FACTORS MODIFYING HOST RESISTANCE TO VIRAL INFECTION

III. Effect of Whole Body X-Irradiation on Experimental Encephalomyocarditis Virus Infection in Mice*

By BRIAN R. MURPHY and LOWELL A. GLASGOW, M.D.

(From the Departments of Microbiology and Pediatrics, The University of Rochester School of Medicine and Dentistry, Rochester, New York 14620)

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The nature of host resistance to virus infections has never been fully defined. A series of investigations currently in progress in this laboratory is aimed at developing experimental models which would permit further delineation of the physiologic mechanisms of host resistance. It has been recognized that a wide variety of both intrinsic and environmental factors may affect the capacity of the host to control and eliminate an infection with a viral agent (1). Preliminary studies have demonstrated an enhanced susceptibility to encephalomyocarditis (EMC) virus infection in male mice, in mice housed alone rather than in the usual grouping of 5–10 per cage, in female animals, during pregnancy or lactation, and in mice receiving immunosuppressive drugs. The mechanisms by which these factors may modify host defenses have been only partially defined.

Baron (2) has recently reviewed the literature pertaining to host defense mechanisms against viral infections and has interpreted the evidence to support the concept that antibody plays only a minor role in the host's resistance to a primary infection with a virus. Friedman and coworkers (3) have demonstrated that whole body X-irradiation combined with methotrexate treatment suppressed both detectable levels of circulating antibody and delayed hypersensitivity response to a localized vaccinia virus infection in mice, but failed to alter the course of virus infection. The present study utilized EMC virus infection in mice, a systemic infection which is characterized by a primary site of replication and viremia followed by seeding of target organs such as heart, brain, and pancreas. This virus-host interaction provides a model of a systemic infection in which antibody might be expected to play a more significant role by restricting or preventing the spread of virus to susceptible target organs in contrast to the situation of a localized infection with vaccinia virus in which antibody might well play a less critical role (4). The purpose of this investigation was to

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further elucidate the respective roles of interferon and the immune response in host resistance to a primary systemic viral infection by studying the effects of whole body X-irradiation on EMC virus infection in mice.

Material and Methods

Animals.—6-wk-old female mice, random bred strain, MLM 1 (derived from an ICR strain) obtained from the Western New York Animal Resources Inc., Ontario, N.Y., were used for all experiments.

X-Irradiation.—Mice were irradiated in Lucite containers placed on a rotating turntable (1 rpm) with a Picker Industrial X-ray machine which has the following specifications: 250 kv, 15 ma, aluminum parabolic and 0.5 mm copper filter, and a dose rate of approximately 20 Roentgens (R) per minute.

Cells.—All assays were carried out in a cloned, continuous line of mouse L-cells obtained from Dr. Piero Balduzzi.

Viruses.—EMC virus was a large plaque variant originally obtained from Dr. K. K. Takemoto, National Institutes of Health. A virus pool (pool 1) containing 1×10^8 plaque-forming units (PFU) per ml was prepared and assayed in L-cells. This pool contained a 30:1 ratio of the small plaque variant to the large plaque variant. A large plaque pool (pool 2) containing 3×10^7 PFU/ml, used for clearance studies, was prepared in L-cells in the presence of 100 μg of heparin/ml (5). A small plaque pool (pool 3) containing 5×10^8 PFU/ml was prepared in L-cells.

Vesicular stomatitis virus (VSV), Indiana strain, used as the challenge virus in the interferon assay, was obtained from the American Type Culture Collection, Rockville, Md. Stock virus was grown and assayed in L-cells.

Media.—All tissue cells were maintained in Eagle's minimum essential medium (MEM) with 5–10% calf serum (Hyland Laboratories, Los Angeles, Calif.) containing 100 units/ml penicillin, 50 μg/ml streptomycin, and 5 μg/ml amphotericin B. Overlay medium contained 0.9% Noble agar (Difco Laboratories, Inc., Detroit, Mich.) or Agarose (Bausch & Lomb Inc., Rochester, N.Y.) in MEM. Agarose was used for all EMC virus assays.

Animal Techniques.—Animals were inoculated intraperitoneally with 0.2 ml of the appropriate dilution of EMC virus. 6 mice were exsanguinated via the retroorbital plexus at each designated time. The blood was anticoagulated with K_2EDTA (Eastman Organic Chemicals, Rochester, N. Y.). 1 aliquot was used for WBC counts; another was immediately frozen in a dry ice–70% alcohol bath, and was stored at −70°C until assayed for virus. The remaining blood was centrifuged and plasma was collected for antibody assay. The hearts and brains of the mice that had been exsanguinated were removed aseptically. The hearts were washed in 0.15 M NaCl to remove blood and were placed immediately in dry ice–70% alcohol bath. Brains were directly frozen. Pooled blood from an additional eight mice was used for the interferon determination.

