

Franco H. Falcone¹,
Clemens A. Dahinden²,
Bernhard F. Gibbs³,
Tobias Noll³,
Ulrich Amon³,
Holger Hebestreit¹,
Ole Abrahamsen¹,
Jens Klaucke¹,
Max Schlaak¹ and
Helmut Haas¹

¹ Forschungszentrum Borstel,
Borstel, Germany

² Institute of Clinical Immunology
and Allergology, University
Hospital, Bern, Switzerland

³ Department for Dermatology,
Medical University of Lübeck,
Lübeck, Germany

Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen

The elevated interleukin (IL)-4 and IgE production in *Schistosoma mansoni* infection seems to be induced essentially by the egg stage of the parasite. The underlying mechanism, however, is not known. Since basophils from human peripheral blood can produce IL-4, we asked, whether soluble *S. mansoni* egg antigens (SEA) would trigger basophils to release IL-4. Basophils from healthy human donors ($n = 32$) without prior history of schistosomiasis were incubated with SEA in the presence of IL-3. In all donors, IL-4 was produced at different concentrations. The IL-4 production was dependent on the dose of SEA, was correlated with the purity of the basophil preparation, and the IL-4 concentration in the culture supernatant was maximal 5 h after stimulation with SEA. In addition to its IL-4-stimulatory effect, SEA triggered basophils to degranulate, thereby releasing histamine and sulfidoleukotrienes. Stripping of receptor-bound IgE from basophils inhibited both SEA- and anti-IgE-induced, but not ionomycin-induced IL-4 production. Moreover, resensitization of stripped basophils with stripping supernatants or human serum restored SEA-induced IL-4 production. This suggests that IgE is involved in the mechanism of IL-4 induction by SEA. Since IL-4 is induced in basophils from nonexposed donors, basophils may play a role as an early source of IL-4 in *S. mansoni* infection.

1 Introduction

Exposure to certain pathogens can stimulate two patterns of cytokine production by T cells [1–3]. The production of interferon- γ (IFN- γ), lymphotoxin and interleukin-(IL)-2 is classified as a T helper type 1 (TH1) response, whereas the production of IL-4, IL-5, IL-10, and IL-13 is called a T helper type 2 (TH2) response [4, 5]. The cytokines that are present during initial antigen priming seem to control the differentiation of the T cells into either TH1 or TH2 cells [6, 7] with IL-4 being a crucial factor controlling the switch towards TH2. Moreover, IL-4 and IL-13 are key factors to switch B cells from the production of IgM to the production of IgE [8, 9]. Although during an ongoing immune response, TH2 cells are a major source of IL-4, they are unlikely to provide the early IL-4 needed for their own differentiation from unprimed T helper precursor cells to TH2 cells. This suggests that other cell populations act as a primary source of IL-4. Major candidates are basophils and mast cells, both of which have been shown to produce IL-4 in mice [10, 11] and humans [12–15]. In mice CD4⁺ NK1.1⁺ T cells [16] and $\gamma\delta$ T cells [17] have also been suggested to play such a role. Natural infestations with parasitic worms cause many characteristics of a TH2 immune response: elevation of the serum IgE levels [18], eosino-

philia and in the case of certain parasites, mastocytosis [19]. Correspondingly, TH2 cytokine patterns have been described as a consistent response to various helminth parasites [2, 20–25]. However, not all stages of the life cycle of helminthic parasites have the potential to induce a TH2, and thus an IgE response. When mice are infected with the trematode *Schistosoma mansoni*, the egg stage has TH2-inducing potential, whereas the schistosomula (the larval stage) induce a TH1 immune response [22]. The IL-4-inducing potential of schistosomal eggs is so pronounced that even naive mice develop a strong TH2 response after being injected with *S. mansoni* eggs [26]. This stage-specific effect seems also to apply to humans [27]. A stage-dependent induction of a TH1 or a TH2 response has also been described during infection with the nematode *Brugia malayi*. The adult female stage of this helminth parasite has IL-4-inducing capacity, whereas the microfilarial stage promotes production of IFN- γ [24]. Due to their IL-4-inducing potential, *S. mansoni* eggs are a suitable means for investigating the basic mechanism of TH2 induction. To study the question of whether *S. mansoni* soluble egg antigen (SEA) might induce the release of IL-4 from basophils of unprimed donors, and thus presumably skew T cell differentiation towards TH2, we stimulated human basophils *in vitro* with SEA and determined the IL-4 concentration in the supernatant.

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Correspondence: Helmut Haas, Immunpharmakologie II, Forschungszentrum Borstel, Parkallee 22, D-23845 Borstel (Fax: +49-45 37-18 84 04)

Abbreviations: NCM: Nitrocellulose membrane; *S. mansoni*: *Schistosoma mansoni* SEA: *Schistosoma mansoni* egg antigen sLT: Sulfidoleukotrienes Tris: 0.1 M Tris HCl pH 7.5, 0.1 M NaCl, 2.5 mM MgCl₂ Tris-T: Tris containing 0.05 % Tween 20

