Parasitological, Hematologic, and Immunologic Responses in Acute and Chronic Infections of Dogs with *Ancylostoma ceylanicum*: A Model of Human Hookworm Infection

Simon M. Carroll and David I. Grove

A model of human hookworm infection has been defined with a strain of *Ancylostoma ceylanicum* that is known to be infective for humans. In the first experiment, dogs were infected with between 150 and 12,150 filariform larvae and followed up for six weeks. A direct relation was found between the size of infecting dose, fecal egg excretion, intestinal adult worm burden, and worm distribution in the bowel. Dogs with heavy infection developed bloody diarrhea and iron-deficient anemia. In the second experiment, dogs infected with 2,000 larvae excreted ova until autopsy 36 weeks after infection. Normocytic anemia and blood eosinophilia coincided with maximal egg excretion. Transitory lymphocyte stimulation was seen with adult worm antigen. The specific IgM antibody response was transient, but IgG antibodies persisted indefinitely. Marked skin immediate hypersensitivity and Arthus (but no delayed) hypersensitivity reactions were seen to injected larval and adult antigens. Thus, dogs develop both hookworm disease and chronic infections similar to those seen in humans.

Hookworm infection is one of the most important of all human helminthic infections, afflicting perhaps one-quarter of the world's population [1]. Nevertheless, current interest and research in this infection are limited. This situation devolves largely from the lack of a suitable animal model of human hookworm infection in which to study the pathophysiology and immune reactions of the host and to evaluate possible interventionist strategies [2].

Three species of hookworm, *Ancylostoma duodenale*, *Necator americanus*, and *Ancylostoma ceylanicum*, complete their development in humans. Several attempts have been made to infect a variety of laboratory animals with these worms. *A. duodenale* infections have been described in chimpanzees [3], but these animals are scarce and costly to use. Schad [4] infected puppies with this parasite, but immunosuppression with corticosteroids was necessary for consistent maintenance of infection. Some success has been claimed for infecting infant rabbits with *A. duodenale* [5] and *N. americanus* [6], but the necessity for infecting neonatal animals has inhibited wide use of these models. Similarly, limited development occurs in infant hamsters infected with *N. americanus* [7].

Whereas *A. duodenale* and *N. americanus* are primarily human pathogens, *A. ceylanicum* has a wider host range, infecting humans [8–11], dogs [8, 10, 12, 13], and cats [8, 10, 13, 14] in nature. For many years there was considerable confusion and controversy over the specific designation of this parasite and its differentiation from *Ancylostoma braziliense*. *A. ceylanicum* was first described in 1911 by Looss [14], who found it in material from a civet cat sent to him by Willey in Ceylon, and shortly thereafter it was reported in humans in India by Lane [8]. Meanwhile de Faria [15] had discovered a hookworm in the intestines of cats and dogs in Brazil that he named *Ancylostomum braziliense*.

Leiper [16] and also Lane [17] considered that these two species were identical, and because de Faria had described his worm first this name held general sway. In 1951, however, Biocca [18] differentiated the two species by morphological criteria: this distinction was then confirmed by Rep et al. [19] in cross-breeding experiments.

Although *A. ceylanicum* is the most infrequently occurring hookworm in humans, its ability to...
infect a number of animal hosts makes it the only one that can be studied easily in experimental animals. Such infections in an animal model would be of greatest value if they resembled closely those seen in humans. An important distinction that must be made in ancylostomiasis is the differentiation of hookworm infection from hookworm disease, the latter being ill health as a consequence of the former; the major feature of hookworm disease is anemia with all of its accompanying symptoms and signs. In this article we first confirm that there is a strong relation between intensity of infection and appearance of disease. Second, we verify that, like humans, dogs develop chronic infections with *A. ceylanicum*, and we define the hematologic and immunologic responses of this species to these worms.

**Materials and Methods**

*Parasite.* *A. ceylanicum* was obtained originally from an infected dog in Malaysia. The acquisition of this worm, maintenance of the life cycle, and methods of infection have been described previously in detail [20]. In brief, dogs were infected percutaneously while under light anesthesia by application of infective larvae to the skin of the shaved inguinal region.

