

Larval exoantigens from ascarid nematodes are potent inducers of histamine release from human blood basophils

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Summary

Histamine release (HR) from washed human blood cells after challenge with the excretory-secretory antigens (ES) of the parasitic nematodes *Toxocara canis* and *Ascaris suum* was studied, employing a recently developed microfibre-based assay combined with hyperosmolar release media for maximal sensitivity.

Blood samples were obtained from 30 patients suspected of parasite infection and 11 healthy volunteers serving as controls. Specific antibodies of IgE, IgG₁ and IgG₄ subclasses were determined by ELISA.

Virtually no HR could be provoked by ES in the 11 controls. In contrast, HR was seen in 16 patients after challenge with *T. canis* ES and in 11 patients with *A. suum* ES. In the majority of these, HR was detectable after challenge with ES protein concentrations of less than 1 ng/ml, and the maximal HR obtained with ES was greater than that seen with optimal concentrations of anti-IgE.

The HR after ES antigen challenge correlated with the amount of specific IgE in patient plasma, and coincided with the presence of specific IgG₁ and IgG₄.

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Introduction

Helminth parasites have a profound effect on health in large areas of the world, constituting a major factor in malnutrition and mortality, especially in childhood. Both helminth infections and allergic disease regularly cause elevated plasma contents of immunoglobulin E (IgE), some of which has been shown to be helminth- or allergen-specific [1,2]. A newly developed histamine release assay, based on the selective binding of histamine to ground glass microfibres, has enabled a sensitive quantitation of basophil histamine release (HR) after allergen challenge, using small volumes of washed blood cells [3-5].

It was the aim of this study to investigate the basophil histamine release after *in-vitro* challenge with antigen from two ascarid nematodes: *Toxocara canis*, causing the visceral larva migrans syndrome in man, and the porcine *Ascaris suum*, whose antigen composition has been shown

to be similar to its human counterpart, *Ascaris lumbricoides* [6].

Histamine release determinations were compared to the presence in serum of IgE, IgG₁ and IgG₄ specific for the parasites, assayed by a newly developed, sensitive ELISA method.

Materials and methods

Study population

The patient group consisted of 30 persons undergoing outpatient clinical examinations at a specialist clinic for tropical diseases. Seventeen patients (group I) were Danish citizens returning from stays in tropical environments, whereas 13 (group II) were refugees, mainly from Asia. In the latter group, medical history was lacking or incomplete with respect to recent anti-helminthic treatment.

A control group, consisting of 11 healthy volunteers with no suspicion of parasitic infections was included. The members of the control group had all been tested

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weekly for 6 weeks in order to determine spontaneous fluctuations in the histamine release to non-specific stimuli, including anti-IgE.

Ascaris suum antigens. Adult body fluid (ABF) was collected by drainage from cut adult worms, following the procedure of Eriksen [7]. ABF contained 37 mg protein/ml, according to the Lowry assay using BSA as a standard. Larval excretory/secretory antigen (L2/L3-ES) was obtained by sterile cultivation of artificially hatched larvae, essentially following the procedure described by Urban and Romanowski [8].

In short, embryonated eggs containing L2-larvae were hatched by slow rotation (50 r.p.m.) with 4.0 mm glass-balls in an Erlenmeyer flask. Sterile Baermannization of the larval suspension in saline took place for 20 hr after which active larvae were washed 10 times in sterile saline and cultivated at 37°C/5%CO₂ in Dulbecco's modified Eagle Medium (DMEM), with added penicillin and streptomycin (20 000 L2/ml). Supernatants from 10-day changes of medium were concentrated 100× by ultra-filtration on an Amicon UM10 membrane. The final solutions contained 0.6 mg protein/ml according to the Lowry assay, using BSA as a standard.

Toxocara canis antigen. Concentrated L2-ES, produced according to de Savigny [9] and Smith [10] was a gift from Dr H. Smith, Stobhill General Hospital, Glasgow. A second batch was produced according to the procedure for *Ascaris* L2/L3-ES. When tested by ELISA for their binding of specific IgE from a panel of eight sera, reactions from the two batches were closely correlated ($r_s=0.90$) and the two preparations were considered diagnostically equivalent.

