Pathophysiology of *Mesocestoides corti* infection in the mouse

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ABSTRACT
Liver histology and serum enzyme and protein changes were studied in two strains of mice showing different initial susceptibilities to infection with *Mesocestoides corti*. The results show an increase in ALT and AST levels during the period of invasion and proliferation in the liver and a decrease in the levels of these two enzymes following encapsulation of the parasite in the liver and liver regeneration. A progressive loss of albumin was accompanied by increases in the levels of the beta- and gamma-globulins. These changes are discussed in the light of our knowledge of the effects of this parasite upon its host.

INTRODUCTION
Invasion and colonization of the host by protozoan and helminth parasites frequently leads to tissue damage in organs that are the sites of predilection or migration of the parasite. The extent and severity of the damage which the parasite inflicts upon its host may be assessed by the techniques of histology and also by the assay of enzymes characteristic of the insulted organ which are released into the blood by damaged cells (Sadun et al., 1965; Bundesen & Janseins, 1971a; Bundesen & Jansens, 1971b; Campbell & Barry, 1970). In addition, alterations in the relative and absolute levels of serum proteins can provide valuable information on the sequence and extent of pathological changes that occur in the host as a result of the activities of the invading organism (Sadun et al., 1965; Wellde et al., 1972).

*Mesocestoides corti* is a cestode with an unusual asexually dividing larval stage, the tetrathyridium. In the mouse, *M. corti* has been shown to proliferate in the peritoneal cavity initiating a severe inflammatory response (Mitchell & Handman, 1977) before invading and migrating through the liver (Specht & Vogt, 1965; Specht & Widmer, 1972; Pollacco et al., 1978) and the lung (Todd et al., 1978; White et al., 1981). In chronically infected animals invasion of the epididymis, testicle and kidney has been reported (Specht & Widmer, 1972; Todd et al., 1978). While proliferation in the lung is limited (White et al., 1981), damage to the liver parenchyma is extensive before the containment and encapsulation of the parasite (Specht & Widmer, 1972).

In this study we correlate changes in serum enzymes and serum protein levels with parasite numbers and the histopathology of the liver in two strains of mice showing different initial susceptibilities to the parasite (White et al., in press) in an attempt to identify progressive pathological changes during the first 60 days of infection.

MATERIALS AND METHODS

**Animals**
The breeding nuclei of CBA/H and C57BL/6 mice were obtained from the John Curtin School of Medical Research (Canberra, A.C.T.). Breeding colonies were established in a minimum disease area. Litters were weaned at three weeks and introduced into conventionally maintained experimental areas at four weeks of age.
Male mice were infected at four to six weeks of age and autopsied at various intervals after infection. No differences in the intensity of the infection are apparent between male and female CBA/H and C57BL/6 mice of this age. (White et al., in press).

Parasite

Tetrathyridia of M. corti were maintained in the laboratory in Quackenbush mice by serially passing the larvae by intraperitoneal inoculation. Tetrathyridia for experimental infections were removed from the body cavity of donor mice and washed twice in Hanks balanced salt solution (HBSS). All mice were infected by the intraperitoneal route with 50 tetrathyridia.

Autopsy

Tetrathyridia were recovered from infected mice by washing out the peritoneal cavity into a petri dish of HBSS. Parasites were counted directly or where necessary tetrathyridia were suspended in 100 ml of saline and stirred mechanically on a magnetic stirrer and the number of parasites estimated from the mean of three 10 ml samples. Tetrathyridia were recovered from the livers following digestion of minced livers in fresh 1% trypsin solution for six hours at 37°C and counted under a dissecting microscope.

Histology

Tissues were fixed in 10% buffered formalin for histology and stained with either haematoxylin and eosin, fast green van Gieson, carbonyl chromotrope or methyl green pyronin.

Serum samples

Normal mouse serum (NMS) was prepared from uninfected age-matched, male CBA/H and C57BL/6 mice and this was used to obtain normal values of serum enzymes and proteins in these two strains of mice. Infected mice were killed and bled on days 14, 35 and 60. Blood was collected from the thoracic cavity and allowed to clot at 37°C for one hour. Serum was separated from the clot by centrifugation and stored at 4°C.

