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Ivermectin-facilitated immunity in onchocerciasis; activation of parasite-specific Th1-type responses with subclinical *Onchocerca volvulus* infection

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SUMMARY

The present study examined the quantitative and qualitative changes registered in the parasite-specific antibody response, cellular reactivity and cytokine production profile in onchocerciasis patients repeatedly treated with ivermectin over a period of 8 years. The densities of *Onchocerca volvulus* microfilariae (mf) in treated patients remained significantly reduced, whereas the number of permanently amicrofilaridermic patients (subclinical infection) increased with repeated treatments. *In vitro* cellular responses to *O. volvulus* antigen (OvAg) were highest ($P < 0{\cdot}01$) in untreated control individuals exposed to infection, but negative for mf of *O. volvulus* (endemic normals). Cellular reactivity in repeatedly treated patients was higher at 84 than at 36 months post initial treatment (p.i.t.); furthermore, the proliferative responses to OvAg, mycobacterial purified protein derivative (PPD) and streptococcal SL-O were greater ($P < 0{\cdot}05$) at 84 months p.i.t. in amicrofilaridermic than in microfilaria-positive onchocerciasis patients. In amicrofilaridermic patients such reactivity approached the magnitude observed in endemic normals. Peripheral blood mononuclear cells (PBMC) from patients and endemic normals produced equivalent amounts of IL-2, IL-4 and interferon-gamma (IFN-γ) in response to mitogenic stimulation with phytohaemagglutinin (PHA); in response to OvAg, however, significantly more IL-2 and IFN-γ were produced by PBMC from subclinical amicrofilaridermic patients or endemic normals than by mf-positive patients. OvAg-specific production of IL-4 by PBMC from treated patients was lower at 84 than at 36 months p.i.t. At three months p.i.t. the titres of circulating OvAg-specific IgG1–3 had increased ($P < 0{\cdot}05$), but they then continuously declined with repeated treatments. Only IgG1 and IgG4 bound to OvAg of mol. wt 2–12 kD at 1 month p.i.t., while recognition of OvAg of mol. wt 10–200 kD by IgG1, IgG2 and IgG4 reached a maximum intensity at 3–6 months p.i.t., with the overall intensity of binding to OvAg gradually weakening thereafter. These results suggest that onchocerciasis-associated immunosuppression is reversible following ivermectin-induced permanent clearance of microfilariae from the skin; and that a vigorous parasite-specific cellular reactivity and a sustained production of IL-2 and IFN-γ in amicrofilaridermic individuals may contribute to controlling *O. volvulus* infection.

Keywords onchocerciasis ivermectin cellular responses cytokines antibody production antigen recognition

INTRODUCTION

Filarial parasites are responsible for millions of chronic infections in humans with cutaneous, lymphatic and ophthalmologic manifestations [1,2]. The severity of chronic disease produced by the parasitic nematode *Onchocerca volvulus* varies widely, ranging from asymptomatic infection to cutaneous involvement and, most severely, to ophthalmologic pathology which may finally cause blindness. In West Africa, control of onchocerciasis by the Onchocerciasis Control Programme (OCP) relies on both vector eradication and mass treatment campaigns with ivermectin [3], the drug of choice for onchocerciasis treatment [4]. These efforts have indeed reduced the risk of infection and blindness [5,6]. Humans chronically infected with the filarial parasite *O. volvulus* demonstrate not only a prominent produc-

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tion of all subclasses of parasite-specific immunoglobulins [7,8], but also depressed cellular reactivity *in vitro* and deficient production of IL-2 in response to *O. volvulus*-specific antigenic (OvAg) stimulation [9–11]. Recent reports suggest that ivermectin temporarily eliminates microfilariae (mf) from the skin and also facilitates cellular immunity in treated patients [12–14]. However, whether onchocerciasis-associated immunosuppression in patients may gradually be converted into an effective immune response remains unanswered. Delayed cutaneous hypersensitivity responses and circulating lymphocyte subpopulations normalized after a single dose of ivermectin, leading to improved *in vitro* cellular reactivity to mitogenic stimulation and to augmented cellular production of several cytokines by peripheral blood mononuclear cells (PBMC) [14]. These changes appeared rather gradually, without inducing severe adverse reactions, but ivermectin-facilitated immune responses controlling microfilaridermia in infected individuals may only reach critical importance after several years. In this study, our observations suggest that parasite-specific cellular immunity of onchocerciasis patients underwent further substantial alterations following repeated treatment; and therefore therapy may be expected to contribute synergistically to effective control of microfilaridermia in already infected individuals, and to induce increased resistance to re-infection.

