Cloning, characterization and DNA immunization of an *Onchocerca volvulus* glyceraldehyde-3-phosphate dehydrogenase (*Ov*-GAPDH)†

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Received 13 August 2004; received in revised form 5 December 2004; accepted 14 December 2004

Available online 5 January 2005

Abstract

In the search for *Onchocerca volvulus* antigens possibly involved in protection against human onchocerciasis, partial amino acid sequence analysis of one of the *O. volvulus* antigens of the serologically identified proteins showed a close relationship to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein family. Subsequent adult worm cDNA library screening and cloning produced a clone of 1650 bp. An open reading frame spans over 1020 bp encoding for a protein of 340 amino acids with an apparent molecular weight of 38000. Comparison of the complete amino acid sequence identified this protein as a member of the GAPDH protein family. The recombinantly expressed protein shows GAPDH enzymatic activity as well as plasminogen-binding capacity. DNA sequence analysis of the corresponding gene revealed the presence of two introns. Using immunohistology *Ov*-GAPDH was observed in microfilariae, infective larvae, and adult male and female worms. Most striking was the labelling of the musculature of the body wall. Labelling was also observed in the pseudocoeloma cavity and in a subset of cell nuclei, suggesting additional, non-glycolytic functions of the *Ov*-GAPDH. Gene gun immunization with the DNA-construct in cattle led to specific humoral immune responses. Thus, the protective potential of the DNA-construct of *Ov*-GAPDH can be evaluated in vaccination trials using animal models such as the cattle/*Onchocerca ochengi* model.

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Keywords: Glyceraldehyde-3-phosphate dehydrogenase; Filaria; *Onchocerca volvulus*; Plasminogen-binding; Protective antigen; DNA immunization

1. Introduction

The parasitic nematode, *Onchocerca volvulus*, is a major cause of blindness and dermal pathology in the tropics. Chemotherapy with the microfilaricidal drug ivermectin, which is the backbone of the present African Programme for Onchocerciasis Control, even in combination with vector control by larvicides, cannot eradicate the parasite reservoir from hyperendemic areas in West Africa [1]. Since bovine onchocerciasis is a vaccine-preventable disease [2], it has been proposed that in combination with these control measures, a vaccine might fully stop transmission as well as disease. Using DNA for vaccination greatly simplifies vaccine development and production, as DNA vaccines remain stable under local conditions, presumably without a cold chain [3]. Several antigens of *O. volvulus*, for example the DNA-construct of the *O. volvulus* chitinase [4], have been shown to induce significant protection in animal models. One of the major vaccine candidates against the human pathogenic trematode *Schistosoma mansoni* was identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [5,6]. GAPDH has been suggested as major therapeutical target in several parasitic diseases, as a vaccine candidate or as a target for chemotherapeutic treatment. This has been primarily attributed to the role of GAPDH as a key enzyme in glycolysis and gluconeogenesis, thus being

† The DNA sequence has been submitted to GenBank under the accession number: Y09455.

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crucial in energy production. Here we report the characterization of the *O. volvulus* GAPDH as well as the immune response of cattle against the DNA-construct of the coding sequence of the GAPDH of *O. volvulus*.

2. Materials and methods

2.1. Study population

After informed consent residents of West African areas endemic for onchocerciasis in Liberia, Benin and Guinea underwent physical and parasitological examinations, essentially as described [7]. The study procedures were in accordance with the Declaration of Helsinki (1975 and its revisions in 1983 and 2000).

2.2. Parasite preparation

Adult *O. volvulus* worms were obtained by collagenase digestion from nodules surgically removed from Liberian patients as previously described [8]. Onchocercomas embedded in paraffin were available from several studies in Liberia, Ghana, and Uganda [9–11]. The extirpation of onchocercomas for research had been approved by the Medical Board, Hamburg, Germany, and by authorities in the African countries. Nodules with adult *Onchocerca ochengi* had been collected from cattle in Ngaoundere in Cameroon (supplied by PD. Dr. A. Renz, University of Tübingen, Germany). For the examination of infective larvae, we used *Simulium yahense* that had been reared from pupae and had been experimentally infected with *O. volvulus* in Guinea (supplied by Dr. T. Kruppa, BNI Hamburg) or *S. soubrense* from Liberia (supplied by PD Dr. G. Strote, formerly BNI Hamburg).

