumenthal M, McSherry D. Allergy and Asthma. In: Blumenthal M, McSherry D, eds. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:1-10.
2. Pirquet CV. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:11-20.
3. Coca AF, Coca PR. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:21-30.
4. Cooke RA. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:31-40.
5. Church M, Church H. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:41-50.
6. Landsteiner K. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:51-60.
7. Wilson JA. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:61-70.
8. López-Torres M. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:71-80.
9. Davies DR. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:81-90.
10. Aalberse HC. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:91-100.
11. Egger M. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:101-110.
12. Jemmersen M. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:111-120.
13. Greenbaum J. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:121-130.
14. Marsh DG. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:131-140.
15. Pierson-Nel A. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:141-150.
16. Kim KE. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:151-160.
17. Pierson-Nel A. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:161-170.
18. Jackola D. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:171-180.
19. Mina H. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:181-190.
20. Bredenoord A. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:191-200.
21. Jackola D. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:201-210.
22. Chapman S. *Textbook of Allergy and Asthma*. Philadelphia: FA Davis Co, 1998:211-220.

DEFINITIONS

Antigens are substances that have immunogenicity, leading to production of antibodies with which the antigens will react.

Allergens are a subclass of antigens that stimulate the production of and combine with the IgE subclass of antibodies.

Haptens are substances that are not immunogenic in/of themselves (cannot stimulate humoral response without the help of carrier substances) but can combine specifically with antibody once it is formed.

Immunogens are substances that stimulate specific immune response such as the production of an antibody.

B-cell epitopes are specific surface areas on antigen toward which the specificity of a single antibody is directed.

T-cell epitope is a proteolytic antigenic fragment (approximately 13 amino acids long) displayed by MHC; MHC-restricted recognition is required for activation of antigen-specific T cells.

T helper cell 2 (T_H2) profile is a specific pattern of effector molecules, of which IL-4 and IL-5 are dominant derived from activated T cells.

T helper cell 1 (T_H1) profile is a specific pattern of effector molecules, where INF γ is dominant, derived from activated T cells.

REFERENCES

1. Blumenthal MN. Historical perspectives. In: Bjorksten B, Blumenthal MN, eds. *Genetics of Allergy and Asthma: Methods for Investigative Studies*. New York: Marcel Dekker, 1996:1-8.
2. Pirquet CV. Allergy. In: Gell PGH, Coombs RRA, eds. *Clinical Aspects of Immunology*. Philadelphia: FA Davis Company, 1963:1457.
3. Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. *J Immunol* 1923; 8:163-182.
4. Cooke RA, vander Veer A. Human sensitization. *J Immunol* 1916; 1:201-205.
5. Church MK, McGill JI. I am atopic, but why don't I develop allergy? The association between atopy and clinical expression of allergic disease. *Clin Exp Allergy Rev* 2005; 5:12-15.
6. Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1935; 61:643-656.
7. Wilson IA, Stanfield RL. Antibody-antigen interactions: new structures and new conformational changes. *Curr Opin Struct Biol* 1994; 4:857-867.
8. López-Torrejón G, Díaz-Perales A, Rodríguez J, et al. An experiment and modeling approach to locate IgE epitopes of plant profiling allergens. *J Allergy Clin Immunol* 2007; 119:1481-1488.
9. Davies DR, Padlan EA, Sheriff S. Antibody-antigen complexes. *Ann Rev Biochem* 1990; 59:439-473.
10. Aalberse RC. Assessment of allergen cross-reactivity. *Clin Mol Allergy* 2007; 5:2.
11. Egger M, Mutschlechner S, Wopfner N, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy* 2006; 61:461-476.
12. Jemmerson R. Epitope mapping by proteolysis of antigen-antibody complexes. Protein footprinting. *Methods Mol Biol* 1996; 66:97-108.
13. Greenbaum JA, Andersen PH, Blythe M, et al. Towards a consensus on datasets and evaluation metrics for developing B-cell epitope prediction tools. *J Mol Recognition* 2007; 20:75-82.
14. Marsh D. Genetics of allergy. In: Sela M, ed. *The Antigens*. New York: Academic Press, 1975:271-359.
15. Pierson-Mullany LK, Jackola DR, Blumenthal MN, et al. Characterization of polyclonal allergen-specific IgE responses by affinity distributions. *Mol Immunol* 2000; 37:613-620.
16. Kim KE, Rosenberg A, Roberts S, et al. The affinity of allergen specific IgE and the competition between IgE and IgG for the allergen in Amb a V sensitive individuals. *Mol Immunol* 1996; 33:873-880.
17. Pierson-Mullany LK, Jackola DR, Blumenthal MN, et al. Evidence of an affinity threshold for IgE-allergen binding in the percutaneous skin test reaction. *Clin Exp Allergy* 2002; 32:107-116.
18. Jackola D, Liebeler C, Blumenthal MN, et al. Allergen skin test reaction patterns in children. *Int Arch Allergy Immunol* 2003; 132:364-372.
19. Mita H, Yasueda H, Akiyama K. Affinity of IgE antibody to antigen influences allergen-induced histamine release. *Clin Exp Allergy* 2000; 30:1582-1589.
20. Bredehorst R, David K. What establishes a protein as an allergen? *J Chromatogr B* 2001; 756:33-40.
21. Jackola DR, Liebeler CL, Blumenthal MN, et al. Random outcomes of allergen-specific responses in atopic families. *Clin Exp Allergy* 2004; 34:540-547.
22. Chapman MD, Pomés A, Breiteneder H, et al. Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* 2007; 119:414-420.

