

A broad Spectrum of IgE-binding Self-Antigens associated to Atopic Eczema

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INTRODUCTION / BACKGROUND

Atopic eczema (AE) is the most common human chronic inflammatory skin disease (1). Various genetic factors, immunologic deviations, altered skin structure, and environmental factors such as allergen exposure are known or assumed to be involved in the pathogenesis of this complex disease (2). In the past years, clear evidence for T and B cell-mediated autoreactivity to self-antigens sharing a high degree of sequence identity to environmental allergens has been demonstrated (3). Recombinant human self-antigens like manganese superoxide dismutase (MnSOD) (4), ribosomal P₂ proteins (5), cyclophilins (6), and thioredoxins (7) have been shown to bind IgE from sera of patients suffering from AE, to induce proliferation of peripheral blood mononuclear cells *ex vivo*, and to elicit Type I hypersensitivity reactions. Moreover, human MnSOD is sufficient to elicit an atopic eczema by simple application of the protein to the skin of AE patients (8), directly demonstrating a prominent role of self-reactivity in the perpetuation of the disease. However, also IgE-mediated reactivity to self-antigens without sequence identity to environmental allergens has been reported (9) raising the question about the size of the self-antigen repertoire. Aim of our project is to estimate the size of the self-antigen repertoire involved in the pathogenesis of AE.

MATERIAL & METHODS

Selection of patients, control subjects, and routine assessments

Adult patients with AE and healthy individuals were recruited and carefully examined regarding clinical history, symptoms, medications, and other treatments as described (8). Pooled serum samples from five AE patients with high total IgE levels (>3000 IU/ml) were used for the isolation of clones expressing putative IgE-binding self antigens.

Construction of a human cDNA library displayed on phage surface

Human cDNA was isolated from a commercial λ -Zap II cDNA library (Stratagene, La Jolla, USA) by *in vivo* excision of the pBluescript phagemid (10). Inserts were isolated as Eco RI / Xho I restricted fragments, subcloned into phagemid pJuFo (11), and displayed on the surface of filamentous phage M13 (12).

High throughput screening of enriched clones

Serum IgE of individuals with AE was captured with solid phase bound anti-human IgE antibodies to produce a specific IgE-ligand surface. 10¹¹ colony forming units of the phagemid library were added to a well, incubated for 2 h at 37°C, and extensively washed to remove unbound phage. Binding phage were eluted and used to infect *E. coli* cells to prepare phagemids for a further round of biopanning (13). After four rounds of affinity selection phage were used to infect *E. coli* cells, and plated onto large square agar plates (230 x 230 mm). Using a picking/gridding robot (14), 21 x 384 single colonies were picked into 384-well

microtitre plates containing complete medium supplemented with freezing mix. After growth at 37°C overnight, plates were replicated into new microtitre plates using 384-pin replicating tools to produce working copies.

High throughput identification of selected genes

cDNA inserts of all picked clones were amplified by high throughput PCR and the amplification products gridded onto 222 x 222 mm membrane filters using a picking/gridding robot as described (15). Filter hybridizations for the identification of clones using DIG-labelled PCR probes of randomly selected inserts were performed as described (14, 15) until all clones could be assigned to a discrete sequence.

