Differences in Resistance to *Trypanosoma musculi* Infection Among Strains of Inbred Mice

JULIA W. ALBRIGHT* AND JOSEPH F. ALBRIGHT

Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809

Received 20 February 1981/Accepted 25 April 1981

Inbred strains of mice were inoculated with *Trypanosoma musculi*, and the course of the ensuing parasitemia was followed. The mouse strains fell into three groups: those displaying high and moderate (fivefold less) parasitemia and C57BL/6 (B/6) mice which had exceptionally low infections. To gain insight concerning the mechanisms responsible for interstrain variations in infections, several types of experiments were performed. Comparison of the ability of spleen cells from the various strains to provide the growth-promoting substances required by *T. musculi* for growth in culture revealed that B/6 cells were deficient; this suggested one mechanism for regulating parasite infections. Exposure of C3H (high parasitemia) and B/6 mice to graded levels of ionizing radiation revealed that B/6 mice have much greater innate resistance to infection than do C3H mice. The effects of treating mice with silica dust or mercaptoethanol indicated that relative resistance to infection is not primarily associated with macrophage activity or limited growth-promoting substances. We conclude that variations in immune responsiveness to parasite antigens (probably not associated with the H-2 complex), possibly in concert with variations in a non-immunological mechanism, account for interstrain variation in resistance to *T. musculi* infections.

Recent investigations concerning the genetic control of resistance of inbred strains of mice to infection with protozoan parasites have demonstrated no major role of genes located within the major histocompatibility complex (MHC) (8–10, 19, 20, 22, 23). For example, infection with leishmaniasis is controlled by genes at a distinct locus, designated *Ish*, situated on chromosome 1 of mice (10). Genes within the MHC may play a secondary role, however, concerned with the duration of infection (7). Evidence that the MHC may be involved in controlling resistance to *Toxoplasma gondii* has been presented (26). In the case of trypanosomes, there is considerable variation among mice in both the magnitude of infection and the ultimate mortality (19, 23). Nevertheless, the MHC plays little or no role in determining resistance to either *Trypanosoma cruzi* or *Trypanosoma congoense* (19, 23).

It remains an open question, therefore, as to whether the genes that regulate resistance act on some aspect of the immune system or on some feature of a different system (see review, reference 25). It is conceivable that certain genotypes, characteristic of certain strains of mice, could control relative resistance to a given parasite without involvement of the immune system. For example, inadequate nutrition seems a possibility in view of the recent demonstrations that the growth of trypanosomes in vitro requires substances elaborated by mammalian cells (1, 2, 12, 13, 24). Another possibility might be the elaboration of inhibitory or toxic substances capable of restricting parasite growth in resistant strains of mice. It is quite possible that macrophages might regulate parasite growth independent of typical immune responses.

In the present communication we describe studies of the relative resistance of various strains of mice to infection with murine-specific *Trypanosoma musculi*. Significant variation in the magnitude of infections was reflected by differences in parasitemia among strains of mice. We have attempted to analyze this variation and the underlying causative mechanisms by: (i) comparing the ability of spleen cells of various strains of mice to support *T. musculi* growth in cultures, including cultures stimulated with 2-mercaptoethanol (2ME); (ii) comparing the effects of graded levels of ionizing radiation on the course and severity of infections in C3H (relatively susceptible) and C57BL/6 (B/6) (relatively resistant) mice; and (iii) assessing the effects of silica dust, an agent toxic to macrophages, and 2ME, an agent that promotes parasite growth in cultures, on the course of infections in intact mice.
MATERIALS AND METHODS

Mice. The following strains of mice were employed in these investigations: A/HeJ, C57BL/6J, and DBA/1J, obtained from the Jackson Laboratory, Bar Harbor, Maine; BALB/c Cum, C57BL/Cum, CBA/Cum, C3H/Anf Cum, and BC3F1 (C57BL/Cum 2 x C3H/Anf Cum 2), obtained from Cumberland View Farms, Clinton, Tenn. All mice were 4 to 6 months of age when used in the experiments.

Irradiation. Gamma radiation was delivered by a 60Co source. Mice were placed in individual compartments of a semicircular plastic chamber and exposed to different amounts of total body irradiation delivered at a rate of 105 rads per min.

Trypanosomes. The origin and maintenance of the T. musculi involved in these studies have been described (6). They have been maintained by passage in normal C3H/Cum mice.

Cultures. The method of culturing T. musculi in vitro and the growth characteristics of the parasite have been reported (1, 2). Cultures were established in medium RPMI 1640 supplemented with 20% fetal calf serum and antibiotics. Some cultures were provided with 5 x 10^-4 M 2ME. Cultures were initiated with 10^7 normal mouse spleen cells from the various donor strains, and the desired number of T. musculi was freshly isolated from the blood of donor mice on days 10 to 12 of infection.