2.3. Identification of the *Ov*-GAPDH cDNA and protein expression

A λ ZapII expression cDNA library, prepared from adult *O. volvulus* mRNA, was screened with a 32P-labelled 970 bp probe coding for *Ov*-GAPDH. The probe was obtained by PCR amplification of *O. volvulus* cDNA using primers derived from conserved regions of known GAPDH nucleotide sequences. After plaque purification inserts were subcloned into phagemids (pBluescript II SK, Stratagene, Heidelberg, Germany) for sequencing using the in vivo excision protocols as supplied by the manufacturer (Stratagene).

PCR was carried out using synthetic oligonucleotides spanning the entire coding region of the *Ov*-GAPDH cDNA. The obtained fragment was digested with the appropriate restriction enzymes and cloned into the pUC45FFlag expression vector modified according to Clos and Brandau [12]. Expression was performed in *Escherichia coli* strain PAPlacO (DE3) according to Clos and Brandau [12]. The further purification of the recombinant protein by Ni2+ chelate chromatography and pH-shift under denaturing conditions was performed according to the pET manual (Novagen, Madison, WI, USA).

2.4. DNA sequence analysis

In order to identify the genomic structure of *Ov*-GAPDH, an *O. volvulus* λ FixII-gDNA library was screened using the same screening method as described above. Manual sequencing was carried out employing the dideoxynucleotide chain termination method of Sanger et al. [13] using the appropriate vector primer and synthetic internal primers deduced from the partial sequence of the clone. Sequence analysis was performed in both orientations. Automated sequencing was performed on an Applied Biosystems automated DNA sequencer. The derived sequence was compared with the public protein and nucleotide database (Genbank) by using the BLASTn and BLASTx algorithms. The DNA sequence of *Ov*-GAPDH was deposited in GenBank (accession no.Y09455).

2.5. Southern blot analysis

Human DNA was prepared from HL60 cells and *O. volvulus* DNA from adult female worms. The isolation and preparation of the DNA was done as described [14]. The Southern blot analysis was carried out by standard methods using the entire cDNA of *Ov*-GAPDH as a probe. The Southern blot was prepared by separation of approximately 10 μg human and adult *O. volvulus* EcoRI and HindIII restricted genomic DNA on an 1% agarose gel. After depurination, denaturation and neutralization, separated DNA was transferred to nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). The filter was hybridized overnight at 55 °C using a radioactively labelled probe.

2.6. Western blotting

The purified recombinant antigen was separated by SDS-PAGE in a 10% acrylamide gel based according to standard methods. Nitrocellulose strips were incubated with sera at different dilutions and a 1:1000 dilution of goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate (HRP) (Biorad, Munich, Germany) and developed using 4-chloro-naphtol/H2O2.

2.7. Enzyme-linked enzyme immunoassay (ELISA)

For antibody analysis, wells of Maxisorb plates (Nunc, Wiesbaden, Germany) were coated at 37 °C with purified *Ov*-GAPDH in carbonate-bicarbonate buffer, pH 9.6, 2 μg/well. After incubation with the primary antibody as secondary reagent, horseradish peroxidase-conjugated goat-anti-rabbit IgG (Sigma, Deisenhof, Germany) was
applied. The substrate was tetramethyl-benzidine (Sigma). The absorbance was read at 450 nm.

2.8. Synthesis of cDNA

Total O. ochengi RNA was isolated with an RNA extraction reagent (TRIzol Reagent, Gibco, Karlsruhe, Germany) and cDNA was obtained by reverse transcription using oligo-dT primers. cDNA from O. volvulus L3 was derived from a ZAPII O. volvulus cDNA library. PCR amplification was primed with a deduced GAPDH primer set, obtained by sequence comparison of highly conserved regions, such as the ATP binding site and NAD+ binding site of members of the GAPDH protein family.

2.9. Enzymatic analysis of the recombinant protein

The activity of the GAPDH enzyme was determined by measuring the decrease in absorbance of NADH at a wavelength of 340 nm. Tests were performed on the recombinantly expressed Ov-GAPDH. Rabbit GAPDH (Sigma) was used as positive and nonrecombinant pJC40 vector as negative control.

