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Experimental *Toxoplasma gondii* Infection in Mice: The Role of the Fifth Component of Complement[1] (38902)

FAUSTO G. ARAUJO, LEON T. ROSENBERG, AND JACK S. REMINGTON

Division of Allergy, Immunology and Infectious Diseases, Palo Alto Medical Research Foundation (F.G.A. and J.S.R.), Department of Medicine, Stanford University School of Medicine (F.G.A. and J.S.R.), and Department of Medical Microbiology, Stanford University School of Medicine, Palo Alto, California 94305 (L.T.R.)

The relative role of humoral and cellular mechanisms in resistance to a primary infection with the intracellular protozoan *Toxoplasma gondii* is unclear (1). Passive transfer with antibody alone has been shown to confer resistance in some models (2) and adoptive transfer of cellular immunity in others (3).

The complement and properdin systems have been studied in relation to their role in Toxoplasma serology, but their relation to antibody- or cell-mediated induced resistance to Toxoplasma infection has not been defined. Earlier work (4) demonstrated that C2, C3, and C4 are components of the so-called accessory factor (5, 6) or activator (6) systems responsible for neutralization of Toxoplasma trophozoites which occurs in the presence of specific antibodies against the parasite. An alteration of the parasite cell wall induced by the activator–antibody complex is suggested as the mechanism for the neutralization (7).

The availability of mice deficient in the fifth component of the complement system (8) and reports indicating that these animals have an impaired resistance to certain infectious organisms (9, 10) prompted us to perform the present experiments in which C5-deficient mice were tested for their resistance to infection with *T. gondii*.

Materials and Methods. Mice. The mice used were outbred Swiss–Webster (Simonsen Laboratories, Gilroy, CA); C5-deficient inbred B10.D2/old line; B10.D2/new line possessing normal activity of the entire complement sequence; hybrids of B10.D2/old line × B10.D2 new line which have an intermediate level of C5; and animals of the strain supermouse which were developed in our laboratory and have a very high level of serum complement (11). These inbred strains were raised in our own laboratory. Complement-deficient DBA/2J and complement-sufficient DBA/1J mice were purchased from Jackson Laboratories, Bar Harbor, ME. Before inoculation with Toxoplasma trophozoites, mice from the noninbred Swiss-Webster stock were tested for C5 component of complement using a microimmunodiffusion technique (12). Groups of noninbred complement-deficient and complement-sufficient animals were assembled according to the results of this test.

Toxoplasma inocula. Toxoplasma trophozoites of the C56 strain were harvested from peritoneal fluid of mice, washed by centrifugation in sterile phosphate-buffered saline (PBS), pH 7.2, and filtered through a sintered-glass filter to yield a suspension of parasites free of contaminant peritoneal cells (13). Challenge was by the intraperitoneal route and mortality of mice was recorded daily for 30 days. During the first 20 days of infection, all mice appeared extremely ill but animals surviving beyond 30 days appeared normal and were shown to be chronically infected. Statistical analysis was performed using the Wilcoxon tests as modified by Gehan (14).

Results. In Table I and Fig. 1A and B are the results obtained in the noninbred Swiss–Webster mice inoculated with 5×10^3 and 5×10^5 Toxoplasma trophozoites. Complement-sufficient mice died earlier than did C5-deficient animals, and over-all mortality at 30 days was also greater in the C5-sufficient mice. The difference in mortality between the two groups of mice was more

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marked with the lower inoculum, and the differences are highly significant.

The inbred B10.D2/old, B10.D2/new, the hybrid B10.D2/old × B10.D2/new and supermouse were challenged with 5×10^2, 5×10^3, and 5×10^4 Toxoplasma trophozoites. The results of the challenge with 5×10^2 organisms are shown in Fig. 2. The highest mortality was noted in the high-complement level line supermouse and the lowest mortality was in the hybrid and B10.D2/old line. The mortality in the B10.D2/new line mice treated with moderate doses was intermediate. No difference in mortality or in time to death between the various strains of mice was detected with the larger inocula.

TABLE I. MORTALITY OF C5-DEFICIENT AND COMPLEMENT-SUFFICIENT MICE AFTER CHALLENGE WITH DIFFERENT INOCULA OF TOXOPLASMA ORGANISMS.

Toxoplasma inoculum	C5 activity[a]	Mortality		*P* value
		Ratio[b]	%	
5×10^3	Pos	18/25	72.0	0.008
5×10^3	Def	11/27	40.7	
5×10^5	Pos	14/14	100.0	0.04
5×10^5	Def	14/16	87.5	

[a] Pos = full complement activity. Def = C5 deficient.

[b] Ratio = $\frac{\text{number dead after 30 days}}{\text{number animals}}$

The results in the DBA/1J and DBA/2J mice are shown in Fig. 3. Over-all mortality and time to death were significantly different in these mice ($P < 0.0004$). The difference was especially notable when the subcutaneous route of infection was employed. DBA/2J, which showed low resistance, is C5 deficient, whereas DBA/1J with a high degree of resistance has a normal complement sequence.