ELISA for IgE antibody to ES-antigens. One hundred microlitres of antigen preparations (*Ascaris* ABF, 4 µg/ml; *Ascaris* L2/3-ES, 6.0 µg/ml and *Toxocara* L2-ES, approx. 10 µg/ml) in 0.1 M sodium carbonate buffer, pH 9.8, were coated onto polystyrene microtitre-trays (Maxisorp F96, Nunc, Denmark) overnight at room temperature. After washing (3×3 min with 0.05% Tween-20 in PBS), blocking with 0.5% BSA in PBS took place for 30 min, followed by overnight incubation with 0.1 ml serum, diluted 1:5 in 0.5% BSA in PBS. After washing, incubation took place for 1 hr with 0.1 ml horse-radish-peroxidase (HRP) conjugated rabbit-anti human IgE (DAKO, code P295), diluted 1:2000, in 0.5% BSA/PBS. After another washing step, 100 µl substrate (3.0 mM hydrogenperoxide/5.0 mM ortho-phenylenediamine in 0.1 M sodium citrate buffer, pH 5.0) was added for 15 min reaction in darkness, after which 0.1 ml 1.0 M sulphuric

acid was added. After another 15 min in the dark, the absorbance was read at 490 nm. All determinations were done in duplicate.

Four-layer assay for IgG₁ and IgG₄ antibody. Coated and blocked ELISA-plates were produced as above. After 3 hr of incubation with sera (diluted 1:300 for assay of IgG₁ and 1:50 for IgG₄ antibody) and washing, monoclonal mouse-anti human IgG₁ (Janssen Biochemica, code 24.148.92), diluted 1:100 000 or monoclonal mouse-anti human IgG₄ (Unipath, code BAM16/6878), diluted 1:50 000, was added for overnight incubation followed by a 1 hr incubation with HRP-conjugated rabbit-anti mouse immunoglobulin (DAKO code P260), diluted 1:5000.

Substrate reaction took place as described for the IgE assay.

Quantitation of total IgE

Polystyrene microtitre plates (NUNC Maxisorp) were coated overnight with monoclonal mouse-anti human IgE (Janssen Biochemica, code 23.881.19), 10 µg/ml in sodium carbonate buffer, pH 9.8. IgE-standard (Pharmacia Diagnostics) in concentrations from 0.1 to 20 U/ml and test sera, diluted 1:50 in BSA/PBS were added, incubating for 3 hr. After washing, HRP-conjugated rabbit-anti human IgE (DAKO, code P295), pre-absorbed by addition of an equal volume of mouse serum and diluted 1:2000 was added for 1 hr of incubation. Substrate reaction and reading of absorption took place as above.

Samples giving readings above the 20 U/ml upper limit of the measurement range were reassayed in dilutions 1:500 and 1:5000. For 10 sera, the measurements correlated well ($r=0.92$) with those obtained with the Pharmacia PRIST-kit.

ELISA cross-inhibition. Seven sera with large IgG₁-reaction to *A. suum*, *T. canis* or both were mixed with *A. suum* L2/L3-ES (final concentration 60 µg/ml), *T. canis* L2-ES (final concentration 20 µg/ml) or buffer and subjected to IgG₁ ELISA for both antigens at a final serum dilution of 1:300 as above.

Histamine release assay

Ten millilitres of blood, anticoagulated with 10 µM of EDTA, was obtained from patients and controls. Plasma was isolated for antibody-ELISA by centrifugation.

The blood cells were washed four times in hyper-osmolar PAGCM (PIPES (Piperazine-*N,N'*-bis[2-ethane-

