Antigenicity and specificity of very low molecular weight *Onchocerca volvulus* polypeptides in the range 2.2–12.5 kD

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Summary

*Onchocerca volvulus* polypeptides in the molecular mass range of 2.2 to 12.5 kD were separated by Tricine-SDS-PAGE and the serological recognition of these very low molecular weight antigens (VLMW-OvAg) was then investigated by immuno-blotting. Sera from 21 onchocerciasis patients as well as from 53 individuals with other filariases were used to determine the sensitivity and specificity of detection of individual VLMW-OvAg. In onchocerciasis patients, up to 16 VLMW-OvAg were recognized predominantly by IgG1 and IgG4, while only few antigens were recognized by IgG2 and IgG3. The antigen recognition pattern varied individually, but 4 VLMW-OvAg of 8.6, 6.2, 5.4, and 5.1 kD, respectively, were bound by IgG4 from more than 90% of the onchocerciasis patients. Six VLMW-OvAg of 7.3, 5.8, 5.4, 4.0, 3.8, and 3.6 kD were recognized exclusively by IgG1 from onchocerciasis patients. In amicrofilaraemic filariasis patients with lymphatic pathology, a strong reactivity of IgG3 to an OvAg of 2.2 kD was observed, indicating a possible contribution of this antigen to the pathogenesis. In the molecular mass range below 13 kD, no specific carbohydrate residues or phosphorylcholine-containing (PC) determinants could be identified by lectin-blotting or PC-specific immunoblotting, respectively. Two-dimensional separation and immunoblotting distinctly resolved more than 40 antigenic polypeptides, the majority focusing at acidic isoelectric points. In *O. volvulus*-infected chimpanzees the IgG1- and IgG4-reactivity against OvAg below 13 kD appeared concurrently with onset of patent infection. These data suggest that some of these VLMW-OvAg might be associated with the production and release of microfilariae from gravid female worms as well as be involved in immune-mediated pathogenesis during filarial infections.

**keywords** *Onchocerca volvulus*, onchocerciasis, lymphatic filariasis, pathogenesis, very-low-molecular weight antigens, Tricine-SDS-PAGE, immunoblotting.

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Introduction

Reliable serodiagnosis of onchocerciasis has been substantially improved by the use of low-molecular weight antigens (Weiss & Karam 1989) and recombinant polypeptides (Lobos et al. 1991; Bradley et al. 1993). Low-molecular weight *Onchocerca volvulus* antigens (OvAg) proved to be more species-specific (Lobos & Weiss 1986), sensitive to detect early patent infection (Lobos et al. 1991) and useful for immunoenzymeological surveys (Weiss & Karam 1989). Previous studies have focused on antigens of 12–36 kD; this lower limit was predetermined by the restrictions imposed by common electrophoretic separation, i.e., the
system described by Laemmli (1970). Until recently, no suitable polyacrylamide-gel electrophoresis (PAGE) system for separating antigens of very low molecular masses was available that permitted them to be investigated for their immunological characteristics. We separated *O. volvulus* polypeptides with molecular masses below 13 kDa by Tricine-SDS-PAGE (Schägger & Jagow 1987); in combination with isoelectric focusing and immunoblotting, we were then able to demonstrate their immunological relevance as well as their diagnostic potential.