*Dogs.* Male mongrel dogs three to five months old were obtained from the general public. Each animal was housed separately and provided with food and water ad libitum. Dogs were washed, treated with the anthelmintics bunamidine hydrochloride and pyrantel pamoate, and immunized with canine measles, parovirus, distemper, and hepatitis vaccines. The feces of all dogs were examined to ensure that they were free of helminths. Infections were initiated four weeks after treatment with anthelmintics.

Venous blood was obtained from the foreleg of each dog at 10:00 A.M., and feces were collected before and at various intervals after infection.

*Experimental designs. Experiment 1.* Dogs were divided as evenly as possible into five groups of two dogs; animals in each group were infected with 150, 450, 1,350, 4,050, or 12,150 filariform *A. ceylanicum* larvae, respectively. Parameters were measured weekly for six weeks, when the animals were killed and the intestines were examined.

*Experiment 2.* Dogs were divided randomly into one group of eight dogs that were infected with 2,000 larvae and another group of four animals that served as uninfected controls. After the 24th week of infection the eight infected dogs were graded according to their fecal egg excretion and divided into four pairs ranging from the highest egg output to the lowest. One dog from each pair was then removed randomly from the study to enter another experiment. Parameters were examined 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32, and 36 weeks after infection; then the animals that had remained in this experiment were killed and the intestines were examined.

*Parasitological parameters.* To measure fecal egg excretion, 1-g aliquots of feces were weighed, broken up with a glass rod in a small quantity of water in 15-ml centrifuge tubes, and suspended in 10 ml of water. Multiple 0.2-ml samples were examined in Sedgewick-Rafter-type chambers. Adult worm burdens, distribution, and sex of worms were assessed six weeks after infection in the first experiment. The animals were killed, and the small bowel and large bowel were removed. The small intestine was divided into eight equal segments, and the large intestine was separated into the appendix-cecum and the remainder of the colon. Each of the 10 segments was opened longitudinally, and all the adult worms were removed, sexed, and counted.

*Hematologic parameters.* Samples of blood, anticoagulated with EDTA, were analyzed with a Coulter Counter S-Plus® (Coulter Electronics, Hialeah, Fla). Hemoglobin concentration, mean corpuscular volume, total white blood cell count, and platelet count were measured. Eosinophils, in blood treated with heparin (50 units/ml), were counted in an improved Neubauer hemacytometer after staining with freshly prepared Carpentier’s stain. Bone marrow smears were prepared at autopsy from the sternum of dogs in experiment 1 and stained for hemosiderin by the acid-ferrocyanide reaction [21].

*Immunologic parameters.* Soluble antigens were prepared for lymphocyte transformation studies from adult worms recovered from the intestines of infected dogs and infective larvae obtained from fecal cultures. The antigens were extracted as described for *Strongyloides ratti* [22] and sterilized by passage through a Millipore GV® filter with 0.22-µm pores (Millipore, Bedford, Mass), and the protein concentration was estimated [23].

Venous blood was anticoagulated with mucous heparin sodium (50 units/ml; Allen and Hanbury,
maximal stimulation occurred when cells were incubated for three days and then pulsed with \(^{[3}H\)thymidine (Amersham International, Amersham, England), 0.5 \(\mu\)Ci per well, for another 24 hr; these incubation periods were used routinely thereafter.

Similarly, in preliminary experiments attempts were made to stimulate lymphocyte transformation by incubation with a range of concentrations of larval and adult hookworm antigens. Subsequently the concentration of both antigens used was 10 \(\mu\)g per well; cells were incubated for three days before pulsing for 24 hr with \(^{[3}H\) thymidine. Cells were harvested with a Skatron\textsuperscript{®} multiwell harvester, and \(^{[3}H\) thymidine uptake was counted in a liquid scintillation spectrometer (Tricarb\textsuperscript{®}, Packard Instruments, Palo Alto, Calif).

