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**The recognition of antigens
on the surface of adult and L4 *Necator americanus*
by human and hamster post-infection sera**

D.I. PRITCHARD, J.M. BEHNKE, A. CARR & CATHERINE WELLS

MRC Experimental Parasitology Research Group, Department of Zoology, University of Nottingham, Nottingham NG7 2RD, UK

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Summary The surface antigens of adult *Necator americanus* were recognized by post-infection hamster sera and resolved at molecular weight 93000, 67000, 46000, 43000, 32000 and 25000. L4 larvae in contrast had one major surface antigen, resolving at 93000. These antigens were also recognized by a range of human sera, although on a differential basis. This suggests that the human sera are immunologically relevant to the human disease state, in that infected hamster recognized the full cuticular antigen spectrum of adult *Necator*. This, at least, gives the experimenter a convenient reference point from which to conduct further experiments incorporating parameters such as re-infection, anthelmintic treatment and genetic variability to study the effect of these modifications on the serological response.

Keywords: *Necator americanus*, antigens, cuticle, post-infection sera.

Introduction

The cuticle of nematodes is a dynamic structure, capable of participating in a variety of functions. It is biochemically active, maintaining the homeostasis of the organism (Lumsden 1975, Lee 1977), performs as an elasticated exoskeleton (Inglis 1983) and presumably represents a barrier against immune attack. It is apparent, however, that this barrier can be overcome and this has served to focus attention on the antigenic properties of the nematode cuticle (Philipp & Rumjanek 1984). Little attention has been paid to nematodes which establish as *chronic* infections of the gastrointestinal tract, and the human hookworm *Necator americanus* is a prime example. The present study was initiated to resolve this situation and to determine: (a) whether the immune system of hosts harbouring *Necator* adults is capable of recognizing epitopes expressed at the parasite's surface; and, (b) whether the laboratory model of hookworm disease, the

Correspondence: Dr D.I. Pritchard, MRC Experimental Parasitology Research Group, Department of Zoology, University of Nottingham, Nottingham NG7 2RD, UK.

hamster, can be considered relevant to the human situation with regard to the recognition of surface antigens.

Materials and methods

PARASITOLOGY

The infective larvae of *N. americanus* were obtained from Dr G. Rajasekariah in 1983 and were from a strain maintained in laboratory hamsters for 69 generations in India. During the course of this work the parasite was passaged a further five times in Nottingham and larvae obtained from these infections have been described by Sen & Seth (1967) and Behnke, Paul & Rajasekariah (1985). Hamsters 2–3 days old were infected percutaneously with 70–150 L3 and the infectivity of the inocula used was determined by worm counts within 3 weeks of infection. L4 and adult worms were hand-picked from the intestines of infected hamsters 17 and 35 days following infection respectively.

ANALYSIS OF ANTIGENS

Worms were labelled with ^{125}I using Iodogen and antigens and immunoprecipitates analysed by SDS-PAGE as described previously (Pritchard *et al.* 1984). Briefly, radiolabelled parasites were homogenised in the presence of protease inhibitors and centrifuged for 30 min at 11 000 g (4°C). Supernates were routinely assayed for labelling efficiency and counts were always between 40–58% TCA precipitable. Antigens and immunoprecipitates were run on slab gels consisting of a 12% running gel and 3% stacking gel. Dried gels were autoradiographed using Kodak X-OMATS film at –80°C.

SOURCE OF SERA

Hamster sera were obtained as shown in Table 1., Human sera were obtained from hookworm infected individuals in the Gambia (P. Hagan) and Calcutta (G. Schad). Normal sera were taken from non-infected controls.

Results

HAMSTER SERA

The different batches of *N. americanus* larvae used in the present work were infective, with 50–70% of the administered dose being recovered within 3 weeks of infection. In all four experiments faecal egg counts were monitored in selected individuals from 5 weeks post-infection when eggs first appeared in the faeces until approximately 20 and 30 weeks in female and male hamsters respectively, when eggs could no longer be detected.