Table 1. Total serum IgE, specific antibodies and histamine release

	Controls (n = 11)		Patients group I (n = 17)		Patients group II (n = 13)	
	Median	Range	Median	Range	Median	Range
Total serum IgE content (IU/ml)	39	6-388	38	8-600	1850	25-18 000
Specific antibodies (ELISA-absorbance)						
<i>T. canis</i> ES						
IgE	0.04	0.02-0.04	0.04	0.01-0.23	0.40	0.02-2.12
IgG ₄	0.03	0.03-0.12	0.05	0.03-0.32	0.98	0.02-2.53
IgG ₁	0.16	0.09-0.96	0.33	0.16-1.06	1.39	0.13-2.81
<i>A. suum</i> ES						
IgE	0.04	0.03-0.07	0.04	0.03-0.15	0.36	0.04-1.43
IgG ₄	0.05	0.03-0.15	0.03	0.02-0.35	0.14	0.03-1.35
IgG ₁	0.22	0.09-0.91	0.18	0.07-1.44	1.79	0.41-2.57
Maximal histamine release (ng/ml blood)						
<i>T. canis</i> ES	0	0-8	2	0-77	33	0-81
<i>A. suum</i> ES	1	0-5	0	0-40	22	0-56
<i>A. suum</i> ABF	0	0-12	1	0-27	30	0-89
Anti-IgE	23	3-93	18	1-80	35	1-79

sulfonic acid]) 10 mM, Na-Acetate 230 mM, K-Acetate 5 mM adjusted to pH 7.40 with Tris-[hydroxymethyl]-aminomethane) buffer containing CaCl 0.6 mM and MgCl 1.1 mM, with added heparin 7.5 IE/ml, glucose 2.8 mM and human serum albumin 0.16 g/l, final osmolarity 497 mOsm/kg H₂O. After the washing steps, the blood cells were resuspended to the original blood volume with PAGCM.

Microfibre-coated 96-well microtitre-plates (P. S. Skov, Reference Laboratory, University of Copenhagen) were employed, prepared as described previously [3,4,11]. Briefly, to each well was added 50 µl of PAGCM, 50 µl of washed blood cells and finally 20 µl of PAGGM containing either anti-IgE, parasite antigen or standard quantities of histamine for generation of standard curves.

Rabbit-anti-human IgE, ε-chain specific (Behringwerke, Marburg, Germany), was used in serial tenfold dilutions (0.04-4000 U/ml), serving as control of the ability of the blood cells to respond to IgE-mediated stimuli.

T. canis L₂/L₃ ES-antigen (TCES) was added in five sequential tenfold dilutions, with antigen protein concentrations (in the microtitre well) of 0.02-200 ng/ml. *A. suum* ES-antigen (AES) was similarly employed at concentrations ranging from 0.05-500 ng/ml, while *A. suum* ABF (AABF) was used at the higher concentrations of 4-40 000 ng/ml.

Histamine release was determined fluorometrically as triplicate measurements, and the results are expressed in nanograms of histamine released per millilitre of blood [3,4,11].

Statistics

Only non-parametric statistic methods were employed, i.e. Spearman's method in calculation of correlations, Kruskal-Wallis' analysis of variance by ranks and Wilcoxon's test for paired comparisons.

Results

The plasma contents of ES-specific immunoglobulins in controls and patients are shown in Table 1. In patient group II, the contents of IgE, IgG₁ and IgG₄ were significantly higher than in patient group I and controls. Only very minimal amounts of specific IgE were seen in the control group, whereas the levels of specific IgG were more variable (Table 1).

The cross-reactivity in the IgG₁-ELISA between the two ES preparations was tested in seven sera by inhibition experiments, showing only insignificant cross-inhibition (Table 2).

Table 2. *A. suum*/*T. canis* cross-inhibition of IgG₁-ELISA

Patient no.	<i>A. suum</i> -coated			<i>T. canis</i> -coated		
	Abs.* -Inh.	%Inh.† Asc.	%Inh. Tox.	Abs. -Inh.	%Inh. Asc.	%Inh. Tox.
1	2.01	92	9	0.65	17	72
3	1.56	94	31	>	—	—
14	1.93	84	7	0.04	—	—
16	2.70	78	0	2.69	2	86
17	2.53	75	2	2.52	3	95
25	2.46	82	8	2.62	5	82
26	2.12	82	5	1.21	17	72

* Absorption reading in ELISA with added buffer in place of inhibiting antigen.

† Calculated as: $(1 - \text{Abs.}_{\text{inh.}} / \text{Abs.}_{\text{inh.}}) \times 100\%$, where Abs._{inh.} and Abs._{inh.} are ELISA-absorptions with and without inhibitor, respectively, corrected for absorption read without added serum.

For comparison with the histamine release results, a presumptive 'normal range' was defined by the highest specific antibody contents observed in the control group.

