

Induction of autoallergy with an environmental allergen mimicking a self protein in a murine model of experimental allergic asthma

Robert Bänder, MD,^{a*} Irene Mittermann, PhD,^{b*} Udo Herz, PhD,^a Margit Focke, PhD,^b Michael Wegmann, BS,^a Rudolf Valenta, MD,^{b,‡} and Harald Renz, MD^{a,‡}
Marburg, Germany, and Vienna, Austria

Background: Allergy and autoimmunity are traditionally considered as 2 exclusive entities related to the development of either T_H2-dominated or T_H1-dominated immune responses. **Objective:** This study investigated whether allergic sensitization to a foreign antigen mimicking a self protein can induce allergy accompanied by an autoimmune response. **Methods:** BALB/c mice were sensitized to human α -NAC, an evolutionary conserved component of the nascent polypeptide-associated complex, recently identified as an IgE-reactive autoantigen in patients with severe forms of atopy. By using nitrocellulose-blotted murine lung and skin extracts, purified recombinant human as well as murine α -NAC and murine α -NAC-derived synthetic peptides, the IgE, IgG1, and IgG2a antibody responses were measured, and their epitope specificity was mapped.

Results: Cross-reactivity of IgE and IgG antibodies with murine α -NAC was found in mice sensitized with human α -NAC. The biological relevance of the antibody response was demonstrated by the induction of immediate skin reactions in sensitized mice and by the fact that skin sensitivity could be passively transferred with serum to naive mice. Antigen challenge of sensitized mice resulted in airway inflammation accompanied by eosinophil and neutrophil accumulation, airway hyperresponsiveness to methacholine and perivascularitis of lung veins.

Conclusion: Our data demonstrate that sensitization with a foreign antigen mimicking self can induce an allergic immune response of a mixed T_H2 and T_H1 profile that is associated with autoreactivity. Cross-sensitization to self may represent an important pathomechanism involved in the maintenance of severe and chronic forms of allergy. (*J Allergy Clin Immunol* 2004;114:422-8.)

Key words: Autoallergy, antigens, peptides, epitopes, autoantibodies, lung inflammation, cross-reactivity

From ^athe Department of Clinical Chemistry and Molecular Diagnostics, University of Marburg; and ^bthe Department of Pathophysiology, Medical University of Vienna.

*These authors contributed equally to this work.

‡These authors contributed equally to this work.

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Reprint requests: Harald Renz, MD, Philipps-University Marburg, Department of Clinical Chemistry and Molecular Diagnostics, Central Laboratory, Baldinger Strasse, 35033 Marburg, Germany. E-mail: renzh@med.uni-marburg.de.

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Abbreviations used

HSA: Human serum albumin

ICHs: Immediate cutaneous hypersensitivity

NAC: Nascent polypeptide-associated complex

UK: United Kingdom

Allergy and asthma have been traditionally considered as primarily IgE antibody-mediated and T_H2 T-cell-mediated diseases,¹⁻³ whereas many autoimmune diseases resemble a classical T_H1 phenotype.^{1,2,4} More recent evidence suggests that chronic manifestations of atopy (eg, atopic dermatitis, asthma) are accompanied by a mixture of T_H1 and T_H2 cytokine production.⁵⁻⁷ Recent evidence indicates that several allergens show a high degree of similarity in sequence and structure with self proteins.⁸ In this context, it has been demonstrated that patients allergic to birch pollen profilin and fungal allergens exhibited IgE cross-reactivity against the homologous self proteins.⁹⁻¹²

Furthermore, patients with chronic manifestations of atopy mount IgE autoantibody responses against self proteins.¹³ Several of these have been identified and cloned by using the patients' IgE autoantibodies.^{14,15} These IgE-reactive autoantigens include squamous cell carcinoma antigen recognized by T cells,¹⁴ cytokeratin type II,¹⁵ and the alpha chain of the nascent polypeptide-associated complex (α -NAC),^{15,16} a highly conserved protein from yeast to human beings.¹⁷ Although IgE recognition of autoantigens reflects tissue damage in these patients, it has remained unclear whether IgE auto-reactivity is just accompanying or contributes to the pathogenesis of atopic dermatitis.^{15,18,19} Furthermore, it is unknown whether the T_H1 component of the immune response in patients with chronic atopy may be linked to an allergic immune response against self. This study was aimed to develop an animal model to test the concept of cross-reactivity between allergens and self proteins.