PATIENTS AND METHODS

Patients and control individuals

The study population was composed of three groups of onchocerciasis patients (33 females, 229 males), and one group of infection-free local control individuals. All patients came from an arboreal savanna area in central Togo where onchocerciasis is meso- to hyperendemic. In this area vector control by the OCP was started in 1988. Patients from Group 1 ($n=150$) were repeatedly treated with ivermectin as described by Heuschkel *et al.* [15]. Briefly, before each ivermectin treatment patients received a thorough physical, parasitological and ophthalmological examination, and the density of *O. volvulus* microfilariae was determined in skin biopsies (mf/mg skin) taken from the right and left calf, hip and shoulder. In 1985 patients from Group 1 received an initial treatment of either 100, 150 or 200 μg/kg ivermectin; 22 or 28 months post initial treatment (p.i.t.) these patients were treated again with the same dose, and were subsequently re-treated with 150 μg/kg at intervals of 12–18 months. The second group of patients (Group 2, $n=51$) initially received placebo, but these individuals were treated with ivermectin (150 μg/kg) at 22 months and subsequently in the same intervals as Group 1. Group 3, which was established in 1989, consisted of 61 previously untreated onchocerciasis patients who annually received ivermectin (150 μg/kg) [14]. A group of local individuals who were exposed to *O. volvulus* infective larvae—but who exhibited no microfilariae in skin biopsies and had had no previous anti-filarial therapy—will be referred to as 'endemic normals' [11].

Preparation of O. volvulus *antigen*

Onchocerca nodules containing adult *O. volvulus* were excised and adult female and male worms were isolated as described by Schulz-Key *et al.* [16]. The still living worms were washed in sterile PBS (pH 7·2–7·4) and snap frozen in liquid nitrogen. PBS-soluble OvAg for cell culture experiments was prepared as described by Greene *et al.* [9]. Briefly, a PBS-soluble antigen extract was prepared under sterile conditions on ice in a Ten Broeck tissue grinder (Fisher Scientific, Pittsburgh, PA), centrifuged at 4°C at 16 000 *g* for 45 min and filtered through a 0·2 μm filter. The protein concentration of this antigen preparation was determined by the Bradford assay (Biorad, München, Germany).

O. volvulus *antigen-specific ELISA*

From patients participating in this study serum samples were collected from before and until 84 months p.i.t. Levels of OvAg-specific IgG and their subclasses were determined by ELISA. Microtitre plates (Maxisorb Nunc, Wiesbaden, Germany) were coated with adult *O. volvulus*-derived antigen (OvAg, 6·4 μg/ml) in coating buffer (bicarbonate buffer pH 9·6) and left overnight at 4°C before being washed with PBS (pH 7·4) containing 0·4% Tween 20. The plates were then stored at −70°C until further use. Sera were diluted to 1:200 in PBS containing 0·4% Tween 20, 0·02% NaN_3 and 0·02% bovine serum albumin (BSA; Enzygnost AP; Behring, Marburg/Lehn, Germany) and 100 μl of each serum were then dispensed in duplicate in each well. After 2 h incubation at room temperature the plates were washed three times with PBS (as above). Total IgG antibodies were detected with alkaline phosphatase-conjugated mouse antibody against human IgG (Sigma, Deisenhofen, Germany; 1:5000); similarly, IgG subclass antibodies were detected with alkaline phosphatase-conjugated subclass-specific mouse MoAb (Zymed, San Francisco, CA; 1:500). After 2 h at room temperature, plates were washed as described above and substrate solution (Enzygnost AP, Behring) was added; the plates were then read at 405 nm in a BioTek (Winooski, VT) microplate autoreader. Both high- and low-antibody titre standard reference sera were included on each plate in order to obtain comparable values for each assay. All sera from one individual were tested in parallel.