The detection limit for the histamine release assay employed here is approximately 3 ng/ml. All 11 control samples, as well as 28 of 30 (93%) patient samples released detectable amounts of histamine upon challenge with anti-IgE, illustrating an equal ability to respond to non-specific IgE-mediated stimuli in the two groups (Table 1).

Only one control person showed detectable histamine release to parasite antigens, yielding a histamine release of 8 ng/ml after challenge with TCES at 200 ng/ml, and 10 ng/ml resp. 12 ng/ml with AABF at 4000 resp. 40 000 ng protein/ml.

The highest histamine releases seen in the control group were adopted as the presumptive 'normal range' for histamine release after challenge with parasite antigen (Fig. 1).

When compared to this 'normal range', samples from 10 patients released significant amounts of histamine after challenge with both ES-antigens, one responded only to AES, whereas six showed histamine release with TCES only. The absence of histamine release in the remaining patients was not caused by an inability to respond to anti-IgE, as indicated in Fig. 2.

As seen from Fig. 1, the occurrence of specific IgE-antibodies towards the parasite antigen coincided with histamine release after challenge with antigen, although the correlation between the absolute amount of histamine released and the ELISA-determinations of specific IgE was only moderate ($r_s = 0.66$, $P < 0.05$ for TCES, $r_s = 0.62$,

$P < 0.05$ for AES in group II). Only 6/17 patients in group I showed histamine release with any ES antigen, precluding a meaningful correlation analysis in this group.

The excretory-secretory antigens from both *A. suum* and *T. canis* were found to be extremely potent secretagogues in the presence of specific IgE in serum. In several patients, a significant histamine release could be induced by antigen concentrations as low as 0.02–0.05 ng/ml. Furthermore, the shape of the HR dose-response curve induced by challenge with the ES antigens was found to be determined by the amount of specific antibody in serum, as exemplified with AES in Fig. 3. When defining the antigen sensitivity of the blood cells as the smallest ES protein concentration capable of causing an HR above the 'normal range', this sensitivity correlated well with the measured amounts of ES-specific IgE in patient serum ($r_s = 0.88$ ($P < 0.01$) for TCES and $r_s = 0.83$ ($P < 0.01$) for AES in group II).

Most patients with high levels of ES-specific IgE in plasma also had elevated total serum IgE. In group II, the plasma levels of ES-specific IgG₁, IgG₄ and HR were closely correlated ($r_s = 0.76$ – 0.85 , $P = 0.01$ – 0.005) for the *T. canis* antigen. In the case of *A. suum*, the contents of specific IgG₁ and IgG₄ were closely correlated ($r_s = 0.79$, $P < 0.01$), whereas there was no correlation between the level of specific IgG and ES-induced HR.

The degree of cross-reactivity between the two ES-preparations in inducing histamine release from the basophils could not be assessed by the method employed.

The *A. suum* ABF antigen, which was available in large quantities, was used in high concentrations in order to uncover non-immunological histamine release, as reported in mast cells [12]. However, the histamine release after challenge with AABF was dose-dependent and correlated well with the HR obtained with AES ($r_s = 0.87$, $P < 0.01$) in group II.

In all patients, triple fecal examinations for parasite eggs or cysts were performed. All fecal examinations were negative for *A. suum* eggs. Unrelated helminth infections were diagnosed in only three patients.

Discussion

Basophil histamine release, as seen in allergic disease, is initiated by the binding of allergen(s) to allergen-specific IgE, situated on high-affinity receptors on the surface of the basophil granulocyte [13]. The presence of very high total IgE contents in plasma from patients with helminth infection has stimulated research into the relation between allergic disease and the prevalence of helminth infection [14–17].

