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IgE antibodies in human onchocerciasis. Application of a newly developed radioallergosorbent test (RAST)

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Summary

A radioallergosorbent test (RAST) was developed to detect IgE antibodies against adult *Onchocerca volvulus* antigens coupled to CnBr-activated Sepharose. Twenty-four out of 25 (96%) onchocerciasis sera were reactive. The lower limit of sensitivity was estimated to be at approx. 3 ng/ml IgE antibodies. Tests of sera from patients with non-filarial helminth infections showed much less cross-reactivity with RAST than with an enzyme-linked immunosorbent assay (ELISA) detecting IgG and IgM antibodies against the same antigen preparation. At a specificity comparable to that of RAST, the sensitivity of ELISA was only 61%. A heterologous antigen, prepared from female *Dipetalonema viteae* worms, was comparatively evaluated with *O. volvulus*. In RAST and ELISA, onchocerciasis sera were less reactive than against the *O. volvulus* antigen. Since sera from patients with non-filarial helminth infections were more reactive in RAST and almost equally reactive in ELISA using the *D. viteae* antigen, sensitivity was 83% for RAST and only 22% for ELISA (compared at the specificity identical to that of the *O. volvulus* RAST).

Key words: onchocerciasis; serology; radioallergosorbent test; enzyme-linked immunosorbent assay; sensitivity; specificity.

Introduction

A wide range of serological methods has been used to detect antibodies in human onchocerciasis (reviewed by Ambroise-Thomas, 1974; Kagan, 1979). Since the supply with the homologous antigen is a major problem, heterologous

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antigens, such as *Onchocerca gutturosa*, *Dipetalonema viteae*, *Dirofilaria immitis* or *Ascaris suum* were used in most studies. Many authors have reported that sensitivity of a given serological method was similar or only slightly less sensitive when a heterologous antigen was used: this was the case for immunodiffusion (Niel et al., 1972), immunoelectrophoresis (Biguet et al., 1963; Gentilini, 1972) and for the indirect immunofluorescent antibody test (IFAT) (Ambroise-Thomas, 1975; Le Bras et al., 1977). For the enzyme-linked immunosorbent assay (ELISA), a high senitivity was reported using *O. gutturosa* or *D. viteae* antigens (Bartlett et al., 1975; Speiser, 1980). The major problem of all serological methods developed so far is their lack of specificity. Homologies between helminth antigens cause the detection of cross-reacting antibodies elicited by infections such as *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Trichinella spiralis* or even *Echinococcus granulosus* (Ambroise-Thomas et al., 1978; Speiser and Weiss, 1979; Speiser, 1980). Recently, *O. volvulus* antigens have been purified using affinity chromatography, gel filtration and isoelectric focusing (Marcoullis et al., 1978). However, the specificity of the isolated antigens has not been evaluated.

Helminth parasites are known to induce highly elevated levels of circulating IgE in man (Sadun, 1972; Kojima et al., 1972). While skin tests have been used for many years to detect homocytotropic antibodies, only recently circulating IgE antibodies were measured in ascariasis (O'Donnell and Mitchell, 1980) and schistosomiasis (Weiss et al., 1978) using radioallergosorbent tests (RAST). In the case of schistosomiasis, the sensitivity and the specificity of RAST were better when compared to the IFAT, even when crude somatic extracts of adult worms were used as antigens. From this fact, we hypothesized that homologies between allergens are less extensive than that for other antigens. To test this hypothesis, we developed a sensitive RAST to detect IgE antibodies in onchocerciasis and subsequently compared its sensitivity and specificity with a enzyme-linked immunosorbent assay (ELISA) detecting IgG and IgM antibodies. For both tests, analogous somatic extracts from adult *O. volvulus* and *D. viteae*, a filarial parasite of jirds (Worms et al., 1961) were comparatively evaluated.

