Polyamine analogues, e.g. *bis*(benzyl)polyamine (MDL 27695), although shown to interfere strongly with both polyamine uptake systems of filarial worms, probably exhibit their filaricidal effects by competing and interacting with polyamine binding sites such as nucleic acids and structural macromolecules.

Inhibition of S-adenosylmethionine decarboxylase, a regulatory and rate-limiting step in the biosynthesis of polyamines which provides the aminopropyl group for the synthesis of spermidine and spermine, results in depletion of polyamines. MDL 73811, an irreversible inhibitor of S-adenosylmethionine decarboxylase, affects both polyamine synthesis and the viability of nematodes maintained *in vitro*.

The strategy proposed here is directed to the interconversion and degradation pathway for polyamines. This is based on the findings that the polyamine oxidase is the rate-limiting step for the back conversion of spermine to spermidine and putrescine and that elevated levels of spermine are lethal for *Brugia* worms maintained *in vitro*. The potential of polyamine oxidase as a target for chemotherapy has been assessed by treatment of *Brugia* and *Ascaris* worms with MDL 72145, an irreversible inhibitor of nematode polyamine oxidase, which resulted in enzyme inactivation and a subsequent accumulation of spermine. By structural analysis of the filarial polyamine oxidase and the isofunctional mammalian enzyme, differences may be identified and exploited for the synthesis of drugs which react selectively with the parasite enzyme.

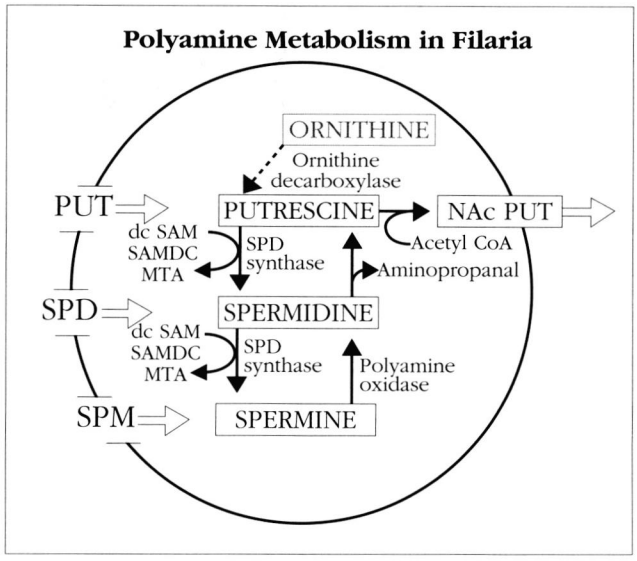

Figure 1.

VECTOR BIOLOGY

HUMORAL DEFENSE MOLECULES IN VECTORS OF FILARIASIS

HAM P.J.*, HAGEN H.E.*, SMITHIES B.M.*, CHALK R. AND ALBUQUERQUE C.M.R.*

KEYWORDS : insect immunity. defense. *Onchocerca*. humoral proteins.

INTRODUCTION

The relationship between the definitive host, man, and filarial parasites, has for long been a subject of intense study, and as this network attests, still is today. However, the interactions between vectors of lymphatic filariasis and onchocerciasis and these nematodes, is less well understood. There is now a considerable body of data describing the innate and acquired resistance of vectors to the parasites they carry (eg. reviewed by Townson and Chaithong, 1993 ; Christensen and Severson 1993 ; Ham, 1992). Blackflies and mosquitoes possess complex immune systems, comprising inter-related cellular and humoral components. Not only is immunity expressed in the haemocoel, but mechanisms of refractoriness also appear to be linked to gut lumen components (see review Ham, 1992) and thoracic tissues (Wattam & Christensen, 1992).

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RESPONSES TO INFECTION

Three generalised responses appear to take place following infection of insects : (1) an alteration in metabolic activity, including protein synthesis, (2) induction of specific immune peptides and proteins some of which have protective functional antibiotic activity, and (3) enhanced haemocytic proliferation. This proliferation maybe associated with increased secretion of molecules in (2) (Figure 1). As well as responses to infection, insects, including vectors of filariasis may exhibit responses to others forms of stress such as cold, overcrowding and physical trauma such as injury (eg Komano *et al.*, 1980).

