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TOXOPLASMIN SKIN TESTS AND ANTIBODY IMMUNE RESPONSES OF GUINEA PIGS INFECTED WITH *TOXOPLASMA GONDII*

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ABSTRACT

Guinea pigs were infected with the RH strain of *Toxoplasma gondii.* Their antibody and toxoplasmin skin test immune responses were followed for 28 weeks. The mean titer response peak (1:8875) for the methylene blue dye test occurred early at 32 days post-infection, but the mean titer response peak (1:1741) for the indirect hemagglutination test was not seen until much later at 188 days post-infection. A cyclic toxoplasmin skin test response was found with three mean skin test peaks (in mm induration) occurring at 24 (15.3mm), 103 (12.2mm) and 197 (11.2mm) days post-infection indicating a premunition call-mediated immune response to the chronic *Toxoplasma* infection.

INTRODUCTION

Toxoplasmosis is caused by a protozoan parasite, *Toxoplasma gondii,* which is widespread throughout the world both geographically and zoologically (Beattie, 1967; Jacobs, 1967; Feldman, 1968; Jacobs, 1976). The purpose of this investigation was to evaluate the dynamics of the immune response to a chronic *Toxoplasma* infection in guinea pigs (GP) with two frequently used serological tests and the skin test during development of the infection. Data from the methylene blue dye (MBD) test which uses live parasites, the indirect hemagglutination (IHA) test and the *in vivo* toxoplasmin intradermal skin test (ST) were compared temporally with each other during a 28 week immune response following infection of GP with the RH strain of *T. gondii.*

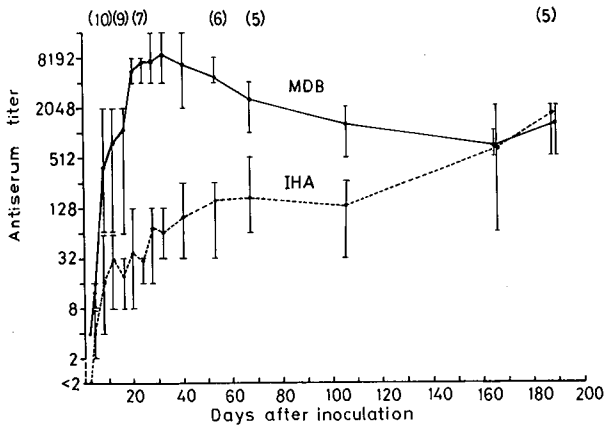

FIG. 1. *Antibody immune response of guinea pigs during* Toxoplasma *infection. The curves show the mean titers obtained by the indirect hemagglutination (IHA) and methylene blue dye (MBD) tests. Vertical bars indicate titer ranges. Number of guinea pigs are at top in parentheses.*

MATERIALS AND METHODS

Infection with Toxoplasma gondii

Ten Hartley strain female GP, each weighing about 400 grams, were infected subcutaneously with peritoneal exudate containing 2 x 10^6 (proliferative form) *Toxoplasma* (RH strain) obtained from mice after a three day infection (Ourth, 1971). Rising MBD test titers of guinea pig antisera indicated that *Toxoplasma* infection was manifest. After 28 weeks post-infection, their brains were removed for examination by Giemsa staining for the presence of *Toxoplasma* cysts.

Toxoplasma Serological Tests

Anti-*Toxoplasma* serum titers were determined by the MBD test (Beverley and Beattie, 1952) and the IHA test (Jacobs and Lunde, 1957) using sheep red blood cells sensitized with a soluble *Toxoplasma* antigen (Behringwerke, Germany). MBD and IHA initial titers were both begun at a 1:2 dilution. The GP were heart-bled 16 times (pre and post-infection) to obtain antisera over a 28 week period of infection.

Preparation of Toxoplasmin Skin Test Antigen

A *Toxoplasma* RH strain soluble ST antigen (toxoplasmin) was obtained by diluting mouse peritoneal exudate containing trophozoites with an equal volume of sterile distilled water followed by freezing and thawing at −80°C and 37°C ten times in succession (Frenkel, 1948; Chordi, Walls and Kagan, 1964; Ourth, 1971). Following centrifugation of the preparation at 6000 rpm for 20 min., protein concentration was determined and the ST antigen was diluted in phosphate-buffered saline, pH 7.2 (PBS) to contain 10 μg in 0.1 ml for intradermal skin testing of the GP.

Toxoplasmin Skin Test

Three uninfected and the ten infected GP were skin-tested 13 times (pre and post-infection) at different skin sites over a 28 week period by intradermal injection of 0.1 ml (10 μg) toxoplasmin ST antigen prepared as previously described. The ST was observed for erythema, and the diameter of ST induration was measured in mm at 4 and 24 hours (Ourth, Lunde and Watson, 1976) for evidence of *in vivo* delayed hypersensitivity (DH). The GP were also skin-tested intradermally with 0.1 ml PBS used as a control and with 0.1 ml normal mouse peritoneal exudate.