Key words: *Schistosoma mansoni* / Basophil / Interleukin-4

2 Materials and methods

2.1 Chemicals, antigens and antibodies

SEA was kindly supplied by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland. Iscove's modified Dulbecco's medium was from Gibco BRL (Paisley, Scotland); FCS was from Boehringer Mannheim (Mannheim, Germany); ionomycin was from Sigma (München, Germany); Ficoll was from Biochrom (Berlin, Germany); Percoll was from Pharmacia (Uppsala, Sweden); rhIL-3 was a

kind gift of Kirin (Gunma, Japan); rhIL-4 was a kind gift from Glaxo (Greenford, GB) or was purchased from Genzyme (Nr. 2181-01, Cambridge, USA); polyclonal rabbit anti-hIgE Ab was from Dako (Nr. A094, Glostrup, Denmark); mouse anti-hIL-4 mAb (clone IL-4I), biotinylated rat anti-hIL-4 mAb (clone MP4-25D2) and alkaline phosphatase-conjugated mouse anti-IgE mAb (clone G7-26) were from PharMingen (Hamburg, Germany); Quantikine™ IL-4 kit was from R&D Systems (Minneapolis, MN) and the CAST ELISA kit was from Bülmann (Allschwil, Switzerland).

2.2 Purification of basophils

Basophils were purified in a three-step procedure including a Ficoll/Percoll discontinuous density gradient, counter-current centrifugal elutriation and negative selection with immunomagnetic beads. EDTA-treated blood (240 ml) freshly drawn from healthy nonatopic or atopic volunteers (laboratory staff) was carefully layered on a 100:6 (v/v) Ficoll/Percoll mixture (density: 1080 g/l). After centrifugation (20 min, 500 × g, 4°C) the interphases were pooled, washed twice and resuspended in elutriation buffer (1 × HBSS, 4.2 mM NaHCO₃, 0.25 % BSA (w/v) without Ca²⁺ and Mg²⁺, pH 7.35–7.40). Elutriation was performed with a J2-21 centrifuge and a JE-6B rotor, equipped with a standard chamber (Beckmann Instruments, Palo Alto, CA). Interphase leukocytes (2 × 10⁷–1.0 × 10⁸) suspended in 50 ml cold elutriation buffer were loaded into the chamber. The rotor speed was held constant at a speed of 3500 rpm (1220 × g) throughout the whole elutriation. The flow rate was progressively raised from 28 to 41 ml/min and several fractions collected. Fractions containing basophils were concentrated by centrifugation (10 min, 500 × g, 4°C) and aliquots were taken for the evaluation of cell concentration, viability (trypan blue exclusion) and basophil purity (May-Grünwald stain). The fractions with the highest basophil purity (ranging from 8%–47%) were pooled, concentrated by centrifugation (see above) and resuspended in 5–7 ml elutriation buffer. Finally, immunomagnetic beads (M-450-anti-CD2, -anti-CD14, -anti-CD19; Dynal, Hamburg, Germany) were added and incubated for 1 h on a sample mixer at 4°C. Thereafter, beads with bound cells were removed with a magnetic particle concentrator for 2 min and the magnet-bound fraction resuspended in 7 ml elutriation buffer. This procedure was repeated three times. Using this protocol, the obtained basophil purity ranged between 25–97%. The mean purity of the basophils used for the experiments in this study was 67%. The mean yield was 2.83 × 10⁶ ± 2.12 × 10⁶ (mean ± SD; range 7 × 10⁵–14.7 × 10⁶).

2.3 Culture and stimulation conditions

Culture medium was Iscove's modified Dulbecco's medium supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 10 % FCS, 5 µg/ml bovine insulin and 50 µg/ml human transferrin. Basophil preparations were resuspended in culture medium at a concentration of 1 × 10⁶ basophils/ml. All incubations were carried out in sterile round-bottom 96-well microtiter plates (Nunc, Roskilde, Denmark) at 37°C in a humidified air containing 6 % CO₂. If not stated otherwise, single determinations were per-

formed. Cell-free supernatants were harvested after 18 h, if not indicated otherwise, and stored at –20°C. Cells were exposed to the different reagents at the following concentrations: SEA: 40 µg/ml; anti-IgE: 0.4 µg/ml (rabbit anti-hIgE); IL-3: 10 ng/ml; ionomycin: 1 µM; *Phleum pratense* (timothy grass) pollen extract (Phl pX): 10 µg/ml and myeloma IgE-PS (a generous gift from Prof. K. Ishizaka, La Jolla Institute of Allergy and Immunology, La Jolla, CA): 100 ng/ml.

2.4 IgE stripping and resensitization of basophils

Receptor-bound IgE was dissociated from the surface of basophils as described by Pruzanski et al. [28]. Briefly, purified basophils (1 × 10⁶–3 × 10⁶) were resuspended in 500 µl 0.01 M lactic acid/lactate-buffered isotonic saline pH 3.9 for 5 min on ice; the pH was then neutralized by adding 14.5 ml cold elutriation buffer. Immediately afterwards, the cells were centrifuged (300 × g, 10 min, 4°C) and the supernatant removed. Stripping supernatants were concentrated to 1 ml by ultrafiltration. Basophils were resensitized with the concentrated stripping supernatants, human serum (native or after heating to 56°C for 30 min) or myeloma IgE-PS for 90 min at 37°C, washed twice and resuspended in medium. Then native, stripped and resensitized basophils were stimulated in parallel with SEA, Phl pX, anti-IgE (each in the presence of IL-3) or ionomycin.