**Material and methods**

**Study population**

Twenty-one onchocerciasis patients (age range 16–60 years), found to be positive for microfilariae (mf) of *O. volvulus* in skin-snips (range 2–333 mf per mg skin), volunteered to participate in this study. All patients came from an arboreal savannah area in central Togo, Africa. Patients were negative for blood-dwelling microfilariae of *Mansonella perstans*, as determined by blood filtration (Soboslay et al. 1992a). Each patient received a thorough clinical examination, including differential and total blood cell counts, and ophthalmological examination. Stool samples were examined for intestinal parasites. Six residents from Lomé and Sokodé (Togo) who were negative for microfilariae of *O. volvulus*, volunteered as local controls. Three individual sera and 1 serum pool (all US inhabitants) were used as non-endemic controls. All participants included in this study were HIV-1 and HIV-2 antibody-negative as determined by IgG ELISA. Additionally, 53 sera from patients with filariasis other than onchocerciasis were kindly donated by the WHO Filariasis Serum Bank (Prof. N. Weiss, Basle, Switzerland), Prof. R. Lucius (Hohenheim, Germany), and Dr. H. Kretschmer (Tübingen, Germany). Sera (numbers in brackets) were obtained from patients infected with *Wuchereria bancrofti* (11), *Brugia malayi* (23), *Mansonella perstans* (1) and patients concurrently infected with *L. loa* and *M. perstans* (3). Seven sera were from patients exhibiting lymphatic pathology but without microfilariae in peripheral blood. All sera from lymphatic filariasis patients came from Southeast-Asia (Philippines, Sri Lanka, Indonesia). Sera from chimpanzees experimentally infected with *O. volvulus* were collected as previously described (Soboslay et al. 1992).

**Antigen preparation**

Onchocercomata were surgically excised from Liberian onchocerciasis patients. Adult *O. volvulus* worms were isolated from nodules by the collagenase technique (Schulz-Key et al. 1977). Isolated live and motile adult worms were washed in sterile phosphate-buffered saline (PBS) (Sigma, Deisenhofen, FRG) at pH 7.2–7.4 and snap frozen in liquid nitrogen. Two batches of isolated worms were used for independent preparations of antigen. Briefly, PBS-soluble extracts were prepared under sterile conditions on ice in a Ten-Broeck tissue grinder (Fisher-Scientific, Orangeburg, USA) and then centrifuged at 20,000 g for 90 min at 4°C. The supernatants were filtered through a 0.2 μm filter (Millipore, Bedford, USA) and the protein concentration determined as described by Bradford (1976). Aliquots of the antigen preparations were stored at −80°C until further use.

**Electrophoresis**

**Gel preparation**

The separation of polypeptides migrating in the molecular mass range of 14–200 kDa was accomplished by a vertical SDS-PAGE system with a linear gradient of 10–17% T (Total monomer concentration) and 2.6% C (% of total monomer contributed by the crosslinker), as described by Laemmli (1970). Polypeptides in the molecular mass range of 1.7–30 kDa were separated by the Tricine-SDS-PAGE system, without urea, as described by Schägger & von Jagow (1987). The slab gels (1.1mm thick, 120mm broad) consisted of three sections: a 2cm stacking gel (4% T, 2.6% C), a 2cm spacer gel (10% T, 2.6% C) and an 8cm separating gel (16.5% T, 6% C). The relative molecular mass (Mr) of the OvAg polypeptides was determined by parallel separation of molecular mass standards (PMW marker kit 1.7–16.9 kDa, Pharmacia, Freiburg, FRG and Dalton Mark IV marker kit 14.3–66 kDa, Sigma Deisenhofen, FRG).

**Sample preparation**

For conventional Glycine-SDS-PAGE, the antigen samples were boiled for 5min in a double volume of sample buffer containing 10% (v/v) glycerol (87%), 5% (v/v) 2-mercaptoethanol (2-ME), 3% (w/v) SDS, 0.01%
(w/v) bromophenol blue, 0.5 M Tris-HCl at pH 6.8, as well as 1% stock solution A and 1% stock solution B. Stock solution A contained 0.01 M EDTA, 0.01 M benzamidine and 0.01 M amino-capronic acid. Stock solution B contained 0.01 M phenylmethylsulphonyl fluoride (PMSF) in ethanol. For Tricine-SDS-PAGE, the samples were boiled for 5 min in a double volume of buffer containing 10% glycercol (87%), 1% SDS, 8 M urea, 1% 2-ME, 0.01 M phosphoric acid and 0.01% bromophenol blue dye; the pH was then adjusted with Tris to pH 6.8.

