LOUSE ANTIGENS RECOGNIZED BY RESISTANT HOSTS: IMMUNOBLOTTING STUDIES

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INTRODUCTION

Vertebrate hosts often acquire resistance after being parasitized by hematophagous arthropods. Resistance can also be induced by injecting hosts with antigenic material from the parasite (1). Resistance is mediated, in part, by the host's production of antibodies against components of the parasite. These antibodies affect the parasite deleteriously when they are taken-in with the blood-meal. A successful example of this approach was reported by Kemp et al. (2). A high degree of resistance was induced against the cattle tick, Boophilus microplus, by immunizing cattle with extracts derived from whole adult female ticks. In an in vitro assay, anti-tick antibodies caused damage to tick gut cells even in the absence of other components of the immune system (3). Thus, antibodies from a resitant host can be used to identify antigenic material from the parasite which, in turn, may be employed in producing an anti-parasitic vaccine.

Anaplura, the sucking lice, is an important group of ecto-parasites. Lice cause both irritation and economic loss to man and livestock around the world (4). Lice are permanent ectoparasites which feed on blood several times a day and their feeding habit makes them particularly susceptible to blood-borne control agents.

We induced a high degree of resistance to the human body louse, Pediculus humanus humanus, by immunizing hosts with louse extracts (5). Lice that fed on the immunized hosts took significantly smaller blood meals than those fed on non-immunized hosts. Lice that were reared on hosts that had been immunized with midgut extract suffered the greatest damage and many of them died from rupturing of the gut. In this study we employed antibodies from lice-resistant hosts to identify potentially protective antigenic proteins in lice extract. Protein immunoblotting (6,7) was used for this purpose.

MATERIALS AND METHODS

Lice and host species. A colony of P.h. humanus has been maintained in our department for the past 5 years and was originally obtained from Dr. Maunder at the London School of Hygiene and Tropical Medicine. All lice were fed on rabbits every other day, and when not feeding, they are kept at 30°C and 70% RH. Outbred male New Zealand white rabbits (2-4 kg BW), with no previous exposure to ectoparasites were used. About 500 lice, of all life stages, were fed on each rabbit for 2 weeks in this study.

Antigen preparation. Extracts were prepared from batches of 300 lice (100 females, 100 males and 100 nymphs of all stages; 48 hrs after last blood meal). Louse whole body extract (LWBE) was prepared by homogenizing lice in 3 ml of ice cold 0.01 M phosphate buffered saline pH 7.2 (PBS) using Polytron^{R} (Kinematica, Switzerland). The homogenate was agitated overnight at 4°C to increase the yield of

soluble material from lice fragments. The homogenate was then centrifuged for 10 min at 6,000 g. The supernatant was filtered (pore size 0.2 um) and its protein concentration was determined using the Lowry method (8). LWBE was used for rabbit vaccination and as antigen in radioimmunoassay (RIA) and immunoblotting. Louse midgut extract (LME) was prepared by dissecting out midguts of 300 lice (as used above) in cold louse-saline (9). Midgut tissue was rinsed 3 times with PBS to remove gut content and homogenized in 0.5 ml ice-cold PBS using a glass tissue grinder. Homogenates were treated in the same manner as the LWBE. Extracts were adjusted to protein concentration of 1 mg/ml and stored at -20°C until needed. Ovalbumin (Sigma, St. Louis, USA) was used to immunize the control rabbits.

Immunization and sera collection. Rabbits were injected with 500 µg protein 3 times at 3 week intervals. Freund's complete adjuvant (DIFCO Laboratories, Detroit, USA) was used for the first injection, given intra-muscularly in both hind legs. Freund's incomplete adjuvant was used for the two subsequent injections given subcutaneously in the neck area. Serum samples were collected before immunization, 2 weeks after the last injection and 1 week after lice feeding had terminated. RIA was used to determine the anti-louse antibody titer.

