

# A glycoprotein from *Schistosoma mansoni* eggs binds non-antigen-specific immunoglobulin E and releases interleukin-4 from human basophils

KARIN HAISCH<sup>1</sup>, GABRIELE SCHRAMM<sup>2</sup>, FRANCO H. FALCONE<sup>3</sup>, CHRISTIAN ALEXANDER<sup>2</sup>, MAX SCHLAAK<sup>2</sup> & HELMUT HAAS<sup>2</sup>

<sup>1</sup>SUNY at Buffalo, Department of Microbiology, Buffalo, NY, USA, <sup>2</sup>Forschungszentrum Borstel, Borstel, Germany and

<sup>3</sup>Institute of Cell, Animal and Population Biology, The University of Edinburgh, Edinburgh, UK

## SUMMARY

We have recently shown that soluble extracts from *Schistosoma mansoni* eggs (SmEA) triggered basophils from nonsensitized donors to rapidly release interleukin (IL)-4. Assuming that this mechanism might play a role in vivo in biasing the immune response towards a Th2 phenotype, we determined basic properties of the IL-4-inducing activity contained in SmEA. Sensitivity to pepsin digestion indicated protein nature. Binding to and specific elution from Concanavalin A-sepharose suggested that this protein contains mannose residues, thus being a glycoprotein. The IL-4-inducing activity was stable for 30 min at room temperature towards shifting the pH between 3 and 10. When incubated at 100°C, it was stable at pH 3, but less stable at neutral and alkaline pH. Electroelution from an SDS-PAGE gel indicated an apparent molecular weight of the IL-4-inducing activity between 31 and 66 kDa. Although binding to purified human immunoglobulin E (IgE) and activating basophils IgE-dependently, SmEA appears to activate basophils in a non-antigen-specific way, since the cells were purified from noninfected donors. Because the IL-4-inducing activity was found to be released from eggs, it could be an important factor in the environment of the eggs skewing the immune response towards the Th2 phenotype.

**Keywords** SmEA, basophils, *Schistosoma mansoni*, IL-4, eggs, IgE

Correspondence: H. Haas, Division of Cellular Allergology, Forschungszentrum Borstel, Parkallee 22, D-23845 Borstel, Germany (e-mail: hhaas@fz-borstel.de).

Received: 2 January 2001

Accepted for publication: 3 April 2001

## INTRODUCTION

Infections with the parasitic trematode *Schistosoma mansoni* are characterized by a pronounced systemic Th2 response following deposition of eggs in both humans and experimental animals (1,2). A more thorough understanding of the mechanisms by which this parasite manipulates the immune system of its hosts may permit general insights into the switch to the Th2 phenotype. Interleukin (IL)-4 is the major cytokine that induces B-cells to switch to immunoglobulin (Ig)E production. Moreover, when present early during an immune response, IL-4 is considered to be a key factor for promoting Th2 differentiation. Several cell types are discussed as a source of early IL-4 (3). Basophils could be such a source because, upon antigen-specific or nonspecific stimulation, these cells quickly release considerable amounts of IL-4 *in vitro* (4–7). As motile cells, they are able to migrate into affected tissues and may provide naive T helper cells with IL-4 during priming. For investigation of basic mechanisms of polarization towards a Th2-response, infections with parasitic helminths are suitable models, since helminths are very reliable triggers of IL-4 and IgE production. The eggs of *S. mansoni* are well known to be potent inducers of IL-4; they can promote a strong Th2-response in naive mice even against ‘bystander’ antigens (8). Therefore, we used *Schistosoma mansoni* eggs (SmEA) to stimulate human basophils in order to investigate the mechanisms of their IL-4 production. As previously published (4), basophils from healthy human donors release IL-4 after incubation with SmEA. We have now characterized several basic properties of the IL-4-inducing activity in SmEA and demonstrate that this activation is dependent on the presence of polyclonal, non-antigen-specific IgE on the surface of basophils.

## MATERIALS AND METHODS

### Materials

SmEA and supernatant from live *S. mansoni* eggs were obtained from Dr F.Yousif (SBSP, Theodor Bilharz Research Institute, Guiza, Egypt). SmEA was prepared according to Boros & Warren (9) and Carter & Colley (10). In brief, eggs isolated from mouse liver were homogenized in 0.01 M phosphate buffer pH 7.4 and centrifuged. The supernatant was ultracentrifuged and the clear supernatant was ultrafiltered and lyophilized. For the excretory/secretory compounds 200 000 isolated eggs were incubated in RPMI 1640 containing FCS for 18 h. IgE(PS) was a kind gift of Dr K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA). Iscove's modified Dulbecco's medium, penicillin and streptomycin were from Gibco BRL (Paisley, UK); FCS was from Boehringer Mannheim (Mannheim, Germany); EDTA and Tween 20 were from Merck (Darmstadt, Germany); ionomycin, bovine insulin, Trypan Blue, May-Grünwald stain, porcine pepsin and human transferrin were from Sigma (Deisenhofen, Germany); Ficoll was from Biochrom (Berlin, Germany); recombinant human (rh)IL-3 was a kind gift of Kirin (Gunma, Japan); rhIL-4 was purchased from Genzyme (No. 2181-01, Cambridge, MA, USA). Hank's balanced salt solution was from PAA Laboratories (Linz, Austria).