Materials and methods

Sera. Sera were collected from 25 onchocerciasis patients who underwent nodulectomy at 'Ad lucem' Hospitals at Mbouda and Bafoussam (United Republic of Cameroon). These patients (aged 23 to 70) were hospitalized for various reasons. In most stool specimens eggs of intestinal nematodes (*Trichuris*, *Ascaris* and hookworm) were found. In addition, sera from uninfected patients and from patients with filarial and other helminth infections were obtained from the Diagnostic Center at the Swiss Tropical Institute (Basel) and from the Laboratory of Parasitic Diseases at the National Institute of Allergy and Infectious Diseases (Bethesda, Md.). All sera were kept frozen in aliquots, shipped on dry ice and stored at –70° C.

Antigens. Onchocerca volvulus adult worms were from fresh nodules which were digested by collagenase as described by Schulz-Key et al. (1977). Living worms were carefully washed from host

tissues and immediately frozen. After homogenization with a tissue grinder, the worms were extracted overnight in phosphate-buffered saline (0.15 M PBS pH 7.4) at 4° C. The supernatant was dialyzed against 0.15 M borate buffered saline (pH 8.4) for coupling to the immunosorbent or dialyzed against 0.06 M sodium carbonate buffer (pH 9.6) for coating microtiter plates. After dialysis, the extracts were ultracentrifuged (12,000 *g*, 4° C, 30 min). A soluble extract of female *Dipetalonema viteae* was prepared exactly the same way. Female adult worms were obtained from infected hamsters. Protein determinations were carried out using a commercial protein assay kit (Biorad Lab., Rochester, N. Y.).

Preparation of immunosorbents. Both antigens were coupled at a concentration of 1.0 mg protein per one milliliter of swollen CnBr-activated Sepharose 4B (Pharmacia, Piscataway, N. J.) following the instructions provided by the manufacturer. More than 99% of the proteins were coupled to the beads as demonstrated by protein measurements of the concentrated supernatants after coupling. The sorbents were stored at 4° C in RAST buffer (PBS pH 7.5 containing 0.4% Tween, 0.2% bovine serum albumin and 0.05% sodium azide). Immunosorbents were washed with RAST buffer before use.

Preparation of radiolabeled anti-IgE antiserum. An affinity purified goat anti-human IgE antiserum was obtained by courtesy of Dr. R. F. Ritchie (Portland, Maine). Fifty micrograms of this preparation were labeled with 1 mCi of ^{125}NaI (Amersham, Arlington Heighs, Ill.) using the chloramine T method (Greenwood et al., 1963). The iodinated protein was then separated from unbound ^{125}I by gelfiltration. Precipitation of the labeled proteins by cold 50% trichloroacetic acid demonstrated 95% bound radioactivity.

Radioallergosorbent test (RAST). Prior to test, all sera were absorbed using a 5% solution of Sepharose 4B (Pharmacia) for at least 4 h at room temperature to remove any anti-Sepharose IgE antibodies (Hamilton, 1980). Sera were diluted in RAST buffer containing 1.25% of normal serum. Sera were tested at a dilution of 1 in 20 or 1 in 80. The reference pool (a mixture of three high titered sera) was titrated starting at 1 in 40. These serum dilutions were choosen to guarantee antigen excess conditions. A 2% suspension of immunosorbent (0.5 ml per 0.1 ml serum) was used throughout all experiments. Thus, the antigen concentration per test tube was 10 μg bound protein.

Tubes were kept rotating for 6 to 8 h at room temperature. After 3 washings with RAST buffer, 0.5 ml of ^{125}I-labeled goat anti-human IgE ($8–10 \times 10^4$ cpm; corresponding to 10–20 ng labeled antibodies) was added per test. After orbital rotation at room temperature (14 to 16 h), unbound marker activity was removed by 4 washings. The reactivity of sera was expressed as percent of maximal binding (B_{max}) which was calculated by the following formula:

$$\% \, B_{max} = \frac{\text{net cpm} \times 100}{B_{max}}$$

B_{max} is the immunoreactive cpm added, determined by incubating the anti-IgE antiserum with excess of insolubilized IgE (Hamilton et al., 1981).

Determinations of total serum IgE. Total serum IgE levels were determined by a paper radioimmunosorbent test (Phadebas-PRIST IgE, Pharmacia).

