

Autoallergy: A pathogenetic factor in atopic dermatitis?

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Long before the discovery of IgE it was reported that human dander extract can elicit immediate-type skin reactions in patients with severe atopy and that this skin sensitivity can be passively transferred with serum. Several recent findings have rekindled the interest in this phenomenon and led to the concept that IgE autoreactivity may play a pathogenetic role in severe and chronic forms of atopy. The elucidation of the nature of several environmental allergens has revealed striking structural and immunologic similarities with human proteins. It was also reported that patients predominantly with severe and chronic manifestations of atopy (eg, atopic dermatitis) contain IgE autoantibodies against a wide variety of proteins expressed in histogenetically unrelated human cell types and tissue specimens. Last, complementary DNAs coding for autoallergens were isolated from human expression complementary DNA libraries and recombinant autoallergens were produced. The autoallergens characterized to date represent mainly intracellular proteins, but some of them could be detected as IgE immune complexes in sera of sensitized patients. We suggest that at least two pathomechanisms could play a role in autoallergy. First, autoallergens may cross-link effector cell-bound IgE autoantibodies and, by release of inflammatory mediators, lead to immediate-type symptoms. Second, IgE-mediated presentation of autoallergens may activate autoreactive T cells to release proinflammatory cytokines, contributing to the magnitude of the allergic tissue reaction. (*J Allergy Clin Immunol* 2000;105:432-7.)

Key words: Autoallergy, IgE autoreactivity, atopic dermatitis, cross-reactivity, complementary DNA cloning, allergy, immune responses, eczema, molecular biology

AUTOALLERGY: AN OLD CONCEPT REVISITED

Between 1920 and 1930 two investigators, Storm van Leeuwen and Keller, reported independently that individuals with severe forms of atopy can exhibit immediate-type reactions on skin testing with aqueous human dander extracts.^{1,2} In 1941 it was demonstrated that the skin-sensitizing factors could be passively transferred in Prausnitz-Küstner tests (PK tests) mainly with sera from patients with atopic dermatitis (AD),³ and a much higher incidence of positive skin reactions to human dander extracts was reported for AD patients than for patients with asthma or allergic rhinoconjunctivitis.⁴⁻⁷ Later it was shown that human skin extracts induced proliferation in leukocytes from patients with AD.⁸ Since then, the concept that autoallergy could play a pathogenetic role in severe forms of atopy fell into oblivion until the more recent discovery that environmental allergens (eg, plant profilins,⁹ dog serum albumin,¹⁰ mold-derived manganese superoxide dismutase¹¹) shared structural and immunologic similarities with human proteins. A search for the presence of IgE autoantibodies in sera from patients with various manifestations of atopy and other autoimmune disorders indicated that IgE reactivity against a variety of autoantigens occurred most frequently in AD patients.¹² The recent isolation and characterization of complementary DNAs (cDNAs) coding for autoallergens from human expression cDNA libraries with serum IgE from AD patients revealed the molecular nature of IgE-defined autoantigens and provided unequivocal proof for the existence of IgE autoimmunity in severe forms of atopy.^{13,14}

SIMILARITIES BETWEEN EXOGENOUS ALLERGENS AND HUMAN PROTEINS

The molecular analysis of allergens by cDNA cloning has revealed striking similarities between environmental allergens and human proteins. A cDNA clone that was isolated with use of serum IgE from a birch pollen-allergic patient coded for profilin, a ubiquitous actin-binding protein.⁹ Recombinant birch profilin was found to share immunologic and sequence similarities with human profilin.⁹ Likewise, it was reported that patients who were allergic to manganese superoxide dismutase and riboso-

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Abbreviations used

AD: Atopic dermatitis
cDNA: Complementary DNA
mRNA: Messenger RNA
 α -NAC: α Chain of the human nascent polypeptide-associated complex
PK tests: Prausnitz-Küstner tests

mal P-2 protein from *Aspergillus fumigatus* exhibited humoral and cell-mediated autoimmune reactions to the human homologs.^{11,15} Several other environmental allergens with similarities to human proteins may be responsible for cross-reactivities. They comprise albumins from animals,¹⁰ calcium-binding allergens from plants and fish,¹⁶ and plant cytochromes.¹⁷ The analysis of the 3-dimensional structure of environmental allergens showed that even allergens with low (eg, birch profilin) or no (timothy grass pollen allergen, Phl p 2) sequence homology with human proteins can closely mimic the 3-dimensional structure of human proteins.^{18,19} Clinical analysis of patients with IgE antibodies against highly conserved environmental allergens ("panallergens") indicates that these patients constitute a group of polysensitized atopic individuals who have allergic symptoms on contact with a great variety of allergen sources.²⁰ However, no apparent association of polysensitization with certain clinical manifestations of atopy has been reported so far.

