Brief Definitive Report

IMMUNE RESPONSE TO STAGE-SPECIFIC SURFACE ANTIGENS OF THE PARASITIC NEMATODE *Trichinella spiralis*

By MARIO PHILIPP,* PATRICIA M. TAYLOR, R. M. E. PARKHOUSE, and BRIDGET M. OGILVIE‡

From the Divisions of Parasitology and Immunology, National Institute for Medical Research, Mill Hill, London NW7 1AA, England

The cuticle of parasitic nematodes has long been known to be a target of the host immune response (1). Infective and newborn larvae of *Trichinella spiralis* are killed in vitro when eosinophils interact with the parasite surface in an antibody-dependent manner (2–5). This antibody-mediated eosinophil adherence assay has been used to show that rats infected with *T. spiralis* mount a stage-specific antibody response to the parasite surface (2). Furthermore, the immune responses leading to host protection in rats and mice are also stage specific (6–8).

For these reasons, the surface proteins of the various stages of *T. spiralis* were recently characterized by a combination of biochemical and immunological procedures (9, 10). Interestingly, we found that the pattern of iodine-labeled surface proteins for each stage was not only restricted in number, but also characteristic of each stage. None of the surface proteins expressed by infective, intestinal, and newborn stages were identical. They differed either in their apparent molecular weight or in binding properties to lectins. As the surface proteins were antigenic in infected rats and mice, it seemed possible that the biochemical differences that were found could be the structural counterpart of antigenic differences among the surface antigens of each stage.

In this paper, we present a study of the serum antibody response to these surface antigens during the course of a primary infection with *T. spiralis* in rats. This study was complemented with cross-adsorption experiments in which sera containing antibodies to two or more stages were adsorbed with living worms of one stage, and tested for reactivity with surface antigen preparations of the homologous and heterologous stages.

We found that antibodies recognizing the surface antigens of one stage do not recognize those of other stages. In addition, the kinetics of appearance of these antibodies and antibodies mediating eosinophil attachment to the parasite surface (2) are remarkably parallel. Hence, during a primary infection with *T. spiralis* in rats, there is an absence of immunological cross-reactivity among the characterized surface antigens of each stage. Furthermore, these could be the targets for antibody-mediated cell adherence to and killing of the parasite.

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‡ Present address: The Wellcome Trust, 1 Park Square West, London NW1 4LJ, England.

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Materials and Methods

Parasites. Infective larvae and intestinal worms were obtained from infected Parkes mice and Sprague-Dawley rats, respectively, as previously described (4). Newborn larvae were collected from intestinal worms (4) but fetal calf serum was not added to the collecting medium. About 10^4 infective larvae, 10^4 intestinal worms, and 10^5 newborn larvae were routinely used for iodination.

Iodination and Detergent Solubilization of the Parasites. Iodination and solubilization of the worms in sodium deoxycholate were carried out as previously described (10), but newborn larvae were disrupted by sonication and not by homogenization. The soluble fractions contained 80–90% of the total radioactivity taken up by infective larvae and intestinal worms, and 50–70% of the radioactivity taken up by newborn larvae. Trichloracetic acid precipitability ranged from 70 to 90%.

Immunoprecipitation and Gel Analysis of Radiolabeled Surface Proteins. Serum samples were collected sequentially between days 1 and 40 of a primary infection in August rats given 4,000 *T. spiralis* infective larvae orally. 5 μl of the appropriate serum and 10^4–10^6 cpm of the detergent extract of radiolabeled worms were diluted to 100 μl with 10 mM Tris-HCl buffer, pH 8.3. The mixture was incubated overnight at 4°C and then an excess (50–200 μl) of a rabbit anti-rat $(Fab')_2$ antiserum was added. Precipitates were allowed to form for 4 h at 37°C and were then centrifuged, washed, counted, and electrophoresed in sodium dodecyl sulphate polyacrylamide gels (10).

Adsorption of Sera on Living Worms. Serum was taken from Wistar rats 389 d after they had been given a primary infection of 4,000 infective larvae orally. Intestinal worms were harvested from rats 48 h after infection and washed in phosphate-buffered saline. 100 μl serum was added to 100 μl of packed worms. The mixture was agitated and then left overnight at 4°C. Sera were adsorbed twice more with fresh worms and then used for immunoprecipitation of various surface antigens. The same procedure was followed for adsorption with infective larvae.