Discussion. In mice, important systems in host defense against infection have been shown to be genetically controlled (15, 16). It is known that antibody response to certain antigens (17), level of complement in serum (18), as well as interferon production (19) and phagocytic activity of the reticuloendothelial system (20) are genetically modulated

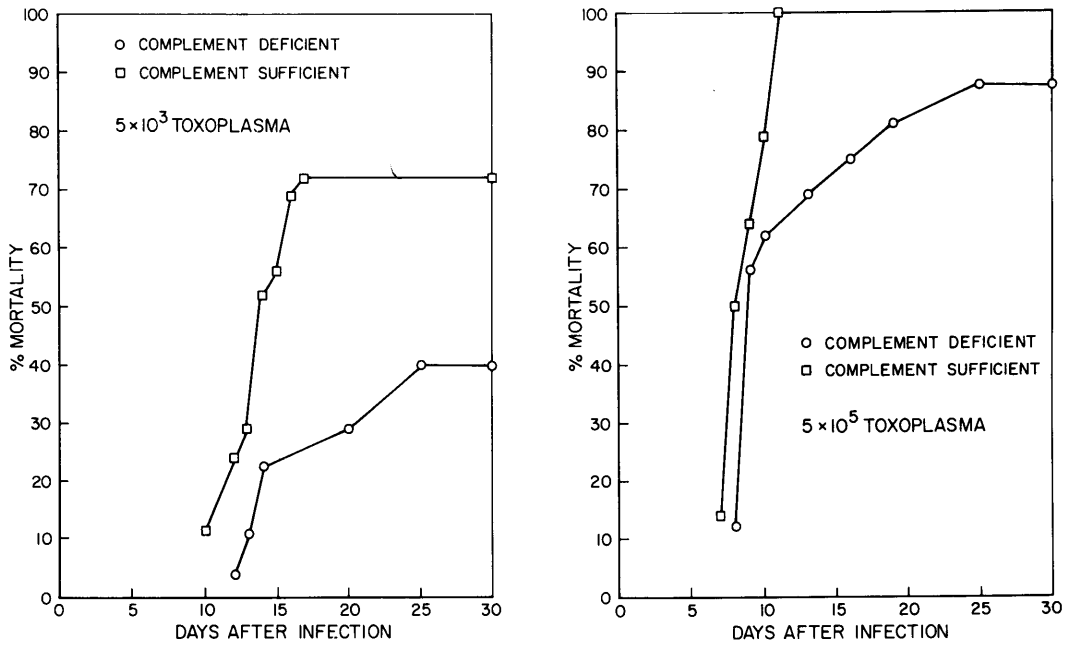

FIG. 1. A. Time to death of C5-deficient and complement-sufficient Swiss-Webster mice after peritoneal infection. Numbers of mice: C5-deficient, 25 mice, complement sufficient, 25 mice. B. Numbers of mice: C5-deficient, 16 mice; complement-sufficient, 14 mice.

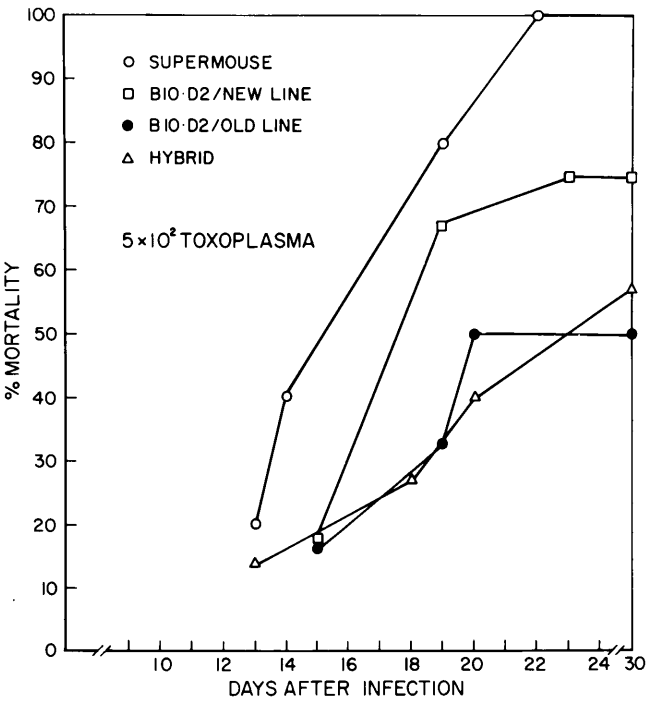

FIG. 2. Mortality in inbred strains of mice after Toxoplasma challenge. Numbers of mice: Supermouse, 5; B10.D2/new line, 12; B10.D2/old line, 6; hybrid, 15.

characteristics of importance in studies of resistance to infectious organisms.