23. Aalberse RC. Structural biology of allergens. *J Allergy Clin Immunol* 2000; 106:228–238.
24. Breiteneder H, Ebner C. Atopic allergens of plant foods. *Curr Opin Allergy Clin Immunol* 2001; 1:261–267.
25. Breiteneder H, Mills C. Molecular properties of food allergens. *J Allergy Clin Immunol* 2005; 115:14–23.
26. Breiteneder H, Mills C. Nonspecific lipid-transfer proteins in plant foods and pollens: an important allergen class. *Curr Opin Allergy Clin Immunol* 2005; 5:275–279.
27. Hewitt CR, Brown AP, Hart BJ, et al. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 1995; 182: 1537–1544.
28. King C, Brennan S, Thompson PJ, et al. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* 1998; 161:3645–3651.
29. Peake HL, Currie AJ, Stewart GA, et al. Nitric oxide production by alveolar macrophages in response to house dust mite fecal pellets and the mite allergens, Der p 1 and Der p 2. *J Allergy Clin Immunol* 2003; 112:531–537.
30. Pomés A. Intrinsic properties of allergens and environmental exposure as determinants of allergenicity. *Allergy* 2002; 57:673–679.
31. Schneeberger EE. The permeability of the alveolar-capillary membrane to ultrastructural protein tracers. *Ann N Y Acad Sci* 1974; 221:238–243.
32. Janeway CA, Medzhitov R. Innate immune recognition. *Ann Rev Immunol* 2002; 20:197–216.
33. Pancer Z, Cooper MD. The evolution of adaptive immunity. *Ann Rev Immunol* 2006; 24:497–518.
34. Woof JM, Kerr MA. The function of immunoglobulin A in immunity. *J Pathol* 2006; 208:270–282.
35. Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Ann Rev Immunol* 2002; 20:495–549.
36. Tada T, Ishizaka K. Distribution of gamma E forming cells in lymphoid tissues of the human and monkey. *J Immunol* 1970; 104:377–387.
37. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004; 118:229–241.
38. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122:107–118.
39. Erwin EA, Platts-Mills TAE. Allergens. *Immunol Allergy Clin N Am* 2005; 25:1–14.
40. Kuehr J, Frischer T, Meinert R, et al. Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994; 94:44–52.
41. Kabelitz D, Medzhitov R. Innate immunity—cross-talk with adaptive immunity through pattern recognition receptors and cytokines. *Curr Opin Immunol* 2007; 19:1–3.
42. Lee HK, Iwasaki A. Innate control of adaptive immunity: dendritic cells and beyond. *Semin Immunol* 2007; 19:48–55.
43. Rijkers GT, Sanders LA, Zegers BJ. Anti-capsular polysaccharide antibody deficiency states. *Immunodeficiency* 1993; 5:1–21.
44. Guernonprez P, Valladeau J, Zitvogel L, et al. Antigen presentation and T cell stimulation by dendritic cells. *Ann Rev Immunol* 2002; 20:621–667.
45. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. *Ann Rev Immunol* 2006; 24:419–466.
46. Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. *Ann Rev Immunol* 2002; 20:825–852.
47. Gay NJ, Gangloff M, Weber AN. Toll-like receptors as molecular switches. *Nat Rev Immunol* 2006; 6:693–698.
48. Kemper C, Atkinson JP. T-cell regulation: with complements from innate immunity. *Nat Rev Immunol* 2007; 7:9–18.
49. Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002; 2:933–944.
50. Weaver CT, Hatton RD, Mangan PR, et al. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Ann Rev Immunol* 2007; 25:821–852.
51. Woodfolk JA. T-cell responses to allergens. *J Allergy Clin Immunol* 2007; 119:280–294.
52. Rothenberg EV. Cell lineage regulators in B and T cell development. *Nat Immunol* 2007; 8:441–444.
53. Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN- γ loci accompany Th1/Th2 differentiation. *J Immunol* 2002; 169:647–650.
54. Soutto M, Zhou W, Aune TM. Cutting edge: distal regulatory elements are required to achieve selective expression of IFN- γ in Th1/Tc1 effector cells. *J Immunol* 2002; 169:6664–6667.
55. Hwang ES, Szabo JS, Schwartzberg PL, et al. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. *Science* 2005; 307:430–433.
56. Stockinger B, Veldhoen M. Differentiation and function of Th17 cells. *Curr Opin Immunol* 2007; 19:281–286.
57. Bacchetta R, Gambineri E, Roncarolo M-G. Role of regulatory T cells and FOXP3 in human diseases. *J Allergy Clin Immunol* 2007; 120:227–235.
58. Ansel KM, Djuretic I, Tanasa B, et al. Regulation of Th2 differentiation and IL4 locus accessibility. *Ann Rev Immunol* 2006; 24:607–656.

