

Autosensitization as a Pathomechanism in Asthma

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ABSTRACT: Recent observations indicate that autoimmune disorders and allergies are more closely connected to each other than traditionally considered. Both diseases are characterized by a break of immunological tolerance and in both Th1- and Th2-driven mechanisms may be involved. Moreover, patients suffering from severe chronic atopic diseases have been shown to develop autoreactive antibodies and vice versa autoreactive IgE antibodies may contribute to the perpetuation of allergic immune responses. However, animal models for the investigation of the underlying mechanisms have not been available so far. Here we demonstrate that allergic sensitization and subsequent airway aerosol challenge of mice to a potential autoantigen leads to an allergic airway inflammation and chronic exposure of sensitized mice to a foreign allergen induces autoreactive antibodies. Thus, autoreactive immune mechanisms may be induced either by molecular mimicry or as a consequence of chronic allergic inflammatory processes and these reactivities may then possibly contribute to the perpetuation of allergic immune responses even in the absence of exogenous allergens and to the development of autoimmune diseases.

KEYWORDS: allergy; autoimmunity; asthma; autosensitization; animal model; chronic inflammatory disease

AUTOIMMUNITY AND ALLERGY: COEXISTENCE OR EVEN MORE?

Autoimmune diseases and allergies have traditionally been considered as two distinct and independent disease entities mainly due to their assumed selective induction by T helper 1 (Th1) or Th2 cell activities, respectively.¹⁻⁴

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However, there is increasing evidence that autoimmune diseases and allergic disorders have more features in common than showing differences, and are therefore more closely connected to each other than formerly proposed. From the epidemiological point of view the prevalence of both disease entities show a paralleled steady increase over the last decades in the developed countries.^{5,6} Immunologically, both groups of disorders represent chronic inflammatory diseases characterized by a break of tolerance that only differs in the origin of the antigen. Whereas in autoimmune diseases tolerance is broken toward self-proteins, in allergies an inflammatory immune response is generated toward foreign antigens—in this case termed allergens—that are normally recognized as harmless. This indicates that mechanisms responsible for the development of immunological tolerance toward the respective antigens are not properly developed. This scenario becomes dramatically evident in patients suffering from the IPEX (immune dysregulation polyendocrinopathy, enteropathy, and X-linked inheritance) or XLAAD (X-linked autoimmunity-allergic dysregulation) syndromes, which develop severe autoimmune reactivities and features of allergic diseases including significantly increased IgE levels and eosinophil-dominated inflammatory reactions at the same time.^{7,8} These syndromes are based on functionally active mutations in the gene for the transcription factor Foxp3 that essentially controls the activity of regulatory T cells.⁹ These cells are known to play an important role in the control of Th1- as well as Th2-driven immune responses, and are therefore involved in the generation of immunological tolerance.¹⁰ Moreover, more recent investigations have shown that autoimmune diseases are not restricted to Th1-dependent mechanisms but may also occur due to autoreactive Th2 cell activities.¹¹ On the other hand, chronic manifestations of allergic diseases (e.g., atopic dermatitis, severe bronchial asthma) have been shown to have a mixture of Th2 and Th1 cytokine production.^{12–14} Furthermore, patients with chronic atopic diseases may mount IgE autoantibody responses. For example, more than 60% of patients suffering from severe atopic dermatitis develop autoreactive IgE antibodies¹⁵ and histamine-releasing autoantibodies could be detected in sera from about 15% of patients with chronic urticaria. In the latter case, 30–60% of these antibodies were directed against the α -chain of the high-affinity IgE receptor on mast cells.¹⁶ Other IgE-reactive autoantigens include squamous cell carcinoma antigen recognized by T cells, cytokeratin type II, the α -chain of the nascent polypeptide-associated complex (α -NAC), the calcium-binding protein Hom s4, cyclophilins, and manganese superoxide dismutase.^{17–22} All these proteins belong to families with phylogenetically highly conserved sequences and structures and were therefore formerly termed pan-allergens. Further evidence came from an animal study, where autoimmunity was induced toward a peptide of a self-antigen and animals developed autoimmunity together with allergic features after subsequent immunization with this antigen finally resulting in an anaphylactic shock to this selfpeptide.²³

Even though IgE recognition of autoantigens may contribute to inflammatory tissue damage in these patients, it is still unclear whether autoreactive immune responses are just accompanying or contributing to chronic allergic immune responses. It has been speculated that cross-reactivity against autoantigens may contribute to the perpetuation of severe atopic disorders, especially in times of absence of exogenous allergen exposure. Vice versa, it is unknown whether Th1 activities in patients with chronic allergic disorders may be linked to an allergic immune response against self.

