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Unique recognition of a low molecular weight *Onchocerca volvulus* antigen by IgG3 antibodies in chronic hyper-reactive oncho-dermatitis (Sowda)

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SUMMARY

Individual human Ig class responses to *Onchocerca volvulus* antigens have been evaluated by Western blotting using sera from cases of generalized onchocerciasis and chronic hyper-reactive onchocerciasis (Sowda). in all cases except IgG3 the patterns of recognition by human antibody classes were similar in Sowda and generalized onchocerciasis. Weak or undetectable responses were seen with IgG1, IgG2 and IgM. The total profiles of antigens recognized by the other Ig classes were different, although in some cases certain bands were commonly identified. The result with IgG3, however, was striking. Here, two major antigens (9 kD and 72kD) were recognized by IgG3 antibodies in Sowda sera but not generalized onchocerciasis sera. Furthermore, these two antigens were not recognised by any other Ig class, either in generalized or Sowda onchocerciasis, nor were they detected by antibodies of any class present in a collection of sera representative of other nematode infections. This difference in the IgG3 response was so pronounced that Sowda sera could be distinguished from generalized onchocerciasis sera by an IgG3-specific ELISA assay with a PBS parasite extract as the antigen. Thus, a correlation has been established between one particular clinical condition of onchocerciasis (Sowda) and a serological response, defined in terms of both the parasite antigens and an immunoglobulin class restricted antibody response.

Keywords antigen, antibody isotypes, *Onchocerca volvulus* Sowda

INTRODUCTION

In common with many nematode infections, onchocerciasis is rarely fatal and infected individuals may show only few and minor signs and symptoms of disease. It is however frequently debilitating due to pathological changes occurring in the skin and the eyes, largely as a result of inflammatory reactions provoked by microfilariae (Bryceson, 1976; Gibson *et al.*, 1976; Connor, 1979; Henson, Mackenzie & Spector, 1979; Gibson, Hekkie & Connor, 1980; Piessens & Mackenzie, 1982; Connor, George & Gibson, 1985; Mackenzie *et al.*, 1985). As in many parasitic infections, then, the interaction between the host's immune system and the parasite, far from eliminating the problem, may indeed exacerbate it. A study of the immune status, both serological and cellular in infected patients, might provide clues as to the basic underlying mechanisms responsible for these inflammatory responses.

In this study we have examined individual human Ig class responses to *Onchocerca volvulus* antigens from patients with generalized onchocerciasis or with chronic hyper-reactive onchodermatitis (also known as localized onchocerciasis or Sowda). In Sowda, there is a well characterized localized dermal and lymphatic pathology associated with low skin microfilarial density and humoral and cellular hyperimmune responsiveness (Bartlett, *et al.*, 1978; Gibson *et al.*, 1976; Buttner *et al.*, 1982; Lucius *et al.*, 1986; Brattig *et al.*, 1987). The reverse is true of generalized onchocerciasis where microfilariae survive in greater numbers, possibly due to a lowered immune responsiveness (Greene, Fanning & Ellner, 1983; Mackenzie & Williams, 1985). Of particular interest was the observation of a selective recognition of two major parasite antigens by the IgG3 class in the Sowda patients.

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MATERIALS AND METHODS

Preparation and fractionation of parasites

Nodules of *O. volvulus* were excised from patients with generalized onchocerciasis of the forest type in Liberia. The related cattle parasite, *O. gibsoni*, was obtained from Dr D.B. Copeman in Australia. Worms were recovered by collagenase digestion (Schulz-Key, Albiez & Buttner, 1977). Males and females were separated by morphological criteria and microfilariae were obtained from chopped females.

A PBS extract of worms was prepared by homogenizing the worms in freshly prepared PBS, pH 7·4, with L-1-tosyl-amide-

2-phenylethyl-chloromethyl Ketone (TPCK) (50 μg/ml), *N-p*-tosyl-L-lysine-chloromethyl Ketone (TLCK) (50 μg/ml) and phenyl-methyl-sulphonyl fluoride (PMSF) (1 mM).