Results

Mean MBD and IHA antibody titers are graphed in FIG. 1. Mean MBD test titers by day (in parentheses) of immune response were: 4(2), 13(4), 405(8), 796(12), 1095(16), 5916(20), 7373(24), 7509(28), 8875(32), 6963(40), 4915(53), 2662(67), 1331(105), 717(165), 1331(188). Mean IHA test titers by day (in parentheses) of immune response were: <2(2), 4(4), 17(8), 31(12), 21(16), 37(20), 30(24), 75(28), 64(32), 105(40), 160(53), 179(67), 134(105), 678(165), 1741(188).

Mean ST diameters in mm of induration are graphed in FIG. 2. Mean toxoplasmin ST diameters in mm induration by day (in parentheses) of immune response were: 5.3(5), 8.7(8), 13.3(16), 15.3(24), 9.9(32), 6.0(40), 7.2(53), 8.0(67), 12.2(103), 8.2(165), 8.4(188), 11.2(197). Mean PBS control ST diameters in mm induration by day (in parentheses) of immune response were: 2.2(5), 3.8(8), 2.6(16), 3.1(24), 2.4(32), 2.1(40), 3.8(53), 3.4(67), 1.6(103), 1.6(165), 1.6(188), 1.8(197).

Sera from prebleeding the GP before *Toxoplasma* infection and sera from the uninfected control GP were negative. Toxoplasmin ST results in GP before infection were negative. No significant ST reactions were observed at 4 hours in infected or uninfected control GP nor at 24 hours in uninfected control GP. Normal mouse peritoneal exudate skin testing showed erythema but not induration to be higher when compared with the PBS control.

During the first 4 weeks of infection, good correlation between the MBD test and the ST was observed but not between the IHA test and ST or between the IHA and MBD tests FIG. 1 and 2). Peak responses were seen at 24 days and at 32 days post-infection, respectively, with the ST and MBD test (Fig. 1 and 2). Although IHA titers were also increasing early during infection, a peak IHA

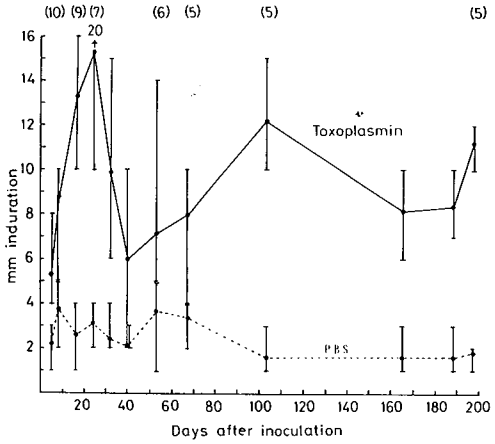

FIG. 2. *Toxoplasmin skin test (cellular) immune response of guinea pigs during* Toxoplasma *infection. The curves show the mean values in mm induration obtained by intradermal skin testing with toxoplasmin and the phosphate-buffered saline (PBS) control. Vertical bars indicate skin test ranges. Number of guinea pigs are at top in parentheses.*

test was not observed until much later at 188 days post-infection (Fig. 1). The MBD test was beginning to peak again at 188 days along with the IHA test and ST (Fig. 1 and 2).

The minimum infective serological titer for toxoplasmosis in man is considered to be 1:64 for the IHA test (Kagan and Norman, 1970) and 1:16 for the MBD test (Feldman, 1968). In this investigation, the greatest mean MBD titer of 1:8875 occurred early at 32 days post-infection, but the greatest mean IHA titer of 1:1741 occurred much later at 188 days post-infection (FIG. 1). The highest MBD and IHA titers found, respectively, were 1:16384 and 1:2048 (Fig. 1).

The GP were skin tested with toxoplasmin 12 times over a period of 197 days post-infection (Fig. 2). Two ST peak responses were observed at 24 and 103 days and a third peak was evidently developing at 197 days following initial infection. 79 days elapsed between the first and second ST peaks and 94 days between the second and third ST peaks. The ST data seemed to indicate a cyclic DH response to the chronic *Toxoplasma* infection (FIG. 2).

In man, Frenkel (1948) considered the toxoplasmin ST to be positive if 10 mm or more of induration were seen after 48 hours using toxoplasmin prepared from mouse peritoneal exudates. In this investigation, the greatest mean of 15.3 mm of induration in GP occurred at 24 days post-infection with a high of 20 mm induration being seen (FIG. 2).

Numerous *Toxoplasma* cysts were found in the five surviving guinea pigs' brains by Giemsa staining. Five of the ten infected GP and one of the three uninfected GP died from heart-bleeding during the investigation.