Quantitation of IgE antibodies against Onchocerca volvulus. Five selected onchocerciasis sera with a high ratio of IgE antibodies to the total IgE level were depleted of IgE antibodies by using a mixture of *Brugia malayi, Dipetalonema viteae* and *Ascaris lumbricoides* sorbents (Weiss et al., in prep.). Absorbed and unabsorbed sera were tested in RAST and PRIST. From the percent binding to *O. volvulus* sorbent and the differences in total IgE levels the amounts of IgE antibodies against *O. volvulus* could be estimated.

Enzyme-linked immunosorbent assay (ELISA). ELISA tests were run at the Diagnostic Center of the Swiss Tropical Institute using a standardized procedure described elsewhere (Speiser, 1980).

Each well of polystyrene microtiter plates (Dynatech 29B) was sensitized with 0.2 ml of the antigen solutions (0.5 μg protein per ml). Sera were tested at a dilution of 1 in 160 against both antigens in the same test. A horseradish peroxidase labeled antiserum was a goat anti-human IgG (heavy and light chain) serum (Miles Lab., Elkhart, Ind.) used at a dilution of 1 in 1500. Optical densities (O. D.) were read at 492 nm on a Multiscan photometer (Flow lab. Rockville, Md.).

Results

For the quantitation of IgE antibodies against *O. volvulus* in individual sera, a reference pool of three high titered sera was used as a standard. The mean binding curve of the reference pool calculated from seven independent RAST experiments is given in Fig. 1. For all assays the same batch of the immunosorbent and the same batch of the anti-IgE antiserum (labeled at two different dates) were used over a 3-month period. The mean coefficient of variation ($\pm$SE) for six serum dilutions tested was $22 \pm 2\%$. A linear binding curve was obtained between 2 and 8% B_{max}. Individual sera with various amounts of IgE antibodies gave nearly parallel binding curves (Fig. 1). Setting the reference