Virus Assay.—Monolayers of L-cells in 60 mm Falcon plastic Petri dishes were exposed to 0.2 ml of a serial 10-fold dilution of blood for 1 hr at 37°C. They were then overlaid with 5 ml of agarose plaque medium. 48 hr later, plates were overlaid with 2 ml of agar medium containing neutral red to make a final concentration of 1:20,000. Brains and hearts were homogenized with a motor driven Teflon pestle. A 10% w/v suspension was prepared using MEM as diluent. Assay of 0.2 ml of the appropriate dilution of the suspension was made as described above.

Antibody Assay.—An equal volume of the appropriate dilution of plasma and EMC virus (100 PFU/0.2 ml) was mixed and incubated at 37° for 30 min and then 0.2 ml of this mixture was assayed as above. The end point was taken as the dilution which caused a 50% reduction in the number of plaques of the EMC virus challenge.

Interferon Assay.—Serum samples were diluted 1:10 with MEM before adjusting the pH to 2 with concentrated HCl. After 2 days at 4°C, the pH was returned to 7 using concentrated NaOH and the samples stored at −20°C until assayed. 2 ml of the appropriate dilution of interferon was allowed to incubate on L-cell monolayers overnight. The concentration of interferon was defined as the reciprocal of the dilution which inhibited 50% of a challenge inoculum of VSV.

Preparation of Immune Serum.—Control mice that recovered from infection with 5000 PFU of EMC virus were infected with 10^7 PFU of EMC virus intraperitoneally. 1 wk after the last inoculation, they were bled and the serum was collected. Approximately 30 ml of serum with a neutralizing antibody titer of 1:50,000 could be collected from 100 mice.

TABLE I

Effect of Dose of EMC Virus on Mortality of X-Irradiated (350 R) and Nonirradiated Mice

Day after challenge with EMC virus	350 R				Nonirradiated		
	10^{4*}	10^3	10^2	10^1	10^6	10^5	10^4
4	0‡	0	0	0	0	0	0
5	0	10	0	0	0	0	0
6	11	10	0	0	10	0	0
7	33	30	0	0	10	0	0
8	66	40	20	11	10	0	0
9	77	40	30	11	10	10	0
10	77	50	50	22	10	10	0
11	88	50	50	22	10	30	0
12	100	60	60	33	20	30	0
13	100	80	60	33	20	30	0
14	100	80	70	33	20	30	0

* PFU per mouse of EMC virus.

‡ Per cent mortality (10–12 mice/group).

RESULTS

Effect of Dosage of EMC Virus on Mortality of Mice Exposed to Total Body X-Irradiation.—Preliminary experiments demonstrated that mice exposed to either 350 R or 650 R of X-irradiation 2 days before EMC virus infection were strikingly more susceptible to EMC virus in comparison to nonirradiated animals. Only the LD_{50} obtained using mice that had received 350 R is presented in Table I. Similar results were obtained at 650 R with the exception of the occurrence of deaths in the control group receiving 650 R of X-irradiation alone. The observed enhancement in susceptibility was characterized by a shortening of the incubation period and by a striking increase in the final mortality. The LD_{50} for the irradiated mice was approximately 5×10^1 PFU, while that for the nonirradiated groups was greater than 1×10^6 PFU. Thus, using a sublethal dose of X-irradiation, 350 R, it is possible to increase the

susceptibility of mice challenged intraperitoneally with EMC virus by a factor of approximately 10^5.

The effect of the dosage of X-irradiation was further defined in groups of mice infected with 10^4 PFU of EMC virus 2 days after receiving 50–650 R of X-irradiation. A difference in mortality was not observed between mice treated with 50 R and nonirradiated mice, but dosages from 200 R to 650 R resulted in an enhancement of mortality which correlated with the increasing dosage of

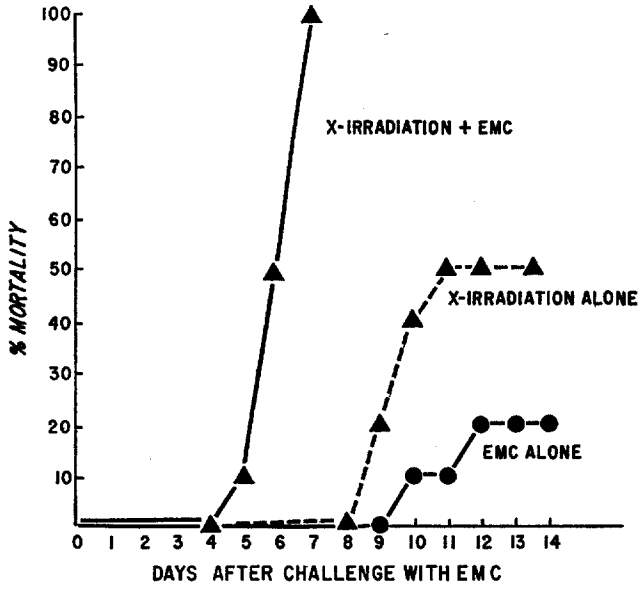

FIG. 1. Mortality of X-irradiated (650 R) and nonirradiated mice challenged with EMC virus.

irradiation. Either 350 R or 650 R were employed in the subsequent studies. Both dosages of X-irradiation had similar effects on the viremia and on the virus titer of the central nervous system (CNS) in EMC virus-infected mice. The results of the experiments using 650 R are representative of these studies and are presented in the following section as more complete data is available at this level of X-irradiation.