### Purification of basophils

Basophils were purified by a three-step procedure as described in Haisch *et al.* (11). Briefly, this procedure consists of a Ficoll density gradient step, counterflow elutriation and negative selection by magnetic cell sorting (MACS). The mean purity of the basophils obtained by this method is 96.7%.

### Culture and stimulation conditions

Purified basophils at  $10^5$  basophils per well in a final volume of 100  $\mu$ l culture medium (Iscove's Modified Dulbecco's Medium supplemented with 100 U/ml penicillin G, 50  $\mu$ g/ml transferrin, 5  $\mu$ g/ml insulin, 100  $\mu$ g/ml streptomycin, 10% FCS) were incubated in round-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) at 37°C in humidified air containing 6% CO<sub>2</sub>. Because IL-3 enhances IL-4-production of basophils (1), all wells were supplemented with IL-3, except the wells to which ionomycin was added. The cells were incubated at the indicated concentrations: SmEA: 40  $\mu$ g/ml; IL-3: 10 ng/ml; ionomycin: 1  $\mu$ M. The supernatants were collected after

18 h and stored at -20°C until IL-4 was assayed by sandwich ELISA.

### Stripping and resensitization of basophils

Basophils were incubated for 5 min on ice in 500  $\mu$ l 0.01 M lactate buffer pH 2.5 to remove bound IgE, washed with 4 ml Hank's balanced salt solution supplemented with 4.2 mM NaHCO<sub>3</sub> and 0.25% BSA, pH 7.4, and resensitized with purified human IgE (200 ng/ml) for 1 h at 37°C in culture medium. Total cell numbers and viability were determined by Trypan Blue exclusion.

### ELISA

Flat-bottomed microtiter plates (Maxisorp F96, Nunc) were coated overnight at 4°C with mouse anti-human (h)IL-4 mAb (clone 8D4-8; Pharmingen, San Diego, CA, USA) diluted 1 : 4000 in Tris (0.1 M Tris, 0.1 M NaCl, 2.5 mM MgCl<sub>2</sub>, pH 7.5). Plates were washed seven times with Tris-T (Tris/0.05% Tween 20) between incubation steps on a rotating platform. Following incubation for 90 min with samples or serial dilutions of rhIL-4 as a standard, biotinylated rat anti-hIL-4 mAb (clone MP4-25D2; Pharmingen) diluted 1 : 4000 in Tris-T/0.5% BSA was added for 60 min. Afterwards the plates were incubated with streptavidin-alkaline phosphatase (1 : 1000 in Tris-T/0.5% BSA) for 60 min. The chromogen solution was 1 mg/ml p-nitrophenylphosphate in 0.1 M Tris, 0.1 M NaCl, 5 mM MgCl<sub>2</sub>, pH 9.5. The absorption was read at 405 nm.

For IgE determination, the procedure was similar to the IL-4-ELISA with the following differences: IgE(PS) was used as standard; for coating mouse anti-hIgE-Ab (clone A 0094; Dako, Hamburg, Germany) was used (1 : 200 in Tris buffer pH 7.5). As a second antibody, biotinylated goat anti-hIgE-Ab (No. 2594; Tago, Brulingame, CA, USA) (1 : 10 000 in Tris/Tween/0.5% BSA) was used.

### SDS-PAGE according to Laemmli (12)

SmEA was added to nonreducing sample buffer [20 mM Tris, pH 6.7, 2 mM EDTA, 2% (w/v) SDS, 25% (v/v) glycerol, 0.02% (w/v) Bromophenol Blue] and separated by discontinuous SDS-PAGE using 5% stacking gels and homogeneous 12% separation gels. The proteins were visualized by silver staining according to Heukeshoven & Dernick (13) or eluted from the gel.

### Electroelution from SDS-PAGE

The gel was stained with Roti-White inverse protein staining (Roth, Karlsruhe, Germany) according to the

manufacturer until the background was white and the protein bands were still transparent. The gel was divided into nine parts, the proteins were mobilized with mobilizing buffer (25 mM Tris, 50 mM EDTA, pH 8.3) and eluted by electroelution in a Centrilotor microelectroelutor (Amicon, Witten, Germany) according to the manufacturer into microconcentrators (cut-off 10 kDa). The samples were concentrated by centrifugation at 2000 *g*.

#### Test of sequestration of the IL-4-inducing activity

The lyophilized supernatant of 10<sup>5</sup> eggs from *S. mansoni* incubated for 18 h in RPMI 1640 containing 10% FCS was resuspended in 1 ml of *aqua bidest* and its IL-4-inducing activity tested on basophils at various concentrations. IL-4-inducing activity was released into the supernatant only when the eggs were cultured in the presence of FCS, but not in its absence.

#### Treatment of SmEA with pepsin

SmEA (50 µg) and 0.08 µg pepsin in 25 µl PBS (137 mM NaCl, 3 mM KCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>) pH 2.2 were incubated at 37°C for 0, 15, 30, 60, 120 and 720 min. For inactivation of the enzyme, all samples were incubated at 70°C for 15 min and subsequently tested for IL-4-inducing activity.