Liver enzyme and serum protein estimations

The activities of serum alanine amino transferase (ALT), aspartate amino transferase (AST) (Henry et al., 1960) and alkaline phosphatase (Wilkinson et al., 1969) and the total serum protein level (Wechselbaum, 1946) were assayed on an automated Gemeni miniature centrifugal analyser (Electro-Nucleonics Inc., Passaic Ave., Fairfield, New Jersey, USA). The activity of the enzymes were measured in International units/litre of serum. Serum electrophoresis on cellulose polycetate (Sepraphore) strips was performed in a Gelman semi-micro electrophoresis chamber using Gelman high resolution buffer at 200 volts (1.5 to 4.0 milliamps) for 25 min. Strips were stained with Ponceau S and decolourized in 5% acetic acid. Levels of individual serum protein fractions were computed using a Gelman AVD-18 automatic computing densitometer.

Experimental design

Eight age-matched male mice of each strain were bled on day 0 to obtain normal serum enzyme and serum protein values. Male mice of both strains were killed on days 14, 25 and 60. The serum from eight of these animals was used for the estimations of serum enzymes and proteins. Samples of liver from these mice were
taken for histology. Student t test was used in the statistical analysis of results and a probability or more than 5% was not considered statistically significant.

RESULTS

Kinetics of proliferation of M. corti in CBA/H and C57BL/6 mice

The proliferation of the tetrathyridia in these two strains of mice and the subsequent parasite burdens on days 14, 35 and 60 are shown in Table I. Significant differences were observed in the number of tetrathyridia in the liver, peritoneal cavity, and in the total parasite burden on days 14 and 35. However no significant differences were observed between the numbers of tetrathyridia found in C57BL/6 and CBA/H mice on day 60.

Histopathology

The sequential histopathological changes following invasion of the livers of CBA/H and C57BL/6 mice were essentially the same as those described by Specchierl & Widmer (1972) in Swiss mice. Furthermore no differences were observed in the cellular changes accompanying migration of the tetrathyridia through the livers of CBA/H and C57BL/6 mice.

In both strains of mice extensive damage to the liver parenchyma was observed around parasite migration pathways. Extensive inflammatory responses were observed around parasites and their migratory paths with the subsequent deposition of collagen which was in evidence on day 14. Marked encapsulation with concomitant collagen deposition was observed on day 35. By day 60 the liver showed extensive regeneration around the encapsulated tetrathyridia. However, even at this late period in the infection unencapsulated tetrathyridia and their migratory tracks were observed. The possible source and significance of these unencapsulated tetrathyridia will be discussed later.

Serum enzymes

The levels of serum alanine amino transferase (ALT) and serum aspartate amino transferase (AST) are shown in Fig. 1. AST levels were significantly raised in both strains of mice by day 14. Levels peaked at day 35 and fell while remaining significantly higher than normal values on day 60. The levels of ALT although lower paralleled those changes already described for AST. No significant difference in the levels of AST or ALT were observed between the two strains of mice examined. The levels of alkaline phosphatase declined throughout the experimental period and no significant differences were observed between C57BL/6 and CBA/H mice (Fig. 1).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time (Days)</th>
<th>No. per Group</th>
<th>Body Cavity</th>
<th>P</th>
<th>Liver</th>
<th>P</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA/H</td>
<td>14</td>
<td>10</td>
<td>153±21</td>
<td>0.05</td>
<td>125±24</td>
<td>&lt;0.01</td>
<td>278±40</td>
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<tr>
<td>C57BL/6</td>
<td>14</td>
<td>10</td>
<td>93±15</td>
<td></td>
<td>47±7</td>
<td></td>
<td>140±19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10</td>
<td>1136±131</td>
<td>&lt;0.001</td>
<td>304±45</td>
<td>&lt;0.05</td>
<td>1439±126</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>35</td>
<td>10</td>
<td>361±38</td>
<td>&lt;0.001</td>
<td>178±23</td>
<td></td>
<td>539±49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CBA/H</td>
<td>60</td>
<td>10</td>
<td>2712±347</td>
<td></td>
<td>523±57</td>
<td></td>
<td>3233±345</td>
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<tr>
<td>C57BL/6</td>
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<td>10</td>
<td>1859±359</td>
<td></td>
<td>510±106</td>
<td></td>
<td>2366±388</td>
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FIG. 1. Levels of A.S.T. (○), A.L.T. (●) and alkaline phosphatase (▲) in CBA/H (solid line) and C57BL/6 mice (broken line) following infection with *Mesocestoides corti*. Normal ranges are shown on day 0.

