Trichinella spiralis: Phospholipase in Sensitized Mice after Challenge

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LARSH, JOHN E., JR., OTTOLENGHI, ATHOS, AND WEATHERLY, NORMAN F. 1975. Trichinella spiralis: Phospholipase in sensitized mice after challenge. Experimental Parasitology 37, 233-238. Mice given three sensitizing infections with Trichinella spiralis and then challenged with 400 larvae showed greatly elevated phospholipase B levels in the small intestine from one through 20 days after challenge; by 25 days after challenge, the enzyme level approached that of the uninfected controls. The high enzyme levels were accompanied, after a lag in time, by greatly increased numbers of eosinophils in the bone marrow. Increases of eosinophils strikingly above those in uninfected controls were noted from 11 through 20 days after challenge. The enzyme and eosinophil increases were parallel to the time course of the intestinal inflammatory response described in a previous study of sensitized mice of about the same age challenged with the same number of larvae. The results are consistent with our hypothesis that inflammation, elevated enzyme levels, and increased production of eosinophils are causally related.

INDEX DESCRIPTORS: Trichinella spiralis; T. spiralis in mice; Intestinal phospholipase; Bone marrow eosinophilia; Intestinal histopathology.

In the first paper of this series (Larsh et al. 1974), greatly elevated phospholipase B levels in the small intestine of mice five through 29 days after an initial infection with T. spiralis were shown to correlate with the onset and intensity of intestinal inflammation described in an earlier study of mice of about the same age (Larsh and Race 1954). Moreover, the rises in enzyme levels were associated, after a lag in time, with greatly increased numbers of eosinophils in the bone marrow. Similar results were noted in rats given a primary infection.

These findings, and the reported close association between phospholipase and eosinophils (Ottolenghi 1970) suggested the hypothesis that the elevated enzyme levels are due to the intestinal inflammation, and that the eosinophils are the source of the enzyme. It was suggested tentatively that memory T-cells provide the stimulus
for increased production of eosinophils by
the bone marrow, and that the eosinophils,
as terminal cells, undergo morphological
alterations in the sites of inflammation and
release their stores of phospholipase.

The present report deals with our first
test this hypothesis. The follow-
ing question was posed. In mice previously
sensitized by stimulating infections, do the
elevated enzyme levels and bone marrow
eosinophilia after a challenging infection
correlate with the known accelerated and
intensified intestinal inflammatory re-

MATERIALS AND METHODS

The Swiss mice used in this study were
from a colony randomly bred in the De-
partment of Parasitology and Laboratory
Practice for more than 30 years. The strain
of *Trichinella spiralis* was isolated originally from
a pig in 1936 and, although it was main-
tained in laboratory rats for seven years,
it has been maintained thereafter in our
Swiss mice.

*Trichinella spiralis* larvae for infection
were obtained from mice infected for not
fewer than 45 days. The techniques used
for collecting larvae, and for standardizing
the infecting inocula, were those described
by Larsh and Kent (1949) and modified
by Weatherly (1970). The methods for
recovering and counting adult worms were
those of Larsh and Kent (1949).

The methods used to prepare tissue
samples for phospholipase B determina-
tions, as well as to estimate the numbers of
eosinophils in the bone marrow, were
previously detailed by Larsh *et al.* (1974).

RESULTS

In this experiment, 100 female mice, at
least four months old but less than seven,
were selected from the departmental
colony. Sixty experimental mice were sen-
sitized three times with 200 *Trichinella spiralis*
larvae each; the sensitizations were sepa-
rated by a period of 21 days. The remain-
ing 40 control mice were not sensitized.
For each sensitizing infection, additional
normal female mice of the same age (vi-
ability controls) were infected with the
same batch of larvae. Counts of adult
worms from the viability controls at 11
days after infection proved the viability of
the larvae used for the three separate
sensitizing infections. After the third sen-
sitization, four experimental mice were se-
lected at random on Days 7, 14, 21, 28, and
35 and the amount of phospholipase in the
small intestines was determined (Table I,
where levels are averaged).

The level of phospholipase in the small
intestines of the four mice was strikingly
high on Days 7, 14, and 21 after the third
infection, and declined thereafter until the
35th day of the observation period, when
the level was within the range of normal
values (i.e., a value not greater than about
3000; Larsh *et al.* 1974).

Five days later, or 40 days after the third
sensitizing infection, the remaining 40 sen-
sitized mice (experimental), as well as
the 40 nonsensitized mice (controls) of the
same age and sex were challenged with
400 *Trichinella spiralis* larvae. Eight mice, four
from each group, were set aside at this
time. Adult worms were counted 11 days
later to determine the sensitivity, hence
immune, status of the mice. The four ex-
perimental mice had an average of 74 adult
worms, the controls 149; hence, the three
sensitizing infections had produced a sig-
nificant degree of immunity (*P* = 0.001).

Four experimental and four control mice,
of the remaining 36 mice in each of the
two groups, were killed on Days 1, 2, 3, 4,
6, 11, 15, 20, and 25 after challenge. The
data on the average phospholipase levels
of the four mice in each group are pre-
sented in Table I.