Levels of serum antibodies to hookworm were measured by a modification of the immunofluorescence technique described for antibodies to Strongyloides [24]; living infective A. ceylanicum larvae were exsheathed by exposure to 1:2,000 hypochlorite [25], incubated with serum, and assayed using fluoresceinated rabbit antisera to IgM and IgG (Cappel Laboratories, Cochranville, Pa).

Dogs were shaved closely on the abdomen, and 50 \(\mu\)l of PBS containing 0, 2, or 20 \(\mu\)g of larval or adult worm antigen was injected intradermally. The diameter of the weals were measured 15 min and 2, 24, and 48 hr after injection.

**Statistics.** All results are expressed as mean \(\pm\) SD values. All tests of significance were performed using Student’s \(t\) test.

**Results**

**Experiment 1. Effects of infective dose on worm burden and severity of disease in dogs with acute infections. Clinical features.** Stools were of normal consistency until between two and three weeks after infection; at this time diarrhea developed in the majority of dogs. Diarrhea persisted for the remaining weeks of observation in those dogs that were infected with \(\geq\)4,050 larvae. Blood and mucus were present in large quantities in the stools of those dogs with the heaviest infections. There was no discernible relation between intensity of exposure and change in body weight of the dogs.

**Parasitological parameters.** Eggs were first seen in the feces three weeks after infection. No
significant differences were seen in fecal egg counts for dogs in each group during weeks 3, 4, 5, and 6. To reduce the effect of sample-to-sample variations counts from these weeks were averaged for each animal. The relation between infective dose and mean fecal egg excretion showed a highly significant linear correlation \((r = .862, P < .01); \) figure 1, top). The relation between infective dose and adult worm burden also showed a highly significant linear correlation \((r = .965, P < .001); \) figure 1, bottom). The mean percentage recovery of adult worms with respect to infecting dose was 17.2\% \pm 7.7\%.

The distribution of worms in dogs with differing intensities of infection is illustrated in figure 2. In dogs with small worm burdens most of the worms were located in the anterior half of the small intestine. In dogs with larger worm burdens, however, worms were more evenly distributed throughout the bowel. In those animals with the heaviest worm burdens about half of the worms were found in the large bowel. About 60\% of the worms recovered from all the dogs were females. This frequency did not vary significantly with increasing dose of infection and ranged between 54\% and 68\% female worms. Similarly, in those dogs with heavy infections in which worms were dispersed throughout the small and large bowel, there was no significant variation in sex distribution in relation to location of worms in the intestines.

There was a linear relation between increasing intestinal adult worm burden and excretion of eggs in the feces \((r = .914, P < .001). \) Similarly, there was a highly significant correlation between increasing female adult worm burdens \((r = .928, P < .001)\) and excretion of eggs in the feces. A measure of fecundity was given by the value for excretion of eggs per gram of feces per female worm; there was no significant variation in fecundity in dogs with differing worm burdens, the mean \pm SD excretion being 20 \pm 11 eggs/g of feces per female worm.

**Hematologic responses.** The changes in hemoglobin concentration in relation to infective dose are illustrated in figure 3, top. In the dogs with the lowest infections there was a 30\% increase in hemoglobin concentration by six weeks after infection. There was a less marked increase in the dogs infected with 450 larvae, and little change was observed in dogs infected with 1,350 and 4,050 larvae. There was, however, a marked 45\% decrease in hemoglobin concentration in dogs with the heaviest infection. A strong correlation existed between the degree of change in hemoglobin concentration six weeks after infection and the infective dose \((r = - .900, P < .001). \)

The erythrocyte corpuscular volume of the 10 dogs before infection was 69 \pm 2.1 \mu m^3. There was no significant variation in this parameter during the six weeks of observation in the dogs infected with 150, 450, 1,350, or 4,050 larvae. There was, however, a marked fall in the mean corpuscular volume in the dogs with the heaviest infections; the average volumes 0, 1, 2, 3, 4, 5, and 6 weeks after infection were 70, 69, 72, 72, 66, 62, and 60 \mu m^3, respectively. There was a marked correlation between the absence of siderotic granules and free-lying hemosiderin in sternal marrow smears and infective dose. Stainable iron was not seen in either of the two dogs with the heaviest infections. There
was, however, stainable iron present in one of the two animals each infected with 4,050, 1,350, or 450 larvae and in both the dogs with the lightest infections of 150 larvae.