2.5 IL-4 assay

IL-4 was determined using a sandwich ELISA. The buffer was 0.1 M Tris-HCl, 0.1 M NaCl, 2.5 mM MgCl₂ pH 7.5 (Tris). All incubation steps were carried out on a rotating platform. Between the steps, plates were washed eight times with Tris/0.05 % Tween 20 (Tris-T). Microtiter plates (Maxisorp F96, Nunc) were coated overnight at 4°C with mouse anti-hIL-4 mAb (1:4000 in Tris). After incubation (90 min) with samples or serial dilutions of rhIL-4, biotinylated rat anti-hIL-4 mAb (1:4000 in Tris-T/0.5 % BSA) was added for 60 min. Next, the plates were incubated with streptavidin-alkaline phosphatase (1:1000 in Tris-T/0.05 % BSA) for 60 min. The chromogen was *p*-nitrophenyl-phosphate (1 mg/ml) in 0.1 M Tris/HCl, 0.1 M NaCl, 5 mM MgCl₂ pH 9.5. The absorption was read at 405 nm. The assay had a sensitivity of 50 pg/ml with a dynamic range of up to 5 ng/ml. In some experiments, IL-4 was determined using the Quantikine™ human IL-4 ELISA kit with similar results.

2.6 Isoelectric focusing and immunoblotting

This was performed as described in detail elsewhere [29]. Briefly, SEA was separated by IEF in 0.8 % agarose with 3 % (w/v) Servalyte 3–10 and 1 % (w/v) Servalyte 4–6 (Serva, Heidelberg, Germany). The separated SEA was transferred by the print technique (capillary blotting) to a nitrocellulose membrane (NCM). After blocking free protein binding sites with Tris-T, strips of the NCM were incubated over night at 4°C with 1.5 ml of individual sera (1:50 in Tris-T) in multichannel plates on a horizontal shaker (the sera were from healthy nonatopic and atopic individuals and from patients with schistosomiasis (each

group, $n = 7$); four of the patients were infected with *S. mansoni*, two with both *S. mansoni* and *S. haematobium*, and one with *S. intercalatum*). After washing the strips four times 10 min in Tris-T, mouse anti-IgE mAb (1:2000 in Tris-T) was added for 2 h. After washing, the strips were developed overnight with nitroblue-tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate.

2.7 Determination of histamine and leukotrienes

The concentration of histamine in supernatants of basophils was determined by using the Pharmacia Methyl-Histamine RIA according to the manufacturer's protocol. The concentration of the sulfidoleukotrienes (sLT) LTC₄, LTD₄ and LTE₄ was measured with a single leukotriene ELISA contained in the CAST ELISA test kit according to the manufacturer's protocol.

3 Results

3.1 IL-4 is released from human basophils after stimulation with ionomycin or anti-IgE

Since basophils produce IL-4 in response to nonspecific activation [10, 13], all basophil preparations were stimulated with 1 μ M ionomycin to assess the functional integrity of the cells. The IL-4 production after stimulation with ionomycin ranged between 1.1 and 12.5 ng/10⁶ basophils (mean $\pm$ SD: 4.5 ± 2.5) in 35 donors. To determine whether our basophil preparations released IL-4 when triggered via the IgE receptor, they were stimulated with anti-IgE at various concentrations and with IL-3. A typical bell-shaped curve was obtained and IL-4 secretion was maximal when anti-IgE was applied at concentrations between 0.2–0.8 μ g/ml with the optimal anti-IgE concentration differing slightly for different donors. The IL-4 production after stimulation with 0.4 μ g/ml anti-IgE was 0.36 ± 0.42 ng/ml (mean $\pm$ SD; range: 0.11–1.1 ng/ml) in five donors (data not shown).

3.2 IL-4 is released from human basophils after stimulation with SEA

Basophils of healthy human donors were stimulated with 40 μ g/ml SEA in the presence of 10 ng/ml IL-3. IL-4 was

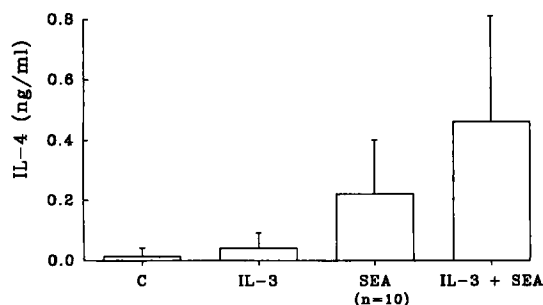


Figure 1. SEA stimulates basophils to produce IL-4. Basophils of healthy blood donors ($n = 32$) were incubated with medium, IL-3, SEA (only 10 donors), or SEA + IL-3. Although there was a high degree of variation between individuals, all donors' basophils produced IL-4 in response to SEA.

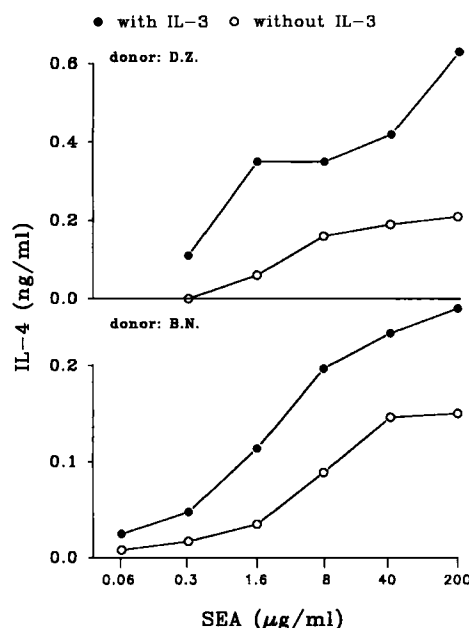


Figure 2. Dose-response curve for IL-4 production by basophils stimulated with various concentrations of SEA in the presence or absence of IL-3 (two representative out of five experiments).

released from basophils of all donors under study ($n = 32$) ranging between 0.1–1.4 ng/ml per 10⁶ basophils (mean $\pm$ SD: 0.41 ± 0.33 ng/10⁶ basophils). Since initial experiments with 10 donors had shown that IL-4 production after stimulation with SEA was higher when IL-3 was added to the culture, stimulation with SEA was in further experiments carried out only in the presence of IL-3 (Fig. 1).