The average phospholipase activity per
gram of wet intestinal tissue from the
thrice-sensitized mice was already elevated
one day after challenge, whereas the en-
TABLE I

Phospholipase Activity per Gram of Intestinal Tissue of Mice Challenged with Trichinella spiralis
(Each Reading is an Average for Four Mice)

<table>
<thead>
<tr>
<th>Treatment of experimental mice</th>
<th>Days after last infection</th>
<th>Phospholipase activity a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experimental (sensitized)</td>
</tr>
<tr>
<td>Three sensitizations with 200 larvae each</td>
<td>7</td>
<td>112,280</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>77,185</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>78,171</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>15,811</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>2,466</td>
</tr>
<tr>
<td>Three sensitizations, plus challenge with 400 larvae b</td>
<td>1</td>
<td>13,027</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15,021</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37,018</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>83,892</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>66,794</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>100,397</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>97,064</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>54,134</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3,745</td>
</tr>
</tbody>
</table>

a Numbers represent μmole of lysolecithin hydrolyzed per g of wet tissue per hr.
b Counts of adult worms from the thrice-sensitized mice averaged 74, whereas the nonsensitized mice harbored an average of 149 worms.

zyme levels in the nonsensitized, challenged mice were within the normal range until four days after challenge. It is worth underlining that all of the 16 sensitized mice tested during the one-to-four-day period after challenge showed phospholipase levels greatly in excess of the values found in normal mice or in sensitized mice at Day 40 after a last challenge. The data also show that the levels of phospholipase activity underwent an earlier decline in the previously sensitized experimental mice.

The data on the numbers of eosinophils in the bone marrow of the experimental and control mice are shown in Table II. Our uninfected mice average between 4.3 and 8.3% of eosinophils (Larsh et al. 1974); hence, the present data do not give a clear indication of marrow eosinophilia until the 11th day, without a detectable difference between the two groups of mice. Also, by this means of comparison, the numbers of eosinophils in the experimental mice remained elevated until the 25th day, whereas the normal range was reached in the control animals on Day 20.

DISCUSSION

A morphological study published years ago (Larsh and Race 1954) described in detail the sequence of histological changes occurring in the small intestine of mice reinfected with T. spiralis and compared this response with the reaction found in mice first exposed to the parasite. The general features of the local response in the two groups were similar, including the cellular components. The distinguishing features were the much earlier appearance of acute inflammation in the previously exposed mice, beginning within the first 12 hr after reinfestation. The acute inflammatory reaction reached its zenith by the fourth day, which, in contrast, was the time of little or no reaction in the intestine of the mice infected for the first time.

The data presented here offer a striking parallel to the morphological findings. As
TABLE II

Average Percentages of Eosinophils in the Bone Marrow of Four Sensitized (Experimental) and Four Nonsensitized (Control) Mice for Each Observation Period after a Challenging Infection with Trichinella spiralis

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Eosinophils*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimentals</td>
</tr>
<tr>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>4.7</td>
</tr>
<tr>
<td>6</td>
<td>8.4</td>
</tr>
<tr>
<td>11</td>
<td>15.5</td>
</tr>
<tr>
<td>15</td>
<td>13.4</td>
</tr>
<tr>
<td>20</td>
<td>10.2</td>
</tr>
<tr>
<td>25</td>
<td>6.1</td>
</tr>
</tbody>
</table>

* Percentage of eosinophils in the total pool of nucleated cells.

noted in the upper part of Table I, the mice sensitized by three infections showed elevated enzyme levels in their intestinal tissues at 7, 14, 21, and 28 days after the third infection. The reading at 35 days is within the range for uninfected mice. It is noteworthy that the peak level at seven days (112,280) is considerably less than that noted at 14 days in mice given only one infection (Larsh et al. 1974). According to our hypothesis that the documented intestinal inflammation is the cause of elevated phospholipase levels, this difference in enzyme level might at first glance appear to be contradictory to the hypothesis. However, it must be recalled that in previously sensitized mice a significant expulsion of adult worms from the small intestine occurs about one week earlier than in nonsensitized mice (Larsh et al. 1952). Since the worms cause the inflammatory response, it is logical to assume that the response, with its associated phospholipase accumulation, would be less severe after the worms have been expelled. In fact, the zenith of the inflammatory response is reached at four days after challenge in previously sensitized mice, and by six days it has diminished considerably in intensity; on the other hand, in nonsensitized mice the zenith is at eight days, and after 11 days, although diminishing in intensity, inflammation is still striking (Larsh and Race 1954). Therefore, it would be expected that the enzyme level of the nonsensitized mice at 14 days (Larsh et al. 1974) would be considerably higher than that noted in sensitized mice at seven days after the third sensitizing infection (Table I).

As shown in the lower part of Table I, within 24 hr after challenge, the experimental mice had an elevated enzyme level. Since time (40 days) was allowed after the third sensitizing infection for the enzyme levels to return to normal, it is clear that accumulations of enzyme occurred promptly after the stimulation produced by the challenging infection. This rapid accumulation of the enzyme is related temporally with the early onset (within 12 hr) of acute intestinal inflammation in similarly treated mice (Larsh and Race 1954).

