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IgE antibodies are more species-specific than IgG antibodies in human onchocerciasis and lymphatic filariasis

N. WEISS, R. HUSSAIN & E. A. OTTESEN *Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.*

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Summary. To explore the relative species specificities of the IgE and IgG antibody responses to helminth infections in man, we studied four pools of sera from patients infected with *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus* or *Ascaris lumbricoides* and ten individual sera from patients with onchocerciasis. IgE antibodies were detected by radioallergosorbent test (RAST) analysis and IgG antibodies by a Staphylococcus protein A radioimmunoassay (Staph A-RIA). Analysis of the binding curves with four different immunosorbents (prepared from antigens of *B. malayi*, *O. volvulus*, *Dipetalonema viteae* and *A. lumbricoides*) in the RAST and the binding curves with these same four antigens in the Staph A-RIA confirmed the relative species specificities for both the IgE and IgG antibody responses. Then determination of these antibody levels after specific absorption of the sera with both homologous and heterologous antigens showed that in all instances there was significantly less cross-reactivity with heterologous parasite antigens (i.e. higher species specificity) in the IgE antibody response to filarial infection than in the corresponding IgG antibody response. Such findings imply that efforts toward developing techniques for specific immunodiagnosis of filarial infections are likely to be particularly successful if focused on the IgE antibody response of exposed individuals.

Abbreviations: B_{max}, maximal binding; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; RAST, radioallergosorbent test; Staph A-RIA, staphylococcus protein A radioimmunoassay.

Present address and correspondence: Dr N. Weiss, Department of Medicine, Swiss Tropical Institute, Socinstrasse 57, CH 4051 Basel, Switzerland.

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INTRODUCTION

One of the greatest obstacles to progress in immunoparasitology has been the existence of extensive cross-reactivity both between the antigens of different parasite species and between the antigens derived from different developmental stages of the same species. Nowhere has this problem been more vexing than in dealing with the chronic helminth infections. The lack of antigen specificity in these infections continues to hamper interpretation of 'specific' immunological findings in populations in which multiple parasites are endemic and to make reliable and accurate immunodiagnosis a persistently elusive goal.

In the human filariases, numerous authors have indicated how extensive this problem of cross-reactivity is (Ambroise-Thomas, 1974; Kagan, 1979). However, since homologous antigens have often proven difficult or impossible to obtain (especially from *Wuchereria bancrofti*, *Loa loa* and *Dipetalonema perstans* but also from *Brugia malayi* and *Onchocerca volvulus*), this cross-reactivity has been exploited to

permit development of serodiagnostic techniques employing heterologous antigens from more easily obtained animal parasites, such as *Dirofilaria immitis*, *Dipetalonema viteae* and *Onchocerca gutturosa* (for review see Ambroise-Thomas, 1974; Kagan 1979). While studies with heterologous antigens have given useful information, the introduction of serological methods with extremely high sensitivities, such as the enzyme-linked immunosorbent assay (ELISA), has accentuated the problem of limited specificity. Indeed, extensive cross reactivities have been found not only with antigens of various filarial species but also with antigens of other nematodes (*Strongyloides*, *Trichinella*, *Ascaris*) and even cestodes (*Echinococcus*) (Ambroise-Thomas, Desgeorges & Monget, 1978; Speiser, 1980.)

Most of the serological techniques used to detect helminth infections have measured IgG or IgM antibodies to the parasites. Recently, the radioallergosorbent test (RAST) has been applied to measure IgE antibodies in helminth infections (e.g. Dessaint, Capron, Bout & Capron, 1975; Weiss, Stuerchler & Dietrich, 1978; O'Donnell & Mitchell, 1980; Hamilton, Hussain, Ottesen & Adkinson, 1981). There has been some suggestion that, despite polyclonal IgE stimulation, there may be greater antigenic specificity in the IgE response to schistosomes than in the IgG or IgM antibody responses (Weiss *et al.*, 1978). Therefore, in the present study we examined this question directly by taking four pools of human sera (from patients with *W. bancrofti*, *B. malayi*, *O. volvulus* or *Ascaris lumbricoides* infection) as well as ten individual sera from patients with onchocerciasis and comparing the relative specificities of the IgE and IgG antibodies to somatic antigens derived from *B. malayi*, *O. volvulus*, *D. viteae* and *A. lumbricoides*. IgE antibodies were measured with the RAST technique (Wide, Axen & Porath, 1967; Adkinson, 1976) and IgG antibodies with a staphylococcus protein A radioimmunoassay (Kessler, 1975). Our findings confirm that there is much less cross-reactivity (i.e. higher specificity) in the IgE response to these helminth parasites than in the IgG antibody response.

