

OBSERVATIONS ON THE EFFECTS OF CYSTICERCOSIS IMMUNE SERA ON CYSTICERCOSIS BY SCANNING ELECTRONMICROSCOPY

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Abstract. Two antigens of *Taenia solium* cysticercus, cystic fluid antigen (CFA) and the culture medium antigen (CMA), were used respectively to immunize rabbits in order to obtain immunosera. The CMA immunoserum added to culture medium with cysticerci limited the activities of the bladder worms. By using the scanning electronmicroscope, we could observe particulate deposits on the surface of the scolices, suckers and necks of the worms. The CFA immunoserum group showed similar changes but the deposit was less than that on the worms in the former group and appeared mainly on the cystic wall. After adding complement to the two groups mentioned above, we found that the microcilia on the surface of the worms were swollen and were seriously damaged. The worms treated with praziquantel were damaged over large area of their surfaces and were affected deep into their tissues. The damaged parts of the worms were quite different between the two groups. CMA is secreted by the living worms and therefore the serum antibodies are more effective than CFA in anti-parasite activity.

INTRODUCTION

Cysticercus cellulosae the larval stage of *Taenia solium* in the human body, causes chronic inflammatory reaction around the parasitic tissues, infiltrated by eosinocytes (Williams, 1979). Specific antibodies into the parasites can be detected. In order to study the effect of the antibodies on parasites we cultured the bladder worms *in vitro* in different culture fluids which contained two immunosera respectively and observed the damage to worms by means of the scanning electronmicroscope.

MATERIALS AND METHODS

The preparation of the antigen

Cysticercus fluid (CFA) was drawn from cysticerci in freshly slaughtered pigs, and cysticercus medium antigen (CMA) was taken from the culture medium of the bladder worms (Shi, 1986). Both antigens were centrifuged, concentrated and their protein contents measured; CFA 7, 4mg/ml and CMA 8, 6mg/ml. After filter sterilization these antigens were divided and stored at 4°C.

Animals

Ten male rabbits, cysticercus antibody negative, (average weight was 1.5kg) were divided into 3 groups: 3 in the CMA group, 3 in the CFA group and 2 as in the control group.

The preparation of the sera

Normal rabbit serum (NS) was prepared from the control rabbits.

CMA immunoserum (F) and CFA immunoserum (S) were prepared according to the method of Yu (1982). The two antigens were used to immunize the rabbits 3 times respectively. When the antibody level was over 1 : 6,400 (by ELISA), the immunosera were separated from heart blood.

Complement (C) was prepared from a guinea pig. The sera, except C, were treated at 56°C for 30 minutes.

The preparation of the samples for the scanning electronmicroscope

Cystic worms were carefully taken from the

freshly slaughtered pork and put into 50% bile for 5 to 10 minutes, making their scolices evaginate. After being washed with normal saline for three changes, and PBS for three changes, the worms were put into the different systems (Table 1)

The preparation of samples for scanning electron-microscopy

After culturing in each system for 2 hours, the worms were taken and washed in 3 changes of PBS for 10 minutes each. Then the walls of the worms were cut open and fixed in 60% alcohol, washed in 2 changes of PBS for 10 minutes each. These were observed under a Hitachi-S 450 scanning electronmicroscope.

RESULTS

Gross observation

Before the operation of the worms in the group, they appeared to be moving: Their scolices moved from side to side, their necks stretched out and drew back and their walls were contracted. Some of them were

attached to the wall of other worms or to themselves by suckers. After culturing in each operation system, the worms showed different changes:

NS group : no changes. F and S group: worms moved and their necks drew back; 2 hours later, worms stopped moving. Fc and Sc groups: the worms stopped moving in 30 minutes and their necks drew back and their bodies swelled. Fp and Sp group: the worms stopped moving in 3-5 minutes. Two hours later, the culture fluid became turbid and the worms sank to the bottom of the culture bottle.

Observing under scanning electronmicroscope:

Ns group: There was no damage or precipitates on the surface of the worms (Fig 1).

F group: There was a lot of flocculate on the surface of the worms' scolices, suckers and necks, The hooks on the scolices were covered with the flocculate. Microcilia on the surface of the worms were adhesive. Fc group: in addition to the above changes, microcillia were swollen and parts of them had fallen off (Figs 2, 3).