Using immune sera from infected hamsters to immunoprecipitate Iodogen-labelled adult *Necator* (SDS-PAGE analysis), it was revealed that the parasite possessed at least six distinct antigens accessible to surface-labelling and that these resolved under reducing conditions at mol wt. approximately 93 000, 67 000 (doublet), 46 000 (doublet), 43 000,

32000 and 25000 (Figure 1). The immune response was qualitatively the same along a time course following infection in that each of these polypeptides was recognized although the degree of recognition increased during the course of infection. This was reflected in the number of ct/min precipitated (Table 1) and, consequently, the intensity of the autoradiography. Sera taken from male and female hamsters recognised the same epitopes on the parasites' surface (Figure 1). It was also interesting to note that there appeared to be an antigen specific to adult female worms, resolving at mol.wt approximately 25000 (Figure 1-arrowed) and that the response to female worms was more pronounced overall.

The recognition of adult surface antigens by post-infection hamster sera was confirmed using pooled sera taken 95, 108 and 125 days after infection (Figure 2—lanes 4, 6, 8). In addition, a major cuticular antigen (93000 mol wt.) was demonstrated on *L4*

Table 1. The efficiency of labelling of and ct/min. precipitated from *Necator americanus* surface antigens, by hamster sera taken along a time course from four different experiments

Experiment No (sex of hamsters)	Day sera taken	Dose of infection	ct/min precipitated	
			Adult male <i>Necator</i>	Adult female <i>Necator</i>
1	117	70	51 880	61 402
2	21	70	776	5298
2	28		2683	19191
3 (male)	24		584	5727
3 (female)	24	150	306	2734
3 (male)	38		2740	6409
3 (female)	38		7871	4616
3 (male)	52		14095	28 121
3 (female)	52		15882	15037
4	4	100	291	2849
4	10		1594	4005
4	14		1800	18418

Necator americanus males (58% TCA pptbl. 111 329 ct/min—10 μ l pptd) and females (53% TCA pptbl. 190 132 ct/min—10 μ l pptd) were homogenized in 10 mM Tris, pH 8.0 containing the protease inhibitors TPCK (50 μ g/ml), TLCK (25 μ g/ml), PMSF (1mM) and 1% (w/v) sodium deoxycholate. Homogenates were cleared and 10 μ l samples of the supernates taken for determination of the incorporation of 125 I into proteinaceous material. Antigens and immunoprecipitates were then analysed by SDS-PAGE as shown in figure 1.

The *L4* antigen used in later studies was 40% TCA pptbl. 21 329 ct/min—20 μ l pptd.