MATERIALS AND METHODS

Sera

The onchocerciasis serum pool was prepared from three selected serum samples obtained from 'Ad Iucem' hospitals in Mbouda and Bafoussam, United Republic of Cameroon. The sera were selected for their high titres of IgE and IgG antibodies against *O. volvulus* (Weiss, Speiser & Hussain, 1981). Diagnosis was established by the presence of adult filariae in nodules excised from each patient. Parasitological examination of these and other patients gave evidence that *Ascaris*, *Trichuris* and hookworm were endemic in that area. Ten other sera from patients with onchocerciasis were analysed individually. Two sera were obtained from Swiss patients diagnosed by skin biopsies, and eight were from Cameroonians diagnosed by nodulectomy.

The brugian filariasis serum pool consisted of eight selected serum specimens collected in Tanah Intan (South Kalimantan, Borneo, where *B. malayi* is endemic), kindly provided by Dr P. B. McGreevy, Armed Forces Institute of Pathology, Washington D.C. Criteria for their selection for the pool were a high IgG antibody titre and high total serum IgE $\geqq$ 5000 i.u./ml. The donors of these sera were not examined for intestinal worms, but *Ascaris*, *Trichuris* and hookworm are endemic in the region.

The bancroftian filariasis serum pool was prepared by mixing seven sera with high titres of IgE and IgG antibodies detected using *B. malayi* antigens as described elsewhere (Hamilton *et al.*, 1981). These sera were collected in Mauke, Cook Islands, and in Madras, India, where *W. bancrofti* is endemic.

The ascaris serum came from a single South African patient of Dr J. R. Joubert. It was selected for its high titre of anti-*Ascaris* IgE antibodies. Human filariasis is not endemic in this region.

All sera were stored in aliquots at $-70°$. To remove any potential anti-Sepharose antibodies, sera were absorbed for at least 4 hr at room temperature with 0·5 ml of a 5% v/v suspension of Sepharose 4B (Pharmacia, Piscataway, N.J.) per 0·1 ml of serum (Hamilton, 1980).

Antigens

O. volvulus. Adult worms were recovered from nodules excised at 'Ad Iucem' hospitals in Mbouda and Bafoussam, United Republic of Cameroon. Fresh nodules were digested by collagenase as described by Schulz-Key, Albiez & Buettner (1977). Living worms were carefully washed from host tissues and immediately frozen. After homogenization, worms were extracted overnight in phosphate-buffered saline (0·15 M PBS, pH 7·4) at 4°. The supernatant was dialysed against borate-buffered saline (pH 8·4) and then centrifuged (12,000 *g*, 30 min, 4°).

B. malayi. The preparation of a PBS extract of approximately equal numbers of adult male and female worms recovered from jirds (*Meriones unguiculatus*) has been described elsewhere (Hamilton *et al.* 1981).

D. viteae. Female worms were obtained from infected hamsters and were stored frozen (−70°). Preparation of the soluble antigens was the same as for *O. volvulus.*

A. lumbricoides. Adult worms, collected from a patient in Guatemala, were kindly provided by Dr D Levy (Johns Hopkins University, Baltimore, Md). The preparation of the crude borate-buffered *Ascaris* extract has been described elsewhere (Hussain, Strejan & Campbell, 1972).

Protein determinations were done colourimetrically after dialysis (Biorad protein assay).

Preparation of immunosorbents

Each of the four antigen preparations was coupled to CNBr-activated Sepharose CL-4B (Pharmacia) according to the instructions provided by the manufacturer. The antigen concentration per millilitre of swollen beads was 1·0 mg protein for the *Onchocerca*, *Brugia* and *Dipetalonema* sorbents, and 2·3 mg for the *Ascaris* sorbent. More than 99% of the soluble proteins were coupled to the beads as demonstrated by protein measurements after coupling. All sorbents were stored in RAST buffer (PBS pH 7·5, containing 0·4% Tween 20, 0·2% bovine serum albumin and 0·05% sodium azide) at 4°. Immunosorbents were washed with RAST buffer before use.

Radioallergosorbent test RAST

The procedure for this solid phase radioimmunoassay has been described in detail elsewhere (Hamilton *et al.*, 1981). Briefly, sera were diluted in RAST buffer containing 1·25% of a Sepharose-absorbed human serum from an uninfected North American control (total IgE: 80 i.u./ml). Half a millilitre of a 2% sorbent solution was added to 0·1 ml of diluted serum. Tubes were kept rotating for 6 to 8 hr at room temperature. After three washings with RAST buffer, 0·5 ml of a ^{125}I-labelled affinity-purified goat anti-human IgE antiserum (kindly provided by Dr R. F. Ritchie, Portland, Maine) was added. This corresponded to 6–12 ng labelled antibody. Total activity added per tube was approximately 0·1 μCi. After orbital rotation overnight, unbound radioactivity was removed by four washings. One batch of labelled antiserum was used so long as maximal binding (B_{max}) to insolubilized IgE (bound to Sepharose) exceeded 30% of the total activity added.