Results and Discussion

The composition of each of the three labeled antigen preparations is presented in Fig. 1, in confirmation of previous work (9). Two main conclusions can be drawn from the gel patterns: first, the number of iodine-labeled surface components for any stage is small; second, the pattern for each individual stage is different from all of the others. The apparent molecular weights of the different components on reduced gels are: 47,000, 55,000, 90,000, and 105,000 for the infective larvae; 20,000, 33,000, 40,000, and 56,000 for the adult worms; and 28,000, 30,000, 58,000, and 64,000 for the newborn larvae. The 56,000 molecule of the adult worms, unlike the similar-sized component of the infective larvae, binds to lentil-lectin. It is interesting that it appears on 1–2 d-old intestinal worms and disappears gradually thereafter (9; Fig. 1, insert). 2-d-old worms were used in the adsorption experiment described later.

The primary antibody response to different surface antigens was studied by the coprecipitation procedure outlined in Materials and Methods.

Three major points emerged from the data. First, the antibodies precipitating all the labeled surface proteins of adult and infective larvae clearly appeared ~5 d before antibodies to surface antigens of newborn larvae. For example, at day 11, when the response to newborn larvae was undetectable, the circulating antibody level to the other two stages was over half the maximum level reached in the first 40 d of infection (Fig. 2). This clearly indicates that there are no shared antigenic determinants between the surface proteins of newborn larvae and the other two stages. Alternatively, shared antigenic determinants may exist but may not be recognized during a primary infection in rats. The second point is that from day 12 onwards, the concentration of antibody to surface antigens of infective larvae and adult worms changed reciprocally,

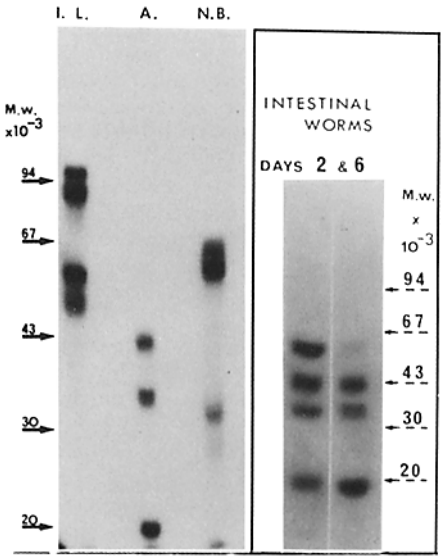

FIG. 1. Radiolabeled surface antigens of three stages of *T. spiralis*: infective larvae (I. L.), adult intestinal worms (A.), and newborn larvae (N. B.). Insert: comparison of surface antigens of intestinal worms 2 and 6 d after infection.

and thus total cross-reactivity between the antigens of these two stages is excluded. An absence of cross-reactivity can, however, be concluded from the finding that adsorption of immune rat sera with intestinal worms removed antibodies to their surface antigens as expected, but not to infective larvae surface antigens (Fig. 3). Conversely, adsorption of sera with infective larvae removed only antibodies to surface antigens of this stage but not to the adult worm surface components (data not shown).

The third point comes from electrophoretic analysis of immune precipitates. Basically, the finding was that whenever antibody to the surface antigens of a given stage was detectable, all the different electrophoretic components were precipitated (Fig. 2). Therefore, immune recognition of all the different surface antigens of a given stage must occur simultaneously. The relative intensity of the response to components of a given set of surface antigens may change during the course of infection. Thus the intensity of the 58,000 and 64,000 mol wt newborn larvae bands increased in relation to the 28,000 and 30,000 mol wt components (Fig. 2A, insert).

The appearance of antibodies to the surface measured by precipitation of labeled surface antigens was compared with the appearance of antibodies to the surface, measured by eosinophil adherence (2; Fig. 2). With the exception of the later portion of the response to infective larvae, the two curves are remarkably parallel, which suggests the possibility that identical antigens are being detected in both assays. The divergence of the two curves during the later response to infective larvae is most reasonably explained by a shift in immunoglobulin classes produced, from largely eosinophil-adherent to eosinophil nonadherent, during the course of infection.

We have shown here that the surface proteins of *T. spiralis* are antigenically stage specific, and it has been shown by others that the immune responses that protect rats and mice from reinfection are also stage specific (6–8). That this should be more than a coincidence remains to be proven, although there is circumstantial evidence that

