Suppression and enhancement of humoral immune response to *Toxoplasma gondii* by passive antibody

K. N. Masihi and H. Werner

Department of Medical Parasitology, Robert Koch Institute, Nordufer 20, D-1000 Berlin 65 (West), 21 March 1977

Summary. Anti-toxoplasma antibodies administered passively to mice may lead to suppression or enhancement (reported for the first time with Protozoan parasites) of subsequent antibody response when these animals are later infected with *Toxoplasma gondii*. The outcome is dependent on infecting strain of Toxoplasma and the antigen-antibody ratio.

Infection by *Toxoplasma gondii*, an intracellular protozoan parasite, is ubiquitous and affects most species of warm-blooded animals. It can be transmitted to humans through improperly cooked meat, by contact with cat faeces containing oocysts and congenitally to the foetus. The latter is generally believed to occur when the maternal infection is primarily acquired during pregnancy. In addition to clinical significance, the socio-economic consequences of this can be considerable. Recently estimated costs of toxoplasmic children in USA alone was put at 31 to 40 million dollars per year[1]. The current diagnosis of congenital infections with *Toxoplasma gondii*, cytomegalovirus, rubella virus and *Treponema pallidum* are based to a large extent on demonstration of immunoglobulin M in the newborn serum. As maternal antibodies in response to these infections may cross in utero, particularly during the later part of gestation, it is pertinent to consider the influence which the passive antibody may exert on the immune response of the foetus and on interpretation of serological tests.

Suppression of Toxoplasma IgM has been shown to occur in newborn rabbits which had been previously injected with IgG antibody[2]. Prior treatment of mice with specific antiserum led to a suppression of the humoral response and could be demonstrated 24 h after infection[3], using a rosette test[4]. Subsequent circulating antibody titres in similarly treated animals were found to be lower for at least 30 days[3,5]. In some cases, no antibody could be detected although the parasite was present in the brain, indicating that a state of tolerance had been induced[5]. We report here that passively acquired antibody may lead not only to suppression but also enhancement of the humoral response depending on the antigen-antibody ratio and the infecting strain of Toxoplasma.

Materials and method. Strains Alt, Gail and Witting which are maintained in our laboratory were used. These are cyst-forming strains and had been originally isolated from human cases. Toxoplasma antiserum for passive immunization was prepared by injecting mice with 10 brain cysts of Gail strain followed 1 week later by 2 mg Sulfamethoxypyrazine + 0.02 mg Pyrimethamine per mouse. Sera were collected after 3 months when chronic infection was established, and stored at −20 °C. The Sabin-Feldman dye test titre of this pool was 1:8000. As the precise quantitation of parasites in cysts is difficult, all further infections were started with trophozoites. To obtain these, brains from chronically infected mice were titurated in saline with glass beads and the homogenate adjusted to contain 200 cysts per 0.3 ml. Mice were injected by i.p. route, and the resulting exudate harvested by washing out the peritoneal cavity on the 6th day. The parasites were separated from the host cells by a simple paper filtration method[6].

Results. 6-week-old female NMRI mice were injected i.v. in groups of 12–15 animals with 0.25 ml of Gail antiserum or controls with saline and 24 h later injected i.p. with 500 Alt or Witting Toxoplasma. Tail blood from 3–4 mice was collected at regular intervals and stored at −20 °C until tested for antibodies by Sabin-Feldman dye test. By the 3rd week, earlier suppression of antibody titres in passively immunized animals infected with Alt strain was overcome, and both this and the control group had similar 1:1000 titre. In the 4th week, the experimental group had higher titres. This enhanced serum antibody level was maintained for at least 45 days, when pooled sera of all animals in each group were tested. By contrast, animals infected with Witting strain showed lowered antibody titres when compared to controls. The effect of passively administered antiserum on antibody formation by homologous strain was observed by using Gail antiserum and subsequent active immunization with 500 parasites of the same strain. All the animals in experimental group tested after 2 weeks showed lower titres than controls. To investigate what influence antigen-antibody ratio may have, the infecting dose was reduced 5fold. Mice were passively immunized with Gail antiserum and infected 24 h later with 100 Toxoplasma of Alt, Gail or Witting strains. No enhancement of antibody response was observed with Alt or Witting strains, but the antibody-treated group infected with Gail strain showed higher titres than controls in the 4th and 6th weeks.

