FOLIA PARASITOLOGICA (PRAHA) 27: 145–149, 1980.

DEMONSTRATION OF CELLULAR HYPERSENSITIVITY IN BOVINE CYSTICERCOSIS BY MIGRATION-INHIBITION TEST

K. BLAŽEK, M. ŠVEC*), J. SCHRAMLOVÁ, and J. KURSA**)

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, *) Military Institute of Hygiene, Epidemiology and Microbiology, Prague, and **) University of Agriculture, České Budějovice

Abstract. Cellular hypersensitivity of bovine leukocytes to cysticercus antigen in experimental bovine cysticercosis was demonstrated by means of the migration-inhibition test. The authors detected lymphokin which is capable of suppressing in vitro the migration of heterogeneous cells of the macrophage type. The maximum value of migration inhibition was 44 %.

Cellular immunity in animals with parasitic diseases has been studied particularly during some protozoal infections, as bovine anaplasmosis (Buening 1973, Carson et al 1977a, b) and less frequently during helminthoses, e.g., intestinal and muscular trichinellosis (Bany 1978, Ljungström 1978), migration phase of ascariosis (*Ascaris suum* in guinea pigs) (Soulsby and Khoury 1975), expulsion of intestinal parasitic nematodes in a model experiment (Lustigman and Wertheim 1978), infection of sheep with larvae of *Taenia hydatigena* and *T. ovis* (Blundell et al. 1969), infection of rabbits with *Taenia pisiformis* (Rickard and Outteridge 1974) and experimental hydatidosis in mice (Bolshakova and Leikina 1977). However, there are no reports dealing with cellular immunity in bovine cysticercosis caused by *Cysticercus bovis*. Some authors described positive allergic skin reactions indicating the hypersensitivity of the organism during this parasitosis (Dewhirst et al. 1960, Bugyaki 1961, Froyd 1963, Lerche 1963).

While studying organ reaction during the early and late phase of *C. bovis* development, an intense and early proliferation of some types of cells was observed around the migrating or already settled larva (Blažek et al. 1980). We have therefore attempted to demonstrate the cellular hypersensitivity of bovine leukocytes to cysticercus antigen by means of the migration-inhibition test (MIT). The mechanism of this reaction consists in the production of biologically active lymphokins as a result of interaction of sensitive lymphocytes with the specific antigen. One of the lymphokins is the migration-inhibition factor (MIF) capable of suppressing the migration of cells of macrophage type from both the normal and sensitive organism (Švejcar et al. 1971, Pekárek and Švejcar 1978). We assumed that the result of this experiment could contribute to a better knowledge of the mechanism of host defence reactions during bovine cysticercosis and that this method, after a modification, could be applied also in intravital diagnosis of this parasitosis.

MATERIAL AND METHODS

Animals. A male calf of red dappled race, at the age of three months, weighing 120 kg was infected orally with 200,000 eggs of *Taenia saginata* (calf No. 2/78). The method of infection is described in detail in another paper (Blažek et al. 1979). The blood for the migration-inhibition test was taken five times, on days 63, 98, 196, 228 and 236 after infection. The result of primary infection was verified by the detection of humoral antibodies and biopsy of the masseter on day 63 after infection.

*) Partly supported by WHO Grant No. VPH/Z2/181/73.

The calf was reinfected with 26,000 eggs of *T. saginata* 228 days after primary infection and the last blood collection was performed 8 days after reinfection. The calf was killed on day 250 after primary infection and on day 21 after reinfection.

A calf of the same sex, race, age and weight and bred under the same conditions (calf No. 6/78) was used as a control. The blood for the MIT was taken twice, at the same time as from the experimental animal. No humoral antibodies have been found in the serum of this animal during the whole period of observation (112 days) and the post-mortem examination was negative.

Blood collection. The blood was taken from vena jugularis into 20-ml plastic syringes containing 0.5 ml of 0.8 % water solution of Chelaton 3 per 20 ml of blood. Every time 60 ml of blood were taken. The blood was placed in a 150-ml NTS bottle with 1 cm high layer of glass beads and defibrinized by shaking for 10—15 min (Carson et al. 1975).