59. Spilianakis
60. Chang S, *interferon-*
61. Krangel M
62. Romagnan *Immunol*
63. Gould HJ, *Immunol*
64. Cobaleda *Immunol*
65. Carrasco Y *ligands. C*
66. Gould HJ, *to immuno*
67. Akdis M, *characteriz*
68. Akdis M, *2006; 18:7*
69. Jackola DE *'selective*
70. Akdis CA, *18:718–72*
71. Blumenth *Genetics o*
72. Le Souëf *asthma. C*
73. Blumenth *Immunol*
74. Blumenth
75. Jackola D *concentra*
76. Hoffman S *14:709–71*
77. Hoffman S *review of*
78. Ober C, *Immun*
79. Blumenth *in three c*
80. Blumenth *Collabora*
81. Laitinen T *related tr*
82. Gohlke H *asthma. A*
83. Dessi T, *increased*
84. Tiki-Erkk *interleukin*
85. van Oost *allergic a*
86. Yang SD *in gender*
87. Ebrahimi *Th-selecto*
88. Ebrahimi *Th-selecto*
89. Ebrahimi *Th-selecto*
90. Ebrahimi *Th-selecto*
91. Ebrahimi *Th-selecto*
92. Ebrahimi *Th-selecto*
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96. Ebrahimi *Th-selecto*
97. Ebrahimi *Th-selecto*
98. Ebrahimi *Th-selecto*
99. Ebrahimi *Th-selecto*
100. Ebrahimi *Th-selecto*