METHODS

Expression and purification of recombinant human and murine α -NAC

Recombinant human α -NAC was expressed as described.¹⁶ For expression of murine α -NAC, RNA was prepared from mouse skin by using a NucleoSpin RNA II extraction kit (Macherey-Nagel, Dueren, Germany). cDNA was synthesized by using a SuperScript One-Step

TABLE I. IgE, IgG1, and IgG2a cross-reactivity of human and murine α -NAC-immunized mice

Reactivity of sera from mice immunized with	Human α -NAC	Murine α -NAC	HSA
IgE reactivity			
Human α -NAC (n = 10)	0.22 $\pm$ 0.007	0.214 $\pm$ 0.003	0.039 $\pm$ 0.004
Murine α -NAC (n = 5)	0.096 $\pm$ 0.02	0.148 $\pm$ 0.06	0.036 $\pm$ 0.004
PBS (n = 10)	0.039 $\pm$ 0.002	0.035 $\pm$ 0.004	0.036 $\pm$ 0.001
IgG1 reactivity			
Human α -NAC (n = 10)	>2.5	>2.5	0.045 $\pm$ 0.007
Murine α -NAC (n = 5)	0.83 $\pm$ 0.03	0.32 $\pm$ 0.08	0.05 $\pm$ 0.004
PBS (n = 10)	0.034 $\pm$ 0.002	0.046 $\pm$ 0.008	0.031 $\pm$ 0.001
IgG2a reactivity			
Human α -NAC (n = 10)	1.064 $\pm$ 0.16	1.804 $\pm$ 0.009	0.032 $\pm$ 0.002
Murine α -NAC (n = 5)	0.38 $\pm$ 0.05	0.16 $\pm$ 0.02	0.06 $\pm$ 0.002
PBS (n = 10)	0.027 $\pm$ 0.002	0.029 $\pm$ 0.001	0.029 $\pm$ 0.001

Mean optical density values $\pm$ SDs are displayed. Antibody levels of NAC-immunized mice are significantly higher than those ($P < .01$) from PBS-immunized mice.

RT-PCR kit (Invitrogen, Paisley, United Kingdom [UK]) with murine α -NAC specific primers: Nde I-NAC, 5'GGGAGTTC CATATGCCCGGTGAAGCCACAGAA 3'; and Eco R I-NAC, 5'CGGAAT7CTAGTGGTGGTGGTGGTGCATTGTTAATT-GCATTGTTAATCCATAATAGCATT3'. The Nde I and Eco R I sites are underlined and italicized, respectively. The RT-PCR product was cut with Nde I/Eco R I, gel-purified, and subcloned into plasmid pET-17b (Novagen, Cambridge, UK). The correct sequence of the construct was confirmed by double-strand DNA sequencing (MWG Biotech AG, Ebersberg, Germany). Recombinant human α -NAC or murine α -NAC was expressed in *Escherichia coli* BL21 (DE3) (Stratagene, La Jolla, Calif) and purified via Nickel affinity chromatography.¹⁶ Purity of proteins was checked by SDS-PAGE.²⁰ Protein concentrations were determined by Micro BCA Protein Assay (Pierce, Rockford, Ill). The endotoxin content of the purified proteins was determined by the limulus-amoebocyte-lysate assay (Bio-Whittaker, Walkersville, Md). An endotoxin content of less than 50 endotoxin units/milligram protein was measured for the recombinant human and murine α -NAC. The endotoxin content of the recombinant proteins was thus lower than that found in allergen extract-based vaccines.²¹

Synthesis and purification of peptides

Twenty-one peptides (15-mers with 5-amino acid overlap) covering the sequence of the murine α -NAC protein were synthesized by using a 9-fluorenyl-methoxy-carbonyl strategy with (2-[1H-benzotriazol-1-yl]1,3,3 tetramethyluronium hexafluorophosphate activation on the Applied Biosystems (Foster City, Calif) peptide synthesizer 433A. Preloaded 9-fluorenyl-methoxy-carbonyl p-hydroxymethylphenoxymethyl polystyrene resins (0.50-0.70 mmol/g) were used as solid phase to build up the peptides. Coupling of amino acids was confirmed by conductivity monitoring. Peptides were cleaved from the resins with 250 μ L distilled water, 250 μ L triisopropylsilan, and 5 mL trifluoroacetic acid for 2 hours and precipitated in tert-butyl methyl ether. Peptides were checked by mass spectrometry and purified by preparative HPLC (piChem, Graz, Austria).