Effect of X-Irradiation on Mice Infected Intraperitoneally with EMC Virus.— 48 hr after being exposed to 650 R, mice were injected intraperitoneally with 10^4 PFU of EMC virus (pool 1). Groups of six mice were bled and sacrificed at 12 hr intervals for the first 4 days, at 24 hr intervals until the 6th day, and every other day until the 17th day.

White blood cell count and spleen weight in the X-irradiated and control animals:

The effect of whole body X-irradiation on the suppression of peripheral white blood cells (WBC) is well documented (6). To confirm these recognized effects of total body X-irradiation under the present experimental conditions, the peripheral WBC count and spleen weights were recorded. A depression of WBC count to less than 1000/mm³ was observed in the X-irradiated mice, and

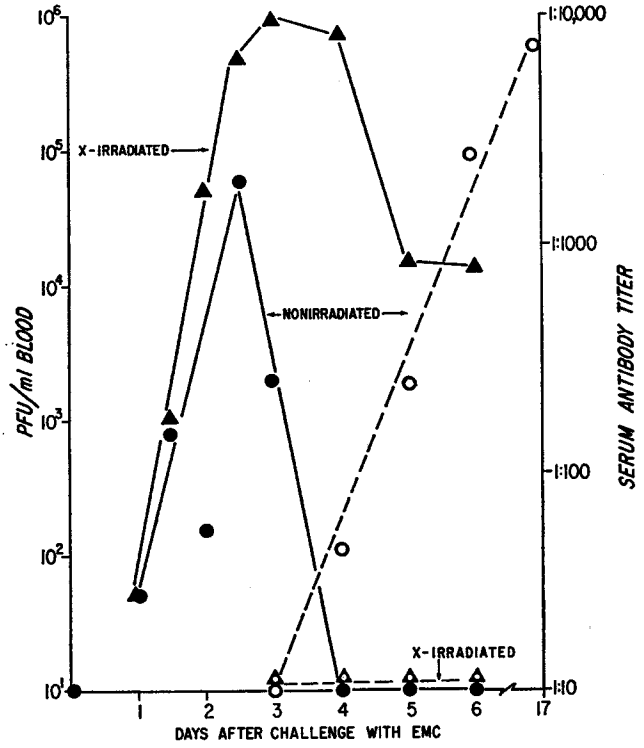

FIG. 2. Virus titer in blood, and antibody response in X-irradiated (650 R) and nonirradiated mice challenged with EMC virus. ●—●—●, virus titer in nonirradiated mice. ▲—▲—▲, virus titer in X-irradiated mice. O—O—O, antibody response in nonirradiated mice. △—△—△, antibody response in X-irradiated mice.

average spleen weights decreased from 114 mg in the nonirradiated control mice to 30 mg in the X-irradiated animals.

Mortality: The data presented in Fig. 1 represent the mortality of mice from one of three similar experiments using 10–15 mice per group in each experiment. All the deaths in the group receiving both 650 R and 10^4 PFU of EMC virus occurred before the first deaths due to either the X-irradiation alone or the virus alone.

Viremia and antibody response: The course of the viremia is shown in Fig. 2.

It is clearly demonstrated that the titer of virus in the blood of the X-irradiated mice was consistently higher and persisted until the death of the animal, whereas the control mice effectively cleared the viremia by the 4th day. This

