RESEARCH NOTE

IMMUNE RESPONSE IN ACUTE *TOXOPLASMA* INFECTION OF BALB/C, ICR AND CBA/J MICE

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Several important variables appear to govern the pathogenicity of naturally acquired and experimental *Toxoplasma* infections. Different host species vary considerably in their susceptibility to *Toxoplasma* infection (Krahenbuhl and Remington, 1982).

Host susceptibility is related to the age of the host (Walters and Claman, 1975), hereditary factors (William *et al,* 1978) as well as the size of the inoculum (Araujo *et al,* 1976). In this study we assessed the effect of mouse strain differences on the humoral immune responses as detected with the enzyme-linked immunosorbent assay (ELISA) using soluble antigen and the indirect fluorescent antibody test (IFAT) using tachyzoites.

Three strains of white mice - Balb/C, CBA/J (both inbred) and ICR (outbred) - all laboratory bred and between the ages of four and six weeks and weighing an average of 25.0 g (22.0 g -27.0 g), were used for the present study. Ninety mice from each strain were each inoculated with 500 tachyzoites of *T. gondii* (RH strain), suspended in 0.5 ml of normal saline, while 10 mice of each strain which served as controls were inoculated with 0.5 ml of normal saline. Mice were given water and laboratory mouse chow *ad libitum.* The progress of the infection and mortality was followed for a period of ten days. On each day after inoculation, ten infected mice of each strain were bled by cardiac puncture. Sera collected were stored in separate vials at -20°C until use. Sera from 10 uninfected mice were used as controls. Peritoneal exudate from each mouse was taken to assess the parasite count. Brain smears from each mouse were also made on glass slides, stained with 10% Giemsa pH 7.2 and examined for cysts microscopically.

A modification of the Indirect ELISA method of Voller *et al* (1976) was used. Protein concentrations of the soluble (sonicated) antigens, ES antigens and uninfected mouse peritoneal exudate (UES) were estimated using the Bio-Rad (R) kit (Cat. No. 500.0006), and were 0.370 mg/ml, 0.096 mg/ml and 0.050 mg/ml, respectively. A preliminary chequer-board titration showed that the optimal working dilution of mouse serum, soluble parasite antigens, ES antigens, UES and enzyme conjugate were 1:200, 1:800, 1:800, 1:1000 and 1:35000, respectively. The wells of the microELISA plates were coated with 200 µl of soluble antigens and UES diluted in coating buffer in alternating columns.

The IFAT according to the method of Chessum (1970) was used. Fluorescein-labelled goat anti-mouse IgG or IgM conjugate (Capel, USA) diluted at 1:100 and 1:50 respectively with 0.2% Evans blue (as counterstain) were then added to each antigen slide.

The most dramatic increase in peritoneal parasites was observed between day 2 and day 6 for the inbred mouse strains and between day 1 and day 6 for the ICR outbred strain (Fig 1). Balb/c mice survived eight days post-inoculation of 500 tachyzoites as compared to seven and six days for CBA and ICR mice respectively. They also had the highest parasite yield, being 6.37×10^7 tachyzoites per ml of peritoneal fluid compared to 5.94×10^7 and 2.26×10^7 tachyzoites per ml for CBA/J and ICR mice, respectively. The importance of inoculum size was also demonstrated by Araujo *et al* (1976) who showed that Balb/c mice were least susceptible at lower doses of *Toxoplasma* but most susceptible when a large inoculum was used. 500 tachyzoites per ml of normal saline was the

