Clin. exp. Immunol. (1992) **88**, 258–263

The profile of IgG and IgG subclasses of onchocerciasis patients

T. H. DAFA'ALLA*, H. W. GHALIB*†, A. ABDELMAGEED‡ & J. F. WILLIAMS§

**US NIH/Sudan Medical Parasitology Research Project, Khartoum, †Department of Microbiology, College of Medicine, University of Juba, Khartoum Centre, ‡Department of Zoology, Faculty of Science, University of Khartoum, Khartoum, Sudan, and §B-305 Clinical Center, Michigan State University, East Lansing, MI, USA*

(Accepted for publication 16 October 1991)

SUMMARY

In this study *Onchocerca gutturosa* was compared with *O. volvulus* in an ELISA test to detect *Onchocerca*-specific IgG and IgG subclasses. The test was developed and standardized to detect *Onchocerca*-specific IgG and IgG subclasses in sera of onchocerciasis patients and endemic controls. *Onchocerca volvulus* and *O. gutturosa* crude water-soluble antigens showed no significant difference in detecting onchocerca-specific IgG antibody ($T=1{\cdot}88$, $P>0{\cdot}05$). The levels of IgG subclasses varied greatly. IgG4 showed the highest detected mean level ($0{\cdot}84\pm0{\cdot}59$) and the other three subclasses showed considerably lower mean levels (IgG1 $=0{\cdot}27\pm0{\cdot}16$, IgG2 $=0{\cdot}24\pm0{\cdot}17$, IgG3 $=0{\cdot}28\pm0{\cdot}12$). The status and score of skin lesions were found to have significant effect on the IgG and IgG subclasses levels (all $P<0{\cdot}001$). IgG4 showed a positive correlation with the microfilarial (Mf) load ($r=0{\cdot}21$, $P<0{\cdot}03$). IgG3 levels have a significant negative correlation with the Mf load ($r=-0{\cdot}23$, $P<0{\cdot}02$). The biological significance of these IgG and IgG subclasses in onchocerciasis is discussed.

Keywords onchocerciasis *Onchocerca volvulus* *Onchocerca gutturosa* IgG IgG subclasses

INTRODUCTION

Onchocerciasis is one of the major tropical parasitic diseases and is a serious cause of morbidity in certain parts of the tropics. It infects over 30 million people with about 1 million blind cases [1]. The disease is endemic in several localities in the Sudan. It presents a spectrum of clinical manifestations ranging from asymptomatic individuals with no skin or eye lesions, to patients with severe disfiguring skin lesions and blindness [2,3].

The variability and severity of the clinical manifestations are probably due to the duration and intensity of infection, and to the degree and nature of the host immune response against the parasite. Clearance of the parasite was suggested to be mediated by an antibody-dependent cell cytotoxicity mechanism (ADCC) [4]. The isotypes involved in the ADCC and the immunopathogenesis of the disease have not been defined, and the parasite antigens responsible for eliciting these mechanisms have not been characterized.

The role of IgG subclasses in parasitic infections remains to be elucidated. In filariasis and schistosomiasis there is evidence of a predominance of IgG4 subclass [5,6]. An apparent association between a form of onchocerciasis identified as asymmetric hyper-reactive 'Sowda' type and enhanced serological reactivity of IgG3 was also observed [7].

In this study we used sera from well-characterized patients stratified in clinical groups based on the degree and severity of skin involvement. We developed an ELISA test to detect the onchocerca specific IgG and IgG subclasses and compared these responses in clinically and parasitologically defined groups of patients representing the spectrum of the disease. The data were analysed in order to find out the relationship, if any, between these levels and the degree of skin involvement and/or parasite load. The significance of the IgG isotype and its subclasses is discussed in relation to parasite load, parasite clearance mechanism and the immunopathology.

Correspondence: H. W. Ghalib, PO Box 1270, Khartoum, Sudan.