**Two-dimensional gel electrophoresis**

Isoelectric focusing (IEF) was performed in a horizontal slab gel system (Pharmacia Freiburg, FRG) using ampholines ranging from pH 3.5 to 9.5, as described by Görg et al. (1980). The gel contained 8M urea with no detergents and was run at 200 V for 4 h. The IEF-separated samples were equilibrated in Tricine-SDS-PAGE sample buffer for 3 min at 80°C, then for 5 min in the same buffer containing 150 M M Dodecamethamide. Subsequently, the samples were embedded in 1% agarose and 30% sample buffer, loaded onto a 16.5% T Tricine-SDS-PAGE gel, and polypeptides were separated as described above.

**Staining and destaining**

Coomassie-staining was undertaken for 40 min in 0.25% (w/v) Coomassie G250, 10% acetic acid and 50% methanol. Destaining was accomplished in 5% acetic acid and 10% methanol. Silver staining of proteins was performed as described by Oakley et al. (1980); while the method of Tsai & Frasch (1982) was used to stain proteins and lipopolysaccharides. The staining of sugar residues by periodic acid-Schiff’s reagent (PAS) was performed as described by Kapitany & Zebrowski (1973).

**Immunoblotting**

Separated proteins were transferred onto nitro-cellulose (NC) according to the semidyrid method of Kyhse-Anderson (1984) employing a discontinuous buffer system. For gradient gels of 10–17% T and 2.6% C a nitro-cellulose (NC) membrane of 0.45mm pore size (Biotec-Fischer Reisenkirchen, FRG) was used. Blotting time was 2.5 h at constant 0.9 mA/cm². For Tricine-SDS-PAGE gels (16.5% T, 6% C) an NC membrane (Biotec-Fischer, Reisenkirchen, FRG with a higher matrix density (0.22 mm pore size) was employed. Blotting time was 2.5 h at constant 2.0 mA/cm². For multiple sample testing the membranes were cut into 3.5mm strips, placed into single-tray incubation containers and rinsed in Tris-buffered saline (TBS; 0.9% NaCl, 10 mM Tris-HCl, pH 7.4). Non-specific binding sites were blocked by 5% bovine serum albumin (BSA)/0.05% Tween 20 in TBS for 2 h at room temperature (RT). Strips were then washed 3 × 5 min in TBS/0.05% Tween 20 prior to addition of serum diluted 1:100 in TBS/5% BSA/0.05% Tween 20. After 2.5 h at RT, strips were washed 3 × 5 min with TBS/0.05% Tween 20, followed by 3 × 5 min in TBS. Next, alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA antibodies (heavy chain-specific Sigma Deisenhofen, FRG) were diluted to 1:1000 in TBS/5% BSA/0.05% Tween 20. For the IgG subclasses, monoclonal antibodies (Zymed San Francisco, USA; clones HP6069 for IgG1, HP6002 for IgG2, HP6047 for IgG3, and HP6025 for IgG4) were diluted 1:830 in TBS/5% BSA/0.05% Tween 20. After 2 h of incubation, strips were washed as above, then substrate solution containing 0.33mg/ml nitroblue tetrazolium chloride (NBT), 0.165 mg/ml 5-bromo-4-chloro-3-indoxyl phosphate (BCIP), 5 mM MgCl₂ and 0.1 M NaCl in 0.1 M Tris-HCl, pH 9.5 was added for 30 min.

**Lectin blotting**

For lectin blotting, non-specific binding sites on NC-membrane strips were first blocked with TBS containing 5% BSA and 0.05% Tween 20 for 2 h. NC-strips were then washed (as above) and incubated for 4 h with one of the following horse radish peroxidase-conjugated lectins (Sigma Deisenhofen, FRG): wheat germ agglutinin (WGA; specific for α-D-glucosamine residues, Concanavalin A (Con A; specific for α-D-mannose, α-D-glucose, and D-fructose), Lotus tetragonolobus (Lotus; specific for α-L-fucose) and soybean agglutinin (SBA; specific for α-N-acetylgalactosamine and α-D-galactose). All lectins were used at a final concentration of 10 μg/ml in TBS. Then strips were washed (as above), substrate solution chloro-1-naphthol and H₂O₂ were added and, after colour development, the strips were rinsed in TBS. To show specificity of lectin binding, lectins were pre-incubated with inhibitory disaccharides: 5% N-acetylgalactosamine for WGA, 5% D-mannose for ConA, 1% L-fucose for Lotus, and 1% N-acetylgalactosamine for SBA.
Detection of phosphorylcholine (PC)-containing determinants

