SHORT ARTICLES

EXPERIMENTAL INFECTION OF ATHYMIC MICE WITH *TOXOPLASMA GONDII*

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PLATE XV

CONGENITALLY athymic mice die after infection with virulent *Toxoplasma gondii* unless they are reconstituted with lymphocytes from normal mice (Lindberg and Frenkel, 1977). Thus effective defence against *T. gondii* in mice depends on the presence of an unimpaired cellular immune system. The purpose of the present study was to examine further, toxoplasma infection in congenitally athymic nude mice with a normally avirulent cyst-producing strain.

MATERIALS AND METHODS

Animals. Three-week-old mice, which were congenitally athymic, nude and heterogeneous in genetic background were maintained with their hirsute littermates with thymus glands in sterile isolators with autoclaved water, food and bedding. Mice were removed only for experimental manipulations, which were performed under aseptic conditions.

Toxoplasma gondii. The MI strain used was originally isolated from a stillborn lamb and maintained by serial passage in mice. The brains of donor mice were removed aseptically and homogenised in Hanks's balanced salt solution as already described (Buxton, Reid and Pow, 1979). Experimental mice were injected intraperitoneally (i.p.) with 0.2 ml of a suspension containing an estimated 100 cysts.

Experiment 1. Groups of nude and hirsute mice were anaesthetised with carbon dioxide at intervals after infection, their chests were incised, and blood was collected from the heart; the serum was separated and stored for future examination for antibody to *T. gondii* by an indirect haemagglutination (IHA) test (Jennis, 1966).

All the mice were examined for the presence or absence of a thymus. Brains were removed, fixed for 14 days, and sliced coronally through the caudate nucleus, thalamus, midbrain and cerebellar peduncles to give a total of five blocks which were processed through to paraffin wax as composite blocks. Sections 6 μ m thick were cut and stained with haematoxylin and eosin. All slides were randomised and coded, and all the *T. gondii* cysts and perivascular inflammatory cuffs seen on each slide were counted. A perivascular cuff was defined as a blood vessel of any size encircled by at least one complete layer of lymphoid cells or a vessel that was at least half surrounded by a minimum of two layers of lymphoid cells.

The sections on the slides were then photographed at a magnification of 10 and the resulting images were cut out and weighed. From the weight per unit area of paper, the area of the cut-outs and hence of the brain sections was calculated. The mean total counts of cuffs were then corrected to numbers of cuffs per cm² of brain section examined.

The diameters of all cysts in the stained sections of brain from 50% of the mice from each group were measured with a calibrated eyepiece graticule.

Experiment 2. This was similar to experiment 1 but, in addition, spleens were taken for histological examination and ascitic fluid, which had been observed in nude mice from day 12

onwards in the first experiment, was collected when the mice were killed. Drops of the ascitic fluid were dried on slides and fixed in methanol for 10 min., rinsed in phosphate-buffered saline and treated with fluorescent anti-*Toxoplasma gondii* reagent (Wellcome Reagents, Beckenham, Kent) and examined.

RESULTS

Clinical signs. All the mice appeared normal until day 12 after challenge when some of the nude and hirsute animals became mildly ascitic. Ascites was very severe in the nude mice after day 16 and necessitated terminating experiment 1 on day 19 instead of day 21 as planned. Hirsute littermates were not severely affected and remained alert throughout the experiments.

Thymus. Thymic tissue was not found in any of the nude mice whereas a thymus of normal appearance was found in each of the hirsute mice.

Spleen. Spleens from hirsute mice were significantly larger than those from nude mice examined on the same day (table I). Nude-mouse spleens remained unaltered throughout the experiment and were of the same order of size as those taken from uninoculated nude mice killed on day 21.

TABLE I
Mean spleen weights from mice in experiment 2

State of mice	Mean* spleen weight (g) (and significance† of difference)			
	on indicated day after inoculation			on day 21 in uninoculated mice
	7	14	21	
Nude	0.08	0.07	0.07	0.10
Hirsute	0.14 ($p < 0.005$)	0.26 ($p < 0.001$)	0.22 ($p < 0.001$)	0.06 (NS)

* The inoculated mice were in groups of eight, and the uninoculated in groups of four.

† In Student's *t*-test (two-tailed); NS = not significant.

Histopathological examination showed marked follicular enlargement in the spleens of hirsute mice infected with *Toxoplasma*. No such changes were observed in the nude mice.

Brain. The results of experiment 1 (table II) and experiment 2 were similar. Few toxoplasma cysts were found in the brains of any mice killed on day 7 or 14 but by day 19 and 21 there were significantly more cysts in the nude mice than in their hirsute littermates. In addition the cysts were usually in large groups and had a mean diameter significantly smaller than that of cysts in hirsute mice, which were present either singly or in pairs. Furthermore, unlike the reaction in hirsute mice, there was negligible inflammation, in the form of perivascular cuffing by lymphoid cells in the brains of nude mice (table II).

Neither inflammation nor toxoplasma cysts were found in the brains of uninoculated nude or hirsute mice.