SDS–PAGE and immunoblotting

Conventional SDS–PAGE was performed, with some modifications, as described by Lüder *et al.* [17] in a horizontal slab gel apparatus (Pharmacia-LKB, Freiburg, Germany). Antigen solutions were diluted with sample buffer (75 mM Tris-HCl pH 8·8, 4% SDS, 65 mM dithiothreitol and 12% glycerol), boiled for 5 min, and then resolved in a 7·5–17·5% T gradient gel with a 4·5% T stacking gel. For the resolution of very low molecular weight antigens, both a Tricine-SDS–PAGE system and a vertical slab gel unit (Hoefer, San Francisco, CA) were used as described previously [18]. The separated antigens were transferred electrophoretically to nitrocellulose (NC) membranes (Biotec-Fischer, Reiskirchen, Germany) using the semidry method as described by Kyhse-Andersen [19]. For multiple sample testing, NC membranes were cut into strips, placed into single incubation containers and saturated (by overnight incubation at 4°C) with 5% non-fat dry milk, 0·2% Tween-20 and 0·02% NaN_3 in TBS (0·9% NaCl, 10 mM Tris-HCl pH 7·4). After being washed three times in TTBS (0·05% Tween-20 in TBS) and TBS, NC strips were probed with 2 ml of a 1:200 dilution of human sera in incubation buffer (5% non-fat dry milk, 0·05% Tween-20, 0·02% NaN_3 in TBS) for 2 h at room temperature. After further washing (as above), strips were either incubated with alkaline phosphatase-conjugated anti-human IgG (γ chain-specific, 1:5000, Sigma) or with anti-human IgG

subclass-specific mouse MoAb (Zymed, 1:750) diluted in incubation buffer for 2 h. After final washing, antigen–antibody binding was visualized by adding a substrate solution which contained 0·33 mg/ml nitroblue tetrazolium chloride, 0·165 mg/ml 5 bromo-4 chloro-3 indoxyl phosphate, 5 mM $MgCl_2$ and 0·1 M NaCl in 0·1 M Tris-HCl pH 9·5.

Isolation of PBMC and cell culture experiments

Heparinized venous blood was collected from onchocerciasis patients and endemic controls, and PBMC were isolated by means of Ficoll–Hypaque (Pharmacia-LKB) density gradient centrifugation. Cell culture experiments were carried out as previously described [14,20]. PBMC were adjusted to 1×10^7/ml in RPMI (GIBCO, Eggenstein, Germany) supplemented with 25 mM HEPES buffer, 100 U/ml penicillin and 100 μg/ml streptomycin, 0·25 μg/ml amphotericin B; they were then immediately used for cytokine production or proliferation assays, or else were cryopreserved for further use. For proliferation assays, freshly isolated cells were seeded at 1×10^5/well in sterile round-bottomed 96-well microtitre plates (Costar, Fernwald, Germany). Cells were suspended in RPMI (as above) containing 10% fetal calf serum (FCS), and kept in 5% CO_2 at 37°C and saturated humidity. For mitogenic stimulation with phytohaemagglutinin (PHA; 1:100, GIBCO) and for antigenic stimulation with OvAg (3·5 μg/ml), streptolysin-O (1:50; Fisher) or mycobacterial purified protein derivative (PPD; 100 μg/ml, Lederle Laboratories) cultures were maintained for 3 or 5 days respectively. For the last 18 h 1 μCi of ^{3}H-thymidine was added; cells were then harvested on glass fibre filters (Wallac, Turku, Finland) and the incorporated radioactivity determined by scintillation spectroscopy (Beta Plate; LKB-Pharmacia). Data were expressed as mean values of triplicate cultures in ct/min minus baseline stimulation.

Cytokine assays

Cells were cultured at a concentration of $3·7 \times 10^6$/ml in RPMI (as above) supplemented with 1% heat-inactivated FCS, in the presence of either *O. volvulus*-derived antigen (3·5 μg/ml) or PHA (1:100; GIBCO) or streptolysin-O (1:50; Fisher) in 5% CO_2 at 37°C and saturated humidity. Culture supernatants were collected after 24 or 48 h and stored in liquid nitrogen. IL-2, IL-4 (Biermann, Bad Neuheim, Germany) or interferon-gamma (IFN-γ) (Holland Biotechnology, Leiden, The Netherlands) were quantified by ELISA. All assays were used as recommended by the manufacturer.