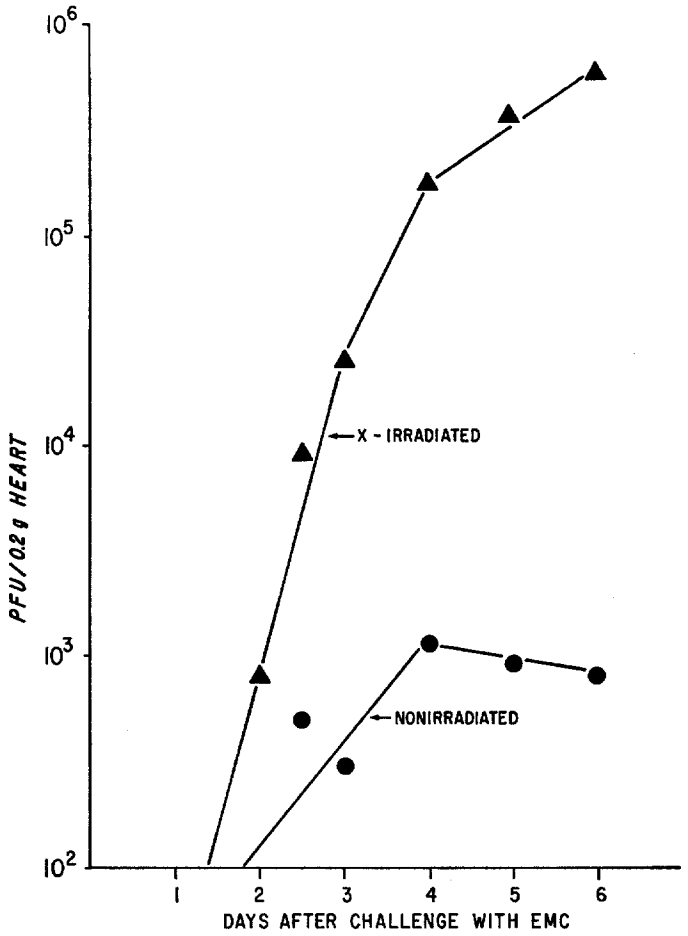

FIG. 3. Virus titer in heart of X-irradiated (650 R) and nonirradiated mice challenged with EMC virus.

clearance could be correlated with the development of neutralizing antibody (Fig. 2), which was detectable in the serum of the nonirradiated mice on the 4th day after infection. In contrast, the X-irradiated animals had a persistent viremia and antibody could never be detected in the serum.

Virus in the heart and brain: The course of infection in the hearts of the X-

irradiated and control mice, as evidenced by virus replication, is shown in Fig. 3. In the X-irradiated group, virus was detectable earlier and its titer steadily increased until the death of the animals. Virus was present in the hearts of the nonirradiated mice but in considerably lower titer. The presence of higher levels of virus in the hearts of the X-irradiated group strongly suggests an increased target organ involvement. Evidence that the virus in the heart

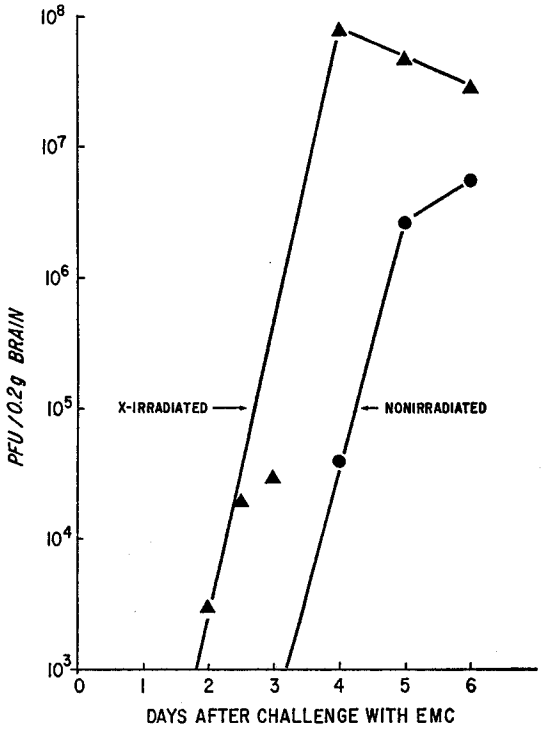

FIG. 4. Virus titer in brain of X-irradiated (650 R) and nonirradiated mice challenged with EMC virus.

was not due to contamination by blood is found in the lack of correlation between the titer of virus in blood and heart in both the X-irradiated and nonirradiated mice.

The course of infection in the brains of the X-irradiated and control mice is illustrated in Fig. 4. The virus is again detected earlier and in higher amounts in the brains of the X-irradiated mice, however the quantity of virus in the brains of both groups was not greatly different by the 6th day after infection.

Serum Interferon Response.—The possibility that X-irradiation caused a

suppression of the interferon response of mice was next investigated. Groups of eight X-irradiated and control mice were sacrificed at daily intervals for determination of the level of serum interferon. The interferon response of the control and X-irradiated groups of animals is presented in Fig. 5. Only on day 5 was a difference in interferon titer detected between the irradiated mice and control mice. On all other days, including days 4 and 6, the titers were similar. The