Non-specific binding for the normal serum (at 1:20 or 1:80 dilution) to the four sorbents ranged from 0·5 to 1·5% of B_{max}. Results are expressed as net percent of B_{max}.

Staphylococcus protein A radioimmunoassay (Staph A-RIA)

All four antigen preparations were labelled with iodine by the chloramine T method (Greenwood, Hunter & Bolver, 1963). Specific activity ranged from 1 to 5 mCi per mg protein. All antigens were absorbed for 1 hr at 37° with a solution of staphylococci (IgGsorb; Enzyme Center, Inc., Boston, Mass.) to reduce non-specific binding. To 0·1 ml of the serum sample diluted in RAST buffer, 0·1 ml of labelled antigen (equivalent to 20–100 ng protein) was added. Immune complexes formed with the labelled antigens were then precipitated by adding 0·1 ml of a 5% staphylococci suspension. After overnight incubation (rotating at room temperature) unbound labelled antigens were removed by four washings with RAST buffer. Results are expressed as mean net c.p.m. (=c.p.m. minus c.p.m. of normal serum diluted 1:80). The four serum pools being compared were always titrated in the same assay.

Determination of total serum IgE

Total serum IgE was determined by a paper radioimmunosorbent test (Phadebas-Prist IgE, Pharmacia).

Depletion experiments

To remove anti-parasite antibodies, sera were extensively absorbed with immunosorbents prepared from antigens of *D. viteae*, *B. malayi* and *A. lumbricoides.* The amount of immunosorbent used corresponded to 10 μg (protein) of *D. viteae* or *B. malayi* antigen and to 23 μg of *A. lumbricoides* antigen for each microlitre of undiluted serum. Sera were depleted overnight under agitation at room temperature. Their final dilution was adjusted to 1:80 with RAST buffer before the immunosorbent was removed by centrifugation. In addition, ten onchocerciasis sera were depleted with a mixture of all three immunosorbents.

The remaining binding after depletion was calculated as percentage binding of the untreated serum at the same dilution (1:80). From the titration curve of the untreated serum (run in the same assay), the dilution (1:Z) which gave a binding identical to that of the depleted serum was determined. The following

equation was used to calculate the remaining binding as a percentage:

$$[(1:Z)/(1:80) \times 100] = [(80 \times 100)/Z].$$

RESULTS

Detection of IgE antibodies

Total IgE levels and approximate amounts of specific IgE antibodies for the four sera used in this study are summarized in Table 1. In all four sera IgE was greatly elevated. IgE antibodies against *O. volvulus* or *B. malayi* ranged from 1 to 3 μg/ml, a level corresponding to 5%–8% of total IgE.

Table 1.

Sera	Total IgE (i.u./ml)	IgE antibodies in % (μg/ml) to
Onchocerciasis pool	20,000	6·3 (~3·0) *O. volvulus**
Bancroftian filariasis pool	11,000	8·2 (~2·2) *B. malayi*†
Brugian filariasis pool	8000	5·2 (~1·0) *B. malayi*‡
Ascaris serum	6700	Not done

* Weiss *et al.* (1981).

† Hussain, Hamilton, Atkinson & Ottesen (manuscript submitted for publication).

‡ Estimated from binding curves (Fig. 1b).

The relative binding of IgE antibodies in the four different serum pools to homologous and heterologous antigens is shown in Fig. 1. Binding of the onchocerciasis, brugian filariasis and ascaris sera to their homologous antigens was at comparably high levels (solid lines in Fig. 1). Binding to the cross-reactive heterologous immunosorbents was, in all cases, lower. The bancroftian filariasis pool, for which no homologous antigen was available, gave highest binding to the *Brugia* sorbent (11·5% B_{max} at 1:80), even higher than the brugian filariasis pool (Fig. 1B). From the two binding curves it can be concluded that this serum contained two and one fifth times more IgE antibodies to *B. malayi* than did the brugian serum pool (Table 1). Interestingly, for the bancroftian filariasis serum pool a higher degree of cross-reactivity to *D. viteae* than to *O. volvulus* was observed (Fig. 1a and C). The onchocerciasis and the two filariasis serum pools, for which past or active *Ascaris* infections cannot be excluded, all reacted to similar (low) degrees with the *A. lumbricoides* sorbent (Fig. 1d). Similarly, only very low binding resulted when the *Ascaris* serum was tested against the filarial antigens (Fig. 1a, b and c).