Growth of trypanosomes in mice. The course of infection in inoculated mice was evaluated by determining levels of parasitemia. Small samples of blood were collected from the tail veins and diluted appropriately in 0.83% ammonium chloride solution to lyse the erythrocytes. Parasite counts were performed by phase-contrast microscopy with the aid of thin hemacytometers.

Treatment with silica and 2ME. Some mice were treated with powdered silica before infection (silica particles were less than 5 μm in size, obtained through the courtesy of M. Reisner, Steinkohlenbergbauverein, 43 Essen-Kray, Germany). The particles were suspended in physiological salt solution at a concentration of 10 mg/ml, and each mouse received 0.5 ml of the suspension intravenously 1 h before parasite inoculation. Control mice received identical injections of saline. The preparation of silica employed was characterized when first received from the supplier and was found to be highly effective in eliminating the ability of spleen cell suspensions of treated mice to perform in vitro immune responses to sheep erythrocytes or to ingest opsonized sheep erythrocytes. To test the effect of 2ME on the course of infection, B/6 mice received repeated injections of 2ME according to a previously determined optimum regimen (16). The first intraperitoneal injection of 1 ml of 5 x 10^-4 M 2ME (diluted in saline) was given 4 days before infection. A second equivalent injection was given 2 days before infection, and the third injection was given on the day of infection.

RESULTS

The course of parasitemia in representative strains of mice inoculated with a constant number of T. musculi is shown in Fig. 1. The levels of parasitemia in these strains fell into two groups, high and moderate, with the exception of strain C57BL/6 (B/6). The plateau levels of parasitemia found on days 8, 10, and 12 in the case of the strains with high parasitemia were averaged to obtain a mean plateau number of 2.1 x 10^9 trypanosomes per ml of blood. Similarly, the mean plateau number of 4.4 x 10^7 trypanosomes per ml of blood was obtained for the strains with moderate infections. The difference between these mean values is highly significant (P < 0.01 determined by Student's t test with 72 degrees of freedom). The growth of parasites in the B/6 strain was slower than in all other strains. The mean plateau number obtained by averaging the parasite counts on days 10, 12, and 14 was 2.0 x 10^7. This mean was significantly less than the mean value for the strains with moderate parasitemia (P < 0.01). These results raised the question of whether differences in parasitemia reflected differences in the ability of strains of mice to sustain the
parasites nutritively or, perhaps, differences in the elaboration of noxious substances. The following experiments were intended to examine such possibilities.

The growth of *T. musculi* in culture is dependent upon substances elaborated by supportive cells, and the availability or production of such growth-promoting substances is enhanced by the addition of 2ME to the cultures (1, 2). The ability of spleen cells from representative strains of mice to support growth of *T. musculi* is depicted in Fig. 2. For comparative purposes a constant, optimum number of $10^7$ supportive spleen cells per culture was used. The cells of most strains were equivalent in supporting parasite growth and equally affected by the addition of 2ME to the cultures. Figure 2 shows representative results from experiments with two strains that developed high parasitemia and two that developed moderate parasitemia. The spleen cells of the B/6 strain supported atypical growth of the trypanosomes. Figure 3 shows the fact that cells of B/6 mice were deficient in supporting parasite growth in comparison to cells of other strains including those of C57BL used for preparation of simultaneous, comparative cultures. The most apparent difference was in the rate of parasite growth. Addition of 2ME to cultures containing B/6 cells resulted in growth at the same rate as in cultures of cells from other strains in the presence of 2ME.

To demonstrate that the immune system plays a significant role in regulating the plateau level of parasitemia, two types of experiments were performed. The first concerned the effects of ionizing radiation. Mice of two strains, C3H (high parasitemia) and B/6 (low parasitemia), were exposed to various levels of gamma radiation. Immediately after irradiation they were injected with approximately $2 \times 10^6$ *T. musculi*. The course of the ensuing parasitemia was followed and compared with that in unirradiated animals of the same strain. The results are displayed in Fig. 4 and 5.

In C3H mice exposed to 400 rads or more (Fig. 4), the parasitemia rose rapidly and the mice died in less than 10 days. Mice exposed to 200 rads developed a significantly higher parasitemia than did unirradiated controls, and the duration of the infection was greatly prolonged. Ultimately some of these mice developed severe anemia and died.