3.3 Dose dependency of SEA-induced IL-4 synthesis

Basophils were incubated with various concentrations of SEA in the presence or absence of IL-3. IL-4 production was found to be dose-dependently induced by SEA, and IL-3 enhanced this effect (Fig. 2). The amount of IL-4 produced in response to different concentrations of SEA differed among individuals.

3.4 IL-4 production correlates with basophil purity

Since IL-4 is produced also by T cells, it seemed possible that T cells contaminating the basophil preparations produced the IL-4. To test this possibility, highly purified basophils (purity 91 %) were reconstituted at various proportions with autologous PBMC and stimulated with SEA in the presence of IL-3 or with ionomycin (Fig. 3). In both conditions, IL-4 production strictly correlated with the purity of the basophils. This indicates that SEA-induced IL-4 is derived from basophils and not from other cells.

3.5 Time course of SEA-induced IL-4 synthesis

Fig. 4 shows the kinetics of SEA-induced IL-4 production by human basophils cultured with IL-3. IL-4 secretion rapidly reached a plateau after approximately 5 h. Similar

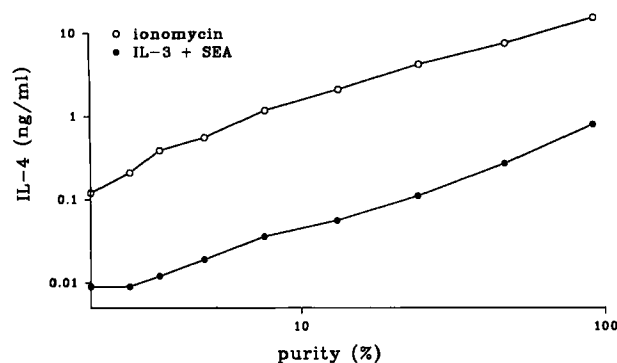


Figure 3. IL-4 production depends on the purity of the basophil preparation. Purified basophils (91 % purity) were reconstituted at various proportions with autologous PBMC and stimulated with SEA + IL-3 or with ionomycin. With both stimuli, IL-4 production was dependent on basophil purity.

kinetics were observed for stimulation with ionomycin. In contrast to basophils, T cells have been reported to release IL-4 at a slower rate when stimulated with the relevant antigen. This further supports the view that IL-4 is produced by the basophils and not by T cells contaminating the basophil preparations.

3.6 SEA induces degranulation of basophils

In addition, we asked whether incubation of basophils with SEA would also trigger the release of mediators other than IL-4. Since, in contrast to IL-4, the release of histamine and sLT is very rapid, we assayed culture supernatants of stimulated basophils 30 min after stimulation for these mediators. Indeed, SEA in combination with IL-3 induced a pronounced release of both histamine and sLT, whereas IL-3 alone had only a marginal effect (Fig. 5).

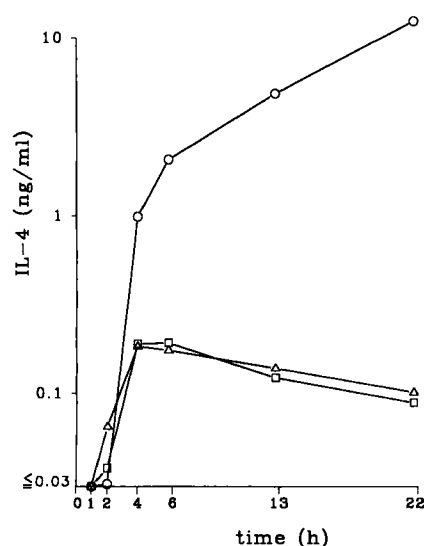


Figure 4. Kinetics of IL-4 production by basophils after stimulation with SEA + IL-3, anti-IgE + IL-3, or ionomycin. After the times indicated, the culture supernatants were collected for IL-4 determination (one representative out of two experiments). The IL-4 measured in the supernatant reached the plateau phase after approximately 5 h, when the cells were stimulated with SEA or anti-IgE.

