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OPEN SESSION

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

HAGEN H.E*. AND HAM P.J.*

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.

* Centre for Applied Entomology and Parasitology, Department of Biological Sciences, Keele University, Staffordshire ST5 5BG, U.K.

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

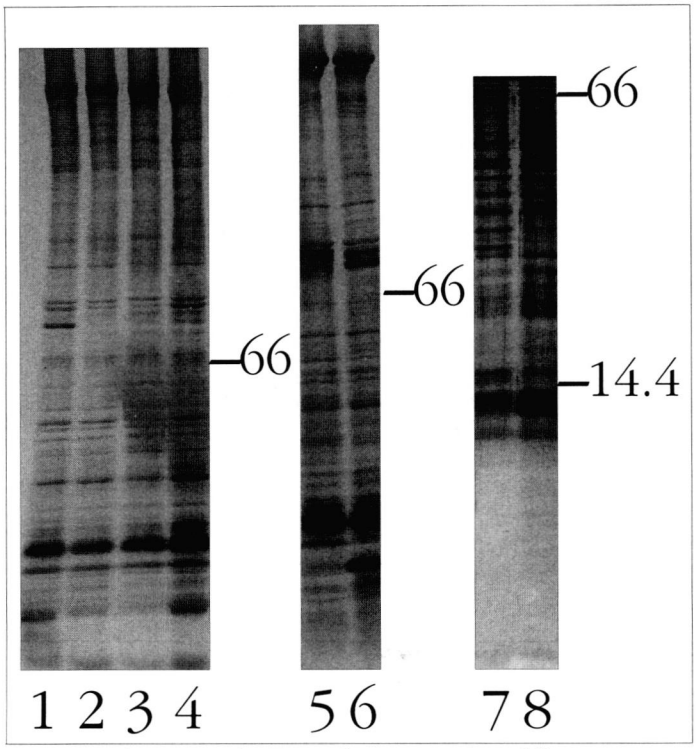

Fig. 1. – *S. damnosum* s.l. : Humoral immune reaction following injection with *O. ochengi* (lane 1), sham inoculation (lane 2) and *O. dukei* (lane 4, control : lane 3) as well as after a blood-meal on *Onchocerca* infected cattle (lanes 5 and 7* : non infected, lanes 6 and 8* infected flies).
* Samples were run on a tricine gel (Schägger & von Jagow, 1987).

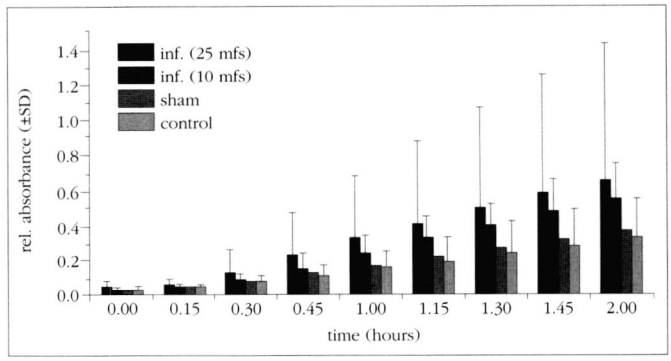

Fig. 2 . – Phenoloxidase activity on *O. ochengi* infected *S. damnosum* s.l.

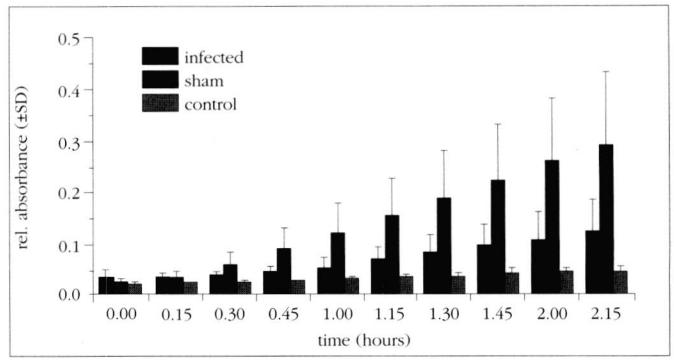

Fig. 3. – Phenoloxidase activity of *O. lienalis* infected *S. ornatum.*

inoculated and control flies did not show any reaction (Fig. 1, lanes 2 and 3). If the blackflies were infected via a blood-meal on *Onchocerca* infected cattle (zebu) the 66 kDa band could be observed (Fig. 1, lanes 6 and 8/control flies lanes 5 and 7). Additional colorimetric *in vitro* assays to monitor the phenoloxidase activity of the same haemolymph samples showed a high activity in those blackflies that had a strong 66 kDa band (Fig. 2).

S. ornatum s.l. : In the haemolymph of European blackflies the 66 kDa band could be detected in *O. lienalis* infected and sham inoculated flies. The intensity was highest in the sham group. Using a modified SDS-PAGE system, the 66 kDa band turned out to represent two proteins of 66 and 64 kDa. However, this could only be detected in the European blackfly species.
Phenoloxidase assays showed a clear correlation between the abundance of the 66 kDa molecule and the enzymatic activity (Fig. 3).

To prove whether or not this molecule represented in fact active phenoloxidase, whole haemolymph was separated on SDS-Page under non-denaturing conditions. The band representing phenoloxidase activity was eluted and run on SDS-PAGE under denaturing conditions. The only band that could be observed had a molecular weight of 66 kDa (Hagen *et al.*, in prep.).

In conclusion it is suggested that the pro-phenoloxidase is activated due to the presence of the parasite. The activated form might act as an opsonin after being deposited on the surface of the parasites, thus bridging between the intruding pathogen and the immune system of the insect. Compatible species like *O. volvulus* in *S. damnosum* s.l. are able to evade this process resulting in a decreased deposition of phenoloxidase hence higher residual phenoloxidase activity *in vitro* accompanied by a greater intensity of the 66 kDa band on the gel.
The factors responsible for the recognition of the parasites by the pro-phenoloxidase activating system have yet to be found. Future work will concentrate on the role of inducible proteases in blackflies.

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