MATERIALS AND METHODS

Study population

The study population comprised 162 subjects from two onchocerciasis endemic foci in the Sudan (Gedarif and Abu Hamed) which were described previously by Ghalib *et al.* [8] and Williams *et al.* [9]. Sera from 57 uninfected controls were included from areas free of human filariasis. The study population was classified into: 'endemic positive' (EP), 'endemic negative' (EN) and '*Onchocerca* negative control' (ONC) groups. The EP group ($n=95$) consisted of subjects who were living in onchocerciasis endemic areas and were parasitologically positive. The EN group ($n=43$) consisted of subjects who were living in onchocerciasis endemic areas but were parasitologically negative and clinically showed no skin or eye lesions. The ONC group ($n=57$) consisted of four subgroups of subjects who all came from areas free of filariasis. The ONC group included

schistosomiasis, malaria, visceral leishmaniasis patients and normal Sudanese coming from an onchocerciasis-free area (Khartoum). These were all *Onchocerca* negative.

The following system was used to classify the patients according to sex, age (10–20, 21–30, 31–40, 41–50, 51–60 and >60 years) and the severity of skin lesions (no lesions = 0, mild lesions = 1, severe hyper-reactive generalized lesions = 2 and severe hyper-reactive localized lesions 'Sowda' = 3). The clinical scores were mainly based on a system of aggregate lesion intensity and distribution as described by Ghalib *et al.* [8].

Parasitological and clinical examinations

Demographic data were gathered and parasitological and clinical examinations were performed on all individuals from the endemic areas. Bilateral skin snips from the hip were obtained to determine the microfilarial (Mf) load, and palpation of nodules and complete dermatological examinations were performed to determine the status of each individual as described previously by Ghalib *et al.* [8].

Sera

Serum samples were collected from all the study population and kept frozen at −40°C until used.

Antigens

Adult *O. volvulus* worms were obtained from nodules removed surgically from Sudanese patients, released by the collagenase digestion method described by Schultz-Key *et al.* [10]. *Onchocerca gutturosa* adult worms were obtained by dissection from fresh connective tissues of the ligamentum nuchae of cattle slaughtered at Omdurman (Sudan) abattoir.

Crude water-soluble antigens were prepared from the collected worms by homogenization, sonication and centrifugation according to the method of Ngu [11]. The protein concentration of the water soluble antigens was determined by the Bradford method [12]. The crude water soluble antigens were then stored in aliquots at −70°C until used.

ELISA

The *O. volvulus*-reactive IgG and IgG subclasses levels of all the individuals included in this study were measured by ELISA. For the detection of the class IgG, *O. volvulus* and *O. gutturosa* water-soluble antigens were used while only the latter was used for the detection of the four IgG subclasses. The ELISA test was performed according to the method of Voller *et al.* [13]. Polystyrene microtitre flatbottom plates (Nunc-Immuno Plate I, Denmark) were used. The appropriate concentrations of antigens, sera, and anti-human serum probes were determined by checkerboard titration. The *O. volvulus* and *O. gutturosa* antigens were applied at concentrations of 4·42 and 1·25 μg/ml respectively (in carbonate–bicarbonate buffer, pH 9·6). The coating of the microtitre plate wells was carried out overnight at 4°C. One percent bovine serum albumin (BSA) (Sigma Chemical Co., St Louis, MO) was added (100 μl/well) to block the free sites of the microtitre plate wells. The plates were then incubated at 37°C for 1 h. All sera were tested in duplicates at a single dilution of 1:100 in PBS with 0·05% Tween 20. The plates were then incubated at 37°C for 1 h. A horseradish-peroxidase labelled goat anti-human IgG serum, Fc conjugated (Sigma) was used at a concentration of 1/3000 ml (in PBS and 0·05% Tween 20) and incubated at 37°C for 1 h. In the case of the IgG subclasses monoclonal mouse anti-human isotypes were used (anti-IgG1, M 895; anti-IgG2, A 012; anti-IgG3, M 631 and anti-IgG4, M 611; ICN, USA) at a concentration of 1/3000 ml (in PBS and 0·05% Tween 20) and incubated for 1 h at 37°C. For detecting the subclasses, peroxidase anti-mouse conjugate was used (E 803, ICN) at a concentration of 1/4000 ml (in PBS and 0·05% Tween 20) and incubated for 1 h at 37°C. *o*-Phenylene diamine dihydrochloride (OPD) (Sigma) substrate was used at a concentration of 0·4% (w/v) in citrate buffer (pH 5) with 0·01% (v/v) H_2O_2. The reaction was stopped by the addition of 20% sulphuric acid (100 μl/well). The period of substrate reaction was determined according to the rate of colour development. The rate varied in the different antibodies tested. Two limiting factors determined the substrate incubation period, the first to get an optical density (OD) within the range of the ELISA reader (0·00–2·20, OD) and the other to get the least background in the blank controls of the test. IgG was stopped after 15 min, IgG1, IgG2 and IgG3 after 30 min and IgG4 after 10 min.