IgE AUTOREACTIVITY IS FREQUENTLY FOUND IN PATIENTS WITH AD

In a study of the frequency and association of IgE autoreactivity with certain diseases or disease manifestations we used nitrocellulose-blotted human cellular protein extracts to search for the presence of IgE autoantibodies in sera from patients with various manifestations of atopy, other immunologically mediated diseases, and healthy individuals.^{12,13} We found that sera mainly from AD patients displayed IgE reactivity against a broad variety of proteins in histogenetically unrelated human cell types. AD is a chronic inflammatory skin disease that resembles other eczematous diseases and exhibits features of a T cell-mediated, delayed-type hypersensitivity reaction.²¹⁻²³ Although many AD patients are sensitized against several environmental allergen sources and total IgE levels are often elevated in AD, the role of IgE in the pathogenesis of AD is not clear and AD patients frequently exhibit disease exacerbation without obvious contact with exogenous allergens.²⁴ We found IgE autoantibodies frequently in sera from AD patients with elevated total serum IgE values, but certain AD patients with low total serum IgE values also displayed strong IgE autoreactivity and others with extremely high total serum IgE levels failed to react with human proteins.^{12,13} In comparing the intensity of IgE reactivity to blotted human epithelial proteins with the severity of skin lesions in an AD patient cohort, we observed that patients

with strong IgE autoreactivity tend to have more severe forms of atopic eczema than those without detectable IgE autoantibodies.¹³ We further observed that, in certain AD patients, disease exacerbation is accompanied by an increased IgE autoreactivity.^{13,25} Taken together, these findings indicate that IgE autoimmunity occurs frequently in AD patients and may be associated with disease activity. Whether certain forms of AD indeed represent IgE-mediated autoimmune diseases represents an open question and will require further experimental work. One way to address this issue would be to study whether epicutaneous or intracutaneous application of autoallergens to sensitized AD patients can cause exacerbation of their eczematous skin lesions.²⁶ The second possibility to study the pathogenetic role of IgE autoimmunity in AD would be to investigate whether experimental sensitization of animals with autoallergens can induce atopic skin symptoms. Both types of investigation will require the availability of defined autoallergens.

cDNA CLONING REVEALS THE NATURE OF AUTOALLERGENS

The sequences of many environmental allergens have been revealed by cDNA cloning techniques during the last few years and recombinant allergens useful for diagnostic and therapeutic purposes have been produced.²⁷ The strategy of using IgE antibodies from atopic individuals for the isolation of allergen-encoding cDNAs has recently been applied also for the isolation of cDNAs coding for autoallergens (Fig 1). A cDNA expression library prepared from human tissue was screened with serum IgE from atopic individuals containing IgE autoantibodies. Phage clones expressing autoallergens are identified by IgE antibodies and enriched to homogeneity by "rescreening." DNA coding for autoallergens was isolated from the IgE-reactive phage and subcloned into plasmid vectors. The sequence analysis of the autoallergen-encoding cDNAs allowed us to determine their nature and the cDNAs can serve as templates for the production of recombinant autoallergens (Fig 1). With use of IgE immunoscreening, several cDNAs coding for autoallergens were recently isolated. These autoallergens included the following: (1) Hom s 1, the autoallergen most frequently recognized by IgE autoantibodies was found to be strongly expressed in skin and to a lesser extent in other tissues.¹⁴ Within the skin, Hom s 1 was detected prominently in the epidermis and occasionally in fibroblasts and endothelial cells of the dermis. Comparison of the Hom s 1 sequence with the sequences deposited in the databases revealed an almost complete sequence identity with SART-1, an antigen reportedly recognized by cytotoxic T cells of a patient with squamous esophageal cancer.¹⁴ (2) Hom s 2, the α chain of the human nascent polypeptide-associated complex (α -NAC), is a protein required for sequence-specific sorting and translocation of intracellular proteins that may also act as a cotranscriptional factor.¹³ (3) Hom s 3, BCL7B, is a putative oncogene.¹³