A total detergent soluble fraction was prepared by homogenizing entire worms in 10 mM tris-2% (w/v) sodium deoxycholate (DOC) containing TPCK, TLCK and PMSF, as given above. Both extracts were centrifuged (25 min, 20,000 *g*) and the supernatants were stored at -20°C.

The PBS extracts were used for sensitizing ELISA plates and the total detergent soluble fraction was used for immunoblot analysis.

Sera

The following sera were used in these investigations and *n* represents the number of individual sera comprising each pool. Normal human serum ($n=2$) was obtained from British subjects known not to have visited Africa or America. A serum pool from patients with all characteristics of Sowda was obtained from Taizz Province in the Yemen Arab Republic ($n=24$) (Buttner *et al.*, 1982), and Liberia ($n=24$) (Albiez *et al.*, 1985). Serum pools from patients with generalized onchocerciasis were obtained from Chiapas, Mexico ($n=40$), Venezuela ($n=80$), Burkino Faso ($n=27$), Liberia ($n=29$) and the Yemen ($n=20$).

Sera ($n=3$) from confirmed cases of *Loa loa* were obtained from the Hospital for Tropical Diseases, London. A serum pool ($n=10$) from people with microfilariae of *Brugia malayi* was obtained from Malaysia. A serum pool ($n=10$) from patients positive for *Wuchereria bancrofti* microfilariae, but negative for *Mansonella ozzardi,* was obtained from Blanchisense, Northern Trinidad.

A serum pool ($n=10$) from patients infected with *M. ozzardi* was obtained from Venezuela. Sera ($n=10$) from parasitologically positive case of *Trichuris, Ascaris* and hookworm were obtained from St. Lucia in the West Indies. All the samples from St. Lucia were from individuals who have had *Schistosoma mansoni.*

Individual sera were collected from patients with Sowda in Liberia, generalized onchocerciasis in Venezuela, *M. ozzardi* infections in Venezuela and *W. bancrofti* in Kenya.

Monoclonal antibodies to human immunoglobulins

Purified mouse monoclonal antibodies against human IgM (145-8), IgA (CH-EB 6-8), IgE (E-RB6-2), IgG3 (C3-8-80) and IgG4 (JDL) were prepared in the laboratories of M.D. Cooper, University of Alabama and labelled with ^{125}I to a specific activity of 10 μCi/μg, using chloramine T (Hunter & Greenwood, 1962).

Class-specific enzyme-linked immunosorbent assay (ELISA)

This was done according to standard procedures using Nunc microtitre plates sensitized with a PBS extract of *O. volvulus* females (20 μg/ml, diluted in PBS, 16 h).

After blocking with BSA (1% (w/v), 30 min), plates were developed by sequential applications of human sera (diluted 1/100 in PBS, 2h), purified monoclonal antibody to mouse human Ig classes (1 μg/ml in PBS, 2 h), goat anti-mouse Ig-phosphatase absorbed on a column of normal human Ig-Sepharose, and substrate (p-nitrophenyl phosphate, disodium salt, 1 mg/ml in 0·9 M diethanolamine pH 9.8).

After 30 min, the reaction was stopped by the addition of 50 μl 1N NaOH and the absorbance was read at 410 nm.

Polyacrylamide gel electrophoresis and Western blotting

Polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed using 5–20% (w/v) gradient gel slabs, made using Pharmacia equipment. The worm extracts were separated by SDS-PAGE, applying a concentration of 50 μg protein/cm of gel width. The separated proteins were electrophoretically transferred to nitrocellulose sheets (Western blotting) and antigens revealed by successive treatments with infected or normal human serum diluted 1:200 and ^{125}I-labelled, purified mouse monoclonal antibodies to human immunoglobulin classes (0·5 μCi/ml, 10·0 μCi/μg) (Towbin, Staehelin & Gordon, 1979). All dilutions and washes were conducted in PBS containing bovine haemoglobin at 30 mg/ml. Negative controls with normal human sera were always done, but they produced negligible background and so are omitted from the figures. The autoradiographs were exposed for 24 h, except for development with anti-IgE, which was done for 6 days.