Protocol of sensitization

Pathogen-free female BALB/c mice 6 to 8 weeks old were sensitized with 10 μ g human α -NAC adsorbed to aluminum hydroxide (1.5 mg) by intraperitoneal injections at days 1, 14, and 21. Control mice were sham-sensitized with PBS and aluminum hydroxide (1.5 mg).²² Skin tests were performed at day 24. All mice received 10 μ g human α -NAC intranasally in 50 μ L PBS at days 26

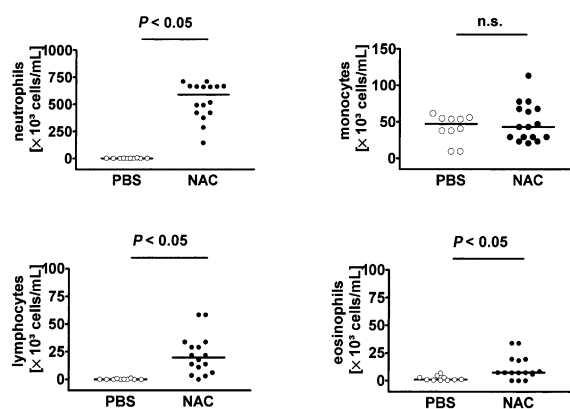


FIG 1. Leukocyte distribution in bronchoalveolar lavage fluids. Bronchoalveolar lavage was performed in PBS (sham; n = 10) and human α -NAC-sensitized mice (n = 16). Depicted are the absolute numbers of eosinophils, neutrophils, macrophages, and lymphocytes per mouse. Circles, PBS mice; black dots, human α -NAC-sensitized mice. Depicted is the median of each group.

and 27. Airway responsiveness to methacholine was assessed 24 hours later. Forty-eight hours later, bronchoalveolar lavage was analyzed. Humoral cross-reactivity between human and murine α -NAC was tested in BALB/c mice, which were sensitized with 10 μ g murine α -NAC adsorbed to aluminum hydroxide (1.5 mg) at days 1, 14, and 21. To test the contribution of adjuvant, selected BALB/c mice were sensitized in the absence of adjuvant.

Antibody measurements

IgE, IgG1, and IgG2a levels in PBS, human or murine α -NAC-sensitized mice were measured by ELISA.²³ Ninety-six-well ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with human α -NAC (5 μ g/mL), murine α -NAC (5 μ g/mL), or 15-mer peptides spanning the murine α -NAC sequence (5 μ g/mL). Antibody isotypes and subclasses were analyzed.²³

Proliferation assay

Mononuclear cells were prepared from spleens of immunized and control mice, washed in RPMI 1640, and cultured in triplicates (2.0×10^5 per well) of 96-well culture plates in culture medium (RPMI 1640 with 5% FCS, 100 μ g/mL penicillin-streptomycin, and 2-mmol/L L-glutamine). Cells were stimulated with either 10 μ g/mL human

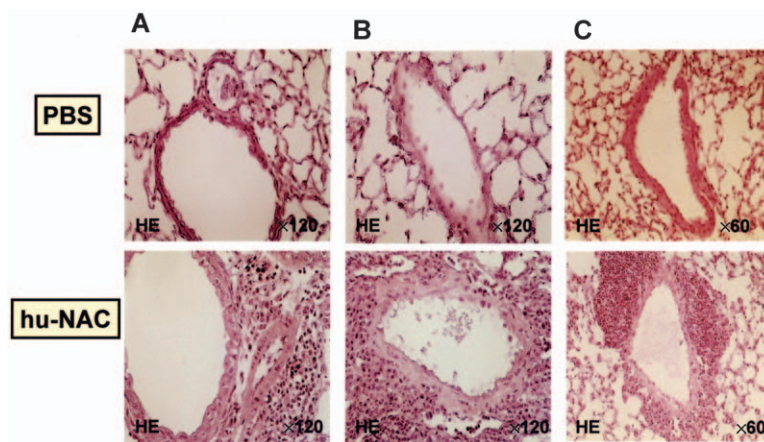


FIG 2. Lung histology. Histologic analysis of representative lung sections obtained from human α -NAC-sensitized and human α -NAC-challenged as well as PBS-treated and human α -NAC-challenged mice. **A**, Peribronchitis. **B** and **C**, Vasculitis of lung veins. HE, Hematoxylin and eosin.