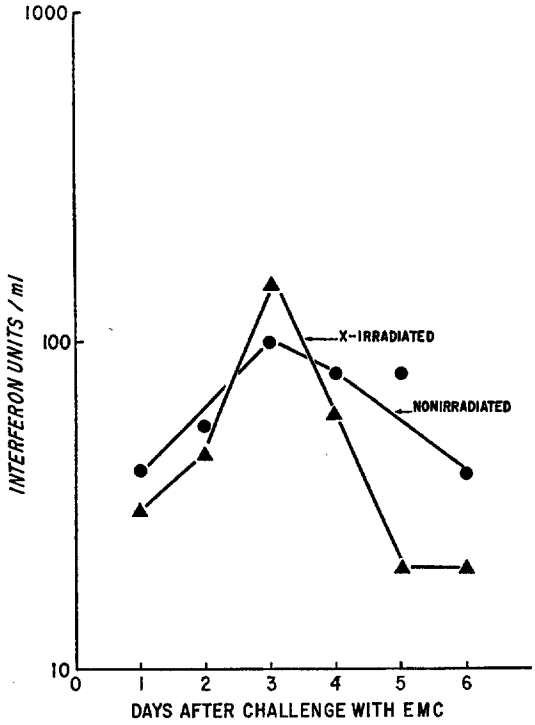

FIG. 5. Interferon response in X-irradiated (650 R) and nonirradiated mice challenged with EMC virus.

results are interpreted to indicate that the observed enhanced susceptibility of the X-irradiated mice is not a result of a decreased interferon response. It is interesting to note that the interferon level in the serum declined in the X-irradiated mice despite a persistent viremia.

Effect of X-Irradiation on the Clearance of EMC Virus in the Nonimmune Mouse.—The clearance of the large plaque variant of EMC virus (pool 2) was studied in X-irradiated and control mice following intravenous inoculation of 10^7 PFU of EMC virus. The large plaque variant of EMC virus was used in the present experiment because previous studies in our laboratory demonstrated

that its rate of clearance, like the large plaque variant of Mengovirus (7), is more characteristic of enterovirus than the very rapid rate of clearance exhibited by the small plaque variant. One group of nine mice received 650 R 5 days before the intravenous EMC virus injection. Three mice in both the treated and control groups were bled at 20, 60, and 120 min after the intravenous inoculation, and virus assays were performed on the pooled blood. The data presented in Fig. 6 demonstrate that the rate of clearance in the nonirradiated and irradiated mice was similar. To rule out the possibility that

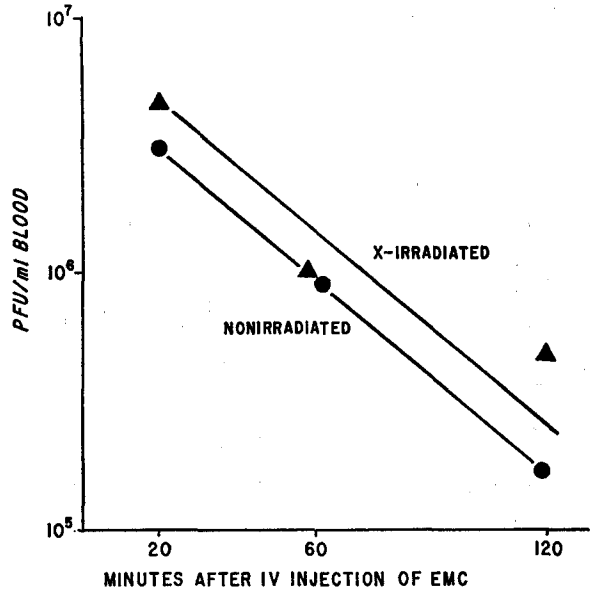

FIG. 6. Clearance of intravenously inoculated EMC virus from the blood of nonimmune mice which have received either 650 or 0 R of X-irradiation.

thermal inactivation or undefined intravascular phenomena contributed to the observed rate of clearance, 10^6 PFU of EMC virus were added to anticoagulated blood collected from nonimmune mice and was incubated at 37°C for 2 hr. No loss of virus could be demonstrated in this control preparation during the 2 hr incubation period. The slightly higher titer of virus in samples from the X-irradiated mice is probably a function of the same dosage of virus being injected into the slightly smaller blood volume of the animals that were subjected to X-irradiation. These results demonstrate that clearance of EMC virus in nonimmune mice is not affected by X-irradiation.

Effect of Antibody Replacement on the Mortality of X-Irradiated, EMC Virus-Infected Mice.—Since inhibition of antibody formation is one of the biological

effects of X-irradiation, information concerning the role of antibody in the pathogenesis of EMC virus infection could be obtained by replacing the endogenous antibody production in X-irradiated, EMC virus-infected mice with exogenous antibody, and then observing the effect this replacement therapy has on the mortality of the mice. 2 days after receiving 350 R, mice were injected intraperitoneally with 2×10^4 PFU of EMC virus (pool 1). A group of 15 mice was inoculated intraperitoneally with a single dose of 0.3 ml/mouse of undiluted hyperimmune anti-EMC virus mouse serum on the day prior to