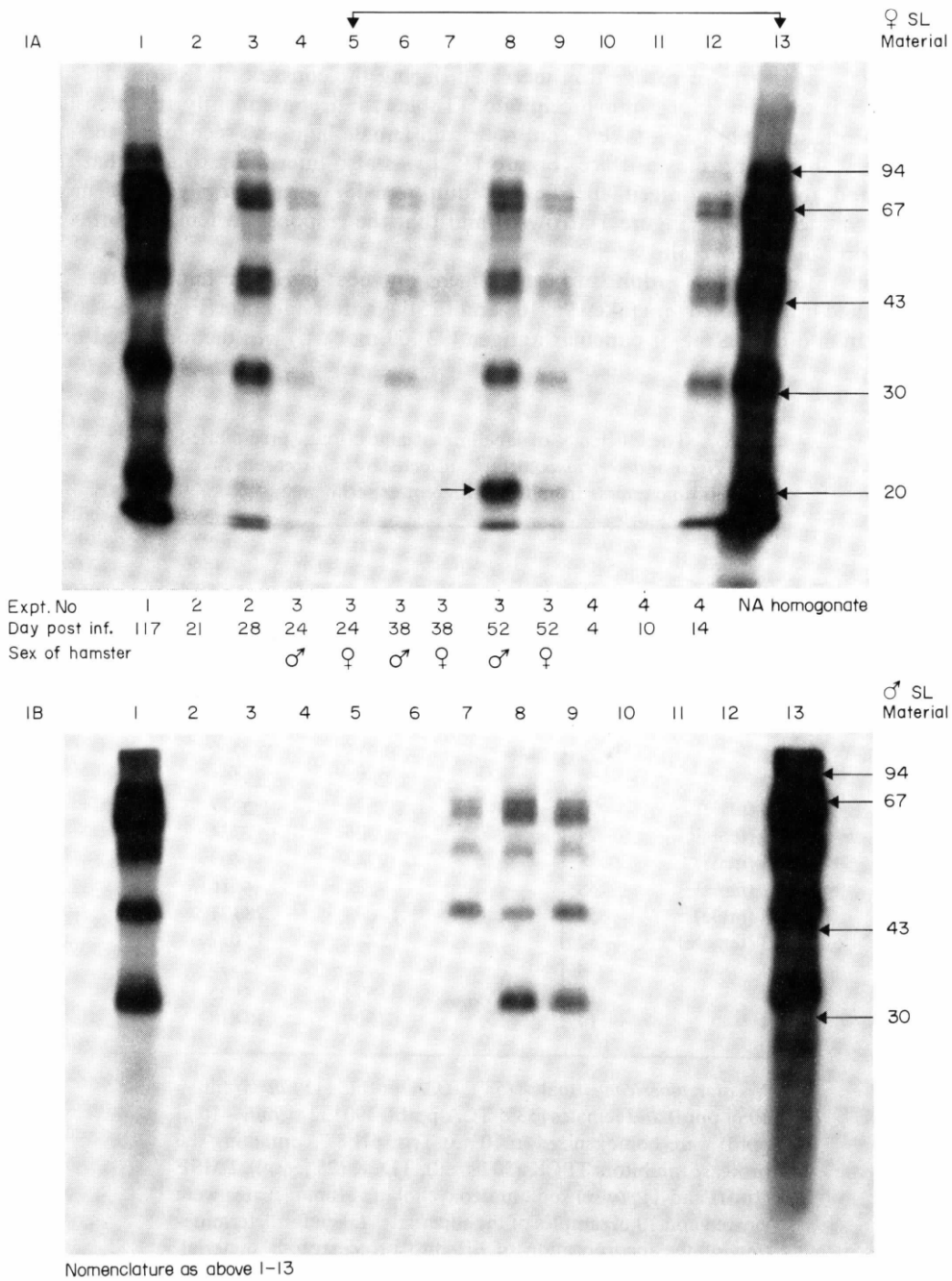


Figure 1. SDS-PAGE profile of the surface antigens of adult *N. americanus* as recognized by post-infection hamster sera. a. female Necator; b, male Necator. Surface labelled material (lane 13) was immunoprecipitated using post-infection hamster sera from four separate experiments (Exp. 1, 2, 3 and 4) and male and female hamsters (see Table 1 for details). The profile of these immunoprecipitates is shown in each of these figures in lanes 1-13. N.A.HOM = total surface labelled material.

larvae using these sera (lanes 3, 5, 7). However, the other major cuticular polypeptide (mol wt. 41000) did not seem to be precipitated under the conditions used in this experiment (lanes 1, 12) (see Discussion Section).

HUMAN SERA

A sample of serum taken from a hookworm positive patient (courtesy of Dr P. Hagan, MRC, Gambia) was also shown to react with the major cuticular antigens of adult *Necator* (Figure 2—lane 10) whilst normal human serum was negative (lane 9). Lane 11 shows the reactivity of 117 day hamster sera for comparison, and the total surface polypeptide profile of each stage is shown in lanes 1, 2 and 12, 13.

Analysis of a greater number of sera from hookworm positive patients (from Calcutta—courtesy of Professor G. Schad) revealed that each was reactive against surface antigens (Figure 3—lanes 3–6 and 8–10) albeit on a differential basis. Hamster sera taken 100 and 117 days post-infection (lanes 1,2) were again shown to react against the full spectrum of surface antigens (shown in lane 7).