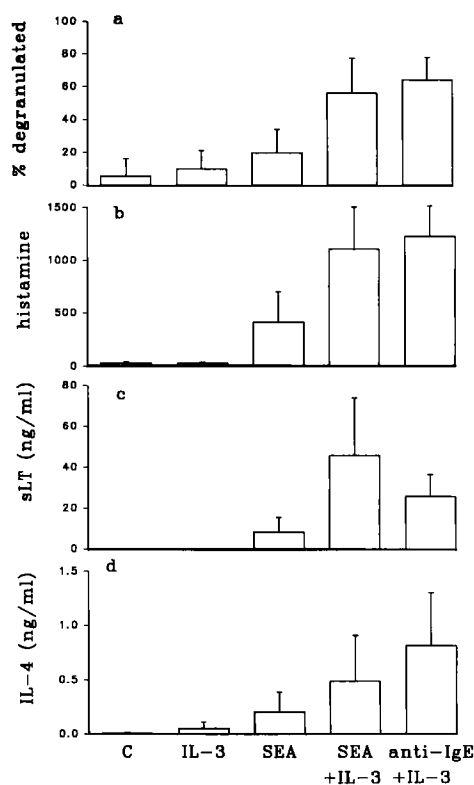


Figure 5. SEA induces degranulation of basophils. Basophils were obtained from four healthy human donors [additional donor in (b) and (d)] and were incubated in the presence of the following agents: medium (C), IL-3, SEA, SEA + IL-3, or anti-IgE + IL-3. After 30 min, the percentage of degranulated basophils (a) and the concentrations in the culture supernatants of histamine (b) and sLT (c) were determined. IL-4 production (d) was assayed after 5 h.

Along with the release of histamine, degranulation of the basophils occurred after treatment with SEA, with the extent of degranulation resembling that after stimulation with anti-IgE (Fig. 6).

3.7 Immunoblot analysis of IgE antibodies to SEA in human sera

Although the individuals included in this study had never been infected with *S. mansoni*, and thus, their sera should not contain SEA-specific IgE, the possibility of cross-reactive IgE antibodies present on their basophils could not be excluded. To assess whether the SEA-induced IL-4 production of basophils is mediated via IgE, we studied human sera by immunoblotting for the presence of IgE antibodies to SEA. SEA was separated by isoelectric focusing and blotted onto NCM. Strips of the NCM were incubated in parallel with the sera of healthy nonatopic and atopic blood donors without prior history of schistosomiasis and of patients with schistosomiasis. In all patients, IgE binding was strong and differed among individuals. In contrast, only one of seven nonatopic and three of seven atopic donors' sera showed a moderate IgE reactivity to SEA components (Fig. 7).

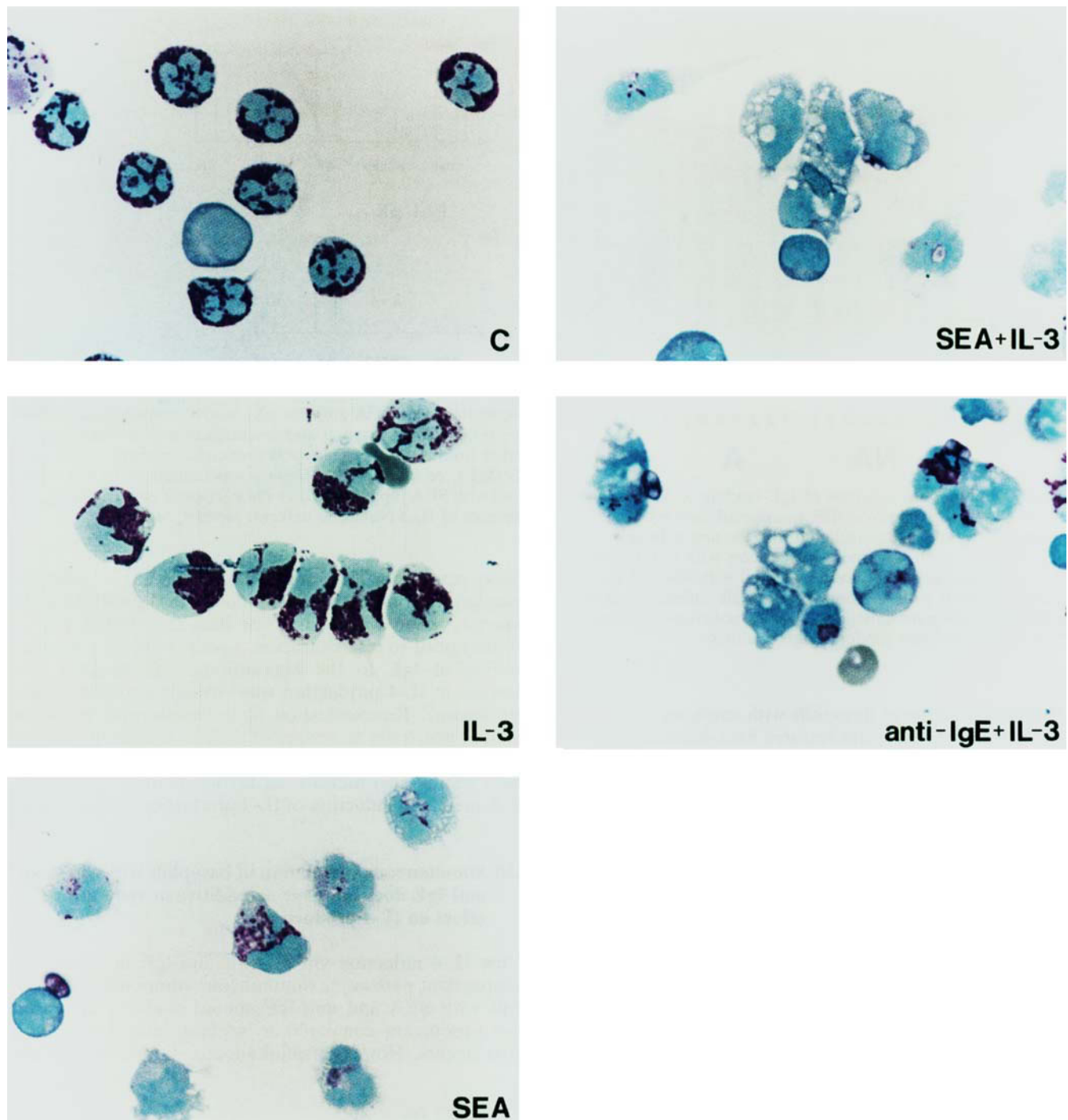


Figure 6. The morphology of basophils in the experimental conditions discussed in the legend of Fig. 5 is shown.