Statistical analysis

Statistical analyses were performed by either Student's *t*-test or the Mann–Whitney test. Analysis of proliferative reactivity and cytokine production was performed on logarithmically transformed data. Microfilarial densities are specified as geometric means.

RESULTS

Parasitological and clinical changes

In all three patient groups the density of microfilariae of *O. volvulus* in the skin decreased drastically after the first ivermectin treatment (Fig. 1), e.g. dropping in Group 1 from 48 mf/mg ($n = 150$) to 1 mf/mg (3 months after therapy); it then remained at 8% and 13% of the initial density at 12 and 34 months p.i.t.

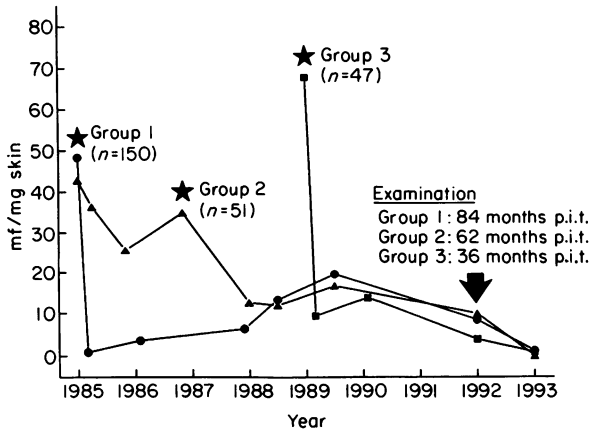

Fig. 1. Density of *Onchocerca volvulus* microfilariae (mf/mg skin) in onchocerciasis patients (Group 1, 2, 3) repeatedly treated with ivermectin. Patients were treated with ivermectin (★) and examined (↓) as described in Patients and Methods. p.i.t., Post initial treatment.

respectively [15]. In Group 1, at 42, 54, 84 and 96 months p.i.t., and before each further ivermectin treatment, microfilarial density was 13·2 mf/mg ($n = 105$), 19·5 mf/mg ($n = 107$), 8·5 mf/mg ($n = 37$) and 1·2 mf/mg ($n = 31$), respectively; during these follow-up examinations, the number of patients without microfilariae in skin biopsies increased from 10/105 to 11/107, 20/37 and 19/31, respectively. Patients negative for microfilariae at 84 months p.i.t. remained amicrofilaridermic at the follow-up examination at 96 months post initial treatment.

Table 1. Proliferative reactivity of peripheral blood mononuclear cells (PBMC) from microfilariae (mf)-positive and mf-negative onchocerciasis patients at 36 and 84 months post initial ivermectin therapy, and from untreated individuals exposed to *Onchocerca volvulus* infection (endemic normals) in response to stimulation with streptococcal antigen (SL-O, 1:50), mycobacterial antigen (PPD, 110 μg/ml) and *O. volvulus*-derived antigen (OvAg, 3·5 μg/ml)

Stimulation (months)	Patients		Endemic normals
	mf-positive	mf-negative	
SL-O			
36	51·5±8·6 (14)	49·1±8·2 (19)	
84	51·7±10·3 (12)	74·7±9·5 (15)*	87·6±2·0 (15)***
PPD			
36	3·1±0·9 (14)	1·9±0·5 (19)	
84	4·8±1·4 (10)	9·5±2·2 (12)**	7·9±2·0 (12)***
OvAg			
36	2·9±0·7 (14)	4·2±0·9 (19)	
84	4·6±1·4 (12)	10·5±2·7 (15)**	14·1±2·5 (15)***

Cellular responses at 36 months post initial treatment (p.i.t.) represent patients from Group 3, and responses at 84 months p.i.t. represent patients from Group 1. Data are indicated as mean (ct/min $\times 10^{-3}$) ± s.e.m. from the number of patients indicated in parentheses. Significantly different **($P < 0·01$), *($P < 0·05$) compared with 36 months (Group 3). Significantly different ***($P < 0·01$) from treated onchocerciasis patients, except for mf-negative patients at 84 months (Group 1).

