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SHORT COMMUNICATION

Immunogenic proteins in the body and faecal material of the human body louse, *Pediculus humanus*, and their homology to antigens of other lice species

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Induced resistance to lice was observed in mice infected with *Polyplax serrata* (Ratzlaff & Wikel, 1990) and in rats infested with *Polyplax spinulosa* (Volf & Grubhoffer, 1991). Resistance induced to the human body louse, *Pediculus humanus* L. (Phthiraptera: Pediculidae), by immunizing rabbits with an extract of louse midgut, was shown by the lice taking smaller bloodmeals, having higher mortality, laying fewer eggs and taking longer to develop, compared to lice fed on control animals (Ben-Yakir *et al.*, 1994).

Polyclonal antibodies raised against a whole-body extract of the rat louse recognized at least eleven antigenic components by the immunoblotting technique (Volf & Grubhoffer, 1991), whereas in sera raised against body lice, up to nine immunogenic antigens were observed (Ben-Yakir & Mumcuoglu, 1989; Ochanda *et al.*, 1995).

The aim of the present study was to identify the immunogenic proteins of the louse midgut by immunoaffinity chromatography and to search for common proteins in louse midgut, louse faeces and in other louse species in order to isolate large quantities of immunogenic proteins by an easier technique than dissection of midguts.

A strain of human body louse, adapted to feed on rabbits (obtained from the colony at the London School of Tropical Medicine and Hygiene, courtesy of Dr J. W. Maunder, and maintained in our laboratory for the last 11 years), was used. The lice were fed on the shaved abdomen of rabbits four times a week. When not feeding, lice were kept at 30 ± 1°C and 70–80% relative humidity.

The head louse, *Pediculus capitis*, was collected from infested children in Jerusalem using a lice comb. The cattle louse, *Haematopinus africanus*, and the goat louse, *Linognathus stenopsis*, were collected from infested animals in an abattoir near Jerusalem.

Outbred male albino rabbits, weighing 3–4 kg, with no previous ectoparasitic infestation were used.

Batches of 150 female and 150 male lice, 24 h after feeding, were dissected in cold phosphate-buffered saline (PBS) (0.01 M, pH 7.2). Louse midguts were removed, separated from adhering tissues including the mycetome and salivary glands, and sliced in several pieces. The gut contents were removed by three PBS rinses and the pieces were homogenized manually in 0.5 ml of 1% Triton X-100 in PBS. The extract was sonicated and centrifuged at 6000 ***g*** for 15 min at 4°C. The supernatant was removed and sterilized by filtration (pore size 0.2 μm). Louse midgut extract (LME) was adjusted with PBS to give a protein concentration of 1 mg/ml measured by the method of Lowry *et al.* (1951).

Faecal pellets of the human body louse which had been deposited during the previous 15 h were collected from containers in which the lice were kept when not feeding. The faecal material was first separated from other louse particles by sieving (0.2 mm mesh); 0.8 g of faecal material was dissolved in 3.3 ml of 0.01 M PBS, centrifuged at 2000 ***g*** for 5 min, and the supernatant was collected. Rabbit blood, diluted 1:2000 in PBS (protein concentration 300 μg/ml), was used for comparison with faecal extracts by electrophoresis and immunoblotting techniques.

One hundred each of body lice, head lice and cattle lice were homogenized in 1ml PBS, and thirty goat lice were homogenized in 0.3 ml PBS using a Polytron homogenizer (Kinematica, Switzerland) and stirred overnight at 4°C. The homogenates were centrifuged at 6000 ***g*** for 10 min and the supernatant was collected.

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Two rabbits were injected with 500 μg LME proteins three times at 3-week intervals. Freund's complete adjuvant (Difco) was used for the first injection given intramuscularly in both hind legs. Freund's incomplete adjuvant was used for the subsequent injections given subcutaneously in the neck area. Serum samples were collected before immunization and 2 weeks after the last injection. Two rabbits were injected with 500 μg whole-body louse extract and two control rabbits with chicken ovalbumin (Sigma) as described by Ben-Yakir *et al.* (1986).

Rabbit anti-louse IgG titre was determined by the ELISA technique (Voller *et al.*, 1979). Unfed first nymphal lice were homogenized in PBS and the antigen was absorbed directly to the plate at a protein concentration of 1 μg/ml. A blocking treatment was followed with 5% bovine serum albumin in PBS-Tween 20. Serum samples (1:10) were added to the microplates. Goat anti-rabbit IgG conjugated with alkaline phosphatase enzyme (Bio-Makor, Rehovot, Israel) was used at a concentration of 1:500. Absorbance, at a wavelength of 405 nm, was measured 30 min after the addition of the substrate. Antibodies in immune serum were concentrated using the ammonium sulphate precipitation method according to Harlow & Lane (1988). Immunogenic proteins were isolated by coupling antibodies to CNBr-activated Sepharose 5MB beads (Pharmacia) and eluting antigens from an immunoaffinity column according to the method described by Harlow & Lane (1988). Polyacrylamide gel electrophoresis (PAGE, 10%) was carried out in the presence of SDS with a discontinuous buffer system (Laemmli, 1970). A mixture of low molecular weight standard mixture (Sigma) was run with each gel. Fractions separated by gel electrophoresis were transferred to nitrocellulose membranes for immunoblotting using antisera to midgut and whole body extracts as described by Burnette (1981).

