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Detection of IgE antibodies in onchocerciasis. Possibility of using allergens from *Dipetalonema viteae* extracts that cross-react with allergenic determinants in crude extracts of *Onchocerca volvulus*

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SUMMARY

The present study reports the presence of *Onchocerca volvulus* specific IgE in the sera obtained from onchocerciasis patients. About 70% of onchocerciasis patients showed a raised level of *O. volvulus* specific IgE compared to patients infected either with other human filarids (*Loa loa*, *Wuchereria bancrofti*, *Brugia malayi*) or with other helminths (*Schistosoma mansoni*, *Ascaris lumbricoides*, *Fasciola hepatica*). The *O. volvulus* specific IgE level was significantly higher in patients exhibiting 'gale filarienne' than in microfilaraemic patients or in endemic controls. The total IgE level was significantly raised in the serum samples of all groups of subjects from endemic areas compared to European controls. There was no significant increase in the level of IgE in the onchocerciasis sera when *O. volvulus* antigen was replaced by the antigens from various helminths in the present assay system (radioallergosorbent test). However, there was a clear evidence of the presence of cross-reacting allergens in the crude extracts from adults of *O. volvulus* and *Dipetalonema viteae* (a rodent filarial parasite) because there was a significant reduction in IgE level in onchocerciasis sera following absorption with either *O. volvolus* or *D. viteae* sorbents. Moreover, the IgE antibodies in onchocerciasis patients sera recognized the allergens which were present in the somatic extracts of *O. volvulus* and *D. viteae* as revealed by radiolabelled anti-IgE.

INTRODUCTION

An increased production of IgE is a frequent concomitant of human infection with helminth parasites (Jarett, 1972; Kojima, Yokogawa & Tada, 1972). In the case of human filarial infections, an increase in serum IgE levels has been shown in bancroftian filariasis (Neva *et al.*, 1975; Ito Sawada & Sato, 1972), Brugian filariasis (Hussain *et al.*, 1981) and in onchocerciasis (Weiss, Speiser & Hussain, 1981). It has been suggested that parasite specific IgE antibodies may play a significant role both in protective immunity to helminth parasites and in the pathogenesis of certain clinical manifestations of these infections (Capron & Dessaint, 1977; Ottesen *et al.*, 1979). The quantitative analysis and the qualitative characterization of IgE responses of infected individuals may provide invaluable clues and insights both into the determinants of protective immunity and into the nature of the pathogenesis of some of the major clinical aspects of these diseases.

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In the present study we have investigated the presence of parasite specific IgE levels in onchocerciasis patients sera and examined the cross-reactivities of allergens present in somatic extracts from adult *Onchocerca volvulus* which parasitizes man and *Dipetalonema viteae* which infects rodents.

MATERIALS AND METHODS

Antigens. O. volvulus adult worms were collected from subcutaneous nodules of patients after nodulectomy. The antigens from *O. volvulus*, *Schistosoma mansoni*, *D. viteae* and *Ascaris lumbricoides* were prepared according to Capron *et al.* (1968).

Sera. Samples of blood were taken from 83 patients with proven infection by *O. volvulus*, the causative agent of onchocerciasis. These patients live in Mali and Cameroon, the two endemic areas for onchocerciasis. Sera from individuals infected with *Loa loa* were collected from the same areas.

Sera from patients infected with *S. mansoni* and *F. hepatica* were also collected. Sera from individuals infected with *B. malayi* came from Malaysia. Sera from patients showing the presence of microfilariae of *Wuchereria bancrofti* in their peripheral blood were obtained from Brazil.

Quantitative measurement of total serum IgE level. The radioimmunosorbent tests (RIST) was performed. The concentration of IgE in serum was evaluated from its capacity to inhibit the binding of ^{125}I-labelled IgE to antibodies (anti-IgE), as compared to a standard. Sera were tested at a dilution of 1/20. Results were expressed in units/ml. The IgE level was considered significantly elevated when over 800 units/ml.

