Infection and immunity in dogs infected with a human strain of *Strongyloides stercoralis*

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Infection and immunity in dogs infected with a strain of *Strongyloides stercoralis* of human origin were investigated. The first dog infected developed a chronic infection lasting at least 15 months. Larvae disappeared from the faeces by three months after infection in another four dogs; these animals were resistant to challenge infection. A further dog developed a chronic infection of low intensity which could not be boosted by repeated heavy infections. These differences may be genetically determined. Immune responses in primary infections were measured in four dogs. A blood eosinophilia occurred in infected animals. Anti-*Strongyloides* antibodies of the IgM class appeared one week after infection, peaked at three weeks then slowly declined in titre while IgG antibodies appeared slightly later and then persisted in high titre. When compared with uninfected control dogs, no significant differences were seen in PHA stimulation of peripheral blood lymphocytes, nor did significant lymphocyte proliferation occur in the presence of *Strongyloides* antigen. Infected dogs showed marked immediate hypersensitivity to antigen injected intradermally, but Arthus and delayed hypersensitivity reactions were not seen. This model of human strongyloidiasis merits further investigation.

Introduction

Animal models available for experimental investigations of the host-parasite relationship in strongyloidiasis are limited. Since *Strongyloides stercoralis* is not well adapted to small laboratory animals, most studies have used rats and mice infected with the closely related species, *S. ratti*. These systems have a major disadvantage, however, as *S. ratti* does not appear to have the ability to autoinfect (Olson & Schiller, 1978; Grove & Dawkins, 1981; Dawkins et al., 1982). It is this capacity of *S. stercoralis* to replicate within the host which determines many of the characteristic features of human strongyloidiasis.

It has been known for many years that dogs may be infected successfully with some strains of *S. stercoralis*. Since canine strongyloidiasis may bear a closer resemblance to the human infection than does *S. ratti* infections of rodents, further examination of this model seems necessary. This paper reports preliminary data on the course of infection and immunological responsiveness in dogs infected with *S. stercoralis*.

Summary

The course of infection and immunological responses in dogs infected with a strain of *Strongyloides stercoralis* of human origin were investigated. The first dog infected developed a chronic infection lasting at least 15 months. Larvae disappeared from the faeces by three months after infection in another four dogs; these animals were resistant to challenge infection. A further dog developed a chronic infection of low intensity which could not be boosted by repeated heavy infections. These differences may be genetically determined. Immune responses in primary infections were measured in four dogs. A blood eosinophilia occurred in infected animals. Anti-*Strongyloides* antibodies of the IgM class appeared one week after infection, peaked at three weeks then slowly declined in titre while IgG antibodies appeared slightly later and then persisted in high titre. When compared with uninfected control dogs, no significant differences were seen in PHA stimulation of peripheral blood lymphocytes, nor did significant lymphocyte proliferation occur in the presence of *Strongyloides* antigen. Infected dogs showed marked immediate hypersensitivity to antigen injected intradermally, but Arthus and delayed hypersensitivity reactions were not seen. This model of human strongyloidiasis merits further investigation.

Materials and Methods

*S. stercoralis*

Worms were recovered from a man who had been infected while a prisoner-of-war in Southeast Asia 35 years previously. A male mongrel pup was infected as described earlier (Dawkins & Grove, 1982a); this animal was the source of worms for subsequent infections. Moisten ed faeces were kept for two weeks at room temperature on a watch glass in a petri dish containing water. Filariform larvae were removed from the water, washed twice in phosphate buffered saline, and adjusted to the desired concentration. When larvae were injected subcutaneously (s.c.), benzyl penicillin 400 i.u./ml and streptomycin sulfate 400 µg/ml were added.

*Dogs*

For the major experiment, eight male mongrel dogs, six to eight weeks old and ranging in weights from 2.7 to 6.5 kg, were obtained from the general public. These animals were comprised of one litter of three pups, two litters of two dogs, and a single pup. The animals were divided into two groups with the litters being split as evenly as possible. Each group was housed separately and provided with food and water *ad libitum*. The other two dogs were also male mongrels obtained from the public. Dogs were washed, treated with the anthelmintics bumidine hydrochloride and pyrantel pamoate, and immunized with canine measles, parvovirus, distemper and hepatitis vaccines. The faeces of all dogs were examined to ensure that they were free of helminths.

