

SHORT COMMUNICATION

INFECTION OF MICE IMMUNIZED WITH FORMOLIZED
CYSTOZOITES OF *SARCOCYSTIS DISPERSA* ČERNÁ, KOLÁŘOVÁ
ET ŠULC, 1978

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Abstract. Twenty mice were immunized with formolized cystozoites of *Sarcocystis dispersa*. Antibodies at the titres up to 1 : 128 were detected by IFAT. The antibody level induced in this way has no protective effect against the infection with the same species.

The immunity in sarcosporidia infection has been only little studied. The papers dealing with this subject only followed the levels of serum antibodies during sarcosporidiosis.

The indirect fluorescent antibody test (IFAT) was used for the detection of sarcosporidia antibodies for the first time by Tadros et al. (1974). Černá (1978) recommends to use IFAT for the detection of antibodies against *Sarcocystis dispersa*. Červa and Černá (1980) developed the indirect haemagglutination test (IHAT) for the detection of antibodies against sarcocysts. Bordjochki et al. (1978) immunized rabbits probably with live zoites of four *Sarcocystis* species obtained from domestic animals and compared the results of two serological methods, complement-fixation test and IFAT. The authors are of different opinions on the question whether the infection present in the host body protects it against a reinfection with the same species. Erber and Geisel (1979) state that after *S. suicanis* infection in pigs no reinfection can occur, whereas Ruiz and Frenkel (1976), who studied *S. muris* infection in mice, are convinced that a repeated infection is possible. This was confirmed also by our own experiment on the repeated infection of mice with *S. dispersa* (Gut and Černá 1980). The aim of the paper was to study the possibility of immunization of mice with muscle zoites of *S. dispersa* and protective effects of antibodies induced in this way.

MATERIAL AND METHODS

Ten males and ten females of SPF mice of ICR strain at the age of 4 weeks were used in the experiment. Three females served as controls.

The mice were fed with a standard diet (DOS-IIb — produced by VELAZ, Lysá n. L.). They were placed in a separate box. Muscle zoites from mice experimentally infected with *S. dispersa* and killed two months after infection were used as the antigen for IFAT and for immunization of mice. Cross-striated muscles were taken from the mice and homogenized in a mixer. The homogenate was trypsinized in 0.25 % trypsin in PBS pH 7.4 for 1 h (Erber 1977). The zoites were then released by digestion, washed 5 times in PBS and centrifuged at 1500 rpm for 5 min. Pure free zoites were fixed in 1 % formalin in PBS for 1 h and then washed 5 times in PBS. The dead antigen was used for the immunization of mice. For IFAT, the zoites were dropped on a slide and after drying they were kept at —18 °C.

IFAT was carried out after Kramář (1963) using a commercial anti-mouse conjugate from the Institute of Sera and Vaccines, Prague. The antigen prepared from cryolyzed cystozoites of *S. dispersa* (Červa and Černá 1980) was used for IHAT.

RESULTS

The mice were immunized with three doses of antigen containing 10⁵ zoites each on days 1, 5 and 28 after the beginning of the experiment. The first two doses were applied intraperitoneally, the third one subcutaneously with the addition of Freund's complete adjuvant mixed with the antigen in 1 : 1 ratio. The dose per mouse was 0.1 ml at intraperitoneal and 0.05 ml at subcutaneous inoculation.

On day 18, blood samples were taken and the IFAT antibodies detected were 1 : 8 to 1 : 16. The control non-immunized mice were negative.

On day 45, the titres were 1 : 32 to 1 : 128 and the control mice were again negative.

On day 48, all mice were infected perorally with high doses of *S. dispersa* sporocysts; males with 10⁴ and females with 10⁵ sporocysts.

Table 1. Immunization of mice with cystozoites of *Sarcocystis dispersa*

Day serological test		1	5	28	45	48	60		67		70
					IFAT			IFAT	IFAT	IHAT	
♂	1	Immunization with 10 ⁵ zoites i.p.	Immunization with 10 ⁵ zoites i.p.	Immunization with 10 ⁵ zoites s.c. + adjuvant		Infection with 10 ⁴ sporocysts			64	256	P
	2				64				256		
	3				128		+				
	4						+				
	5				64				128	640	
	6								128		
	7				32				256		
	8						+	512			
	9				64				128		P
	10				64				512		
♀	1	Immunization with 10 ⁵ zoites i.p.	Immunization with 10 ⁵ zoites i.p.	Immunization with 10 ⁵ zoites s.c. + adjuvant		Infection with 10 ⁵ sporocysts			128	1280	P
	2				128				256	2560	
	3								128	1280	
	4				128				256	640	
	5								256	1280	
	6								256	640	P
	7				64				512	1280	
	8								512	2560	
	9								256	2560	P
	10				128				512	1280	
C	1				0	Infection with 10 ⁵ sporocysts			32	1280	P
	2				0				0	80	
	3				0				32	2560	

+ — dead mouse, P — finding of muscle cysts at dissection, C — control

On day 60, three males died. Strongly enlarged and hæmorrhagic livers were found at the dissection. Blood sample was taken from a male immediately before dying and the IFAT titre was 1 : 512.