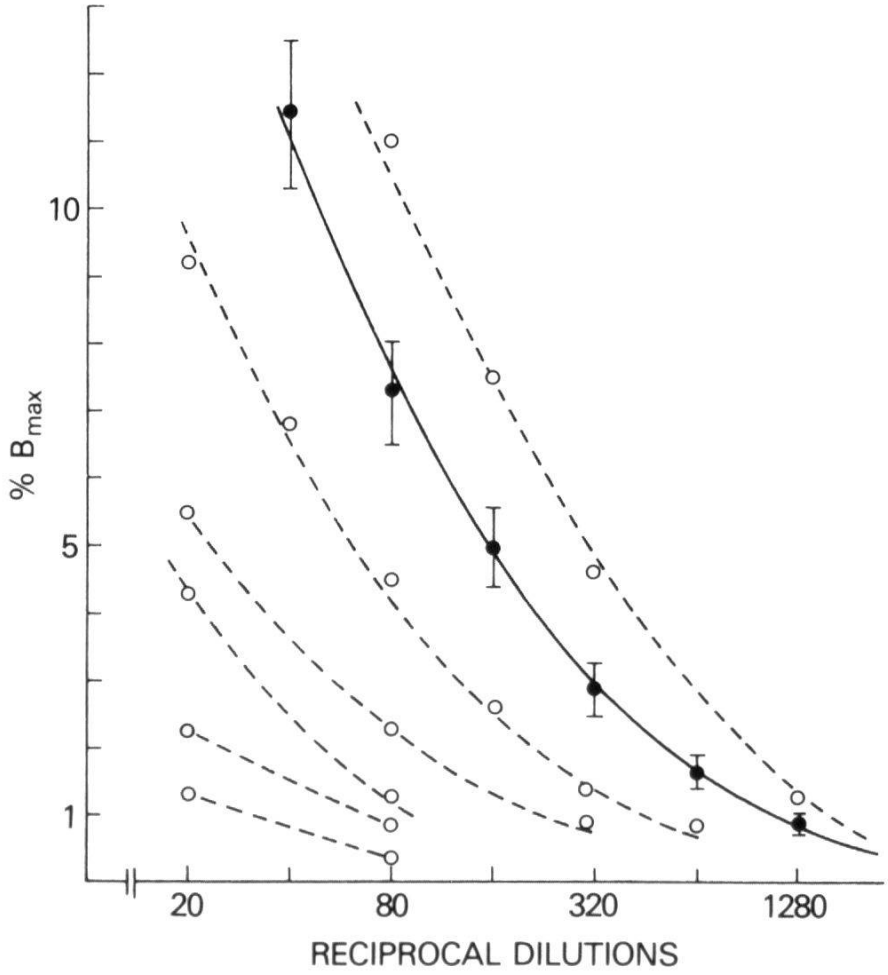

Fig. 1. Binding characteristics of IgE antibodies to *Onchocerca volvulus* sorbent measured by RAST for 6 individual sera (broken lines) and for the reference serum pool (solid line). Bars indicate mean $\pm$SE of binding obtained in 7 independent experiments.

serum pool as 100 arbitrary units (U) of anti-*O. volvulus* IgE antibodies per ml of undiluted serum and by taking only the linear part of the reference curve, IgE antibodies can be quantitated from 5 to 30 U/ml (at a serum dilution of 1 in 20) or from 20 to 120 U/ml (at 1 in 80). The lower end of the reference curve (at about 1% B_{max}; equals about two-times background) allowed measurements of 0.1 U/ml of IgE antibodies.

For 25 individual onchocerciasis sera the amount of anti-*O. volvulus* IgE antibodies and the total serum IgE levels were determined (Fig. 2). Only one serum had less than 2 U/ml of IgE antibodies. Values ranged from 1 to 220 U/ml (geometric mean: 11.4). The amount of IgE antibodies detected by RAST correlated with the total IgE level ($r = 0.77$, $p < 0.01$). Total IgE values ranged from 150 to 35,000 IU/ml (geometric mean: 3,200 IU/ml).

From RAST and PRIST values of 5 selected sera (previously absorbed with filarial and *Ascaris* antigens) it was calculated that 1 U/ml equals about 30 ± 7 ng IgE (mean $\pm$ SE). Thus, for the 25 onchocerciasis sera given in Fig. 2, between 1 and 40% of the total IgE (median: 3.3%) reacted with the *O. volvulus* sorbent. The reproducibility of RAST was determined using 8 sera which were run in 2 or 3 assays. When results were expressed as U/ml, the mean coefficient of variation ($\pm$ SE) was $21 \pm 5\%$.