3.8 Low-pH lactate stripping of IgE from basophils reduces SEA-induced IL-4 production

To determine whether SEA-induced IL-4 production is mediated via IgE, basophils were stripped of surface IgE by incubating them in lactate-buffered saline at pH 3.9. After washing with elutriation buffer, the cells were stimulated with SEA, anti-IgE (both in the presence of IL-3) and ionomycin as a control. Compared to untreated cells, IgE-stripped basophils released lower amounts of IL-4 when stimulated with SEA or with anti-IgE. It is

noteworthy that the IL-4-reducing effect of the stripping treatment was less pronounced when the cells were stimulated with anti-IgE. This might indicate that even a small number of IgE molecules per cell might be sufficient to mediate basophil degranulation when unspecifically cross-linked [30]. In contrast, stripping did not influence IL-4 release after stimulation with ionomycin, which indicates that the stripping treatment left the cells functionally intact. These results suggest that IL-4 induction by SEA from basophils is IgE-dependent (Fig. 8).

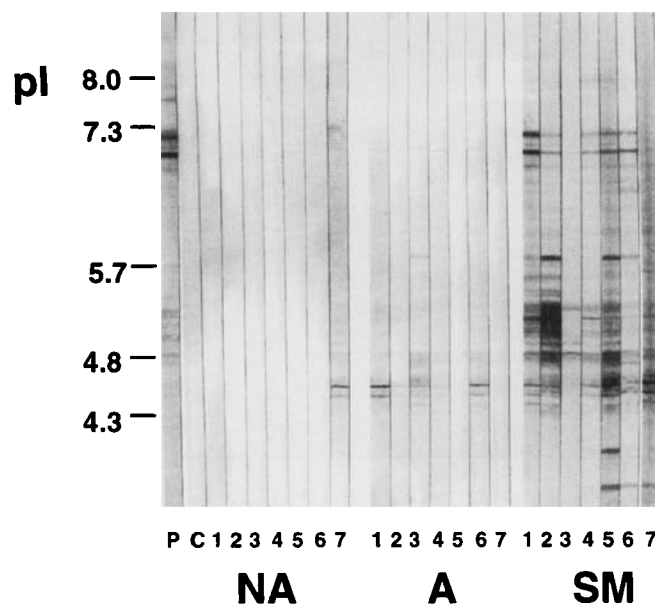


Figure 7. Immunoblot analysis of IgE-binding to SEA components. SEA was separated via IEF, transferred onto NCM (P: protein stain with India ink), incubated with the sera of healthy nonatopic (NA), atopic (A) donors, or patients with schistosomiasis (SM) (each group $n = 7$) and developed with labeled anti-IgE mAb (C: control without serum). While all patients' sera show strong IgE-binding patterns, one of seven nonatopic and three of seven atopic sera show moderate IgE reactivity.

3.9 Resensitization of basophils with stripping supernatants or serum restores SEA-induced IL-4 production

To determine whether the effect of the stripping treatment could be reversed, basophils were resensitized with myeloma IgE-PS, stripping supernatants, or serum from a grass pollen-allergic donor. In Fig. 9, IL-4 production from native, stripped and resensitized basophils from five donors stimulated by SEA and Phl pX is compared. Resensitization of basophils with serum from a timothy grass pollen-allergic donor clearly restored SEA- and Phl pX-induced IL-4 production. Resensitization with concen-

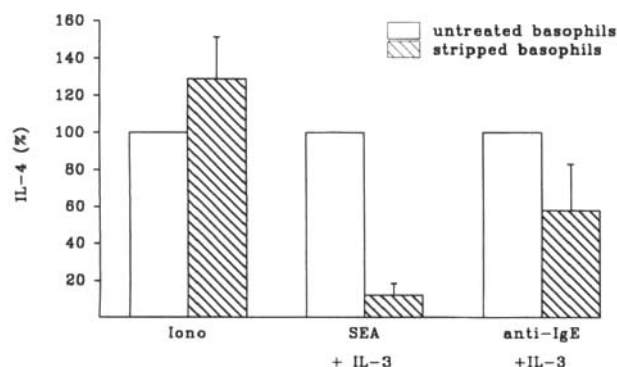


Figure 8. Stripping of IgE from basophils reduces SEA- and anti-IgE-, but not ionomycin-induced IL-4 production. Basophils of seven donors were left untreated (open bars) or were treated with lactate-buffered saline pH 3.9 to remove surface bound IgE (hatched bars). Cells were incubated with SEA and IL-3, anti-IgE + IL-3, or with ionomycin (iono) as control (mean $\pm$ SD).