The immunization of the rabbits with louse midgut or whole body extracts both induced a high titre (1:10,000) of specific IgG, when tested by the ELISA technique 2 weeks after the last injection of the rabbits. Anti-louse antibodies were not detected in the two control rabbits.

Eight fractions were isolated from the midgut extracts of the human body louse by immunoaffinity chromatography. In fractions 1–6 and 8 no protein bands were detected by 10% SDS-PAGE electrophoresis. However, in fraction 7 three main proteins with a mol. wt of 17 kDa, 29 kDa and 35 kDa were present (Fig. 1).

The electrophoresis of the faecal material and the subsequent immunoblotting using the sera of the rabbits immunized with a midgut extract showed that antibodies recognized eight faecal proteins with a mol. wt of 17 kDa, 21 kDa, 29 kDa, 35 kDa, 63 kDa, 69 kDa, 78 kDa and 86 kDa. In the blood of a control rabbit, only one band with a mol. wt of 63 kDa was observed.

The electrophoresis of the extracts from human body lice, human head lice, cattle lice and goat lice and the subsequent immunoblotting with sera raised against human body lice showed the presence of at least four proteins with a mol.wt of 38 kDa, 39 kDa, 42 kDa and 44 kDa which were common to all extracts (Fig. 2).

Immunization of rabbits with midgut antigens of the human body louse, revealed at least nine immunogenic proteins of relative mol. wt of 17–117 kDa when tested by the Western blot technique (Ochanda *et al.*, 1995). In the present study, three proteins with a mol. wt of 17 kDa, 29 kDa and 35 kDa were isolated from a midgut extract by immunoaffinity chromatography. These proteins were similar to those recognized by the Western blot technique (Ochanda *et al.*, 1995).

In the extracts prepared from louse faecal pellets, eight immunogenic antigens were recognized when sera against louse midgut antigens were used. Four of these proteins have been previously identified in the louse midgut extract (Ochanda *et al.*, 1995). The 63 kDa protein seems to derive from the rabbit blood

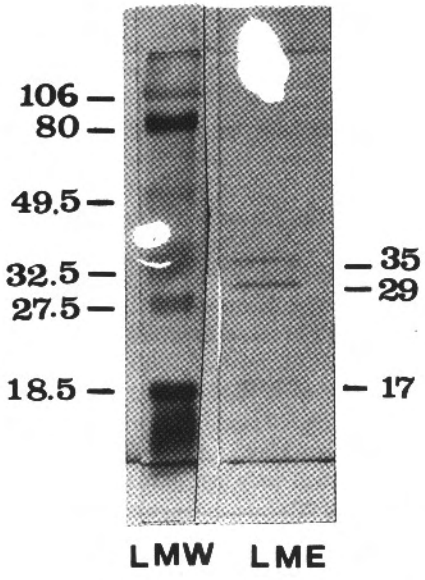

Fig. 1. SDS-PAGE fractionated *P.humanus* midgut extracts (LME) after separation by immunoaffinity chromatography.

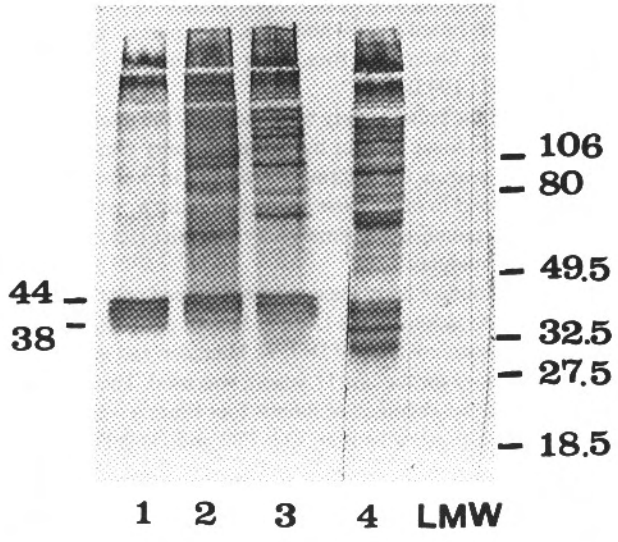

Fig. 2. Immunoblots of SDS-PAGE fractionated *L.stenopsis* (1), *H.africanus* (2), *P.capitis* (3) and *P.humanus* (4) whole-body extracts probed with serum of rabbits immunized with a body louse extract.

ingested by the louse. The others could be polymers of lower molecular weight proteins.

The present results show that faecal material, as well as different lice species, share common antigens. Therefore, simple colonization of human body lice (Culpepper, 1948) could provide the source of antigens for immunization of domestic animals against their specific lice.

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