To detect PC-containing determinants, the *O. volvulus*-containing NC-strips were blocked and washed as described above. Then the PC-specific myeloma protein TEPC 15 (Sigma Deisenhofen, FRG) – diluted 1:1000 in TBS, 5% BSA and 0.05% Tween 20 – was added for 2.5 h. After washing, the NC-strips were incubated with alkaline phosphatase-conjugated anti-mouse kappa chain-specific antibody (Southern Biotechnology Birmingham, USA) diluted 1:1000 in TBS, 5% BSA and 0.05% Tween 20, followed by washing and incubation in substrate solution (NBT, BCIP, as above). A parallel control was performed with TEPC 15, pre-incubated with 1mM sodiumphosphoryl-choline chloride for 24 h.

Analysis of serological reactivity

Recognition of single VLMW-OvAg by antibodies from onchocerciasis patients is indicated by the percentage of positively reacting sera. Intensity of recognition of *O. volvulus* by antibodies was quantified visually as follows: vaguely visible (0), weakly visible (+), clearly visible (++), and strongly visible (+++). Specificity of single OvAg in the range 2.2–12.5 kD was calculated as the percentage of negatively reacting sera from patients with filariasis other than onchocerciasis.

Results

Separation of low-molecular weight OvAg

In conventional Glycine-SDS-PAGE the mobility of *O. volvulus*-derived antigens (OvAg) below 13 kD was not linear and separation was poor, as shown by the concurrent use of molecular mass standards. However, resolution improved with the Tricine-SDS-PAGE and the linearity of the relative mobility of the OvAg between 2.2–12.5 kD allowed a reliable determination of their molecular masses (Fig. 1, Lane A). Following Tricine-SDS-PAGE and Coomassie-staining, three major OvAg (12.5, 8.4, and 4.9 kD) and five minor bands of 9.9, 9.3, 7.4, 6.3, and 3.7 kD could be detected. More sensitive silver-staining visualized additional polypeptides of 10.6, 9.7, and 6.4 kD (Lane B). The incubation of the samples with 1% iodoacetamide for 15 min prior to PAGE did not change the banding pattern of VLMW-OvAg (Lane C). Comparison of the two antigen preparations revealed identical staining patterns (data not shown). Only one of the two *O. volvulus*-derived PBS-extracts was then used for the immunoblot analyses.

Carbohydrate and phosphorylcholine residues in VLMW-OvAg

Silver staining according to Tsai & Frasch (1982) visualized glycoproteins or lipopolysaccharides (Fig. 1, Lane D). Non-specific detection of carbohydrate residues by PAS revealed a diffuse staining in the molecular mass range of 1.5–2.2 kD (Lane E). However, specific detection of sugar moieties by lectin blotting was restricted to *O. volvulus* glycoproteins above 17 kD, dominated by strong Con A binding, while WGA
detected only few bands with lower intensity. Lotus and SBA did not show any remarkable binding. Reactivity to OvAg kD was inhibited by their corresponding sugars. The phosphorylcholine-binding myeloma protein TEPC 15 did not detect PC-conjugated OvAg below 12.5 kD, but OvAg of higher molecular masses were recognized. Again, pre-incubation with phosphorylcholine-chloride inhibited reactivity (data not shown).

Antigen recognition by sera of onchocerciasis patients

In contrast to IgM and IgA, which bound to OvAg only in the range of 15–200 kD (Fig. 2, Lanes A and B), total IgG (Lane C) and IgG subclasses (Lanes D–G) recognized several antigens below 13 kD. Although antigen recognition varied considerably between individual sera, some general features were evident (Fig. 3).

