STUDIES ON ECHINOCOCCOSIS XXII

CHANGES OF SERUM TITERS IN MICE INFECTED EXPERIMENTALLY WITH *E. MULTILOCULARIS*

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INTRODUCTION

Over half a century has passed since the method of serological test was introduced in the diagnosis of echinococcosis, and many reports have been published up to the present. However, concerning the immune response of the host in company with the development of the larval *E. multilocularis*, little information is available.

In this paper, the reactivity of a circulating antibody in the infected mice is reported correlatively with the development of the larval *E. multilocularis*.

MATERIALS AND METHODS

Origin of *E. multilocularis* Eggs of *E. multilocularis* from a naturally infected fox caught at Nemuro, Hokkaido, and from a dog infected experimentally with Alaskan strain were used for inocula.

Experimental animals Inbred CF#1 mice were used. The mice were inoculated orally with 200 eggs of Nemuro strain, and were exsanguinated after a suitable period; 1, 2, 3, 4 and 7 months after the inoculation, respectively. The mice inoculated with 200 eggs of Alaskan strain were killed 11 months after infection.

The individual serum was separated from the blood clot, and heated at 60°C for 20 minutes after 4-fold diluting with 0.9% saline solution.

Sera from several normal mice were also used as a control.

For histological study, the liver, after weighing, was fixed with formalin, and paraffin sections were stained with hematoxylin-eosin.

Antigen preparation Cystic fluid of the liver foci at 7 months after infection was used for antigens. The liver foci were homogenized gently with a Waring Blendor and the centrifugal supernatant was used as cystic fluid, as described in the previous paper (ORIHARA, 1967).

Assay for antigen-antibody reaction Reactivity of the circulating antibody was measured by a complement fixation test, using KOLMER's method (ORIHARA, 1967).

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RESULTS

The liver of the infected mice was histologically examined from the viewpoints of a) multilocular vesiculation, b) brood capsule formation and c) protoscolex formation. The result is summarized in tables 1~6.

Histological findings were classified and represented as follows:

a) Degree of multilocular vesiculation

+ The number of the cysts on each section was less than 10. The size of the cyst was less than 100 μ in diameter.

++ The number of the cysts on each section was 10~20. The size of the cyst was 600 μ, although, sometimes, large cysts (1~2 mm in diameter) were observed. Exogenous budding was observed in small cysts.

+++ Large numbers of cyst were seen. A few large cysts (2 mm in diameter) were seen, but almost all the cysts were small.

++++ The hepatic lobes were replaced by the echinococcal foci. Numerous cysts were seen. The germinal layer was thick and reticular.

b) Degree of brood capsule formation

− No brood capsule formation

+ A few brood capsules were observed in few cysts, but almost all the cysts had no brood capsule.

++ Brood capsules were formed in the large cysts, and the number of them on each section was more than 10.

+++ Almost all the cysts had brood capsules.

c) Degree of protoscolex formation

— No protoscolex formation

+ A few protoscolices were formed, but almost all of them were immature.

++ Mature and immature protoscolices were observed, and brood capsules lined the cyst wall in one layer, and the number of protoscolices on each section was less than 20.

+++ Cysts were filled with protoscolices of which the majority were mature.

1 Cases after one month

The results of the complement fixation test and histological observation are shown in table 1.

In this group, the weight of the liver was less than 1.7 g in all cases. The development of larval echinococcus was at an early stage of multilocular vesiculation and no brood capsule or protoscolex formation was observed.

Complement fixation titers were mostly less than 1 : 4.

2 Cases after two months

In the cases observed two months after infection, increased weight of the liver was observed compared with that of the above group. In half the cases, large cysts were observed (1~2 mm in diameter), although their number was few, and exogenous budding was conspicuously seen in small vesicles.