Table 1

The composition of the culture medium and its effect on cysticerci *in vitro*.

Composition and amount (ml)	F	S	Fc	Sc	Fp	Sp	Ns
CMA immunoserum	1.5	-	15	-	1.5	-	-
CFA immunoserum	-	1.5	-	1.5	-	1.5	-
Normal rabbit serum	-	-	-	-	-	-	1.5
1640 culture fluid	8.5	8.5	7.0	7.0	8.5	8.5	8.5
Complement serum	-	-	1.5	1.5	-	-	-
Praziquantel (µg)	-	-	-	-	20	20	-

F : CMA immunoserum group

Fc : CMA immunoserum + complement

Fp : CMA immunoserum + praziquantel

Ns : normal rabbit serum

S : CFA immunoserum group

Sc : CFA immunoserum + complement

Sp : CFA immunoserum + praziquantel

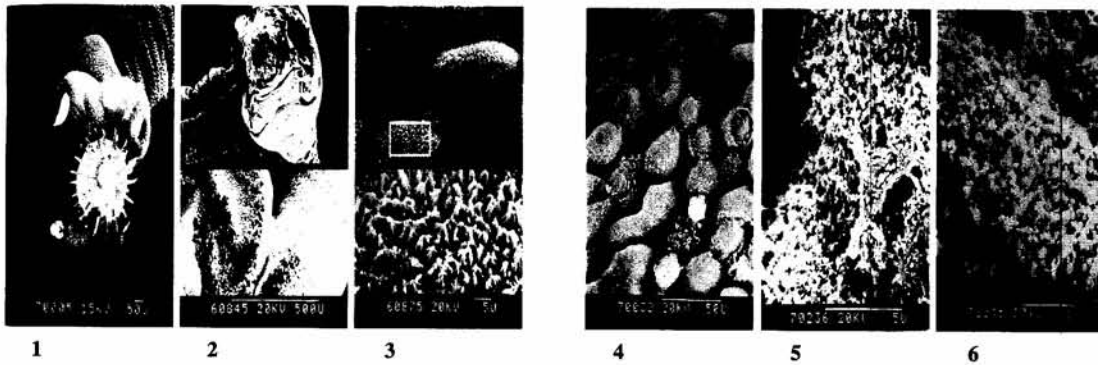


Fig 1 - NS group; no change; no precipitates on the body.

Figs 2, 3 - F group; precipitates or flocculates on the body of the worms.

Figs 4, 5 - S group; precipitates on the body especially on the microcilia.

Fig 6 - Sp and Fp groups; worm tissue shows a great deal of damage.

S. group: There was a lot of precipitate deposited on the worms' bodies.

Sc group: microcilia were adhesive and parts had fallen off. Less flocculate on the scolices and necks with no damage to the structure on the scolices and neck with no damage to the structure (Figs 4, 5).

Sp and Fp groups: The tissues of the worms were seriously damaged. In Sp group: The damage was mainly on the walls but in Fp group the damage was mainly on the scolices and necks of the worms (Fig 6).

DISCUSSION

Chen (1956) had reported that when *T. taeniaformis* was exposed to immunoserum, its body became shrunken and it collapsed. He considered that immunoserum could damage the cystic wall. This paper reports similar results. It was a reasonable fact that the worms were seriously damaged in the group of immunoserum with complement (Fc and Sc), which suggested that antibodies themselves could harm the worm or could activate the complement to cause a series of anti-parasitic immunoreactions in the host's body. In addition to the above, the antibodies probably influenced the normal metabolism of worms.

From the different deposits on the worms in F and S groups, we observed different specific properties of the two antigens. CMA was the metabolite from the living worms. Scolices and necks were the life center of the worms which had their own complex

structure. The worms which had only the scolex and neck left could still develop into a whole adult. Therefore, the metabolic activities in these parts could be very vigorous. Being the internal metabolic environment, the cystic fluid could contain metabolites of the worms. However, we found by the analysis of the protein electrophoresis that CMA contained at least more than two kinds of characteristic protein compared to CFA. It has been reported that the metabolic antigens of hydatid cysts played a more important role than that of the body antigens (Kwa, 1977). Consequently, the finding that antibodies caused by CMA deposits flocculates on the main parts of the worm warrants further study.

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