The effects of the combined insults (irradiation and *T. musculi* infection) on B/6 mice were quite different (Fig. 5). Mice exposed to 600 or 800 rads developed substantially higher infections than did the other groups of mice. Nevertheless, there occurred a plateau in the level of parasitemia around days 10 to 12 after parasite inoculation. This phase was followed by a period of slowly rising parasitemia culminating in death. Mice exposed to 400 rads developed a level of parasitemia that was moderately, but significantly, higher than that displayed in the mice exposed to 200 rads or in unirradiated controls. In addition, the duration of infection was substantially prolonged. B/6 mice exposed to 200 rads displayed a plateau of parasitemia slightly higher than the controls and, again, infection was prolonged. Mice exposed to 200 rads eventually cleared the infection around day 24 postinoculation; an even longer time, approximately 42 days, was required by the mice exposed to 400 rads.

The role of macrophages in regulating *T. musculi* infections was investigated by injecting silica dust to inactivate these cells. Table 1 shows the fact that macrophage inactivation was without effect on the course of parasitemia in B/6 mice. In mice of the C3H strain, as well as in BC3F1 hybrids, silica treatment resulted in significant elevation of parasitemia. This is illustrated by the data presented in Table 1 repre-

---

**Fig. 2. Growth of *T. musculi* in cultures containing $10^7$ normal spleen cells from the following strains of mice: (□) A/He, (○) C57BL, (○) C3H, (△) DBA/1. Parallel cultures prepared with or without $5 \times 10^{-5}$ 2ME. Standard error bars omitted for clarity; six to eight samples per point.**
senting the plateau of parasitemia in control and silica-treated BC3F1 mice.

Finally, in view of the marked enhancement of parasite growth that occurred in cultures containing 2ME, it was of interest to determine the effects of 2ME on the course of parasitemia in intact mice. Injection of 2ME occurred before and at the time of inoculation of T. musculi. As shown in Table 1, there was no significant effect of 2ME on the course of parasitemia in B/6 mice.

DISCUSSION

Major questions which this and other recent publications address are: (i) what mechanisms are responsible for natural resistance to infections by various parasites, (ii) are they accessible to cytological and biochemical analysis, and (iii) can the underlying genetic control of these mechanisms be analyzed by reasonably straightforward genetic techniques? Recent studies of resistance among inbred strains of mice to several protozoan parasites, viz., Plasmodium berghei (6), Leishmania donovani (7-10), Trypanosoma congoense (18, 19), Trypanosoma cruzi (23), and Trypanosoma lewisi (5), strongly suggest that answers to these questions may soon be obtained. It is clear already that resistance to L. donovani, T. congoense, and T. cruzi is not, to any major extent, under the control of genes within the MHC. The involvement of genes within the MHC in resistance to parasite infections has been thoughtfully discussed in two recent reviews (17, 22), and the conclusion has been drawn that MHC genes are not involved. One exception to this conclusion may be the case of murine resistance to T. gondii (26).

The present studies of the magnitude of parasitemia in various inbred strains of mice suggest that control of infections by the natural parasite, T. musculi, also is not centered in genes within the MHC. Final proof of this conclusion, however, requires appropriate crosses and use of congenic lines of mice. The strains that we have studied fell roughly into two groups, those with high parasitemia and those having a mean parasite count fivefold lower (moderate parasitemia). The B/6 strain appeared to be exceptional, displaying a mean parasitemia significantly lower than the strains that developed moderate parasitemia.

We sought insight into mechanisms of resistance by analyzing the ability of cells from various inbred strains of mice to provide the

FIG. 3. Curves comparing the growth of T. musculi in cultures containing 10⁵ spleen cells from C57BL mice, either with (○) or without (□) 2ME, or 10⁵ spleen cells from C57BL/6 mice, either with (●) or without (□) 2ME. Vertical bars represent 1 standard error; five or six samples per point.
growth-promoting substances that *T. musculi* require. The analysis included the growth-enhancing effect of 2ME which is exerted on *T. musculi* indirectly by way of the supportive spleen cells (2). The cells of most strains were equivalent in their ability to support *T. musculi* growth in vitro and equivalent in supporting rapid parasite growth after stimulation with 2ME. The results with B/6 cells were exceptional and suggested that parasite growth, both in vitro and in the intact mice, might be limited, in part, by the availability of essential growth-promoting substances. A deficient rate of production of such substances, coupled with even a typical, normal rate of their catabolism, could result in limitation of growth of the parasites in vivo. The rate of growth was enhanced by 2ME treatment of cultures of B/6 cells as it was in the case of cells of other strains.

