THE EFFECTS OF IMMUNE RAT SERUM ON CLONORCHIS SINENSIS IN VITRO

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Abstract

The present study was performed to clarify the effect of immune rat serum on Clonorchis sinensis, liver fluke, adult worms, excysted juvenile worms, metacecariae, and eggs in vitro.

The adult and juvenile C. sinensis incubated in infected rat serum (IRS) developed tegumental precipitate which seemed to have an effect on the viability of parasites. No such reactions were observed in normal rat serum (NRS). Metacecariae and eggs cultured in immune serum did not show the precipitate on tegument.

Heat inactivation of IRS at 56°C, 30 min. diminished the precipitate dramatically on adult worms. But the ability of precipitate formation against adult worms regained upon addition of NRS.

From the results of the present study, it was suggested that the precipitate on tegument and in the medium have parasiticidal effects on C. sinensis adults and young worms, and is a specific immunoglobulin(s) and complement-dependent.

INTRODUCTION

It has been shown that the effective host immunity to a number of parasitic flukes develops after exposure to immune serum (Howell, Sanderman and Rajasekarah, 1977; Hayes, Bailar and Mitrovic, 1972; Lang, 1974; Jung et al, 1985). The host immunity involves of both cellular and humoral factors in elimination of worms (Corba et al, 1971; Armour and Dargie, 1974; Butterworth et al, 1977). The nature and mechanism of antiparasites immunity is, however, very complex one.

Sun (1969) attempted the effect of immune sera on adult Clonorchis sinensis in vitro. Precipitates were constantly demonstrated when living parasites were incubated immu-
nized sera heated or not. But, closely related species, *Opisthorchis viverrini* showed no precipitate in heat inactivated serum (Flavell, 1981).3)

The present study was undertaken to demonstrate whether the precipitate formed and complement-dependent. Furthermore, this paper attempted to clarify the effects of immune rat serum both on *C. sinensis* adult worms and on metacercariae, excysted juvenile worms, and eggs.

**MATERIALS AND METHODS**

**Experimental infections**

A group of 50 female spargue-dawley (SD) rats initially received 20 *C. sinensis* metacercariae per os by esophageal intubation, and thereafter a further 20 metacercariae at 15 days intervals. Animals were housed 10 cages and fed a standard rats chew diet and water ad libitum.

**Test sera**

Infected rat serum (IRS) was obtained from the above group of animals at death. Rats were exsanguinated 4 months later. Blood was collected and allowed to clot and centrifuged at 4,000 rpm for 30 min., stored at -20°C until required.

**Adult worms**

*C. sinensis* adult worms were obtained from the bile ducts of infected SD rats. Worms were washed three-times in M-199 (Gibco, U.S.A.) containing 100 units/ml penicillin and 100 μg/ml streptomycin, and then transferred in group of about 20 to culture flask (Bellco, U.S.A.) containing 5ml of M-199. Fresh worms for immediate use were held in culture medium at room temperature.

**Young Adults**

Excysted juvenile worms were obtained by metacercariae in excystation solution (trypsin 0.5g, sodium bicarbonate 0.2g, saline 50ml). Metacercariae were recovered from enzymatic digestion of infected *Pseudorasbora parva*.

Excysted metacercariae were rinsed several times in M-199, and then transferred in group of 50 to sterile culture plastic dishes (Bellco, U.S.A.) contained 1ml of culture medium.

**Eggs**

To obtain the eggs, adult worms were incubated in Tyrode solution for 12 hours at 37°C. The eggs were collected under a stereomicroscope and transferred to culture flask containing M-199 solution.

**Experimental Protocol**

Adult worms, excysted juvenile worms and metacercariae were tested against two concentration of serum (20%, 50%) as shown in Table 1. Heat inactivation effect was summarized in Table 2.

Single adult worms were placed in a 24-well culture plate (Bellco, U.S.A.) and 20% or 50% culture fluid was added, and incubated at 37°C. Also 5 metacercariae and 2 excysted juvenile adults were placed in each well. The eggs were contained in culture dishes about a group of 50.

Parasites in individual wells were observed and photographed under an inverted microscope at 2, 6, 10, 16, 24 and 48 hours and then once daily for further several days.

Formed precipitate around parasites and in the culture medium were indicated arbitrary,
Table 1. Results for reactions of adults worms, excysted juvenile worms, metacercariae, and eggs incubated in IRS with survival times of adult parasites

<table>
<thead>
<tr>
<th>Concentration of sera</th>
<th>Adult worms ppt</th>
<th>Juvenile worm ppt</th>
<th>Metacercariae ppt</th>
<th>eggs ppt</th>
<th>Survival time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% IRS*</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>50% IRS</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>20% NRS*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>50% NRS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
</tbody>
</table>

a. IRS: infected rat serum
b. NRS: normal rat serum
c. ppt: precipitate

Table 2. Results for reaction of adult worms, excysted juvenile worms, metacercariae, and eggs incubated in IRS heat inactivated at 56°C and reconstituted with NRS

<table>
<thead>
<tr>
<th>Type of sera</th>
<th>Adult worm ppt</th>
<th>Juvenile worm ppt</th>
<th>Metacercariae ppt</th>
<th>Egg ppt</th>
<th>Medium ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI* 50% IRS</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>+ 50% NRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI 50% IRS</td>
<td>++</td>
<td>+</td>
<td>NDc</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

a. HI: heated inactivated (56°C, 30 min.)
b. NRS: normal rat serum
c. ND: not done

such as +++ was scored maximum state, and + was minimum state.