In this study, controls and patients were equally able to

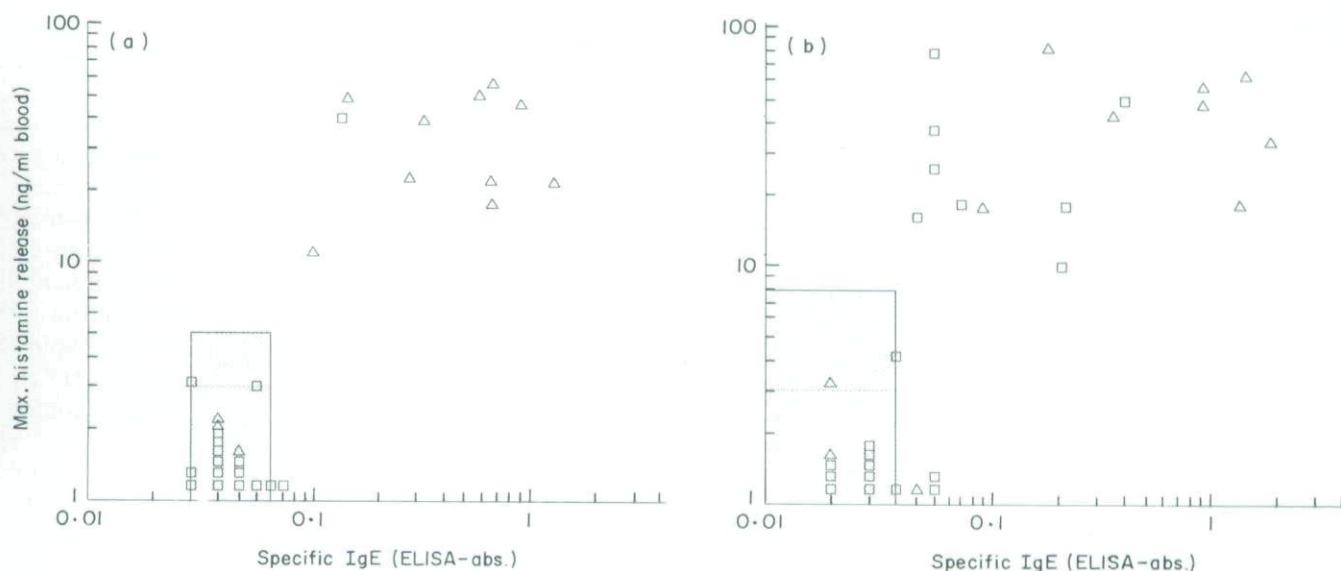


Fig. 1. Specific IgE-antibodies against (a) *A. suum* ES and (b) *T. canis* ES related to maximal histamine release after antigen challenge. Shaded area delimits the values observed in the control group for the two antigen preparations. Histamine release is given as ng/ml blood, IgE content in arbitrary ELISA-absorbance units. □, group I; △, group II; ---, detection limit.