- 228–238.
- Immunol 2001; 1:261–267.
- Clin Immunol 2005;
- pollens: an important
- the immunoglobulin E
- 1995; 182:1537–1544.
- cytokine release from
- ecular macrophages in
- Der p 2. J Allergy Clin
- as determinants of
- structural protein
- 20:197–216.
- 2006; 24:497–518.
- 2008:270–282.
- Inflammation. Ann Rev
- of the human and
- microflora by Toll-
- of symbiotic bacteria
- 5:1–14.
- incidence of specific
- through pattern
- and beyond. Semin
- deficiency states.
- T cell stimulation by
- coreceptors. Ann Rev
- Rev Immunol 2002;
- Rev Immunol 2006;
- immunity. Nat Rev
- Immunol 2002; 2:933–944.
- expanding diversity of
- 280–294.
- Immunol 2007; 8:441–444.
- the IL-4 and IFN- γ loci
- required to achieve
- 4–6667.
- by kinase-mediated
- Opin Immunol 2007;
- 23 in human diseases.
- IL4 locus accessibility.
59. Spilianakis CG, Flavell RA. Epigenetic regulation of *Ifng* expression. *Nat Immunol* 2007; 8:681–683.
60. Chang S, Aune TM. Dynamic changes in histone-methylation ‘marks’ across the locus encoding interferon- γ during the differentiation of T helper type 2 cells. *Nat Immunol* 2007; 8:723–731.
61. Krangel MS. T cell development: better living through chromatin. *Nat Immunol* 2007; 8:687–694.
62. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004; 113:395–400.
63. Gould HJ, Sutton BJ, Beavil AJ, et al. The biology of IGE and the basis of allergic disease. *Ann Rev Immunol* 2003; 21:579–628.
64. Cobaleda C, Schebesta A, Delogu A, et al. Pax5: the guardian of B cell identity and function. *Nat Immunol* 2007; 8:463–470.
65. Carrasco YR, Batista FD. B cell recognition of membrane-bound antigen: an exquisite way of sensing ligands. *Curr Opin Immunol* 2006; 18:286–291.
66. Gould HJ, Takhar P, Harries HE, et al. The allergic march from *Staphylococcus aureus* superantigens to immunoglobulin E. *Chem Immunol Allergy* 2007; 93:106–136.
67. Akdis M, Verhagen J, Taylor A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567–1575.
68. Akdis M. Healthy immune response to allergens: T regulatory cells and more. *Curr Opin Immunol* 2006; 18:738–744.
69. Jackola DR, Pierson-Mullany LK, Liebler CL, et al. Variable binding affinities for allergen suggest a ‘selective competition’ among immunoglobulins in atopic and non-atopic humans. *Mol Immunol* 2002; 39:367–377.
70. Akdis CA. Allergy and hypersensitivity; mechanisms of allergic disease. *Curr Opin Immunol* 2006; 18:718–726.
71. Blumenthal MN. Overview of genetic factors in the allergic response. In: Liggett SB, Meyers DA, eds. *Genetics of Asthma: Lung Biology in Health and Diseases*. New York: Marcel Dekker, 1996:479–494.