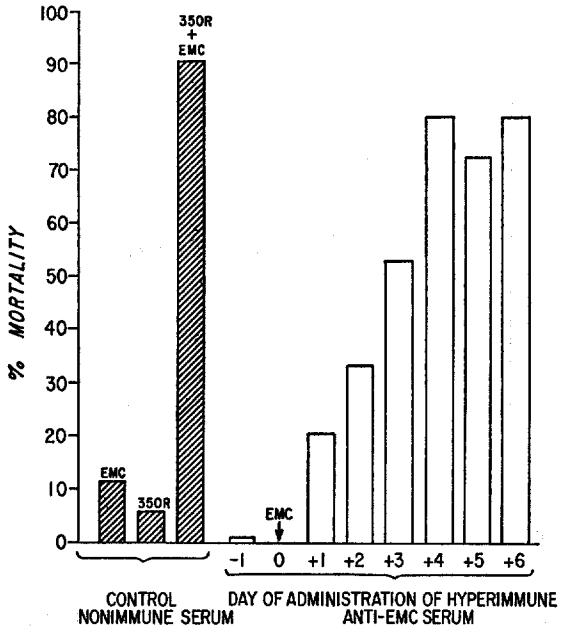

FIG. 7. The effect of time of administration of anti-EMC immune serum on the mortality of X-irradiated (350 R), EMC virus-infected mice.

EMC virus inoculation. Other groups received an identical inoculation on the 1st, 2nd, 3rd, 4th, 5th, or 6th day after EMC virus challenge. The separate groups of mice were followed for mortality for 17 days after EMC virus injection. The neutralizing antibody titer of the hyperimmune serum used in this experiment was 1:50,000 and the titer achieved in the serum of noninfected mice 24 hr after the intraperitoneal administration of 0.3 ml of antiserum was 1:2000, a level which approximates that reached after recovery from primary exposure to EMC virus. Three control groups that received 350 R alone, EMC virus alone, or a combination of these, were injected with undiluted normal mouse serum on the same day that the EMC virus inoculation was given.

The data from one of two similar experiments employing groups of 25 mice are presented in Fig. 7 and illustrate that after the 3rd day of EMC virus infection, passively administered antibody is no longer able to prevent the enhanced mortality observed in X-irradiated mice. The fact that antibody is effective only until the 3rd day after EMC virus challenge is interpreted to indicate that

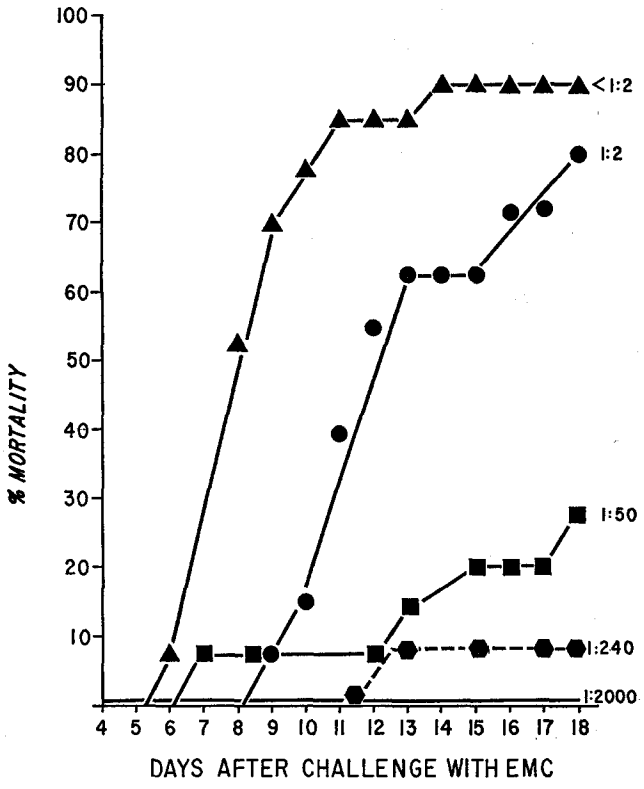

FIG. 8. Relation between titer of serum anti-EMC antibody and the mortality of X-irradiated (350 R), EMC virus-infected mice.

if antibody is to alter the pathogenesis of EMC virus infection, it must be present early in the course of the infection.

Effects of the Serum Anti-EMC Virus Antibody Titer on the Mortality of X-Irradiated, EMC Virus-Infected Mice.—The level of anti-EMC virus antibody required to restore a normal degree of host resistance to EMC virus in X-irradiated mice was next determined. Mice were irradiated with 350 R and 2 days later were challenged intraperitoneally with 2×10^4 PFU of EMC virus (pool 1). Different dilutions of antibody were administered 8 hr after the virus

challenge. This time interval would permit adsorption and approximately one cycle of multiplication of EMC virus. It is also the time after primary poliovirus infection in rabbits at which Svehag (12) could detect specific neutralizing antibody in the serum. Groups of 15 mice which had received both 350 R and EMC virus were injected intraperitoneally with 0.3 ml/mouse of a 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilution of hyperimmune anti-EMC serum. To determine the levels of antibody achieved by these inoculations, groups of two uninfected, X-irradiated (350 R) mice were bled 24 hr after injection, and the antibody titer of the serum from their pooled blood was measured. Dilutions of 10^0, 10^{-1}, 10^{-2}, 10^{-3}, and diluent MEM produced anti-EMC virus antibody titers of 1:2000, 1:240, 1:50, 1:2, and <1:2, respectively. The groups were followed