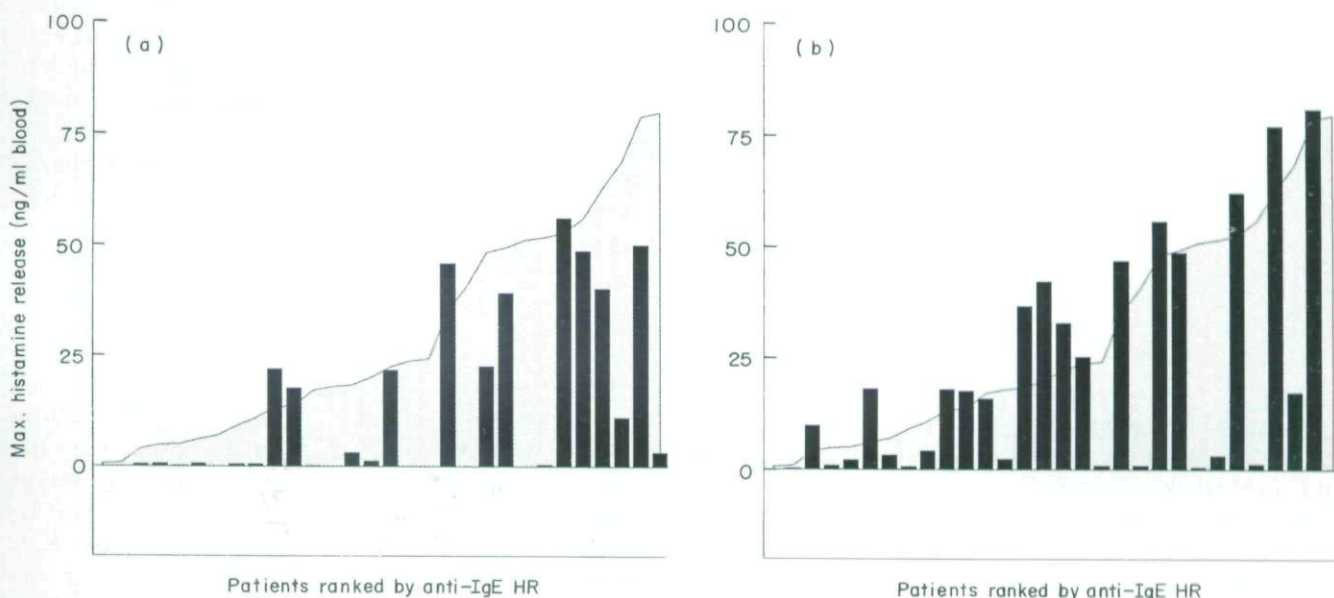


Fig. 2. Maximal histamine release after challenge with (a) *A. suum* ES antigen and (b) *T. canis* ES related to the maximal histamine release obtained with anti-IgE. Patients from groups I and II have been ranked according to their anti-IgE histamine release, showing a uniform distribution of responses. The lack of histamine release after antigen challenge in the majority of patients is not caused by an inability to respond to non-specific IgE-mediated stimuli. ■, TCES. ▨, anti-IgE.

respond to a non-specific IgE-mediated stimulus for histamine release (anti-IgE). In contrast, the ES antigens could only induce HR in patients with detectable levels of ES-specific IgE-antibodies in their plasma (Fig. 1). Furthermore, a significant HR could be induced by very

dilute antigen, i.e. containing less than 0.1 ng protein per ml in patients with high levels of specific IgE. In these patients, the maximal histamine release obtained with antigen was often higher than that seen with optimal concentrations of anti-IgE.

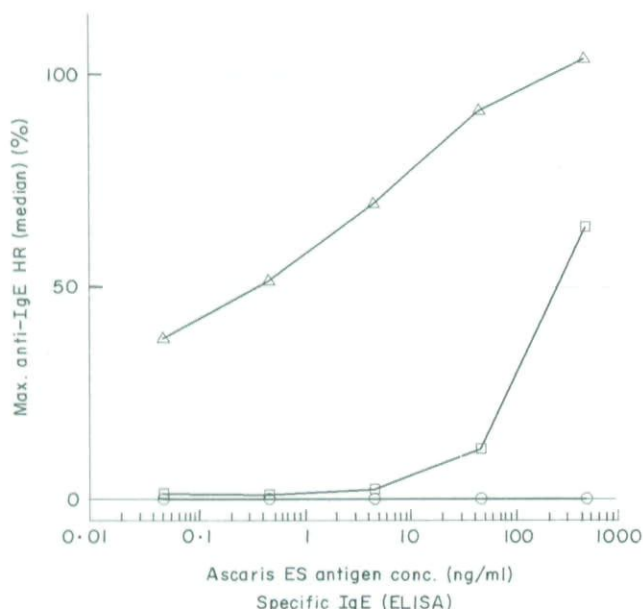


Fig. 3. Subdividing the patients from both group I and II according to the amount of *A. Suum* ES-specific antibody in plasma, histamine release with different concentrations of antigen (abscissa) is shown relative to the maximal histamine release seen with anti-IgE (100%) for each patient. O, <0.1 ($n=19$); □, 0.1–0.4 ($n=5$); Δ >0.4 ($n=6$).

These findings indicate, that ES antigens activating the cell via the F(ab)2 portion of the cell-bound IgE is a more potent stimulus for basophil degranulation than the Fc-crosslinking by anti-IgE. Furthermore, the ES antigens are likely to present themselves to the cells as polyvalent antigens, a more potent stimulus for HR than the divalent anti-IgE.

In allergic patients with elevated serum IgE content, a good correlation between the number of IgE molecules bound to the basophil cell surface and the total plasma IgE content was found. The median total plasma IgE content seen in group II (1850 IU/ml) was higher than that reported to cause saturation of the IgE receptors on the basophil cell surface (≈ 400 –1200 IU/ml), irrespective of the absolute number of IgE receptors on the cell surface [13].