TABLE 1 *Relationship between the serum titers and the development of echinococcus; one month*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.*1	B. C.*2	P.*3	
1	1.7	++	−	−	1 : 8
2	1.6	++	−	−	1 : 4
3	1.4	++	−	−	1 : 4
4	1.3	+	−	−	1 : 4
5	1.3	+	−	−	1 : 4
6	1.3	+	−	−	1 : 4
7	1.3	+	−	−	1 : 4
8	1.2	+	−	−	1 : 4
9	1.2	+	−	−	1 : 4
10	1.2	+	−	−	1 : 4
11	1.2	+	−	−	1 : 4
12	1.2	+	−	−	1 : 4

*1 Multilocular vesiculation
*2 Brood capsule formation
*3 Protoscolex formation

TABLE 2 *Relationship between the serum titers and the development of echinococcus; two months*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.	B. C.	P.	
1	3.1	++	+	−	1 : 32
2	1.9	++	−	−	1 : 16
3	1.9	+	−	−	1 : 4
4	1.8	++	−	−	1 : 32
5	1.7	++	−	−	1 : 16
6	1.6	+	−	−	1 : 4
7	1.6	++	−	−	1 : 4
8	1.5	+	−	−	1 : 4

Variety in complement fixation titers was observed as shown in table 2; from 1 : 4 to 1 : 32. Considerably higher titers were found in the cases where the liver was 1.7 g or more in weight, with one exception where a liver-weight of 1.9 g and a titer of 1 : 4 (tab. 2) were observed.

3 Cases after three months

The weight of the livers varied ranging from 2.0 to 6.2 g. In almost all the cases, multilocular vesiculation was more advanced, and a small number of brood capsules was formed. In a few cases, immature protoscolices were also observed.

In all cases, higher serum titers were recognized ranging from 1 : 16 to 1 : 32.

TABLE 3 *Relationship between the serum titers and the development of echinococcus; three months*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.	B. C.	P.	
1	6.2	###	+	−	1 : 32
2	5.6	###	##	+	1 : 32
3	5.1	###	+	−	1 : 16
4	4.1	###	+	−	1 : 16
5	3.3	###	+	−	1 : 32
6	3.0	###	+	+	1 : 16
7	2.4	##	−	−	1 : 32
8	2.0	##	−	−	1 : 32

4 Cases after four months

The livers were conspicuously enlarged, and were almost entirely occupied with echinococcal tissue. Large cysts were filled with transparent fluid and were soft, but a small number of comparatively hard foci composed of numerous minute cysts was also observed. Most cysts had brood capsules and immature protoscolices. Higher serum titers were recognized in all cases, ranging from 1 : 64 to 1 : 128 (tab. 4).

TABLE 4 *Relationship between the serum titers and the development of echinococcus; four months*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.	B. C.	P.	
1	18.6	####	+	+	1 : 128
2	16.0	####	##	##	1 : 64
3	15.0	####	##	##	1 : 128
4	14.2	####	##	##	1 : 64
5	13.6	####	##	##	1 : 128
6	13.2	####	##	+	1 : 64
7	12.1	####	##	+	1 : 64

5 Cases after seven months

The weight of the liver had further increased in comparison with cases above-noted. Foci protruded on the liver surface showing an irregular granular appearance. The multilocular structure became very complicated, and many brood capsules with fully developed protoscolices were found.

Complement fixation titers kept the same level as cases after four months (tab. 5).

TABLE 5 *Relationship between the serum titers and the development of echinococcus; seven months*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.	B. C.	P.	
1	21.4	###	##	##	1 : 64~128
2	20.4	###	##	##	1 : 64
3	18.7	###	##	##	1 : 64
4	18.6	###	##	##	1 : 128
5	16.0	##	##	+	1 : 64
6	15.8	###	##	##	1 : 128
7	13.2	###	##	##	1 : 128
8	7.7	###	##	##	1 : 64

TABLE 6 *Relationship between the serum titers and the development of echinococcus; eleven months*