The total white blood cell counts in selected groups of dogs are illustrated in figure 3, bottom. No significant changes in white blood cell counts were seen in dogs infected with 150–1,350 larvae. A leukocytosis was noted, however, in dogs with heavier exposure, with the most marked increases in white blood cell counts being observed in the dogs with the heaviest infections. Eosinophilia was noted in all dogs with a fourfold increase in blood eosinophil levels, but there was no apparent relation between intensity of infection and degree of eosinophilia. There was no significant change in platelet counts in relation to intensity of exposure.

**Immunologic responses.** The mean spontaneous and mean phytohemagglutinin-induced [H]-thymidine uptakes by lymphocytes for the 10 dogs before infection were 690 ± 480 and 6,100 ± 3,600 disintegrations per minute (dpm), respectively; there were no significant changes in these parameters in relation to either intensity of exposure or duration of infection.

Titers of serum antibodies of the IgM and IgG classes to hookworm were measured before infection and at two and six weeks after infection. No antibodies were detected before infection. There were no significant correlations between intensity of infection and IgM antibody titer at both these times. Similarly, there was no correlation between intensity of infection and IgG antibody titer two weeks after infection. At six weeks after infection, however, there was a significant positive correlation \( r = .703, P < .05 \) between exposure to increasing numbers of infective larvae and increasing titer of IgG antibody.

**Experiment 2. Development and course of chronic hookworm infections.** **Parasitological parameters.** Egg excretion over the 36 weeks of infection is shown in figure 4. Eggs were first seen in the stools three weeks after infection. For the first 12 weeks of infection fecal egg output was relatively constant. Thereafter there was a steady decline in egg numbers. By 36 weeks after infection
egg excretion was minimal, being ~2% of that seen at the height of infection. Four dogs were examined at autopsy 36 weeks after infection. The numbers of adult worms recovered from these dogs were 3 (2 males and 1 female), 3 (2 males and 1 female), 5 (3 males and 2 females), and 30 (23 males and 7 females), respectively.

**Hematologic responses.** The hemoglobin levels in infected dogs and control dogs are shown in figure 5, top. Initially there was no significant difference between the two groups. A reduction in hemoglobin concentration was evident in infected dogs two weeks after infection and became significant by four weeks after infection ($P < .02$). This difference was maintained at six ($P < .05$) and eight ($P < .025$) weeks after infection. Thereafter the hemoglobin level in infected dogs slowly increased, and there was no significant difference between the two groups of animals 12 weeks after infection.

The initial red blood cell corpuscular volumes, white blood cell counts, and platelet counts in infected and control dogs were 69 ± 1.4 and 72 ± 2.8 $\mu$m$^3$, 14.7 ± 5.3 and 17.0 ± 5.4 x 10$^6$ cells/liter, and 350 ± 190 and 430 ± 180 x 10$^9$ platelets/liter, respectively; these differences were not statistically significant. Similarly, no significant differences were found for any of these parameters thereafter.

The eosinophil levels in infected and control dogs are illustrated in figure 5, bottom. Initially there was no significant difference between the two groups of animals. By two weeks after infection there was significant eosinophilia in infected dogs ($P < .05$). This significance was maintained at four ($P < .01$) and six ($P < .01$) weeks after infection. Thereafter, there was persistent, although not statistically significant, eosinophilia in the infected dogs.