RESULTS

In a first series of experiments the immunoblot technique was used to establish the individual human Ig isotype responses to *O. volvulus* antigens, using a Sowda serum pool (Fig. 1). Weak, or undetectable responses were seen in the IgM (Fig. 1a), IgG1 (Fig. 1c), and IgG2 (Fig. 1d) classes. The total profiles of antigens recognised by IgG3 (Fig. 1e), IgG4 (Fig. 1f), IgA (Fig. 1g) and IgE (Fig. 1h) antibodies were different, although in some cases some bands were commonly identified.

The survey was then extended by similar Ig class development of immunoblots treated with a variety of sera representative of generalized onchocerciasis. In all cases, except IgG3 antibodies, the patterns of recognition by human antibody classes were similar in Sowda and generalized onchocerciasis. The result with IgG3 however was striking. Here two major antigens (molecular weights 9 kD and 72 kD) were recognized by IgG3 antibodies in pools of serum from the Yemen (Fig. 2g) or Liberia (Fig. 2h), but not with pools of sera from generalized onchocerciasis (Fig. 2 b-f) or other nematode infections (Fig. 2i–k).

The difference observed was so dramatic that the same sera were tested in human Ig class-specific ELISA assays for IgG3 (Fig. 3) and IgG4 (Fig. 4) antibodies. Once again, the Sowda sera (Fig. 3 b,c) were easily distinguished from the other sera (Fig. 3 d–i) using IgG3 development, but not using the IgG4 development (Fig. 4). In both Fig. 3 (IgG3) and Fig. 4 (IgG4) the simultaneously performed total IgG ELISA values are included for comparison. As expected, these largely mirrored the results obtained with IgG4 and also demonstrated the familiar cross-reactions observed between nematodes when soluble antigen extracts are used as diagnostic tools.

An important question next addressed was the extent to which the 9 kD and 72 kD antigens were recognized by individual sera. This was examined by both immunoblot (Fig. 5) and IgG3-specific ELISA systems (Fig. 6). As can be seen, with the exception of two Sowda sera (Fig. 5b,i, 6e) which were taken from mild cases of Sowda, all other samples contained IgG3 antibodies to the 9 kD components and also scored positive in the ELISA assay. Antibodies of the IgG3 class were variably present to the 72 kD component (e.g. compare Fig. 5c with 5g).

