

Brief communication

Production of IgE antibodies against the 22 kDa tegumental membrane-associated antigen of schistosomes is directed by the antigen itself

G.J.WAINE, D.R.MAZZER & D.P.McMANUS

Molecular Parasitology Unit, Tropical Health Program, The Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Queensland, Australia

SUMMARY

It has previously been reported that the predominant target of immunoglobulin E (IgE) recognition in sera from humans infected with Schistosoma japonicum in The Philippines or with S. mansoni in Kenya, is a 22 kDa tegumental membrane-associated schistosome antigen. In the present study, we demonstrate that the 22 kDa antigen can direct the production of antigen-specific IgE antibodies independently of schistosome infection and in the absence of any other parasite components or adjuvant. Three strains of mice were immunized using the purified, recombinant 22 kDa antigen of S. japonicum without the use of any adjuvant. Sera from all three strains of immunized mice, but not control animals, generated IgE antibodies specific for the native 22 kDa schistosome antigen in Western blots. Thus, the 22 kDa antigen itself must contain signals (presumably encoded by the primary amino acid sequence or by the secondary or tertiary structures of the molecule, or by a combination of these) which are sufficient to direct the isotype switch required for production of antigen-specific IgE.

Keywords Schistosomiasis, immunoglobulin-E (IgE), 22 kDa antigen, antibody isotype switching, immune response mechanisms

INTRODUCTION

Human schistosomiasis, principally caused by *Schistosoma japonicum*, *S. mansoni* or *S. haematobium*, is characterized by high levels of schistosome-specific IgE. It has recently been demonstrated that a native 22 kDa antigen is a predominant target of the human IgE response both to *S. japonicum* infection in The Philippines and *S. mansoni* infection in Kenya (Webster *et al.* 1997). However, the reason for the pre-eminent recognition of the 22 kDa antigen by host IgE antibodies is not known.

The 22 kDa antigen recognized by serum IgE from *S. mansoni* patients has been cloned and characterized (Webster *et al.* 1996), and found to be 100% identical to Sm22.6, a tegumental membrane-associated antigen cloned twice previously, first by Stein & David (1986), and then by Jeffs *et al.* (1991). We previously cloned the homologous antigen from *S. japonicum*, Sj22.6, and showed that Sj22.6 and Sm22.6 share 71% identity in their amino acid sequence (Waine *et al.* 1994). In that report (Waine *et al.* 1994) we described the production of highly purified recombinant Sj22.6 protein (reSj22.6), and also identified an EF-hand motif, characteristic of a Ca²⁺-binding domain, within the Sj22.6 sequence. A similar EF-hand motif is present in the Sm22.6 homologue (Francis & Bickle 1992). We have recently identified the EF-hand motif as a dominant B-cell epitope recognized by IgG antibodies in sera from mice immunized with reSj22.6 (Waine *et al.* in press).

Elevated levels of IgE are also a characteristic hallmark of asthma and other allergic diseases in humans. Sj22.6 and Sm22.6 exhibit several characteristics in common with the major birch pollen (*Betula verrucosa*) allergen, Bet v III. A cDNA coding for this allergen was isolated by screening an expression cDNA library using serum IgE from a patient who was allergic to pollen (Seiberler *et al.* 1994). The

Correspondence: G.J.Waine

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deduced amino acid sequence, with a calculated molecular weight of 23.7 kDa, encoded a polypeptide sharing significant sequence homology with Ca^{2+} -binding proteins, primarily in regions found to encode EF-hand domains. Thus, the pollen allergen and the schistosome antigens share common features, being a target of human IgE recognition, having similar molecular mass, and in having EF-hand Ca^{2+} -binding domains. Further, we have identified similar hydrophilicity/hydrophobicity profiles for the three antigens (data not shown).

With the evidence of numerous similarities between Sj22.6, Sm22.6 and the birch pollen allergen Bet v III, and after examining published Western blots (Dunne *et al.* 1992, Webster *et al.* 1997) and our own data (Waine *et al.* unpublished) which show that the IgE response in human schistosomiasis is overwhelmingly targeted to the 22.6 kDa schistosomal antigen, we hypothesized that the 22.6 kDa antigen alone (possibly structure- or sequence-related) may be sufficient to direct the IgE isotype switch required for production of antigen-specific IgE in schistosome infections. Antigen-specific isotype switching (to IgG4 and IgE) has recently been described for two different recombinant proteins of another helminth parasite, *Onchocerca volvulus* (Garraud *et al.* 1996), thus strengthening this hypothesis.

To test the hypothesis, we immunized mice using purified recombinant Sj22.6, thus eliminating the possibility of contamination with other parasite proteins which could occur if an antigen purified from adult worms was used. Mice were chosen as the animal model, firstly, because they are a natural host for *S. japonicum*, and secondly, because it has recently been demonstrated, using mice with a targeted deletion of the IgE gene, that IgE participates in the immune

response to schistosomiasis infection in the murine host (King *et al.* 1997), thus validating the use of the murine model of schistosomiasis to better understand the mechanism of IgE-mediated protective immunity *in vivo*.

