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IMMUNE RESPONSE IN RABBITS EXPERIMENTALLY INFECTED WITH ASCARIS SUUM

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Abstract. Immune response of rabbits experimentally infected with *Ascaris suum* was studied by indirect haemagglutination method. The animals were infected with the doses of 5,000, 10,000 and 20,000 infective eggs per animal. Positive reactions were observed from days 5—11 p.i., maximum reactions on days 11—19 p.i. A reinfection with the same doses (1× or 2× after 35 and 65 days) increased the antibody titre. The strongest individual reaction was recorded on day 19 p.i. in the group infected with the highest dose (titre 1 : 4096). The increased antibody titres persisted till the end of the experiment (82th day p.i.) in all groups.

The attempts to diagnose ascaridiasis by serological methods have been made for a long time. Soulsby and Gilles (1965) used for the first time double diffusion in gel and indirect haemagglutination for the diagnosis of larval ascaridiasis. Experimental aspects of this disease were studied by, e.g., Poletaeva and Fedorova (1972), Benková and Borošková (1976) and Borošková (1981) in rabbits with various intensity of infection. The results did not provide a picture of the development of immune response of both specific and nonspecific hosts to the migration of ascarid larvae.

The present paper deals with the development of antibody response of a nonspecific host, rabbit, to the migration of *A. suum* larvae in simple and repeated infections at various intensities using the method of indirect haemagglutination.

MATERIAL AND METHODS

Chinchilla rabbits (VELAZ) weighing 2.5 kg on the average were used in the experiments. They were fed with KO 16 mixture. Before the experiment, a control sample of blood was taken and the animals were examined coprologically for the presence of helminth eggs.

The rabbits were divided into three groups including 10 animals each (sex ratio 1 : 1). They were infected per os with infective eggs of *A. suum* cultured after the method described by Prokopič and Klabanová (1980). The infectivity of eggs was previously verified in mice. Three different doses of eggs, 5,000, 10,000 and 20,000 per rabbit were used. The blood for examination was taken from the ear vein at 7-days' intervals up to the 82th day of infection when the experiment was terminated. The sera were freezed and stored at —20 °C until examination.

One third of each group of experimental rabbits was used as a control with a single infection (3 specimens) and the remaining animals were reinfected on day 35 with the same dose as that used for the first infection. Three of them were infected for the third time in the same manner on day 65. Negative control sera were obtained from a group of 10 noninfected rabbits.

Haemagglutination was carried out after Boyden (1951) in the modification by Uhlíková (1973). Sheep erythrocytes were modified with tannin (Lachema) in the concentration of 1 : 20,000. The extract from *A. suum* adults in saline, pH 7.2 was used as antigen, the initial concentration being 4.25 mg/ml protein. The working concentration was determined by titration against the control hyperimmune serum. Dynatech microtitrator with type U plates was used for the test.

Titre values for statistical evaluation (mean and standard deviation) were transferred to logarithms computed to the base of 2 (Vennes et al. 1957) and expressed in graphs.