Effect of passively administered antibody on humoral immune response to *Toxoplasma gondii*

Immunization		Reciprocal Sabin-Feldman dye test titre				
		Weeks after infection				
Passive	Active	1	2	3	4	6
Yes	500 Alt	128	128	1000	8000	16000
No	500 Alt	64	1000	1000	2000	4000
Yes	100 Alt	128	64	256	1000	64000
No	100 Alt	64	1000	2000	2000	256000
Yes	500 Gail	64	128	256	1000	1000
No	500 Gail	32	1000	2000	2000	2000
Yes	100 Gail	128	64	256	8000	4000
No	100 Gail	32	1000	1000	2000	2000
Yes	500 Witting	256	64	64	1000	64000
No	500 Witting	64	1000	1000	4000	256000
Yes	100 Witting	64	64	256	512	64000
No	100 Witting	32	1000	1000	4000	256000

Animals were injected i.v. with Toxoplasma antiserum or saline and 24 h later infected i.p. with *Toxoplasma gondii*.

1 J. K. Frenkel, Biol. Sci. *23*, 343 (1973).
2 F. G. Araujo, and J. S. Remington, J. Immun. *115*, 335 (1975).
3 K. N. Masihi and H. Werner, Zentbl. Bakt. ParasitKde. Abt. I *237*, 405 (1977).
4 K. N. Masihi and H. Werner, Infect. Immun. *13*, 1678 (1976).
5 F. G. Araujo and J. S. Remington, J. Immun. *113*, 1424 (1974).
6 K. N. Masihi, Zentbl. Bakt. ParasitKde. Abt. I *233*, 556 (1975).

Discussion. These experiments suggest that passively administered antiserum may suppress or enhance antibody formation to Toxoplasma. The outcome seems to be dependent on a number of variables including the infecting strain and the antigen-antibody ratio. Previous studies with sheep red blood cells have also shown that prior treatment of animals with IgG or IgM can both enhance and suppress antibody production[7]. The suppression in present experiments may have been due to action of passive antiserum on antibody-forming cells. This was indicated in a recent study of kinetics of antibody-mediated suppression of humoral immune response to Toxoplasma at a cellular level[8]. A marked reduction in the number of spleen rosette-forming cells was revealed in antibody-treated group. Using defined lymphoid populations, majority of the rosette-forming cells in Toxoplasma immunized mice have been identified as B-cells (Masihi and Werner, unpublished observations). The mechanism of enhancement is at present not completely clear. However, much experimental data suggest that T- and B-lymphocytes are heterogenous in their composition and that T-cells consist of helper and suppressor subpopulations. The exact conditions under which these subpopulations may be triggered are not fully elucidated. It is of interest that differences in the degree of lymphocyte stimulation by 11 strains on challenge with virulent Toxoplasma have been observed in mice[8]. Maternal antibody passively acquired by the newborn is catabolized within a few months. To what extent this antibody may influence the immune response of human foetus or newborn to Toxoplasma infection is not known. In a recent study with rats, Toxoplasma IgM antibody response could not be detected by immunofluorescent test during the 7-week observation period in newborns infected with the parasite 1–2 days after birth from chronically infected mothers[9]. In the offsprings from normal mothers, high IgM antibody titres were present 2–3 weeks after similar infection. Newborn rats from mothers with chronic infections had high Sabin-Feldman and IgG immunofluorescent test titres. This suggests that their IgM response may have been suppressed by passively acquired maternal IgG antibody. In the case of humans, it is not unlikely that the alteration of immune response to suppression or enhancement could occur but would depend on time and magnitude of foetal and maternal infections, amount of circulating antibody formed by the mother and the infecting strain of Toxoplasma.