It is clear from the studies of the effects of irradiation that the immune system plays a significant role in regulating levels of parasitemia and, thus, in determining relative resistance to infection by the mouse-specific trypanosome. In both the C3H and B/6 mice exposure to 600 or 800 rads resulted in marked increase in parasitemia. The infection increased rapidly in the C3H mice, and these animals died prior to the appearance of overt signs of radiation damage, presumably from the massive infections. In B/6 mice exposed to the highest levels of radiation there was also rapid trypanosome growth which ended abruptly in a plateau of parasitemia lasting about 3 days. Subsequently there was further gradual increase in parasitemia correlated with visible manifestations of radiation damage. These mice died from the combined effects of irradiation and infection. The C3H mice exposed to 400 rads also died promptly of fatal infections, whereas similarly irradiated B/6 mice survived and displayed moderate infections. C3H mice exposed to 200 rads developed severe parasitemia which appeared to be brought under control by the recovering immune system; however,
some of these mice ultimately died from the stress of the combined irradiation and infection. Moderate parasitemia of prolonged duration was sustained by the B/6 mice exposed to 200 rads. The differences between the infections displayed by C3H mice exposed to 200 and 400 rads and those displayed by B/6 mice, similarly irradiated, are open to at least three possible interpretations.

First, the differences might be quantitative, rather than qualitative, reflecting a substantially greater capacity for immune response to T. musculi in B/6 mice compared to C3H mice. The effect of an equivalent radiation exposure would result in a greater, more severe, infection in C3H mice. Such a difference might be related to the well-known interstrain variation with respect to the lethality of ionizing radiation (27); such variation must reflect, at least in part, interstrain differences in the numbers and potentialities of lymphohematopoietic stem cells (although the actual proof of this has been difficult; see reference 27).

Second, the differences between C3H and B/6 mice with regard to severity of infections after moderate radiation might reside in the macrophage populations. This possibility was raised clearly by a report concerning the effects of silica...
treatment of C57BL/10 mice on infections with T. cruzi (23); in this case, higher parasite infections were observed in silica-treated mice than in mice exposed to 450 rads. In our experiments, treatment of B/6 mice with a single, intravenous injection of silica failed to alter the course of T. musculi infection. In contrast, silica treatment of both C3H and BC3F1 hybrid mice resulted in an approximate threefold increase in the magnitude of parasitemia. Jaroslow (15) reported no increase in parasitemia in CF-1 mice subjected to macrophage blockade by India ink before infection with T. musculi; in contrast, 550 rads delivered to mice in the interval between 8 days before and 4 days after infection with T. musculi resulted in marked increases in parasitemia (14). Blockade with India ink did, however, result in a transient increase in the proportion of dividing forms present in the blood. When both irradiation and India ink blockade were employed the effects were additive. These results suggested that macrophages might play a protective role in restricting reproduction during the early course of infection whereas trypansocidal activity was associated with the more radiosensitive immune system. Brooks and Reed (11) demonstrated that trypan blue inactivation of macrophages in a subline of BALB/c mice resulted in a significant, early increase in parasitemia after inoculation of T. musculi; however, similar mice exposed to 550 rads of gamma radiation displayed a much greater, much more rapid rise in blood parasites. The differences between the effects of macrophage inhibitors on infections with T. cruzi and T. musculi are probably related more closely to the fact that the former are intercellular and the latter are free-living than to any fundamental differences in resistance to infections by the parasites.

A third possible explanation of the differential effects of moderate radiation on infection in the C3H and B/6 mice concerns the availability of essential growth-promoting substances in B/6 mice. Perhaps the effects of moderate radiation were masked by an already limiting supply of such substances that restricted parasite growth. The recovering immune system would be able to cope with a moderate number of parasites. This explanation seems inept and is not supported by the fact that injection of 2ME was without effect on the course of parasite infection in B/6 mice, an effect which would be expected if growth-promoting substances were limited in vivo.

Two fundamental questions are raised, but not completely answered, by the present investigation. First, is interstrain variation in resistance to T. musculi a reflection of a mechanism that is not typically immunological and which varies in potency among different strains? Second, is this the same mechanism, displayed in a milder or modified form toward T. musculi, that is responsible for the solid resistance of mice to a heterospecific trypansome such as T. lewisi? At the moment it cannot be argued convincingly that interstrain variation in resistance to T. musculi involves an unfamiliar mechanism. The differential effects of radiation on the course of infections in C3H and B/6 mice can be explained quantitatively by invoking a substantially greater potential for immune response in B/6 mice; the combined effects of moderate levels of radiation and the immunosuppressive activity of the trypansomes (3, 4) would be less severe in B/6 mice than in C3H mice. With respect to the second question it appears that there may be basic differences in the resistance encountered in mice by T. musculi and T. lewisi, e.g., resistance to T. lewisi is not affected by exposing mice to ionizing radiation whereas it is inhibited by treatment with substances such as silica that interfere with the functions of macrophages and granulocytes (21).

ACKNOWLEDGMENTS

This work was supported by grant PCM 78-15875 from the National Science Foundation and by grants from the Eagles’ Cancer Research Fund and the Lee Foundation.

We thank Steven Fortuna and Shirley Ware for their dedicated assistance in the performance of the experiments.

LITERATURE CITED