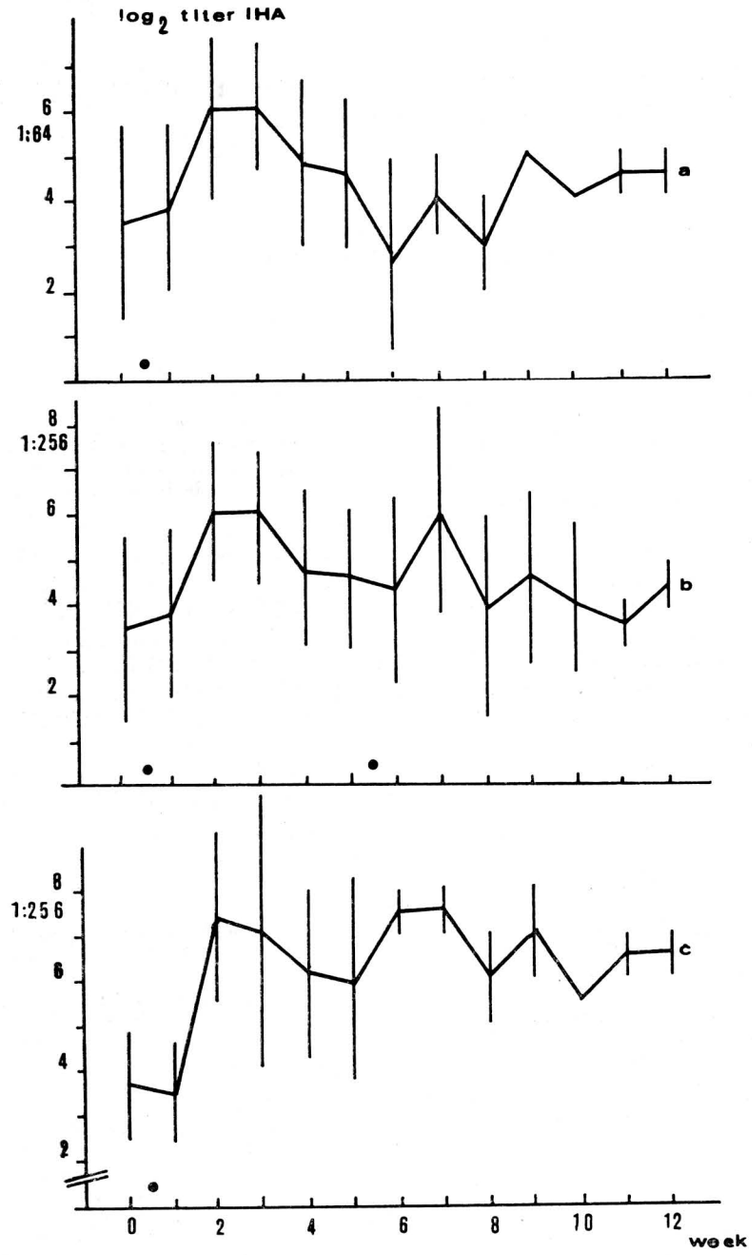

Fig. 1. Titres of antibody response to *Ascaris suum* infection in rabbits. a — 5,000 eggs, single infection; b — 5,000 eggs, repeated infection; c — 20,000 eggs, single infection. Mean titres and standard deviations detected by indirect haemagglutination.

RESULTS

The antibody levels in the control groups without infection were as follows: 1 : 2 titre in 15 cases, 1 : 4 titre in 11 cases, 1 : 8 titre in 4 cases, 1 : 16 titre in 6 cases and 1 : 32 titre in 4 cases. The results of haemagglutination of sera collected from control groups of rabbits (26) before the experiment were negative in 4 cases, 1 : 2 titre was found in 2 cases, 1 : 4 titre in 6 cases, 1 : 8 titre in 3 cases, 1 : 16 titre in 4 cases, and 1 : 32 titre in 7 cases.

The single infection of rabbits with the dose of 5,000 eggs of *A. suum* resulted in a slight increase in antibody level on days 5—11 p.i. and a marked increase from day 11 with maximum on day 19. Then the titres decreased (Fig. 1a). Up to the end of the experiment, the antibody level was slightly increased, compared to the original mean. After a reinfection with the same dose on day 35 p.i., the titres increased already on day 5 and the increased level persisted till the end of the experiment. The second reinfection resulted in a sudden increase in the mean antibody titre on days 11—18 after the second infection (Fig. 1b).

The course of infection with the dose of 10,000 eggs was similar. The antibody titres started to increase on day 5 p.i., maximum levels were recorded on day 19 p.i and the mean titre values decreased till the end of the experiment. After the reinfection with the same dose of eggs, the maximum titres were observed on day 47. The second reinfection resulted in a slight increase in the titres which persisted till the end of the experiment.

The infection with the maximum number of eggs (20,000) was manifested in a sudden increase in the mean antibody titre on day 11, with maximum on day 19, and the increased level persisted till the end of the experiment. In the group reinfected on day 35 p.i., in contrast to the previous one, no increased titre appeared and the curve slightly decreased till the end of the experiment. The second reinfection resulted in an increase in titres and this tendency persisted till the end of the experiment (Fig. 1c).