Electrophoresis. LWBE (25 ug/lane) was resolved by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS), with a discontinuous buffer system (10). Stacking gel was 3.5% acrylamide and the separating gel was 7.5% acrylamide. Molecular weight standards (SDS-6H, Sigma, St. Louis, USA) and rabbit IgG (as positive control) were included with each run. Polyacrylamide slab gels (160 x 170 x 1.5 mm) were run at 20 mamp until samples reached the separating gel, at which point the current was increased to 30 mamp; the temperature was kept at 10°C.

Western blotting. Electrophoretic transfer of polypeptides to nitrocellulose membrane (NCM) and subsequent immune localization were performed according to Burnett (7). Blocking was done overnight at 4°C with 5% BSA in PBS containing 0.05% Tween 20. Rabbit serum was used at 1:250 dilution. Rabbit antibodies were localized by peroxidase labelled goat anti-rabbit IgG (Bio-Makor, Rehovot, Israel) at a dilution of 1:400, followed by reaction with precipitable substrate 4-chloro-1-naphthol. Polypeptides transferred to NCM were stained with 0.1% naphthol blue black in 7% acetic acid and 20% ethanol.

RESULTS AND DISCUSSION

Immunization with louse extracts induced resistance in all rabbits with no adverse effects. The anti-louse titers were tested by RIA and were clearly positive even at a dilution of 1:50,000. Hyperimmune serum of rabbits immunized with LWBE reacted strongly with about 10 polypeptides and weakly with 15-20 polypeptides ranging in molecular weights from 20 to 210 KDa (Figure 1). Hyperimmune serum of rabbits immunized with louse midgut extract reacted strongly with a polypeptide at 95 KDa and weakly with a polypeptide

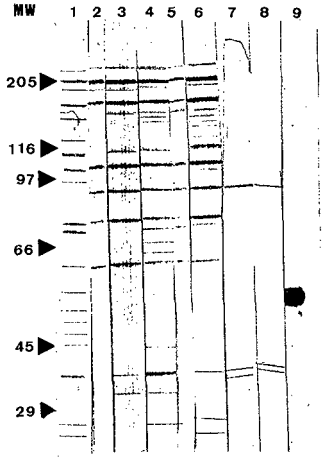

Figure 1. Polypeptide profite and immunoblots of SDS-PAGE fractioned P. h. humanus whole body extracts probed with serum of resistant rabbits.
lane 1. Transferred polypeptides stained with naphtol blue black.
lanes 2 to 6. Used serum of rabbits hyperimmunized with louse whole body extract.
lanes 7 and 8. Used serum of rabbits hyperimmunized with louse midgut extract.
lane 9. Rabbit Immunoglobulin G (3 ug/lane)

at 35 KDa. No rabbit antibodies were detected in the LWBE as indicated by the absence of homologous bands to the rabbit IgG.

In humans, intense skin irritation after repeated feeding of

P. h. humanus was reported by several investigators (1). The best studied interactions between a louse and its host are those of Plyplax serrata and the mouse. Bell et al. (11) proposed that the decrease in louse burden after 3 weeks of infestation, even when grooming was restricted, was due to the development of host-resistance. Ratzlaff (12) investigated the immunological basis for anti-louse resistance, which was found to be mediated by lymphoid cells and manifested most markedly by louse-specific skin hypersensativity. However, he did not find anti-louse antibodies in the serum of resistance mice. In addition, some mice strains did not develop resistance against lice at all. In our laboratory no resistance against P. h. humanus has been noted in rabbits that have been used to feed lice once a week, over several years. Despite this fact, we limited the length of infestation in this study (2 weeks) in order to avoid the possible effects of skin hypersensativity reaction. We did not detect antibodies that react with LWBE in both sera of control rabbits in this study and rabbits used to feed lice for over a year. Although we still lack the direct proof that artificially induced anti-louse antibodies were responsible for the observed resistance, there are strong indications from this and other studies (2,3) to suggest that resistance is mediated by host humoral antibodies.

We are presently conducting immunoblotting studies using antigens from specific louse tissues and organs in order to further localize the origin of the antigens identified in this study. We are planning to raise specific antisera to the 95 and 35 KDa polypeptides and study the effect of feeding lice on these antibodies. We are also studying the cross antigenicity between the human body louse used in this study and the human head louse, which at present is an important public health problem worldwide.

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