#### Determination of temperature stability at low and high pH

SmEA (2 mg/ml PBS) was adjusted to pH 3, pH 7.4 and pH 10 with 1 M HCl or 1 M NaOH and 20 µl aliquots incubated at 20–100°C for 30 min in a water bath. Subsequently, the denatured proteins were removed by centrifugation (10 min, 1000 *g*) and the supernatants tested for IL-4-inducing activity.

#### Affinity chromatography of SmEA with Concanavalin A (Con A)

Con A-sepharose (0.5 ml; Amersham-Pharmacia, Freiburg, Germany) packed into a 1-ml syringe onto glass fibre filters (Schleicher & Schuell, Dassel, Germany) was washed with 10 ml buffer I (0.01 M Tris, 0.5 M NaCl, 1 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, pH 8). Since Con A stimulates basophils to release IL-4, the sepharose was first 'eluted' with 2.5 ml buffer II (buffer I with 0.2 M methyl- $\alpha$ -D-mannopyranoside) to determine if Con A leaches from the sepharose under elution conditions. Afterwards, the affinity gel was equilibrated with 10 ml buffer I and 25 µl SmEA (2 mg/ml) was applied onto the column. Subsequently, the column

was washed with 5 ml buffer I (effluent) and eluted with 2.5 ml buffer II. Effluent, eluate and control eluate were concentrated and washed with PBS pH 7.4 at 2000 *g* using microconcentrators (cut-off 30 kDa, Amicon).

#### Purification of polyclonal human IgE

Polyclonal human IgE was purified by a three-step procedure from human plasma with high concentrations of IgE. To 120 ml of ice-cold EDTA-plasma, 36 ml saturated ammonium sulphate solution were added dropwise and incubated overnight at 4°C while stirring. The precipitated proteins were removed by filtration (cut-off 1 µm). The filtrate was concentrated and washed with 10 volumes of Tris/NaCl (0.05 M Tris with 0.1 M NaCl, pH 8) in an ultrafiltration chamber (cut-off 100 kDa; Amicon). The filtrate was further purified by affinity chromatography with anti-IgE sepharose (monoclonal mouse anti-hIgE antibodies HP 6029 and HP 6061, Hybridoma Reagent Laboratory, Baltimore, MD, USA; 0.5 mg of each antibody being coupled to each ml packed sepharose). Before elution with 6 ml 0.05 M glycine buffer, pH 2.5, the sepharose was washed six times with 0.05 M Tris pH 8 and twice with 0.05 M Tris pH 4. To neutralize the eluate, 600 µl of 0.5 M Tris pH 8 were added immediately. The purity of the IgE was evaluated by SDS-PAGE with subsequent silver staining and the amount was determined by sandwich ELISA. The SDS-PAGE showed two bands (at 190 kDa and in a higher molecular weight range) that bound anti-hIgE following blotting onto nitrocellulose membrane, with the higher molecular weight-band presumably corresponding to complexed IgE.

#### Affinity chromatography of SmEA with IgE

Mouse anti-hIgE-Ab (1 mg) (clone A 0094; Dako) was coupled to 172 mg CNBr-activated sepharose 4 B and packed into a 1-ml syringe onto glass fibre filters. Purified human IgE (157 µg) was incubated on the column and washed five times with 1 ml PBS pH 7.2. Thereafter, 400 µg SmEA was applied prior to washing five times with 1 ml PBS. Subsequently, the column was eluted with 0.05 M glycine buffer pH 2.5. The content of IgE of the eluate was measured by ELISA.

## RESULTS

#### The IL-4-inducing activity is released from *S. mansoni* eggs

To determine whether the IL-4-inducing activity is released from the schistosome eggs, basophils were stimulated with

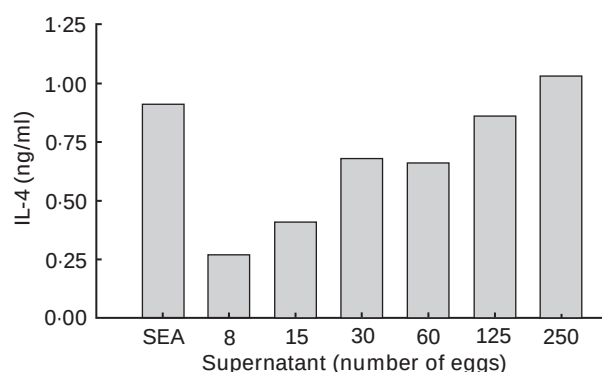


**Figure 1** The IL-4-inducing activity is released by *S. mansoni* eggs. Basophils were stimulated with 18 h culture supernatant from various numbers of eggs. The IL-4-production from  $10^6$  basophils following stimulation with supernatant from 125 eggs was equivalent to the amount released upon stimulation with  $40 \mu\text{g}$  SmEA. Spontaneous release of IL-4 was subtracted from all data.

culture supernatant of various numbers of *S. mansoni* eggs. As shown in Figure 1, IL-4 was released dose-dependently. This observation indicates that the IL-4-inducing activity is not a structural component of the eggs such as lacto-N-fucopentaose III, which has been reported to switch the immune response towards Th2 (14). Importantly, the IL-4-inducing activity belongs to the excretory/secretory compounds released from *S. mansoni* eggs.