7 D. S. Pearlman, J. exp. Med. *126*, 127 (1967).
8 K. N. Masihi and H. Werner, Z. ParasitKde., in press (1977).
9 Y. Omata and N. Suzuki, Res. Bull. Obihiro Univ. *9*, 265 (1975).

Villus growth and cell replacement in the small intestine of the neonatal pig

M. W. Smith and L. G. Jarvis

ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (England), 3 May 1977

Summary. Cells produced in the crypts of newborn pig ileum migrate onto villi during development. There is little or no corresponding loss of cells from villus tips during the first week of postnatal life. Villus growth during this period is largely responsible for the slow rate of cellular renewal seen to take place.

Intestinal absorption of immunoglobulins in the pig at birth is associated with an immediate increase in sodium transport[1]. This is followed 5–24 h later by a marked inhibition[2]. Immunoglobulin brought into contact with the newborn pig intestine initially increases the rate of oxygen consumption but this effect disappears some 4 days later[3]. Removing piglets from the sow overnight restores sodium transport to levels found in the newborn animal. This effect disappears 10 days after birth[2]. The purpose of the present work was to try to establish which if any of these changing parameters might be associated with the physical appearance of a different population of cells upon the surface of the developing intestinal villus.

Materials and methods. Pigs taken at birth were injected s.c. with an aqueous solution of [6-^{3}H] thymidine obtained from The Radiochemical Centre, Amersham, England (1 μCi/g b.wt, 20,000–30,000 mCi/mmole). Piglets were then returned to the sow to be killed 2, 24, 48, 96, 144 and 192 h later. The small intestine was dissected out, portions of the mid-intestine being fixed, prior to embedding, in phosphate-buffered formalin, pH 7.0. Sections taken at 2 μm, coated with Ilford K2 emulsion, were allowed to develop for 127 days before being stained with haematoxylin and eosin. Total and radioactive cell counts were then carried out from base of crypt to tip of villus.

Results and discussion. Villus height and crypt depth, together with the mean position of the fastest migrating thymidine labelled cells, is shown for different times following injection of thymidine in the table. The depth of crypts remained virtually constant up to 6 days after birth. Additional measurements on 10-day-old pig intestines taken from the same region show a crypt depth of 24.9 ± 1.1 cells. The height of villus plus crypt showed a 3fold increase over the same time period (80–231 cells). Thymidine labelled cells were confined initially to the crypts. They moved onto the villus after 24 h reaching cell position 189 some 7 days later. Cells produced by

Villus growth and cell migration in the pig intestine measured during the first 8 days of postnatal life

Time after birth (h)	2	24	48	96	144	192
Height of villus and crypt	80.3 ±4.6	82.3 ±4.6	99.5 ±3.7	136.9 ±5.7	215.2 ±8.7	231.0 ±7.6
Depth of crypt	24.7 ±0.9	26.2 ±1.1	27.1 ±1.2	27.0 ±1.0	28.2 ±1.8	33.5 ±1.4
Distance from crypt base to leading labelled cell	11.9 ±0.7	26.4 ±1.7	46.1 ±2.4	78.1 ±4.2	172.8 ±7.6	189.2 ±7.5

Numbers give cell counts from base of crypts. Each value gives the mean ± SE of 12 determinations.

1 C. Henriques de Jesus and M. W. Smith, J. Physiol., Lond. *243*, 225 (1974).
2 C. Henriques de Jesus and M. W. Smith, J. Physiol., Lond. *243*, 211 (1974).
3 M. W. Smith, E. A. Munn, K. A. Burton and C. Henriques de Jesus, in: Materno-foetal Transmission of Immunoglobulins, p. 381. Ed. W. A. Hemmings. Cambridge University Press 1975.