INFECTION AND IMMUNITY, May 1976, p. 1528-1530
Vol. 13, No. 5

Printed in U.S.A.

Strain-Dependent Differences in Murine Susceptibility to Toxoplasma

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Received for publication 8 December 1975

Susceptibility of seven inbred and outbred strains of mice to infection with trophozoites of *Toxoplasma gondii* at two different doses was determined. Striking strain differences in susceptibility and changes of susceptiblity with dosage change were seen. The data are consistent with the hypothesis that this may be due to genetic factors.

Toxoplasma gondii, a ubiquitous intracellular parasite of man and other animals, infects virtually all species of mammals. Although the differences in host susceptibility that have been demonstrated can be due to proven differences in virulence among different strains of toxoplasma, the role of genetic characteristics of the host has not been adequately explored (2; J. S. Remington and J. L. Krahenbuhl, *In* S. Cohen and E. Sadun, ed., Immunology of Parasitic Infections, in press). The resistance of certain species of animals to this parasite, in fact, suggests that genetic factors may play an important role in host susceptibility to toxoplasma. No data are available to allow for further characterization of these genetic factors. To obtain such data, we considered it necessary first to establish a model for study, and then we set out to determine if there are significant differences in susceptibility to a single strain of toxoplasma among different inbred strains of mice.

The strains of mice employed in the experiments were DBA/1, DBA/2, BALB/c, C57B1/6J, B10.D2, white SW/SIM (outbred), and C3H/Bi from Jackson Laboratories, Bar Harbor, Me., Simonsen Laboratories, Gilroy, Calif., or local breeder colonies at Stanford University School of Medicine. They were females 6 to 8 weeks of age, except for confirmatory experiments in which BALB/c and C57B1/6J males of the same age were used. All were negative in the Sabin-Feldman dye test prior to experimental toxoplasma infection.

Trophozoites of the C56 strain of *T. gondii* were used in all experiments. This strain was isolated in 1961 from ovary and oviduct of a chicken by Jacobs and Melton at the National Institutes of Health. Trophozoites were obtained from the peritoneal fluid of Swiss Webster mice (Simonsen Laboratories, Gilroy, Calif.) inoculated 5 to 6 days earlier with brain obtained from Swiss Webster mice chronically infected with the C56 strain (3).

Mice were inoculated intraperitoneally (i.p.) or subcutaneously either with an inoculum of 5×10^3 or 1×10^5 toxoplasma trophozoites in 0.2 ml of Hanks balanced salt solution. There were 15 animals in each group of inbred mice in each experiment. Mice were given water and laboratory mouse chow ad libitum, and no medication was provided either in water or food in any experiment. The progress of the infection and mortality was followed for 30 days; mortality was recorded daily. Any animal dying during this period, as well as survivors, was examined for the presence of toxoplasma as previously described (6).

The Sabin-Feldman dye test was performed as described by Frenkel and Jacobs (1).

Statistical analysis was performed by the chi-square method.

The mortality and the time to death in seven different strains of mice infected i.p. with 1×10^5 toxoplasma trophozoites is shown in Fig. 1. Mice of the BALB/c and DBA/2 strains were most susceptible to this inoculum of toxoplasma; mortality in these two strains was 100% on day 12 and day 13 of infection, respectively. Although 100% of mice of the B10.D2 strain also died, the time to death in this strain (100% by day 19) was prolonged, compared with BALB/c and DBA/2. Mortality in C57B1/6J and C3H/Bi mice was intermediate, 87 and 80%, respectively. Time to death in these two strains was prolonged when compared with the BALB/c and DBA/2 strains. Mice of the DBA/1 and white SW/SIM strains were most resistant, with a mortality of 67% at 30 days. All survivors had evidence of infection, as toxoplasma

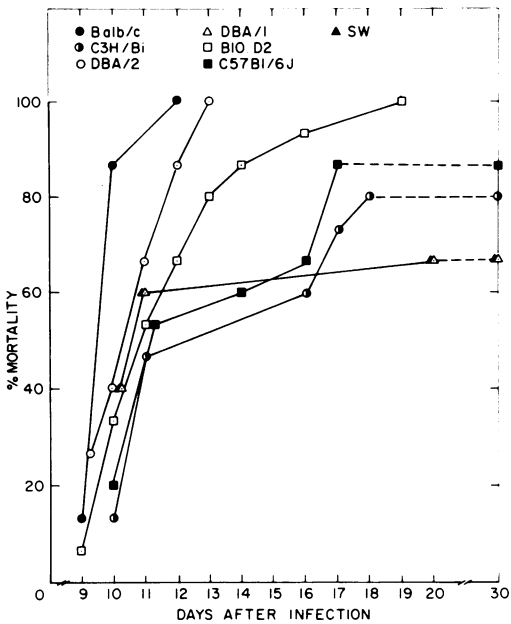

FIG. 1. *Mortality in different strains of mice following intraperitoneal infection with 1 × 10^5 toxoplasma trophozoites of the C56 strain. There were 15 animals in each group of inbred mice.*