**Immunologic responses.** The initial mean $[^3]H$[thymidine uptakes by lymphocytes when incubated in the absence of antigen or mitogen for infected and control dogs were 420 ± 110 and 420 ± 210 dpm, respectively. When incubated with phytohemagglutinin the initial mean uptakes were 6,000 ± 4,200 and 12,000 ± 11,000 dpm, respectively. The differences between groups of animals for each parameter were not statistically significant. Similarly, no significant differences were found for either parameter thereafter.
The stimulation indices of lymphocytes incubated with larval antigen before infection were 1.0 ± 0.4 and 1.2 ± 0.3 for infected and control dogs, respectively. The stimulation index is the ratio of dpm in the stimulated cultures to dpm in the unstimulated (spontaneous uptake) cultures. When cells were incubated with larval antigen, there was a suggestion that stimulation might be occurring between weeks 2 and 4 in infected dogs, but this difference did not reach statistical significance ($P < .10$). When cells were incubated with adult worm antigen (figure 6, top), stimulation was seen during the same period; this difference was significant four weeks after infection ($P < .02$).

The levels of serum antibodies of the IgM and IgG classes to hookworm are shown in figure 6, bottom. No antibodies were detected before infection. IgM antibodies appeared transiently; they were first detected two weeks after infection, were maintained at this level until the third week, and then declined and disappeared by eight weeks after infection. IgG antibodies also appeared two weeks after infection but persisted in high titer for 36 weeks after infection.

Four infected dogs and four control dogs were skin tested at the end of the experiment (table 1). Markedly positive immediate hypersensitivity reactions were seen in infected dogs 15 min after injection of 2 or 20 μg of both larval and adult worm antigen. Five hours after injection significant reactions were seen only at the sites of injection of 20 μg of both antigens. No reactions were seen 24 and 48 hr after injection at the sites of injection of antigens. Similarly, no reactions were found at any time after injection with PBS.

**Discussion**

Although this species of hookworm was obtained originally from an infected dog, we have since shown that it is capable of complete development in human subjects (authors unpublished observation). The first ideal for the development of a suitable animal model of human hookworm infection.
Table 1. Skin reactivity 15 min and 5 hr after injection of hookworm antigens in infected and control dogs.

<table>
<thead>
<tr>
<th>Time, antigen</th>
<th>Control</th>
<th>Infected</th>
<th>P</th>
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<tr>
<td>15 min</td>
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<td>Larval</td>
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<td>2 μg</td>
<td>0.5 ± 1.0</td>
<td>13 ± 3.6</td>
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<td>20 μg</td>
<td>0.8 ± 1.5</td>
<td>21 ± 3.1</td>
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<td>Adult worm</td>
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<tr>
<td>2 μg</td>
<td>0.5 ± 1.0</td>
<td>16 ± 3.2</td>
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<tr>
<td>20 μg</td>
<td>0.8 ± 1.5</td>
<td>23 ± 0.5</td>
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<td>5 hr</td>
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<td>20 μg</td>
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<td>27 ± 2.6</td>
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<td>Adult worm</td>
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<td>2 μg</td>
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<td>20 μg</td>
<td>1.3 ± 1.5</td>
<td>31 ± 1.9</td>
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NOTE. Data are mean ± SD skin reactivity (in mm). There were four dogs per group. NS = not significant.

is an ability for the human parasite to develop normally in a laboratory animal. Second, the course of such infections and the clinical manifestations produced by them should resemble those seen in infected humans. In the present study dogs infected with this strain of *A. ceylanicum* have clearly fulfilled both of these requirements.

A marked relation between worm burden and anemia has been well quantified in humans infected with *N. americanus* by Roche and Layrisse [26], who demonstrated a strong correlation between fecal egg output and daily loss of blood in the stools. The first aim of the present study was to delineate under controlled conditions the relation between intensity of infection and severity of disease in dogs infected with *A. ceylanicum*. Increasing numbers of hookworm ova were recovered from the feces of dogs in direct proportion to the number of infective larvae given. Subsequent direct observation six weeks after infection confirmed that this result was the consequence of greater numbers of adult worms in the intestines.

This examination disclosed marked variation in the distribution of hookworms in the intestine depending on the worm burden. It appears that the desired niche for these worms is the anterior half of the small bowel because most of the worms in dogs with light infections were found in this location. In dogs with heavier infections, however, there was a spillover of worms into the posterior part of the small bowel and into the large intestine.