One week later, one group of four dogs was lightly anaesthetized and the inguinal region of each animal shaved. They were then infected with 1500 filariform larvae in 0.5 ml saline percutaneously plus a further 1500 larvae injected s.c.

*Antigens*

Soluble antigens were prepared from *S. ratti* and *S. stercoralis* filariform larvae as described earlier (Dawkins & Grove, 1982b) and then sterilized by passage through a 0.45 µm filter (Millipore Corporation, Bedford, Massachusetts, USA).

*Faecal worm counts*

Dogs were isolated at intervals and the faeces were collected. One gram aliquots of faeces were weighed, soaked and homogenized in 10 ml water. The numbers of larvae in multiple 0.5 ml samples were counted in Sedgwick-Rafter-type chambers.

*Blood eosinophils*

Venous blood was collected from the forearm into heparinized tubes at 9.00 a.m. Eosinophils were counted using Carpentier's stain.
Fig. 1. Faecal larval counts at various times after primary infection of four dogs with 3,000 filariform larvae.

Fig. 2. Faecal larval counts at various times after repeated infection of a single dog with varying numbers of filariform larvae. The times of challenge are indicated by arrows.

Fig. 3. Blood eosinophil counts in infected (●) and control (○) dogs at varying times after primary infection.

Fig. 4. Serum anti-Strongyloides antibodies of the IgM (○) and IgG (●) classes at various times after primary infection.
**Serum antibody**

Venous blood was collected into non-heparinized tubes and the serum separated. Anti-Strongyloides antibodies were measured using fluoresceinated rabbit anti-IgM and anti-IgG antisera (Cappel Laboratories, Cochranville, Pennsylvania, USA) and living filiform S. stercoralis larvae as described elsewhere (Grove & Blair, 1981).

**Lymphocyte transformation**

Venous blood was anticoagulated with mucous heparin sodium (Allen and Hanbury's, Boronia, Victoria, Australia), 50 units/ml, then diluted with two volumes of RPMI 1640 (Gibco, Grand Island, New York) containing 10% foetal bovine serum (FBS, Flow Laboratories, Stanmore, Victoria, Australia). Lymphocytes were separated on a Ficoll-Paque® column after centrifugation at 4,000 g for 20 min at 20°C. Total and differential white cell counts were made and viability determined by trypan blue exclusion. Lymphocytes were diluted to 5 × 10⁶ cells/ml in RPMI-FBS then dispersed in 200 µl aliquots into Microtest trays (Nunc, Roskilde, Denmark). In preliminary experiments using a wide range of mitogen concentrations, maximal stimulation was observed when cells were incubated with 1-10 µg PHA (Bacto-phytohaemagglutinin P, Difco Laboratories, Detroit, Michigan, USA); this concentration was used routinely thereafter. Further studies indicated that maximal stimulation occurred when cells were incubated for 24 hours then pulsed with ³H thymidine (Radiochemical Centre, Amersham; England) 0-5 µCi per well for another 24 hours; these incubation periods were used routinely thereafter. Attempts were made to stimulate lymphocyte transformation by incubation with S. ratti antigen in concentrations ranging from 1-0 to 30 µg per well for 24 hours followed by pulsed with ³H thymidine for a further 24 hours. The trays were harvested using a Scatron multi-well harvester and ³H thymidine uptake was counted in a Packard Tricarb® liquid scintillation spectrometer.

**Skin tests**

Dogs were lightly anaesthetized and the abdomen shaved. 50 µl volumes of PBS containing 0, 1-5 or 15 µg S. stercoralis antigen were injected intradermally. The diameters of the weals were measured with a ruler after 15 min, 5, 24 and 48 hours. The net reactivity was calculated by subtraction of the diameter of the weal induced by PBS from that of the weal in response to injected antigen.