The enzyme levels in the experimental mice reached a peak on Day 11 and remained elevated above normal for at least 20 days after challenge (Table I). These elevations correlate well with the initiation and development of the acute and later subacute or chronic inflammatory responses in the proximal portions of the small intestine of sensitized mice of about the same age challenged with the same number of larvae (Larsh and Race 1954). The striking temporal relation between the inflammation and elevated enzyme levels supports our hypothesis that the former results in the latter (Larsh et al. 1974). The fact that the enzyme levels remained elevated for at least 20 days after challenge, when it is known that some worms are still present (Larsh et al. 1952), suggests that within this period the inflammation had not run its course. To substantiate this,
Histopathologic observations need to be extended until after the worms are lost and the tissues have returned to normal.

The increased numbers of eosinophils in the bone marrow of the experimental (sensitized, challenged) mice occurred, as expected, with a time-lag after the rise in enzyme levels in the intestinal tissue (Table II); both responses were accelerated in comparison with those noted after challenge of nonsensitized mice with the same number of larvae (Larsh et al. 1974). This would appear to provide more than circumstantial evidence that the intestinal inflammation, elevated enzyme levels, and bone marrow eosinophilia are causally related.

It is noteworthy that the controls in the present study given only the challenging infection showed greatly elevated enzyme levels at four days after the challenging infection and that by 25 days they had returned almost to normal (Table I), since these observations differ from those of similarly infected mice in our first study (Larsh et al. 1974). In the earlier study, the enzyme levels were first elevated strikingly at five days after infection, and returned to normal between 29 and 31 days. Also, the levels of enzyme and peak numbers of eosinophils in the bone marrow were greater in the mice of the first study. Although 400 larvae were used for infecting the mice in the two studies, the average numbers of adult worms recovered varied greatly, i.e., 260 in the first study, 149 in the present instance. Therefore, since the numbers of worms that establish after challenge affect the severity of the inflammatory response (Larsh and Race 1975), it is probable that the much smaller number of worms in the mice of the present experiment accounted for milder inflammation and lower phospholipase levels.

Basten and Beeson (1970), in a study of the mechanism of eosinophilia in trichinellosis, demonstrated eosinophilia in normal rats exposed to sensitized lymphocytes in diffusion chambers implanted intraperitoneally. In view of the evidence that immunity against the adults of T. spiralis is cell-mediated (Larsh and Weatherly 1974, 1975), this suggested the possibility that the diffusible factor is released from memory T-cells. The recent findings of Colley (1973) in studies of Schistosoma mansoni in mice support this view. Migration of eosinophils was stimulated by a product (tentatively considered to be a lymphokine) released from sensitized lymph node cells after interaction with either specific antigen or phytohemagglutinin. With this new information, our hypothesis can be logically extended as follows.

After sensitized mice have been challenged, as in the present instance, the interaction of antigen with memory T-cells in the tissues of the small intestine causes the release of lymphokines, possibly including Colley's "eosinophil stimulation promoter" (ESP), and local tissue injury triggers the inflammatory response (Larsh and Race 1975). The ESP stimulates eosinophils to migrate to the inflamed areas, into which in time their contents of phospholipase are released. This sequence continues until the worms are lost and the inflammation has run its course. The continued recruitment of eosinophils during this time produces a gradual and striking expansion of the eosinophil proliferating compartment to account for the great increases in eosinophils in the bone marrow (Spry 1971). When the inflammation subsides, the stimulus for bone marrow proliferation is reduced, and a declining trend develops in the enzyme levels in the tissues of the small intestine with a delay probably reflecting the life span of the eosinophils in these tissues.

Intensive histopathologic studies by use of a variety of experimental designs have shown: (1) a close, direct association of a characteristic pattern of acute intestinal inflammation and expulsion of adult
worms of *T. spiralis*; and (2) a similar association between the degree of sensitivity of the host at challenge and the timing and intensity of inflammation and loss of worms (Larsh and Race 1975). Therefore, since the phospholipase levels in the present experiment correlated so well with the accelerated and intensified inflammatory response noted earlier in similarly treated mice, the question is raised whether the enzyme itself is responsible for expulsion of the worms. Although this is a tempting conclusion, no evidence is yet available to support it directly.

The close association between the presence of worms in the tissues, and increased levels of phospholipase in the same tissues was first demonstrated in mice infected with *Hymenolepis nana* (Ottolenghi 1973). This now has been confirmed in mice and rats after an initial infection with *T. spiralis* (Larsh et al. 1974), and in the present instance in sensitized mice after challenge. This suggests the possibility that this association might exist in a wide variety of host–parasite models. In fact, this has been confirmed in studies with *Nippostrongylus brasiliensis* and *Angiostrongylus cantonensis* in rats, the details of which are to be reported separately.

ACKNOWLEDGMENTS

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REFERENCES