This phenomenon may be a consequence of overcrowding of worms with a deterioration of the local milieu in the anterior half of the small bowel. The mechanisms by which the local environment is altered are obscure. If this effect could be achieved with immunization, it would provide a valuable means for reducing the worm burden in infected individuals.

Hemoglobin levels of dogs infected with 1,350 and 4,050 larvae showed no significant change over the six weeks of infection. The hemoglobin concentration in dogs with lighter infections, however, rose significantly during this period. The increase in hemoglobin level in these dogs may reflect improved nutritional opportunities after admission to the animal house. When this factor is taken into account, it is clear that there is a direct inverse relation between hemoglobin level and worm burden, with the anemia becoming progressively more marked in dogs with increasing intensities of infection. In the most heavily infected dogs the mean corpuscular volume of red blood cells was reduced by ~15% six weeks after infection, an observation suggesting that the anemia was of the iron deficiency type. This supposition was confirmed by examination of the bone marrow, which revealed an absence of iron stores in the most heavily infected dogs. The appearance of this anemia coincided with the onset of blood and mucus in diarrheic stools of dogs with the heaviest hookworm infections.

In addition to determining the relation between worm burden and effects on the host as evidenced by the severity of anemia, we have also attempted to determine whether the intensity of infection influences in any way host responses to those worms. Dogs with light and heavy infections responded in a similar manner during the first six weeks of infection with respect to blood eosinophilia, spontaneous and phytohemagglutinin-induced lymphocyte transformation, and production of serum IgM antibodies to hookworm. However, the titer of serum IgG antibody to hookworm were significantly greater in dogs with heavier infections six weeks after infection; it is possible that the greater and continuing antigen load in these animals may have accelerated antibody production. These largely negative findings should not be entirely unexpected because the direct relation between infective dose and subsequent adult worm burden that we have observed indicates
that these reactions have conferred no protective immunity. These observations were made in dogs with primary infection, however, and it does not necessarily follow that responses in secondary infection are nonprotective.

No information is available yet concerning the duration of *A. ceylanicum* infections in humans. Infections with *A. duodenale* and *N. americanus* may last for several years, but there have been suggestions that reductions in worm burdens occur within a few months to a year of infection [27]. For example, Mhaskar [28] followed the intensity of hookworm infection in a population of prisoners in an Indian jail who were not exposed to reinfec-
tion; he noted that adult worm numbers declined by 13%, 36%, and 50% after 1, 3, and 12 months incarceration, respectively. In the present study the maximal egg output in infected dogs was noted soon after infection and was maintained at this level for the first three months or so. Thereafter there was a relatively rapid decline in egg output, which was succeeded by chronic excretion of ova in low numbers. The decreased egg output could have reflected either reduced fecundity of female worms or partial expulsion of adult worms. That the latter proposal is more likely is indicated by the small numbers of worms recovered at autopsy 36 weeks after infection. Two possible mechanisms for this disappearance of worms are apparent. First, the inherent life span of most adult worms may be only several months. Alternatively, the dogs may have developed partial protective immunity after several months, which resulted in expulsion of the majority of hookworms.

Not only did these dogs have chronic infections with *A. ceylanicum*, but disease also developed. We noted significant anemia in infected dogs between four and eight weeks after infection. The anemia we observed was normocytic and transitory, and occurred at the same time as maximal egg excretion, when the greatest blood loss might be expected. Thus in contrast to dogs with the heaviest infections in the first experiments, this anemia is consistent with excessive loss of erythrocytes but without depletion of body iron stores.

Blood eosinophilia has long been recognized as an accompaniment of hookworm infections in humans. In these infected dogs eosinophilia was noted two weeks after infection and then persisted indefinitely. This observation undoubtedly reflects the continued direct interaction between the heads of the worms and the gastrointestinal mucosa. Furthermore, we have observed many eosinophils in the small intestinal lamina propria of dogs in close proximity of adult hookworm [29]. The roles of these cells in this infection remain enigmatic. It seems likely, however, that they play a dual role in host defenses, by direct destruction of worms and containment of immediate hypersensitivity reactions invoked by released helminth antigens [30].