#### The IL-4-inducing activity is a protein

SmEA is a complex mixture of different compounds (10). To investigate whether the IL-4-inducing activity is a protein, SmEA was digested for various time intervals with pepsin. Following pepsin digestion, the samples were heated for 15 min at  $70^\circ\text{C}$  in order to inactivate the pepsin. Separation of the samples by SDS-PAGE showed that after 15 min incubation with the protease, the majority of the proteins in SmEA was degraded. After digestion for 60 min or longer, only small peptides were left (Figure 2a). When these samples in which the proteins were completely degraded were incubated with human basophils, no IL-4-inducing activity was detected (Figure 2b). The pepsin sensitivity indicates protein nature of the IL-4-inducing activity.

#### Temperature and pH stability of the IL-4-inducing activity

To further characterize the IL-4-inducing activity, its temperature stability at different pHs was investigated. Aliquots of SmEA at pH 3, pH 7.4 and pH 10 were incubated at temperatures between  $20^\circ\text{C}$  and  $100^\circ\text{C}$  for 30 min. At pH 3,

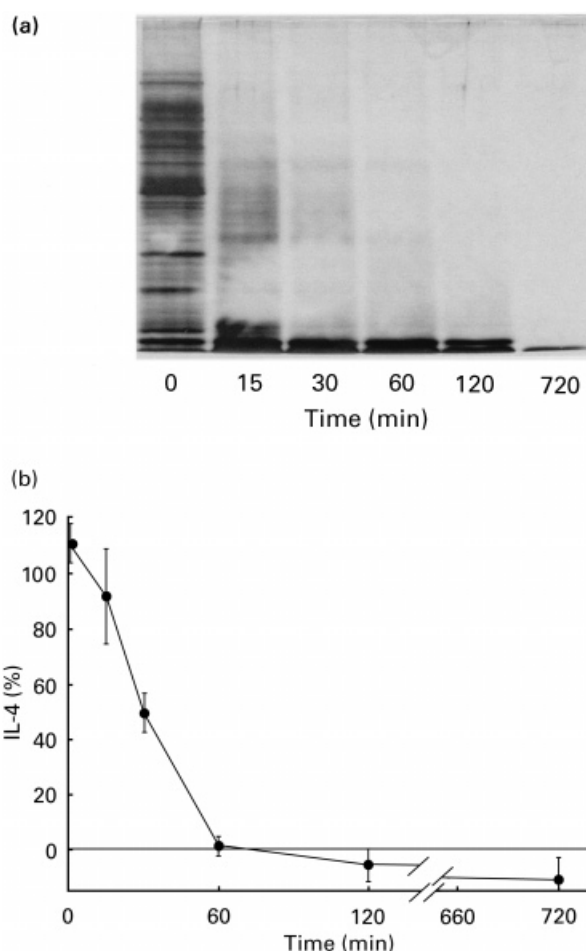


**Figure 2** The IL-4-inducing activity is digested by pepsin. SmEA at pH 2.2 was incubated for 0, 15, 30, 60, 120 and 720 min. Subsequently, all aliquots were heated for 15 min at  $70^\circ\text{C}$  to inactivate pepsin and separated by SDS-PAGE followed by silver staining (a). Furthermore, all aliquots were incubated with basophils from three human donors (b). No IL-4-inducing activity was detectable after 60 min (values in percent; arithmetic means  $\pm$  SEM). At this time, all proteins were digested by the pepsin. Spontaneous release of IL-4 was subtracted from all data.

the activity was thermoresistant up to  $100^\circ\text{C}$ ; at pH 7.4 up to  $70^\circ\text{C}$ , whereas at pH 10, the activity was stable only up to  $50^\circ\text{C}$ . At pH 10, only half of the activity was measurable after incubation at  $60^\circ\text{C}$  (Figure 3). In conclusion, the IL-4-inducing activity proved to be most stable at acidic pH, where it was found to resist boiling for 30 min.

#### The IL-4-inducing activity binds to Con A

Some already characterized proteins in SmEA are glycoproteins that can be enriched by Con A-affinity chromatography (15–17). Con A is a lectin that preferentially binds mannose (6). To investigate whether the IL-4-inducing

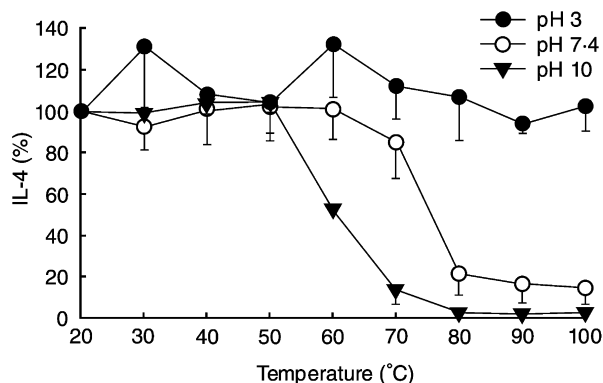


**Figure 3** Temperature stability of the IL-4-inducing activity at acidic, neutral and alkaline pH. Basophils from seven healthy human donors were stimulated with aliquots incubated for 30 min at the temperatures and pHs indicated. At pH 3, the IL-4-inducing activity was completely stable up to 100°C; at pH 7.4 up to 70°C and at pH 10 only up to 50°C (values in percent; arithmetic means  $\pm$  SEM). Spontaneous release of IL-4 was subtracted from all data.