Table 2. Production of IL-2, IL-4 and IFN-γ by peripheral blood mononuclear cells (PBMC) from microfilariae (mf)-positive and mf-negative onchocerciasis patients at 36 and 84 months post initial treatment (p.i.t.) with ivermectin, and from untreated individuals exposed to *Onchocerca volvulus* infection (endemic normals) in response to antigenic stimulation with *O. volvulus*-derived antigen (OvAg, 3·5 μg/ml)

	Patients		
OvAg-stimulation (months)	mf-positive	mf-negative	Endemic normals
IL-2 (pg/ml)			
36	3·5 ± 1·7 (9)	13·2 ± 4·5 (16)**	
84	4·6 ± 1·7 (9)	16·5 ± 10·8 (11)**	69·9 ± 27 (14)***
IL-4 (pg/ml)			
36	35·4 ± 17·4 (10)	60·3 ± 51·6 (12)*	
84	9·7 ± 7·5 (7)	32·0 ± 2·4 (6)**	39·3 ± 20·6 (15)
IFN-γ (pg/ml)			
36	26·6 ± 9·3 (11)	88·2 ± 60·3 (18)**	
84	59·2 ± 34·4 (7)	228·0 ± 76·6 (9)**	3683·7 ± 1713 (11)***

Cellular production of cytokines at 36 months p.i.t. represents patients from Group 3, and cytokines produced at 84 months p.i.t. represent patients from Group 1. Data are indicated as mean pg/ml ± s.d. from the number of patients indicated in parentheses.

Significantly different **($P < 0·01$), *($P < 0·05$) compared with mf-positive; significantly different ***($P < 0·01$) from treated onchocerciasis patients.

Cellular reactivity and cytokine production

Proliferative responses of freshly isolated PBMC were examined in repeatedly treated patients from Group 1 (84 months p.i.t.) and Group 3 (36 months p.i.t.), and these responses were then compared with a local group of untreated individuals exposed to *O. volvulus* infection but negative for microfilariae of *O. volvulus* (endemic normals) (Table 1). The cellular reactivity to OvAg was highest ($P < 0·01$) in endemic normals. In onchocerciasis patients from Group 1 and 3, OvAg-specific responses increased with repeated ivermectin treatments; furthermore, cellular responses to OvAg at 84 months p.i.t. were greater ($P < 0·05$) in mf-negative patients than in those with persisting microfilaridermia. Likewise at 84 months p.i.t., cellular responses to mycobacterial PPD and streptoccocal SL-O antigens were higher ($P < 0·05$) in mf-negative than in mf-positive patients, approaching the magnitude observed in endemic normals. Cellular responses to mitogenic stimulation with PHA were similar in all groups (data not shown).

Production of IL-2, IL-4 and IFN-γ by PBMC from treated patients (Groups 1 and 3) and from endemic normals was measured after stimulation with PHA or OvAg (Table 2). In response to PHA, the amounts of all three cytokines produced by cells from treated patients and endemic normals were similar (data not shown). However, PBMC from mf-positive individuals produced less IL-2 and IFN-γ in response to OvAg than was the case with cells from mf-negative patients or endemic normals ($P < 0·01$). At 84 months p.i.t., cells from mf-negative patients (Group 1) secreted three times more IL-2 and IFN-γ in response to OvAg than was the case with mf-positive individuals, while secretion was respectively four and 16 times higher in cells from endemic normals than in those from subclinical mf-negative patients. OvAg-specific production of IL-4 in Group 1 at 84 months p.i.t. was statistically lower than in Group 3 patients at 36 months p.i.t., while cells from mf-negative patients produced equivalent amounts of IL-4 to endemic normals.

Quantification of O. volvulus*-specific immunoglobulins*

After the initial ivermectin dose, significant increases in circulating OvAg-specific IgG1–3 subclasses were registered at 3 months after treatment, while parasite-specific IgG4 peaked at 6 months p.i.t. (Fig. 2a, Group 1). Such changes were not observed in patients from Group 2 who had received a placebo (Fig. 2b). Thereafter, levels of circulating *O. volvulus*-specific IgG1–3 antibodies gradually decreased in repeatedly treated patients (Group 1), registering statistically lower titres at 48 months p.i.t. than before initiation of therapy. In this group the OvAg-specific total IgG and IgG4 also declined, and were significantly reduced in patients at 84 months p.i.t. Circulating OvAg-specific immunoglobulins of all subclasses did not differ among patients who received repeatedly either 100, 150 or 200 μg/kg ivermectin. In subclinical amicrofilaridermic patients, circulating IgG of all subclasses were only slightly reduced compared with mf-positive individuals. At 84 months p.i.t., circulating OvAg-specific IgG of all subclasses were still clearly higher ($P < 0·01$) in repeatedly treated patients than in local control individuals (endemic normals) (Fig. 2c).