On day 67, blood was taken from all mice and IFAT titres of 1 : 64 to 1 : 512 were detected. The antibody titre in the control non-immunized mice was 1 : 32 and one

mouse was negative. The antibody titres detected by IHAT were 1 : 640 to 1 : 2 560 and in the control mice 1 : 80, 1 : 1 280 and 1 : 2 560.

On day 70, six mice were killed and well developed muscle cysts were found in all of them.

The results of the experiment are summarized in Table 1.

DISCUSSION

The results show that a high antibody level can be induced by the immunization of formolized zoites of *S. dispersa*. Specific antibodies up to the titre of 1 : 128 were detected by IFAT after immunization. These results are consistent with the data by Černá and Kolářová (1978) who detected the maximum antibody level on days 70—120 after experimental infection of mice with *S. dispersa*. The maximum IFAT titre was 1 : 80. IFAT was carried out using the antigen from muscle tissue sections with *S. dispersa* cysts and the antigen from free zoites.

It is evident that the immunization of mice with formolized zoites can provoke the formation of specific antibodies at the same level after a real infection with *S. dispersa*. These artificially evoked specific antibodies have no protective effect against *S. dispersa* infection. This is in agreement with the observation that the infection of mice with *S. dispersa* does not protect the animals against a reinfection (Gut and Černá 1980).

An interesting phenomenon is the death of three males on day 12 after infection. At this time, the transition of developmental stages from the liver to muscles is terminated and an intensive development of young muscle cysts begins (Černá 1977). We assume that this was the reason why some infected specimens became weak and died. Most probably the anaphylactic reaction induced by the developing zoites in young muscle cysts was involved. These stages are antigenically identical with the zoites used for the immunization. The previous developmental stages, liver schizonts, may differ antigenically from the cystozoites and the anaphylactic reaction occurs only when the muscle cystozoites are formed.

Acknowledgements. The author thanks Dr. Ž. Černá for providing the infection material of *S. dispersa*, Dr. L. Červa and Dr. V. Kozojed for a consultation and Mrs. M. Jírová for a technical assistance during serological reactions.

ЗАРАЖЕНИЕ МЫШЕЙ, ИММУНИЗИРОВАННЫХ ОБРАБОТАННЫМИ ФОРМАЛИНОМ ЦИСТОЗОИТАМИ *SARCOCYSTIS DISPERSA*
ČERNÁ, KOLÁŘOVÁ ET ŠULC, 1978

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Резюме. 20 мышей иммунизировали обработанными формалином цистозоидами *Sarcosystis dispersa*. Антитела в титрах до 1 : 128 выявлены при помощи IFAT. Антитела вызванные этим образом не оказывают защищающего действия против заражения тем-же видом.

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Received 2 December 1980.

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FOLIA PARASITOLOGICA (PRAHA) 29: 288, 1982.

I. V. Merkusheva, A. F. Bobkova: Gelminty domashnykh i dikikh zhivotnykh Byelorussii (Helminths of domestic and wild animals of Byelorussia). Publ. House Nauka i tekhnika, Minsk 1981, 120 pp. Price 1.10 R.

The Soviet science presents to the public another important book comprising the data on parasitic worms of domestic and free-living vertebrates living in the territory of the Byelorussian SSR. Though it is in fact a catalogue summarizing the parasite species, their hosts and localities, it is an evidence of the great development of the scientific work performed in the Byelorussian SSR after the Great October Socialist Revolution and the Great Patriotic War. As it follows from the historical survey, the first helminthological studies in this territory were performed in the years 1864 and 1880. A further development of scientific investigations in this branch was based on the scientific expeditions in 1925—1937 and the following expeditions realized after the year 1945. The literary survey comprising more than 400 citations indicates that the scientific school of Academician Skryabin has become deeply rooted in the Byelorussian SSR. As it is registered in the catalogue, 563 helminth species parasitic in fishes, amphibians, snakes, birds and mammals

have been found in this region. Besides the purely theoretical papers, a majority of studies deal with practical problems. For example, 20 helminth species have been recorded in mammals of the family Suidae, 37 species in cattle, 39 species in sheep, 24 species in goats, 7 species in horses, 14 species in domestic cats and 15 species in dogs. The investigations include also domestic birds (66 helminth species in geese and ducks and 13 species in poultry) and fishes (127 helminth species in 41 species of fishes). There are also many records of helminth larvae in various intermediate hosts.

A great part of the book is devoted to a survey of individual host species and their helminths. It is concluded by a carefully arranged alphabetic list of species and their genera.

The significance of this book lies also in the fact that it summarizes numerous data which will be of use to biologists, veterinarians and pedagogs engaged in the studies of parasitology and zoology.

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