activity belongs to these glycoconjugates, Con A-affinity chromatography was performed. No IL-4-inducing activity was detectable in the effluent, indicating that the IL-4-inducing activity completely bound to Con A. Methyl- $\alpha$ -D-mannopyranoside was successful in elution, strongly suggesting the presence of glycostructures in the IL-4-inducing activity, presumably including mannose residues. Since Con A itself can activate basophils to release IL-4 (6,18), the control eluate (eluate of Con A-sepharose to which no SmEA had been applied) was also tested for IL-4-inducing activity. It did not activate the basophils, excluding leaking ConA as a source of artificially positive results (Figure 4a). Together with the pepsin sensitivity, these results indicate a glycoprotein nature of the IL-4-inducing activity in SmEA.

#### The molecular weight of the IL-4-inducing activity lies between 31 and 66 kDa

To assess the molecular weight of the IL-4-inducing activity, the Con A-eluate was separated by SDS-PAGE and the gel was cut into nine pieces. After electroelution, two adjacent fractions corresponding to the molecular weight range between 31 and 66 kDa were shown to contain the IL-4-inducing activity (Figure 4b).

#### SmEA-induced cell activation depends on the presence of IgE

Basophils and mast cells can be activated in two ways: IgE-independently, e.g. by ionophores or complement factors

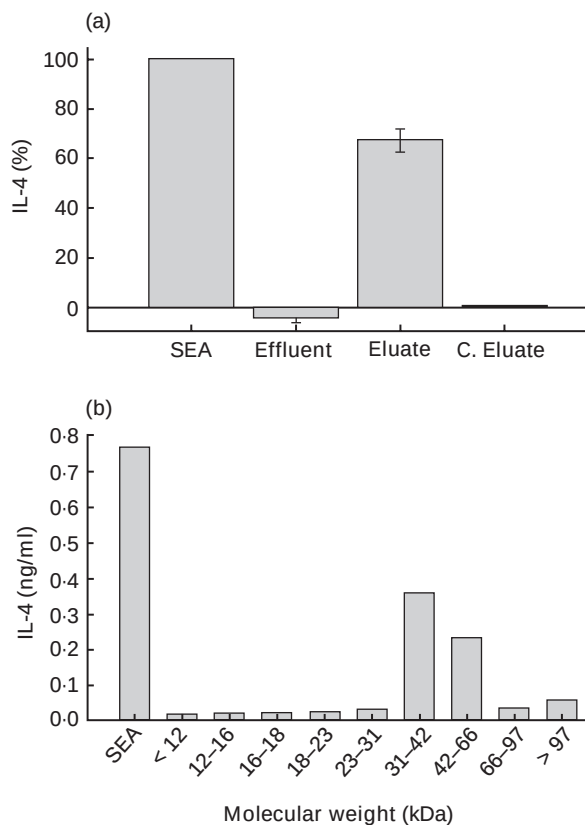


**Figure 4** (a) The IL-4-inducing activity binds to Con A-sepharose. Basophils of three human donors were incubated with effluent, eluate and control eluate (C. Eluate: eluate of Con A sepharose prior to incubation with SmEA) of the Con A affinity chromatography of SmEA. Nearly 70% of the activity were eluted by methyl- $\alpha$ -D-mannopyranoside; in the effluent and the control eluate no activity was detectable (values in percent; arithmetic means  $\pm$  SEM). Spontaneous IL-4 release was subtracted from all data. (b) Determination of the approximate molecular weight of the IL-4-inducing activity. The IL-4-inducing activity, enriched by Con A affinity chromatography, was separated by SDS-PAGE. Subsequently, the gel was sliced into nine pieces and subjected to electroelution. The activity was detected in two adjacent fractions which correspond to molecular weights of 31–66 kDa. Spontaneous release of IL-4 was subtracted from all data.

(19,20), and IgE-dependently, e.g. by allergens and specific lectins (6,18,21). To determine whether the activation of basophils by SmEA was dependent on IgE, surface IgE was stripped from the basophils at low pH prior to resensitization with purified IgE. Native, stripped and resensitized basophils were stimulated with SmEA (Figure 5). While, in stripped basophils, SmEA-induced IL-4 release was clearly reduced nearly to the level of the spontaneous release, it recovered in resensitized basophils. Stimulation with ionomycin showed unimpaired IL-4-release under all conditions, demonstrating functional integrity of the cells. These results indicate that the activation of human

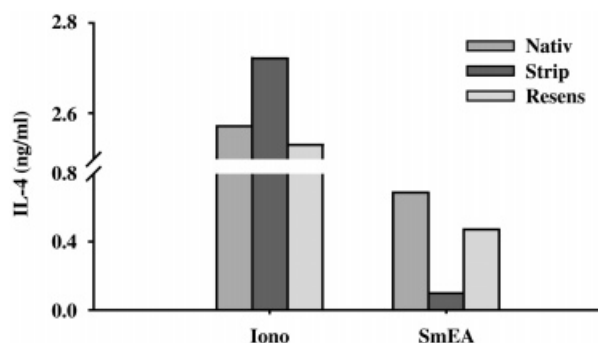


**Figure 5** Resensitization of basophils with purified human IgE restores SmEA-induced IL-4-secretion. Native basophils were stripped with lactate buffer pH 2.5 (Strip) and resensitized (Resens) with purified human IgE and subsequently incubated with ionomycin (Iono) or SmEA. Following stripping, SmEA-induced IL-4-release was dramatically reduced. This effect could be reversed by resensitization with purified IgE. IL-4-production after stimulation with ionomycin as functional control was not altered, indicating functional integrity of the basophils. Spontaneous release of IL-4 was subtracted from all data.

basophils is dependent on the presence of IgE on the surface of the cells.