2.10. Plasminogen-binding assay

Binding of human plasminogen (Sigma) was performed using human plasminogen followed by anti-plasminogen antibodies and secondary antibodies conjugated with peroxidase. In blot-overlay assays plasminogen-binding activity was detected by incubation of the membrane with a substrate solution containing 1 mg/ml 4-chloro-1-naphthol (Sigma) and 0.1% H2O2 in PBS. Control experiments were a substrate solution containing 1 mg/ml 4-chloro-1-naphthol precipitated onto gold beads (1.6 μm diameter) at a ratio of 1 μg/mg gold. In all experiments rabbits and cattle were bled before DNA immunization to collect preimmune samples for antibody assays. Rabbits were DNA immunized on day 1 and boosted on days 33 and 69. Twenty-four shots of 0.2 μg Ov-GAPDH-pcDNA3.1(+) were applied into the shaved back using a Helios gene-gun (Biorad). Following the protocol of the manufacturer, a pressure of 300 psi was used. A similar protocol was applied for gene gun immunization of two calves using for each immunization 1.25 μg/mg gold. In all experiments rabbits and cattle were bled before DNA immunization to collect preimmune samples for antibody assays. Rabbits were DNA immunized on day 1 and boosted on days 33 and 69. Twenty-four shots of 0.2 μg Ov-GAPDH-pcDNA3.1(+) were applied into the shaved back using a Helios gene-gun (Biorad). Following the protocol of the manufacturer, a pressure of 300 psi was used. A similar protocol was applied for gene gun immunization of two calves using for each immunization on days 1, 27, and 66, ten shots of 1.25 μg Ov-GAPDH-pcDNA3.1(+) and a pressure of 300 psi. The shots were applied intradermally into the shaved skin of the neck.

3. Results

3.1. Cloning and sequence analysis of Ov-GAPDH

To identify O. volvulus antigens possibly involved in protection, partial amino acid (AA) sequence analysis of an O. volvulus antigen showed a close relationship to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein family. Subsequent screening of an O. volvulus adult
worm cDNA library using the GAPDH-specific PCR fragment resulted in a clone of 1650 bp. An open reading frame spans over 1020 bp coding for a protein of 340 AA with an apparent molecular weight of 38,000.

Comparison of the deduced AA sequence indicated a high level of identity to members of the GAPDH protein family (74% to human GAPDH) [16]. Furthermore, Genbank comparison as well as the presumptive AA

-391
T GTC TTT CAAA N GG CN NA TCA T GGT GTC TAC
-360
T GAC TAC TT C TCC CAG CAA TTA CAC ACA T AAT GGG TGA GTT GAT TTT TGT AAA TTC ACC CAT
-330
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT
-300
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT
-270
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT
-240
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT
-210
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT
-180
T GT TAC TG TTT AC GAA GGC AT TT CCA CAG TAT CAA CAC AAT TTT CCT GCT TCA TCA TCA TCA A TGA ACC CAT

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Fig. 1. Nucleotide sequence of the Ov-GAPDH gene with the 5' and 3' flanking regions. The coding region starts at nt 1 and ends at nt 1394 and contains two introns (underlined). A putative promotor sequence TGTTG at position –40 to –36 is also underlined.
sequences for an ATP and NAD\textsuperscript{+} binding site and S-loop confirmed the relationship of the deduced AA sequence to the GAPDH family. Finally, the proof of the enzymatic activity and plasminogen-binding capacity of the recombinant protein allowed designation of cDNA clone \textit{Ov}-GAPDH.

3.2. Genomic Structure

Following a 5\textprime;-noncoding region of bps 391, the coding region of \textit{Ov}-GAPDH is interrupted by 2 introns, with the length of bps 173 of intron I and bps 202 of intron II, followed by a 3\textprime;-noncoding region of bps 36 (Fig. 1). The untranslated region contains a CAATT sequence at position –87 to –83, an AT-rich region at position –40 to –21 and a TGTTG promoter sequence at position –40 to –36, indicating the presence of the complete gene. The introns indicate the eukaryotic origin of the \textit{Ov}-GAPDH sequence.