Quantitation of specific IgE antibodies to O. volvulus. The test used for quantification of specific IgE antibodies was the Phadebas RAST i.e. a radioallergosorbent test (Pharmacia diagnostics) described by Wide, Bennich & Johansson (1967). Soluble antigens from *O. volvulus*, *D. viteae*, *S. mansoni* and *A. lumbricoides* were coupled respectively to separate lots of discs of Whatman paper No. 1 CNBr pre-activated. Specific IgE present in patients sera reacted with antigen. After elimination of non-specific IgE, ^{125}I-labelled anti-IgE (Pharmacia, Phadebas RAST) was added to form complexes. The radioactivity of that complex was determined by a γ-counter. The percentages of counts were directly compared to those obtained with a maximal binding.

$$\% \text{ B max} = \frac{\text{No. c.p.m. bound}}{\text{B max}} \times 100$$

B max is the radiolabelled anti-IgE corresponding to 4 ng/50 μl added in test tube. Tests were done in triplicate.

Inhibition test. Immunoadsorbents prepared with antigens from *O. vovulus* (*O.v.*) and *D. viteae* (*D.v.*) were performed to demonstrate that the IgE binding to the *O.v.* sorbent or *D.v.* sorbent was specific.

The antigens from *O. volvulus* and *D. viteae* were each coupled to CNBr activated Sepharose 4B (Pharmacia, Sweden). The efficiency of coupling was assessed by measuring the optical density (OD) of antigen supernatant obtained before and after incubation with CNBr–Sepharose.

The demonstration of specificity of IgE binding to the *O.v.* sorbent or *D.v.* sorbent was performed as follows: 0·5 ml of *O.v.* sorbent or *D. v.* sorbent was added to the test tube containing 0·5 ml of serum with high level of specific IgE antibodies. Samples were rotated for a night at room temperature followed by centrifugation at 3,500 rpm for 20 min to sediment the sorbent. Each supernatant was then run in RAST to determine the specific IgE content. Unabsorbed sera were simultaneously evaluated.

Radioimmunoelectrophoresis (RIEP). Immunelectrophoresis was carried out according to Grabar & Williams (1953) in 1% agarose gel plate (8 × 8 cm). Antigenic extract (20 mg/0·1 μl) was submitted to electrophoresis in 0·1 M veronal buffer pH 8·2 for 2·45 hr at 19 V. Hyperimmune sera were concentrated three times by lyophilization. After 48 hr of diffusion at room temperature and three washes in saline (24 hr), the plate was incubated at room temperature. Saline was poured over the gel. A patient's serum (150–300 μl) was then added and incubated overnight at room temperature. The gel was washed at least three times with saline containing 1% Tween 20 for 3 min.

Finally, incubation buffer, with 0·25 μg ^{125}I-labelled (1 μCi) anti-human IgE rabbit antibodies (Pharmacia, Uppsala, Sweden), was added and left at room temperature until the next day. The incubation buffer consisted of 0·05 M phosphate buffer, pH 7·5, 0·3% bovine serum albumin, 0·9% sodium chloride, 0·1% sodium azide and 0·1% EDTA. Subsequently, the gel was washed with incubation buffer saline, and distilled water for periods of 30 min then pressed and dried. Autoradiography was performed on a Kodirex film (Kodak, Sevran, France), the film was exposed for up to a week.

RESULTS

Quantification of total IgE in patients sera

The total serum IgE is greatly elevated in onchocerciasis patients sera. Only five out of 83 onchocerciasis patients showed a level of total serum IgE less than 1,000 units/ml. The results are presented in the Fig. 1. The control sera from Africans who recently came from endemic areas also showed higher level of total IgE when compared to the sera obtained from European controls ($P < 0·001$).

