Echinococcus multilocularis: mouse strain difference in hydatid development

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Abstract

Female mice of nine inbred strains (A, AKR, BALB/c, C3H, C57BL/6, C57BL/10, CBA, DBA/1 and DBA/2) and H-2 congenic B10.D2, at 9-10 weeks of age, were infected with larval Echinococcus multilocularis by trans-portal injection of hydatid homogenate. Parasitized livers were histologically examined 9 or 13 weeks after infection. Hydatid development was quite different among mouse strains. Multivesiculation was prominent in C57BL/10, DBA/1, C57BL/6 and BALB/c. Protoscoleces were well developed in DBA/2, AKR, DBA/1 and CBA. H-2 congenic B10.D2, which has the background genes of C57BL/10 except for the H-2d gene of DBA/2, resembled C57BL/10 in prohibiting the development of protoscoleces. These data suggest that the qualitative difference in hydatid development may be regulated by non-H-2 gene(s).

Introduction

Alveolar echinococcosis (AE) due to larval Echinococcus multilocularis is one of the most lethal helminthic zoonoses and is often misdiagnosed as hepatic cancer. In general, protoscolex production is rare in most human patients. In order to establish laboratory animal models for human AE, many species of rodents have been investigated by oral inoculation of eggs or intraperitoneal injection of hydatid homogenate (Yamashita et al., 1958). There are substantial but somewhat controversial reports on host susceptibility using murine models infected by intraperitoneal injection of hydatid homogenate (Ali-Khan, 1974; Ali-Khan et al., 1983; Kroeze & Tanner, 1987). In these studies, susceptibility was assessed by infection rate, parasite burden (cyst weight) or immune response, although the quality of parasite development was not evaluated.

In this report, we compared parasite development in nine inbred strains of mice qualitatively. We used trans-portal injection of hydatid homogenate to establish the hepatic lesion (Ohnishi, 1984; Nakao et al., 1990), since liver infection with alveolar hydatid is rational as a model for human AE. In addition, using H-2 congenic mice, we examined whether the host’s major histocompatibility complex is related to the qualitative difference of hydatid development.

Materials and methods

Female mice of nine inbred strains (A/J, AKR/N, BALB/c, C3H/HeJ, C57BL/6J, C57BL/10Sn, CBA/J, DBA/1J and DBA/2J) were used for the first experiment. For the second experiment, C57BL/10, DBA/2 and H-2 congenic B10.D2/n were used. H-2 haplotypes of these strains are shown in table 1. They were purchased from CLEA Japan, Inc., Charles River Japan, Inc. and Japan SLC, Inc. as specific pathogen-free mice and were housed under conventional conditions throughout the experiments. All mice used in these experiments were 9-10 weeks old.

Larval E. multilocularis was isolated from a naturally infected vole of Clethrionomys rufocanus captured in Hokkaido, Japan, in 1985. This isolate was maintained by intraperitoneal passages using the Mongolian gerbil, Meriones unguiculatus, at Asahikawa Medical College.

Metacestode tissue of E. multilocularis was removed from the peritoneal cavity of infected gerbils and minced well with scissors. Minced pieces were suspended in sterile phosphate-buffered saline (PBS, pH 7.4) containing kanamycin sulphate at 60 µg/ml and passed through a 210 µm mesh. The sediment containing minute vesicles, protoscoleces and calcareous corpuscles was washed three times with PBS. We prepared 5% suspension (sediment volume/total volume) with PBS and 0.1 ml of this suspension was injected into the upper mesenteric vein of each mouse (Nakao et al., 1990).
In the first experiment, the number of mice used was 7–14 of each strain; however, several mice died before examination. All surviving mice were killed 13 weeks after infection. In the second experiment, 5–7 mice of each strain were used and killed earlier at 9 weeks post-infection in order to avoid the loss of mice. After taking photographs, the infected liver of each mouse was removed and fixed in 10% neutral-buffered formalin (pH 7.4). Pieces of infected tissue were dehydrated and embedded in paraffin-wax. Sections at 5µm thickness were stained with haematoxylin and eosin (H & E) or periodic acid/Schiff (PAS). We used one of the sections from each strain and DBA/2 as representative of a vesicular developing strain. In all mice, vesicles were surrounded by a granulomatous infiltration with epithelioid cells, macrophages, giant cells, eosinophils and lymphocytes. Fibrosis was found in all strains.