The detected OD readings of all the samples were corrected to the set reference positive according to the method of Voller *et al.* [13]. A reference positive serum was used in all plates and only inter-assay variation of less than 10% was accepted [14,15]. The discrimination level between positive and negative individuals was set at the mean of normal controls plus two standard deviations from the mean [16].

Statistical analysis

Student's *t*-test (paired), Anova, Scheffe, χ^2 and correlation analyses were applied.

RESULTS

Comparison of O. volvulus *and* O. gutturosa *whole worm water-soluble antigens*

Figure 1 shows the mean IgG levels (OD) detected by *O. gutturosa* and *O. volvulus* antigens in the various age groups of the study population. No significant differences was detected in the recorded levels by either of the two antigens ($T = 1·88$, $P > 0·05$).

The detecting power of ELISA

As shown in Table 1, the ELISA positivity expressed as a percentage varied within the different antibodies in the various groups of the study population. In the EP group the percent positivity (ELISA sensitivity) is high, reaching 94·7% with IgG1 and IgG4. However, in this group no significant difference was observed in the percent positivity between any of the antibodies studied. The ONC group showed very low percent positivity (percent of false positives), ranging between 0% for IgG1 to 7·0% for IgG3. Within all the antibodies tested the EN group showed percent positivities higher than those recorded in the ONC group and lower than those recorded in the EP group. IgG1 recorded the highest percent positivity in this group (88·4%). This percent positivity was significantly higher than those recorded with the IgG ($\chi^2 = 25·3$, $P < 0·001$), IgG2 ($\chi^2 = 28·5$, $P < 0·001$), IgG3 ($\chi^2 = 20$, $P < 0·001$) and IgG4 ($\chi^2 = 11·9$, $P < 0·001$).

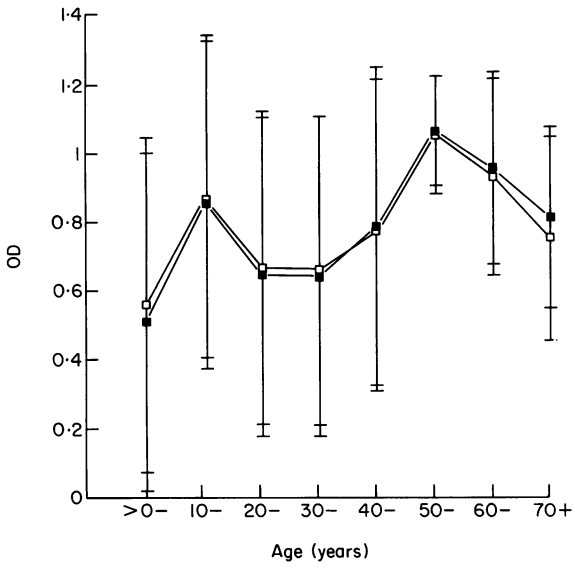

Fig. 1. Comparison of IgG responses in the different age groups detected by water soluble antigens of *Onchocerca volvulus* (■) and *O. gutturosa* (□) adult worms. Optical density read at 490 nm.

Table 1. Percent positivity* of the different study groups, and different negative control subgroups

Group	IgG	IgG1	IgG2	IgG3	IgG4	Total (*n*)
Endemic positives	92·6	94·7	89·5	91·6	94·7	95
Endemic negatives	34·9	88·4	32·6	41·9	55·8	43
Onchocerca negative controls	5·2	0	1·7	7·0	5·3	57
Total						219
Subgroups						
Schistosomiasis	7·7	0	0	7·7	7·7	13
Malaria	0	0	0	0	0	9
Leishmaniasis	7·7	0	7·7	15·4	15·4	13
Normals	4·5	0	0	4·5	0	22
Total						57

* Positive individuals were determined according to the ELISA cut-off values of 0·42, 0·02, 0·04, 0·09 and 0·08 OD for IgG, IgG1, IgG2, IgG3 and IgG4 respectively.