Detection of O. volvulus *specific IgE antibodies in sera from onchocerciasis patients*

The highest *O. volvulus* specific IgE was observed in patients with clinical sign of 'gale filarienne' (Fig. 2) and this level was significantly different from patients showing the presence of high or low level of microfilariae in the skin and from endemic controls ($P < 0·001$). The specificity of the IgE response in onchocerciasis has been tested in the present assay system by making two different approaches. First, the sera from patients with proven infections of other helminths such as *S. mansoni*, *A. lumbricoides*, *F. hepatica* and of other human filarids (*L. loa*, *W. bancrofti* and *B. malayi*) were tested in the RAST in which *O. volvulus* antigen was coupled to the paper discs. The mean values in % Bmax of the patients infected with these helminths and filarids was significantly lower than that of onchocerciasis patients ($P < 0·001$). Few of the sera obtained from patients infected with human filarids have demonstrated slightly augmented values. The results are shown in

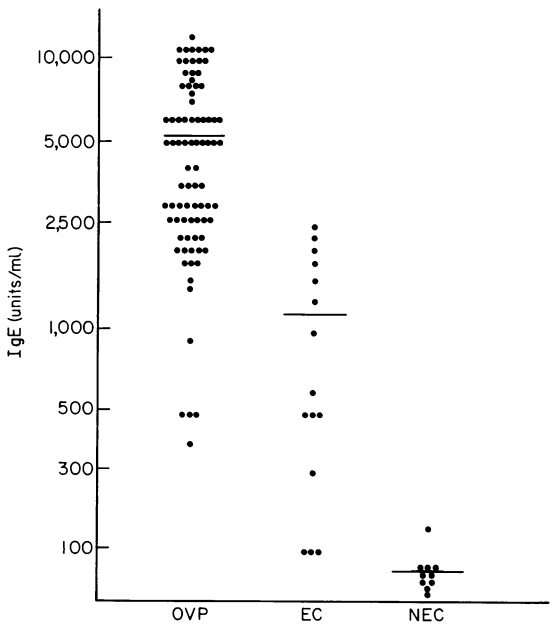

Fig. 1. Quantitation of total IgE in onchocerciasis sera (OVP), in endemic controls (EC) and in endemic European controls (NEC).

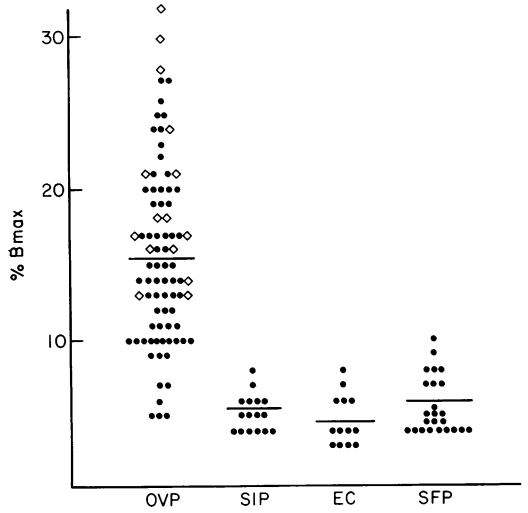

Fig. 2. Detection of *O.v.* specific IgE in the sera of onchocerciasis patients (OVP, ◊ 'gale filarienne') using homologous *O.v.* antigens, (SIP) in sera from patients infected with various helminth parasites (*A. lumbricoides, S. mansoni, F. hepatica*), (SFP) in sera from patients infected with other filarids (*W. bancrofti, L. loa, B. malayi*), (EC) in endemic controls.

the Fig. 2. Second, the antigens of other helminths were coupled to the paper discs and sera of onchocerciasis patients were assayed for the presence of IgE antibodies in the RAST. There was no significant increase in % Bmax value when antigens from *S. mansoni* or *A. lumbricoides* were used (Fig. 3). Only few of the onchocerciasis sera have shown slightly increased value against these two antigens. However, onchocerciasis patients sera gave almost the same value with antigens from *D. viteae* as obtained with antigens from *O. volvulus*. Using *D.v.* antigen in the RAST, the sera from patients infected with such helminths as *S. mansoni. A. lumbricoides, F. hepatica* or with human filarids (*W. bancrofti, L. loa, B. malayi*) did not show a significant higher value in % Bmax value