HUMORAL DEFENSE MOLECULES

A number of defense peptides have been observed in insects, and many of these are known to be induced by bacterial pathogens such as *Escherichia coli*, as well as filariae (see Figure 2 which shows immune and non immune

blackfly haemolymph protein profiles, arrowed bands may represent some of the molecules described below). Included in these are the bacteriolytic cecropins (~4KDa) ; an insect defensin (~4KDa) ; the bacteriostatic attacins (20-23KDa), and digestive lysozyme (~15KDa). Of these, defensin has been found in *Aedes aegypti* (Chalk *et al.*, 1994), and cecropin-like and lysozyme-like molecules have been observed in blackflies (Smithies, pers. comm.). Other defense molecules such as inducible haemolymph proteases are also known in blackflies (Ham, 1992). A number of these are activated in response to infection, and comprise both serine and cysteine type species. It is believed that these molecules may play a role in activation of other immune proteins such as phenoloxidases (the subject of an additional abstract, Hagen and Ham, in this series). In addition to activation, the proteases may also be responsible for breakdown of excessive levels of immune molecules, and indeed may have a direct immune killing effect in their own right (Ham, 1992).

Phenoloxidase (~66KDa+/-) is an enzyme that acts in a cascade mechanism, which is known to be activated by serine proteases, by cleavage of the inactive larger pro-enzyme. Evidence indicates that as well as the more well known result of PO activity, that of filariae melanisation, this enzyme may also act as a non-self recognition molecule or an opsonin, bridging the parasite and an effector molecule. A further group of humoral molecules, the insect lectins, are also believed to carry out this role, by binding specifically to carbohydrate molecules on the parasite surface. Indeed PO may possibly act as a lectin. There is strong evidence to show that lectins are induced by *Onchocerca* infections in blackflies (Smail and Ham, 1989) and that carbohydrate residues exist on the surface of the filariae that are specific for such lectins (Ham *et al.*, 1988).

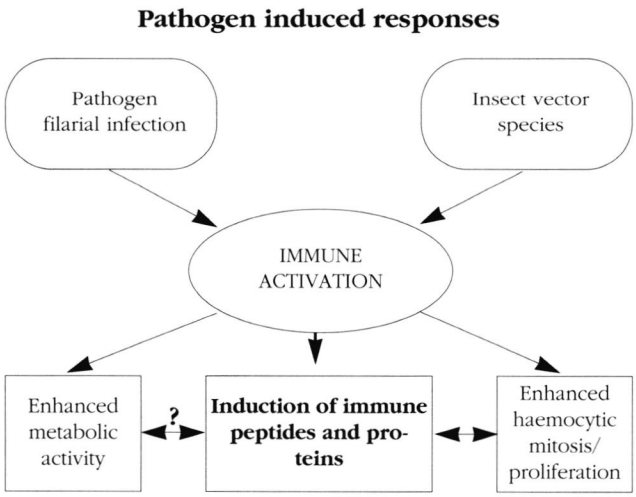

Figure 1. – Insect responses to infection and other forms of stress.

There are therefore a number of candidate molecules for the immune killing of filariae which have been observed in vectors, both *in vivo* and *in vitro*. This killing has been shown by incubation of microfilariae in immune haemolymph, by the passive transfer of immune haemolymph to naive susceptible recipient insects rendering them refractory, and by resistance during multiple consecutive infections within the same individual insect. Experiments in British simuliids have shown a resistance to reinfection with *Onchocerca lienalis*, a bovine filariae, as well as reduction in the development of the pre-existing infection following secondary infection. In Cameroon, secondary infection with *O. ochengi* resulted in the reduced development of a primary *O. ochengi* infection when compared with flies given an heterologous primary infection with *O. volvulus* (Figure 3). It appears, therefore, that the immune defenses of the vectors are able to distinguish at some level, between parasites of different species, within the same genus.

The fundamental understanding of immune systems in the insects, and how they affect parasite development, is of importance in our development of novel as well as more informed control strategies. The intermediate host has received much less research attention in terms of basic biology, than the definitive host. We are attempting to redress that balance a little by targetting an area of vector physiology, that plays an important part in determining whether an insect is a vector of filariasis or not.

