

Screening of a Genomic DNA Library and Recombinant Expression: A New Approach to the Question of Allergic Responses to Bacterial Proteins

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Key Words

Genomix DNA library screening · Bacterial proteins ·
Staphylococcus aureus · Atopic dermatitis

Introduction

For many years, allergists used a microbial extract that included proteins derived from *Staphylococcus aureus* in skin testing and immunotherapy protocols for patients with allergic conditions including rhinitis and asthma. More recently, this practice has greatly decreased owing to a lack of evidence supporting a role for immediate hypersensitivity to bacterial antigens in allergic conditions. However, this may result, in part, from a lack of availability of appropriate antigens for investigation. *S. aureus* infection is recognized as an important trigger for exacerbation of skin disease in patients suffering from atopic dermatitis (AD) [1]. Serum IgE antibodies to *S. aureus* have been demonstrated in AD patients using whole bacteria or purified cell wall and cell lysate preparations. In general, the specificity of these antibodies and their effects in vitro and in vivo has not been determined [2, 3]. More recently, IgE to staphylococcal superantigens was demonstrated in sera from patients with AD [4].

In recent years, molecular cloning techniques have been used to define the primary structures of allergens (e.g. pollens, dust mites, animal danders, cockroaches) [5, 6]. We have been interested in investigating the pathogenicity of staphylococcal proteins in AD. This study describes the identification of an IgE-binding protein derived from *S. aureus* by screening a genomic DNA library with sera from patients with AD.

Material and Methods

Genomic DNA Cloning

A clone of *S. aureus* from a culture of the skin of a patient hospitalized with severe AD was used to prepare a genomic DNA library in the ZAP Express™ phagemid vector (Stratagene). The library was screened with pooled human sera (1:20 dilution) from 5 AD patients with high-titer total IgE antibodies (mean IgE 37,000 IU/ml) and a serum pool (1:20 dilution) obtained from 8 AD patients with mid-range-titer total IgE (mean IgE 3,300 IU/ml). Sera were preabsorbed with *Escherichia coli* lysate.

Expression of Recombinant Proteins in *E. coli*

Recombinant putative proteins were expressed in *E. coli* as hexahistidine-tagged recombinant proteins using the pET-21b expression vector (Novagen). The competent *E. coli* strain BL21[DE3] (Novagen) was transformed and expression of proteins was induced with 1 mM IPTG at 37 °C. Recombinant proteins were purified from cell lysates by affinity chromatography using immobilized nickel chelate followed by cation exchange using a Mono S column.

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1018–2438/01/1243–0097\$17.50/0

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Production and Characterization of Monoclonal Antibodies

A/J mice were immunized with 200 µg of HPLC-purified putative *S. aureus* proteins in CFA. Mice were boosted with antigen at 2 and 4 weeks. A final intrasplenic injection of purified proteins was given to the mouse demonstrating the highest IgG antibody titer. These mice were killed and spleen cells were fused with myeloma cells. Hybrids producing antistaphylococcal IgG antibodies were cloned by limiting dilution. Secreted protein and cell lysate fractions of whole organisms from *S. aureus* (a clinical isolate used for producing genomic DNA library) were prepared. The binding of monoclonal antibodies and *S. aureus* proteins was tested using immunoblot analysis.

Serological Responses to a Novel S. aureus Putative Protein

IgE and IgG antibodies to putative protein were measured in sera obtained from 61 patients with AD and 59 non-atopic controls using an antigen-binding radioimmunoassay. Diluted sera were incubated with radiolabeled protein and precipitated with sheep anti-human IgE and IgG. IgE myeloma serum diluted 1:200 was used as carrier for the IgE assay. Precipitates were washed with borate-buffered saline and counted in a γ -counter.

Results

Genomic DNA Cloning

Ten positive plaques were identified after screening ~60,000 plaques. Nucleotide sequence analysis confirmed that 9 of the 10 clones encoded the same protein which was identified as Staphylococcus protein A. Additional screening using sera preabsorbed with protein A identified 9 positive clones with an insert size of approximately 1,900 bp containing the same nucleotide sequence; this sequence was the same as that encoded by the single non-protein A clone from the initial screen. This clone, designated SA3B, contained two open reading frames (ORF) preceded by a Shine-Dalgarno sequence, with sizes of approximately 870 and 470 bp. The longer ORF encoded for 289 amino acids with an estimated molecular mass of 31,727 kD and a pK value of 2.25, and this putative protein was designated SA3B-1. Searches of the protein databases for sequence similarity revealed that SA3B-1 was similar to proteins of unknown function derived from bacteria belonging to different genera including *Bacillus subtilis*, *E. coli* and *Haemophilus influenzae*. The highest degree of identity was between SA3B-1 and the *B. subtilis* hypothetical 30.2-kD protein YXEH (Smith-Waterman score 363, 30.4% amino acid identity). The second ORF encoded a 156-amino acid putative protein (designated SA3B-2) with an estimated molecular mass of 17,117 kD and a pK value of 8.07. Sequence similarity searches showed that SA3B-2 was homologous to bacterial hypothetical proteins of unknown function. The

highest degree of sequence identity was between SA3B-2 and the *B. subtilis* hypothetical 17.8-kD protein YAAJ (Smith-Waterman score 646, 61.7% identity).

Production and Characterization of Monoclonal Antibodies

Putative *S. aureus* proteins were produced in a bacterial expression system, and monoclonal antibodies to recombinant proteins were produced. Monoclonal antibodies to rSA3B-1 and rSA3B-2 were tested for binding to supernatant and cell lysate fractions of *S. aureus* cultures by Western blot. Anti-rSA3B-1 bound to a protein of a molecular mass consistent with natural SA3B-1 in both culture fractions. Antibodies to rSA3B-2 showed no reactivity with *S. aureus* culture fractions.

Serological Responses to a Novel S. aureus Putative Protein

Sera from 61 AD patients and 58 non-atopic controls were tested for IgE and IgG antibody binding to rSA3B-1 by antigen binding radioimmunoassay. Forty-eight percent of AD patients had detectable IgE antibody to rSA3B-1 while only 2% of controls had detectable IgE ($p < 0.0001$). The majority of AD patients (77%) had detectable IgG antibody to rSA3B-1 compared with only 33% of controls ($p < 0.0001$). Linear regression analysis showed a significant quantitative correlation between IgE and IgG anti-rSA3B-1 antibodies ($r = 0.78$, $p < 0.0001$).

Conclusion

We have cloned two putative proteins, SA3B-1 and SA3B-2, from *S. aureus* by screening a genomic DNA library with sera from patients with AD. Antibody binding studies suggest that natural SA3B-1 is present in *S. aureus* cultures. The titer of IgE to SA3B-1 was significantly higher in sera of patients with AD than in non-atopic controls. Library screening, a technique commonly used to define conventional allergens, may prove useful in identifying immunogenic proteins for investigating allergic diseases such as AD or chronic rhinosinusitis where bacteria are commonly cultured.

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