DISCUSSION

The passive transfer of *Toxoplasma* (RH strain) immune serum has been shown to afford little protection in GP, mice and rabbits when they were subsequently challenged with a lethal dose of the RH strain of *Toxoplasma* (Eichenwald, 1949; Foster and McCulloch, 1968); Gill and Prakash, 1970). Frenkel (1948) first demonstrated DH in human toxoplasmosis by toxoplasmin skin testing. Later Frenkel (1967) demonstrated the importance of cell-mediated immunity (CMI) in protection against *Toxoplasma* (RH strain) infection. He found that normal hamsters when injected with spleen and lymph node cells from infected hamsters resisted a *Toxoplasma* challenge infection that was otherwise fatal to normal animals. These observations suggest that long-term resistance to a chronic infection of toxoplasmosis is most likely due to CMI rather than to anti-*Toxoplasma* antibodies ,at least when considering infection with the RH strain.

A previous investigation demonstrated DH by macrophage migration inhibition and skin testing at 4, 8, 12 and 17 weeks and by lymphocyte transformation at 4, 12 and 17 weeks after infection of GP with the C-37 strain of *Toxoplasma gondii* (Ourth, Lunde and Watson, 1976). Macrophage migration inhibition and lymphocyte transformation were both most pronounced at 4 and 17 weeks post-infection (Ourth, Lunde and Watson, 1976). These results correspond closely with the peak ST responses found at 24 days and 103 days post-infection in this investigation (Fig. 2). Positive MBD and IHA test titers from 4 through 17 weeks were also found (Ourth, Lunde and Watson, 1976).

Several investigators including Thiermann *et al.* (1964) have previously compared the MBD and IHA tests and have found an earlier, more rapid appearance of MBD test antibodies in patients with acute *Toxoplasma* infections (Jacobs, 1976). IHA test antibodies appear later and are more useful in diagnosing chronic infections (Jacobs, 1976). MBD test antibodies are presumably first produced to *Toxoplasma* outer cell membrane antigens and later, following immune lysis, internal antigens are released that elicit production of IHA test antibodies (Jacobs, 1976). For these reasons, it is thought that the two tests detect different antibody responses and therefore must employ different test antigen preparations (Beattie, 1967; Jacobs, 1967; Jacobs, 1976; Thiermann, Knierim and Niedmann, 1964). The immune response data from this investigation also showed an early MBD test titer peak with a much later developing IHA test titer peak to the *Toxoplasma* infection by the GP (Fig. 1).

Reduction with 2-mercaptoethanol was not done on the guinea pig antisera to distinguish 19S from 7S antibody during the immune response to *Toxoplasma* infection in this investigation. However, Uhr and Finkelstein (1963; 1967) previously found by 2-mercaptoethanol reduction that 19S antibody formation had ceased within 10 days after injection of GP with *E. coli* bacteriophage and was replaced by 7S antibody.

The ST is an *in vivo* indicator of a *Toxoplasma* CMI response (Frenkel, 1948; McCluskey and Leber, 1974). The first and greatest peak (mean of 15.3 mm induration) of the DH response according to the ST data occurred at 24 days following initial infection with *Toxoplasma* (Fig. 2). During approximately the first 40 days after initial infection, the GP demonstrated their ability to control and thus survive the infection (Fig. 1 and 2). A second ST peak response occurred at 103 days and a third ST peak response was apparently developing at 197 days after initial infection (Fig. 2). These three ST peaks, seemingly cyclic in nature, are most likely a host response to the chronic infection comparable to premunition. The latent *Toxoplasma* infection thus becomes active intermittently as indicated by the three ST peak responses. These three ST peaks would in turn represent specific T-cell responses or the CMI response that would be continually needed to control a chronic infection and that would therefore be necessary for animal survival. Antibody may also be important in establishing a premunition response together with T-cells in mice infected with an avirulent strain of *Toxoplasma* (Hafizi and Modabber, 1978).

The cyclic nature of the ST data cannot be completely explained (Fig. 2). It could represent a DH response to the release at certain times in the infected GP of trophozoites from cysts thereby giving a sudden antigenic stimulus. These events could represent a regular, periodic release of antigen as happens for instance in malaria or be caused by a random, unpredictable release of antigen. However, it seems more likely that the cyclic nature of the ST would be caused by a random release of antigen due to the chronic, long-term infection of the host animal.

An investigation of human sera suggested that circulating antigens are found only during the short, active time phases of an acute *Toxoplasma* infection and when reinfected (van Knapen and Panggabean, 1977). Thus circulating antigen would be present only at certain periods during infection to promote an immune response. This would corroborate the cyclic ST data associated with the chronic *Toxoplasma* infection as seen in this investigation (Fig. 2). The cyclic ST response could represent the duration in time of a specific T-cell response to released *Toxoplasma* that would then be replaced after another antigenic stimulation by another specific T-cell population to *Toxoplasma*.

Whatever the reasons may be, specific T-cells would be stimulated at certain times following the initial infection giving the cyclic ST response pattern as indicated by the data (Fig. 2). It seems that the guinea pigs' ST response to toxoplasmin would remain cyclic so long as the chronic infection continued which likely would be for a lifetime.

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