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Isolation of cDNAs Coding for IgE Autoantigens: A Link between Atopy and Autoimmunity

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Key Words

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Introduction

While in many manifestations of atopy, environmental allergens (low-molecular-weight proteins from pollen, mites, animal dander, fungi) are responsible for the IgE-mediated activation of allergic effector cells, the pathogenetic mechanisms for certain more chronic and/or severe forms of atopy (atopic dermatitis, atopic asthma) are less clear. Atopic dermatitis patients frequently experience exacerbation of the disease even in the absence of environmental allergen contact and suffer from chronic manifestations [1, 2]. Autosensitization against human proteins has been considered very early as a possible mechanism and a number of publications provide evidence for IgE reactivity against human proteins and skin test activity of human dander extracts in severely atopic patients [3–6]. Further evidence for the hypothesis that IgE autoimmunity might play a role in the pathogenesis of atopy came from the observation of immunological and structural similarities among environmental allergens and human proteins such as profilins, which represent ubiquitous eukaryotic actin-binding proteins [7, 8], albumins from animals [9] and fungal superoxide dismutase [10]. It could recently be shown that a high percentage of sera from atopic dermatitis patients contained specific IgE autoantibodies against Western blotted proteins from histogenetically different human cells and cell lines [11]. To re-

veal the nature of the IgE autoantigens, we have chosen an approach which has led to the molecular characterization of many important environmental allergens, i.e. IgE immunoscreening of expression cDNA libraries [12]. Sera from patients with severe atopic dermatitis containing IgE autoantibodies (fig. 1) were used to screen an expression cDNA library constructed from the epithelial cell line A431 (fig. 2) [13, 14]. In the current contribution, we describe the isolation of cDNAs coding for human IgE autoantigens, the expression of one of these autoantigens (ara 1) in *Escherichia coli*, its specific IgE-binding capacity and ultrastructural localization.

Results and Discussion

We have screened a cDNA library constructed from the human epithelial cell line A431 with serum IgE from patients with atopic dermatitis. The cDNAs coding for IgE autoantigens represented important intracellular proteins, among them the α -chain of the nascent polypeptide complex, cytokeratin type II, a novel calcium-binding protein and the BCL7B protein [14]. The most frequently recognized IgE autoantigen, ara 1 [13], is a novel cytoplasmic protein of approximately 55 kD which was expressed in different cell types but was found most abundantly expressed in human epidermis. The cDNA coding for ara 1 was expressed as β -galactosidase fusion protein in *E. coli* Y1089. Figure 3 shows that affinity-purified recombinant ara 1 is

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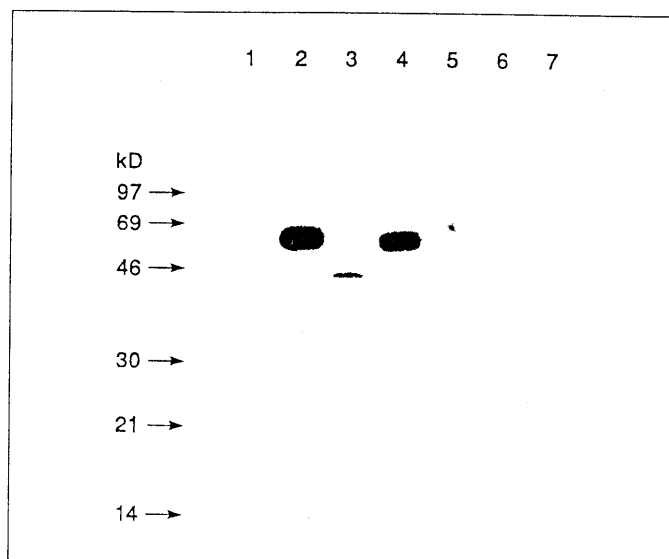


Fig. 1. IgE reactivity of sera from patients with severe atopy with nitrocellulose blotted human proteins. Protein extracts from the human epithelial cell line A431 were separated by SDS-PAGE and blotted onto nitrocellulose. Nitrocellulose strips were incubated with serum from an atopic patient without atopic dermatitis (lane 1), with serum IgE from patients with atopic dermatitis (lanes 2–4), nonatopic controls (lanes 5–6) or buffer without addition of serum (buffer control, lane 7). Bound IgE antibodies were detected with ^{125} I-labeled antihuman IgE antibodies.