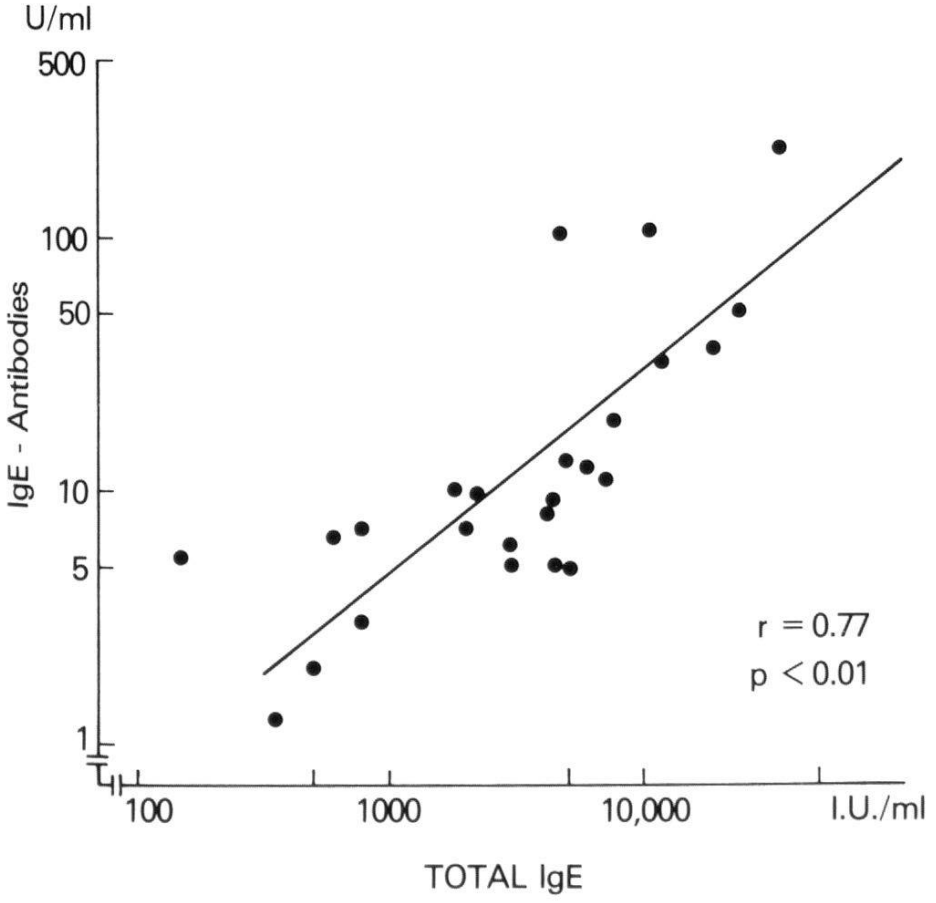

Fig. 2. IgE antibodies against *O. volvulus* and total IgE levels of 25 onchocerciasis sera.

High RAST values were obtained from 5 Swiss patients with onchocerciasis (64, 80, 210 U/ml) and loiasis (7, 14 U/ml). For a serum pool of patients with Bancroftian filariasis 38 U/ml were determined. Twelve non-parasitized North American controls and 8 with strongyloidiasis as well as 5 Swiss patients with proven schistosomiasis and 4 with hydatid disease were unreactive (<2 U/ml). This was also the case for a myeloma serum (total IgE: 38,000 IU/ml), which was kindly provided by Dr. L. Yman (Pharmacia AB, Uppsala, Sweden). However, one serum with a high titer of anti-*Ascaris* IgE antibodies from a South African patient gave 6 U/ml. IgE content of this serum was 6,700 IU/ml.

The results of 23 out of 25 onchocerciasis sera obtained by RAST and by ELISA are plotted in Fig. 3. High titers of IgG/IgM antibodies did not correlate with high titer of IgE antibodies.

Since the supply with *O. volvulus* antigens is rather limited, we evaluated the use of a heterologous filarial antigen, *D. viteae* for RAST and ELISA. The binding curves against both antigens determined in the same RAST or ELISA experiments were parallel (Fig. 4). Binding to the heterologous antigen, however, was one doubling dilution lower for RAST and two doubling dilutions lower for ELISA. Using the corresponding reference curves for quantitation, we found a strong correlation ($r = 0.87$, $p < 0.01$) between the RAST values of onchocerciasis sera obtained with the *O. volvulus* and the *D. viteae* sorbents (Fig. 5 A). Two sera with low levels of IgE antibodies were not reactive to *D. viteae*. Five sera from patients with *Loa loa* (3), *D. perstans* (1) and *Wuchereria bancrofti* (1 serum pool) were more reactive against the *D. viteae* than the *O.*