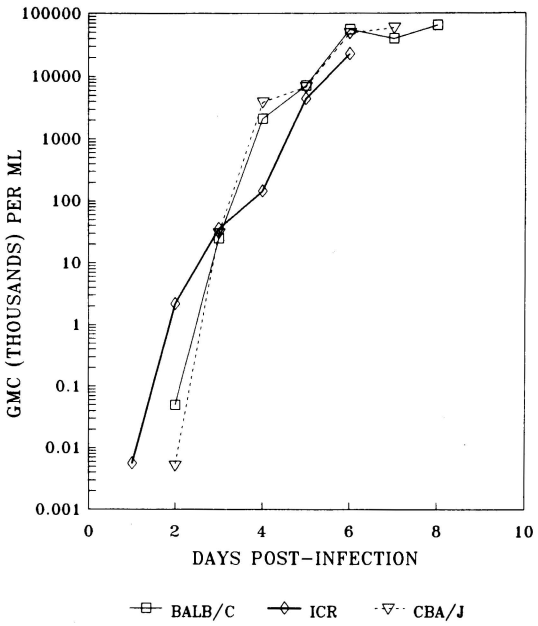

Fig 1—Geometric mean parasite count (GMC) in peritoneal exudate of Balb/c, ICR and CBA/J mice infected with 500 tachyzoites intraperitoneally, by days post-infection.

optimum inoculum size in the present study in terms of survival time of the mice.

The inbred mouse strains Balb/c and CBA/J showed a similar pattern of increase in parasitemia, while the outbred ICR mice not only showed a slightly different pattern of increase in parasitemia but also a shorter survival time (Fig 1). Araujo *et al* (1976) reported clearly significant differences in susceptibility to *Toxoplasma* infection of the C56 strain among inbred strains of mice. William *et al* (1978) showed that resistance in mice to *Toxoplasma* infection was under multigenic control. The expression of the different genes determining the resistance was markedly influenced by the infective dose since at high doses all strains of mice succumbed rapidly and at low doses almost all the mice of each strain survived the infection.

There were positive correlations between the duration of infection (days post-infection) and the production of IgM ($r = 0.93, t = 8.070, p < 0.001$) and IgG ($r = 0.57, t = 1.835, 0.2 > p > 0.1$) antibodies against soluble antigens in infected Balb/c mice (Fig 2). The IgG antibody production peaked on day 6 and day 8 post-infection. The correlations in ICR mice were $r = 0.78$ ($t = 2.790$, $0.05 > p > 0.02$) for IgM and $r = 0.63$ ($t = 1.810$, $0.2 > p > 0.1$) for IgG (Fig 3). They were $r = 0.99$ ($t = 17.19$, $p < 0.001$) for IgM and $r = 0.35$ ($t = 0.915$, $0.4 > p > 0.3$) for IgG in CBA/J mice (Fig 4). IgM antibodies against soluble parasite antigens appeared first, increased until day 4 and then decreased whereas the production of IgG antibodies peaked on day 5 post-infection. IgG antibody production was slower and only peaked on day 6, 5 and 5 in Balb/c, CBA/J and ICR mice respectively. Poorer correlations were obtained in the case of increase in IgG antibody production with the duration of infection as compared to increase in IgM antibody production.

Antibodies to surface antigens detected by the

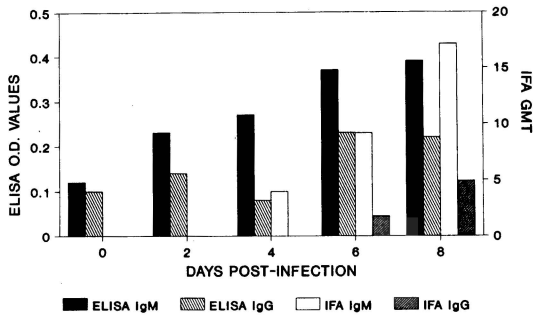

Fig 2—Enzyme-linked immunosorbent assay (ELISA) antibody levels at 492 nm optical density (OD) readings, to *Toxoplasma* soluble antigens and indirect fluorescent antibody geometric mean titer (IFA GMT) to surface antigens, in Balb/c mice, by days post-infection.