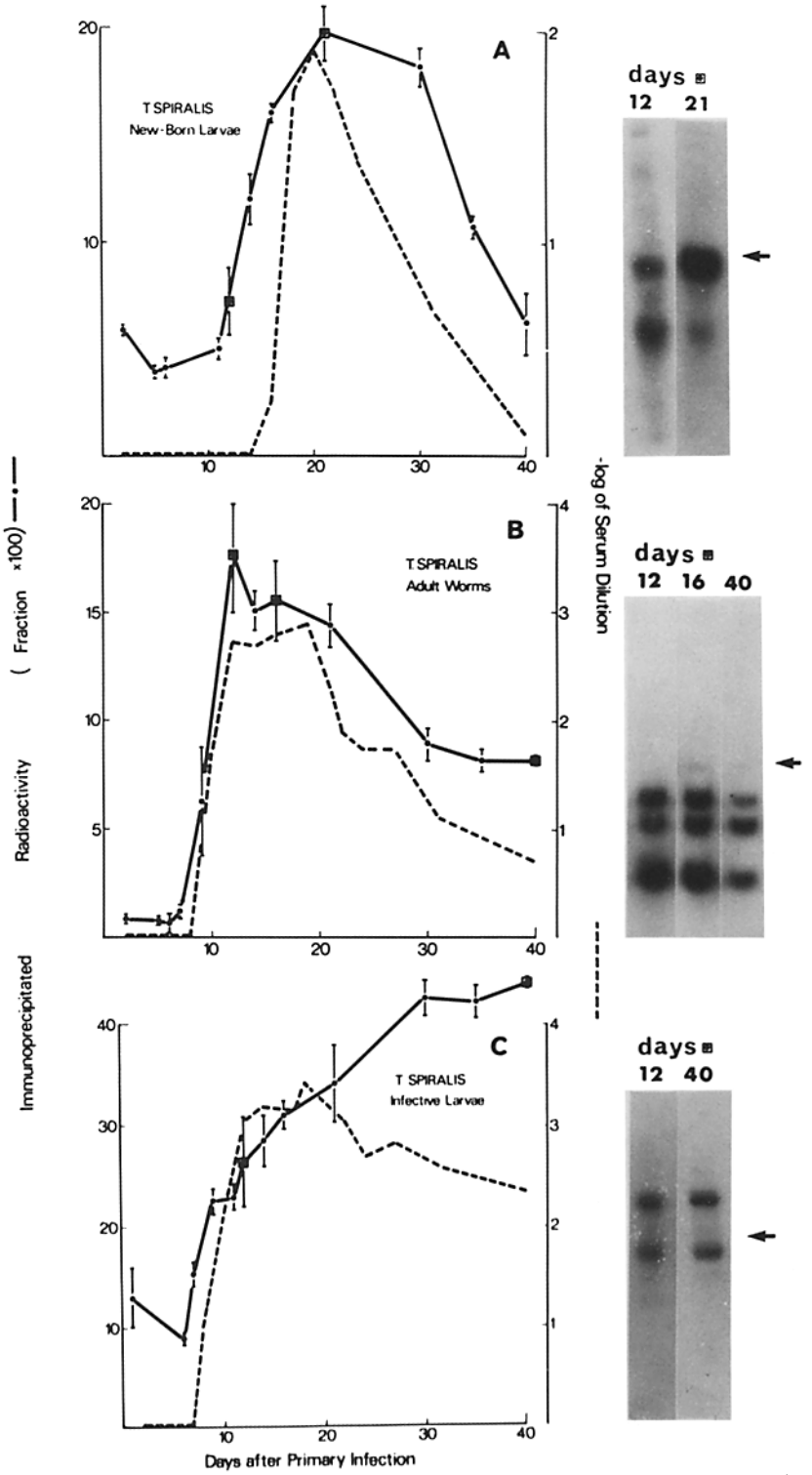

FIG. 2. Time-course of appearance of antibodies to the surface of *T. spiralis* in sera of primary infected rats. Solid line: measured by immunoprecipitation of radiolabeled surface antigens. Dotted line: measured by eosinophil adherence to the parasite surface (2). Insert: surface antigens of different stages precipitated by sera taken at days 12 and 21 (newborn larvae, A); days 12, 16, and 40 (adult worms, B); and days 12 and 40 (infective larvae, C). The arrows indicate the position of a 67,000 mol wt standard protein.

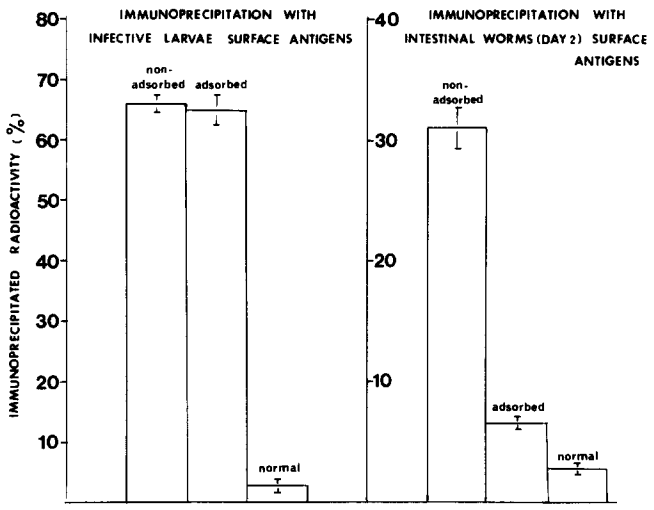

FIG. 3. Immunoprecipitation of radiolabeled surface antigens of infective larvae and intestinal worms (day 2), with serum of infected rats adsorbed on living intestinal worms (day 2), the same unadsorbed serum, and with normal uninfected rat serum. All antigens of each stage were precipitated with both the adsorbed and unadsorbed sera, the total amount precipitated changing as shown in the figure.