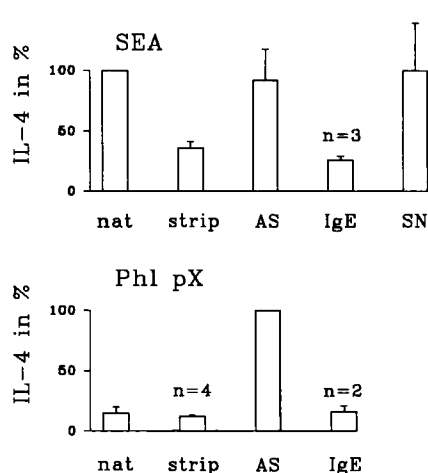


Figure 9. Resensitization of stripped basophils and subsequent stimulation with SEA and Phl pX. Native basophils (nat) from five donors were stripped and resensitized with medium (strip), serum from a timothy grass pollen-allergic individual (AS), IgE-PS (IgE), or autologous stripping supernatant (SN) and stimulated with SEA (upper panel) or Phl pX (lower panel), both in the presence of IL-3 (values in percent; mean $\pm$ SEM).

trated autologous stripping supernatants also resulted in complete restoration of SEA-induced IL-4 production. In contrast, when the serum was heat inactivated (56°C , 30 min) prior to resensitization, a process which abrogates binding of IgE to the high-affinity IgE receptor, the increase in IL-4 production was virtually abolished (data not shown). Resensitization of basophils with myeloma IgE-PS, which has an irrelevant antigen specificity, did not increase SEA- or Phl pX-induced IL-4 production. Thus, these results also indicate an involvement of IgE in the SEA-mediated induction of IL-4 production by basophils.

3.10 Simultaneous stimulation of basophils with SEA and anti-IgE does not have an additive or synergistic effect on IL-4-production

If the IL-4 induction via SEA or anti-IgE involved two independent pathways, simultaneous stimulation of basophils with SEA and anti-IgE should result in enhanced IL-4 production compared to separate stimulation with those agents. However, simultaneous stimulation did not

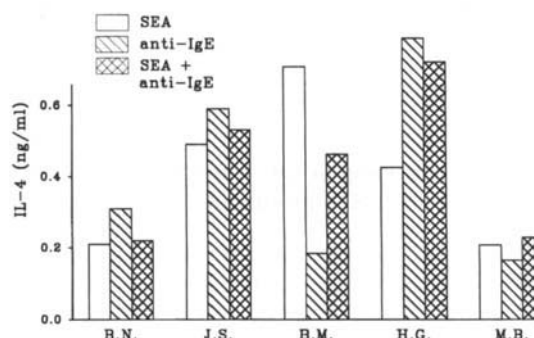


Figure 10. Simultaneous stimulation of basophils with SEA and anti-IgE does not enhance IL-4 production. Basophils of five donors were stimulated with SEA, anti-IgE, or SEA + anti-IgE, each in the presence of IL-3.

lead to increased IL-4 production. This indicates that SEA and anti-IgE act via a common pathway, thereby further supporting the concept of an IgE-dependent mechanism of the SEA effect (Fig. 10).

4 Discussion

Here, we report that SEA triggered the release of IL-4 from basophils of all healthy human donors studied. It is noteworthy that the donors ($n = 32$) had not before been infected with *S. mansoni*. The SEA-induced IL-4 production in the presence of IL-3 ranged between 0.1 and 1.4 ng/10⁶ basophils in individual donors. The response was dose dependent and was complete after 5 h. Reconstitution experiments showed that the IL-4 production after stimulation with SEA strictly correlated with the purity of the basophil preparation, which indicates that the basophils and not other contaminating cell populations were the source of IL-4.

The exact mechanism how SEA triggers the release of IL-4 from basophils is not known. Since the basophils in this study were obtained from healthy individuals who had not been infected before with *S. mansoni*, a specific IgE reactivity to *S. mansoni* was not likely. Moreover, a non-IgE-mediated induction of IL-4 has recently been reported for mouse mast cells that had been stimulated with mercuric chloride [31] or enzymatically active bee venom phospholipase A2 [32]. Therefore, our original hypothesis was that the mechanism of SEA-induced IL-4 production is not IgE-mediated.

However, the results of the IgE stripping and resensitization experiments clearly indicated that IgE is involved in SEA-induced IL-4 production. Thus, after stripping of IgE from basophils, the SEA-induced IL-4 production was reduced to an even greater extent than the anti-IgE-induced IL-4 production. A functional impairment of the basophils by the stripping treatment as a reason for the reduced IL-4 production after stripping can be excluded, since the ionomycin-induced IL-4 production was not reduced after stripping and since resensitization could restore IL-4 production. The supposition of an IgE-mediated mechanism was substantiated by the positive effect of resensitizing stripped basophils with serum of a grass pollen-allergic individual or with autologous stripping supernatants, which reconstituted SEA- and pollen allergen-induced IL-4 production. Evidence for an involvement of IgE was also obtained by heating the serum to 56°C, which abrogates the IgE binding to the high-affinity IgE receptor. Further evidence was obtained by the results of the simultaneous stimulation of basophils with SEA and anti-IgE. The simultaneous stimulation with both agents did not have an additive or synergistic effect on the release of IL-4. This, however, would have been expected if two independent pathways were responsible for stimulation with anti-IgE and SEA.