Detection of IgG antibodies

When the serum pools were analysed for IgG antibodies against the same four antigens, the rank order of the binding curves for each antigen was almost identical to those found for the RAST (Fig. 2). With the *O. volvulus* and the *A. lumbricoides* antigens, highest reactivity was detected in the homologous sera (Fig. 2a and d). Again the bancroftian filariasis serum pool contained more IgG antibodies to *B. malayi* than did the brugian filariasis serum pool (Fig. 2b). Because there were differences in the specific activities of the radiolabelled antigens, reactivities of individual serum pools cannot be quantitatively compared for the different antigens.

Comparison of the extent of cross-reactivity for IgE and IgG anti-parasite antibodies

To evaluate the degree of cross-reactivity of IgE and IgG antibodies to the heterologous helminth antigens, the serum pools were first depleted with *B. malayi*, *D. viteae* or *A. lumbricoides* sorbents before titres of the remaining IgE and IgG antibodies were compared with the titres in undepleted serum. Because of the limited amount of *O. volvulus* antigen, this sorbent could not be used for absorption experiments. Figure 3 summarizes the results of these depletion experiments for the onchocerciasis, brugian and bancroftian filariasis serum pools. The depletion itself was very effective with binding to the depleting antigen being reduced at least 90%, and usually 95%. Similar effectiveness of absorption was also seen with *A. lumbricoides* sorbent which removed 95% of anti-*Ascaris* IgE and IgE antibodies in all three filarial serum pools (results not shown).

For all three pools, the heterologous sorbents removed much more IgG than IgE antibody. Thus, a greater proportion of the IgG antibody was cross-reactive with other parasite species or conversely a higher proportion of IgE antibodies was specific for the homologous antigens. For example, depletion of the brugian filariasis sera (□) with *A. lumbricoides* or *D. viteae* antigens reduced binding of IgE antibodies to the homologous *B. malayi* antigen (Fig. 3, middle

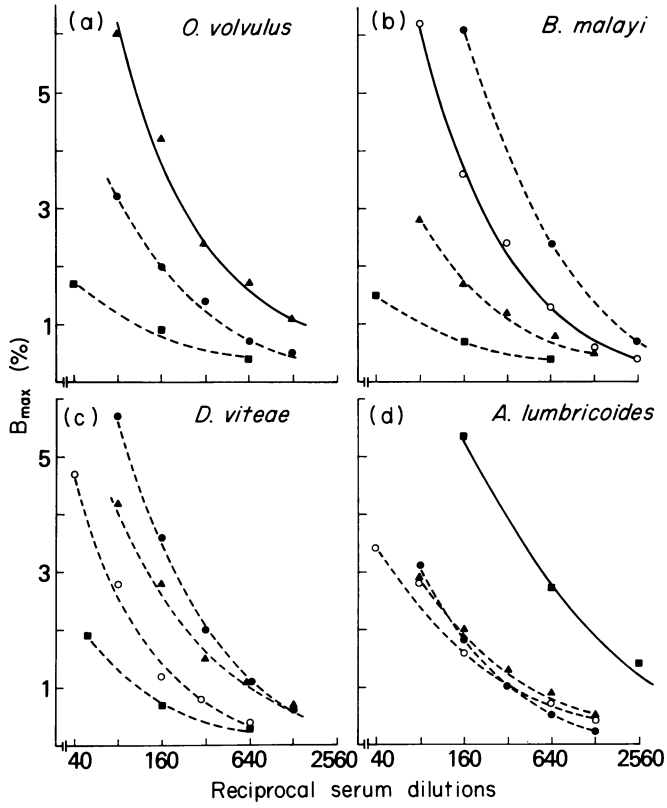

Figure 1. IgE antibodies detected by RAST against *O. volvulus* (a), *B. malayi* (b), *D. viteae* (c) and *A. lumbricoides* (d) antigens. Binding curves are given for the four serum pools tested: onchocerciasis pool (▲), brugian filariasis pool (○), bancroftian filariasis pool (●) and ascaris serum (■). Binding to the homologous antigen is shown with a solid line.

panels) to 76% and 64%, respectively, of the preabsorption values, whereas the remaining binding of IgG antibodies was only 22% and 17%. When the bancroftian filariasis pool (▨) was depleted in the same way, though the percentage of the remaining IgE antibody to *B. malayi* was still much greater than the remaining IgG antibody, it was more reduced than for the brugian filariasis pool (Fig. 3), probably because *B. malayi* is a heterologous antigen. *D. viteae* and *A. lumbricoides* sorbents were almost equally effective in removing cross-reacting IgG antibodies, but *D. viteae* antigens exhibited a higher degree of homology with allergens of the human pathogenic filariae. Depletion of the onchocerciasis serum confirmed this fact, as reduction in IgE binding to *O. volvulus* was greatest using the *D. viteae* sorbent (Fig. 3a). Thus, there also appeared to be a higher degree of homology between *O. volvulus* and *D. viteae* allergens than between *O. volvulus* and *B. malayi* allergens.