Antibody responses in the different study groups

As shown in Fig. 2, there were differences between the various groups in the levels of all the antibodies tested. All these differences were significant at the 0·05 level. The EP group had the highest mean antibody level for IgG, IgG1, IgG2, IgG3 and IgG4. The mean levels of IgG1, IgG2 and IgG3 within the EP group were lower than that of IgG4. The same was observed for the EN group where IgG4 was higher than IgG1, IgG2 and IgG3.

The antibody levels of the ONC subgroups are given in Table 2. The IgG, IgG1, IgG2 and IgG4 mean levels of schistosomiasis, leishmaniasis and malaria groups of patients were not significantly different from those of the normal control group. Two of the leishmaniasis patients had IgG3 levels relatively higher than the cut-off level (0·11 and 0·12 OD).

Relationship between antibody levels and sex, age and the degree of skin involvement (skin score)

In the EP group, significant differences due to sex or age were detected in the class IgG (due to sex, $F=7·01$, $P<0·01$) and IgG2 (due to age, $F=3·34$, $P<0·01$). No significant effect due to sex or age were observed in IgG1, IgG3 and IgG4. The degree of skin involvement showed significant relation with the antibody level of IgG ($F=9·84$, $P<0·001$), IgG1 ($F=10·02$, $P<0·001$), IgG2 ($F=16·12$, $P<0·001$), IgG3 ($F=13·67$, $P<0·001$) and IgG4 ($F=9·48$, $P<0·001$). As shown in Fig. 3, the levels of the different antibodies increased with the rank of severity of skin lesions. The onchocerciasis patients with severe generalized or localized hyper-reactivity skin lesions recorded the highest levels of all the antibodies tested. Table 3 shows the skin score groups between which significant differences were detected, at the 0·05 level (Scheffee test).

Relationship between antibody levels and Mf load

As shown in Table 4, there were inverse relationships between the Mf load and the levels of IgG, IgG1, IgG2 and IgG3, where these antibodies increase with the decrease of the Mf load. IgG4 showed a tendency to increase with the increase in Mf load. These relationships were statistically significant for IgG2, IgG3 and IgG4.

DISCUSSION

The only source of *O. volvulus* antigen for serological analysis is the worms obtained from nodules excised from onchocerciasis patients. The difficulty in obtaining large numbers of nodules or Mf from humans prompted us to search for an alternative source of antigen. The selection of *O. gutturosa* worm for this comparative study was based on: its high prevalence in the Sudanese cattle [17]; its capability of infecting man and developing skin nodules [18–20]; its usefulness in demonstrating antibodies by IHA and in producing skin reactions by intradermal test in onchocerciasis patients [21,22] and a high probability of cross-reactive epitopes with *O. volvulus* [23–26].

Our study showed that *O. volvulus* and *O. gutturosa* share enough antigenic similarities (common antigenic epitopes) for detection of IgG antibodies by enzyme immunoassays. There was no significant difference in the ability of *O. gutturosa* and *O. volvulus* water-soluble antigens to detect onchocerca specific antibodies. This conforms with the observations of Bradley *et al.* [27] who showed that *O. volvulus* and *O. gutturosa* share common antigens. The utilization of *O. gutturosa* antigens to detect the onchocerca specific antibodies remains adequate until new and more specific *O. volvulus* antigens are developed by recombinant DNA technology.

The onchocerciasis study areas (Sundus and Abu Hamed) were apparently free of other human filariasis [8,9], and the detected antibodies could well be related to *O. volvulus* exposure or infection.