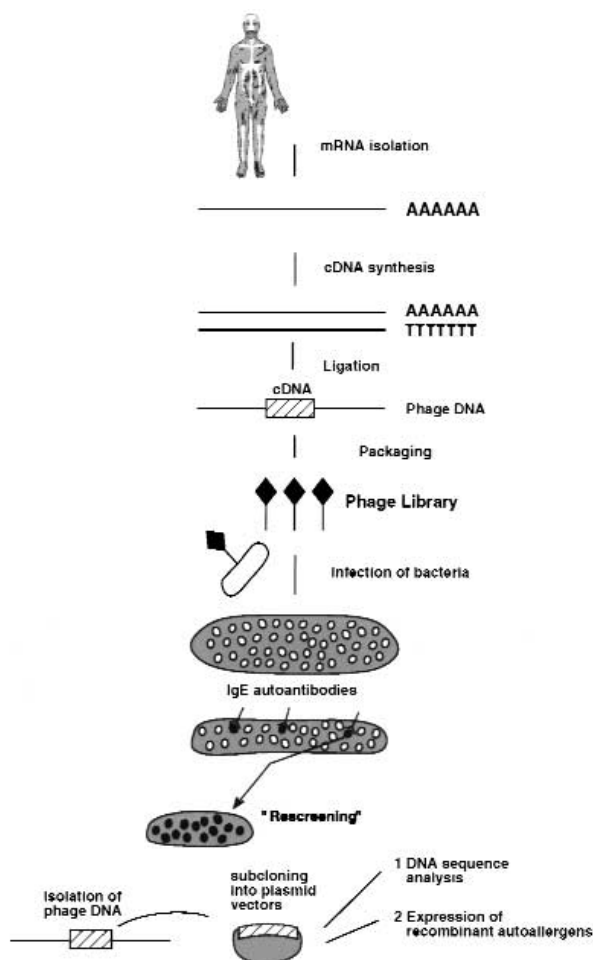


FIG 1. Isolation of cDNAs coding for autoallergens. An expression cDNA library is constructed from messenger RNA (mRNA) that has been isolated from human cells or tissue specimens. The mRNA is reversely transcribed into cDNA and ligated in phage DNA. Infectious phages are obtained by in vitro packaging of the phage DNA. After infection of bacteria, phages can produce recombinant proteins, which allows the isolation of autoallergen-expressing phage clones with IgE antibodies. The IgE-reactive phage clones are purified to homogeneity by "rescreening" and the autoallergen-encoding cDNAs are subcloned into plasmid vectors for sequence analysis as well as for the production of recombinant autoantigens.

(4) Hom s 4, a protein of unknown function, contains a typical calcium-binding motif.¹³ (5) Hom s 5 is human cytokeratin type II.¹³ One of these autoallergens, Hom s 2, showed sequence similarity with antigens present in yeast, plants, animals, and bacteria (eg, α -NAC)¹³ (Mossabeh and Valenta, unpublished data), whereas the other autoallergens seemed to be restricted to man and animals only. All 5 cloned autoallergens represent primarily intracellular proteins,^{13,14} thus raising the question how auto sensitization can occur in atopic individuals and how IgE autoimmunity can contribute to atopic tissue inflammation.

ORIGIN AND COMMON FEATURES OF AUTOALLERGENS

Components of an allergen extract that are recognized by more than 50% of patients are termed major allergens.²⁸ Factors that may determine whether a protein will be recognized as a major allergen are (1) abundant presence in aqueous allergen extracts, (2) high immunogenicity, (3) stability, and perhaps (4) certain intrinsic properties that could facilitate sensitization. In the case of autoallergy, Western blotting experiments showed that sensitized patients reacted with a great variety of different autoallergens and there was no evidence that certain components act as major autoallergens.¹² Although certain of the autoallergens (eg, Hom s 1) were expressed preferentially in the target organs of atopy, others were found in a wide variety of cell types and tissues.^{13,14} Results gained from the molecular and histochemical characterization of autoallergens as well as from subcellular fractionation experiments indicated that most of them represented intracellular proteins.^{13,14,29} Evidence that intracellular autoallergens can be released and occur in the circulation of atopic individuals complexed to IgE autoantibodies comes from the experimental setup exemplified in Fig 2. If IgE autoantibodies contain bound autoallergens, it should be possible to copurify IgE autoallergen immune complexes by affinity to monoclonal antihuman IgE antibodies. With use of this technology, two autoallergens were detected with specific antibody probes in the purified antihuman IgE precipitates of reactive patients. Circulating IgE autoallergen immune complexes may reach target organs of atopy and become immobilized by Fc ϵ receptors to effector and inducer cells of atopy.³⁰ In this context, it is noteworthy that sera from patients with severe atopy were reported to contain IgE-dependent histamine release factors. Such sera, termed IgE+ sera, were identified by their ability to induce histamine release from basophils without addition of allergens, a property that could be also explained by the presence of IgE-autoallergen immune complexes in these sera.³¹⁻³³