In order to confirm that the antibody activity remaining after depletion reflected specific antibody, the onchocerciasis pool was first depleted with a mixture of *B. malayi*, *D. viteae* and *A. lumbricoides* sorbents. The IgE binding to *O. volvulus* antigen was reduced by this procedure to 54% and the IgG binding to 25% of initial (Table 2). To show that the remaining binding activity was due to anti-*O. volvulus* antibodies, the depleted serum incubated with various amounts of soluble *O. volvulus* antigen before RAST and staphylococcus protein A radioimmunoassay (Staph A-RIA) were run. This antigen effectively inhibited binding of serum antibody in both tests with approximately five times more antigen being necessary to inhibit the RAST to the same extent as the Staph A-RIA (Table 2).

These findings of greater IgE specificity than IgG specificity were next extended from studies using serum pools to observations on individual patient

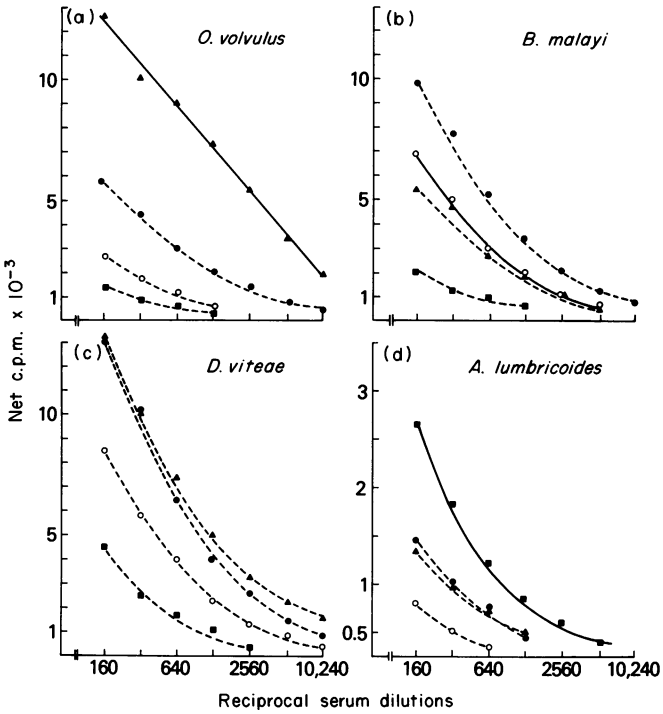

Figure 2. Staph A radioimmunoassay (Staph A-RIA) using *O. volvulus* (a), *B. malayi* (b), *D. viteae* (c) and *A. lumbricoides* (d) antigens. Symbols are the same as in Fig. 1.

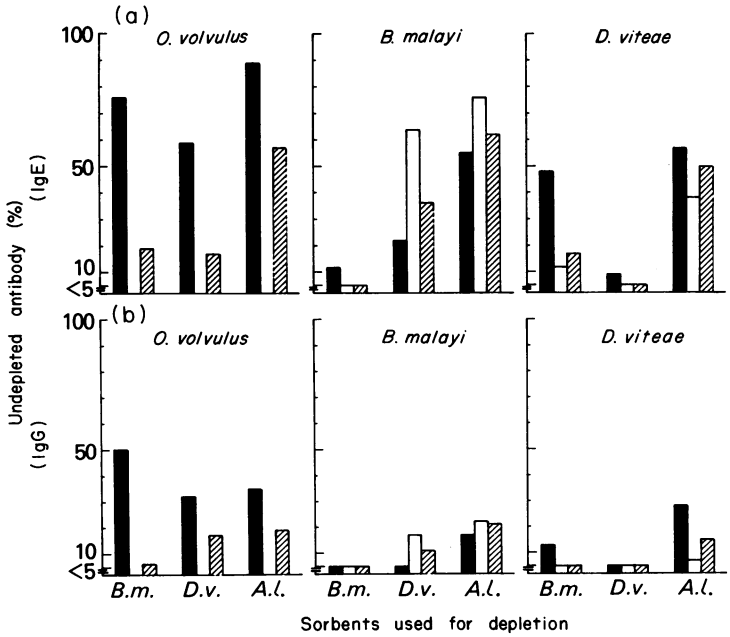

Figure 3. Remaining antibody binding to *O. volvulus*, *B. malayi* and *D. viteae* antigens in RAST (a) and Staph A-RIA (b) as a percentage of undepleted serum. Onchocerciasis serum pool (■), brugian filariasis serum pool (□) and bancroftian filariasis serum pool (■). Sorbents used for depletion are given at the bottom of the columns. The Brugian serum pool was not tested against *O. volvulus*.