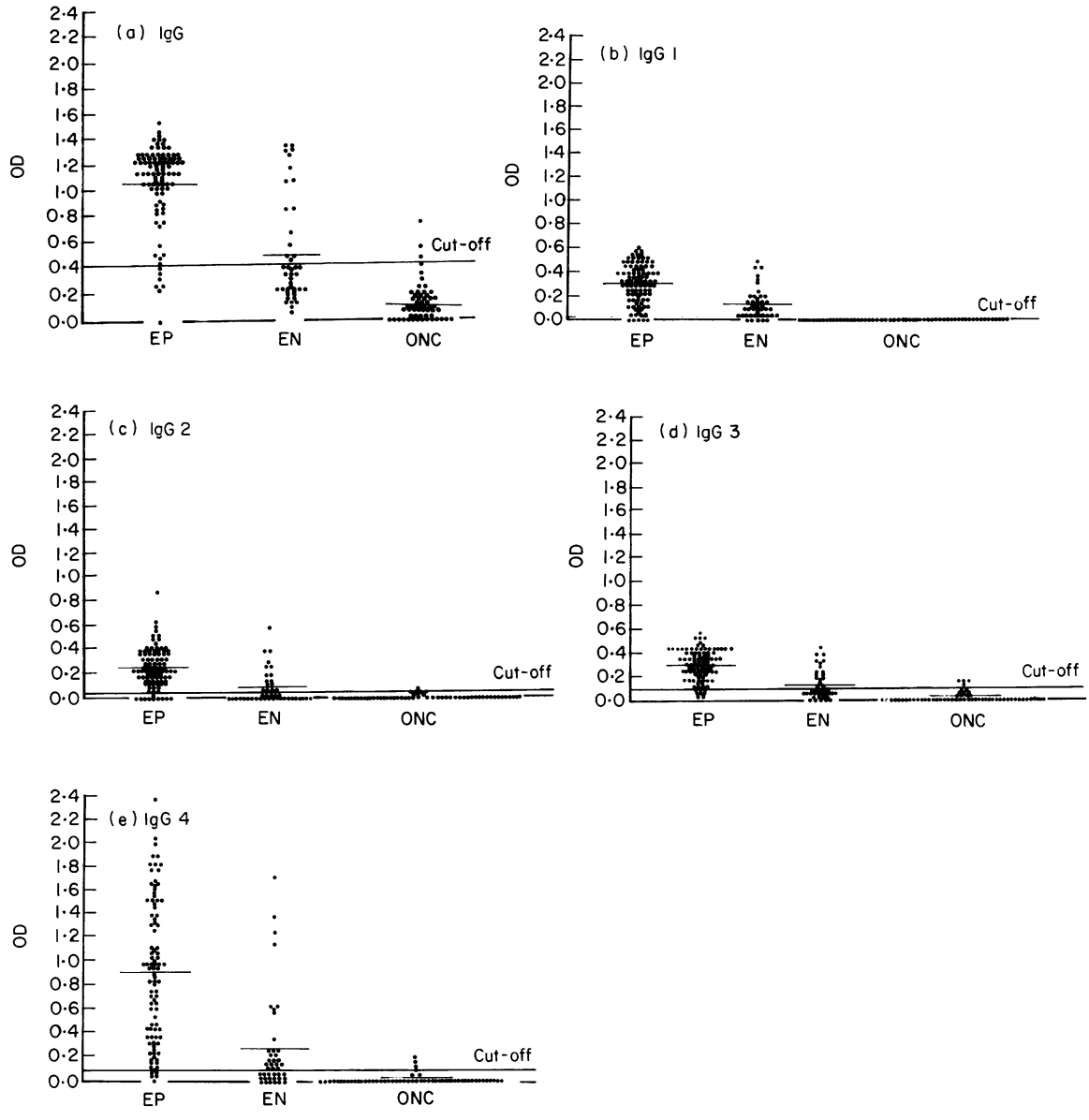

Fig. 2. Scatter diagrams of IgG and IgG subclasses in onchocerciasis endemic positives (EP), endemic negatives (EN) and uninfected controls (ONC) groups. Optical density read at 490 nm. The horizontal lines mark the arithmetic mean of each group.

Table 2. Mean antibody levels (±s.d.) of the different negative control subgroups

Antibody	Malaria	Schistosomiasis	Leishmaniasis	Onchocerca negative control
IgG	0·04±0·04	0·13±0·17	0·13±0·19	0·13±0·12
IgG1	0·00±0·00	0·00±0·00	0·00±0·00	0·01±0·01
IgG2	0·00±0·00	0·01±0·01	0·01±0·02	0·01±0·01
IgG3	0·00±0·00	0·01±0·03	0·04±0·04	0·01±0·03
IgG4	0·00±0·00	0·01±0·05	0·03±0·05	0·01±0·01

The common intercurrent parasitic infections in most of the onchocerciasis endemic areas in the Sudan are mainly malaria, leishmaniasis and schistosomiasis. We found that these infections do not seriously cross-react or interfere with the detection of *O. volvulus*-specific antibodies.