Characterization of the genomic structure of \textit{Ov}-GAPDH by Southern blot analysis revealed a hybridization pattern of two bands (Fig. 2, lanes 1 and 3). Control experiments showed no hybridization of an \textit{Ov}-GAPDH cDNA probe to human genomic DNA (Fig. 2, lanes 2 and 4), confirming the parasite origin of the GAPDH. Since the cDNA contains single sites for the used restriction enzymes \textit{Eco}RI as well as \textit{Hind}III, it suggests the existence of a single copy gene. This is supported by analysis of the gene structure obtained from nine clones of a \textit{FiXII-gDNA} library of \textit{O. volvulus} which shows also no isoforms of the enzyme.

Since the \textit{Ov}-GAPDH will be used as a vaccine candidate antigen in the \textit{O. ochengi}/cattle model, a corresponding GAPDH cDNA clone of \textit{O. ochengi} was isolated and characterized for DNA and AA sequence comparison. The deduced \textit{O. ochengi} AA sequence shows a 99.1\% identity to the AA sequence of \textit{Ov}-GAPDH.

3.3. Analysis of enzymatic activity of \textit{Ov}-GAPDH and binding analysis of human plasminogen

To analyze whether the purified recombinant \textit{Ov}-GAPDH is biologically active, assays for GAPDH enzymatic activity were performed. The NADH-enzyme activity was determined at 740 µM/min/mg GAPDH, representing a moderate activity level.

Since GAPDH has been localized on the surface of bacteria and was shown to bind plasminogen [15,17], the plasminogen-binding activity of the purified recombinant \textit{Ov}-GAPDH was also examined. The plasminogen-binding property of group A streptococci and pneumococci and subsequent activation facilitate the penetration of the pathogen during the invasive infection process [18]. The plasminogen blot-overlay assay revealed binding activity of \textit{Ov}-GAPDH as demonstrated in Fig. 3.
3.4. Immunolocalization

Immunohistological expression of Ov-GAPDH was demonstrated in adult male (Fig. 4A) and female O. volvulus (Fig. 4B–F), in microfilariae in nodule tissues (Fig. 4G) and in infective larvae in blackflies (Fig. 4H–J). Both the serum from the immunized rabbit as well as the affinity purified antibodies produced a distinct labelling pattern, while no labelling was observed after application of the preimmune serum or the human AB-serum (not shown).

In all sections of adult filariae, most striking was the labelling of the afibrillar compartment of the muscles of the body wall, where the mitochondria are (Fig. 4A–B), and of the uterus (Fig. 4D). The hypodermis was usually labelled to a lesser extent whereby often only the outer zone was labelled where the folding of the cell membrane forms a labyrinth in the vicinity of the cuticle (Fig. 4A). Occasionally, the outer as well as the inner labyrinths of the hypodermis were labelled (Fig. 4C). Labelling of some nuclei was also observed in the uterus epithelium (Fig. 4E). Labelling of the fluid in the pseudocoeloma cavity was detected in cross sections of a greater number of male worms and of some females (Fig. 4F), possibly indicating release of Ov-GAPDH into the extracellular space. The epithelia of the genital tracts of both male (Fig. 4A) and female worms (Fig. 4D) were clearly labelled in some worms. Differences in labelling were also observed within one worm section whereby one of the uterus branches was labelled but the other one not. Thus, differences in labelling appear to correlate with the actual presence of Ov-GAPDH and are not due to a variation in the fixation or preservation of the examined tissues. Rarely, weak labelling of the

Fig. 4. Immunolocalization of Ov-GAPDH in adult O. volvulus and microfilariae labelled with rabbit immune serum and in infective larvae labelled with affinity purified antibodies. (A) Cross section of a male worm showing strong labelling of the afibrillar compartment of the body wall muscles (arrow) and of the epithelium of the vas deferens (arrowhead). Sperms are not labelled. (B) Female worm with Ov-GAPDH in the muscles (arrow). (C) Cross section of a female worm with labelling of the outer and inner hypodermal labyrinths (arrowheads). (D) Cross section of the uterus with labelling of the uterus muscles (arrows) and the epithelium of one branch of the uterus. Oocytes positive (arrowhead). (E) Longitudinal section of a female worm with labelled hypodermal nuclei (arrow) and one nucleus not labelled (arrowhead). (F) Female worm with labelled fluid in the pseudocoeloma cavity (asterisk) and labelled muscles (arrow) and outer hypodermal labyrinth (arrowhead). (G) Labelled microfilariae in the tissue of an onchocercoma. (H) Labelled infective larvae in the thorax muscles of S. yahense (arrows). (I) Cross section of an infective larva with thick cuticle (arrow head) showing well-labelled muscles (arrow) in the head of S. yahense. Scale bar=20 μm for A–H and 5 μm for J.
intestine was observed. No specific labelling was detected in the cuticle, in the fibrillar compartment of the body wall muscles, in all stages of sperms (Fig. 4A) and in the Wolbachia endobacteria. A similar labelling pattern was observed in adult *O. ochengi* (data not shown).