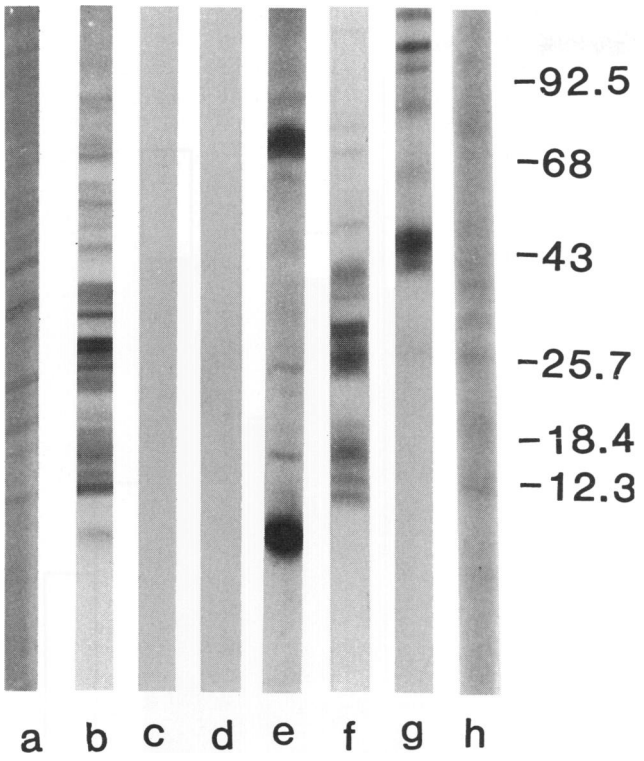

Fig. 1. Antigens in total detergent soluble extract of *O. volvulus* females recognized by different human immunoglobulin classes in Sowda serum pool. The extract was separated by SDS-PAGE, electrophoretically transferred to nitrocellulose paper, and the antigens were revealed by sequential treatment with the Sowda serum pool from the Yemen and ^{125}I-labelled mouse monoclonal antibodies to (a) IgM, (b) IgG, (c) IgG1, (d) IgG2, (e) IgG3, (f) IgG4, (g) IgA and (h) IgE. The autoradiographs were exposed for 24 h, except for development with anti-IgE, which was done for 6 days. All the negative controls with normal human serum were done, and all were uniformly negative. One example, developed with anti-total IgG is given in Fig. 2a. Molecular weight markers ($\times 10^{-3}$) are inserted on the right of the figure. Details in materials and Methods.

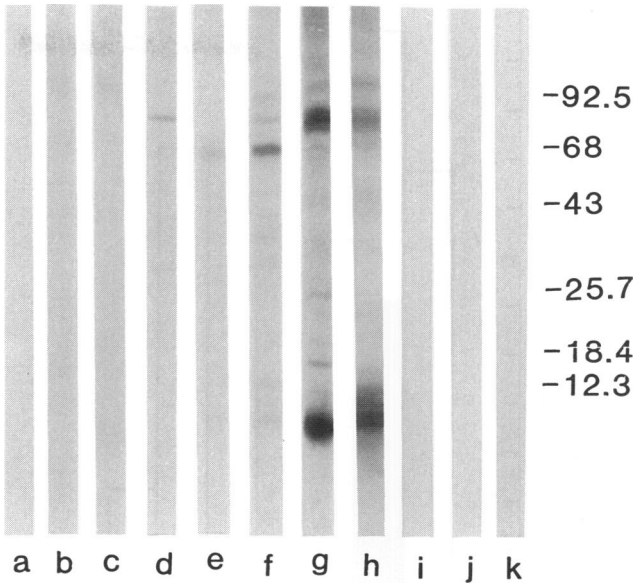

Fig. 2. Unique recognition of antigen in total detergent soluble extract of *O. volvulus* females by IgG3 antibodies present in Sowda sera. The extract was separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper. The antigens were revealed by treatment with pools of normal human serum (a), generalized onchocerciasis from Venezuela (b), Mexico (c), the Upper Volta (d), Liberia (e) and the Yemen (f), Sowda from the Yemen (g) and Liberia (h), intestinal nematode infections (i), *B. malayi* (j) and *W. bancrofti* (k). The bound human IgG3 antibodies were revealed by ^{125}I-labelled mouse MoAb anti-human IgG3. Molecular weight markers ($\times 10^{-3}$) are inserted on the right of the figure. Details as in Materials and Methods.

Finally, since Sowda is associated with low microfilariae densities in the skin, it was important to demonstrate the presence of 9 kD antigen in microfilariae as well as in adult worms. By Western blotting of *O. volvulus* microfilariae this proved to be the case (data not shown). In addition, the antigen was present in males as well as females of *O. gibsoni* and *O. volvulus* (data not shown).

DISCUSSION

The clinical spectrum in onchocerciasis is a consequence of the differences in individual immune responses. The morbidity of onchocerciasis, which is associated with dermatitis (pruritus, itching, papular eruptions) and blindness results from reactions to microfilariae (Bryceson, 1976; Gibson *et al.*, 1980; Mackenzie *et al.*, 1985; Connor *et al.*, 1985). Thus the onset of active immune recognition of the parasite is associated with high levels of pathological changes. Conversely, low expression of the immune responses to microfilariae is not characterized by disease exacerbation (Connor *et al.*, 1985; Bartlett *et al.*, 1978; Buttner *et al.*, 1982; Mackenzie *et al.*, 1985). A particularly good example here is the relationship between active, severe, localized dermatitis (Sowda) and high immune responses. Patients with Sowda show an active cell-mediated immune response detected by delayed hypersensitivity reactions (Bartlett *et al.*, 1978), a higher number of helper-inducer T cells and increased numbers of DR antigen-positive cells. Furthermore, high Ig levels, particularly IgG and IgE are observed (Lucius *et al.*, 1986; Brattig *et al.*, 1987).