O. volvulus*-specific antigen recognition*

In untreated onchocerciasis patients a multitude of OvAg of mol. wt 10–200 kD were bound by circulating IgG subclasses, and within this mol. wt range the number of OvAg recognized by immunoglobulins did not change following repeated ivermectin treatment. However, stronger binding to OvAg by circulating IgG1, IgG2 and IgG4 was observed 3–6 months p.i.t.; thereafter the overall intensity of binding to OvAg by these IgG subclasses gradually weakened. In contrast, IgG3 continuously recognized OvAg with the same intensity until 48

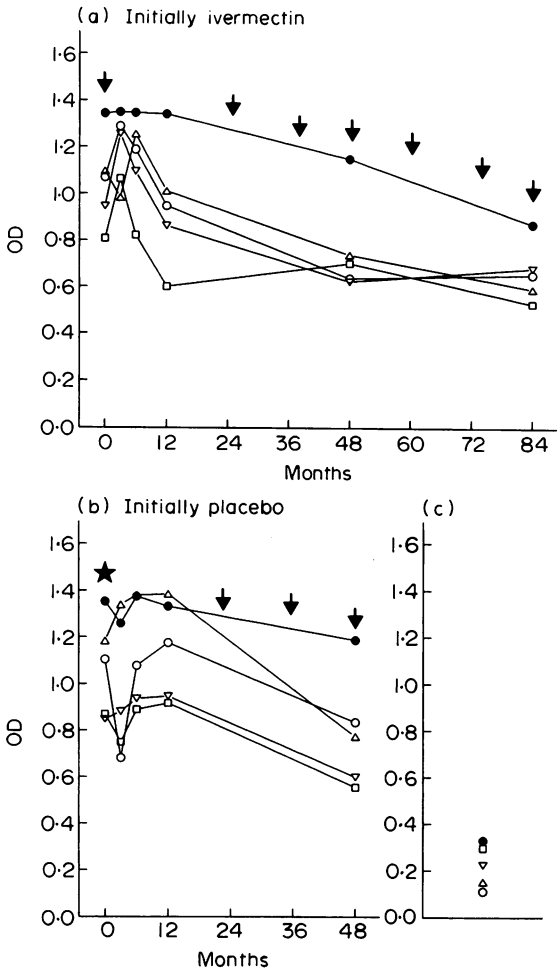

Fig. 2. Paired observations of the *Onchocerca volvulus* antigen (OvAg)-specific total IgG and IgG subclasses in (a) onchocerciasis patients (Group 1, $n=55$) repeatedly treated with ivermectin (⬇) (100–200 μg/kg), (b) onchocerciasis patients (Group 2, $n=21$) receiving placebo (★) at the beginning of this study, and (c) individuals ($n=15$) exposed to *O. volvulus* but without patent infection and without ivermectin treatment were determined in parallel. Titres of OvAg-specific IgG (●), IgG1 (○), IgG2 (▽), IgG3 (□) and IgG4 (△) were determined by ELISA. Patients were treated with ivermectin (↓) or placebo (★) as described in Patients and Methods.

months p.i.t. (data not shown). Four weeks after initial treatment, IgG1 and IgG4 bound increasingly to OvAg of very low mol. wt 2–12 kD; these low mol. wt OvAg had been resolved by Tricine-SDS-PAGE (Fig. 3). More than 15 low mol. wt OvAg in this mol. wt range were recognized by IgG1 and IgG4; the former were not bound by IgA, IgM, IgG2 and IgG3.

