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The Immune Response of Congenitally Athymic (Nude) Mice to the Intestinal Nematode *Nippostrongylus brasiliensis*[1] (38412)

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With the exception of a few intensively studied host–parasite systems, there is relatively little known regarding the mechanisms mediating functional immunity to parasitic helminths (1). In recent reviews on immunity to parasitic helminths, Ogilvie and Jones (2) and Kelly (3) have emphasized the need for systematic studies of naturally acquired host resistance to helminthic infections. Many observations have been made on immunoparasitological phenomena in a variety of host–parasite systems (4, 5). However, the number and complexity of antigens of individual helminths, the relative lack of adequate *in vitro* correlates, and the complexity of helminth life cycles all have contributed to the relative paucity of comprehensive studies on the host immunological response to helminthic infections.

One system which has received intensive study is the immune response of rats to the intestinal nematode *Nippostrongylus brasiliensis* (2, 6). It has been established that the immunological rejection of adult *N. brasiliensis* from the intestine of rats requires at least two separate and sequential steps. The first is the action of antibody on worms (7), which results in severe gut cell structural degeneration (8) and changes in the isoenzymes of acetylcholinesterase and acid phosphatase of the worm (9). Step 2 is lymphocyte dependent (10, 11) and does not occur until worms have been damaged by antibody (12). Recently, it was observed that a third undefined cellular component of bone marrow origin was necessary for worm expulsion (13).

Mice generate active immunity to *N. brasiliensis* even more rapidly than rats, as evidenced by a worm expulsion time of 10–12 days post-larval-inoculation, compared with 12–14 days in rats. Administration of homologous antiserum to rats causes an accelerated expulsion of worms, but the same treatment of mice does not accelerate worm expulsion even though worm damage occurs earlier in passively immunized mice than in controls (14). Although step 1 of the immune mechanism occurs in mice presumably much the same as in rats, the lack of accelerated worm expulsion in passively immunized mice remains an enigma. It is our long-term objective, therefore, to systematically examine the immune response to *N. brasiliensis* and other parasitic helminths by utilizing the congenitally athymic (nude) mouse host as an aid in defining those factors necessary for worm expulsion. We report here that *N. brasiliensis* is not expulsed from nude mice during the lifetime of the mice. However, the expulsion mechansim is generated in nudes which have received thymus implants. The nude mouse, therefore, is an ideal host for use in determination of those factors required for protective immunity to helminthic infection.

Materials and Methods. Animals. Congenitally athymic mice, hereafter designated nude, and their phenotypically normal littermates (NLM) were selected from a stock which has been backcrossed into the BALB/c strain.

Thymus implants. Recipient nude mice, 4 weeks old, were each implanted in the axillary region with thymus glands from two neonatal BALB/c donors. To confirm thymic function, the responses of mice to allografts of CBA skin, grafted as previously described (15), and a single

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intraperitoneal injection of 0.2 ml of a 20% suspension of sheep erythrocytes (SE) were determined. The magnitude of the response to SE was determined 5 days after immunization by a slide modification (16) of the localized hemolysis in gel technique.

Preparation and administration of larvae. Third-stage larvae (L_3) of a mouse-adapted strain of *N. brasiliensis* were obtained from feces–charcoal cultures (17). Larvae were filtered through cellulose tissue (Kimwipes Type L-900, Kimberly-Clark Corp., Neenah, WI) in a standard Baermann apparatus, washed thoroughly in normal saline, and counted by a dilution technique. Each mouse was inoculated subcutaneously in the inguinal region with 300 L_3 on Day 0 of each experiment.

Estimation of fecal worm egg counts and worm burdens. The number of worm eggs per gram of feces (EPG) was estimated by using a modified McMaster technique (18). Total worm counts were made on intestinal contents at necropsy. Intestinal tissue was pressed between glass plates and examined for attached or embedded worms.

Results. In four experiments, a total of 26 nude mice inoculated with 300 L_3 maintained high worm egg counts (usually $>$ 10,000 EPG) throughout their lifetime, whereas NLM egg counts usually dropped to low levels (below 1000 EPG) prior to Day 10. Results from a representative experiment are shown in Fig. 1A. Nude mice died and equal numbers of NLM were sacrificed at intervals from Day 15 to 55 of the experiments. Adult worm burdens averaged 52 ± 23 (SD) in nude mice; no worms were found in any of the NLM controls.