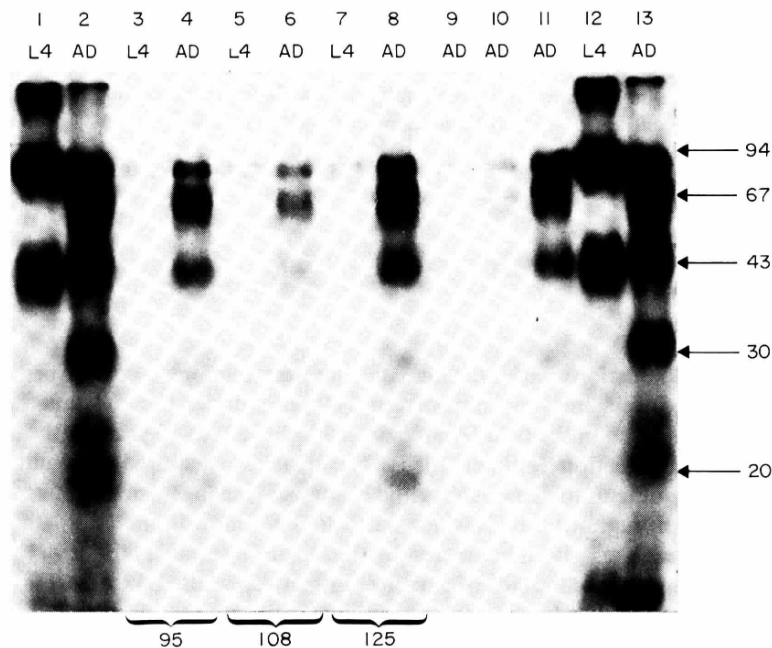


Figure 2. SDS-PAGE profile of the surface antigens of adult and L4 *N. americanus* as recognized by post-infection hamster and human sera. Pooled surface labelled material from L4 (lanes 1, 12) and adult (lanes 2, 13) *Necator* was immunoprecipitated using post-infection (days 95, 108, 125) hamster serum (lanes 3–8), normal human serum (lane 9) and post-infection human serum (lane 10). Lane 11 shows the reactivity of 117 day hamster serum for comparison. The control lane for L4 antigens (normal serum) is not shown in this figure but was of equivalent intensity to lane 9.

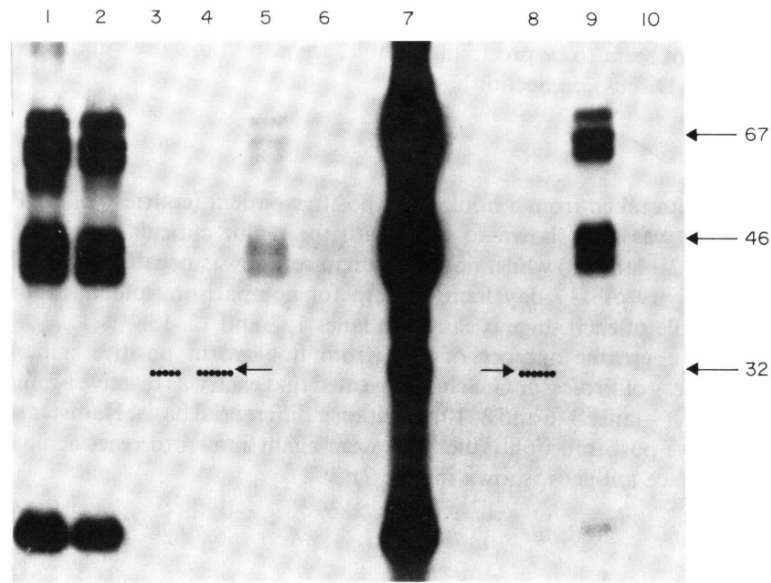


Figure 3. The differential recognition of surface antigens of adult *N. americanus* by a panel of human sera. Pooled surface-labelled material from adult *Necator* (lane 7) was immunoprecipitated using post-infection human (lanes 3–6, 8–10) and hamster (lanes 1, 2) sera.