The immune status, both cellular and serological, of infected patients representative of the disease spectrum seen in onchocerciasis could provide valuable clues as to mechanisms involved in the pathology. As was stated in the Introduction, the serological immune status of infected patients at the level of immunoglobulin class responses may have an important effect on the ultimate clinical outcome in infectious diseases. Thus, an essential first step towards the understanding and eventual immunological control of undesirable immunopathological reactions is the identification of the responsible parasite antigen-host antibody system(s). Unfortunately, to date, most serological investigations have used crude extracts and the few cellular studies have not investigated responses at the single cell level to defined antigens.

Recent studies utilizing biochemically defined antigens have begun to reveal correlations between expression of individual Ig isotype and the progression of the disease. In lymphatic filariasis in patients with elephantiasis for example, IgG1 and IgG3 antibodies preferentially react with a 68 kD somatic antigen of *Brugia malayi*. On the other hand, in patients with microfilaraemia, IgG1 and IgG3 antibodies largely recognize antigens with

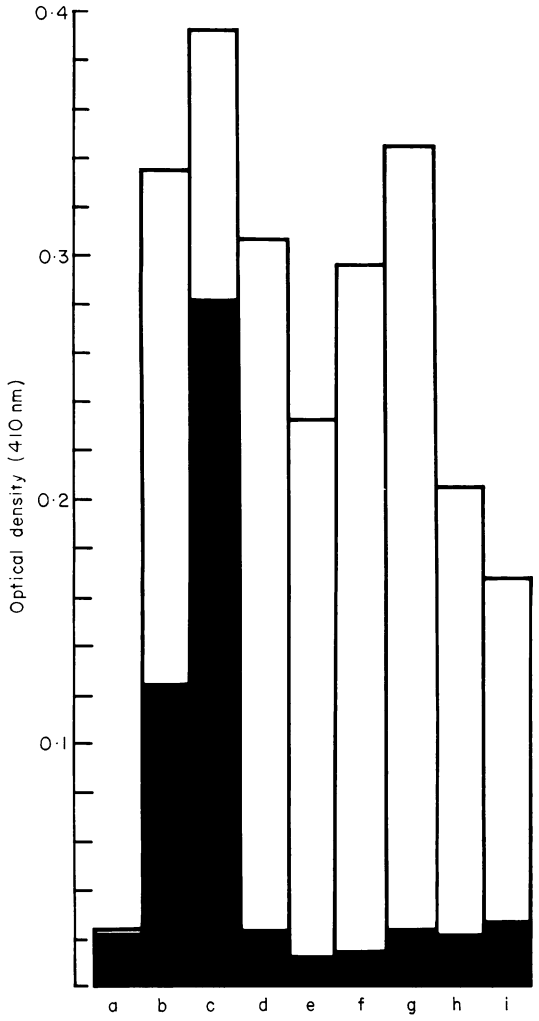

Fig. 3. Class specific ELISA assay for human antibodies to antigens present in a PBS extract of *O. volvulus* females. Microtitre plates were sensitized with the parasite extract and then probed with pools of normal human serum (a), Sowda from the Yemen (b) and Liberia (c), generalized onchocerciasis from Venezuela (d), Mexico (e) and Liberia (f), *B. malayi, W. bancrofti L. Loa* (g), *M. ozzardi* (h) and intesfinal nematode infections (i). The bound human antibodies were revealed by applying either a monoclonal mouse antibody to total IgG (□) or to IgG3 (■). The plate was developed finally with goat anti-mouse Ig-phosphatase and *p*-nitrophenyl phosphase. Details in Materials and Methods.