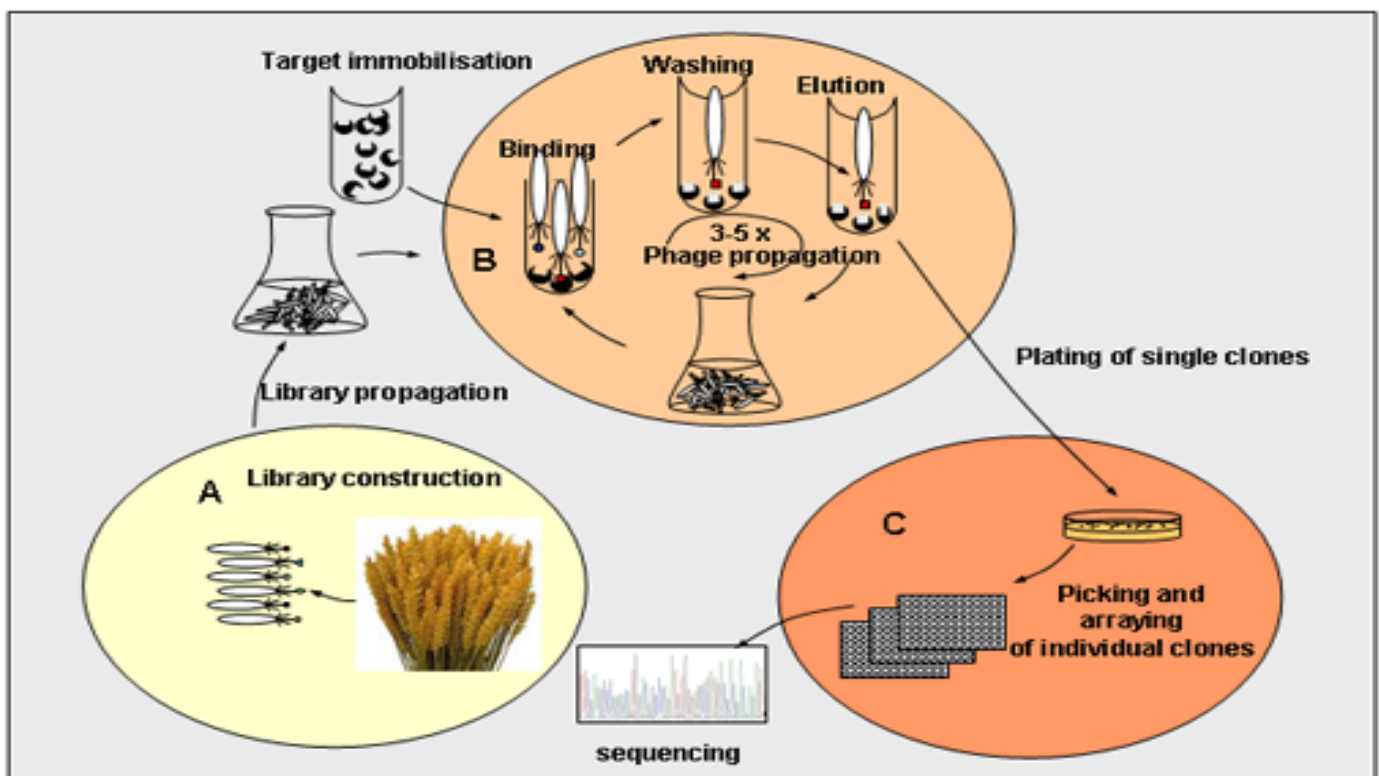
Protein expression and IgE-binding assays

Six selected inserts were subcloned into a high level expression vector and used to express the corresponding 6His-tagged recombinant proteins in *E. coli* (14). The IgE-binding capacity of the proteins was demonstrated by Western blot analysis and ELISA as described (4-7).

RESULTS

A human cDNA library constructed in phagemid pJuFo was displayed on the surface of filamentous phage M13 (Figure 1). Affinity selection against pooled serum IgE from patients suffering from severe AE yielded a large population of phage potentially expressing IgE-binding self antigens. High throughput screening of 8064 arrayed clones revealed 151 complete or truncated sequences encoding human proteins.

Figure 1: Construction and screening of phage surface displayed cDNA libraries



Among the sequences identified cyclophilin, MnSOD, thioredoxin, ribosomal P₂ protein, already described as IgE-binding self-antigens, were present. A common characteristic of these proteins is that they share extended sequence homology to environmental allergens. To demonstrate the specificity of the cloning procedure we have randomly chosen six further human proteins (ribosomal L3 protein, α -actin, α -tubulin, eIF6, HLA-DR- α , and RP1),

subcloned the corresponding inserts into high level expression vectors, and produced the recombinant proteins in *E. coli*. ELISA and Western blot experiments confirmed the capability of all proteins to bind serum IgE from AE patients, demonstrating their allergenic potential. In contrast, serum from healthy controls do not showed detectable IgE specific for the selected human proteins.

Flow cytometry experiments demonstrated that self-antigen mediated cross-linking of receptor-bound IgE on the surface of basophils from AE patients led to degranulation and concomitant up-regulation of CD63 and CD203c on the cell surface. Moreover the recombinant self-antigens were able to induce the proliferation of peripheral blood mononuclear cells of AE patients, convincingly demonstrating the allergenic nature of these self-antigens for AE patients.

CONCLUSIONS & OUTLOOK

By high-throughput screening of a human phage surface displayed cDNA library with immobilized serum IgE from AE-patients more than hundred new IgE-binding self-antigens were identified. Some of the newly identified self-antigens, like ribosomal L3, or α -tubulin are phylogenetically highly conserved and show sequence homology to environmental allergens from the mould *Aspergillus fumigatus* (16) or the storage mites *Lepidoglyphus destructor* (17), respectively. These self-antigens are cross-reactive with the homologous environmental allergens based on shared structural features. Interestingly other IgE-binding self-antigens such as HLA-DR- α or RP1 do not share any homology with so far described allergens but clearly bind IgE from patients' sera. It remains to be elucidated if these proteins are able to induce a primary sensitization or if they are recognized by IgE antibodies originally generated against not yet identified antigens. However, it is already clear that binding of self-antigens to receptor-bound IgE on the surface of basophils and mast cells can induce degranulation and mediator release, thus promoting and perpetuating an already existing skin inflammation.

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