The ELISA percent positivity (sensitivity) in the EP group has almost the same level in all the antibody types and subtypes tested, and in the EN group varied with the antibody type. IgG1 had a significantly higher ELISA percent positivity (88·4%) than all the other antibodies. Since this antibody was 100% specific and did not show in any person of the ONC subgroups as false positive, it is justified to consider the recorded positives as real *O. volvulus*-infected cases that were in a prepatent stage or as seroconverters. Two out of the 13 visceral leishmaniasis patients used as controls had IgG3 levels higher than the cut-off

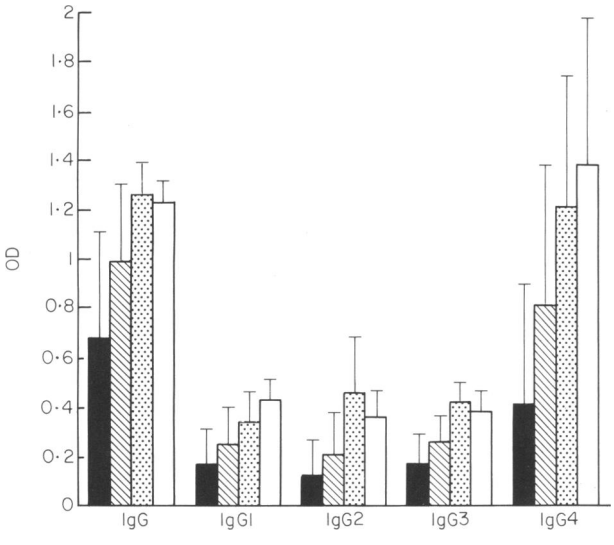

Fig. 3. Mean antibodies levels in the different subgroups of *O. volvulus* infected individuals. Group 0 = asymptomatic (■); group 1 = with mild lesions (▧); group 2 = with severe generalized hyper-reactive skin lesion (▨); group 3 = with severe localized hyper-reactive skin lesions (□). Optical density read at 490 nm.

Table 3. Differences (at 0·05 level) of IgG, IgG1, IgG2, IgG3 and IgG4 levels between groups of *O. volvulus* infected individuals ranked according to degree of skin lesions

Immunoglobulin	Detected variation
IgG	0 vs 1; 0 vs 2; 0 vs 3
IgG1	0 vs 1; 0 vs 2; 0 vs 3; 1 vs 3
IgG2	0 vs 1; 0 vs 2; 0 vs 3; 1 vs 2; 1 vs 3
IgG3	0 vs 1; 0 vs 2; 0 vs 3; 1 vs 2; 1 vs 3
IgG4	0 vs 1; 0 vs 2; 0 vs 3; 1 vs 3

0 = Asymptomatic infected individuals; 1 = patients with mild skin lesions; 2 = patients with severe generalized skin lesions and; 3 = patients with severe localized hyper-reactive skin lesions (Sowda).

The differences were detected by using Scheffee test.

Table 4. Correlation of microfilarial load with the levels of IgG and IgG subclasses in onchocerciasis endemic positives (EP) group

Immunoglobulin	Correlation coefficient (*r*)	Significance (*P*)
IgG	−0·07	0·24
IgG1	−0·16	0·07
IgG2	−0·21	0·02
IgG3	−0·28	<0·01
IgG4	0·18	0·04

−, negative correlation.

level. These two cases lived in an onchocerciasis endemic focus (Sundus, Gedarif) and again these levels could be *O. volvulus* specific.

In the three study groups the mean levels of the reactive antibodies varied significantly. The EP group had the highest levels of all the antibodies studied. The EN group had significantly higher levels of all the antibodies than the ONC group.

IgG4 significantly predominated the other IgG subclasses in the EP group. The predominance of IgG4 was observed previously in bancroftian filariasis and schistosomiasis [5,6].

Since sex and age may have an effect on the immune response of the individual, we tested the relationship between these factors and the onchocerca specific antibody levels. Sex showed a significant effect on the class IgG antibody level, but this effect was not detected in any of the four subclasses of this isotype. This implies that the effect was not real and may be due to a non-specific *O. volvulus* factor that interfered with the detection of the IgG level. The level of IgG2 was significantly different in the various age groups of the EP individuals. This finding could indicate an age-related effect on all the detected levels of IgG2. In turn all associations between this antibody and the clinical score or the parasitological findings in this study were subjected to this age-related effect. No effect due to sex or age was observed in IgG1, IgG3 and IgG4.