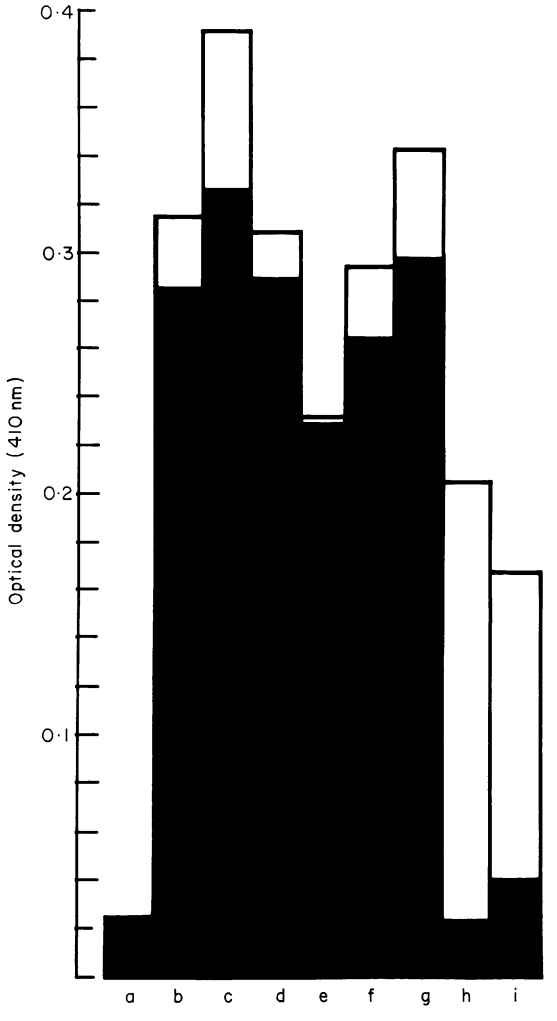

Fig. 4. Class specific ELISA assay for human antibodies present in a PBS extract of *O. volvulus* females. Details of the procedure and the sera used are exactly as in the legend to Fig. 3, except that here the plate was probed with monoclonal mouse antibodies to total IgG (□) or IgG4 (■).

molecular weights less than 68 kD. Similarly, in patients with chronic pathology (elephantiasis) there are low levels of IgG4 antibodies reacting with a polydisperse range of antigens, whereas the IgG4 response of microfilaraemia patients is more pronounced and tends to recognize antigens over a wider range of molecular weights (Hussain, Groge & Ottesen, 1987).

In this study, therefore, a systematic analysis has been made of the individual human Ig class responses to *Onchocerca* antigens from clinically characterized Sowda cases to electrophoretically transferred (immunoblotting), and thus defined, antigens of *O. volvulus*. Sera from generalized onchocerciasis patients were used as a baseline for comparison. A remarkable observation emerged. Sera from establised Sowda cases contained IgG3 antibodies to a high (72 kD) and low (9 kD) molecular weight antigen. These antigens were not recognized by antibodies of any other Ig class by the Sowda patients, nor were they detectable by antibodies of any class present in a collection of sera representative of generalized onchocerciasis and other nematode infections.

A number of other less dramatic observations were recorded. Although the different human Ig classes recognized a different total profile of *Onchocerca* antigens, IgG3 was the only class where the response in Sowda and generalized onchocerciasis clearly were different. Of the IgG subclasses, negligible responses were made by IgG1 and IgG2. The IgM antibody response was also low, presumably since sera were taken from established infections where primary or IgM immune responses would not be expected.

The uniqueness of the IgG3 response in Sowda was so pronounced that an IgG3-specific ELISA assay, using a crude PBS extract to sensitize the ELISA plates was able to provide a Sowda-specific signal. Sera representative of generalized onchocerciasis failed to yield a significant score in this assay.