In developmental stages of *O. volvulus* labelling of oocytes (Fig. 4D) and developing embryos in the uterus varied. In infective larvae the afibrillar compartment of the muscles was always strongly labelled whereas the hypodermis was labelled to a lesser extent (Fig. 4J). In several sections distinct labelling of some nuclei of the hypodermis was observed whereas other adjacent nuclei were not labelled.

### 3.5. DNA immunization of rabbits and cattle

The gene gun immunization of rabbits and cattle was well tolerated; the sites of immunization showed no adverse reactions. To examine the antibody reactivity of rabbits and cattle to the *Ov*-GAPDH-pcDNA3.1(+) DNA, sera of gene gun-immunized rabbits and cattle were obtained and analysed by Western blot and by ELISA. The sera of the immunized rabbits showed production of high levels of specific IgG antibody detectable at high serum dilutions by Western blot (Fig. 5A) as well as by ELISA (Fig. 5B). Antibody levels were comparable to those obtained after DNA immunization with schistosome antigens in mice [19]. Cattle sera showed specific IgG antibody responses after week 15, which increased by week 19 (Fig. 6A) with predominance of IgG2 subclass (Fig. 6B).

![Fig. 5. IgG antibody response against recombinant *Ov*-GAPDH in the serum of a rabbit intradermally immunized with *Ov*-GAPDH-pcDNA 3.1(+)m measured by immunoblot (A) and ELISA (B). (A) IgG antibodies detected in serum obtained at day 98 after immunization used at dilutions 1:50, 1:100, 1:250, 1:500, 1:1000, 1:2500 and 1:5000 (lanes 1–7) and in preimmune serum used at a dilution of 1:50 (lane 8); recombinant *Ov*-GAPDH detected with mouse antibody to FLAG M2 (lane 9) as positive control. (B) ELISA using serum at dilutions 1:80, 1:320 and 1:1280 before (lanes 1–3) and at day 98 after immunization (lanes 4–6).](image)

![Fig. 6. IgG antibody response against *Ov*-GAPDH in the sera of two cattle immunized with *Ov*-GAPDH-pcDNA 3.1(+)m measured by ELISA. (A) Specific anti-*Ov*-GAPDH IgG antibody titers in two animals before (white columns) and at weeks 15 (light grey columns) and 19 (dark grey columns) after immunization. (B) Specific anti-*Ov*-GAPDH IgG1 and IgG2 antibody titers in one animal before (white column) and at week 23 after immunization (grey column).](image)
4. Discussion

Here we report the isolation, characterization and expression of a full length *O. volvulus* cDNA sequence that encodes a 38 kDa protein. The deduced AA sequence shows high similarity with GAPDH sequences from other organisms, and the analysis of its enzymatic activity indicates that the *Ov*-GAPDH represents a glyceraldehyde-3-phosphate dehydrogenase. The gene of *Ov*-GAPDH is split by two introns. Southern blot and sequence analysis of nine independent clones indicate the presence of only one single copy of the gene in the genome of *O. volvulus*. This is in contrast to the genome of *Caenorhabditis elegans* where four GAPDH genes have been identified [20,21]. In addition to its function in glycolysis, GAPDH was shown in various tissues of other species to be involved in functions unrelated to glycolysis. These include the bundling and unbundling of microtubules in brain tissue [22]. GAPDH has also been shown to exhibit protein kinase-like activity, leading to the phosphorylation of transverse tubule proteins which may be involved in the assembly of the junctional triads [23].