DISCUSSION

A single dose of ivermectin facilitated cellular immunity in patients chronically infected with *O. volvulus* [14]. Observations presented in this study suggest that repeated ivermectin treatment further supported cellular reactivity to *O. volvulus*-derived antigens and improved the production of IL-2 and IFN-γ, i.e. Th1-type cytokines. These changes occurred after the microfilariae of *O. volvulus* had permanently cleared from the skin, since diminished cellular reactivity and low production of IL-2 and IFN-γ by PBMC persisted in mf-positive patients. It was still noted, however, that both OvAg-specific cellular reactivity and cytokine production in endemic normal individuals were greater than in repeatedly treated and subclinical mf-negative patients. Circulating OvAg-specific immunoglobulins gradually decreased in repeatedly treated patients, indicating a diminishing microfilarial burden; whereas the serological recognition of *O. volvulus*-derived antigens underwent transient alteration only.

Putatively immune or endemic normal individuals have been described in cases of both lymphatic filariases [21] and onchocerciasis [22], who seem to have a successful immunologic defence against patent infection, i.e. a vigorous parasite-specific cellular reactivity together with a high production of IL-2 and IFN-γ. This fits in with our results which demonstrate a high OvAg-specific cellular reactivity and a sustained production of Th1-type cytokines in subclinical onchocerciasis patients as well as in endemic normals. Moreover, these observations indicate that onchocerciasis-associated immunosuppression [9] is reversible following ivermectin-induced clearance of microfilariae from the skin; and such immune responses may contribute to a lasting control of microfilaridermia. However, the presence of circulating antigen and parasite antigen-specific IgG4 in putatively immune individuals can be regarded as evidence of viable adult worms [23]. In the present study, the endemic normals had low OvAg-specific circulating IgG4 and were therefore considered to have a subclinical *O. volvulus* infection. Similarly, in experimental onchocerciasis of chimpanzees [20], a group of infected animals remained mf-negative (non-patent), demonstrating high cellular reactivity and sustained IL-2 production in response to OvAg. Moreover, increased specific cellular reactivity was measured in patent chimpanzees that had cleared *O. volvulus* microfilariae from the skin. Such clearance of microfilariae has also been observed in human lymphatic filariasis [24] and also in animal filariases [25,26]; but the underlying components and mechanisms, which may also induce immunopathology, still require identification.

In the onchocerciasis patients studied circulating anti-filarial IgG changed after ivermectin treatment in a similar manner to that observed in bancroftian filariasis [27]. The early increase of circulating IgG following initial reduction of microfilariae may indicate a surplus of parasite-specific IgG or transiently activated B cells. Thereafter antibody production diminished only gradually, due to the reduced microfilarial load but continuous stimulation of OvAg-specific B cells by few viable parasites. This is in contrast to antifilarial treatment with diethylcarbamazine (DEC), after which parasite-specific IgG declined more rapidly [27]—which may be due to a complete elimination of microfilariae. Following ivermectin treatment a portion of *O. volvulus* microfilariae could be shown to migrate into deeper layers of the skin [28]. Circulating IgG continuously recognized a multitude of OvAg, and no striking differences in antigen recognition became apparent within the mol. wts of 10–200 kD; however, several very low mol. wt OvAg (< 12 kD) were recognized by IgG1 and IgG4 shortly after initial treatment. The prompt and intense recognition of these OvAg, which were accessible to immune recognition only after therapy, may have primed memory T cells ($CD4^+CD45RO^+$) and activated specific cellular responses. Indeed, $CD4^+CD45RO^+$ T cells were increasingly found in treated patients [14]. The immunogenic potential