CASE NO.	WEIGHT OF LIVER	DEVELOPMENT OF ECHINOCOCCUS			CF-TITER
		M. V.	B. C.	P.	
1	6.5	###	+	+	1 : 32
2	5.1	###	##	##	1 : 32
3	3.9	###	##	##	1 : 16
4	2.5	###	+	+	1 : 16
5	2.3	###	##	##	1 : 8
6	2.1	##	−	−	< 1 : 4
7	2.0	##	−	−	1 : 8
8	1.9	+	−	−	1 : 8
9	1.9	##	−	−	< 1 : 4
10	1.8	##	−	−	1 : 4
11	1.6	###	##	##	1 : 4
12	1.5	##	−	−	< 1 : 4

6 Cases after eleven months

The cases had been inoculated with Alaskan strain of *E. multilocularis*. In many cases, the hepatic foci were small in size, although a long period of time had elapsed after the infection. Cases Nos. 6 and 8~12 had particularly small foci, in which the development of larval echinococcus was delayed and degenerative changes were often found. The result is shown in table 6.

The cases with the liver of light weight, with inconspicuous multilocular vesiculation of the foci, or without brood capsules or protoscolices, showed especially low titers.

The control mice showed a liver weight of 1.2 to 1.3 g and gave a negative reaction in the complement fixation test.

DISCUSSION

IIDA et al. (1961) reported that mice infected with *E. multilocularis* showed positive results in the complement fixation test from only five months after infection. They compared their data with the histological observation reported independently by YAMASHITA et al. (1958) and concluded that positive serum titers could not be detected until the time when "apparent" echinococcus tissue was observed.

In the present study, conducted more minutely, the infected mice offered positive sera in half of the cases two months after the infection, and at this time no formation of brood capsules or protoscolices was recongnized. The serum titer, thereafter, increased gradually as time elapsed and reached a maximum after approximately four months.

In the cases in which larval echinococcus developed normally, multilocular vesiculation progressed by means of exogenous budding or herniation, and then brood capsule formation took place, followed by protoscolex formation. As shown in tables 2 and 3, a detectable amount of antibody could be demonstrated at a stage of infection earlier than the stage when brood capsule or protoscolex formation had not yet occurred. The formation of brood capsule or protoscolex, therefore, seemed not to be an essential factor for producing antibodies. This fact was also supported by the fact that in human multilocular echinococcosis, the protoscolex formation was very rare, whereas the serum titer was very high.

As a reason why a lower titer of antibodies was observed in the early stage of infection, it could be considered that the amount of antigens necessary for stimulating antibody production was insufficient. Antigenic stimulation for antibody production might start at the time of infection, when the parasite invaded the liver through the portal circulation system. Antigens in this stage, however, were small in quantity, and moreover, the release of the antigens might be limited more or less by the existence of granulation tissue surrounding the echinococcal

foci. This supposition might be also applied for explaining the fact that the mice having degenerative cysts surrounded by fibrous tissue showed a considerably low titer.

In the present experiment, the cystic fluid was only used as antigens and the complement fixation method was employed as the serologic test. Further experimentation using different antigens such as cyst or protoscolex extract, should be expected. And different procedures for serological test, such as the hemagglutination test introduced to the field of echinococcosis by GARABEDIAN et al. (1957) and KAGAN et al. (1959), or the gel diffusion test, should be expected.

SUMMARY

By the complement fixation test using cystic fluid of *E. multilocularis* as antigens, the increases of the serum titers in company with the development of the parasite were investigated in mice infected experimentally with *E. multilocularis*.

In the mice in which larval echinococcus normally developed, the complement fixation titer became clearly positive (1 : 16 or more) until three months elapsed after the infection.

In the mice showing the considerably higher titers, corresponding animals having liver foci of extending lesion or large cysts, in which multilocular vesiculation advanced, were usually observed. Almost all the mice with the positive reaction, showed a liver-weight of 1.7 g or more. On the other hand, the mice with reduced foci, in which the larval echinococcus manifested delayed development or degeneration, showed low serum titers in the complement fixation test.

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