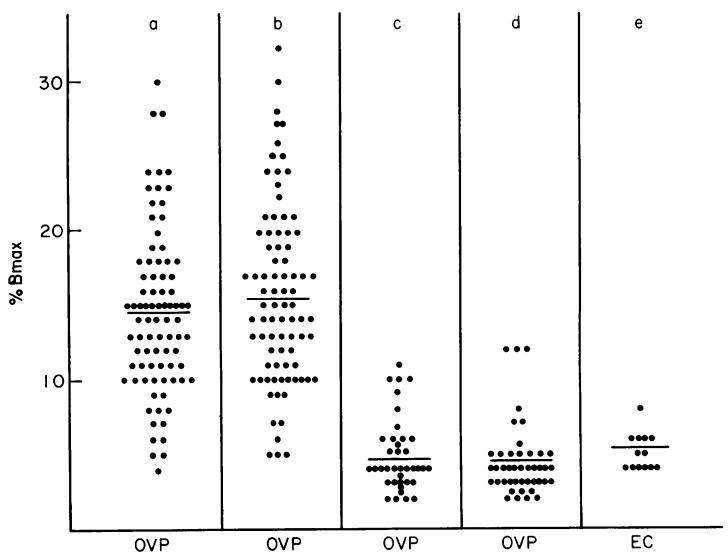

Fig. 3. Determination of parasite specific IgE in the sera of onchocerciasis patients by using antigens from different parasites; (a) *D. viteae*; (b) *O. volvulus*; (c) *S. mansoni*; (d) *A. lumbricoides*; (e) endemic control.

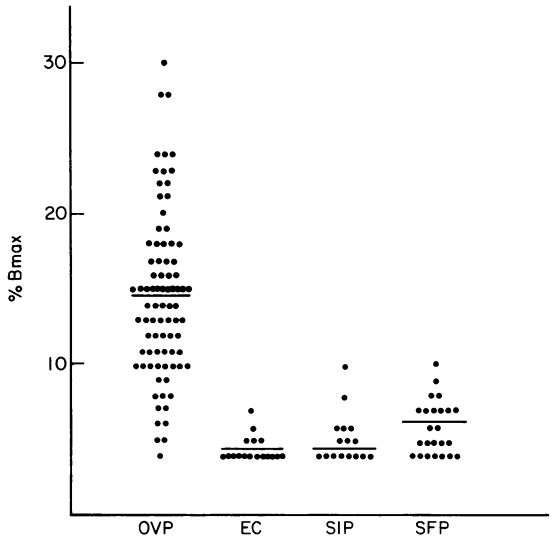

Fig. 4. Utilization of heterologous *D. viteae* antigen in the determination of IgE in onchocerciasis patients sera (OVP), in sera from patients infected with various helminth parasites (SIP), in sera from patients infected with other filarids (SIF), in endemic controls (EC).