Table 2. Reactivity of the onchocerciasis serum pool to *O. volvulus* antigens after depletion and inhibition

	Binding (%)	
	RAST	Staph A-RIA
Untreated onchocerciasis serum	100	100
Depleted with *B. malayi* (10 μg*), *D. viteae* (10 μg) and *A. lumbricoides* (23 μg) sorbents	54	25
Inhibition of depleted serum with		
0·4 μg *O. volvulus*†	36	16
2·0 μg *O. volvulus*	16	3
8·0 μg *O. volvulus*	8	1

* Amount (protein) per microlitre of undiluted serum.
† Soluble antigen per microlitre of undiluted serum.

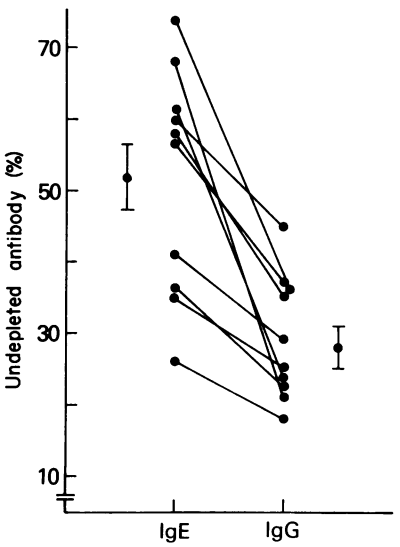

Figure 4. Remaining anti-*O. volvulus* antibody bindings of ten individual onchocerciasis sera after depletion with *B. malayi*, *D. viteae* and *A. lumbricoides* sorbents. Bars indicate arithmetric mean ± standard error.

sera. Ten individual onchocerciasis sera were depleted with a mixture of three immunosorbents (as above) and the remaining binding of IgE and IgG antibodies to *O. volvulus* antigen determined. In every case (Fig. 4) a greater proportion of IgG antibodies was removed by the heterologous antigens than IgE antibodies (paired t test: $t = 5{\cdot}73$, $P < 0{\cdot}01$). The proportion of remaining binding was independent of the initial amount of antibody present.

DISCUSSION

Our studies indicate that there is much less cross-reactivity with heterologous parasite antigens (i.e. higher specificity) in the IgE antibody response to filarial infection than in the corresponding IgG antibody response. This finding was first made when pools of serum from patients with onchocerciasis, bancroftian filariasis, brugian filariasis or ascariasis were tested against somatic antigen extracts of *O. volvulus*, *B. malayi*, *D. viteae* and *A. lumbricoides*; it was later confirmed with ten individual sera from patients with onchocerciasis in whom the proportion of IgE antibodies specific for *O. volvulus* was found always to be significantly greater than the proportion of specific IgG antibodies. These conclusions are based on the comparative analysis of binding curves for the four different immunosorbents in the RAST (for IgE) and Staph A-RIA (for IgG) and on the analysis of specific IgE and IgG antibody before and after these sera were absorbed with homologous and heterologous antigen immunosorbents.

RAST is the current method of choice for quantifying specific IgE antibodies (Adkinson, 1976). The RAST assays for the four parasite antigens used in this study have been previously standardized, and all sera were used in dilutions shown to be optimal for the tests (i.e. for guaranteeing both antigen excess and comparable antigen–antibody binding curves). Furthermore, because there is inherent test-to-test variability in the accuracy of the RAST (Evans, 1980), comparisons between antibody levels in depleted and undepleted sera were always run in the same assays.

The major limitations of the Staph A-RIA are that the antigens had to be radioiodinated (and are thus subject to some alteration) and that while IgG1, IgG2, and IgG4 subclasses bind to the staphylococcus protein A, IgG3 antibodies do not and are therefore not detected. However, since IgG3 antibodies generally constitute only about 8% of the total IgG response (Stanworth & Turner, 1973), their omission from our analysis probably has little bearing on our conclusions. Indeed, the effects of specific absorption of IgG antibodies by homologous immunosorbents has been confirmed with the ELISA technique which employs an enzyme-labelled antibody to the Fc portion of all subclasses of IgG antibody (data not shown).