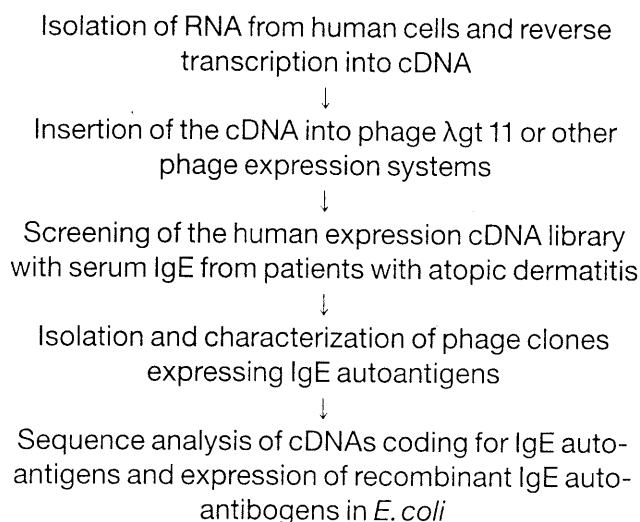


Fig. 2. Isolation of cDNAs coding for IgE autoantigens by IgE immunoscreening of human cDNA libraries.

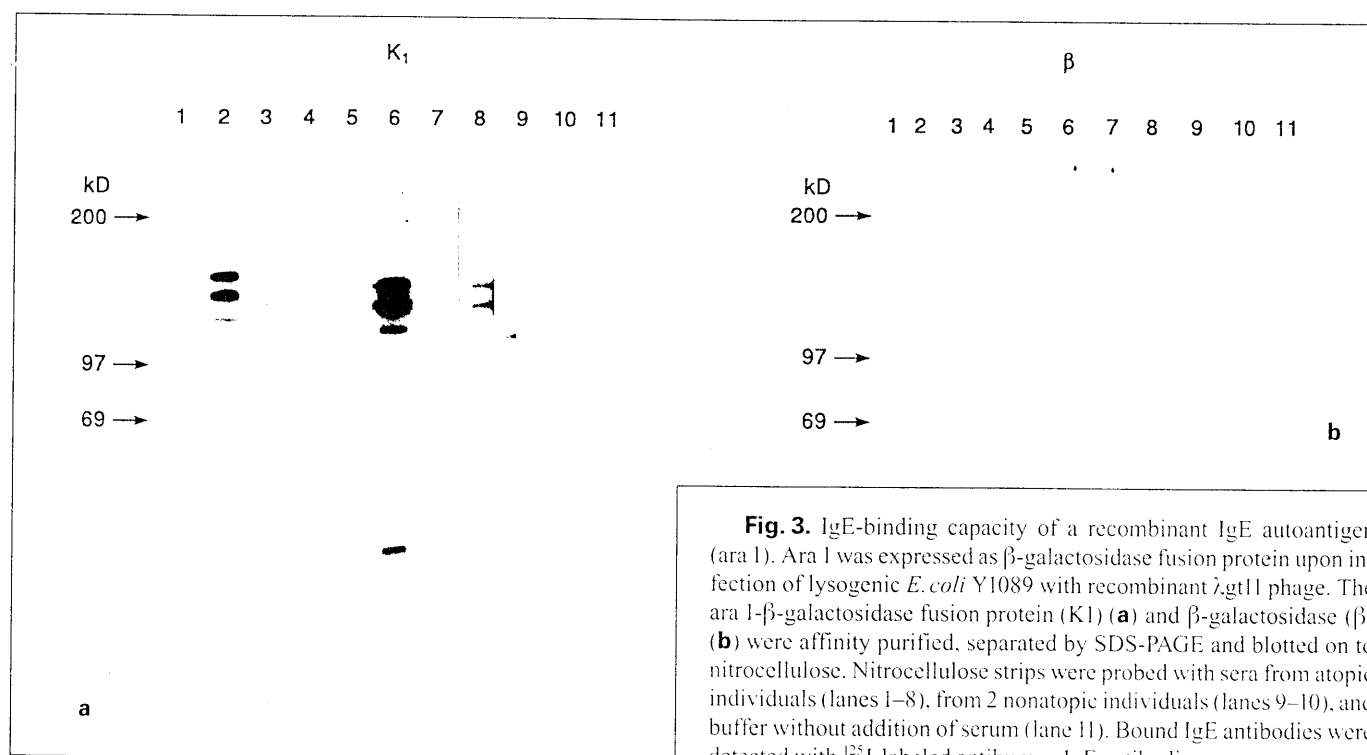


Fig. 3. IgE-binding capacity of a recombinant IgE autoantigen (ara 1). Ara 1 was expressed as β -galactosidase fusion protein upon infection of lysogenic *E. coli* Y1089 with recombinant λ gt11 phage. The ara 1- β -galactosidase fusion protein (K1) (a) and β -galactosidase (β) (b) were affinity purified, separated by SDS-PAGE and blotted on to nitrocellulose. Nitrocellulose strips were probed with sera from atopic individuals (lanes 1–8), from 2 nonatopic individuals (lanes 9–10), and buffer without addition of serum (lane 11). Bound IgE antibodies were detected with ^{125} I-labeled antihuman IgE antibodies.