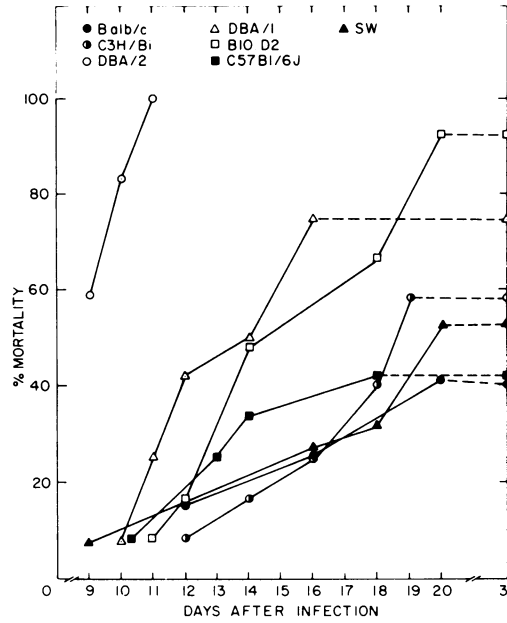

FIG. 2. *Mortality in different strains of mice following intraperitoneal infection with 5 × 10^3 toxoplasma trophozoites of the C56 strain. There were 15 animals in each group of inbred mice.*

cysts were present in their brains and their sera were positive in the dye test.

To determine whether a lower inoculum size would reveal more distinct differences in resistance among the vaious strains, an experiment employing an i.p. inoculum of 5 × 10^3 trophozoites was performed. Mortality and time to death are shown in Fig. 2. Mortality in DBA/1, DBA/2, and B10.D2 mice showed no significant differences from the previous experiment in which an inoculum of 1 × 10^5 toxoplasma organisms was used as challenge. Mortality in C57B1/6J, white SW/SIM, and C3H/Bi mice was considerably reduced.

The most dramatic reduction in mortality and prolongation of time to death was observed in mice of the BALB/c strain. When infected with 1 × 10^5 toxoplasma, the mortality at day 12 was 100%, whereas with the inoculum of 5 × 10^3, the mortality at day 12 was only 17%. This effect of dosage reduction was reproducible in additional experiments. In this experiment, at 30 days of infection, 58% of BALB/c mice receiving the lower inoculum were still alive, whereas all of the mice receiving higher inoculum had died. When compared with the results obtained with the inoculum of 1 × 10^5, time to death was prolonged in all strains except DBA/2, which remained highly susceptible with 100% mortality at 11 days of infection. Similar prolongation of time to death and decreased mortality in BALB/c mice with reduction of dosage was observed in experiments employing male mice.

The data in Fig. 1 and 2 were statistically analyzed by comparing percentage mortalities for each strain at various points in time, and results are consistent with the obvious differences seen in the figures. For example, as shown in Fig. 1, on day 12, BALB/c is significantly different from B10.D2 ($P < 0.02$), DBA/1 ($P < 0.008$, SW ($P < 0.008$), C57B1/6J ($P < 0.003$) and C3H/Bi ($P < 0.001$).

The results described above clearly demonstrate significant differences in susceptibility to toxoplasma infection among the inbred strains of mice studied. Selection of the size of inoculum was critical because, with the doses employed, some strains showed almost no change in susceptibility (e.g., DBA/1, DBA/2), whereas susceptibility of other strains showed striking changes (e.g., BALB/c). The importance of dose of inoculum was also reflected in the change of rank order of susceptibility in going from the lower to the higher dose. For example, whereas BALB/c was the least susceptible strain with the lower dose of toxoplasma, it was the most susceptible strain when the large inoculum was used.

Although alternate explanations may be possible, the data presented here are consistent with the hypothesis that genetic factors may

determine the observed differences of susceptibility, as has been shown for other murine diseases (4, 5, 7). The change in rank order observed with 5×10^3 organisms could also be postulated as secondary to multigenic effects operating at different inoculum challenges. Speculation of this sort, however, as well as speculation about possible differences in immune mechanisms among strains, must await further studies.

This work was supported by Public Health Service grants no. AI 04717 and AI 00260 from the National Institute of Allergy and Infectious Diseases.

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