Studies of the IgE response to helminth infection have shown that only part of the IgE produced possesses helminth specificity, the majority resulting from non-specific induction of IgE synthesis [18]. Nevertheless, the basophils from the sensitized patients in this study possessed sufficient surface-bound IgE to recognize and respond even to minute quantities of ES antigen. A similar finding has been reported in allergic disease, in

which maximal HR can be induced by allergen, in spite of the allergen-specific IgE contributing less than 1% of the patients' total plasma IgE [19].

The HR results presented here indicate that the responses elicited by the basophil granulocytes after challenge with *A. suum* ES antigens *in vitro* are very similar to the responses seen in blood from allergic patients after challenge with the appropriate allergen. In contrast, immediate-type allergic reactions are not usually seen as part of the clinical manifestations in *A. suum* or *T. canis* infection in man [20], although anaphylactic reactions are known to occur as complications to treatment of helminthic infections, for instance in hydatid disease.

The finding *in vitro* that antigens actively excreted or secreted by living, infective ascarid larvae are highly capable of triggering basophil histamine release in sensitized individuals, points to the existence of regulatory mechanisms hindering anaphylactic reactions in patients harbouring the living helminths [21,22].

In human filariasis, IgE and IgG₄ have been reported to recognize identical antigenic structures [23], indicating that specific IgG₄ may serve as a 'blocking antibody' by being present in much larger quantities than specific IgE. However, in allergic disease the IgG₄ subclass has been described as a reaginic antibody, although attempts to passively sensitize basophils with IgG₄ have not generally been successful [24].

In this study, high levels of IgG₄ were found primarily in patients with high levels of *T. canis*-specific IgE, especially in those responding with greater HR after challenge with ES-antigen than with anti-IgE. As these *in vitro* HR experiments were performed in washed blood, they give no indication as to whether IgG₄ is blocking or enhancing the HR after challenge with ES antigen.

The absence of ascaris eggs in the fecal examinations is in contrast with the serologic evidence of infection obtained in this study, but may be explained by the paucity of anamnestic information concerning recent infection or anti-helminthic treatment in the group II. The high frequency of specific antibodies or HR to *A. suum* ES in this group was not surprising, since the majority of inhabitants in tropical countries have harboured infections with *A. lumbricoides* [25]. Reports on the seroprevalence of *Toxocara* spp. in tropical/subtropical countries are relatively scanty, with figures ranging from 5–86% [20]. In the group II there was a high frequency (9/13) of strongly positive reactions to *Toxocara* ES, indicating that infection of humans with dog roundworms may be extensive in certain areas, clearly pointing to future investigations involving larger study groups.

The possibility of utilizing measurement of specific IgE, as well as various basophil histamine release tests, for the diagnosis of helminth infections has been studied by several groups [26,27]. In this study, the basophil granulocytes in peripheral blood from sensitized individuals were found to be very sensitive to allergen challenge, offering perspective for a diagnostic application of the histamine release test in helminth infection when combined with species-specific antigens.