**Results**

**Faecal larval in primary infections**

Rhabditiform larvae were found in the faeces of the four infected dogs two weeks after infection (Fig. 1.). Peak counts were seen at three weeks after which worm numbers fell dramatically, then tailed off slowly over the ensuing seven weeks. No larvae were found 12 weeks or more after infection. These results contrast with the course of infection in the original dog which provided the source of worms; in that animal, larvae were found repeatedly in low numbers (<100 larvae per gram faeces) until it was killed 15 months after infection.

**Table I—Faecal larval counts in four dogs which received a primary infection and four dogs which had been infected six months previously**

<table>
<thead>
<tr>
<th>Weeks after infection</th>
<th>Primary infection Mean ± S.D.</th>
<th>Secondary infection Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-5</td>
<td>64 ± 52</td>
<td>0</td>
</tr>
<tr>
<td>3-5</td>
<td>52 ± 57</td>
<td>0</td>
</tr>
</tbody>
</table>

S.D. = standard deviation.

**Faecal larvae in secondary infections**

Six months after primary infection and three months after worms had disappeared from the faeces, these four dogs together with the four previously uninfected control dogs were challenged with 5,000 filariform larvae. Patent infections with low worm burdens developed in previously uninfected dogs, but larvae were not seen in challenged animals (Table I, P = 0.05, Wilcoxon's sum of ranks test).

In order to observe the effects on faecal worm excretion of repeated infections given during a patent infection, a further male pup was infected with 400 filariform larvae. A small patent infection developed, then the dog was challenged repeatedly with large numbers of larvae beginning six weeks after primary infection (Fig. 2). This was followed by a rapid increase in faecal larval excretion after the first challenge but thereafter worm numbers declined and then persisted at a low level until the dog was killed 20 weeks after the primary infection.

**Blood eosinophil levels**

There was no significant difference in blood eosinophil counts between the two groups of dogs before infection and one week after infection (Fig. 3). By two weeks after infection, however, blood eosinophils increased in animals given a primary infection with S. stercoralis, this difference being significant at six weeks (P<0-05). The elevation in blood eosinophils in infected dogs persisted until about the 16th week of infection after which it declined.

** Serum antibody titres**

Anti-Strongyloides antibodies of the IgM class were present in the serum in relatively high titres as early as one week after infection (Fig. 4). The highest titres were found two to three weeks after infection. Thereafter, titres slowly declined and IgM antibodies had almost disappeared 20 weeks after infection. The appearance of antibodies of the IgG class was slightly delayed; they were easily detectable two weeks after infection. Maximal levels were reached by six weeks after infection and then antibodies persisted in high titre for at least the first 20 weeks of infection.

**Lymphocyte transformation**

The spontaneous uptake of ³H thymidine by unstimulated peripheral blood lymphocytes from infected and uninfected dogs were measured 0, 1, 2, 3, 6, 8, 12, 16 and 20 weeks after infection. No significant differences between the two groups of animals were seen at any time.
Table II—Transformation of peripheral blood lymphocytes from infected and control dogs after exposure to PHA. The results are expressed as the stimulation index (see text for definition)

<table>
<thead>
<tr>
<th>Weeks after infection</th>
<th>Infected dogs</th>
<th>Control dogs</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38 ± 47</td>
<td>78 ± 50</td>
<td>N.S.</td>
</tr>
<tr>
<td>1</td>
<td>32 ± 11</td>
<td>52 ± 25</td>
<td>N.S.</td>
</tr>
<tr>
<td>2</td>
<td>10 ± 7</td>
<td>37 ± 7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>3</td>
<td>24 ± 34</td>
<td>19 ± 18</td>
<td>N.S.</td>
</tr>
<tr>
<td>4</td>
<td>22 ± 15</td>
<td>43 ± 17</td>
<td>N.S.</td>
</tr>
<tr>
<td>6</td>
<td>8 ± 8</td>
<td>29 ± 29</td>
<td>N.S.</td>
</tr>
<tr>
<td>8</td>
<td>7 ± 3</td>
<td>21 ± 19</td>
<td>N.S.</td>
</tr>
<tr>
<td>20</td>
<td>7 ± 3</td>
<td>21 ± 23</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