A major purpose of the present study was to characterize some of the immunologic responses of dogs to defined infection with *A. ceylanicum*. Antibodies of the IgM class to *Ancylostoma* infective larvae first appeared two weeks after infection and then subsided rapidly in level. Thus the presence of these antibodies can be used to demonstrate recent infection with *A. ceylanicum*. Antibodies of the IgG class were detected in higher titer and persisted indefinitely. A similar persistence of antibodies to hookworm has also been observed in humans infected experimentally with *N. americanus* [26]. Nevertheless, the biologic roles of these antibodies is uncertain and requires further investigation.

The responsiveness of peripheral blood mononuclear cells to the T cell mitogen phytohemagglu-
tinin was examined. Although hyporesponsiveness has been noted in a wide range of infections [31], we did not observe any such suppression of cells from dogs with ancylostomiasis.

Similarly, we could find no effect of ancylostoma infection on the spontaneous background activity of peripheral blood lymphocytes in the absence of any mitogen or specific antigen. When cells were incubated with larval antigen, no definite stimulation was observed, although there was a suggestion that lymphocytes may have been activated between two and four weeks after infection. Lymphocytes were stimulated significantly during this period of infection by antigen derived from adult worms. Taylor and Turton [32] found specific antigen-induced blastogenesis 17 and 51 days after experimental infection of a human with *N. americanus*. The reasons for the absence of antigen-stimulated lymphocyte transformation in the later stages of infection are uncertain, but specific inhibition of lymphocyte proliferation has been noted in a number of other chronic parasitic infections, including filariasis [33], schistosomiasis [34], and leishmaniasis [35], as a consequence of the presence of serum suppressive factors, adher-
ent phagocytic suppressor cells, and suppressor T cells [36]. Further studies are required to delineate and characterize these events in this model.

Differentiation of several types of immunologic responses is permitted by measurement of skin reactivity to injected antigen. Immediate (15 min) and Arthus (5 hr) hypersensitivity reactions were observed after injection of both larval and adult antigens, but no significant delayed (24 and 48 hr) hypersensitivity reactions were noted. Thus no stage specificity of antigens was detected by skin reactivity. Nevertheless, although we have no data yet on the specificity of the reactions, their presence does provide the potential for development of a skin test to diagnose the presence of infection with *Ancylostoma*.

Thus there is clear evidence of humoral immune responses with the demonstration of serum antibodies and immediate hypersensitivity and Arthus hypersensitivity skin reactions. We have not provided convincing evidence of cell-mediated immune reactions because delayed hypersensitivity skin reactions were absent and it is uncertain whether the lymphocytes stimulated by adult worm antigen were T cells, B cells, or both. It is clear that these responses do not provide significant protective immunity for the first three months of infection, and some worms continue to evade them thereafter. The mechanisms by which these parasites evade the host defenses are unknown. Clearly if they could be identified and modified, such tactics would provide a means for controlling these infections.

Despite the obvious potential of this parasite for providing an animal model of hookworm infection and disease, little attention appears to have been paid to it apart from the studies of Rep [37], who first demonstrated that individual cats and dogs could be infected with *A. ceylanicum*. He and his co-workers were then more concerned with determining the distribution of worms in the intestine [38], assessment of the effects of infection on blood loss [39–41], and differentiation of *A. ceylanicum* from *A. braziliense* [19].

In conclusion, we have shown that adult hookworm burdens are directly proportional to the size of the infective dose. Diarrhea and microcytic anemia develop in dogs with greater adult hookworm numbers, thus confirming the relation between severity of hookworm disease and intensity of infection. Furthermore, like humans, dogs become chronically infected. Immune responses develop, but their roles in the containment of infection are uncertain. Thus this model provides an opportunity to investigate further the factors contributing to the production of hookworm disease. Moreover, it offers a system in which the acquisition of any resistance to reinfection can be assessed.

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