The effectiveness of the immunosorption procedures was at least 90% and generally over 95% (Fig. 3). Non-specific binding of either normal sera to the antigen sorbents or patient sera to uncoupled Sepharose was negligible.

The partial antigenic homology between human and animal filariae has been the basis for many serological assays to detect IgG or IgM antibodies (reviewed by Ambroise-Thomas, 1974; Kagan, 1979). Similarly, this homology has been exploited to measure antifilarial IgE antibodies in skin tests using heterologous antigens. While there is evidence from both *in vivo* observations (Grove, Cabrera, Valeza, Guinto, Ash & Warren, 1977) and *in vitro* studies (Ottesen, Neva, Paranjape, Tripathy, Thiruvengadam & Beaven, 1979) to show the superiority of homologous antigen in detecting IgE anti-filarial antibodies, our present study is the first attempt to determine the degrees of functional antigenic homology for both the allergens and IgG-inducing immunogens of three human filarial species and *A. lumbricoides*. With cross-reacting IgG antibodies as the criterion, extensive antigenic homologies were detected among *B. malayi*, *D. viteae* and *A. lumbricoides* adult worm somatic antigens. From depletion experiments with the onchocerciasis serum pool, it appears that *O. volvulus* antigens exhibit more specific determinants than the other antigens (Fig. 3b), but conclusive evidence is lacking because the reciprocal experiments absorbing the bancroftian and brugian pools with onchocercal antigens could not be carried out.

Comparing these IgG data with results from the RAST analyses, we conclude that allergens of the different filarial species are much more distinct from each other (Fig. 3a). *D. viteae* showed a relatively higher degree of homology to *Brugia* and *Onchocerca* than to *Ascaris*, and from the binding curves (Fig. 1) and depletion experiments (Fig. 3a) it was evident that homology is greater between *O. volvulus* and *D. viteae* and between *B. malayi* and *D. viteae* than between *O. volvulus* and *B. malayi*. The relationships between filarial allergens are obviously quite complex. Recently, Turner, Fisher & McWilliam (1980) carried out similar studies of the homologies between *A. lumbricoides*, *Ascaris suum* and the hookworm *Necator americanus* by analysing cross-reacting human IgE antibodies. By RAST inhibition experiments a high degree of homology was found between the two *Ascaris* species but homology was only minimal between *Ascaris* and *Necator*. In that study, however, IgG antibodies were not analysed

From the practical point of view, our findings of greater specificity of IgE antibodies than IgG antibodies suggest that efforts toward developing techniques for specific immunodiagnosis of filarial infections might be particularly successful if focused on the IgE antibody response of exposed individuals. The use of excretory/secretory products or surface antigens instead of crude worm homogenates might be another possibility to reduce cross-reactions in serodiagnostic tests. A high specificity has been reported in an enzyme immunoassay using secretory antigens from *Toxocara* larvae (de Savigny, Voller & Woodruff, 1979). Analysis of surface antigens of *Trichinella spiralis* (Philipp, Parkhouse & Ogilvie, 1980) and *Dipetalonema viteae* (Weiss & Tanner, 1981) have demonstrated marked stage specificity. Recently, the possible application of hybridoma technology in parasitic diseases has been discussed (UNDP/World Bank/WHO, 1980). Monoclonal antibodies should be particularly useful for the identification of the species-specific antigens or allergens and for the development of more reliable and specific immunodiagnostic methods.

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REFERENCES

ADKINSON JR N.F. (1976) Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: *Manual of Clinical Immunology* (Ed. by N.R. Rose and H. Friedman), p. 590. American Society of Microbiologists, Washington D.C.

AMBROISE-THOMAS P. (1974) Immunological diagnosis of human filariasis: present possibilities, difficulties and limitations (a review). *Acta Trop.* **31,** 108.

AMBROISE-THOMAS P., DESGEORGES P.T. & MONGET D. (1978) Diagnostic immuno-enzymologique (ELISA) des maladies parasitaires par une microméthode modifiée. 2. Résultats pour la toxoplasmose, l'amibiase, la trichinose, l'hydatidose et l'aspergillose. *Bull. Wld Hlth Org.* **56,** 797.

DE SAVIGNY D.H., VOLLER A. & WOODRUFF A.W. (1979) Toxocariasis: serologic diagnosis by enzyme immunoassay. *J. clin. Path.* **32,** 284.

DESSAINT J.P., CAPRON M., BOUT D. & CAPRON A. (1975) Quantitative determination of specific IgE antibodies to schistosome antigens and serum IgE levels with schistosomiasis (*S. mansoni* or *S. haematobium*). *Clin. exp. Immunol.* **20,** 427.