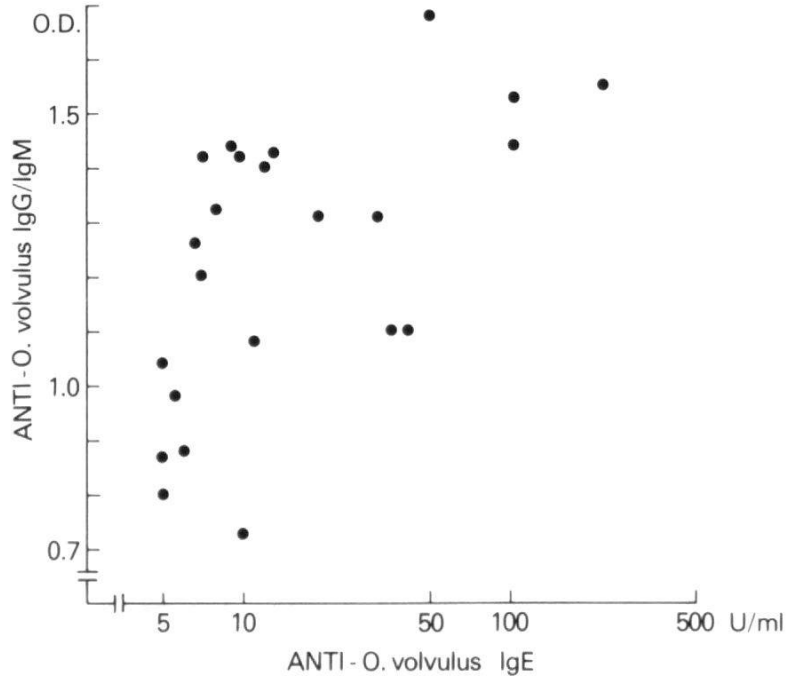

Fig. 3. Levels of IgG/IgM and IgE antibodies against *O. volvulus* as measured by ELISA and RAST for 23 onchocerciasis sera.

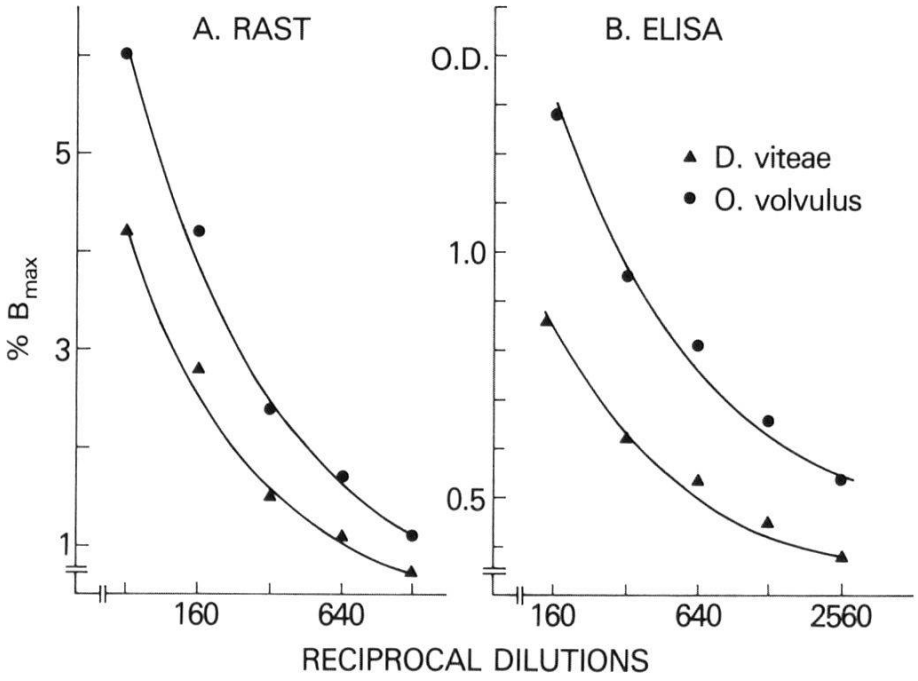

Fig. 4. Binding curves of the reference onchocerciasis serum pool against *O. volvulus* (closed circles) and *D. viteae* (triangles) antigens in RAST (A) and ELISA (B).