### The IL-4-inducing activity binds to IgE

The binding of the IL-4-inducing activity to human IgE was demonstrated by affinity chromatography of SmEA with purified human IgE. No activity could be detected in the effluent of an anti-hIgE sepharose saturated with purified human IgE which was incubated with SmEA (Figure 6). After elution with glycine buffer, pH 2.5, 33% of the

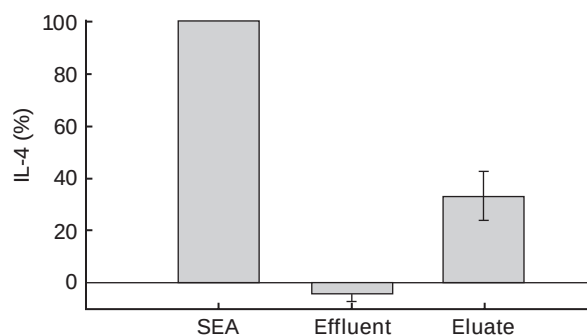


**Figure 6** Affinity chromatography of SmEA with human IgE bound to anti-IgE sepharose. A column with anti-hIgE-antibodies coupled to CNBr-activated sepharose was saturated with purified polyclonal human IgE, incubated with SmEA and the effluent collected. Subsequently, the column was eluted with 0.05 M glycine buffer pH 2.5 (Eluate). After incubation with basophils from six human donors, IL-4-inducing activity was only detected in the eluate fraction (values in percent; arithmetic means  $\pm$  SEM). Spontaneous IL-4 release was subtracted from all data.

applied IL-4-inducing activity could be detected in the eluate. The activity seemed to be eluted together with bound IgE, since approximately 40% of the bound IgE were also eluted. In an additional approach, affinity chromatography was performed with purified IgE directly coupled to sepharose and incubated with SmEA. Again, the effluent did not induce IL-4 from basophils. When the column was eluted with acidic buffer (pH 2.5), no IL-4-inducing activity could be detected either (data not shown). Since a conventional antigen-antibody interaction would have been disrupted under these conditions, this observation suggests that the IL-4-inducing activity binds to IgE in an antigen-independent manner.

### DISCUSSION

In this study, important properties of the IL-4-inducing activity of SmEA were characterized. It appears to be a glycoprotein, with a molecular weight lying between 31 and 66 kDa, which is secreted from the eggs. It is highly stable against heating at low pH and binds to IgE.

The IL-4-inducing activity was not only found in the extract, but also in the culture supernatant from live *S. mansoni* eggs, indicating that it is an excretory/secretory product. This suggests that, following deposition, *S. mansoni* eggs secrete this activity into the environment, which may bias the immune response against the eggs towards the Th2 phenotype. Since SmEA is a complex mixture of different proteins, glycoproteins, glycolipids, carbohydrates, DNA and RNA (10,22–24), the substance class responsible for induction of IL-4 in basophils was determined. The sensitivity towards pepsin digestion indicates that this activity is a protein, and Con A-affinity chromatography suggests the presence of carbohydrate structures. Since ConA preferentially binds to terminal  $\alpha$ -D-mannose, branched mannose or glucose, and the activity could be eluted with methyl- $\alpha$ -D-mannopyranoside, it most likely is a glycoprotein containing mannose residues, which are common constituents of N-linked glycans (25). Several already characterized, immunologically relevant compounds of SmEA belong to the Con A-binding fraction (22,26,27).

The assessment of the molecular weight of the IL-4-inducing activity by SDS-PAGE, and subsequent electroelution, resulted in two adjacent fractions corresponding to a molecular weight between 31 and 66 kDa which contained the IL-4-inducing activity. Future investigations will determine whether the size of the IL-4-inducing activity ranges around the dividing line between the two gel slices or whether its molecular weight stretches over the whole range. Several other compounds derived from *S. mansoni* have been described displaying a broader range of molecular weight rather than an exact value as, for

example, Major Serological Antigen 3 (MSA3) (15), Cathodic Protein  $\kappa$  3 (28), both components of SmEA, and Circulating Cathodic Antigen (CCA) from the digestive tract of adult worms (29).

The activation of basophils by SmEA is dependent on the presence of IgE on the surface of the basophils. High levels of schistosome-specific IgE are associated with resistance against infections with schistosomes in humans (30,31). As reported earlier, low-pH stripping of IgE from basophils results in reduced SmEA-induced IL-4-production. Subsequent resensitization with IgE-containing serum restored the SmEA-induced IL-4-release (4). However, resensitization with myeloma IgE(PS) did not restore SmEA-induced IL-4-release of the basophils. The reasons for this observation are not known. It could be due to the purity of IgE(PS) in case the SmEA-induced IL-4-release was restored by a component of the serum (e.g. complement factors). Another possibility could be that IgE(PS) had an irrelevant specificity or belonged to the 'wrong' type of IgE, since a heterogeneity of IgE has been shown (32–34). To clarify the role of IgE in the stimulation of human basophils by SmEA, purified human polyclonal IgE was used for resensitization instead of serum. The results confirmed the assumption that IgE is required on the surface of the basophils for stimulating these cells by SmEA.