In this study, immunohistology showed that *Ov*-GAPDH is present in the nuclei of the hypodermis and the uterus epithelium of the worm, whereby only a small percentage of the nuclei were labelled. Interestingly, the percentage of labelled nuclei appears to be higher in older worms, an observation which needs further analysis (Erttmann et al., in preparation). This indicates that the *Ov*-GAPDH of the nucleus might have functions other than glycolysis, such as DNA replication and gene activation [24,25], which may be relevant in protection. GAPDH has been shown to be involved in apoptosis [26] and is viewed as a putative molecular target for the development of antiapoptotic therapeutic agents for certain neurodegenerative diseases [27].

In this study, *Ov*-GAPDH was identified based on its potential involvement in protection against the parasite. Based on the same strategy, we have previously identified and characterized an *O. volvulus* protein named *Ov*-E1, which is associated with the neuronal system of the parasite and is related to the apoptotic “death domain” proteins [28]. In view of the possible apoptotic functions of GAPDH and its involvement in neurodegeneration, it is intriguing to speculate that as suggested previously one point of the attack for the human immune response is the neuronal system of the parasite. This may represent a new aspect of the role of GAPDH as a therapeutic target in helminth infections. The identification of other potential vaccine candidates suggested targeting the glycolytic pathway of the parasite, such as the *O. volvulus* fructose-1,6-bisphosphate aldolase [29]. Recently, another glycolytic enzyme of *O. volvulus*, α-enolase, has been cloned [30]. This enables the combination of these three enzymes to be tested as components of a multivalent vaccine.

The immunolocalization of *Ov*-GAPDH observed in this study adds new aspects regarding its role in protection. While the muscular localization of *Ov*-GAPDH in adult worms is consistent with its role in glycolysis and has also been reported for the *O. volvulus* aldolase [29] and enolase [30], its localization in the body cavity of adult worms observed in this study as well as the detected plasminogen binding activity may indicate extracellular functions as also described in other organisms. Thus, streptococcal GAPDH has been reported on the bacterial surface [15] and appears to be involved in bacterial adhesion to host cells [31]. In schistosomes GAPDH was detected on the surface of schistosomula [32]. In *O. volvulus* *Ov*-GAPDH was detected in the larval stage by light microscopy, however, immunoelectron microscopy may be necessary to examine its more precise localization, such as in the region of the cuticle involved in larval molting, as reported for the *O. volvulus* aldolase [29].

We found that *Ov*-GAPDH is immunogenic in natural infections with *O. volvulus* by analyzing the antibody response of individuals exposed to *O. volvulus* against r*Ov*-GAPDH. The results show that *Ov*-GAPDH is a target of the immune response of putatively immune individuals as well as of a subgroup of infected individuals (Erttmann et al., manuscript in preparation). Its potential as a protective antigen is supported by the fact that it was also identified as a protective antigen against infection with schistosomes [6]. It is of interest that it has been also identified based on its association with protective antibody responses and resistance to reinfection in humans [33]. The protective potential of GAPDH in schistosomiasis has been studied in detail [34], and antigenic determinants have been identified which can induce protective immunity [19,33,35].

Vaccination strategies against schistosomes include the testing of DNA-based vaccines. Field testing of *S. japonicum* DNA vaccines in cattle in China showed that each of the vaccine groups could induce partial resistance [36]. In ongoing efforts to optimize immune responses associated with protection, DNA immunization regimens are being developed for several schistosome antigens in mice, showing the induction of significant B-cell and T-cell responses [19]. Our data also indicate that significant antibody responses as well as T-cell responses (Erttmann et al., in preparation) can be elicited in cattle using the *Ov*-GAPDH DNA immunization protocol described here. To conduct similar protection studies in *Onchocerca* infection using the *O. ochengi/cattle model, we have cloned the *O. ochengi* GAPDH. The sequence is almost identical to the *Ov*-GAPDH and therefore greatly facilitates protection studies in cattle [2]. Further studies aimed at improvement of immunization strategies are needed to achieve an appropriate level of protection for control of *O. volvulus* in endemic areas.

Acknowledgements

We thank Silke van Hoorn and Manfred Krömer for excellent technical assistance. This work was supported in
part by the Bundesministerium für Bildung und Forschung, Germany.

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