72. Le Souëf PN. Using twin studies to determine genetic and environmental components of allergy and asthma. *Clin Exp Allergy* 2006; 36:1353–1354.
73. Blumenthal MN. The role of genetics in the development of asthma and atopy. *Curr Opin Allergy Immunol* 2005; 5:141–145.
74. Blumenthal JB, Blumenthal MN. Genetics of asthma. *Med Clin North Am* 2002; 86:937–950.
75. Jackola DR, Liebler CL, Lin C-Y, et al. Evidence that negative feedback between antibody concentration and affinity regulates humoral response consolidation to a non-infectious antigen in infants. *Mol Immunol* 2005; 42:19–30.
76. Hoffjan S, Ober C. Present status on the genetic studies of asthma. *Curr Opin Immunol* 2002; 14:709–717.
77. Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. *Respir Res* 2003; 4:14.
78. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 2006; 7:95–100.
79. Blumenthal MN, Langefeld CD, Beaty TH, et al. A genome-wide search for allergic response (atopy) genes in three ethnic groups: Collaborative Study on the Genetics of Asthma. *Hum Genet* 2004; 114:157–164.
80. Blumenthal MN, Ober C, Beaty TH, et al. Genome scan for loci linked to mite sensitivity: the Collaborative Study on the Genetics of Asthma (CSGA). *Genes Immun* 2004; 5:226–231.
81. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science* 2004; 304:300–304.
82. Gohlke H, Illig T, Bahnweg M, et al. Association of the interleukin-1 receptor antagonist gene with asthma. *Am J Respir Crit Care Med* 2004; 169:1217–1223.
83. Pessi T, Karjalainen J, Hukkonen J, et al. A common IL-1 complex haplotype is associated with an increased risk of atopy. *J Med Genet* 2003; 40:e66.
84. Joki-Erkkila VP, Karjalainen J, Hukkonen J, et al. Allergic rhinitis and polymorphisms of the interleukin 1 gene complex. *Ann Allergy Asthma Immunol* 2003; 9:275–279.
85. van Oosterhout AJM, Deurloo DT, Groot PC. Cytotoxic T lymphocyte antigen 4 polymorphisms and allergic asthma. *Clin Exp Allergy* 2004; 34:4–8.
86. Yang KD, Liu C-A, Chang J-C, et al. Polymorphism of the immune-braking gene CTLA-4 (+49) involved in gender discrepancy of serum total IgE levels and allergic diseases. *Clin Exp Allergy* 2004; 34:32–37.
87. Bourgain C, Hoffjan S, Nicolae R, et al. Novel case-control test in a founder population identifies P-selectin as an atopy-susceptibility locus. *Am J Hum Genet* 2003; 73:612–626.
88. Basehore MJ, Howard TD, Lange LA, et al. A comprehensive evaluation of IL4 variants in ethnically diverse populations: association of total serum IgE levels and asthma in white subjects. *J Allergy Clin Immunol* 2004; 114:80–87.
89. Vercelli D. Learning from discrepancies: CD14 polymorphisms, atopy and the endotoxin switch. *Clin Exp Allergy* 2003; 33:153–155.