We have shown that the IL-4-inducing activity binds directly to the IgE molecule. Schroeder *et al.* (34) and Wantke *et al.* (35) have shown in their binding studies with the recombinant human histamine releasing factor (HrHRF) that an IgE-dependent factor does not necessarily bind to IgE. The direct binding of the IL-4-inducing activity to IgE was demonstrated in two different ways: First, by the binding of this activity to purified human IgE bound to an anti-IgE sepharose and by the subsequent elution of this factor together with the bound IgE and, second, by binding to human IgE coupled to sepharose. With respect to the mechanism of action, the IL-4-inducing activity could bind to IgE on the surface of basophils and crosslink it, leading to activation of these cells and consequently IL-4-production. Since all basophil preparations were derived from healthy blood donors without a prior history of schistosomiasis, the basophils in this study could not be expected to carry schistosome-specific IgE. A possibility is cross-reactivity to other *Schistosoma* species such as *Trichobilharzia* which normally parasitize waterfowl. When cercariae of these species penetrate the human skin, they are killed by a local immune response, causing the so-called 'swimmer's itch' in sensitized persons. However, cross-reactivity is unlikely, since it is improbable that the majority of the blood donors came in contact with these parasites. Furthermore, binding of antibodies to cross-reactive epitopes is normally weaker than binding to the nominal antigen. Since the activity

could not be eluted from the affinity column by the usual elution methods from the affinity column, such a strong binding is unlikely to be caused by cross-reactivity. Similar to a variety of lectins, the IL-4-inducing activity might exert its effect by binding to carbohydrate side chains. As an alternative, the IL-4-inducing activity could bind to the protein part of IgE including superallergens such as protein A from *Staphylococcus* (SpA) or protein Fv (pFv) which crosslink all classes of immunoglobulins not only from humans, but also from other mammals, birds, reptiles, amphibians and fish (36,37).

Taken together, the IL-4-inducing activity in SmEA appears to be a glycoprotein with a molecular weight lying between 31 and 66 kDa. It was shown to be released from the eggs and to bind to IgE, presumably activating basophils by crosslinking of receptor-bound IgE. Further characterization of the IL-4-inducing activity should facilitate future investigations in the field of IL-4-induction.

#### ACKNOWLEDGEMENTS

We would like to thank PD U.Zähringer for helpful discussions and Dr T.Goldmann for his help in preparing this manuscript.

#### REFERENCES

- 1 Doenhoff MJ, Hassounah O, Murare H, Bain J, Lucas S. The schistosome egg granuloma: immunopathology in the cause of host protection or parasite survival? *Trans Royal Soc Trop Med Hygiene* 1986; **80**: 503–514.
- 2 Okano M, Satoskar AR, Nishizaki K, Abe M, Harn DA Jr. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J Immunol* 1999; **163**: 6712–6717.
- 3 Haas H, Falcone FH, Holland MJ *et al.* Early interleukin-4: its role in the switch towards a Th2 response and IgE-mediated allergy. *Int Arch Allergy Immunol* 1999; **119**: 86–94.
- 4 Falcone FH, Dahinden CA, Gibbs BF *et al.* Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen. *Eur J Immunol* 1996; **26**: 1147–1155.
- 5 Arock M, Merle-Beral H, Dugas B *et al.* IL-4 release by human leukemic and activated normal basophils. *J Immunol* 1993; **151**: 1441–1447.
- 6 Haas H, Falcone FH, Schramm G *et al.* Dietary lectins can induce *in vitro* release of IL-4 and IL-13 from human basophils. *Eur J Immunol* 1999; **29**: 918–927.
- 7 Mueller R, Heusser CH, Rihs S, Brunner T, Bullock GR, Dahinden CA. Immunolocalization of intracellular interleukin-4 in normal human peripheral blood basophils. *Eur J Immunol* 1994; **24**: 2935–2940.
- 8 Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine responses to a non-parasite antigen. *J Immunol* 1992; **148**: 3264–3270.
- 9 Boros DL, Warren KS. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble

- factor isolated from *Schistosoma mansoni* eggs. *J Exp Med* 1970; **132**: 488–507.
- 10 Carter CE, Colley DG. An electrophoretic analysis of *Schistosoma mansoni* soluble egg antigen preparation. *J Parasitol* 1978; **64**: 285–290.
  - 11 Haisch K, Gibbs BF, Korber H *et al.* Purification of morphologically and functionally intact human basophils to near homogeneity. *J Immunol Methods* 1999; **226**: 129–137.
  - 12 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–685.
  - 13 Heukeshoven J, Dernick R. Improved silver staining procedure for fast staining in PhastSystem Development Unit. I. Staining of sodium dodecyl sulfate gels. *Electrophoresis* 1988; **9**: 28–32.
  - 14 Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by B220+ cells from schistosome-infected mice: a mechanism for regulation of CD4+ T-cell subsets. *Proc Natl Acad Sci USA* 1994; **91**: 18–22.
  - 15 Pelley RP, Pelley RJ, Hamburger J, Peters PA, Warren KS. *Schistosoma mansoni* soluble egg antigens. I. Identification and purification of three major antigens, and the employment of radioimmunoassay for their further characterization. *J Immunol* 1976; **117**: 1553–1560.
  - 16 Lustigman S, Hamburger J. *Schistosoma mansoni*: radiometric assay of lectin binding specificities of the major egg glycoprotein and its carbohydrate-rich fragment. *Exp Parasitol* 1985; **59**: 59–67.
  - 17 Dunne DW, Bain J, Lillywhite J, Doenhoff MJ. The stage-, strain- and species-specificity of a *Schistosoma mansoni* egg antigen fraction. (CEF6) with serodiagnostic potential. *Trans Royal Soc Trop Med Hygiene* 1984; **78**: 460–470.
  - 18 Magro AM, Bennich H. Concanavalin A induced histamine release from human basophils in vitro. *Immunology* 1977; **33**: 51–58.
  - 19 Lichtenstein LM. The mechanism of basophil histamine release induced by antigen and by the calcium ionophore A23187. *J Immunol* 1975; **114**: 1692–1699.
  - 20 Dahinden CA, Rihs S, Ochsenberger B. Regulation of cytokine expression by human blood basophils. *Int Arch Allergy Immunol* 1997; **113**: 134–137.
  - 21 Sebahoun G, Vervloet D, Charpin J, Carcassonne Y. Histamine release reaction of basophils in chronic granulocytic leukaemia. Induction by heterologous anti-immunoglobulin E, concanavaline A, and phytohaemagglutinin; effect of heavy water. *J Clin Pathol* 1978; **31**: 749–752.
  - 22 Carter CE, Colley DG. Partial purification and characterization of *Schistosoma mansoni* soluble egg antigen with Con A-sepharose chromatography. *J Immunol* 1979; **122**: 2204–2209.
  - 23 Weiss JB, Magnani JL, Strand M. Identification of *Schistosoma mansoni* glycolipids that share immunogenic carbohydrate epitopes with glycoproteins. *J Immunol* 1986; **136**: 4275–4282.
  - 24 Boctor FN, Nash TE, Cheever AW. Isolation of a polysaccharide antigen from *Schistosoma mansoni* eggs. *J Immunol* 1979; **122**: 39–43.
  - 25 Merkle RK, Cummings RD. Lectin affinity chromatography of glycopeptides. *Meth Enzymol* 1987; **138**: 232–259.
  - 26 Owhashi M, Horii Y, Imai J, Ishii A, Nawa Y. Purification and physicochemical characterization of *Schistosoma mansoni* egg allergen recognized by mouse sera obtained at an acute stage of infection. *Int Arch Allergy Appl Immunol* 1986; **81**: 129–135.
  - 27 Harrison DJ, Carter CE, Colley DG. Immunoaffinity purification of *Schistosoma mansoni* soluble egg antigens. *J Immunol* 1979; **122**: 2210–2217.
  - 28 Dunne DW, Bickle QD. Identification and characterization of a polysaccharide-containing antigen from *Schistosoma mansoni* eggs which cross-reacts with the surface of schistosomula. *Parasitology* 1987; **94**: 255–268.
  - 29 Deelder AM, Kornelis D, van Marck EA, Eveleigh PC, Van Egmond JG. *Schistosoma mansoni*: characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamster, and human infections. *Exp Parasitol* 1980; **50**: 16–32.
  - 30 Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti larval IgE levels. *Eur J Immunol* 1991; **21**: 2679–2686.
  - 31 Dunne DW, Webster M, Smith P *et al.* The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate that IgE responses against the antigen(s) it contains are associated with human resistance to reinfection. *Parasite Immunol* 1997; **19**: 79–89.
  - 32 MacDonald SM, Lichtenstein LM, Proud D *et al.* Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987; **139**: 506–512.
  - 33 Robertson MW, Liu FT. Heterogeneous IgE glycoforms characterized by differential recognition of an endogenous lectin (IgE-binding protein). *J Immunol* 1991; **147**: 3024–3030.
  - 34 Schroeder JT, Lichtenstein LM, MacDonald SM. An immunoglobulin E-dependent recombinant histamine-releasing factor induces interleukin-4 secretion from human basophils. *J Exp Med* 1996; **183**: 1265–1270.
  - 35 Wantke F, MacGlashan DW, Langdon JM, MacDonald SM. The human recombinant histamine releasing factor: functional evidence that it does not bind to the IgE molecule. *J Allergy Clin Immunol* 1999; **103**: 642–648.
  - 36 Silverman GJ, Pires R, Bouvet JP. An endogenous sialoprotein and a bacterial B cell superantigen compete in their VH family-specific binding interactions with human Igs. *J Immunol* 1996; **157**: 4496–4502.
  - 37 Silverman GJ, Roben P, Bouvet JP, Sasano M. Superantigen properties of a human sialoprotein involved in gut-associated immunity. *J Clin Invest* 1995; **96**: 417–426.