Our intent in undertaking this study was to assess the importance of the fifth component of complement (and inferentially those components which are affected consequent to C5 activation) to resistance to Toxoplasma infection in mice. The results we have obtained suggest that the contribution of the terminally acting complement component in Toxoplasma infections is quantitatively not striking. Other factors, variously distributed in inbred mouse lines and at present entirely uncharacterized are important determinants of the status of susceptibility to Toxoplasma infection. Nevertheless, with an appropriate experimental design, one can show that C5 deficiency—as expressed in a particular genetic background—offers a small but measurable and statistically significant advantage to a mouse challenged with Toxoplasma. The complexities involved are illustrated by the contrasting results obtained in DBA 1J, DBA 2J, and the B10.D2 lines.

We believe the increased resistance of

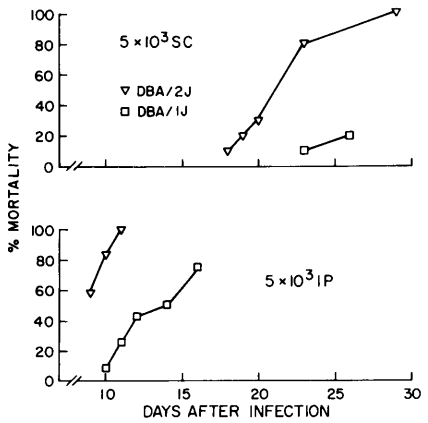

FIG. 3. Difference in mortality in C5-deficient (DBA/2J) and complement-sufficient (DBA/1J) mice after subcutaneous or intraperitoneal challenge. Numbers of mice: SC, 10; IP, 12.

complement-deficient animals, where it is observed, is attributable to the complement difference. The significance of the observation is enhanced because it is seen in outbred mice, as well as in a set of "congenic" mouse

strains. In the outbred line, the differences in resistance were observed between groups of animals preselected for complement type. This tends to exclude other factors, which are likely to be randomly distributed in the experimental groups. The significance of the observations in the related inbred lines depends upon their degree of relatedness. While this is difficult to assess, in this laboratory, we have periodically intercrossed, and thus enhanced, the genetic similarity of the B10.D2 C^+ and C^- stock in order to minimize their tendency to drift apart.

The strain designated supermouse has been inbred and crossed to the B10.D2 stock for too few generations to expect that it approximates congenicity, and skin transplants substantiate this point. Nevertheless, the results with the inbred B10.D2 related lines and supermouse are congruent and suggest that C5 sufficiency may be slightly disadvantageous.

The situation with the two DBA lines, which have been separated for many tens of generations is quite different. They differ in complement type, in histocompatibility genes, and in unknown numbers of other characteristics. They resemble each other in name and coloration. Comparison of the susceptibilities to Toxoplasma of these strains shows only that genetic differences exist. The differences cannot be attributed to only one distinguishing gene. Thus, the relatively resistant C^+ DBA/1J when compared with the complement-deficient DBA/2J is not reasonably attributable to the complement-determining gene H_c, one of the many expressed differently in the two lines.

Since C5 deficiency is very common in laboratory-reared mice, instances in which C5 deficiency is advantageous should be expected. For if this were not true, there would be no explanation for the persistence of C5 deficiency.

The mechanisms responsible for C5 deficiency being advantageous with respect to Toxoplasma challenge are unclear. The only previous report of a situation in which the complement-deficient mouse appeared better able to resist a stress occurred when mice were injected with anti-mouse kidney serum (21). As in that instance, it may be that inflammatory injury during the course of Toxoplasma infection is somewhat less in C5-deficient animals. A detailed histopathologic study of the course of the disease in C5-sufficient and deficient animals would be required to support such a notion, and this we have not done.

Another hypothesis to explain why C5 deficiency might be beneficial is that a block in the sequence at C5 might well favor the local accumulations of C3-derived biologically active intermediates which facilitate phagocyte function. Where C5 is present, the complement cascade might continue to its termination, and this may be irrelevant to the pathogenetic process in this disease. To sustain such an hypothesis would require analysis of the complement intermediates generated in local lesions.

The lessening of resistance of C5-deficient mice by passive administration of C5 would constitute proof that the difference observed is caused by the presence or absence of C5. We have not undertaken such an experiment because the progress of the infection is slow and C5 is a strong antigen (12) in deficient animals and would be eliminated within the time period of the infection.

Summary. Experiments performed to determine the influence of the C5 component of complement in experimental Toxoplasma infection revealed that mice deficient in C5 had reduced mortality due to acute toxoplasmosis. Similar results were noted when inbred congenic mice of known complement type, as well as random-bred mice selected for complement type, were used. In both, mice with high complement activity were less resistant to Toxoplasma than were mice deficient in C5. However, many factors must interact in susceptibility to infection with *T. gondii*. Thus, lower resistance to Toxoplasma was noted in C5-deficient DBA/2J mice, whereas a high degree of resistance was noted in DBA/1J mice, which are not related to DBA/2J mice and which possess a normal sequence of complement. This accentuates the importance of using both random-bred and where possible cogenic lines in assessing the importance of individual factors in infectious immunity.

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