ment makes ivermectin applicable for community-based mass treatment campaigns (White *et al.*, 1987). In most areas of the OCP the combined control measures have indeed reduced the risk of *O. volvulus* infection and onchocerciasis-associated blindness. However, in borderline areas, such as central Togo, the risk of re-infection and evolution of ocular pathology still remains high (De Sole *et al.*, 1992).

In order to study the feasibility, compliance and efficacy of mass treatment in the field we performed annual mass treatment campaigns in six meso- to hyperendemic villages in central Togo. More than 3,000 inhabitants received annually a blanket treatment with 150 µg ivermectin/kg body weight. The effect of this control strategy on prevalence of onchocerciasis, on community microfilarial load (CMFL) and on the evolution and progression of ocular pathology induced by *O. volvulus* infection, has been evaluated over a period of 4 years. In addition, since 8 years, a group of 200 onchocerciasis patients from the same villages, who volunteered to participate in an ivermectin dose-finding study, have been treated and examined annually.

Under consideration of exclusion criteria, disease and refusal about 70 % of the population received the annual treatment with ivermectin. The densities of *O. volvulus* microfilariae (mf) in treated patients remained significantly reduced, and the number of patients with a subclinical infection, i.e. permanently negative for mf of *O. volvulus*, increased following repeated ivermectin therapy. The number of treated patients per village with moderate or severe side reactions decreased following each annual treatment. In those patients who received ivermectin regularly during the last 8 years onchocerciasis-associated ocular pathology clearly diminished (e.g. punctate keratitis, sclerosing keratitis, uveitis, limbitis) or remained at pre-treatment levels (e.g. chorioretinitis, papillitis). In contrast, in those patients who received ivermectin treatment only occasionally the onchocerciasis-associated ocular pathology progressed often to irreversible eye damage, and in some cases to blindness.

In summary, despite the location of our study in an area of the OCP where, however, the risk of re-infection and blindness remains high, we conclude that progression of ocular pathology in chronic onchocerciasis can only be prevented by regular treatments with ivermectin.

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USE OF THE *LITOMOSOIDES SIGMODONTIS* - MOUSE MODEL IN DEVELOPMENT OF AN *ONCHOCERCA* VACCINE. I - MOLECULAR OF *O. VOLVULUS* ANTIGENS

TAYLOR D.W.*, BRAUN G.*, ENGELBRECHT F.*, SALINAS G.* AND SINHA K.*

KEYWORDS : *Onchocerca volvulus*. vaccine. recombinant antigens. *Litomosoides sigmodontis*. white mouse.

Five *Onchocerca volvulus* recombinant antigens are currently being investigated for their capacity to evoke protective responses against *Litomosoides sigmodontis* in mice. Details of the molecular cloning techniques employed are provided by Braun *et al.* (1991).

Ov1.9 : a 19,000 Mr antigen identified using a rabbit serum raised against material extracted from the surface of adult worms by treatment with mercaptoethanol (Engelbrecht *et al.*, 1991). The 512 bp insert has been sequenced and no homology could be found with any other cloned molecule. Measurement (by ELISA) of anti-Ov1.9 antibodies in sera collected from onchocerciasis patients revealed a correlation between high IgG4 levels and presentation of skin disease (Engelbrecht *et al.*, 1991). These IgG4 responses appeared to be enhanced after treatment with ivermectin.

Ov2.5 : a 50,000 Mr antigen which was selected on the basis of its reactivity with a rabbit antiserum against material extracted from the surface of adult worms by treatment with mercaptoethanol (Engelbrecht *et al.*, 1991). No sequence homology could be found with any other cloned molecule.

Ov3.11 : a 42,000 Mr antigen identified using a rabbit sera raised against *O. volvulus* L3 larvae.

OvHSP : The heat-shock protein 70 of *O. volvulus*. This cDNA clone (410bp, all coding) codes for the C-terminal end of the protein. Sequence analysis revealed almost 100 % homology with *Brugia malayi* HSP70 ; 76 % homology with human cognate HSP70 ; and 75 % identity with human HSP70. Despite the overall high homology of parasite and host HSPs, a short sequence of 12 amino acids unique to the parasite was identified. It is predicted that this region, which is proximal to the C-terminus contains 2 non-conserved changes and 4 conserved changes, has a secondary structure and B cell epitope not found in host HSPs.

The OvHSP sequence was sub-cloned into the expression vector pTrcHis, a vector that allows purification of fusion proteins over Ni affinity columns through the interaction with a polyhistidine sequence contained in the carrier peptide.

A rabbit antiserum raised against the recombinant OvHSP was used in histochemical experiments to localise the antigen in adult worm sections. The most heavily stained structures were ova.

Human antibody responses directed against the recombinant HSP70 molecule AND a synthetic peptide representing the 12 amino acid filarial specific region of HSP70 are being investigated. As shown in Figure 1, ELISA performed using

* Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP.

recombinant HSP70 demonstrated, in descending order, IgG1, IgG3, IgA, IgG4 and IgM responses, although all isotypes were detected. When the synthetic peptide was used as the target antigen the most prominent isotype detected was IgA followed by IgM, IgG3 and IgG1 ; IgG4 was not detected (Figure 2). Such a result may indicate that incorporation or selection of specific epitopes in recombinant antigens may enable the balance of synthesis of individual isotypes to be determined through vaccination.

Ov.GST : The Glutathione-S-transferase (GST) of *O. volvulus*. A cDNA clone encoding a pi class (identified by sequence) glutathione-S-transferase was isolated using a rabbit antiserum raised against affinity purified glutathione binding proteins and which was also preabsorbed against total *O. volvulus* antigens depleted of glutathione binding proteins. The clone contains a 470bp sequence of which 300bp encode the C-terminal end of the molecule. Sequence analysis demonstrated a 60 % homology with the pi class GST of *Caenorhabditis elegans*. A full length clone was subsequently isolated using PCR techniques.

The cDNA insert was subcloned into the pTrcHis expression vector and the corresponding recombinant antigen affinity purified over a Ni chelating column. A rabbit antiserum raised against this recombinant was used in immuno-histochemical experiments to localise the GST : the antigen was detected in the hypodermis of adult worms but could not be found in uterine microfilariae.

Two dimensional isoelectric focusing-SDS polyacrylamide gel electrophoresis of *O. volvulus* glutathione-S-transferase resolved at least 10 isoenzymes of which two have now been cloned.

This investigation was supported by Science and Technology for Development Programme of European Community, Contract n° TS3 CT9-0037 and By The Edna McConnell Clark Foundation.

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Figure 1. – Anti-*O. volvulus* HSP70 responses in a pool of serum from onchocerciasis patients. Murine isotype specific monoclonal antibodies were used as detecting reagents in these ELISA.

Figure 2. – Anti-*O. volvulus* synthetic HSP70 responses in a pool of serum from onchocerciasis patients. Murine isotype specific monoclonal antibodies were used as detecting reagents in these ELISA.