

Experimental Cryptococcosis in Normal and B-Cell-Deficient Mice

D. P. MONGA,† R. KUMAR,^{1*} L. N. MOHAPATRA,¹ AND A. N. MALAVIYA²

Departments of Microbiology¹ and Medicine,² All-India Institute of Medical Sciences, New Delhi-110016, India

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B-cell-deficient mice were prepared by administration of rabbit anti-mouse- μ antiserum to newborn animals within 12 h of birth onwards. Such immunodeficient animals, along with the normal controls, were infected intravenously with *Cryptococcus neoformans*. There was no difference in the mortality pattern, viable count of cryptococci in different organs, delayed-type hypersensitivity reaction, and antigen level in the sera of control and B-cell-deficient animals. Antibodies were absent in B-cell-deficient animals but were present in low titers in control animals. It is concluded that antibodies are not involved in protection of mice infected with *C. neoformans*.

The protective role of antibodies in cryptococcal infection is questionable. Several workers were unable to protect experimental animals from a challenge infection with *Cryptococcus neoformans* after passively transferring cryptococcal antibodies (3, 10, 11, 17). However, there are other reports where passive transfer of antibodies increased the survival time of infected animals (8, 9). Recently, antibody-dependent killing of *C. neoformans* by human peripheral blood mononuclear cells has been demonstrated (2, 4). In human disseminated cases of cryptococcosis, antibodies are often absent or are present only in very low titers (1).

Our earlier study on cryptococcal infection in thymectomized animals indicated that antibodies alone did not influence the course of infection (unpublished observations). The present study was undertaken to investigate the role of antibodies in experimental cryptococcosis in mice by using normal and B-cell-deficient animals.

MATERIALS AND METHODS

Organism. A strain of *C. neoformans* isolated from cerebrospinal fluid of a patient in the Mycology Laboratory, Department of Microbiology, All-India Institute of Medical Sciences, New Delhi, was used in the present study. The inoculum for infecting the animals consisted of a 24-h growth of *C. neoformans* on Sabouraud dextrose agar and was harvested in sterile physiological saline. It was washed three times in sterile saline, and the organisms were counted in a hemocytometer. Simultaneously, a viable count was made on Sabouraud dextrose agar.

Preparation of B-cell deficient mice. Swiss al-

bino mice of either sex, bred randomly and raised in the departmental animal house, were used in this study. The pregnant mice were kept separately in individual cages and inspected twice a day for birth of a litter. The method of preparing B-cell-deficient mice has been described earlier (P. K. Maity, D. P. Monga, R. G. S. Murthy, R. Kumar, A. N. Malaviya, and L. N. Mohapatra, *Ind. J. Med. Res.*, in press). Briefly, within 12 h of birth, newborn mice were injected intraperitoneally with 0.05 ml of rabbit anti-mouse- μ antibodies with a 30-gauge needle. The dose was gradually raised to 0.1 ml given twice a week during the first 2 weeks and then once a week until the completion of the experiment. Control animals were similarly treated with normal rabbit serum in place of anti- μ . B-cell deficiency in treated animals was demonstrated by their inability to produce antibodies to sheep erythrocytes, absence or a marked reduction in the number of B cells in their spleens, and depletion of lymphocytes from B-cell-dependent areas in histology of spleen and lymph nodes. The B-cell-deficient animals rejected the skin allograft just like normal animals.

Serological tests. Sera of the infected animals were tested for cryptococcal antibodies by the tube agglutination (25) and indirect hemagglutination (14) tests, as described previously but modified so as to use cryptococcal skin test antigen for sensitization of sheep erythrocytes. Sera were also tested for circulating cryptococcal antigen by the gel diffusion test (21) against hyperimmune anticryptococcal serum raised in rabbits.

Delayed-type hypersensitivity. The skin test antigen was prepared by the method described by Murphy et al. (20). Essentially, it was culture filtrate in which *C. neoformans* was grown for 72 h. Delayed-type hypersensitivity reaction to skin test antigen was determined by measuring the increase in footpad thickness after 24 h following injection of 0.03 ml of antigen.

Infection of animals. B-cell-deficient mice, along with control animals, were infected intravenously with

† Present address: Department of Veterinary Bacteriology, College of Veterinary Sciences, Haryana Agricultural University, Hissar-125004, India.

5×10^3 *C. neoformans* cells. Ten animals from each group were kept separately for the study of mortality. Of the remaining animals, five from each group were studied at various intervals for delayed-type hypersensitivity, viable count of cryptococci in different organs, and levels of circulating cryptococcal antigen and antibodies. For viable counts of cryptococci, the organs of sacrificed animals were homogenized individually, and serial dilutions in normal saline were plated in duplicate on Sabouraud dextrose agar containing diphenyl and chloramphenicol.

RESULTS

Mortality. To determine mortality after cryptococcal infection, the animals were observed for 8 weeks. During this period, 40% of the animals from the B-cell-deficient group and 30% of those from the control group died.