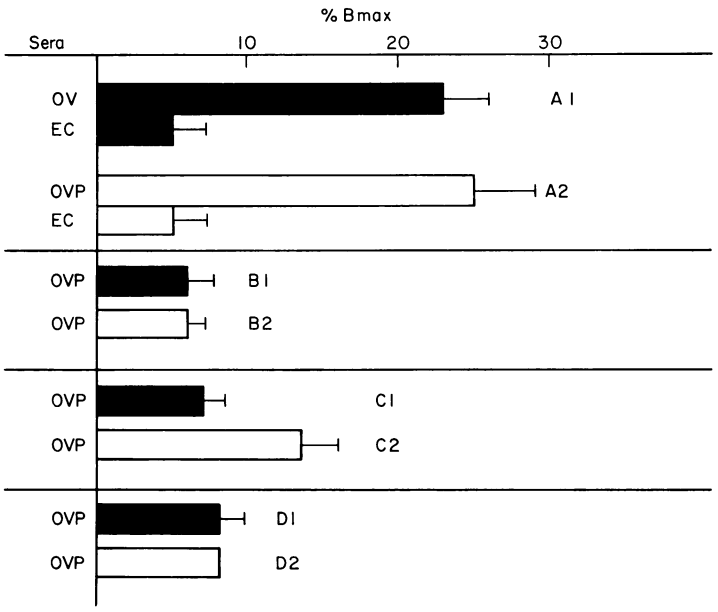

Fig. 5. (□) RAST with antigen *O.v.* (■) RAST with antigen *D.v.* The effect of depletion of IgE antibodies present in the sera from onchocerciasis patients by immunoadsorption on the values in RAST; A_1A_2 non-absorbed; B_1B_2 absorbed with *O.v.* antigens; C_1C_2 absorbed with *D.v.* antigens; D_1D_2 absorbed with *D.v.* antigens and subsequently with *O.v.* antigens.

compared to sera from onchocerciasis patients (Fig. 4). The latter result suggests the presence of cross-reacting allergens in crude extracts of *O. volvulus* and *D. viteae*.

Evidence for the presence of cross-reacting allergens in the somatic extracts of both D. viteae *and* O. volvulus

In the inhibition experiment, the onchocerciasis patients sera were absorbed with immunosorbents prepared from antigens of *O. volvulus* or of *D. viteae*. There was a significant diminution in the IgE binding to *O. volvulus* when the patients sera were absorbed with antigens either from *O. volvulus* or *D. viteae* compared to untreated onchocerciasis sera ($P < 0{\cdot}001$). The depletion was greater with the homologous *O. volvulus* antigens than with the heterologous *D. viteae* antigens ($P < 0{\cdot}02$). The results are summarized in Fig. 5. Furthermore, in radioimmunoelectrophoresis the IgE antibodies in onchocerciasis patients sera recognized the allergens which were present in the crude extracts of *O. volvulus* and of *D. viteae* as revealed by radiolabelled anti-IgE (Fig. 6). The position of the electrophoretic band precipitated by the reaction between IgE antibodies present in onchocerciasis patients serum and *O. volvulus* antigens is clearly different from that shown by the reaction between IgE antibodies and *D. viteae* antigens. This indicates the presence of allergens specific for *O. volvulus*. One of the two bands revealed by radiolabelled anti-IgE in the reaction between onchocerciasis patient's serum and *D. viteae* antigens showed the identical electrophoretic position as that of a major band previously described by Capron *et al.* (1968) as band 8.

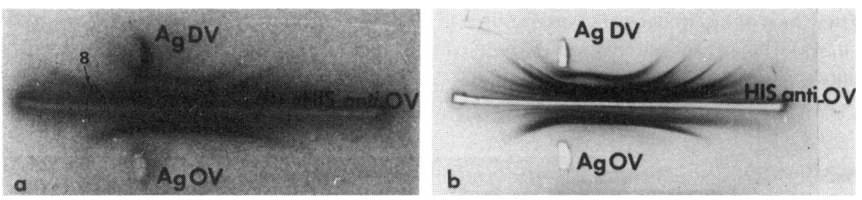

Fig. 6. Recognition of allergens in the crude extracts of *O.v.* and *D.v.* by IgE antibodies present in the sera from onchocerciasis patients. (a) radioimmunoelectrophoresis; (b) immunoelectrophoresis; His anti-*O.v.*, anti-*O.v.* hyperimmune rabbit serum, Ag *D.v.*, antigens from *D. viteae*; Ag *O.v.*, antigens from *O. volvulus*; *D.v.*-8, electrophoretic position of an arc previously identified as band 8.