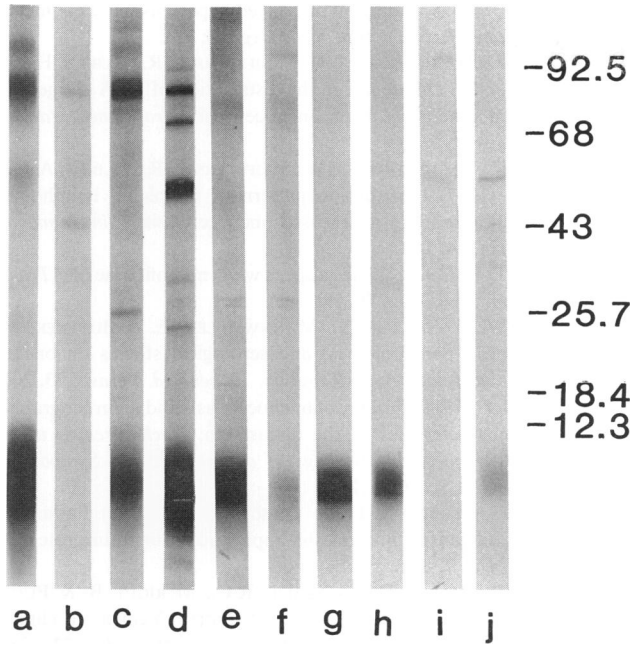

Fig. 5. Unique recognition of antigen in total detergent soluble extract of *O. volvulus* females by IgG3 antibodies present in individual Sowda sera. The parasite extract was separated by SDS-PAGE and electrophoretically transferred to nitrocellulose paper. The antigens were revealed by treatment with the Liberian Sowda pool (a) or individual sera from cases of Sowda (b–j). The bound human IgG3 antibodies were revealed by ^{125}I-labelled mouse monoclonal anti-human IgG3. Molecular weight markers ($\times 10^{-3}$) are inserted on the right of the figure. Details as in Materials and Methods.

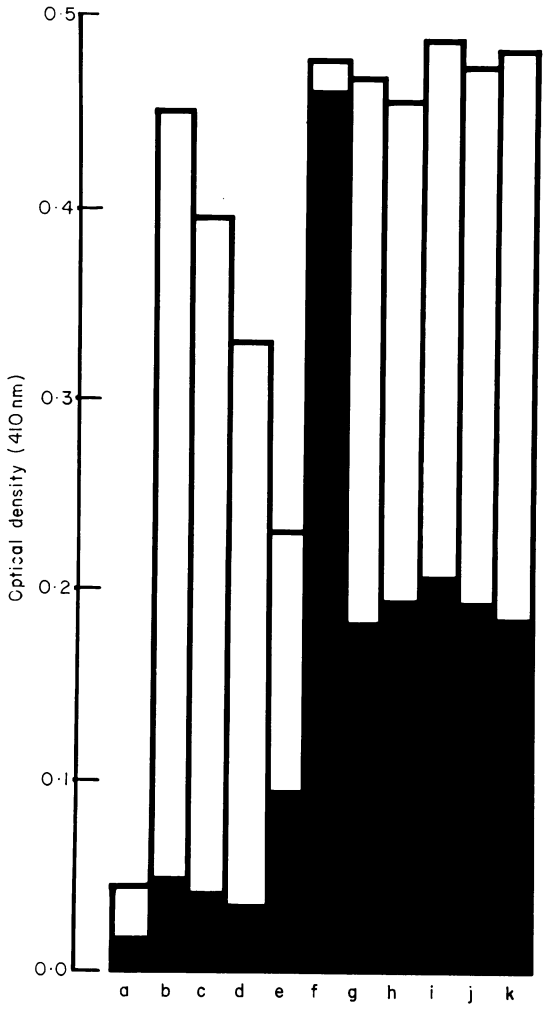

Fig. 6. Class-specific ELISA assay for human IgG3 antibodies to antigens present in PBS extract of *O. volvulus*. Analysis of individual sera. Microtitre plates were sensitized with the parasite extract and then probed with individual human sera from (a) a normal (b) generalised onchocerciasis, Venezuela, (c) *W. bancrofti* Kenya, (d) *M. ozzardi* and (e–k) Liberian Sowda. The bound total IgG (□) and IgG3 (■) antibodies were revealed by sequential application of mouse monoclonal anti-human Ig, goat anti-mouse Ig-phosphatase and *p*-nitrophenyl phosphate. Details in Materials and Methods. Serum samples e–k correspond exactly to those in positions b–h in Fig. 5.