Preliminary studies using nude mice bearing long-standing ($>$ 5 months) thymus implants indicated that worm egg production ceased on about Day 12 as it did in NLM. To confirm and extend this observation, six nude mice were given thymus implants on Day -30. On Day 0, these mice, seven nude mice, and six NLM were inoculated with 300 L_3. Worm egg production in nudes remained about 15,000 EPG for the duration of the experiment whereas it dropped rapidly in thymus-implanted (TI) nude and NLM mice (Fig. 1B). At necropsy, an average of 1.7 ± 2.9 worms was recovered from TI nudes as compared with a burden of 69 ± 54 worms from nudes. Again, no worms were found in any NLM. To confirm generation of thymic function in TI nudes, skin allografts were made on all mice on Day 21. Five days after grafting, healing in was accomplished in all mice. The grafts of NLM and TI mice had become indurated and inflamed by Day 10 postgrafting and by Days 12–13 rejection was complete. At no time did grafts on nude mice show signs of rejection, and the grafts remained healthy for the lifetime of the recipient. On Day 28, all of the mice were injected with SE. On Day 33, the PFC response of nude mice and NLM was 33 ± 26 and 517 ± 277 PFC/10^6 spleen cells, respectively. Thymus-implanted mice responded with 283 ± 155 PFC/10^6 spleen cells.

Discussion. These data present direct evidence for the thymus dependency of *N. brasiliensis* explusion from mice and indicate the potential usefulness of the nude mouse, compared with other hosts rendered immunodeficient by chemical and/or physical means, for studies on mechanisms of immunity to helminthic infections. Previous studies have been carried out on the thymus dependency of resistance to helminths and protozoans (5). However, the immunosuppressive techniques used, such as X-irradiation and splenectomy, were considered by Targett (5) to have had nonspecific effects on systems in addition to those determining the immune response and, therefore, could have affected parasite burdens nonspecifically. Because the immune response of nude mice to *N. brasiliensis* is naturally impaired, the expulsion mechanism may possibly be generated by injection of specific antisera or sensitized cells without the interference of an acquired immune response. Experiments currently underway in our laboratories should define the thymus dependency of both steps 1 and 2 of the worm expulsion mechanism in mice. Extensive work with rats infected with *N. brasiliensis* lends strong evidence for the thymus dependency of step 2, but evidence for thymus dependency of antibody-mediated worm damage (step 1) is lacking (2). With systematic selective reconstitution *i.e.*, with antisera of a specific class and/or selected lymphocytic cell types, it is possible that determination of factors responsible for direct effects upon the worm, resulting in worm expulsion, may be defined using the nude mouse system. Furthermore, the *N. brasiliensis*–nude mouse system is not encumbered with technical difficulties that accompany neonatal thymectomy, nor are the results subject to the extreme

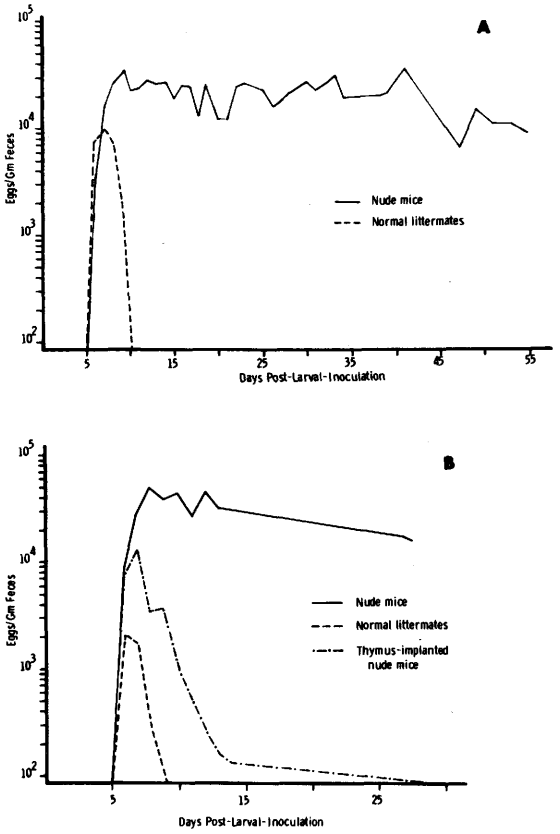

FIG. 1. (A) Worm egg production (expressed as eggs/g of feces) in nude mice and normal littermates. (B) Worm egg production in nude mice, normal littermates, and thymus-implanted nude mice. All mice were inoculated with 300 L_3 on Day 0.

variability (19) and partial effects (19–21) that often accompany such experimental manipulations. The application of this approach to other host–parasite systems should be an aid in understanding the complexity of resistance to parasitic infections.

Summary. Phenotypically normal mice expelled the intestinal nematode *Nippostrongylus brasiliensis* by Day 10–12 post-larval-inoculation. Congenitally athymic (nude) mice maintained, for their lifetime, infections of this parasite. The worm expulsion mechanism was generated by nude mice which had been given thymus gland implants 30 days prior to larval inoculations. The nude mouse may prove most useful in studies on mechanisms of immunity to helminthic infections.

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