suggests that surface antigens could be involved in stimulating host protection. Thus, material released in vitro by adult and infective stages, partially protects mice from reinfection (11). Such material contains, among others, proteins released from the surface of the worms (9, 10). Therefore, although components isolated from the stichosome, a secretory organ of infective and adult stages, have been shown to stimulate protective immunity in rodents (12), there could be a similar and perhaps complementary role for antigens that originate at the worm surface.

Summary

Rats were infected with the nematode *Trichinella spiralis* and the primary serum antibody response to antigenic surface proteins of infective larvae, intestinal worms, and newborn larvae was studied. 1 wk after infection, the sera contained antibodies to surface antigens of both infective larvae and intestinal worms. These early sera, however, failed to react with newborn larvae surface antigens. In addition, adsorption of sera with living intestinal worms or infective larvae removed antibodies to surface antigens of the homologous stage only. Finally, the time-course of appearance of antibodies to the surface antigens mirrored the time-course of appearance of antibodies that mediate eosinophil adherence to the surface of each stage of the parasite.

We concluded that in a primary infection in rats, the surface proteins of *T. spiralis* used in this study are antigenically stage specific. Furthermore, they could be targets for the stage-specific, antibody-dependent eosinophil-mediated destruction of this parasite, known to occur in vitro.

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References

1. Ogilvie, B. M., M. Philipp, M. Jungery, R. M. Maizels, M. J. Worms, and R. M. E. Parkhouse. 1980. The surface of nematodes and the immune response of the host. *In* The Host-Invader Interplay. H. Van den Bossche, editor. Elsevier-North Holland Biomedical Press, Amsterdam. 99.
2. Mackenzie, C. D., P. M. Preston, and B. M. Ogilvie. 1978. Immunological properties of the surface of parasitic nematodes. *Nature (Lond.)*. **276:**826.
3. Kazura, J. W., and D. I. Grove. 1978. Stage-specific antibody-dependent eosinophil-mediated destruction of *Trichinella spiralis*. *Nature (Lond.)*. **274:**588.
4. Mackenzie, C. D., M. Jungery, P. M. Taylor, and B. M. Ogilvie. 1980. Activation of complement, the induction of antibodies to the surface of nematodes and the effect of these factors and cells on worm survival *in vitro*. *Eur. J. Immunol.* **10:**594.
5. Kazura, J. W., and M. Aikawa. 1980. Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil mediated destruction of newborn larvae *in vitro*. *J. Immunol.* **124:**355.
6. James, E. R., and D. A. Denham. 1975. Immunity to *Trichinella spiralis* VI. The specificity of the immune response stimulated by the intestinal stage. *J. Helminthol.* **49:**43.
7. James, E. R., A. Moloney, and D. A. Denham. 1977. Immunity to *Trichinella spiralis*. VII. Resistance stimulated by the parenteral stages of the infection. *J. Parasitol.* **63:**720.
8. Bell, R. G., D. D. McGregor, and D. D. Despommier. 1979. *Trichinella spiralis*: mediation of the intestinal component of protective immunity in the rat by multiple phase-specific anti-parasitic responses. *Exp. Parasitol.* **47:**140.
9. Philipp, M., R. M. E. Parkhouse, and B. M. Ogilvie. 1980. Changing proteins on the surface of a parasitic nematode. *Nature (Lond.)*. **287:**538.
10. Parkhouse, R. M. E., M. Philipp, and B. M. Ogilvie. 1981. Characterization of surface antigens of *Trichinella spiralis* infective larvae. *Parasite Immunol. (Oxf.)*. In press.
11. Vernes, A. 1976. Immunization of the mouse and mini-pig against *Trichinella spiralis*. *In* Biochemistry of Parasites and the Host-Parasite Relationship. H. Van den Bossche, editor. Elsevier-North Holland Publishing Co. Amsterdam. 319.
12. Despommier, D. D. 1974. The stichocyte of *Trichinella spiralis* during morphogenesis in the small intestine of the rat. *In* Trichinellosis. C. Kim, editor. Intext Educational Publishers, New York. 239.