This assay could be useful in endemic areas where the definition of Sowda cases may lack the feature of typical lesions. For example, in Southern Sudan some remarkably reactive severe dermatitis cases with low microfilariae counts do not show asymetrical lesions and/or enlarged lymph nodes typical of Sowda (J.F. Williams, personal communication). Buttner *et al.* (1982) pointed out that in Yemen the diagnosis of Sowda as a localized form of onchocerciasis is not always possible to confirm accurately because onchodermatitis and microfilaria are difficult to detect. Another important feature to consider in Sowda is that this condition may develop in a generalized form after several years, or after extirpation of the femoral node (Buttner *et al.*, 1982; Connor *et al.*, 1983).

The observation of a Sowda-specific IgG3 response to a Sowda-specific antigen, raises the question of the relevance of this antigen-antibody system to the disease state of Sowda. The role of T cells and the IgG3 subclass in the pathological expression of Sowda is not known although the IgG3 subclass is efficient in fixing complement and binding to platelets and monocytes (Turner, 1977; Schur, 1987; Shakib & Stanworth, 1980). High levels of IgG3 have also been reported in patients with farmer's lung (Shakib & Stanworth, 1980), lupus glomerulonephritis (Shur, 1987) and in patients with lymphatic filariasis with chronic obstructive pathology and elephantiasis (Hussain *et al.*, 1987). The common feature of these pathological conditions is the expression of tissue damage, probably mediated by IgG3 antibodies in type III immunological reactions.

In chronic lymphatic filariasis and in chronic severe onchodermatitis the role of immunocomplexes has been associated with tissue damage (Ottesen, 1980; Connor *et al.*, 1983; Greene *et al.*, 1983; Sisley *et al.*, 1987). Although in onchocerciasis this association is concluded from indirect evidence (rather than direct detection of complexes *in situ* in the affected tissue), it is tempting to propose that complement fixing IgG3 forms immunocomplexes with antigens from degenerating microfilariae. These complexes might then initiate a type III allergic reaction, accounting for the severe papular dermatitis and fibrinoid material observed in the upper dermis of patients with Sowda (Connor *et al.*, 1983; Albiez *et al.*, 1985). The antigen recognized by IgG3 antibodies was appropriately present in somatic extracts of microfilaria. Extracts from adult worms were also a source of the same antigen. Thus the Sowda-related

antibody-antigen system is not stage-specific. The IgG3 probably does not play a critical role in the immune recognition of microfilaria surfaces since Sowda serum antibodies did not bind to the surface of living microfilariae (Parkhouse, R.M.E. & Taylor, D. unpublished observations). The release of somatic antigens from degenerating or dead microfilariae therefore forms the basis for immunocomplex formation and subsequent complement activation and immunopathology

In Sowda the pronounced cellular immune response to microfilariae has been associated with high levels of IgE (Brattig *et al.*, 1987; Duke, 1987). Whether the reduction in the numbers of microfilariae depends on the granulocyte adherence promoted by a particular IgG subclass or IgE remains to be established.

With regard to protective immunity Sowda patients may express a partial immunity against infective larvae. The conclusions derive from the frequent occurrence of unilateral lesions of either one leg or one arm over the years in Sowda. Here then the presence of an infection clearly in one limb precludes establishment of the parasite in the other limb (Duke, 1987). This observation raises the possibility of cross-reacting protective antigens shared by microfilariae and infective larvae. An important implication for vaccine development is the desirability of elimination of infective larvae or microfilariae prior to arrival of microfilariae in the skin in order to avoid pathological complications.

In summary, the study presented here has established an immunochemical correlation between one particular clinical condition of onchocerciasis (Sowda) and a serological response, defined in terms of a unique recognition profile of *Onchocerca volvulus* antigen by one IgG subclass. Future work may define the relationship of these components to the pathology of the disease and, in addition, preliminary findings of genetic differences between generalized and localized forms of onchocerciasis (Brattig *et al.*, 1986).

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