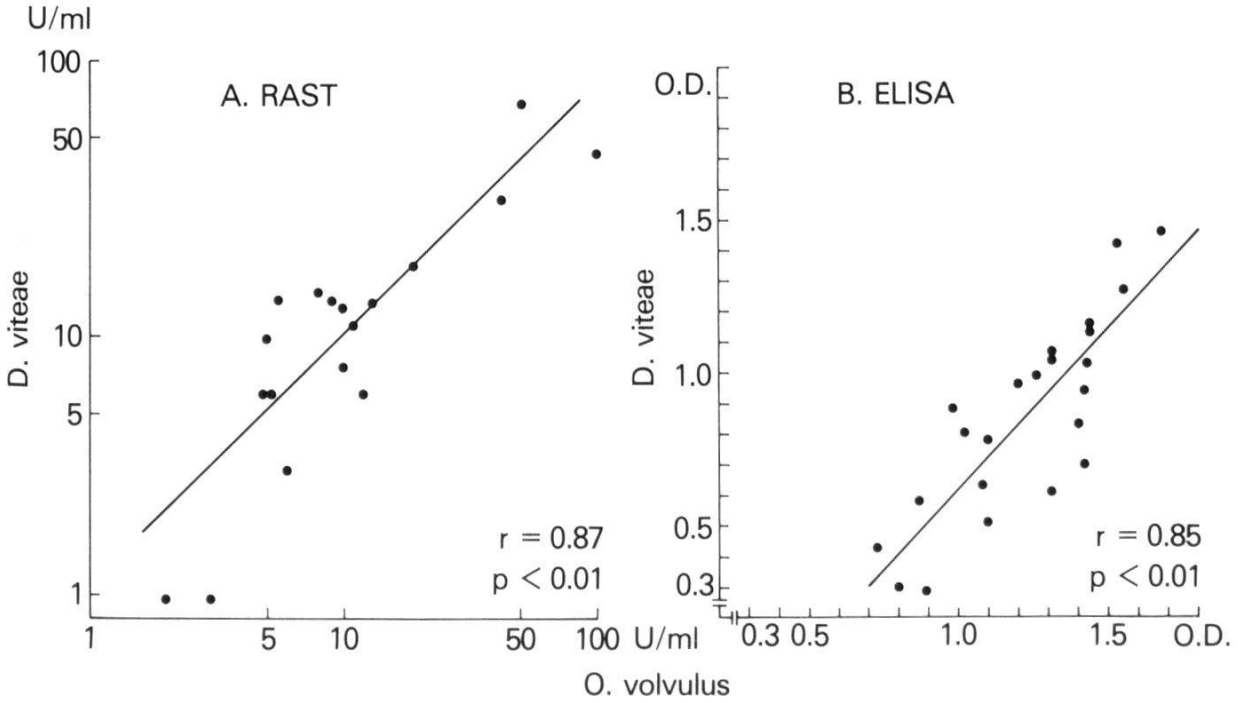

Fig. 5. Correlation of RAST (A) and ELISA (B) results between *O. volvulus* and *D. viteae* antigens for 18 and 23 individual onchocerciasis sera.

volvulus sorbent. This was also the case for some sera from patients with non-filarial helminth infections *(Strongyloides, Schistosoma, Echinococcus)* for which cross-reacting IgE antibodies with *D. viteae* were detectable at low levels (3 to 6 U/ml). The high titered *Ascaris* serum had 19 U/ml of IgE antibodies compared to 6 U/ml using the *O. volvulus* sorbent.

Good correlation ($r = 0.85$, $p < 0.01$) was also observed between ELISA values obtained with *O. volvulus* or *D. viteae* antigens (Fig. 5 B). As an average, O. D. values were 0.4 higher with the homologous antigen. The reactivity of 14 filariasis sera (9 *W. bancrofti*, 4 *L. loa*, 1 *D. perstans*) to both antigens was similar (mean O. D. $\pm$SD:0.92 $\pm$0.39 vs. 0.97 $\pm$0.43). To evaluate the specificity, 60 sera from patients with non-filarial helminth infections were tested. Specificity for the *O. volvulus* antigen was even lower than that already reported for *D. viteae* (Speiser, 1980): 9 out of 60 sera (4 *Echinococcus*, 2 *Trichinella*, 1 *Schistosoma* and 2 intestinal helminths) gave O. D. values over 0.7 with the *O. volvulus* antigen (mean $\pm$SD: 1.02 $\pm$0.12). Seven of these sera reacted also with the *D. viteae* antigen (mean $\pm$SD:0.85 $\pm$0.15).