TABLE II

Relation between Serum Anti-EMC Antibody Titer and Clearance of EMC from the Blood

Dilution of anti-EMC antibody injected i.p.	Anti-EMC titer in serum	EMC remaining in blood 30 min after i.v. inoculation of EMC*	Per cent‡ cleared
10^0	1:2000	0	100
10^{-1}	1:240	0	100
10^{-2}	1:50	3.4×10^4	98
10^{-3}	1:2	1.4×10^6	47
10^{-4}	Not done	2.6×10^6	0
MEM	<1:2	2.4×10^6	0

* Expressed as PFU/ml blood.

‡ $100 - \frac{\text{PFU remaining at 30 min}}{\text{PFU remaining in MEM control at 30 min}} \times 100.$

over a 17 day period and mortalities were recorded. The data presented in Fig. 8 indicate that very low serum levels of antibody exert a protective effect on the pathogenesis of EMC virus infection in X-irradiated mice, characterized at the 1:2 level by a lengthening of the incubation period and at the 1:50, 1:240, and 1:2000 levels by a decrease in the final mortality. Thus, the effects of X-irradiation on EMC virus infection, which include a shortening of the incubation and an enhancement of mortality, were reversed when the level in the serum reached a titer of 1:50. These results indicate that low levels of antibody present early during the course of infection can alter the pathogenesis of EMC virus infection.

Relationship between the Serum Anti-EMC Antibody Titer and Clearance of Virus from the Blood.—Since low levels of antibody present early in the infection may affect the course of a virus infection, it was of importance to examine the relationship between these levels of antibody and the clearance of virus from the blood. 24 hr after receiving an intraperitoneal inoculation of 0.3 ml of a 10^0, 10^{-1}, 10^{-2}, 10^{-3}, or 10^{-4} dilution of anti-EMC virus antibody titering 1:50,000 or diluent MEM, groups of three mice were injected intravenously with $1 \times$