The discovery of autoreactive components in atopic diseases leads to the question about the underlying pathomechanisms mainly with regard to the mechanisms of autosensitization. Two major possibilities have to be taken into account. Due to the high sequence homology of a variety of proteins to which autoreactive antibodies have been shown, the autoimmune component may be the result of cross-reactivity between the exogenous antigen and a corresponding self-protein. Alternatively, the development of an autoimmune component may be the result of the ongoing chronic inflammatory process that causes tissue damage and necrosis. This may lead to the exposure of hidden intracellular antigens. In the context of the present proinflammatory signals providing adequate costimulation, an autoimmune response against these self-antigens may develop. This autoimmune response is then expected to serve as an amplifier of the allergic inflammation.

To prove these concepts and to further investigate the underlying mechanisms, appropriate animal models are necessary. Based on long-lasting experience in the development of mouse models of allergic bronchial asthma, we adapted well-established models for the investigation of these new questions. Moreover, most human data were derived from patients suffering from severe atopic dermatitis, whereas the relationship between autoimmune and allergic reactivities in patients with other chronic atopic diseases, such as chronic allergic asthma, is less intensively investigated. Therefore, further insights into these interactions should be obtained by use of experimental allergic asthma models.

ALLERGIC AIRWAY INFLAMMATION TO A POTENTIAL AUTOANTIGEN

Our first approach was chosen to investigate inflammatory reactions in sensitized animals toward an already known potential autoantigen.²⁴ As mentioned above, a variety of antigens have been described in human atopic dermatitis patients to which an autoreactive IgE response is directed. Among them is the α -NAC protein, which acts as a transporter protein for newly evolved intracellular proteins. The α -NAC is a highly conserved protein from yeast to human beings and therefore the human α -NAC shows a high degree of sequence and

structural similarities to the corresponding mouse protein. Despite this fact, an allergic sensitization of Balb/c mice in the presence of aluminum hydroxide as adjuvant resulted in the induction of an α -NAC-specific T cell response as indicated by a strong proliferative response of *in vitro* α -NAC-stimulated mononuclear spleen cells. Additionally, human α -NAC-specific antibodies of the subclasses IgE, IgG1, and IgG2a, indicative for Th2 (IgE, IgG1) as well as Th1 (IgG2a) immune responses, were found to be induced. Interestingly, human α -NAC-specific IgE antibodies also reacted with linear peptide epitopes in the genuine autoantigen murine α -NAC; even in sequence regions where both proteins, human and mouse α -NAC, were completely identical. Further evidence for the cross-reactivity of human α -NAC-specific antibodies came from the observation that IgG antibodies from human α -NAC-immunized mice were able to bind to murine α -NAC protein derived from mouse skin and lung tissue samples, and preincubation of the respective sera with recombinant human α -NAC was able to inhibit this autoreactivity. Moreover, these cross-reactive antibodies have been proven to be functionally active since mice immunized solely to the human α -NAC protein developed immediate cutaneous hypersensitivity reaction to the human—as expected—but also to the murine α -NAC protein. Most interestingly, intranasal challenges of human α -NAC immunized mice induced a pronounced peribronchial and perivascular inflammation in the airways. Analysis of the bronchoalveolar lavage of these animals revealed significantly increased numbers of neutrophils and eosinophils. These inflammatory changes finally resulted in an increased airway hyperresponsiveness to methacholine, which represents an important feature of allergic bronchial asthma.²⁴ Since α -NAC is an intracellular antigen that is normally not accessible to the immune system, it is speculated that release of intracellular proteins in the course of tissue damage, e.g., as consequence of an allergic inflammatory reaction, is necessary to induce the generation of an autoreactive immune response against this and perhaps other autologous antigens.