RESULTS

The results of reactions of adult, excysted juvenile worms, metacercariae and eggs incubated in various serum are summarized in Table 1.

In adult worms incubated in 50% infected rat serum (IRS) after six hours in culture, a precipitate began to develop on the tegument, particularly in the vicinity of the excretory pore (Fig. 3). In the course of time, large amounts of precipitate were also found free in the medium and enclosed the entire parasites (Fig. 2). This appeared to be derived from the tegument precipitate by dislodgment. These precipitate was seemed to restrict parasite movement. After 24 hours, all 12 adult worms incubated in 50% IRS appeared moribund (maximum state, ++++) and dead after four days (Fig. 4).

After worms induced in 20% IRS showed minimum state (+) precipitate after 24 hours, but the extent and intensity of reaction was markedly below that encountered in 50% IRS. Moreover, all adult worms were alive and healthy in appearance after 10 days (Fig. 1).
Adult parasites incubated in 50% normal rat serum (NRS) showed no precipitate and all 20 parasites survived for more than 10 days. 56°C 30min. inactivated IRS showed minimum state (+) precipitate. Adult worms incubated in 56°C heat inactivated 50% IRS combined with 50% NRS showed precipitate after 12 hours and reach maximum state (+ + +) before 24 hours (Table 2).

Excysted juvenile worms already died in medium state (+ + +) precipitate two days later (Fig. 5).

No precipitate was found in metacercariae cultured with various serum, only 20 metacercariae showed trace precipitate among 100 metacercariae in 50% IRS (Fig. 6).

No precipitate was observed around eggs and in culture medium incubated in various concentration sera (Table 1, Fig. 7).

Freeze-thaw killed parasites incubated in 50% IRS showed a slight precipitate (trace) on their teguments, but the reaction did not proceed any further.

**DISCUSSION**

The present study shows that immune rat serum collected from infected rat has a competence to parasiticidal effects and it form the precipitate on living *C. sinensis* adult worms and juvenile worms in vitro. These results are in agreement with previous works (Sun, 1969; Flavell, 1981).\(^{7,18}\)

Sun (1969) demonstrated parasiticidal effects and precipitin formation of immune sera collected from infected rabbit and human on *C. sinensis* adult worms in vitro. But, he stated that precipitin was consistently seen surrounding the flukes whether the sera heated or not. The present study, however, represented that adult worms incubated in 56°C 30 min. inactivated 50% IRS showed minimum state (+) precipitate. Moreover, 56°C 30 min. inactivated IRS mixed with normal rat serum (NRS) apparently showed maximum state precipitate. These results consist with the work of Flavell (1981) who reported that heat inactivation of chronically infected serum at 56°C 30 min. was resulted in the total loss of precipitate surrounding the worms and in the culture medium. So it seemed probable that it is a specific immunoglobulin(s), and both precipitate and parasiticidal activities of immune serum against parasites appeared to be complement-dependent activity being remarkably diminished by heat inactivation of IRS at 56°C but regained upon addition of normal rat serum as a source of complement.\(^{5} \)\(^{8}\)

When *C. sinensis* eggs were incubated in the culture medium, there was no reaction whatever kind of sera was mixed. Hou (1955) described that adenomatous formation in the wall of bile ducts can hardly be ascribed to the action of *C. sinensis* ova.\(^{5}\) However, closely related liver fluke, *O. viverrini* eggs were demonstrated the granulomatous reaction in the livers of experimentally infected hamsters (Bhamarapravati et al., 1978; Flavell et al, 1980).\(^{5,14}\) These distinctions agree with the results of study on the precipitate formation of eggs in vitro.

Howell et al (1977) reported that the study of *Fasciola hepatica* in vitro also revealed similar results.\(^{10}\) But metacercariae of *F. hepatica* showed a lot of precipitate on the tegument and in culture medium. These differences exhibit the dissimilarity between *C. sinensis* and *F. hepatica*, namely *F. hepatica* penetrate the parenchymal tissues in the liver.
and these effects of immune sera can count upon in vivo. *C. sinensis* and *O. viverrini*, however, harbor in biliary passages and these effects cannot rely upon in vivo (Sun et al, 1968)\(^6\).

Although it is difficult to say that lymphoid cells can come into direct contact with the fluke when resident within the bile ducts, it is possible that metabolic products or antigenic structural components shed from the parasites surface may diffuse through the bile ducts and into the surrounding tissues, where they may elicit an immune response (Flavell et al, 1979).\(^7\) These problems will be subjects to further investigations.

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**REFERENCES**


12. Lang BZ: Host parasite relationships of *Fasciola hepatica* in the white mouse. VI. Studies on the effects of immune and normal sera on the viability of young worms transferred to normal recipients. J Parasitol., 60 : 925, 1974


Fig. 1. *C. sinensis* adult worm in 20% IRS after 8 hrs. It seems healthy in appearance. (×40)

Fig. 2. Adult parasite enclosed in a precipitate in 50% IRS after 72 hours. (×40)

Fig. 3. Adult parasite after 18hrs incubation in 50% IRS showing attached precipitate on tegument surface. (×40)

Fig. 4. Filament-like precipitate cultured in 50% IRS for 36hrs. (×40)
Fig. 5. Excysted juvenile worm in 50% IRS after 24 hrs showing precipitate and moribund. (×40)

Fig. 6. Metacercariae incubated in 50% IRS for 48 hrs. Note that the absence of precipitate on the tegument.

Fig. 7. *C. sinensis* eggs incubated in 50% IRS for 24 hrs, no precipitate on egg surface and in the medium. (×100)