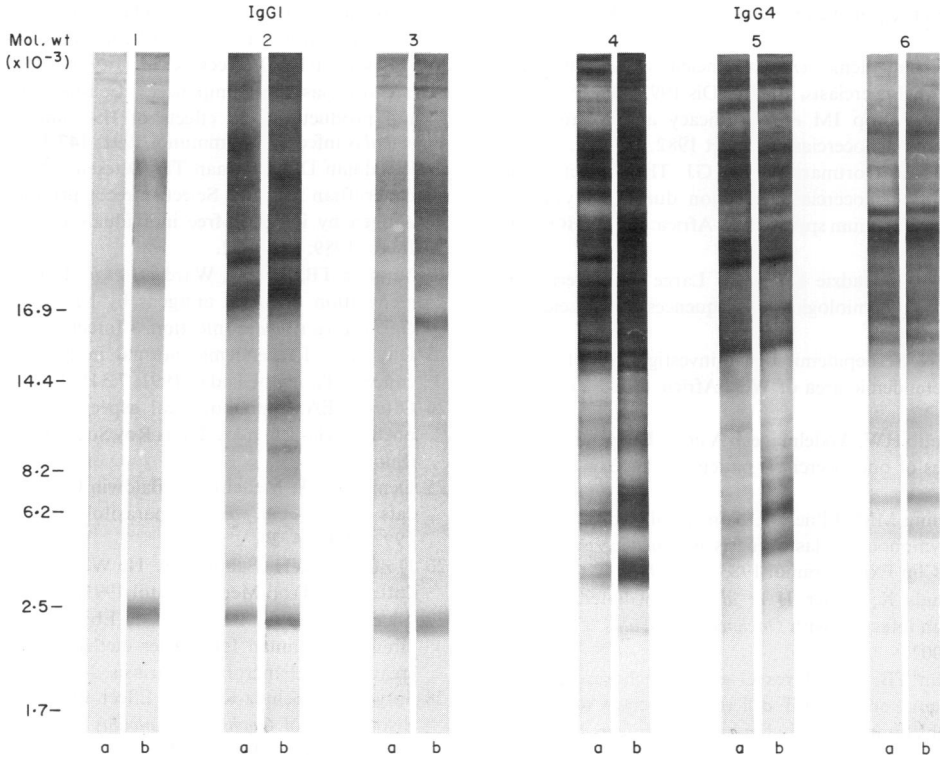

Fig. 3. Recognition of low molecular weight *Onchocerca volvulus* antigens (OvAg, mol. wt 2–12 kD) by IgG1 (lanes 1, 2, 3) and IgG4 (lanes 4, 5, 6) from onchocerciasis patients (Group 3) before (lane a) and 4 weeks after (lane b) initial treatment with 150 μg/kg ivermectin. Resolution of low mol. wt OvAg by Tricine-SDS–PAGE and immunoblotting was done as described in Patients and Methods.

of these low molecular weight OvAg is currently undergoing further investigation [29].

In human filariases, the mechanisms are not understood whereby long lasting immunity to infection, re-infection or overt disease is generated and maintained; but there is widespread agreement that effective immunity requires immunological memory. Recently it was suggested that T and B cell memory was short-lived and dependent on the persistence of antigen [30,31]; in onchocerciasis this would require constant exposure to infective larvae—or the presence of a few viable parasites—to maintain a state of active immunity. The present study was located within the vector controlled area of the OCP, where transmission of L3 of *O. volvulus* has been reduced but where risk of re-infection [32] and blindness [33] still remains high. However, diminished transmission of L3 may have affected parasite-specific immunity of patients. Despite application of these combined control measures, microfilaridermia still persisted in more than half of the patients several years p.i.t. Thus while ivermectin did not disrupt the reproductive capacity of adult *O. volvulus*, augmented OvAg-specific cellular reactivity as well as IL-2 and IFN-γ production in mf-negative (compared with mf-positive) onchocerciasis patients may have facilitated elimination of microfilariae from the skin. Usually, the chronicity of helminthic infections favours the expansion of Th2-type cells, i.e. production of IL-4 and IL-5, which induce IgE secretion and eosinophilia. On the other hand, the Th1-type responses are markedly reduced, i.e. they promote DTH, T cell proliferation and IL-2 and IFN-γ production [34,35]. Th1-type responses are likely to generate protective immunity to intracellular parasites [36], but the importance of Th1 *versus* Th2-type responses in helminthic infections remains controversial [37]. The activation of parasite-specific cellular reactivity, together with the predominance of Th1-type cytokines in both mf-negative onchocerciasis patients and endemic normals, suggest that such immunity could effectively operate against disease recrudescence. To what extent IFN-γ and IL-2 contribute to conferring beneficial immunosurveillance—as opposed to conferring immunopathology of the skin or inducing the granulomatous inflammation that surrounds adult *O. volvulus*—awaits further clarification.

In conclusion, our observations suggest that repeated treatment with ivermectin facilitated parasite-specific cellular immunity in onchocerciasis patients, and that this may reduce the serious morbidity of chronic *O. volvulus* infection.

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