90. Kabesch M, Carr D, Weiland SK, et al. The association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. *Clin Exp Allergy* 2004; 34:340-345.
91. Moffatt MF, Faux JA, Lester S, et al. Atopy, respiratory function and HLA-DR in Aboriginal Australians. *Hum Mol Genet* 2003; 12:625-630.
92. Hoffjan S, Stemmler S. On the role of the epidermal differentiation complex in ichthyosis vulgaris, atopic dermatitis and psoriasis. *Br J Dermatol* 2007; 157:441-449.
93. Cookson W, Moffatt M. Making sense of asthma genes. *N Engl J Med* 2004; 351:1794-1796.
94. Van Eerdewegh P, Little RD, Dupuis J, et al. Association of the ADAM-33 gene with asthma and bronchial hyper-responsiveness. *Nature* 2002; 418:426-430.
95. Allen M, Heinzmann A, Noguchi E, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003; 35:258-263.
96. Zhang Y, Leaves NI, Anderson GG, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat Genet* 2003; 34:181-186.
97. Jang N, Stewart G, Jones G. Polymorphisms within the PHF11 gene at chromosome 13q14 are associated with childhood atopic dermatitis. *Genes Immun* 2005; 6:262-264.
98. Laitinen T, Polvi A, Rydman P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science* 2004; 304:300-304.
99. Howard TD, Postma DS, Jongepier H, et al. Association of disintegrin and metalloprotease 33 (ADAM 33) gene with asthma in ethnically diverse populations. *J Allergy Clin Immunol* 2003; 112:717-722.
100. Werner M, Herbon N, Gohlke H, et al. Asthma is associated with single-nucleotide polymorphism in ADAM 33. *Clin Exp Allergy* 2004; 34:26-31.
101. Lind DL, Choudhry S, Ung N, et al. ADAM 33 is not associated with asthma in Puerto Rican or Mexican population. *Am J Respir Crit Care Med* 2003; 168:1312-1316.
102. Raby BA, Silverman EK, Kwiatkowski DJ, et al. ADAM33 polymorphisms and phenotype associations in childhood asthma. *J Allergy Clin Immunol* 2004; 133:1017-1078.
103. Blumenthal MN. Epidemiology and genetics of asthma and allergy. In: Kaplan AP, ed. *Allergy*. Philadelphia: W.B. Saunders, 1997:407-420.
104. Leonardi S, Miraglia del Giudice M, La Rosa M, et al. Atopic disease, immune system, and the environment. *Allergy Asthma Proc* 2007; 28:410-417.
105. Kalinski P, Moser M. Consensual immunity: success-driven development of T-helper-1 and T-helper-2 responses. *Nat Rev Immunol* 2005; 5:251-260.
106. Strachan DP. Hay fever, hygiene, and household size. *Br Med J* 1989; 299:1259-1260.
107. Stern DA, Riedler J, Nowak D, et al. Exposure to a farming environment has allergen-specific protective effects on TH2-dependent isotype switching in response to common inhalants. *J Clin Allergy Immunol* 2007; 119:351-358.
108. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347:911-920.
109. Celedón JC, Milton DK, Ramsey CD, et al. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J Allergy Clin Immunol* 2007; 120:144-149.
110. Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J Allergy Clin Immunol* 2006; 117:969-977.
111. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000; 55:688-697.
112. Yabuhara A, Macaubas C, Prescott SL, et al. TH2-polarized immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; 27:1261-1269.
113. Vercelli D. Mechanisms of the hygiene hypothesis—molecular and otherwise. *Curr Opin Immunol* 2006; 18:733-737.
114. Romagnani S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J Allergy Clin Immunol* 2007; 119:1511-1513.
115. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288:963-972.
116. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* 2003; 3:169-176.
117. Chen W, Khurana-Hershey GK. Signal transducer and activator of transcription signals in allergic disease. *J Allergy Clin Immunol* 2007; 119:529-541.
118. Liu AH, Murphy JR. Hygiene hypothesis: fact or fiction? *J Allergy Clin Immunol* 2003; 111:471-478.
119. Liu AH, Leung DY. Renaissance of the hygiene hypothesis. *J Allergy Clin Immunol* 2006; 117:1063-1066.
120. Lympay PA, Lee T. Inflammation. In: Bjorksten B, Blumenthal MN, eds. *Genetics of Allergy and Asthma: Methods for Investigative Studies*. New York: Marcel Dekker, 1996:241-280.
121. Barnes P. Inflammation. In: Weiss EB, Stein M, eds. *Bronchial Asthma: Mechanisms and Therapeutics*. Boston: Little, Brown and Co., 1993:80-94.

HISTORICAL

As with most time when all techniques, and of the investigation phenol extract 1960s, ion exchange first allergen (one of five proteins in Ouchterlony) was shown to be an important indicator that allergens were was led by the University, Baye grass allergen studies of hay (Cat-I) (7) and

The state in *The Antigen* factors that in studies of IgE definition of induced immune allergen, to v generally regulated. With the immunoelectrophoresis Scandinavia. CIE CRIE peptide allergen based peaks were given allergens were was also known needed.

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