S.D. = standard deviation
N.S. = not significant

Table III—Mean skin test reactivity in millimetres at various times after injection of 1.5 µg or 15 µg of antigen in infected and control dogs

<table>
<thead>
<tr>
<th>Time</th>
<th>Infected dogs</th>
<th>Control dogs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 µg</td>
<td>11.8 ± 3.8</td>
<td>1.8 ± 2.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>15 µg</td>
<td>11.5 ± 3.1</td>
<td>2.0 ± 2.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 µg</td>
<td>1.0 ± 2.0</td>
<td>1.3 ± 1.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>15 µg</td>
<td>11.0 ± 3.6</td>
<td>5.8 ± 2.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>24 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 µg</td>
<td>0.5 ± 1.0</td>
<td>2.5 ± 2.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>15 µg</td>
<td>8.0 ± 1.8</td>
<td>3.0 ± 4.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>48 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 µg</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>N.S.</td>
</tr>
<tr>
<td>15 µg</td>
<td>8.3 ± 1.7</td>
<td>5.3 ± 6.1</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

S.D. = standard deviation
N.S. = not significant

When PHA-induced stimulation in the two groups of dogs was measured before infection, there was a significant difference (P<0.02, “t” test), the mean ± S.D. disintegration per minute (d.p.m.) being 26,000 ± 7,800 and 5,850 ± 6,950 for the subsequent control and infected groups, respectively. Consequently, the results of PHA-induced stimulation are reported as the stimulation index, i.e., the ratio of d.p.m. in the stimulated culture to d.p.m. in the unstimulated culture. Lymphocyte transformation was measured at various times after infection (Table II). A significant difference between the two groups of dogs was seen on only one occasion.

No significant stimulation of lymphocyte transformation by antigen was seen at any time after infection. The stimulation indices of both infected and control dogs ranged between 1.0 and 2.0.

Skin tests

The four infected dogs were skin tested six months after infection together with the four uninfected control animals. The results are shown in Table III. Markedly positive immediate hypersensitivity reactions were seen in infected dogs 15 min after injection of both 1.5 and 15 µg antigen whereas no reaction was seen in the control dogs. No significant differences were noted between infected and control dogs 5, 24 and 48 hours after injection. At these times, however, a nonspecific reaction was seen in all animals at the site of injection of the higher concentration of antigen.

Discussion

The susceptibility of dogs to infection with *S. stercoralis* has been known since 1914 when Fülleborn reported both the discovery of worms indistinguishable from this species in dogs and the demonstration that parasites of human origin will mature in dogs. Nevertheless, the physical restraints and financial costs of housing these large animals have restricted the use of this model, particularly in recent years. The same constraints have applied in the present study, with limitations of space precluding us from housing as many dogs as we would have desired.

Early investigators noted considerable variability in the susceptibility of dogs to infection with *S. sterco-
These variations may reflect alterations in the characteristics of the infecting parasite, the host animal, or both. FüLBORN (1914) was the first to recognize that strains of S. stercoralis from different geographical regions differed in their ability to infect dogs. Using human strains, he was able successfully to infect dogs with worms of Chinese origin but found that they were completely refractory to worms from East Africa. Furthermore, when patent infections were established, the course of infection depended upon the source of helminths. FüLBORN (1914) found that dogs infected with the Chinese strain lost their infections within a few weeks, whereas Galliard determined that self-cure hardly ever occurred in dogs in Indochina. These observations led GALLIARD (1967) to conclude that there were several “biotypes or geographical biological races” of S. stercoralis which vary in their pathogenicity. Nevertheless, the host also plays a dominant role in determining the evolution of infection.