Taken together these data demonstrate that the human α -NAC protein represents a prototypic foreign allergen to which mice can be sensitized. The adaptive immune response to this protein shows cross-reactivity to the corresponding self-antigen murine α -NAC. Moreover, sensitization to this antigen induces at the same time components of an allergic immune response and an immune reactivity against self, thus representing a model of autoallergy. It is noteworthy that Th2 and Th1 immune responses coexist in this model as characterized by the simultaneous occurrence of the different immunoglobulin isotypes and the mixed phenotype of the airway inflammation, which resembles in many aspects the situation in human patients where a shift from Th2-dominated to a rather mixed phenotype is a hallmark of severe and chronic manifestations of atopy. For example, it is well known that chronic skin lesions of atopic dermatitis patients contain a mixed population of Th2 and Th1 cells^{12,13} and the bronchial inflammation in patients with severe chronic

asthma is characterized by the simultaneous occurrence of eosinophils and neutrophils in the inflammatory infiltrate.^{25,26}

INDUCTION OF AUTOANTIBODIES BY ACUTE AND CHRONIC ALLERGIC AIRWAY INFLAMMATION

To test the hypothesis that autoreactive immune mechanisms may be induced in the course of an ongoing allergic inflammatory response, we investigated the occurrence of autoreactive antibodies in an established experimental mouse model of allergic bronchial asthma toward ovalbumin (OVA) as a model foreign allergen. This model was based on an allergic sensitization of Balb/c mice, which was achieved by intraperitoneal application of the allergen in the presence of the Th2-driving adjuvant aluminum hydroxide. Following OVA aerosol-challenged mice developed typical signs of an acute allergic inflammatory response in the lungs characterized by a peribronchial and perivascular infiltrate mainly consisting of eosinophils, goblet cell hyperplasia, and increased mucus production, increased total cell and eosinophil numbers as well as Th2 cytokine concentrations in the bronchoalveolar fluid and airway hyper-reactivity to methacholine.²⁷ To mimic the situation in patients suffering from chronic bronchial asthma more closely we recently adapted this model toward a chronic model by extending the challenge phase over a period of 12 weeks with two challenges on consecutive days per week. Lungs of mice that underwent this protocol showed a different inflammatory pattern in the airways dominated by a mixed cellular infiltrate consisting mainly of lymphocytes, eosinophils, and neutrophils, which was present not only in the proximal large airways, but also in the distal small airways as it is observed in human patients. Goblet cell hyperplasia and airway hyperresponsiveness were still present and, most importantly, typical signs of airway remodeling, such as significantly thickened lamina propria, collagen deposition, and occurrence of α -smooth muscle, actin-positive myofibroblasts became evident.²⁸ Airway remodeling is considered to be a consequence of tissue destruction due to sustained inflammatory processes. Thus, having these two models of acute and chronic allergic airway inflammation available we could investigate the influence of a chronic allergic inflammatory process on the development of autoreactive antibodies. Therefore, we investigated the sera of mice from the different treatment groups in comparison to sham-sensitized animals (10 mice per group) using the technique of indirect immunofluorescence on a standard cell (HEp-2 cells) and rat tissue (kidney, stomach, liver, and heart) array (Euroimmun, Lübeck, Germany)—as it is used for human autoimmune diagnostics—with adaption for the analysis of murine IgG. As summarized in TABLE 1, allergic sensitization and acute lung challenge with a foreign antigen already induced autoreactive IgG antibodies in a remarkable number of animals. However, the

TABLE 1. Number of acute and chronically allergen-exposed mice with positive autoreactive IgG staining patterns