Ascitic Fluid Cells. Tachyzoites of *T. gondii* were often found in the cytoplasm of cells, which had the morphology of macrophages, in the ascitic fluid from nude mice (figure) in experiment 2 whereas parasitised cells were not found in fluid taken from hirsute mice (table III). However, some macrophages from hirsute mice did contain fluorescent vesicles in their cytoplasm.

Serology. The nude mice produced no detectable IHA antibody to *T. gondii* at any stage in either experiment. Antibody was not found in hirsute mice in experiment 1 on day 7 but by day 14 the geometric mean of the titres was 8 and by day 19 it was 55. The results were comparable in experiment 2 except that the titres were lower. Antibody was not detected in uninoculated mice.

TOXOPLASMA GONDII IN ATHYMIC MICE

FIGURE.—*Toxoplasma gondii* tachyzoites in the cytoplasm of a cell in the ascitic fluid of a nude mouse from experiment 2, 21 days after inoculation. Stained with fluorescent antiserum. $\times 1800$.

TABLE II
Number and size of toxoplasma cysts and degree of inflammation in brains of mice from experiment 1

State of mice	Mean* number of cysts/cm ² of section (and significance† of difference) on indicated day after inoculation				Mean* diameter (µm) of cysts (and significance† of difference on day				Mean* number of perivascular cuffs/cm ² of section (and significance† of difference) on day			
	7	14	19		7	14	19		7	14	19	
Nude	0	10	93		0	6.6	6.0		0	1	1	
Hirsute	0	2	4		0	9.1	9.5		1	15	25	
	(NS)	(NS)	(p < 0.001)		(NS)	(NS)	(p < 0.01)		(NS)	(p < 0.001)	(p0.001)	

* There were originally 10 animals in each group; for the nude mice, the groups were reduced to eight animals by days 14 and 19.
† Mann-Whitney U-test (two-tailed); NS = not significant.

TABLE III

Number of mononuclear cells in ascitic fluid that contained tachyzoites of Toxoplasma gondii

State of mice (and number examined)	Day	Percentage of cells parasitised	Mean number of tachyzoites per cell
Nude (1)	14	35	3
Hirsute (1)	14	0	0
Nude (8)	21	74	2
Hirsute (5)	21	0	0

DISCUSSION

Infection of immunologically competent mice with a cyst-producing strain of *T. gondii* stimulated an inflammatory reaction in the brain, a marked enlargement of the spleen and the production of specific IHA antibody within 21 days; these changes indicated a normal immunological response to *Toxoplasma*. However in similarly treated nude mice the spleens were not enlarged and circulating antibody was not detected at any time. There was also an absence of cuffing by lymphoid cells in the brain. These features indicate the vital role of the thymus in the mounting of an inflammatory reaction against *Toxoplasma*. In addition toxoplasma cysts in brain were smaller but more numerous in nude mice suggesting that they could multiply faster in this host. These findings support and extend previous observations that congenitally athymic nude mice could not develop resistance to *Toxoplasma* while littermate controls with thymus glands could become resistant and produce specific circulating antibody (Hof *et al.*, 1976; Lindberg and Frenkel, 1977).

The role of specific antibody in protection against infection with *T. gondii* is not certain. Masihi and Werner (1978) demonstrated that the passive transfer to immunologically competent mice of immune serum by the intraperitoneal route, before infection with a cyst-producing strain of *T. gondii* resulted in a much reduced mortality and a marked reduction in numbers of brain cysts. They also showed that immune serum given after infection with *Toxoplasma* could give a survival rate higher than in controls but permitted increased numbers of toxoplasma cysts to develop in brain, a finding that they suggested might have been due to immunological enhancement. However it has been reported that resistance against virulent *Toxoplasma* (RH strain) is not due to humoral immune mechanisms (Huldt, 1966). Furthermore Lindberg and Frenkel (1977) demonstrated that specific anti-toxoplasma antibody did not protect congenitally nude athymic mice against *Toxoplasma* but that mice were protected and produced antibody after they received thymus cells from hirsute littermates.

There is evidence that thymus-derived lymphocytes stimulate macrophages to inhibit or kill *Toxoplasma* (McLeod and Remington, 1977). In the present study tachyzoites of *Toxoplasma* of normal morphology were found in the cytoplasm of mononuclear cells present in the ascitic fluid taken from nude mice but not in similar cells from hirsute mice. These observations suggest that tachyzoites might have been killed by macrophages in the hirsute mice while the macrophages of the nude mice, in the absence of thymic cells, were unable to kill the organisms. However more work is required to establish this. What can be said is that the thymus plays an essential role in the development of immunity to a cyst-producing strain of *Toxoplasma* in mice and in its absence neither humoral nor cellular protective mechanisms can develop.

SUMMARY

Congenitally athymic nude mice and their hirsute littermates with thymuses were infected with a normally avirulent cyst-producing strain of *Toxoplasma gondii*. The nude mice were much less able to cope with the infection. Unlike their hirsute littermates they failed to produce any detectable antibody and apparently allowed faster multiplication of cysts in the brain where the normal inflammatory response was absent. It was concluded that the thymus plays an

essential role in the development of immunity to a cyst-producing strain of *Toxoplasma* in mice and in its absence neither humoral nor cellular protective mechanisms can develop.

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