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References

- Jarrett EEE, Miller HRP. Production and activities of IgE in helminth infection. *Prog Allergy* 1982; 31:178-233.
- Lynch NR, Pérez M, López RI, Turner KJ. Measurement of anti-*Ascaris* IgE antibody levels in tropical allergic patients, using modified ELISA. *Allergol Immunopathol* 1987; 15:19-24.
- Stahl Skov P, Norn S, Weeke B. Histamine release and pathological states. A new method for measuring histamine release. *Agents Actions* 1984; 14:414-16.
- Stahl Skov P, Mosbech H, Norn S, Weeke B. Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. *Allergy* 1985; 40:213-8.
- Nolte H, Schiøtz PO, Stahl Skov P. A new glass microfibre-based histamine analysis for allergy testing in children. Results compared with conventional leukocyte histamine release assay, skin prick test, bronchial provocation test and RAST. *Allergy* 1987; 42:363-72.
- Kennedy MW, Quereshi F, Haswell-Elkins M, Elkins DB. Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. *Clin Exp Immunol* 1987; 67:20-30.
- Eriksen L. Host parasite relations in *Ascaris suum* infection in pigs and mice. Thesis, The Institute of Internal Medicine of the Royal Veterinary and Agricultural University, Copenhagen, 1981.
- Urban JF, Romonowski RD. *Ascaris suum*: protective immunity in pigs immunized with products from eggs and larvae. *Exp Parasitol* 1985; 60:245-54.
- de Savigny DH. *In vitro* maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larva migrans. *J Parasitol* 1975; 61:781-82.
- Smith HV. A rapid method for hatching infective eggs of *Toxocara canis*. *Trans Roy Soc Trop Med Hyg* 1988; 82:215.
- Søderberg U, Windelborg Nielsen B, Larsen CS, Schiøtz PO, Thestrup-Pedersen K. Time-course studies of immediate and delayed immune reactivity in patients with atopic dermatitis treated with herbal drugs. *Allergy* 1990; 45:559-65.
- Uvnäs B, Wold JK. Isolation of a mast cell degranulating polypeptide from *Ascaris suus*. *Acta physiol Scand* 1967; 70:269-76.
- Conroy MC, Adkinson NF Jr., Lichtenstein LM. Measurement of IgE on human basophils: Relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977; 118:1317-21.
- Larrick JW, Buckley CE III, Machamer CE, Schlagel GD, Yost JA, Blessing-Moore J, Levy D. Does hyperimmunoglobulinemia-E protect tropical populations from allergic disease? *J Allergy clin Immunol* 1983; 71:184-8.
- Turner KJ, Sumarmo, Matondang-Siahaan C. Precipitating factors in respiratory allergic disease in Indonesian children. *Clin Allergy* 1978; 8:145-54.
- Genta RM, Ottesen EA, Poindexter R, Gam AA, Neva FA, Tanowitz HB, Wittner M. Specific Allergic sensitization stronglylodes antigens in human stronglylodioidiasis. *Lab Invest* 1983; 48:633-8.
- Weil GJ, Hussain R, Kumaraswami V, Tripathy SP, Phillips KS, Ottesen EA. Prenatal allergic sensitization to helminth antigens in offspring of parasite-infected mothers. *J Clin Invest* 1983; 71:1124-9.
- Nutman TB, Volkman DJ, Hussain R, Fauci AS, Ottesen EA. Filarial parasite-specific T cell lines: induction of IgE synthesis. *J Immunol* 1985; 134:1178-84.
- Pruzansky JJ, Patterson R. Limiting concentrations of human basophil-bound IgE antibody required for histamine release. *Immunology* 1988; 64:307-10.
- Barriga OA. A Critical Look at the importance, prevalence and control of Toxocarasis and the possibilities of immunological control. *Vet Parasitol* 1988; 29:195-234.
- Lightowlers MW, Rickard MD. Excretory-secretory products of helminth parasites: effects on host immune responses. *Parasitology* 1988; 96:123-66.
- Hofstetter M, Fasano MB, Ottesen EA. Modulation of the host response in human schistosomiasis. IV. Parasite antigen induces release of histamine that inhibits lymphocyte responsiveness *in vitro*. *J Immunol* 1983; 130:1376-80.
- Hussain R, Ottesen EA. IgE responses in human filariasis. IV. Parallel antigen recognition by IgE and IgG4 subclass antibodies. *J Immunol* 1986; 136:1859-63.
- Nakagawa T, de Weck AL. Membrane receptors for the IgG4 subclass on human basophils and mast cells. *Clin Rev Allerg* 1983; 1:197-206.
- World Health Organization. Intestinal protozoan and helminthic infection. Technical Report Series 666, WHO, Geneva.
- Vuitton DA, Bresson-Hadni S, Lenys D, Flausse F, Liance M, Wattre P, Miguet JP, Capron A. IgE-dependent humoral

immune response in *Echinococcus multilocularis* infection: circulating and basophil-bound specific IgE against *Echinococcus* antigens in patients with alveolar echinococcosis. Clin Exp Immunol 1988; 71:247-52.

27 Aceti A, Celestino D, Teggi A, Caferro M, Pennica A, Grilli A, Sebastiani A, de Rosa F. Histamine release test in the diagnosis of human hydatidosis. Clin Exp Allergy 1989; 19:335-9.

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