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 6—11 p.i.; maximum reactions on days 11—19 p.i. A reinfection with the same doses (1 X or 2 X after 35 and 65 days) 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 ascariasis 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 ascariasis. Experimental aspects of this disease were studied by, e.g., Poletaeva and Fedorova (1972), Bonková and Borosková (1976) and Borosková (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 10 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 Prokopčík and Klabinová (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 frozen and stored at —20°C until examination.

One third of each group of experimental rabbits was used as a control with a single infection 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 (1931) in the modification by Uhlírková (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 (Venner et al. 1957) and expressed in graphs.
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 reinoculated 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, Ferenczi 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 Boroková (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 than indirect immunofluorescence. The author successfully detected antibodies till
ИММУННЫЙ ОТВЕТ КРОЛИКОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЗАРАЖЕНИИ НЕМАТОДОЙ ASCARIS SUUM

III. Лункен

Резюме. Методом инъекционной гемагглютинации изучали иммунный ответ кроликов на экспериментальное заражение нематодой Ascaris suum. Животных заражали инъекционными дозами в дозах 5,000, 10,000 и 20,000 яиц на кролика. Половозрелые реакции наблюдались от 5-го до 11-го дня после заражения с медианным в 11-й — 19-й день. Повторным заражением одинаковыми дозами (через 35 и 65 дней) повышался титр антител. Самая сильная индивидуальная реакция наблюдалась в 19-й день после заражения в группе, зараженной наивысшей дозой (титр 1:4,960). Повышение титров антител продолжалось до конца эксперимента (до 82-го дня) во всех группах.

REFERENCES


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