Liver Lesions and Serum Antibodies in Mice Surviving Tyzzer's Disease

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ABSTRACT. In the liver of mice surviving for 2 weeks or more after intravenous inoculation of Tyzzer's organisms, MSK strain, were observed giant cell granulomas with intracellular deposition of mucopolysaccharide and calcium, while bacterial antigen was not detectable. Serum antibody was positive in all cases already at 2 weeks postinoculation and high-titered antibody remained at 9 weeks of infection. -KEY WORDS: giant cell granuloma, mouse, Tyzzer's disease.


Tyzzer's disease of mice was enhanced by treating with glucocorticoids (GC), and infected and GC-treated mice died of fulminant necrotizing hepatitis in a few days postinoculation (p.i.) [3, 4]. Without GC-treatment most of infected animals survived having postnecrotic granulomatous scar and high antibody titers [4]. This note is to describe repair process of liver lesions in infected mice without GC-treatment during a period from 2 to 9 weeks p.i.

Four-week-old female ICR mice weighing 17 to 21 g were from Charles River Japan, Atsugi, Kanagawa, and given sterilized pellets (Oriental Yeast, Tokyo) and tap water ad libitum.

Forty-two mice received intravenous (i.v.) injection of severely infected mouse liver homogenate in phosphate buffered saline, pH 7.4 (PBS) (0.2 ml), containing 2.5×10⁶ organisms of the MSK strain [2]. Five of 42 mice died on Day 5 or 6 p.i. showing fulminant hepatic necrosis. The remaining 37 mice were killed by fours or fives at 2 to 9 weeks p.i. and examined for liver lesions.

After gross examination liver and spleen tissues were fixed in 10% neutral buffered formalin solution. Paraffin sections were made by a routine procedure and stained with hematoxylin and eosin (HE), alcian blue and periodic acid Schiff (ALB-PAS) and von Kossa's silver impregnation. Another sample of the liver was fixed in Bouin's solution without acetic acid and subjected to immunoperoxidase stain (IPS) for the bacterial antigen [6]. Briefly, deparaffinized sections were first treated with IgG from an MSK-immunized rabbit, and then stained by avidin-biotin-peroxidase-complex (ABC) method, using biotinylated anti-rabbit IgG goat serum, ABC reagent (Vectastain ABC System, Vector Lab. Inc., USA) and diaminobenzidine.

Anti-MSK IgG antibody titers were determined by indirect immunofluorescent antibody technique (IIFA) using target organisms in infected mouse liver homogenate [2].

As shown in Table 1, fine white coagulative necrosis or slightly concaved scarred lesions were seen on the surface of the liver of only one or two mice at each examination (Fig. 1). With or without liver lesions all mice had a markedly enlarged spleen with clearly visible follicles. No gross changes were observed in other organs.

Histopathology of liver lesions was similar regardless of time interval p.i., showing some
Table 1. Liver lesions and antibody titers

<table>
<thead>
<tr>
<th>Weeks p.i.</th>
<th>Liver lesion a)</th>
<th>Antibody titer b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1/4 c)</td>
<td>25, 100* d), 100, 200</td>
</tr>
<tr>
<td>3</td>
<td>2/4</td>
<td>200, 800*, 800, 3,200*</td>
</tr>
<tr>
<td>4</td>
<td>2/5</td>
<td>400*, 400*, 400, 800, 800</td>
</tr>
<tr>
<td>5</td>
<td>2/5</td>
<td>200*, 400, 400, 800*, 1,600</td>
</tr>
<tr>
<td>6</td>
<td>1/5</td>
<td>100, 200*, 200, 400, 1,600</td>
</tr>
<tr>
<td>7</td>
<td>1/5</td>
<td>100, 400*, 400, 400, 800</td>
</tr>
<tr>
<td>8</td>
<td>2/5</td>
<td>1,600*, 1,600, 1,600, 1,600, 1,600, 3,200*</td>
</tr>
<tr>
<td>9</td>
<td>1/4</td>
<td>100, 100, 1,600*, 1,600</td>
</tr>
</tbody>
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a) Inoculated i.v. with 2.5×10⁶ MSK organisms.
b) Reciprocals of individual IIFA titers.
c) Positive/Examined.
d) * Mice with liver lesion.

granulomas with multinucleated giant cells. In the cytoplasm of the giant cells, were observed basophilic or weakly eosinophilic homogenous materials stained blue or reddish purple with ALB-PAS and black with Kossa. Connective tissues were proliferated around the cells (Figs. 2 and 3). Macrophages, lymphocytes and plasma cells were focally accumulated in the granulomatous lesions of some cases. By IPS bacterial antigen was not detectable in the liver of all cases examined at 2 to 9 weeks p.i. Regardless of the presence or absence of liver lesions splenic follicles were markedly enlarged.

Sera of infected mice showed anti-MSK IgG antibody titers of 1:25 to 1:200 and 1:100 to 1:3,200 at 2 week and 3 to 9 weeks p.i., respectively. There was no correlation between the antibody titers and the severity of liver lesions (Table 1).

In this study, some mice died on Day 5 or 6 p.i., indicating that the inoculum dose was large enough to produce liver lesions in all cases. Already within 2 weeks p.i. deposition of mucopolysaccharides and/or calcium was seen at the necrotizing center of the affected liver (data not shown), and bacterial antigen [6] was rather detected around the lesions. In surviving mice most necrotic lesions in the liver were ephemerally produc-
ed with increase in bacterial antigen [5, 6]. They might have disappeared within 2 weeks p.i., leaving giant cell granulomas with calcium deposition even at 9 weeks p.i. when high-titered antibody was detected. Mice were shown to have antibody at 1 week p.i. [4] and to be resistant to reinoculation at 3 weeks after the primary inoculation [1]. The present observation confirmed that persistent resistance to Tyzzer’s disease should be acquired after an ephemeral growth of the bacteria within hepatocytes. Individual difference of the resistance to the infection among mice remains to be studied.

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REFERENCES

1. Fujiwara, K., Kurashina, H., Maejima, K., Tajima,
要 約

Tyzzer 病感染耐過マウスの肝病変と血清抗体価の推移 (速報)：中山裕之・井上 智・二井愛介・安田彰典・岡田信彦・藤原公憲（東京大学農学部家畜病理学教室）——Tyzzer 菌 MSK 株を静脈内に接種後マウスの肝病変を 2 〜 9 週にわたってしらべた。生き残った一部のマウスの肝に、ムコ多糖類・石灰を貪食した多核巨細胞からなる肉芽腫がみられたが、酵素抗体法で菌抗原は観察されなかった。免疫応答法による血清抗体価 2 週で全例陽性を示し、抗体価は 9 週まで 1 : 100〜1 : 3,200 であった。