DISCUSSION

Different techniques for measuring IgE have been described, single radial immunodiffusion (Rowe, 1969), double antibody radioimmunoassay (Gleich, Averbick & Swedlund, 1971), solid phase antibody radioimmunoassay (Bennich & Johansson, 1971) and enzyme linked immunosorbent (Hamilton *et al.*, 1981). However, solid phase (Sepharose) radioallergosorbent test (RAST) is now clearly established as the method of choice for serological determination of IgE antibody because of its high sensitivity, reproducibility and simplicity. The drawback of RAST is that it measures the activity of IgE antibodies which depends on both their concentration and their affinity, but not their quantity in weight. Unless sera have similar binding affinities, they would not be comparable in the RAST analyses. To circumvent this disadvantage several sera were titrated extensively. Parallel slopes were observed with individual serum indicating that the binding affinities were comparable and thus validating the use of a single reference serum for quantitating *O.v.* specific IgE (data not shown). Hussain *et al.* (1981) have reported similar results in their studies with *B. malayi* specific IgE.

The results of the present study show that total IgE was markedly increased in the sera obtained from patients who were parasitologically positive for *O. volvulus*. It should be stressed that onchocerciasis patients may be infected with other helminth parasites which are frequently present in onchocerciasis endemic areas of West Africa. Many helminths are potent stimulators for IgE production. The observation that people apparently without clinical or parasitological signs of *Onchocerca volvulus* infection had also raised total IgE level emphasize the importance of measurement of filaria specific IgE levels.

O. volvulus specific IgE levels were found to be raised in most of the patients (74%) but no correlation with the level of microfilaraemia was observed. The absence of filaria specific IgE in about 30% of patients sera raises the question whether some regulatory mechanisms are involved in the inhibition of production of parasite specific IgE antibodies in these individuals. Furthermore, it is not known whether these individuals were treated with drugs because following chemotherapy the level of IgE antibody may be decreased as has been observed with schistosomiasis (Ito *et al.*, 1972; Dessaint *et al.*, 1975). In the present test system low affinity IgE antibodies to onchocercal antigens may have been discarded and the IgE quantified would be of highest affinity antibodies to *O. volvulus*.

In addition, helminth infection may potentiate the existing reagin production against other antigens (Kojima & Ovary, 1975; Jarrett, Haig & Bazin, 1976). It is possible that following chemotherapy circulating parasite specific IgE antibodies will accumulate at the site of dying or dead microfilariae in the tissues enhancing inflammatory reaction which involve such inflammatory cells as basophils, eosinophils. The most remarkable observation in the present study was the presence of cross-reacting allergens in the crude extracts from adults of *O. volvulus* and of *D. viteae* which infect man and rodent respectively. This confirms the earlier report by Weiss *et al.* (1981) of high cross-reactivity of *D. viteae* crude extracts with IgE antibodies in onchocerciasis sera. The results of immunoabsorption experiments in which onchocerciasis patients sera were absorbed with immunosorbents prepared from antigens of *O. volvulus*, *D. viteae* clearly show that the reduction in IgE binding to *O. volvulus* was significant, using the *D. viteae* sorbent. The depletion was maximum when onchocerciasis sera were absorbed with homologous *O. volvulus* sorbent. This indicates the presence of allergens in somatic extracts from *O. volvulus* specific for this parasite. Indeed, the presence of a specific band revealed by radiolabelled anti-IgE in the reaction between onchocerciasis patient's serum and *D. viteae* antigens further supports this possibility.

The results of radioimmunoelectrophoresis strongly confirm the sharing of allergens between *O. volvulus* and *D. viteae* crude extracts. The pattern of electrophoretic precipitins indicates that ^{125}I-radiolabelled anti-IgE recognizes one of the major bands which was previously described as band '8' (Capron *et al.*, 1968) using *D. viteae* crude extracts as antigen and onchocerciasis patients serum as antisera. The presence of common allergens in crude extracts from *O. volvulus* and *D. viteae* has stimulated further research in our laboratory for isolation and purification of relevant allergens from crude extracts of *D. viteae* which may be useful in the detection of *O. volvulus* specific IgE. Such an approach is of relevance if one considers the difficulties in obtaining *O. volvulus* antigens from infected humans from endemic areas.

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