Separation. The defibrinized blood was diluted with VEL (Sevac) medium at pH 7.2—7.4 (containing 100 units/ml of penicillin and 100 μg/ml of streptomycin) in the ratio of 1.5 : 1 (Pekárek and Švejcar 1978). The blood processed in this way was then carefully placed into 50-ml tubes (22 ml of diluted blood in each tube containing 15 ml of separation mixture) and centrifuged at 2,000 rpm for 30 and 55 min. The quality of leukocyte separation was controlled during the centrifugation. The separation mixture was prepared from 360 ml of Ficoll 400 and 85 ml of 60 % Verografin (Spofa). The density was adjusted to 1.085 by adding redistilled apyretic water.

After centrifugation, the cell suspensions in the layer above the mass of erythrocytes were collected, united and washed three times with VEL medium. Before the third washing the cells were divided into two portions: one of them was used for the cultivation with antigen and the other, cultivated without antigen, was used as a control. The cell viability was assessed by vital staining with 1% trypan blue and ranged between 11×10^3 and 16×10^3 in 1 ml.

Antigen. The somatic antigen from the bladder and scolex of *C. bovis*, of Czechoslovak origin,*) No. 01303 B, lyophilized and containing 1 mg N in 1.0 ml was used. After rehydration with 1.0 ml of redistilled water, the antigen (100 and 200 μg) was applied in the culture.

Incubation. The incubation of cell suspensions with antigen and of control suspensions was carried out in stoppered slant tubes at the temperature of 37 °C for 20—24 hours. The activity of supernatants after cell incubation with and without antigen was detected by means of migration of macrophages from fragments of spleen of a healthy rabbit. Spleen samples measuring about 1 mm^3 were prepared by cutting the spleen on a paraffin base previously devoid of surface fascia and washed with VEL and then they were placed into culture chamber for cultivation with the respective supernatant (Švejcar et al. 1971, Pekárek and Švejcar 1978). The chamber consisted of a frame from silicon vaseline on a microscopic slide. It was filled with the medium and five fragments, covered with a cover glass and cultured in a thermostat at 37 °C for 22 hours.

Evaluation. Culture chambers were projected on the screen of Dokumator DL-5.2 (Zeiss, Jena) at 21-fold linear magnification and the picture of fragments with growth zones was drawn on paper.

Eight lines were drawn from the margin of the fragment to the margin of the growth zone in the picture of each culture. The length of the lines was measured by a rule and the calculated average served as a value of migration activity (MA). Every MA value was the result of ten cultivations of individual fragments. The stage of migration inhibition was determined by a migration index (MI), i.e., ratio between MA under experimental conditions and MA under control conditions. Experimental MI and control MI were used for the calculation of the decrease of MA percentage in relation to MA of control cultures which ranged from 0.82 to 1.09.

Percentage of inhibition $= 1 - \frac{MI_e}{MI_c} \times 100$. Values lower than 0.8 represent the inhibition of migration. A positive result is obtained in case of at least 20% migration inhibition (Pekárek and Švejcar 1978).

RESULTS

Cellular hypersensitivity to a given antigen was determined in five sets of examinations with *C. bovis* antigen in the concentrations of 100 μg/ml and 200 μg/ml of cell suspension of bovine leukocytes performed in parallel with control examinations without antigen and control migration of spleen cells in VEL medium. The results are summarized in Table 1.

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*) The antigen was prepared and supplied by Dr. M. Uhlíková, C.Sc. of the Postgraduate Medical Institute in Prague to whom our thanks are due.

Table 1. Results of MIT in the experimental (2/78) and control (6/78) calves

Days after infection	Sample	ATG µg/1 ml	⌀ MA	MI	Inhibition %
63	2/78	100	119	0.74	30
		0	169	1.06	—
	6/78	100	148	0.92	—
		0	153	0.96	—
	VEL	—	160	—	—
98	2/78	100	125	0.69	16
		200	113	0.62	24
		0	149	0.82	—
	6/78	100	176	0.97	—
		200	190	1.05	—
		0	169	0.93	—
	VEL	—	181	—	—
196	2/78	100	148	0.76	19
		200	103	0.53	44
		0	182	0.94	—
	VEL	—	194	—	—
228 reinfection	2/78	200	112	0.66	39
		0	186	1.09	—
	VEL	—	170	—	—
236 8 days after reinfection	2/78	200	83	0.47	43
		0	155	0.89	—
	VEL	200	145	0.83	—
		0	175	—	—