In all patients IgG4 reacted strongly not only to antigens in the range 14–200 kD but also to antigens below 13 kD (Fig. 3, Lane D). In some sera IgG1 also bound to several OvAg smaller than 13 kD, predominantly bands in the range 8.6 to 5.4 kD, and also weakly to an OvAg of 2.2 kD (Lane A). IgG2 recognized only few antigens below 13 kD (Lane B). In one third of the onchocerciasis patients IgG3 reacted strongly with a very-low-molecular weight OvAg of 2.2 kD (Lane C). However, there was no clear association with clinical status or microfilarial level of the patients. A positive correlation was found between the intensity of immunoblotted VLMW-OvAg (IgG4-reactivity) of different patients and the corresponding OD-values obtained by parasite-specific IgG4-ELISA using crude worm-extracts (p-value > 0.01; data not shown).

The serological reactivity of IgG1 and IgG4 to 16 OvAg in the range 2.2–12.5 kD is summarized in

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**Figure 2** Serological recognition of *O. volvulus* antigens (OvAg) in the molecular mass (Mr) range of 15–200 kD by immunoglobulins from a 40 year-old female onchocerciasis patient (39 mf per mg skin). The immunoblotting of OvAg was performed as described in Material and methods. Lanes represent serological reactivity by A, IgM; B, IgA; C, total IgG; D, IgG1; E, IgG2; F, IgG3; G, IgG4.

**Figure 3** Serological recognition of *O. volvulus* antigens (OvAg) in the molecular mass (Mr) range of 1.5–30 kD by immunoglobulins from a 40 year-old female onchocerciasis patient (39 mf per mg skin). Lanes indicate the serological reactivity by A, IgG1; B, IgG2; C, IgG3; D, IgG4.
Fig. 4. Compared with total IgG (not shown), the reactivity of IgG4 was more sensitive and also displayed better resolution, e.g., serum from an onchocerciasis patient found to be non-reactive against VLMW-OvAg by total IgG showed distinct recognition of two OvAg of 5.1 and 5.4 kD by IgG4. The OvAg of 5.4 kD was recognized by 20 of 21 onchocerciasis sera, i.e., the sensitivity of detection was 95%. In 2 of 6 endemic controls IgG4 reacted with several OvAg below 13 kD, and in one endemic control IgG1 bound to these VLMW-OvAg as well. Antibodies from non-endemic controls did not recognize any of these OvAg.

Species specificity of OvAg below 13 kD

Twenty-one onchocerciasis sera and 53 additional sera from patients with other filariases were used to investigate the species specificity of VLMW-OvAg. Six OvAg with molecular masses of 7.3, 5.8, 5.4, 4.0, 3.8, and 3.6 kD were recognized exclusively by IgG1 from onchocerciasis patients (Fig. 4). However, several other VLMW-OvAg were recognized by IgG4 from patients with respective W. bancrofti, B. malayi and L. loa infection as well (Fig. 5, Lanes 14, 15, and 16). In most lymphatic filariasis patients serum antibodies of the IgG1 subclass strongly bound to the 2.2 kD OvAg (Lane 6). Interestingly, in the IgG3 subclass this 2.2 kD OvAg was recognized preferentially by sera from amicrofilaraemic patients with lymphadenopathy and/or elephantiasis (Lane 12, arrowhead). Reactivity of IgG3 in filariasis patients to other OvAg below 13 kD was mostly weak. Antibodies of patients patently infected with M. perstans reacted only weakly to VLMW-OvAg (Lanes 5, 11, and 17). Since IgG2-reactivity to OvAg below 13 kD was found to be generally weak in onchocerciasis patients, the cross-reactivity of the corresponding subclass from lymphatic filariasis patients was not examined further.
Antigen recognition by chimpanzee sera

In chimpanzees experimentally infected with *O. volvulus*, serological reactivity to VLMW-OvAg markedly increased shortly before microfilariae were detected in skin biopsies, i.e., at the onset of patency (Fig. 6). The IgG1 and IgG4 subclasses disclosed enhanced binding to OvAg of 3.0, 3.8, 4.0, 5.1, 5.4, 6.2, 7.3, 8.6 and 9.6 kD compared to pre-patency. The OvAg of 2.2 kD was recognized by IgG1 and IgG4 early after infection, but intensity increased with onset of patency.