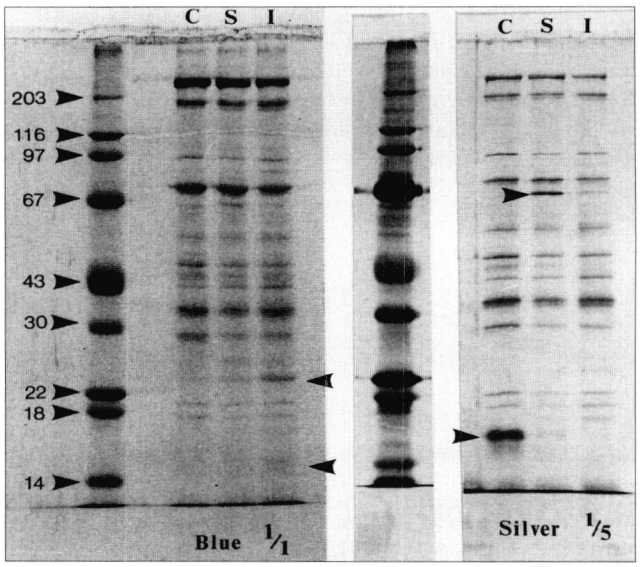

Figure 2. – Haemolymph protein profiles following SDS PAGE separation and staining of haemolymph from *Simulium ornatum* removed 4 days after infection with *Onchocerca* microfilariae (I), or non specific "sham" trauma (S) or no treatment (C). Gels are stained with Coomassie Blue or Silver reagent. Some of the molecules of interest are arrowed.

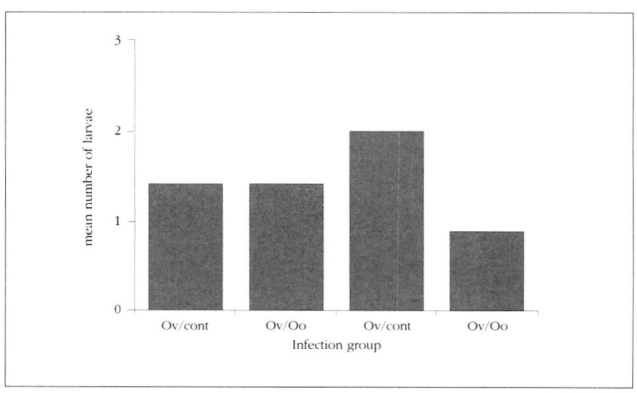

Figure 3. – Recoveries of third-stage larvae of a primary *O. ochengi* (Oo/Oo) or *O. volvulus* (Ov/Oo) infection from *S. damnosum* sl, following a secondary infection with O. ochengi (Ov/cont) and (Oo/cont) are primary infection control groups.

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OPEN SESSION

PHENOLOXIDASE ACTIVITY IN *SIMULIUM DAMNOSUM* S.L. AND *S. ORNATUM* S.L. FOLLOWING AN *ONCHOCERCA* INFECTION

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KEYWORDS : *Simulium*. *Onchocerca*. immunity. phenoloxidase

INTRODUCTION

Immune reactions of blackflies (Diptera : Simuliidae) as in other insects can be divided into innate and acquired resistance to fungal, bacterial as well as parasitic infections. As to acquired immunity, haemolymph transfer experiments showed that if naive recipient blackflies received haemolymph from infected specimens prior to an *Onchocerca* infection, the rates of parasite development were reduced (Ham 1986). Similar effects can be seen in superinfected European and African blackflies. Repeated infection with *Onchocerca* spp. (Nematoda : Filarioidea) had a strong limiting effect on the development of the parasites (discussed elsewhere in more detail). Having shown over the past years that blackflies have an efficient immune apparatus the question arises whether or not this immune system is also contributing to the innate resistance to filarial infections in blackflies. The different populations and (cyto-) species of *Simulium* have a varying transmission capacity of *Onchocerca* parasites as has been observed in endemic areas.

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As there are no laboratory strains of blackflies featuring different degrees of susceptibility towards the parasite, *S damnosum* s.l. collected from a breeding site in North-Cameroon was infected with several *Onchocerca* species, which are either adapted to this vector such as *O. volvulus* (Blacklock, 1926), or like the bovine species *O. ochengi* and *O. dukei*, are not naturally transmitted by this vector species (Denke & Bain, 1978 ; Wahl & Renz, 1991). By comparing the reactions of *S. damnosum* s.l. to infections with these species it was hoped to look for candidate molecules responsible for the regulation of filarial infection in blackflies.

The flies were infected by intrathoracic injection (Nelson, 1962). The microfilariae were recovered from skin-biopsies of onchocerciasis patients in North-Cameroon *(O. volvulus)* or from bovine hides collected at the abattoir in Ngaoundere, North-Cameroon (*O. ochengi* and *O. dukei).* Additional infection experiments on a European model, *S. ornatum* and *O. lienalis*, were carried out 24 hours post infection the haemolymph of the infected, sham inoculated (only medium injected) and untreated control was taken, stored in liquid nitrogen, and subsequently analysed.

RESULTS

S. damnosum s.l. : The haemolymph of infected blackflies showed a 66 kDa band. The amount of this molecule varied according to the *Onchocerca* species injected. While the *O. ochengi* and *O. volvulus* infected blackflies had a strong 66 kDa band (Fig. 1, lane 1) *O. dukei* infected specimens showed a decreased intensity (Fig.1, lane 4). Sham