The ALT/AST ratio remained unchanged throughout the infection period in both strains of mice (Fig. 2 (A)).

**Total protein**

A significant increase was observed in the serum total protein for both strains at day 35 (P < 0.01) and day 60 post-infection (P < 0.001) (Fig. 2(C)). No significant differences were observed in the levels of total serum protein between the two strains.

**Electrophoretic values**

A significant decrease in albumin levels was observed in CBA/H mice throughout the period of infection (P < 0.001) (Fig. 3). Falling albumin values in C57BL/6 mice on day 14 (P < 0.001) were followed by a return to normal values on day 35 and a further decline on day 60 (P < 0.001). A slight increase in alpha-2-globulin levels was observed in both strains on day 14 (Fig. 3). Subsequently this level was maintained in CBA/H mice for the remainder of the experimental period. In C57BL/6 mice levels had fallen to those of CBA/H mice by day 35. Statistically significant values were obtained for C57BL/6 mice on day 14 (P < 0.01).

Alpha-1-globulin levels showed a slight decline in level in both strains following infection with *M. corti* (Fig. 3). Significant differences were apparent for C57BL/6 mice on day 14 (P < 0.01). Beta-1-globulin levels were slightly increased in CBA/H mice at the end of the experimental period (day 60, P < 0.001). Values in C57BL/6
mice showed a slight decline on day 14 (P < 0.01) before returning to normal levels by day 35 (Fig. 4(A)).

Beta-2-globulin levels were significantly raised at 14 days post-infection (P < 0.001) in CBA/H mice and maintained this level until day 35. A further increase was observed on day 60 (Fig. 4(A)). In C57BL/6 mice a significant (P < 0.001) progressive increase was seen throughout the infection (Fig. 4(A)). Significant differences between the two strains were observed on days 14 (P < 0.001), 35 (P < 0.001) and 60 (P < 0.001). A progressive significant (P < 0.001) increase in gamma-globulin levels was observed in both strains of mice throughout the experimental period (Fig. 4(B)). No significant differences were observed in the gamma-globulin levels of C57BL/6 and CBA/H mice. A progressive decline was observed in the albumin/globulin ratio throughout the infection in both strains (Fig. 2(B)).

DISCUSSION

The entry of the tetraphyridia into the liver and the migration and proliferation of the tetraphyridia in this organ with the concomitant destruction of liver parenchyma was reflected in the increased serum levels of AST and ALT on day 14 (Fig. 1). The containment and encapsulation of the tetraphyridia in this organ appeared complete by day 35, most of the tetraphyridia being enclosed in granulomata. AST and ALT levels peaked on day 35 following encapsulation of the tetraphyridia in the liver and showed a marked decline on day 60 during the period of liver regeneration. These
results support the observations made in this study on C57BL/6 and CBA/H mice as well as those of other workers (Specht & Widmer, 1972; Pollaco et al., 1978) that tissue destruction is maximal before encapsulation, and the encapsulation of the parasite in the liver greatly limits tissue destruction in this organ. The statistically significant differences in the numbers of tetrathyridia observed in the livers of CBA/H and C57BL/6 mice on days 14 and 35 post-infection were not reflected in the levels of AST and ALT during this period of the infection.