Seroological reactivity of VLMW-OvAg after two-dimensional separation

Two-dimensional separation of low-molecular weight OvAg by isoelectric focusing, together with Tricine-SDS-PAGE and subsequent IgG-immunoblotting with pooled sera from two onchocerciasis patients, revealed more than 40 antigens (Fig. 7). Most antigenic polypeptides had an acidic isoelectric point (pI), with a predominant seroreactivity detectable at a pI of 3.9 to 5.0. Only a few antigens were recognized by IgG in the pI range of 5.5 to 7.4. Those OvAg manifesting an apparently strong antigenicity in the one-dimensional IgG-immunoblot (Lane B) were separated by 2D-immunoblotting into several polypeptides of different pI. Remarkably, several immunodominant very-low-molecular weight OvAg focused at a similar pI.

![Figure 5](image-url)
Discussion

The present work extends our knowledge of the significance of low-molecular weight *O. volvulus* antigens in serodiagnosis of onchocerciasis, as well as their role in inducing immune-mediated pathogenesis in filarial infections. Furthermore, this is the first study in which *O. volvulus* antigens below 13 kD (VLMW-OvAg) were distinctly resolved by Tricine-SDS-PAGE, and analyzed for their isotype-specific serological reactivity.

Although developed 10 years ago (Schägger & von Jagow 1987) the Tricine-SDS-PAGE is still rarely used in helminth immunology and biochemistry. It allows the separation of small peptides or lipopolysaccharides with molecular masses as low as 400 D (Too et al. 1994) and a more reliable determination of molecular masses, particularly in the range below 20 kD. Tricine-SDS-PAGE has also been used successfully for detection of radiolabeled antigen fragments bound to MHC class II molecules (Davidson et al. 1991). This finding clearly demonstrates the prospective potential of this technique to identify T cell epitopes of parasitic helminths.

Recently Frank et al. (1996) used Tricine-SDS-PAGE to determine the molecular mass of a cloned larval protein of *Dirofilaria immitis*.

By the use of Tricine-SDS-PAGE and in combination with immunoblotting we identified 16 antigens bound predominantly by IgG1 and IgG4. Some of these OvAg were recognized by immunoglobulins produced concurrently with onset of patent *O. volvulus* infection, while the *O. volvulus*-derived antigen of 2.2 kD cross-reacted strongly with IgG3 from lymphatic filariasis patients with severe pathology.

In previous studies the serological diagnosis of onchocerciasis was improved by depleting cross-reactive antigenic determinants such as glycoproteins and phosphorylcholine-containing proteins (Garate et al. 1990), by selecting surface-associated antigens (Cabrera & Parkhouse, 1986), by using species-specific recombinant antigens (Lucius et al. 1992; Bradley et al. 1990), and by focusing on the parasite-specific IgG4 reactivity (Weil et al. 1990). Low-molecular weight filarial antigens, either isolated from crude worm extracts or produced as recombinant polypeptides, have been proposed for reliable, early serodiagnosis (Weiss & Karam 1989; Lobos et al. 1991; Ramachandran 1993), as well as for the classification of different clinical forms of disease (Cabrera et al. 1988; Gallin et al. 1989). In support, we found a higher specificity of VLMW-OvAg compared to antigens of higher molecular masses. Since carbohydrate and PC residues are considered to mediate serological cross-reactivity (Lal et al. 1991), the striking lack of such epitopes in VLMW-OvAg might explain their increased specificity as well as their weak binding to serum IgG2, which is mainly induced by glycosylated antigens.