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A large area of each hepatic lesion was sectioned and measured. Numbers of cross-sectioned vesicles and protoscoleces were counted in each lesion. All values were expressed as mean±S.D. Asterisks indicate a significant decrease compared with the highest value in each experiment by the Mann-Whitney U test.

### Table 1. Development of larval Echinococcus multilocularis in the livers of nine inbred and one H-2 congenic mice.

<table>
<thead>
<tr>
<th>Strains (H-2 haplotypes)</th>
<th>No. mice</th>
<th>Areas examined in mm²</th>
<th>No. vesicles per mm²</th>
<th>No. protoscoleces per mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first experiment: killed 13 weeks after infection</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>A (a)</td>
<td>6</td>
<td>140.2±26.0</td>
<td>1.763±0.466 *</td>
<td>0</td>
</tr>
<tr>
<td>AKR (k)</td>
<td>6</td>
<td>123.0±10.1</td>
<td>1.275±0.903 *</td>
<td>0.164±0.204</td>
</tr>
<tr>
<td>BALB/c (d)</td>
<td>7</td>
<td>124.9±42.6</td>
<td>2.771±0.690</td>
<td>0.002±0.003 *</td>
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<tr>
<td>C3H (k)</td>
<td>10</td>
<td>126.8±23.0</td>
<td>3.137±0.515 *</td>
<td>0</td>
</tr>
<tr>
<td>C57BL/6 (b)</td>
<td>9</td>
<td>139.2±17.1</td>
<td>2.793±1.025</td>
<td>0.002±0.003 *</td>
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<tr>
<td>C57BL/10 (b)</td>
<td>5</td>
<td>102.6±16.5</td>
<td>4.137±0.776</td>
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<tr>
<td>CBA (k)</td>
<td>6</td>
<td>118.3±19.5</td>
<td>1.733±0.663 *</td>
<td>0.087±0.213</td>
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<td>DBA/1 (q)</td>
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<td>164.3±47.8</td>
<td>2.850±1.399</td>
<td>0.137±0.227</td>
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<tr>
<td>DBA/2 (d)</td>
<td>14</td>
<td>126.8±27.6</td>
<td>1.605±0.875 *</td>
<td>0.285±0.225</td>
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<tr>
<td>The second experiment: killed 9 weeks after infection</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>B10.D2 (d)</td>
<td>7</td>
<td>77.0±23.8</td>
<td>2.761±1.332</td>
<td>0</td>
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<tr>
<td>C57BL/10 (b)</td>
<td>5</td>
<td>58.4±16.0</td>
<td>3.294±0.967</td>
<td>0</td>
</tr>
<tr>
<td>DBA/2 (d)</td>
<td>5</td>
<td>90.4±19.3</td>
<td>0.923±0.605 *</td>
<td>0.086±0.091</td>
</tr>
</tbody>
</table>

A large area of each hepatic lesion was sectioned and measured. Numbers of cross-sectioned vesicles and protoscoleces were counted in each lesion. All values were expressed as mean±S.D. Asterisks indicate a significant decrease compared with the highest value in each experiment by the Mann-Whitney U test.

Results

All mice used in the first and second experiments were found to be infected. Macroscopically, the development of multivesicles occurred exclusively within the liver. No metastases developed in the other organs of the peritoneal and thoracic cavities. All mice showed moderate splenomegaly. Livers were enlarged and a large number of translucent or whitish cloudy alveolar vesicles were discernible on the surface of the liver lobes in all mice (fig. 1A,B). The sizes of these stripped vesicles were highly variable.