The clinical severity is directly related to the IgG and all the IgG subclasses response. These antibodies could be involved in the Mf clearance or they may have increased as a result of Mf destruction followed by increased antigenic stimulation or both. Microfilarial destruction is considered as a key event in the pathogenesis of onchocerciasis [28]. Since the Mf load varied in persons who have similar clinical symptoms and even though there was a direct relation between antibody level (of all the tested antibodies) and the degree of skin involvement (severity of skin lesions), we could not associate the levels of these antibodies with any protective or blocking activity.

Microfilarial load (live Mf/mg body wt) did not correlate positively with the antibody levels except for IgG4. The high IgG4 antibody level in individuals with high Mf load (as in the asymptomatic cases) may suggest a role for this isotype in blocking Mf clearance or destruction. On the other hand this suggests that the high IgG4 level in some patients with low Mf load may be induced by antigen release from live or dead worms or Mf. There could be a role for the subclass IgG4 in blocking antibody adherence to Mf and the ADCC mechanisms involved in Mf destruction. Hence this antibody may have the same binding pattern as IgE and was thought to play a role in obstructing the action of IgE-mediated immunopathology [29]. IgG3 and IgG2 showed negative correlation with the Mf load, which may suggest their involvement with the clearance of the Mf in the tissues leading to a significant drop in their circulation levels, but the variation due to age in IgG2 weakens its clear association with Mf destruction mechanisms.

The groups of onchocerciasis patients with severe generalized or localized (Sowda) skin lesions recorded the highest levels of antibodies. Sowda patients, even though low in Mf load, had high levels of IgG4 and were not deficient in antibody production. The reactive IgG3 level was not peculiar for Sowda patients since it was not significantly different from that of patients with severe generalized skin lesions. This does not conform with the observations of Cabrera *et al.* [26] who

reported a specifically high IgG3 in Sowda patients. These different observations may be due to ethnic variation between the study groups and/or the parasite genotype.

These subclasses and their immune modulation may be partly responsible for the variable spectrum of the disease and may all be regulated independently. Again the biological function of these subclasses vary greatly with their biochemical differences. Of particular importance is the high IgG1 and IgG3 in complement fixation and ADCC, and IgG4 being a poor complement fixer and not efficient in ADCC mechanisms. Delineation of the modulation in the level of these antibodies with the immunopathology is essential and would be possible in long term follow-up of onchocerciasis following chemotherapy.

Further work to define the exact function of each IgG subclass is of great importance. It is also of paramount importance to define the association of these antibodies with defined parasite-specific antigens.

ACKNOWLEDGMENTS

We thank Dr A. M. El-Hassan and Dr S. M. Sulaiman for reviewing the manuscript and their valuable suggestions. This work was supported by the USNIH/Sudan MRC Medical Parasitology Research Project, grant AI-16312.