Three strains of mice, BALB/c, CBA, and C57BL/6, were immunized with purified reSj22.6 without any adjuvant. Control animals were immunized with an *E. coli* lysate of the same strain of bacteria from which the recombinant Sj22.6 was expressed and purified, or with another *S. japonicum* recombinant antigen, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following immunization protocol was used. Five mice of each strain were immunized with 25 µg of reSj22.6 on week 0 and subsequently boosted on weeks 2 and 4 with an equal amount of reSj22.6. Control mice were immunized and boosted with 25 µg of *E. coli* lysate or recombinant GAPDH, at the same time intervals. For collection of sera, all mice were bled from the tail vein on week 8.

Sera from mice immunized with Sj22.6, *E. coli*, or GAPDH were tested for specific IgE production by reacting individual sera with whole parasite adult worm extracts in Western blot analysis. All sera were assayed for IgE production using identical conditions, including sera dilutions. Briefly, whole parasite extracts were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes and blocked overnight with 5% skim milk in phosphate buffered saline/0.05% Tween-20 (PBST) as previously described (Waine *et al.* 1993). Sera from the immunized mice (diluted 1:10 in PBST containing 0.5% skim milk) were individually reacted with the nitrocellulose strips at 37°C for 90 min. After washing the nitrocellulose three

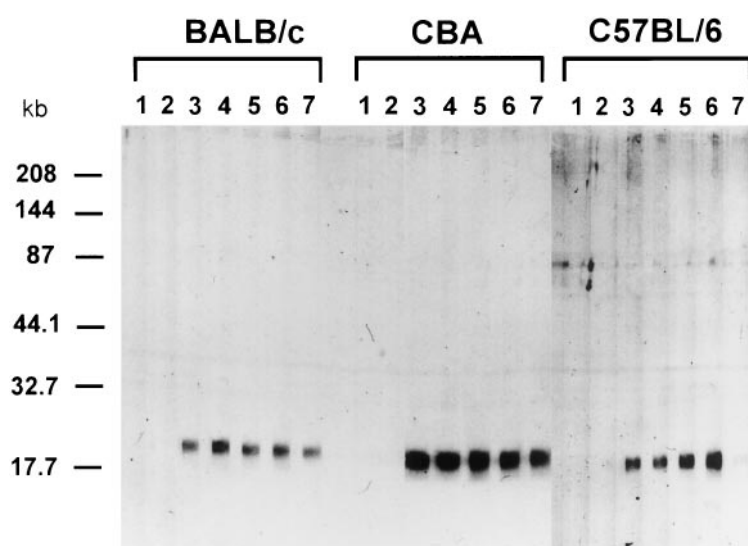


Figure 1 Lanes 1 and 2; control mice immunized with *E. coli* lysate. Lanes 3–7; mice immunized with recombinant Sj22.6. Antigen was whole *S. japonicum* adult worm extract separated on SDS-PAGE. Fractionated antigens were transferred to nitrocellulose and probed with vaccinated mouse sera and goat anti-mouse IgE.

times with PBST, goat anti-mouse IgE (Epsilon chain) (ICN Biomedicals) was added at a dilution of 1 : 30 in PBST, and incubated at room temperature for one h. After washing again, rabbit anti-goat IgG (H + L) conjugated to horse-radish peroxidase (Bio-Rad) was added (1 : 1000 in PBST) and incubated at room temperature for 1 hour. The nitrocellulose was washed 3 times in PBST and colour developed using 4-chloro-naphthol.

The results of the Western blot analysis are shown in Figure 1. The results show conclusively that all of the mice immunized with the recombinant Sj22.6 antigen generated an IgE antibody response and that this response was directed exclusively at a single parasite antigen with a molecular mass of approximately 22.6 kDa. In contrast, none of the sera from the control mice showed IgE reactivity to any schistosome antigen. Mice immunized with the recombinant Sj22.6 antigen, but not control mice, also produced IgG antibodies specific for the 22.6 kDa antigen (data not shown). To confirm that the native 22.6 kDa antigen recognized by IgE in whole parasite extracts was indeed Sj22.6, sera were reacted with reSj22 in Western blots. The pattern of IgE recognition was identical to that shown in Figure 1, when whole parasite extract was used as the antigen. As expected, IgE antibodies from mice immunized with reSj22.6, but not the control mice, recognized the recombinant antigen. As a further control, five mice were immunized with a different *S. japonicum* recombinant antigen, glyceraldehyde-3-phosphate dehydrogenase (Waite *et al.* 1993). These mice did not produce any detectable IgE antibodies, despite specific IgG antibodies of similar titre to those obtained for Sj22.6 being produced (data not shown), indicating that the specific IgE reaction observed for Sj22.6 was not due to cross-reactivity with an excess of IgG antibodies. The data therefore indicates that the ability to induce IgE production by the host is a characteristic of the 22 kDa antigen.

These results are, we believe, significant as antigen-specific IgE was generated without the use of an adjuvant and in the absence of parasite infection or any other parasite component, thus clearly demonstrating that all the signals required for production of antigen-specific IgE antibodies by the host immune system are contained within the 22 kDa antigen itself. Presumably these signals must be encoded by the primary amino acid sequence or by the secondary or tertiary structures of the molecule, or by a combination of these. Given the high sequence homology between the 22 kDa antigens of *S. japonicum* and *S. mansoni*, it is likely that the signals present in Sj22 that direct the specific IgE response, will also be present on Sm22 and homologues of the other schistosome species.

Further molecular studies aimed at defining the signals encoded in the 22 kDa antigen, which are responsible for directing host IgE production, are in progress.

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