In the first experiment, multivesicular development was different among mouse strains (Kruskal-Wallis test; df=8, H=33.26) (table 1). Multivesiculation was prominent in C57BL/10, DBA/1, C57BL/6 and BALB/c. The development of protoscoleces also differed among mouse strains (df=8, H=30.86) and protoscoleces were well developed in DBA/2, AKR, DBA/1 and CBA. We noticed that mouse strains with higher multivesicular development tended to show lower productivity of protoscoleces except DBA/1; however, C3H and A strains exhibited poor development of both vesicles and protoscoleces. No significant differences in inflammatory responses were observed among mouse strains (fig. 1C,D).

In all mice, vesicles were surrounded by a granulomatous infiltration with epithelioid cells, macrophages, giant cells, eosinophils and lymphocytes. Fibrosis was found in all strains.

Based on the data of the first experiment, we selected C57BL/10 as representative of a vesicular developing strain and DBA/2 as a protoscolex-developing strain. In the second experiment, comparison among C57BL/10, DBA/2 and their H-2 congenic B10.D2 was made. As shown in table 1, multivesicular development was not different between C57BL/10 and B10.D2. Both strains did not support the production of protoscoleces.

Discussion

Larval development of E. multilocularis has two phases; the first is the proliferation of multivesicles, and the second is the production of protoscoleces within the vesicles. In hepatic lesions of human AE, most vesicles are sterile. By
Fig. 1. Liver lesions of DBA/1 and C57BL/10 mice at 13 weeks post trans-portal injection with hydatid homogenate of *Echinococcus multilocularis*. A, liver surface of DBA/1; B, liver surface of C57BL/10; C, fertile (f) and sterile (s) vesicles in DBA/1 (H & E stain); D, sterile vesicles in C57BL/10 (H & E stain).
contrast with the human lesion, many fertile vesicles develop in suitable intermediate hosts such as microtine voles (Yamashita et al., 1958). The data presented in this report confirmed that there is a variation in larval development of *E. multilocularis* among different strains of mice (Liance et al., 1984; Kroeze & Tanner, 1987; Gottstein et al., 1994). C57BL/10, A and C3H mice appeared to be resistant strains due to the absence of protoscolex production, indicating that they may be suitable models for human AE.

Using intrahepatic injection of hydatid homogenate, Liance et al. (1984) demonstrated that AKR and C57BL/6 were susceptible strains with a 100% rate of infection and C57BL/10 was a resistant strain with a 32% rate of infection. On the other hand, using oral inoculation of eggs, Yamashita et al. (1958) reported that AKR, C57BL/6, A, BALB/c and C3H showed 100, 79, 71, 60 and 76% rates of infection, respectively. In our experiment, all mice of each strain could be infected by trans-portal injection of hydatid homogenate. However, our assessment of susceptibility on the basis of productivity of protoscoleces was well in agreement with the above mentioned results of infection rate. The discrepancy of infection rates between Yamashita’s and our results suggests that intestinal immunity may be important in the establishment of infection.

Recently, Gottstein et al. (1994) analysed *E. multilocularis*-specific cellular and humoral immune responses using susceptible strains (AKR and C57BL/6) and a resistant strain (C57BL/10). Their data suggested that those immune responses were controlled by non-H-2 gene(s). Similar speculation was presented by Kroeze & Tanner (1987) who compared intraperitoneal hydatid weights and humoral immune responses among C57L, SJL, C57BL/6, C57BL/6 (bgJ) and BALB/c mice. They concluded that the susceptibility or resistance to *E. multilocularis* in mice is probably controlled by non-H-2 gene(s). The result of our study using H-2 congenic mice suggests that qualitative differences in hydatid development may also be regulated by non-H-2 gene(s).

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**References**


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