All samples obtained from the experimental animal (No. 2/78) infected with larvae of *T. saginata* were positive. If 200 µg of antigen were used in 1 ml of suspension of bovine leukocytes, a marked decrease of the migration activity was observed in all cases, i.e., between 63rd and 236th day after infection. The maximum rate of migration inhibition was 44 %. The leukocytes from the blood of the control animal (No. 6/78) did not exhibit any sensitivity to the specific antigen and the MI values corresponded to the control range. This also indicates that the antigen is not toxic in the doses used.

The biopsy from the masseter and the post-mortem examination of the infected animal revealed that a massive infection was involved and that most of the cysts contained living, quite developed cysticerci. Moreover, solitary (6) foci with excessive proliferation of lymphocytes and histiocytes were found around the necrosis containing sometimes young stages of cysticerci. These foci were therefore considered to be directly related with the reinfection.

DISCUSSION

The erythrocytes from ruminants can be uneasily separated from other blood elements. The methods used for the separation of human leukocytes cannot be used at all. Neither the method recommended for the separation of leukocytes from erythrocytes in bovine blood for cultivation purposes described by Ulbrich and Wiegand (1963) was found to be suitable. We have therefore used the method of Carson et al. (1975) based on the centrifugation of blood in a gradient of the density of 1.066 or 1.070. However, the result was not satisfactory, since the yield of living cells was very low. Since the leukocyte separation should yield at least 10^4 of living cells in 1 ml of the suspension, we have performed separation experiments with blood samples from healthy animals at various densities of the mixture Verografin-Ficoll. The optimal density was found to be 1.084 to 1.090. In our experiments we have always used the separation mixture of the density of 1.085.

Although some authors successfully used the migration-inhibition test for the detection of cellular immunity in bovine anaplasmosis (Carson et al. 1977a, b), the determination of the migration-inhibition factor has not been used in the demonstration of cellular hypersensitivity during helminthoses. Rickard and Outteridge (1974) demonstrated cellular immunity in rabbit infection with *Cysticercus pisiformis* by in vitro transformation of labelled lymphocytes. Bolshakova and Leikina (1977) observed increase sensitivity of spleen lymphocytes to antigenic stimulus in experimental echinococcosis of mice using the method of the formation of rosette structures. Blundell et al. (1969) studied the possible participation of cellular immunity in the inducement of resistance. They tried to transfer the immunity transferring leukocytes from an immunized animal to a non-immune recipient, but their results did not confirm this possibility.

In our experiments, the hypersensitivity of leukocytes from the infected animal was observed between days 63 and 236 after infection. The percentage of migration inhibition of heterogeneous macrophages varied only insignificantly and remained constant even for one week after the reinfection. Consequently, the cellular hypersensitivity during the infection with *Taenia saginata* larvae was thus confirmed. For the time being it cannot be said at what time after infection the MIT positivity begins, how long it persists and whether or not the MIF production is proportional to the infection level. Neither the role of cellular immunity in the formal pathogenesis of bovine cysticercosis and its participation in the resistance of infected animals to reinfection can be assessed. These questions will be answered only after evaluation of the results of our further experimental work which is being performed at present.

ОБНАРУЖЕНИЕ КЛЕТОЧНОЙ СВЕРХЧУВСТВИТЕЛЬНОСТИ ПРИ ЦИСТИЦЕРКОЗЕ СКОТА С ПОМОЩЬЮ ТЕСТА ТОРМОЖЕНИЯ МИГРАЦИИ

К. Блажек, М. Швец, Я. Шрамлова и Й. Курса

Резюме. Авторы доказывали при помощи теста торможения миграции клеточную сверхчувствительность лейкоцитов к антигену цистицерка при экспериментальном цистицеркозе скота. Доказали продукцию лимфокина, обладающего способностью тормозить in vitro миграцию гетерогенных клеток типа макрофага. Максимальная величина торможения миграции была 44 %.

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Received 12 March 1979.

K. B., Parasitologický ústav ČSAV,
Flemingovo n. 2, 166 32 Praha 6,
ČSSR