Discussion

The longevity of infections with *N. americanus* in man remains a controversial subject with reports varying in their estimates from 2–4 years under the constant reinfection conditions encountered in the field (Anderson & May 1982) to 15 years in the case of an experimental self infection (Palmer 1955). Nevertheless, acquired immunity against *Necator* has been difficult to demonstrate and humans living in endemic areas may expect to carry worms throughout life (Nawalinski, Schad & Chowdhury 1978). In addition, studies of the disease have been hampered by the lack of a suitable laboratory model for immunological studies and the lack of parasite antigen, especially L4 stages. However, the hamster has been recognized as a suitable candidate for parasite maintenance (Sen & Seth 1967, Ogilvie, McLaren & Worms 1975, Behnke, Paul & Rajasekariah 1985) and from the results described in the present study it would appear that the model may have some immunological relevance also.

The serological response to *Necator americanus* in humans is well documented (Otto, Schugam & Groover 1942; Lewis, Salimonu & Osunkoya 1978, Ball & Bartlett 1969, Schad, Soulsby, Chowdhury & Gilles 1975, Ogilvie *et al.* 1978) and includes antibody responses against L3 and adult stages. In the present study, the full spectrum of adult

cuticular antigens recognized by the infected hamster was also recognized by a range of human sera, adding support to the relevance of the model to the human situation. Therefore this infers that the laboratory strain of *N. americanus*, which has been maintained for 74 generations in hamsters, still retains major cuticular antigens recognized by humans infected with field strains of this parasite. However, the alteration or deletion of surface antigens with possible retention of conserved determinants remains a possibility. The experimental model also has the advantage of being under the control of the investigator, and despite the relatively short period of parasite patency encountered in the hamster model compared with man, the model has much to offer in terms of providing life cycle stages hitherto unavailable for research and an opportunity to analyse host-protective responses.

The major adult cuticular antigens recognized by the immune system resolved at mol wt. 93000, 67000, 46000, 43000, 32000 and 25000, although the latter might be peculiar to female parasites. The differential recognition of surface antigens by sera from hookworm positive patients could reflect differences in the degree of longevity of infection and accompanying parasitological and clinical data will become increasingly important as these studies progress. Cross-reactivity with other parasites and the genetic heterogeneity of the host population are also parameters which will warrant close scrutiny.

To consider the antigens themselves in greater detail, it is relevant to note that a mol wt. antigen 67000 is also produced in measurable amounts by adult *Necator* during *in vitro* culture (Carr & Pritchard, unpublished), indicating that the cuticle of *Necator* could be involved in both antigen presentation and secretion in a manner similar to that described by Maizels, de Savigny & Ogilvie (1984). In this work, *Toxocara canis* larvae were shown to shed 25% of their surface antigens in 1 h of culture (an estimated 200 µg of ES protein/larva/day). Support for a cuticular contribution to ES comes from the demonstration that adult *Necator* sheds significant quantity of its surface antigen into the surrounding medium during (*in vitro*) culture (Pritchard *et al.* 1986).

Alternatively, ES antigens may be adsorbed onto the surface. Such a mechanism has been suggested to be operative for an excretory-secretory antigen of *Trichinella spiralis* (Silberstein & Despommier 1985) on the basis of reactivity of defined monoclonal reagents against ES, the cuticular surface and the lining of the gut.

It will also be interesting to determine whether epitopes resolving around mol.wt 32000 bear any resemblance to the major accumulated antigens of adult *Necator* encountered by Western blotting (30000–35000 mol wt.—unpublished) and the proteolytic enzyme (37000) recently described in *Ancylostoma caninum* by Hotez *et al.* (1985). Finally, the recognition of an antigen epitope at 93000 on L4 larvae by long-term infection sera paves the way for studies on antigenic homology between larval and adult stages. It is also worth noting that the failure of long term infection sera to precipitate the 41000 surface antigen of L4 larvae is consistent with the observation that only short term (day 17) post-infection sera recognized a 42000 L4 stage specific ES product (Carr & Pritchard—unpublished). Monoclonal reagents should reveal any homology which might exist between ES products and cuticular epitopes on both L4 and adult stages.

Therefore, in conclusion, it is felt that the results described above indicate that the hamster model bears immunological relevance to the human situation, at least as far as adult surface antigens are concerned. However, a major requirement to complete these studies in the provision of human post-infection sera with accompanying parasitological and clinical data.

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