Discussion

We have developed a highly sensitive solid phase (Sepharose) radioallergosorbent test (RAST) for the quantitation of IgE antibodies to somatic extracts of *Onchocerca volvulus*. A similar RAST procedure has already been successfully used to quantitate IgE antibodies in lymphatic filariasis (Hamilton et al., 1981; Hussain et al., in preparation). One of the major advantages of using Sepharose as solid phase over paper discs is the considerably reduced amount of antigen required per test (Weiss et al., 1978 and unpublished results). This was particularly desirable since *O. volvulus* antigens are extremely difficult to procure. The lowest antigen concentration which gave adequate binding in our system was 10 μg protein per test (Fig. 1). From studies with other parasite extracts we can assume that IgE antibodies are directed to only a limited number of antigens in the crude extract. Since, in addition, IgE levels were considerably elevated, appropriate serum dilutions were needed to guarantee antigen excess conditions. For most onchocerciasis sera dilutions of 1 in 20 or 1 in 80 were adequate. Due to the limited availability of the homologous antigen, we determined the reactivity of a more easily available heterologous antigen, a somatic extract of female *D. viteae*. High cross-reactivity of this antigen was demonstrated with IgE antibodies in onchocerciasis sera (Fig. 4A, 5A). With *D. viteae* antigen, IgE antibodies were detected in 16 out of 18 (= 89%) of the sera tested (Fig. 5 A). In contrast, only 54% sensitivity was reported in RAST using *Dirofilaria immitis* antigen on paper discs (Somorin and Heiner, 1976). However, cross-reacting IgE antibodies were also demonstrated in sera from patients with non-filarial helminth infections. Thus, to assure a comparable level of specificity as with *O. volvulus*, the cut-off point for the *D. viteae* RAST has to be set at 6 U/ml. Sensitivity would then be only 83% compared to 96% for the homologous antigen (Fig. 5 A, Fig. 2).

Both antigen preparations were also used in an enzyme-linked immunosorbent assay (ELISA). The potential value and the limitations of the *D. viteae* antigen for filariasis serology have been reported elsewhere (Speiser and Weiss,

1979; Speiser, 1980). The reactivity of onchocerciasis sera was much higher to the homologous antigen (Fig. 4 B, 5 B). In contrast to RAST, extensive cross-reactivities were observed with antibodies of the IgG or IgM class induced by non-filarial helminth infections. To guarantee a comparable specificity to that of RAST, only O. D. values over 1.2 (for *O. volvulus)* and 1.1 (for *D. viteae*) could be considered as positive results. Sensitivity of ELISA would then be 61% for the homologous and only 22% fot the heterologous antigen in comparison to 96 and 83% for RAST (Fig. 5 A, 5 B). RAST was also more sensitive than an indirect immunofluorescent antibody test (IFAT) using frozen section of *D. viteae* worms (Weiss and Degrémont, 1976). Nine out of 11 onchocerciasis sera with insignificant antibody titers in IFAT (lower than 1 in 320) were positive ($\geqslant$6 U/ml) in the *D. viteae* RAST (data not shown).

Considering better sensitivity at comparable specificity, RAST is favoured above ELISA. The use of a crude *O. volvulus* antigen does not exclude binding of cross-reacting IgE antibodies induced by loiasis or Bancroftian filariasis. However, we recently found that antigenic homologies detected by IgE antibodies between *O. volvulus, D. viteae, Brugia malayi* and *Ascaris lumbricoides* are less extensive than those detected by IgG antibodies of human sera (Weiss et al., in preparation). For absolute specificity the isolation of the species-specific antigens is needed.

From the technical point of view, ELISA offers several advantages over RAST: The labeled anti-immunoglobulin marker is more easy to store and to handle, and the test procedure is simpler. This might explain the better reproducibility of ELISA (coefficient of variation between tests $<10\%$ compared to 22% for RAST). Attempts to quantitate IgE antibodies to *B. malayi* using an ELISA method were only minimal successful (Hamilton et al., 1981). It was suggested that the limited amount of antigen bound to the surface of the microtiter plate and interference with IgG antibodies caused low sensitivity. Recently, a sensitive ELISA method for the detection of IgE antibodies was described using a double antibody modification (Sepulveda, 1980). Such a method in combination with a (partial) purification of parasite allergens might give the basis to adapt ELISA to the detection of parasite specific IgE.

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