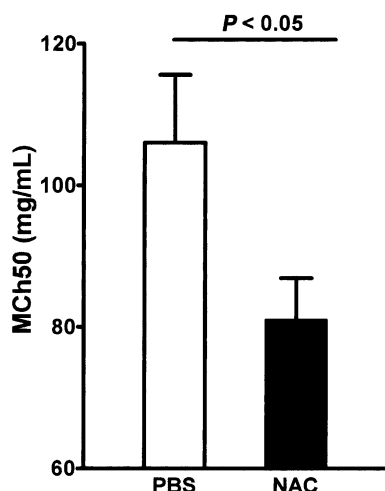


FIG 3. Assessment of airway reactivity. Airway reactivity to methacholine was assessed by head-out body plethysmography. Bars represent the concentration of methacholine causing a 50% reduction in expiratory airflow (MCh50). White bar, PBS mice, $n = 9$; black bar, human α -NAC-immunized mice, $n = 11$. Depicted are medians $\pm$ SDs.

Measurement of airway reactivity

Head-out body plethysmography was performed 24 hours after the last allergen/sham challenge. Airway reactivity was measured in spontaneously breathing mice simultaneously during methacholine aerosol exposure.²² Baseline values were determined for 15 minutes. Each dose of methacholine (up to 200 mg/mL) was aerosolized for 2 minutes. The next higher dose was administered once the respiration values had returned to baseline. The methacholine concentration that caused a 50% reduction in expiratory airflow was calculated.

Inhibition and competition experiments

For inhibition experiments, nitrocellulose membranes containing murine skin and lung proteins extracts were prepared. Skin and lung tissues were removed from naive mice, homogenized in SDS-sample buffer, and boiled for 10 minutes. To remove insoluble particles, extracts were centrifuged at 14,000 rpm for 10 minutes at 4°C. Approximately 100 μ g protein extract per centimeter of gel was separated by preparative 12.5% SDS-PAGE and blotted onto nitrocellulose (Schleicher & Schuell, Dassel, Germany). Sera from human α -NAC-sensitized mice were diluted 1:1000 in buffer (50-mmol/L sodium phosphate, pH 7.5, 0.5% wt/vol BSA, 0.5% vol/vol Tween-20, 0.05% wt/vol NaN_3) and preincubated with either recombinant human α -NAC (5 μ g/mL serum dilution) or, for control purposes, with human serum albumin (HSA; 5 μ g/mL serum dilution; Aventis, Frankfurt, Germany) overnight at 4°C. The nitrocellulose stripes were then incubated with the human α -NAC or HSA-preincubated mouse immune sera overnight at 4°C. After washing, bound IgG antibodies were detected with ¹²⁵I-labeled sheep anti-mouse IgG antibodies (Amersham, Buckinghamshire, UK). Nitrocellulose stripes were washed, dried, and exposed to autoradiography films at -70°C for 24 hours.

In addition, IgE competition studies were performed by ELISA inhibition assay. Sera from human and murine α -NAC-sensitized mice were diluted 1:10 in PBS containing 0.5% wt/vol bovine serum albumin and 0.05% vol/vol Tween and were preincubated with recombinant human or murine α -NAC (30 μ g/mL serum dilution) and, for control purposes, with an equal amount of recombinant Bet v 1 or buffer alone overnight at 4°C. Recombinant human or murine α -NAC-coated (5 μ g/mL) plates were exposed to the preincubated sera, and bound IgE was detected.²³

α -NAC or 2.5 μ g/mL concanavalin A and cultured for 4 days at 37°C in a 90% humidified air, 10% CO_2 atmosphere. Twenty-four hours before harvesting, ³H-methylthymidine was added to the cultures (0.5 μ Ci per well). Cells were harvested onto fiberglass filters, and incorporated ³H-methylthymidine was measured by scintillation counting.