POSSIBLE PATHOMECHANISMS OPERATIVE IN IgE-MEDIATED AUTOIMMUNITY

Two observations support the assumption that autoallergy is initiated or at least preceded by a sensitization against environmental allergens. None of the atopic individuals with IgE autoantibodies was sensitized exclusively against autoallergens but also reacted with environmental allergens.¹²⁻¹⁴ Those individuals who exhibited cross-reactivity between environmental and endogenous proteins (eg, plant profilins, manganese superoxide dismutase) showed much stronger IgE and cellular responses to the environmental allergens than to their endogenous counterparts.^{9,11} It is thus conceivable that tissue damage induced by environmental allergens (Fig 3, orange asterisks) in the target organs of atopy (skin, eyes, respiratory tract, gastrointestinal tract) represents an important start-

Isolation of IgE-autoallergen complexes

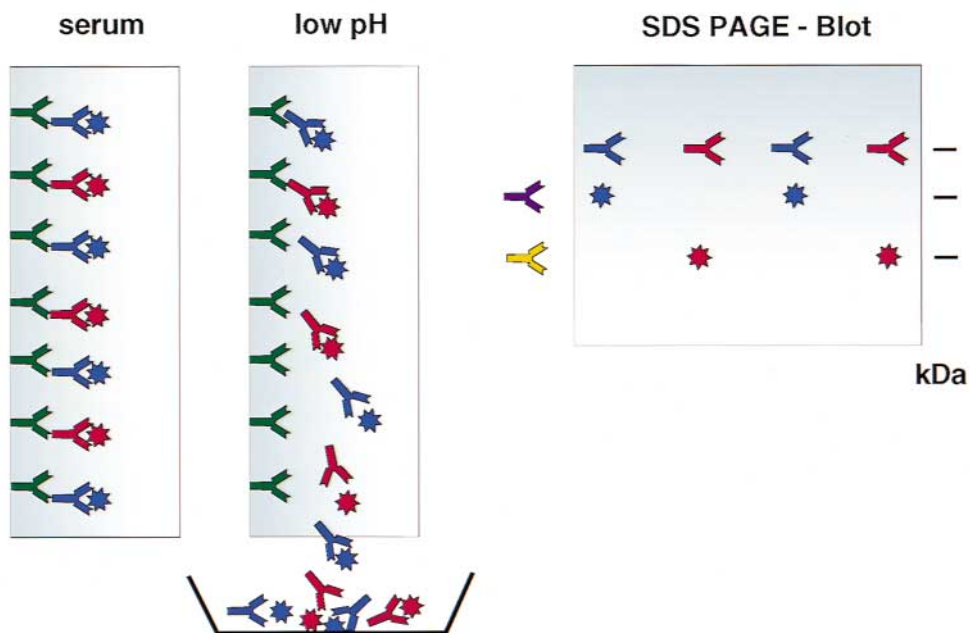


FIG 2. Detection of autoallergens in IgE isolates. IgE antibodies (red, blue) and bound autoallergens (red, blue asterisks) can be affinity purified from sera with use of immobilized monoclonal anti-human IgE antibodies (green). Bound IgE autoallergen immune complexes are eluted at low pH conditions and separated by SDS-PAGE. Autoallergens can then be detected in the nitrocellulose-blotted anti-IgE precipitates with specific antibody probes (violet, yellow).

ing event for the release of autoantigens (Fig 3, blue asterisks). In the context of a T_H2 -prone microenvironment, B cells will tend to produce de novo IgE antibodies against the released autoantigens. It is equally well possible that autoallergens that share similarities with environmental allergens will boost the production of crossreactive IgE antibodies.^{9,13,25}