However, this study has confirmed the observations of Washington, Truscott, Stewart & Nicholas (1979; Proceedings of the Australian Society for Parasitology, Leura, New South Wales), that unencapsulated tetrathyridia are present in the livers of mice during the later stages of infection. Increases in the parasite burden of the liver were evident in both CBA/H and C57BL/6 mice following encapsulation (Table I). There is no histological evidence to suggest that parasites later emerge from existing granulomata and it is likely that tetrathyridia from the peritoneal cavity population, which continues to undergo proliferation throughout the infection period (White et al., in press) is the source of these unencapsulated cestodes. It is likely that continued invasion, migration, encapsulation and liver regeneration occurs.
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![Graph](image)

**Fig. 4.** (A) levels of beta-1-globulins (○) and beta-2-globulins (●) and (B) gamma-globulins (●) in CBA/H (solid line) and C57BL/6 mice (broken line) following infection with *Mesostictodes corti*. Normal ranges are shown on day 0. Protein concentration is measured in grams/decilitres of serum.

Throughout the infection period but to a lesser extent than during the initial invasion of the liver.

Alkaline phosphatase levels were reduced in both strains of mice throughout the period of observation (Fig. 1) and therefore was not an index of tissue damage in this infection. It is of interest that Sadun et al. (1965) reported moderate reductions in the level of this enzyme in mice following infection with *Plasmodium berghei*, a result which they found difficult to interpret.

The decrease of albumin levels seen in both strains of mice (Fig. 3) may have been the result of one othersynthesis of the serum protein which is disturbed in liver dysfunction. However, mice with *M. corti* infections experience a chronic progressive peritonitis and it is likely that this serum protein is part of the inflammatory exudate entering the body space.

Slight decreases were seen in the levels of the alpha-1-globulins and slight increases in the levels of the alpha-2-globulins (Fig. 3). However, these acute phase proteins may also be lost into the peritoneal cavity and the observed levels may not accurately reflect any increased synthesis.

CBA/H mice showed significant progressive increases in the levels of the beta-1 and beta-2-globulins (Fig. 4(A)). The levels of these serum proteins were consistently increased over the levels observed in C57BL/6 mice throughout the experimental period. C57BL/6 mice failed to show significant alterations in the level of the beta-1-globulins although a progressive moderate increase in the levels of the beta-2-
globulins was observed. Both strains of mice showed a progressive increase in the levels of the gamma-globulins (Fig. 4(B)) throughout the infection. Higher levels were apparent in CBA/H mice on day 60. The increased levels of the beta-1, beta-2, and gamma-globulins are likely to be due to antibody synthesis as a result of infection with *M. corti* since antibodies have a wide range of electrophoretic heterogeneity. The predominance of the beta-2-globulin levels over the gammaglobulin levels in CBA/H mice may indicate that most of the antibodies produced as a result of infection with *M. corti* migrate with a different electrophoretic mobility in the two strains of mice.

Mitchell et al. (1977) have demonstrated that an IgG hypergammaglobulinemia is associated with *M. corti* infections in the mouse, a portion of which is specific for surface antigens on the cestode. However, recent evidence suggests that larval cestodes may possess substances capable of stimulating polyclonal lymphocyte proliferation (Dixon et al., 1978; Lorez et al., 1980) which could result in the synthesis of large amounts of non-specific immunoglobulin. The progressive fall in the albumin/globulin ratio in both the C57BL/6 and the CBA/H mice (Fig. 2(B)) is the result of increased globulin synthesis and albumin loss from the serum. An increase in total protein was observed in both strains (Fig. 2(C)) and is the result of increased globulin synthesis.

The present investigation demonstrates that increases in the levels of serum liver enzymes can be correlated with the parasite-related damage to the liver before encapsulation of the tetrahyridia in this organ. On the basis of this study it is unlikely that liver failure is a major contributory factor in the frequently seen death of chronically infected mice as extensive liver damage is not progressive in this infection. Other pathological trends include the progressive loss of some serum proteins which may be related to either liver dysfunction or the chronic peritonitis observed in this infection. In addition there is a hypergammaglobulinemia associated with infection to *M. corti*.

REFERENCES


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