EVANS R. III (1980) Standardization of diagnostic *in vitro* techniques. In: *Advances in Allergology and Clinical Immunology* (Ed. by A. Oehling *et al.*), p. 579. Pergamon Press, Oxford.

GREENWOOD F.C., HUNTER W. & BOLVER S. (1963) The preparation of ^{131}I labeled human growth hormone of high specific radioactivity. *Biochem. J.* **89,** 114.

GROVE D.I., CABRERA B.D., VALEZA F.S., GUINTO R.S., ASH L.R. & WARREN K.S. (1977) Sensitivity and specificity of skin reactivity to *Brugia malayi* and *Dirofilaria immitis* antigens in bancroftian and malayan filariasis in the Philippines. *Am. J. trop. Med. Hyg.* **26,** 220.

HAMILTON R.G. (1980) Quantitative measurements of human immunoglobulins using solid phase radioimmunoassay technology. Ph.D. thesis, Johns Hopkins University, Baltimore, Maryland.

HAMILTON R.G., HUSSAIN R., OTTESEN E.A. & ADKINSON JR N.F. (1981) The quantitation of parasite-specific human IgG and IgE in sera: evaluation of solid phase RIA and ELISA methodology. *J. immunol. Meth.* **44,** 101.

HUSSAIN R., STREJAN G. & CAMPBELL D.H. (1972) Hypersensitivity to *Ascaris* antigen. VII. Isolation and partial characterization of an allergen. *J. Immunol.* **109,** 638.

KAGAN I.G. (1979) Diagnostic, epidemiologic, and experimental parasitology: immunologic aspects. *Am. J. trop. Med. Hyg.* **28,** 429.

KESSLER S.W. (1975) Rapid isolation of antigens from cells with a staphylococcal protein A-antibody absorbent: parameters of the interaction of antibody–antigen complexes with protein A. *J. Immunol.* **115,** 1617.

O'DONNELL I.J. & MITCHELL G.F. (1980) An investigation of the antigens of *Ascaris lumbridoides* using a radioimmunoassay and sera of naturally infected humans. *Int. Archs. Allergy appl. Immunol.* **61,** 213.

OTTESEN E.A., NEVA F.A., PARANJAPE R.S., TRIPATHY S.P., THIRUVENGADAM K.V. & BEAVEN M.A. (1979) Specific allergic sensitization to filarial antigens in tropical eosinophilia syndrome. *Lancet*, **ii,** 1158.

PHILIPP M., PARKHOUSE R.M.E. & OGILVIE B.M. (1980) Changing proteins on the surface of a parasitic nematode. *Nature (Lond.)*, **287,** 538.

SCHULZ-KEY H., ALBIEZ E.J. & BUETTNER D.W. (1977) Isolation of living adult *Onchocerca volvulus* from nodules. *Tropenmed. Parasitol.* **28,** 428.

SPEISER F. (1980) Application of the enzyme-linked immunosorbent assay (ELISA) for the diagnosis of filariasis and echinococcosis. *Tropenmed. Parasitol.* **31,** 459.

STANWORTH D.R. & TURNER N.M.W. (1973) Immunochemical analysis of immunoglobulins and their subunits. In: *Handbook of Experimental Immunology*, 2nd edn (Ed. by D.M. Weir), p. 10.1. Blackwell Scientific Publications, Oxford.

TURNER K.J., FISHER E.H. & MCWILLIAM A.S. (1980) Homology between roundworm (*Ascaris*) and hookworm (*N. americanus*) antigens detected by human IgE antibodies. *Aust. J. exp. Biol. med. Sci.* **58,** 249.

UNPD/World Bank/WHO (1980) *Hybridoma technology with special reference to parasitic diseases.* WHO, Geneva.

WEISS N., STUERCHLER D. & DIETRICH F.M. (1978) Radioallergosorbent and indirect immunofluorescent antibody tests in immunodiagnosis of schistosomiasis. *Lancet*, **ii,** 1231.

WEISS N., SPEISER F. & HUSSAIN R. (1981) IgE antibodies in human onchocerciasis. Application of a newly developed radioallergosorbent test (RAST). *Acta Trop.* **38,** 353.

WEISS N. & TANNER M. (1981) Immunogenicity of the surface of filarial larvae (*Dipetalonema viteae*). *Trans. Roy. Soc. trop. Med. Hyg.* **75,** 179.

WIDE L., AXEN R. & PORATH J. (1967) Radioimmunoassay for proteins. Chemical couplings of antibodies to insoluble dextran. *Immunochemistry*, **4,** 381.