Bronchoalveolar lavage and lung histology

Lungs were rinsed twice, each time with 800 μ L fresh PBS,^{24,25} and obtained cells were counted in the Neubauer chamber. Fifty microliter aliquots of lavage fluids were spun down on an object slide, and cells were microscopically differentiated. For analysis of lung inflammation, lungs were fixed with 4% formaldehyde (wt/vol). The lung was removed and stored in 4% formaldehyde. Paraffin-embedded sections, 3 μ m, were stained with hematoxylin and eosin.²⁵



FIG 4. Alignment of the amino acid sequences of human α -NAC and murine α -NAC and IgE epitope mapping. Amino acids in the murine α -NAC sequence that are identical to human α -NAC are indicated by *dashes*. Every tenth amino acid is indicated by an *asterisk*. IgE-reactive mouse α -NAC-derived 15-mer peptides are *boxed*.

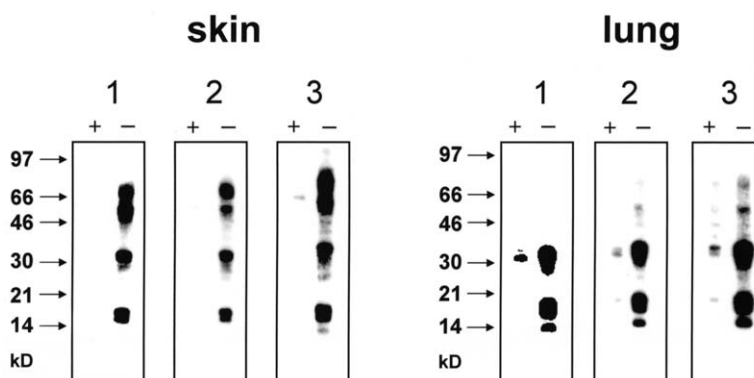


FIG 5. Recombinant human α -NAC inhibits IgG autoreactivity to skin-derived and lung-derived murine α -NAC. Sera from 3 human α -NAC-sensitized mice (1, 2, 3) were preabsorbed with recombinant human α -NAC (lanes+) or HSA (lanes-) and probed with nitrocellulose-blotted mouse skin and lung extracts. Molecular weights (kd) are displayed in the *left margin*.

Immediate cutaneous hypersensitivity reactions in human α -NAC-sensitized mice

Mice received 100 μ L 0.5% (wt/vol) Evans blue solution by intravenous injection. Intracutaneous skin tests were performed with 50- μ L aliquots of compound 48/80 (5 μ g/mL; positive control), PBS (negative control), or human α -NAC (5 μ g/mL).²⁵ Fifteen minutes later, vascular leakage was assessed on inverted skin by a blinded investigator. Indicated are numbers of mice that developed blue flare reactions $\geq$ 3 mm in diameter.²⁵

Assessment of immediate cutaneous hypersensitivity reactions in passively sensitized mice

Naive BALB/c mice were shaved on the back, followed by intracutaneous injections (50 μ L each) of 1:2 PBS-diluted sera from human α -NAC-sensitized or control mice. Two hours later, mice

received 100 μ L 0.5% (wt/vol) Evans blue solution by intravenous injection. Skin tests were performed at the site of sera transfer.²²

RESULTS

Mice sensitized with human α -NAC develop allergic lung inflammation indicative of a mixed T_H2/T_H1 immune response

T_H2 high-responder BALB/c mice were immunized with aluminum hydroxide-adsorbed human α -NAC by using a standard sensitization protocol.²² Human α -NAC-sensitized mice developed a humoral and cellular immune response against human α -NAC, as shown by a strong proliferation response of splenic mononuclear cells. The median counts per minute (cpm) of PBS-immunized mice ($n = 14$) was 732 cpm, versus 11215 cpm in human α -NAC-immunized mice ($n = 13$).

TABLE II. ICHS reactions

Active sensitization with	Positive ICHS reaction to		Human α -NAC	Murine α -NAC
	PBS	Compound 48/80		
Human α -NAC (n = 11)*	0/11	11/11	11/11	ND
PBS (n = 7)	0/7	7/7	0/7	ND
Passive sensitization with sera of human NAC-immunized mice				
ICHS after 2 hours				
Human α -NAC (n = 8)†	0/8	8/8	8/8	8/8
PBS (n = 8)†	0/8	8/8	0/8	0/8
ICHS after 2 days				
Human α -NAC (n = 3)‡	0/3	3/3	3/3	3/3
PBS (n = 5)‡	0/5	5/5	0/5	0/5

ND, Not done.