It is well established that exposure to environmental allergens stimulates the rise of allergen-specific antibody production and increases the sensitivity to the given allergen.³⁴ Support for the assumption that release of autoallergens may also act as a stimulus for the production of IgE autoantibodies comes from the following observations. (1) Increases of IgE antibody levels to α -NAC were found in an AD patient during and shortly after exacerbation of his skin manifestations.¹³ (2) Increased IgE autoreactivity to nitrocellulose-blotted human proteins was found in AD patients who exhibited aggravation of their skin symptoms in response to contact with environmental allergens.²⁵ (3) Reduction of IgE antibody reactivities to human proteins were observed in an AD patient after successful treatment of his skin manifestations with cyclosporin A (Kinacyan et al, unpublished data). Should further clinical studies demonstrate

that IgE autoreactivity is paralleled by tissue damage, the measurement of IgE autoantibody levels may turn out to be a useful tool to measure the extent of tissue destruction occurring during the atopic immune response. Whether IgE autoimmunity can also play a role in the pathogenesis of allergic tissue inflammation has not yet been demonstrated but there are at least two possibilities. First, autoallergens may cross-link effector cells of atopy (eg, mast cells, Fig 3) and by the release of inflammatory mediators cause immediate-type symptoms. Second, IgE-mediated presentation of autoallergens via dendritic cells or monocytes may lead to T cell proliferation and cytokine release and thus induce delayed-type reactions (Fig 3).³⁰ Both events may take place at the site of autoallergen release but also at organ sites distant from the antigenic source because of the formation of immune complexes that can be transported by the vascular system (Fig 3, vessel). The latter assumption is supported by our finding that rabbit antisera raised against two autoallergens detected these autoallergens in anti-IgE precipitates of sensitized individuals.^{13,14}

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Mechanisms of autoallergy

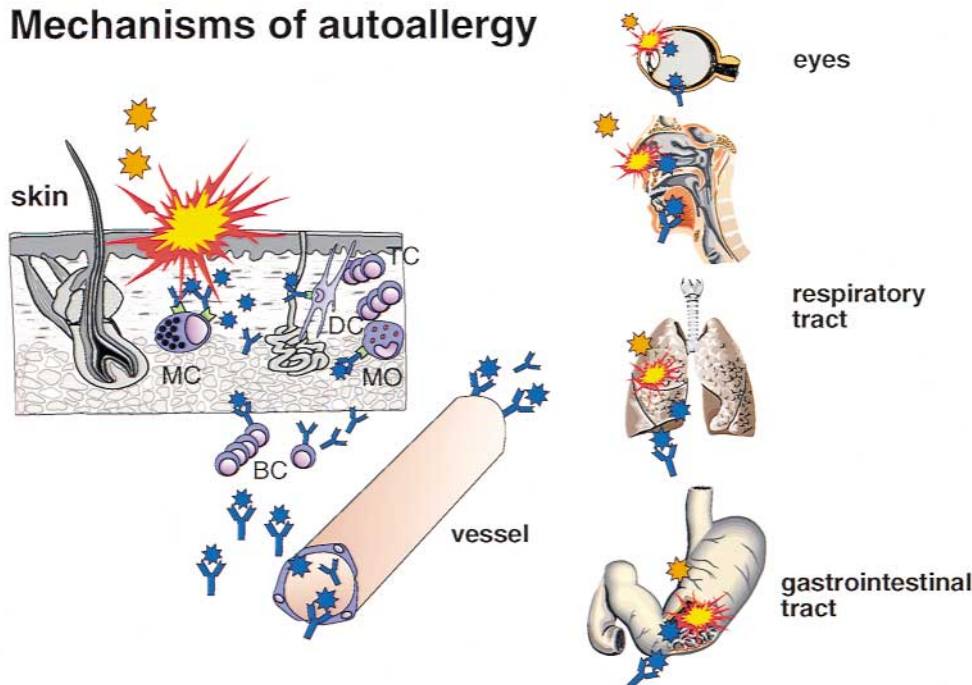


FIG 3. Mechanisms of autoallergy. In sensitized atopic individuals contact with exogenous allergens (orange asterisks) may lead to tissue damage and release of autoantigens (blue asterisks). Particularly in patients with a strong atopic predisposition a T_H2 -prone environment will allow B cells (BC) to produce IgE autoantibodies (blue) on contact with autoantigens. IgE autoantibodies with or without bound autoallergens can be distributed through the circulation (vessel) to cells containing receptors for IgE (eg, $Fc\epsilon R1$; green). Cross-linking of mast cell (MC)-bound IgE autoantibodies may cause release of biologic mediators. IgE-mediated presentation of autoallergens by dendritic cells (DC) or monocytes (MO) may lead to chronic T-cell (TC) activation.

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