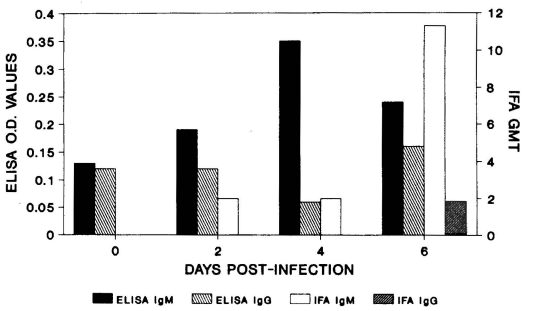

Fig 3—Enzyme-linked immunosorbent assay (ELISA) antibody levels at 492 nm optical density (OD) readings, to *Toxoplasma* soluble antigens and indirect fluorescent antibody geometric mean titer (IFA GMT) to surface antigens, in CBA/J mice, by days post-infection.

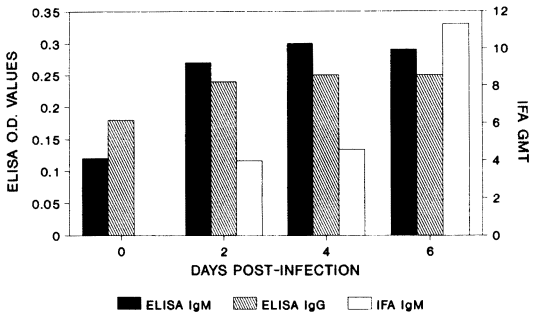

Fig 4—Enzyme-linked immunosorbent assay (ELISA) antibody levels at 492 nm optical density (OD) readings, to *Toxoplasma* soluble antigens and indirect fluorescent antibody geometric mean titer (IFA GMT) to surface antigens, in ICR mice, by days post-infection.

IFAT increased with duration of infection ($r = 0.75, t = 3.000, 0.02 > p > 0.01$ for IgM and $r = 0.82, t = 3.790, 0.01 > p > 0.001$ for IgG antibodies) in infected Balb/c mice. In infected ICR mice, the IFAT detected increasing IgM antibodies ($r = 0.94, t = 6.170, p < 0.001$) with duration of infection. In infected CBA/J mice, IgM and IgG antibodies increased with duration of infection ($r = 0.84\ t = 3.790, 0.01 > p > 0.001$ and $r = 0.71, t = 2.469, 0.05 > p > 0.02$ respectively). IgG antibodies to surface antigens began to appear only on Day 4 in Balb/c mice and on Day 6 in CBA/J mice. IgM antibodies against surface antigens detected by the IFAT appeared from day 1, 2 and 3 in infected ICR, CBA/J and Balb/c mice respectively whereas IgG antibodies were detected from day 6 onwards in infected Balb/c and CBA/J mice. These results suggest that antibodies to surface antigens appear later than antibodies to soluble antigens which are detected by Day 1 post-infection. In the present study the relatively high levels of IgM and IgG antibodies produced against surface and soluble antigens in Balb/c mice compared to the other strains of mice, could be a factor responsible in prolonging the survival time to 8 days compared to 7 and 6 days in CBA/J and ICR mice respectively. The observation supports the above findings that the humoral antibody response could play a role in conferring protection in acute infection. These findings agree closely with those of Handman and Remington (1980). They studied the course of antibody response in mice infected with the C37 and C56 strains of *T. gondii* and showed that the appearance of IgM antibodies preceded that of IgG antibodies. They also showed that in mice these IgM antibodies were not transient but persisted for at least 50 days, by which time the infection was in its chronic state. Studies by these authors also showed that parasites from the peritoneal cavities of mice were coated with IgM antibodies as early as 24-48 hours after the initial infection.

Cysts were not found in the brain smears of all three strains of mice throughout the period of infection.

ACKNOWLEDGEMENTS

We thank Dr M Jegathesan, Director, Institute for Medical Research, Kuala Lumpur for his encouragement and permission to publish this paper. This study received financial support from the SEAMEO-TROPMED project and the National Biotechnology Programme of the Ministry of Science, Technology and Environment, Malaysia.

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