Viable count. The viable count of *C. neoformans* in spleen, lungs, and brain of B-cell-deficient and control groups of animals is summarized in Table 1. There was no difference in viable counts between the two groups of animals in the organs studied ($P > 0.05$) at any stage of infection.

Delayed hypersensitivity. There was no marked difference in delayed hypersensitivity reaction between B-cell-deficient and control animals. Both groups showed a peak reaction (0.39 and 0.32 mm, respectively) on day 24 postinfection. This reaction fell slightly on day 36.

Serum antibody level. Both indirect hemagglutination and tube agglutination tests failed to detect the presence of antibody in the sera of B-cell-deficient mice challenged with *C. neoformans* at any stage of infection. However, control infected mice, sacrificed on days 12 and 24, showed indirect hemagglutination titers of 1:8 and 1:4, respectively, but the tube agglutination test was negative; on day 36 postinfection, their sera were negative for antibody by both indirect hemagglutination and tube agglutination.

Serum cryptococcal antigen. The presence of circulating cryptococcal antigen in the sera of B-cell-deficient mice could be detected in those animals sacrificed on day 36, whereas sera of

control animals were positive for antigen only on day 24 postinfection.

DISCUSSION

Anti- μ treatment of newborn mice suppresses the development of B cells (15, 18, 19). Such immunodeficient mice would be unable to produce antibodies. The results of the present study show that the course of cryptococcal infection in B-cell-deficient and normal animals was similar. The development of delayed hypersensitivity in B-cell-deficient animals was also comparable to that in normal animals. The levels of antibodies and antigen in sera of infected animals were not regular. None of the B-cell-deficient animals showed the presence of antibody (as expected), whereas in the control group the antibodies were present but the titer was low. Since the presence of antibodies in normal animals gave them no advantage over B-cell-deficient animals, it can be safely concluded that antibodies did not influence the course of cryptococcal infection. Several reports are available on the failure of protection by passive transfer of antibodies against lethal cryptococcosis in experimental animals (3, 10, 11, 17) and in humans (16). However, Gadebusch (8), and Gadebusch and Gikas (9) claimed that passive antibody transfer protected against a lethal *C. neoformans* challenge. The protection was, however, limited to the period of immunization and was type specific. As immune serum and challenge dose were given intraperitoneally within 2 h of each other, it appears likely that immune serum eliminated the organisms by agglutination before they had a chance to attack the host.

Diamond (2) reported that human peripheral blood leukocytes in the presence of specific antibody could kill *C. neoformans* by a nonphagocytic mechanism. It was further observed that nonphagocytic lymphoid cells (lymphocytes) had this fungicidal capacity (4). But no such activity by these cells was seen in the presence of normal serum (2). The present study suggests that such an in vitro phenomenon may not have

TABLE 1. Serial viable counts in spleen, lungs, and brain of B-cell-deficient and control mice after intravenous infection with 5×10^3 viable *C. neoformans* cells

Organ	Mean viable counts (log) ^a \pm standard error at days after infection:								
	12			24			36		
	NRS ^b	Anti- μ ^c	P	NRS	Anti- μ	P	NRS	Anti- μ	P
Spleen	3.47 \pm 0.18	3.01 \pm 0.11	>0.05	4.08 \pm 0.09	4.32 \pm 0.12	>0.05	5.53 \pm 0.05	5.24 \pm 0.25	>0.05
Lungs	3.74 \pm 0.18	3.41 \pm 0.18	>0.05	3.67 \pm 0.24	3.91 \pm 0.26	>0.05	5.19 \pm 0.32	5.50 \pm 0.12	>0.05
Brain	4.72 \pm 0.05	4.91 \pm 0.09	>0.05	5.68 \pm 0.07	5.88 \pm 0.05	>0.05	6.14 \pm 0.06	6.12 \pm 0.10	>0.05

^a Each value represents a mean of five animals.

^b NRS, Normal rabbit serum-treated animals.

^c Anti- μ , animals treated with rabbit anti-mouse- μ antiserum.

much in vivo significance. In a later study, Diamond (3) himself could not demonstrate any evidence of protective immunity in guinea pigs after passively transferring immune serum.

The presence of cryptococcal antibody in the sera of patients with cryptococcosis has been correlated with a favorable prognosis (5, 24). The presence of antibodies, however, may not be directly influencing the course of infection. A lesser amount of free antigen in milder disease, not sufficient to neutralize the antibodies, would account for their presence being correlated with a good prognosis. Development of disseminated cryptococcosis in humans is usually associated with abnormalities in cell-mediated immune responses (6, 7, 12, 22).

Graybill and Mitchell (13) did not find any difference in mortality patterns of normal and cyclophosphamide-treated mice after challenge with *C. neoformans* unless very high doses of cyclophosphamide were administered. Cyclophosphamide is known to have a predominant effect on B lymphocytes in mice (23). The present results clearly suggest that antibodies have no protective role in this disease.

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