*Human α -NAC and sham (PBS)-immunized mice were tested for ICHS to human α -NAC. PBS served as negative and compound 48/80 as positive control. Naive mice were passively sensitized with sera from human α -NAC or PBS control mice. ICHS was tested to human α -NAC and murine α -NAC after †2 hours and ‡2 days as described in Methods.

Human α -NAC-specific antibodies indicative of a T_H2 (ie, IgE/IgG1) as well as of a T_H1 (IgG2a) immune response ($P < .01$) were induced. NAC-specific antibodies were absent in PBS-sensitized mice (Table I) and in the preimmune sera (data not shown). Furthermore, human α -NAC-sensitized mice developed airway inflammation after intranasal challenges with human α -NAC. This inflammatory response was characterized by the influx of eosinophils and lymphocytes and by a high number of neutrophils in bronchoalveolar lavage fluids of human α -NAC-sensitized but not in sham-sensitized and human α -NAC-challenged mice. The numbers of macrophages were similar in both groups (Fig 1).

The lung histology confirmed the airway inflammation, which was dominated by an influx of neutrophils followed by lymphocytes and eosinophils into the bronchial mucosa of human α -NAC-sensitized and airway allergen-challenged animals (Fig 2, A). In addition, a strong perivascularitis was present around larger and middle-sized lung veins (Fig 2, B and C). This inflammatory response was accompanied by airway hyperresponsiveness to methacholine, as indicated by a decrease of the methacholine dose causing a 50% reduction of the expiratory airflow as assessed by head-out body plethysmography in human α -NAC-sensitized and intranasally challenged animals (Fig 3).

Sensitization of mice with human α -NAC in the absence of adjuvant resulted also in an IgG1, IgG2a, and IgE antibody reactivity to human α -NAC indicative of a mixed T_H2/T_H1 immune response: the mean optical density values $\pm$ SDs were 0.95 ± 0.21 for IgG1, 0.476 ± 0.14 for IgG2a, and 0.14 ± 0.04 for IgE, compared with 0.07 ± 0.003 , 0.07 ± 0.004 , and 0.07 ± 0.003 , respectively, for PBS-immunized mice.

Sensitization with human α -NAC induces a cross-reactive immune response to both foreign and self NAC

B-cell epitope mapping for IgE reactivity was performed with 21 overlapping 15-mer peptides spanning the

sequence of the murine α -NAC. This analysis revealed sequential IgE-reactive B-cell epitopes at the N-terminus and C-terminus of murine α -NAC. Two of the 3 IgE-reactive 15-mer peptides (murine α -NAC aa 2-16 and aa 152-166) showed complete sequence identity between human and murine α -NAC, and the third IgE-reactive peptide (murine α -NAC aa 142-156) differed in only 2 amino acids from the human α -NAC sequence (Fig 4).

Data in Table I demonstrate that sera from human α -NAC sensitized mice contained IgE antibodies, which react with human and murine α -NAC.

Sensitization with murine α -NAC induced considerably lower antibody levels (IgE, IgG1, IgG2a) than sensitization with human α -NAC (Table I). Cross-reactivity of the IgE antibodies was confirmed by IgE ELISA competition experiments demonstrating that preincubation of sera from human α -NAC-sensitized mice with murine α -NAC, but not with an irrelevant antigen or buffer alone, caused a more than 70% inhibition of IgE binding to human α -NAC (data not shown). Cross-reactivity of the induced antibody response was also demonstrated by the fact that preincubation of sera from human α -NAC-sensitized mice with human α -NAC inhibited IgG antibody reactivity to skin and lung tissue-derived murine α -NAC (Fig 5).

The biological relevance of the induced immune response was investigated by testing for immediate cutaneous hypersensitivity (ICHS). In mice sensitized to human α -NAC, ICHS responses were elicited by intradermal administration of human α -NAC (Table II). Control mice sham-sensitized with PBS did not respond to human α -NAC, and PBS administration failed to induce a positive skin response in human α -NAC-sensitized mice. Furthermore, the ICHS response is induced with murine α -NAC in naive mice passively sensitized with sera from human α -NAC-sensitized mice after 2 hours and after 2 days, the latter indicating an IgE-mediated ICHS. Passively sensitized mice showed no cutaneous reactivity to PBS (negative control), and all mice responded to compound 48/80 (positive control) (Table II).