Substrate	Staining pattern	PBS	OVA acute	OVA chronic
HEp-2 cells	Vimentin-like	0	5	9
	Pearl-string	0	3	1
Kidney	Vessels	1	6	8
	Glomeruli	0	3	3
Stomach	Muscles	1	7	7
	Vessels	0	6	4
	Plexus	1	3	6
	Parietal Cells	1	3	7
Liver	Bile ducts	2	8	4
	Dotted pattern	0	4	8
Heart	Diffuse pattern	0	6	6
Total number		6	54	63

NOTE: Ten Balb/c mice in each group were either sham-sensitized (PBS) or sensitized to OVA and subsequently aerosol-challenged for 1 week (OVA acute) or 12 weeks (OVA chronic), respectively. Autoreactive IgG antibodies were detected using mouse IgG-adapted indirect immunofluorescence on an HEp-2 cell and rat tissue array (Euroimmun). The number of sera showing a positive reactivity with the given staining pattern at the respective substrates is recorded.

number of animals showing different autoreactivities and also the intensity of the observed staining patterns (data not shown) was further increased after chronic allergen challenge of the animals. This observation was further proven by Western blot analyses where the reactivity of sera was tested toward blotted whole cellular protein from the murine T-cell line EL-4. Whereas sera from sham-sensitized and sensitized and acutely challenged animals did not show any reactivity, individual sera from mice chronically exposed to the allergen for at least 8 weeks revealed the occurrence of autoreactive IgG antibodies (data not shown). Unfortunately, the detection system was not sensitive enough to demonstrate autoreactive antibodies of the IgE isotype. Finally, by ELISA analysis we were able to show that sera from chronically exposed animals were reactive to whole proteins from different mouse cell lines of different origins (EL-4, NIH-3T3) and, interestingly, also α -NAC-reactive antibodies could be detected.

SUMMARY AND CONCLUSION

Taken together, using mouse models of allergic bronchial asthma our data clearly indicate that allergic sensitization and/or airway challenge may lead to break of tolerance against self-proteins. Autoreactive immune cells and antibodies may be induced either by molecular mimicry or as consequence of chronic allergic inflammatory processes. Moreover, challenge of airways with potentially cross-reactive antigens leads to an inflammatory reaction with

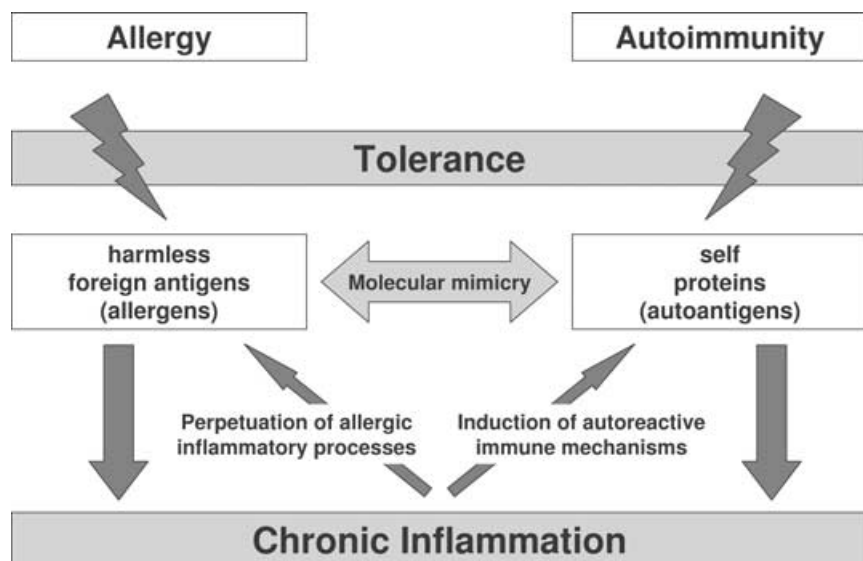


FIGURE 1. Current concept of the relationship between allergic inflammatory disorders and autoimmune diseases.

allergic components, such as eosinophilia and airway hyperresponsiveness. On the basis of the now available animal models future investigations may address the questions whether autoreactive antibodies and T cells really contribute to a sustained allergic inflammation even in times of allergen absence and whether chronic allergic inflammation supports the development of autoimmune diseases (see Fig 1).

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