Although these data indicate that the IL-4-inducing effect is mediated via IgE, presumably via IgE receptor cross-linking, it is not yet clear whether IgE binds to SEA in an antigen-specific or an alternative manner, *i.e.* whether the antigen-binding site or other regions of IgE, such as carbohydrate side chains, mediate the binding. Since IL-4 was

induced in all donors, IgE of all donors should bind to SEA. This was not the case, at least when assessed by immunoblotting. Although all schistosomiasis patients' sera, *i.e.* the positive controls, reacted strongly with SEA, only one of seven nonatopic and three of seven atopic donors' sera showed IgE reactivity to SEA components. Possible explanations are either that immunoblotting is not sensitive enough to detect minor amounts of SEA-bound IgE or that the IgE-binding principle in SEA is not bound by NCM. In the latter case, the bands detected by the healthy donors' sera could represent IgE cross-reactive with SEA. Such cross-reactive IgE might, for instance, be induced by parasites related to *S. mansoni* like the bird schistosome *Trichobilharzia szidati*, which causes swimmer's itch and is endemic in middle Europe.

Our observation that SEA triggers the release of IL-4 from basophils *in vitro* might also have relevance for the situation *in vivo*. In this context, Williams et al. [33] reported that Fcε receptor⁺ non-B, non-T cells from spleens of *S. mansoni*-infected mice are a major source of *S. mansoni* antigen-induced IL-4. When these Fcε receptor⁺ non-B, non-T cells were stained with alcian blue, apart from nongranulated cells, both mature mast cells and cells resembling basophils were observed. Moreover, injection of schistosome eggs into nude mice has been found to induce a transient IL-4 burst in peritoneal cells after 2–12 h, supporting the view that a non-TH cell may promote the development of egg-induced TH2 responses [34].

The site of the postulated interaction between schistosome egg products and basophils *in vivo* is not known yet. During natural infection, the eggs are released within the mesenteric venules, where they may have transient contact with basophils. Then, the eggs penetrate the vessel wall, giving rise to the formation of inflammatory egg granulomas. These granulomas are composed of eosinophils (more than 50 % of leucocytes [35]) and other inflammatory cells [36]. According to the literature, metachromatic cells, *i.e.* basophils or mast cells, seem to be only a minor constituent of *S. mansoni* egg granulomas. This is surprising, since basophils are attracted by the same β chemokines (RANTES and MCP-3) as eosinophils, which are a major constituent of the egg granulomas. We have found that basophils degranulate when incubated with SEA *in vitro* (see Fig. 5 and 6). Moreover, the degranulation of basophils attracted to *S. mansoni* egg granulomas may be enhanced by the potent basophil-activating chemokine MCP-1, whose production in these granulomas correlates with the leucocyte accumulation and granuloma size [37]. Since degranulated basophils are difficult to identify on tissue sections (ghost cells), the actual proportion of basophils in *S. mansoni* egg granulomas might be higher than assumed so far. We think therefore that schistosomal egg-derived products trigger the release of IL-4 from basophils *in vivo*, with the schistosome egg granuloma being a putative site of interaction.

It has been discussed by several authors that mast cell- or basophil-derived IL-4 is possibly involved in TH2 generation [38–40]. Huels et al. found that co-activation of naive mouse CD4⁺ T cells with bone marrow-derived mast cells resulted in the development of TH2 cells [41]. This effect was abrogated by neutralizing anti-IL-4 antibodies. Furthermore, mast cells from IL-4 knock-out mice failed to

support TH2 differentiation, suggesting a key role of IL-4 in mast cell-mediated TH2 development. However, since the IL-4 production by mast cells is triggered via the binding and bridging of antigen-specific IgE antibodies, which are not expected to be present in naive animals, the authors questioned a role for mast cells as a primary source for IL-4 during the initiation of a TH2 immune response. Similar to [42], they suggest that IL-4 produced by activated mast cells in the course of an allergic reaction could perpetuate and enhance an ongoing TH2 response. Considering our findings that basophils from nonimmune donors are reactive with SEA, this objection does not seem to apply for infection with *S. mansoni*.

Recently, it has been proposed that signals other than IL-4, which may be derived from accessory cells, might switch unprimed T cells to IL-4 production [43–46]. Kalinski et al. [43] found that IL-4-producing T helper cells can be generated *in vitro* from naive T helper cells in the absence of any pre-existing source of IL-4 by stimulating the cells with solid-phase anti-CD3 and IL-2 in the presence of anti-CD28 or IL-1 β , *i.e.* accessory cell-related signals. Analogous results were obtained by other groups [47, 48]. In line with these findings, Kuchroo et al. [44], using anti-B7 antibodies in an experimental encephalomyelitis model, could demonstrate that B7-1 and B7-2 co-stimulatory molecules differentially activate the TH1/TH2 developmental pathways. Schmitz et al. [45] could generate IL-4-producing T cells after reconstituting IL-4 knock-out mice with naive congenic wild-type CD4⁺ lymphocytes, *i.e.* in the putative absence of basophils and mast cells, suggesting that factors other than IL-4 are able to promote the primary onset of IL-4 production in Ag-responsive TH cells. Moreover, mouse dendritic cells, as primary antigen-presenting cells, were found to provide all the signals required for effective priming of naive cells both for IFN- γ and IL-4 production [46]. We do not think that these observations contradict the concept that the presence of IL-4 at the time of priming is a crucial factor for the development of IL-4-producing T cells as shown by many groups [6, 7, 39, 49, 50]. They merely indicate that there may be several not mutually exclusive ways to bias T helper cell differentiation towards TH1 or TH2.

In conclusion, we have found that SEA triggers basophils from healthy unprimed human donors to release IL-4 *in vitro*. This effect, which seems to be IgE-mediated, might also play a role in *S. mansoni* infection by providing the early IL-4 needed to switch T cell differentiation towards TH2.

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