IMMUNOMODULATION OF EXCRETORY SECRETORY MATERIALS FROM THE FILARIAL PARASITE *SETARIA DIGITATA*

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ABSTRACT

ES materials released along with the release of mf showed immunosuppression as evidenced by a decrease in T lymphocyte levels in the immunised animals. Lipid fractions of the mf associated ES materials and protein fractions of the detergent soluble materials were found to cause suppression of immune response in the host. Further study revealed that the suppression associated with the lipid fractions is actually the end result and that mf associated ES materials is actually a mixture of immunosuppressive and immunopotentiating components and the release of the components is most likely regulated.

KEY WORDS : *Setaria digitata*, Excretory Secretory materials, Immunopotential Immunosuppression

Filariasis is a chronic infection in which host and parasite establish a fairly stable relation for many years. In order to survive, the parasite must be able to survive in a sensitised host. Filarial worms are considered to accomplish this by actively stimulating their host's normal regulatory mechanism and at the same time suppressing those immune reactions directed against them. Clear evidence to these speculations including the agents that might be responsible for these phenomena are still lacking. Filariae release a variety of molecules into the host environment, which are collectively called excretory secretory (ES) materials. ES materials released by living organisms have been shown to be highly immunogenic molecules in many nematode infections (1). ES materials most probably arise from a variety of anatomical sources, including specialised structures, such as the intestinal tract and the parasitic cuticle where there is turnover of surface components. They may constitute the majority of ES products. In the case of adult filariae and microfilariae (mf), ES products include a complex mixture of shed surface molecules and materials released during the hatching of embryo. The importance of ES materials from parasites are widely accepted today both in re-

lation to diagnosis and in immunisation in diseases such as Chagas disease, Toxocariasis, Onchocerciasis and Bancroftian filariasis. Working with a model filarial system, the *S. digitata*, Kaleysa Raj and group showed recently that the major source of ES materials is the materials released during the hatching process for the release of mf (2). and that the antigenicity of the ES products is proportional to the development of embryo to mf (3). The main problem in studies using materials of parasites of humans is its nonavailability and hence attention has been turned to heterologous materials derived from similar parasites to different hosts. In fact *S. digitata* is a cattle filarial parasite recommended by WHO as a model system for studies on experimental filariasis.

EFFECT OF HATCHING ASSOCIATED MATERIALS

ES materials for immunological studies were separated from the incubation medium after incubating the *S. digitata* under in vitro conditions in Tyrode medium at 37°C for a period of 4 hr using 2 ml per worm with change by fresh medium at the end of each hr. The separated materials were tested using BALB/C mice as the host. The major fractions used and the maximum effect observed are given in table 1.

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Table 1

Material	Suppression on the 10th day when the effect was maximum (splenocytes)*
Crude ES Material	34
Es Protein fraction	10
Heat inactivated ES material	32
ES lipid fraction.	28
Detergent soluble surface materials	50
Detergent soluble lipid fractions	4
29kD protein fractions from detergent soluble materials	65
Heat inactivated 29kD protein	11
Excretory secretory materials in the presence of DEC	+ 3 (negligible) (In blood)
Excretory secretory materials in the absence of DEC	15

From the results it is clear that the immunosuppression observed with whole ES materials was not due to proteins but due to lipids, as the activity was retained in the heat inactivated sample, but was lost in the delipidated sample. Infact, the lipid extract showed the original activity showed in the crude ES materials. The observation which was originally made by changes in total lymphocyte level was further confirmed by separation into monocytes, B and T cells. The confirmatory studies clearly indicated that in all the cases, the suppression was due to a fall in T cell level (4).

In two separate experiments it could also be shown that the ES materials collected in the presence of diethyl carbamazine, an antifilarial drug known to block the release of mf did not show immunosuppression, but on the other hand showed immunopotentiality and when the ES materials showed suppression, the embryonic fluid showed immunopotentiality (5). Relevant results are given in table 1.

As the immunosuppression observed with ES materials was found associated with the lipid fraction, it was further fractionated to individual components. The fractions separated and studied included free and bound fatty acids, triacyl gly-

cerides, glycolipids, phospholipids and nonsaponified lipids. Of these free fatty acids, triacyl glycerols and glycolipids exerted only very negligible effect on the immune system of the host. Bound fatty acids and nonsaponifiable lipids showed suppression and phospholipids showed potentiation of immune response (5).

EFFECT OF SURFACE MATERIALS

Similar study carried out with the detergent soluble surface components isolated from the intact worms after exposure, on the other hand showed an entirely different pattern. The protein fraction of the surface isolated showed suppression while the lipid fraction did not have effect on the immune system. The surface preparation was further fractionated and the purified protein with a molecular weight of 29 kD was shown to possess all the effect observed with the detergent soluble materials (6). The results obtained with the crude detergent soluble preparations the purified 29kD protein and the heat inactivated materials are also given in table 1. As in the case with ES materials, the suppression (5). observed with the detergent soluble materials was also due to a fall in T cell level.

It is proposed that there is a fine control in the filarial parasite systems causing a switching on and off of the release of materials for the general stimulation of the immune response of the host and for the specific inhibition of the host response against the parasite and thus condition persists until the filarial reactions sets in when the total balance is lost. Perhaps this could be a

general pattern in most tropical diseases.

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