The absolutely strongest individual reaction occurred in the group infected with one dose of 20,000 eggs in the third week of the experiment. The titre increased to 1 : 4096, then decreased to 1 : 128 and then it remained almost unchanged till the end of the experiment, the values being 1 : 128—256.

In the 7th—8th weeks after infection, a slight increase in titres could be observed in all experimental groups. This increase seems to be due to the release of the antigen during the disintegration of larvae in the host body.

DISCUSSION

Indirect haemagglutination with erythrocytes treated with tannin or other substances belongs to the most sensitive routine reactions used in serological laboratories at the present time (Malberg 1980, Ferenčík 1980). The method was derived from the original method described by Boyden (1951) and is now widely used. Kagan (1974) and Kagan et al. (1967), however, do not consider the indirect haemagglutination to be a suitable test for the detection of larval ascaridiasis in man.

In our experiments, the method was verified with a rough, non-purified antigen of *A. suum* in rabbits. The test was found to be suitable for the study of antibody response of a non-specific host to the migration of *A. suum* larvae. A similar result was obtained by Borošková (1981) who found that the method of indirect haemagglutination is at least by one dilution of serum more sensitive during the whole course of infection then indirect immunofluorescence. The author successfully detected antibodies till

the 70th day of infection. Our results show that at all intensities of infection (5,000 to 20,000 eggs per rabbit) the antibodies can be detected by indirect haemagglutination from days 5—11, with maximum on days 11—19 p.i.

Borošková (1981) detected maximum titres on days 28—35 p.i. in all variants of the experiment. The absolute level of titres corresponded to the intensity of infection. Poletaeva and Fedorova (1972) using indirect haemagglutination managed to detect maximum level of antibodies in rabbits earlier, on days 23—30 p.i. Our results correspond to the lower value of the above ranges. Like in Borošková's (1981) experiments, maximum titres were detected in the group infected with the largest dose.

Of importance is the detection of the high number of increased antibody levels in the control sera collected before the experiment. Since the rabbits might have got in contact with ascarid antigens before the experiment, even a specific reaction may be considered. Due to the fact that many of rabbit breeds are infected with coccidiosis or toxoplasmosis, which was reported by Čatár et al. (1982) in almost 40 % of rabbits from both private farms and laboratory breeds, the possibility of a cross-reaction cannot be excluded. Čatár et al. (1982) detected toxoplasmosis by indirect immunofluorescence in 36.3 % of cases. Literary data on antigenic relations among helminths and the mentioned protozoans are lacking. For technical reasons this hypothesis could not be verified by a parallel test.

It can be concluded that the model system rabbit-*Ascaris suum* can be successfully studied by indirect haemagglutination. It was demonstrated that the dynamics of specific antibodies is not related with the infection dose of nematodes used in the experiment. On the contrary, the intensity of antibody response is directly proportional to the infective dose of eggs. The results obtained will be used in further studies of the parasite-host relationship in tissue helminthoses.

ИММУННЫЙ ОТВЕТ КРОЛИКОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЗАРАЖЕНИИ НЕМАТОДОЙ *ASCARIS SUUM*

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Резюме. Методом непрямой гемагглютинации изучали иммунный ответ кроликов на экспериментальное заражение нематодой *Ascaris suum*. Животных заражали инфекционными яйцами в дозах 5.000, 10.000 и 20.000 яиц на кролика. Положительные реакции наблюдались от 5-го до 11-го дня после заражения с максимумом на 11-й — 19-й день. Повторным заражением одинаковыми дозами (через 35 и 65 дней) повысился титр антител. Самая сильная индивидуальная реакция наблюдалась на 19-й день после заражения в группе, зараженной наивысшей дозой (титр 1 : 4.096). Повышение титров антител продолжалось до конца эксперимента (до 82-го дня) во всех группах.

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