SANDGROUND (1928) used a single American strain of S. stercoralis to infect a number of dogs. Chronic infections occurred in approximately one third of animals while self-cure with resistance to reinfection developed in the other two thirds. Our observations are reminiscent of those of SANDGROUND (1928). The first dog we infected was only given a single infection; a persistent infection for 15 months was noted, probably indicating that autoinfection occurred. The next four dogs developed a spontaneous self-cure within 12 weeks of primary infection and were profoundly resistant to challenge infection. The final dog developed a persistent infection until it was killed at 20 weeks, but the intensity of infection in the last few weeks was low and could not be boosted by repeated infections with large numbers of larvae. A similar sequence of events happened in the dog studied by AUGUSTINE & DAVEY (1939).

Thus, approximately one third of dogs in both SANDGROUND’s and our series appeared to be incapable of completely eliminating infection with S. stercoralis. This incapacity is presumably genetically controlled. Similar genetic determinants may operate in man. It is of interest that approximately 30% of ex-servicemen who were prisoners of war in Southeast Asia during World War II were still infected 35 years later (GROVE, 1980). While it could be argued that the remaining 70% were not exposed to infection, this seems unlikely since they lived for a long period in extremely unhygienic conditions. It is tempting to speculate that, like dogs, some humans are not genetically endowed with the capacity to eliminate Strongyloides infection.

A major purpose of this study was to characterize some of the immunological responses of dogs to infection with S. stercoralis. Since the four animals which were examined in this way, by chance, developed a sterilizing immunity, it cannot be assumed that similar reactions would have occurred in dogs which maintain persistent infections.

As expected in a helminth infection in which tissue migration occurs, infected dogs developed a mild eosinophilia which subsided after disappearance of the worms. Strongyloides antibodies of the IgM class first appeared one week after infection, reached a peak around two to three weeks, then slowly subsided. Thus, the presence of antibodies of the IgM class can be used to demonstrate recent infection with S. stercoralis. Antibodies of the IgG class were seen in higher titre. They were first noted two weeks after infection, reached a peak around six weeks, then persisted at this level until at least 20 weeks after infection. The biological role of these antibodies is uncertain. SANDGROUND (1928) claimed that passive immunity is not transferred in canine strongyloidiasis, but immunity has been transferred by immune serum in rats (MURKELL, 1981) and mice (DAWKINS & GROVE, 1981) infected with S. ratti.

The responsiveness of peripheral blood mononuclear cells to the T cells antigen, PHA, was examined. Although hyporesponsiveness has been noted in a wide range of infections (SCHWAB, 1975), we did not observe any such suppression of stimulation of cells from dogs with strongyloidiasis. We were not able to demonstrate significant stimulation of peripheral blood lymphocyte by Strongyloides antigen. Specific inhibition of lymphocyte proliferation has been noted in a number of parasitic infections including filariasis (OTTESON et al., 1977), various forms of schistosomiasis (OTTESON, 1979; BARRAL-NE?TO et al., 1982) and leishmaniasis (PETERSEN et al., 1982).

Various mechanisms have been been demonstrated including serum suppressive factors, adherent phagocytic suppressor cells and suppressor T cells (NUSSENZWEIG, 1982). Suppression by a serum factor was not operative in our study since cells were washed and resuspended in foetal bovine serum. Further investigations are required to delineate the role of other possible mechanisms.

Measurement of skin reactivity to injected antigen permits differentiation of several types of immunological responses. We did not see significant Arthus (five hour) or delayed hypersensitivity (24 and 48 hour) reactions, but did observe marked immediate hypersensitivity reactions 15 min after injection of antigen. The biological significance of this reaginic antibody has yet to be defined, but its presence offers the potential for the development of a skin test to diagnose the presence of Strongyloides infection.

Further studies of this model are indicated. It will be important to determine whether measurable humoral and cell-mediated immune responses in dogs which develop chronic strongyloidiasis differ from those which eliminate infection. It may be possible to simulate human overwhelming strongyloidiasis if massive autoinfection occurs in immunosuppressed dogs. Finally, the actions of newer anthelminthics can be intensively investigated in canine strongyloidiasis.

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References


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