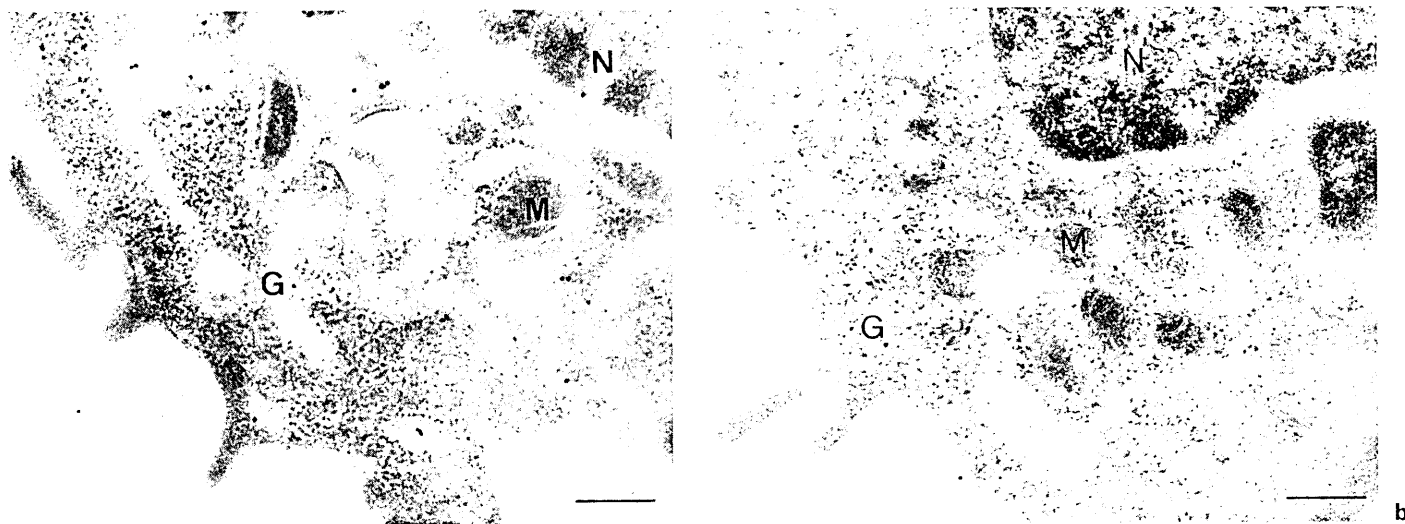


Fig. 4. Localization of ara I by immunogold electron microscopy in human A431 cells. A431 cells were fixed in aqueous paraformaldehyde and embedded; ultrathin sections were stained using an affinity-purified rabbit anti-ara I antiserum (**a**) and as a negative control with the affinity-purified preimmune serum (**b**). Bound antibodies were detected with a gold-labeled antirabbit Ig antiserum. G = Golgi; N = nucleus; M = mitochondrion. The bars represent 0.25 μ m.

specifically detected by IgE autoantibodies from patients with severe atopy (atopic dermatitis, asthma) while no reactivity was observed to β -galactosidase (negative control). To further characterize ara I at the ultrastructural level, a rabbit antiserum was raised against purified recombinant ara I and used to detect the protein on ultrathin sections of A431 cells by immunoelectron microscopy (fig. 4). In these experiments, ara I was localized in the cytoplasm. As the other above-mentioned IgE autoantigens, ara I is an intracellular protein. None of the described IgE autoantigens has been reported as an autoantigen before and therefore IgE autoantigens represent a novel class of autoantigens. Formation of IgE autoantibodies is proposed to occur during tissue damage, as is frequently observed in chronic forms of atopy (atopic dermatitis), in a Th2-favored environment, probably

induced by exogenous allergens. Binding of IgE autoantibodies to autoantigens may then contribute to inflammation by activation of allergic effector cells (mast cells, basophils) and subsequent mediator release. Focussing of IgE autoantigen complexes via Fc ϵ receptors on professional antigen-presenting cells may further lead to chronic T cell activation. IgE autoimmunity would therefore explain chronicity or exacerbation in severe forms of atopy (atopic dermatitis, asthma) even in the absence of exogenous allergens.

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