The detection of distinct VLMW-OvAg in IgG1 immunoblotting showed the highest specificity, while IgG4 was more sensitive in binding to individual VLMW OvAg. The OvAg of 5.1, 5.4, and 6.2 kD, which combined highest specificity with a maximum of sensitivity, are promising candidates for reliable

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**Figure 6** Recognition of very-low-molecular weight *O. volvulus* antigens by IgG1 and IgG4 from chimpanzees experimentally infected with 250 infective thirdstage larvae of *O. volvulus*, as described by Taylor et al. (1988). Seroreactivity of a patently infected animal (allocation no. 177) is shown. Separation of VLMW-OvAg and immunoblotting were accomplished as described in Material and methods. The arrowhead indicates onset of patent infection, i.e., microfilariae of *O. volvulus* were detected in skin biopsies.
serodiagnosis of *O. volvulus* infection. Further characterization including large-scale fractionation from crude parasite extracts, NH2-terminal amino acid sequencing and morphological localization of these antigens are currently being done.

The obvious increase of recognition of VLMW-OvAg by serum IgG4 and the appearance of new VLMW-OvAg at the onset of patency in *O. volvulus*-infected chimpanzees suggests that some of these antigens are associated with production and release of microfilariae from gravid female *O. volvulus*. This hypothesis is also supported by the fact that serological recognition of VLMW-OvAg increased dramatically shortly after treatment with the microfilaricidal drug ivermectin. (Soboslay et al. 1994). Earlier, increasing serological reactivity to low-molecular weight *O. volvulus* antigens was reported in infected children and chimpanzees (Weiss & Karam 1989; Soboslay et al. 1992b). Dafa’Alla et al. (1992) found in onchocerciasis patients from Sudan a positive correlation between parasite-specific IgG4 levels in ELISA, microfilaridermia, and the score of skin lesions. The striking IgG3 reactivity to the OvAg of 2.2 kD in lymphatic filariasis patients with severe pathology indicates the immunopathogenic potential of this antigen. Earlier, Cabrera et al. (1988) found exclusive recognition of a 9 kD OvAg by IgG3 in patients with chronic hyperreactive onchodermatitis (sowda); however, due to the use of conventional Glycine-SDS-PAGE in this study, the molecular mass of this OvAg may be lower than calculated and both antigens may be identical. Recently, Gallin et al. (1995) described in sowda patients the preferential serological recognition of a 2.5 kD antigen derived from crude extracts of *O. volvulus*. Subsequently this antigen was identified as human defensin, a major constituent of neutrophil granules and was considered to mediate auto-reactive responses in these patients. In our study, one third of the onchocerciasis patients also reacted to the 2.2 kD OvAg which could be indeed the 2.5 kD human defensin. Since no sowda patients were included in our study the putative association of the 2.2 kD OvAg with this particular manifestation of disease could not be investigated. However, as observed in our work, serological reactivity to this antigen in lymphatic filariasis patients with severe pathology strongly suggests a similar mechanism of pathogenesis in other human filariases as well.

In addition, low-molecular weight OvAg have been considered important in acquired immunity to *O. volvulus*. In Guatemalan individuals with no clinical signs of infection but living in an area endemic for onchocerciasis (‘putative immunes’), a preferential recognition of low-molecular weight OvAg by IgG3 was observed (Boyer et al. 1991). Interestingly, peripheral...
blood mononuclear cells (PBMC) from non-patent humans exposed to O. volvulus-infection (‘endemic normals’) responded most strongly to low-molecular weight O. volvulus-derived polypeptides while PBMC from onchocerciasis patients failed to proliferate when stimulated with these antigens (Lüder et al. 1996). Our study demonstrates the relevance of O. volvulus antigens below 13 kD. These antigens contain polypeptides which appear to be not only suitable for improved serodiagnosis of onchocerciasis, but also decisively involved in immune-mediated pathogenesis of filarial infections.

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