DISCUSSION

We demonstrate that allergic sensitization to a foreign protein with homology to a self antigen induces an allergic immune response and an immune response against self. Our study is the first describing the induction of autoallergy via cross-reactivity in an experimental animal model. The following arguments support the assumption that molecular mimicry/cross-reactivity is the underlying pathogenetic mechanism for this process. First, it is demonstrated that IgE as well as IgG antibodies recognizing identical 15-mer peptides on the foreign and self protein are induced by sensitization to the foreign protein. Second, it is shown that IgE as well as IgG antibodies exhibit cross-reactivity between the foreign protein and the self antigen. The biological significance of this humoral immune response is documented by *in vivo* elicitation of immediate hypersensitivity reactions by both the foreign and the self antigen in mice sensitized only to the foreign protein. Human α -NAC and murine α -NAC belong to a family of highly conserved eukaryotic proteins sharing a more than 90% sequence identity.^{16,17} Therefore, it may be assumed that the induction and maintenance of autoimmune responses escape the surveillance of the immune system, allowing the adaptive immune system to mount a specific cellular and humoral response against self. The fact that immunization of mice with human α -NAC without adjuvant also induced a strong immune response indicates that NAC is a highly immunogenic protein. Normally, NAC is an intracellular protein that is not available to the immune system. It may therefore be speculated that the release of the protein in the course of tissue damage may initiate an autoimmune response.

Another interesting finding of our study is that in our model of autoallergy, T_H2 and T_H1 autoreactive responses coexist. The phenotype of the human α -NAC-induced immune response resembles in many aspects the immune response in patients with severe and chronic allergic disease. For example, it is well established that chronic skin lesions of atopic dermatitis patients contain a mixed population of T_H2 and T_H1 T cells.^{5,6}

The latter response is also accompanied by recruitment of mast cells and eosinophils as well as of neutrophils. The immunogen in our model, human α -NAC, was identified as an autoantigen by using IgE autoantibodies of patients with atopy.^{15,16} Similar observations have been made for severe and chronic forms of bronchial asthma, in which the influx of neutrophils into the airways is a well known additional, unexplained component.⁷

The T_H2 component of this autoallergic immune response is defined by antigen-specific IgE and IgG1 antibodies, by the elicitation of ICHS, airway hyper-responsiveness and the recruitment of eosinophils. In addition, the perivascularitis contains an eosinophilic component. The T_H1 component of the immune response is characterized by the presence of high titers of antigen-specific IgG2a antibodies and by the recruitment of an

unusually high number of neutrophils into the airway mucosa and within the inflammatory infiltrate found around middle-sized and larger lung veins. The fact that both recombinant protein preparations (ie, human α -NAC, murine α -NAC) contained less endotoxins than standardized allergen extract-based vaccines²¹ suggests that the induction of the T_H1 immune response was not caused by endotoxin. On the other hand, we have shown that immunization with human α -NAC without the T_H2 -driving adjuvant aluminium hydroxide induced also a mixed T_H2/T_H1 immune response. It is therefore likely that α -NAC represents an antigen with an intrinsic property to induce a mixed T_H2/T_H1 immune response. A shift from a T_H2 -dominated to a T_H1 immune response is one hallmark of severe and chronic manifestations of atopy.⁵⁻⁷ Independently from the latter notion, it has been found that patients with severe and chronic manifestations of atopy exhibit IgE reactivity to a variety of self proteins such as NAC.¹³⁻¹⁵ For several allergens, sequence and structural similarities with human proteins have been revealed as the basis for cross-reactivity,⁸⁻¹² and it may be assumed that promiscuous recognition of a great variety of epitopes present on foreign and self antigens may be involved in the induction and maintenance of an autoallergic component in severe forms of atopy.

On the basis of our results, we suggest that the shift from T_H2 to T_H1 and development of autoimmune responses may be linked to each other and may underlie the transition from allergy to foreign antigens to self-sustained autoallergy in at least a subgroup of atopic patients. This mechanism may explain disease progression and persistence of the inflammatory response despite therapeutic strategies of successful allergen avoidance. In summary, autoreactivity and allergy were induced by a foreign allergen (human α -NAC) mimicking a self protein (murine α -NAC) in an animal model of atopy, demonstrating that allergy and autoimmunity are 2 disease entities that are not mutually exclusive and that may coexist in the same individual.

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