REFERENCES

1 World Health Organization. Epidemiology of onchocerciasis. WHO Tech Rep Ser 1976; No. 597:1–94.
2 Salih SY. Clinical onchocerciasis; Dermatological and other presenting signs. Sud Med J 1985; **21**(Supplement):29–31.
3 El Sheikh HA, Ghalal I, O'Day J, *et al.* Ocular onchocerciasis in Sudan. Sud Med J 1985; **21**(Supplement):33–40.
4 Williams JF, Ghalib WH, Mackenzie CD, *et al.* Cell adherence to microfilariae of *Onchocerca volvulus*: a comparative study. Filariasis. Wiley, Chichester (Ciba Foundation Symposium 127) 1987:146–63.
5 Ottesen EA, Skvaril F, Tripathy SP, *et al.* Prominence of IgG4 in the IgG antibody response to human filariasis. J Immunol 1985; **134**(4):2707–12.
6 Jassim A, Hassan K, Catty D. Antibody isotypes in human schistosomiasis mansoni. Parasite Immunol 1987; **9**:627–50.
7 Cabrera Z, Buttner DW, Parkhouse RM. Unique recognition of a low molecular weight *Onchocerca volvulus* antigen by IgG3 antibodies in chronic hyper-reactive oncho-dermatitis (Sowda). Clin Exp Immunol 1988; **74**:223–9.
8 Ghalib HW, Mackenzie CD, Kron MA, *et al.* Severe onchocercal dermatitis in the Ethiopian border region of Sudan. Ann Trop Med Parasitol 1987; **81**(4):405–19.
9 Williams JF, Abu Yousif AH, Mallard M, *et al.* Onchocerciasis in Sudan: the Abu Hamed focus. Trans R Soc Trop Med Hyg 1985; **79**:464–8.
10 Schutz-Key H, Albiez EJ, Buttner DW. Isolation of living adult *Onchocerca volvulus* from nodules. Tropenmed Parasit 1977; **28**:428–30.
11 Ngu JL. Immunological studies on onchocerciasis. Varying skin hypersensitivity and leucocyte migration inhibition in clinical spectrum of the disease. Acta Trop (Basel) 1978; **35**(3):269–79.
12 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein-dye-binding. Anal Biochem 1976; **72**:248–54.
13 Voller A, Bidwell DE, Bartlett A. The Enzyme Linked Immunosorbent Assay (ELISA). A Guide with abstracts of microplate applications. Guernsey: Dynatech, Europe, 1979.
14 Denmark JR, Chessum BS. Standardization of ELISA and the detection of toxoplasma antibody. Med Lab Sci 1978; **35**:227–32.
15 Kurstak E. Progress in enzyme immunoassays: production of reagents, experimental design, and interpretation. Bull WHO 1985; **63**(4):793–811.
16 Richardson MD, Turner A, Warnock DW *et al.* Computer-assisted rapid enzyme-linked immunosorbent assay (ELISA) in the serological diagnosis of aspergillosis. J Immunol Methods 1983; **56**(2):201–7.
17 El Sinnary K. Studies on *Onchocerca gutturosa* (Neumann, 1910) in Sudanese and British cattle. PhD Thesis, University of London, 1981.
18 Collins RC. Onchocerciasis of horses in southeastern Louisiana. J Parasitol 1973; **59**:1016–20.
19 Beaver PC, Horner GS, Bilos JZ. Zoonotic onchocercosis in a resident of Illinois and observations on the identification of *Onchocerca* species. Am J Trop Med Hyg 1974; **23**:595–607.
20 Ali-Khan Z. Tissue pathology and comparative microanatomy of Onchocerca species from Canada and the U.S.A. Ann Trop Med Parasitol 1977; **71**:469.
21 Mwaiko GL, Mkufya A. Demonstration of antibodies in people infected with *Onchocerca volvulus* using adult *Onchocerca gutturosa* antigen. East Afr Med J 1977; **54**(12):680–3.
22 Muller JC, Mitchel DW, Garcia-Monza GA, *et al.* Evaluation of a skin test for onchocerciasis in Guatemala. Am J Trop Med Hyg 1973; **22**:337–342.
23 Speiser F, Weiss N. Comparative evaluation of 7 helminth antigens in the enzyme-linked immunosorbent assay (ELISA). Experientia 1979; **35**:1512–4.
24 Speiser F. Application of the Enzyme-Linked Immunosorbent Assay (ELISA) for the diagnosis of filariasis and echinococcosis. Tropenmed Parasit 1980; **31**:459–66.
25 Weiss N, Speiser F, Hussain R. IgE antibodies in human onchocerciasis. Application of a newly developed radioallergosorbent test (RAST). Acta Trop 1981; **38**:353–362.
26 Cabrera Z, Parkhouse RM. Identification of antigens of *Onchocerca volvulus* and *Onchocerca gibsoni* for diagnostic use. Mol Biochem Parasitol 1986; **20**(3):225–31.
27 Bradley JE, Gregory WF, Bianco AE, *et al.* Biochemical and immunochemical characterization of a 20-kilodalton complex of surface-associated antigens from adult *Onchocerca gutturosa* filarial nematodes. Mol Biochem Parasitol 1989; **34**(3):197–208.
28 Ghalib HW, Mackenzie CD, Williams JF, *et al.* Immunology and the development of pathology in onchocerciasis; studies in Sudan. Sud Med J. 1985; **21**(Supplement):59–64.
29 Hussain R, Ottesen EA. IgE responses in human filariasis. IV. Parallel antigen recognition by IgE and IgG4 subclass antibodies. J Immunol 1986; **136**:1859–63.