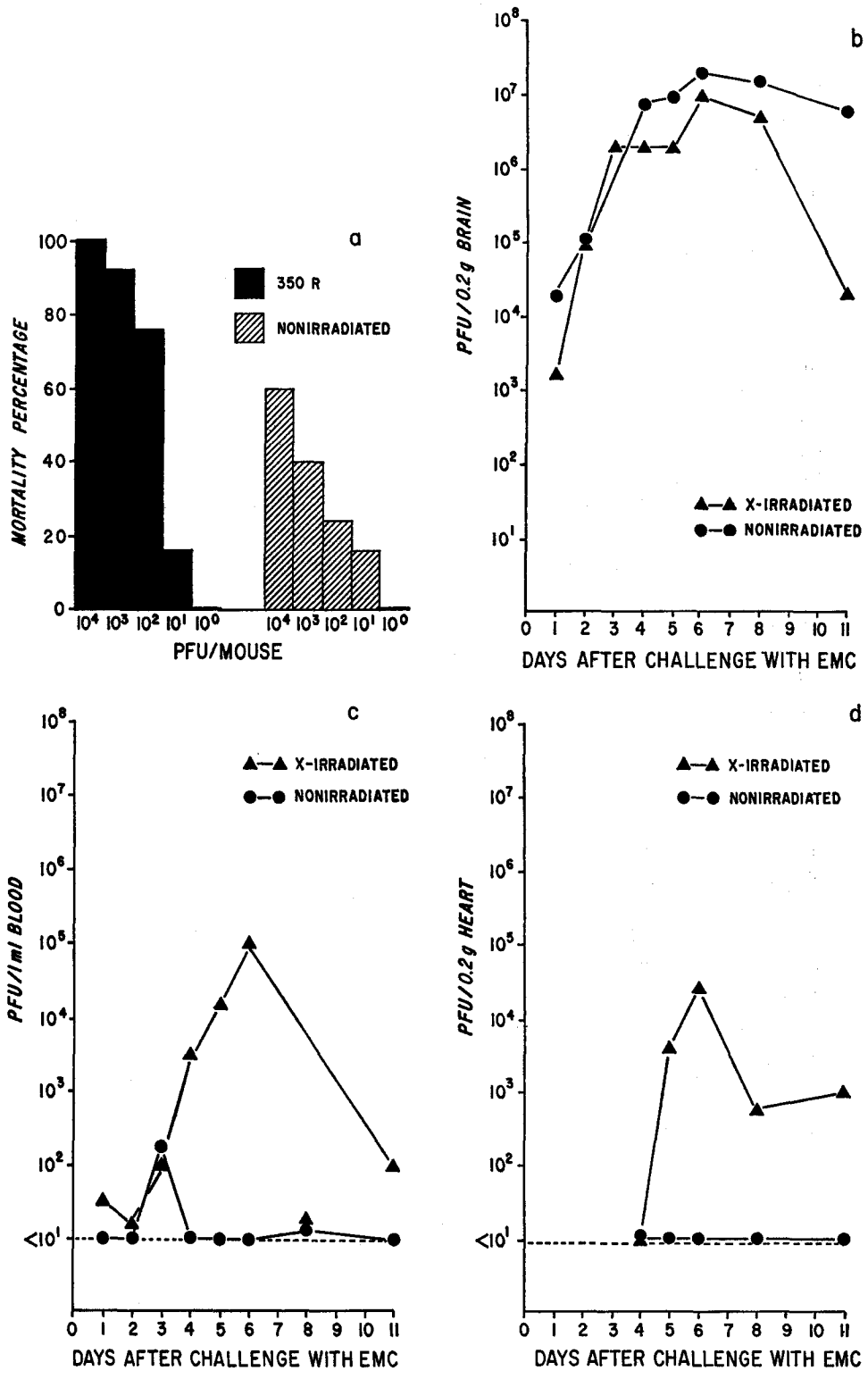

FIG. 9. Effect of X-irradiation (350 R) on the infection of mice inoculated intracerebrally with EMC virus.

10^7 PFU of the large plaque variant of EMC virus (pool 2) and these were then bled 30 min later. The virus titer in the pooled blood was determined. The results summarized in Table II indicate that low levels of antibody may contribute to the clearance of large amounts of virus from the blood stream.

Effect of X-Irradiation (350 R) on EMC Virus Infection of Mice by the Intracerebral Route.—Preliminary experiments demonstrated that the infection of mice with the small plaque variant of EMC virus by the intracerebral route resulted in an infection which remained largely confined to the CNS and which thereby provided a model which simulated a localized infection in a target organ. In order to determine if X-irradiation alters the resistance of a target organ to infection, mice which had received 350 R of X-irradiation and matched, nonirradiated control animals were inoculated by the intracerebral route with the small plaque variant of EMC virus. In this experiment, 350 R of X-irradiation was employed rather than 650 R for the following reasons: (*a*) to determine the LD_{50}, it was necessary to utilize a nonlethal dose of X-irradiation; and (*b*) the effects of 350 R of X-irradiation on virus replication in the brains of mice inoculated with EMC virus by the intraperitoneal route were similar to those of 650 R (see Fig. 4). The LD_{50} was determined in these groups by the inoculation of serial 10-fold dilutions of the small plaque variant (pool 3) 2 days after the test group of mice had received 350 R of whole body X-irradiation. Virus assays of brain, blood, and heart were carried out in other matched groups of mice that had received 10^3 PFU of EMC virus by the intracerebral route. The data presented in Fig. 9 summarize the results of these experiments. Although the levels of virus and the duration of infection in the brains of control animals receiving X-irradiation were not significantly different, a 100-fold difference in the LD_{50} was demonstrated. This increased susceptibility of the X-irradiated mice was correlated, however, with a higher level of virus in the blood and heart tissue of these animals. It should be noted that this difference is significantly less than the 100,000-fold difference in the LD_{50} which was found when similar groups of animals were inoculated by the intraperitoneal route. The evidence that there was no difference in the replication of virus in the CNS after intracerebral inoculation suggests (*a*) that whole body X-irradiation did not have a deleterious effect on local defense mechanisms in this specific target organ, and (*b*) that the difference in levels of virus in the brains of mice that had received X-irradiation followed by infection by the intraperitoneal route was the result of alteration of host defense mechanisms other than that of local organ resistance.

DISCUSSION

The present investigation demonstrates that whole body X-irradiation decreases the resistance of mice to an experimental infection with EMC virus. Infection in mice receiving 650 R is characterized by (*a*) a shorter incubation

period and an enhanced mortality, (*b*) a higher peak level of virus in the serum and persistence of the viremia in the absence of a detectable serum antibody response, (*c*) increased levels of virus in heart and brain, and (*d*) a level of interferon in the serum similar to that of control mice. The decreased resistance is dependent on the dosage of X-irradiation employed. The enhanced susceptibility of X-irradiated mice as compared with control mice is manifested by greater than 10^5 difference in the LD_{50} when EMC virus is administered by the intraperitoneal route. In the present investigation, EMC virus was inoculated intracerebrally into X-irradiated and control mice to determine if the greatly enhanced mortality of the irradiated mice observed after intraperitoneal injection of EMC virus was a result of an alteration in resistance of the CNS to infection with EMC virus. The fact that similar levels of virus were achieved in the brains of both the X-irradiated and the control mice after intracerebral inoculation of EMC virus suggests that the greatly increased susceptibility observed when EMC is administered by the intraperitoneal route cannot be explained solely on the basis of a decreased resistance at the target organ level and suggests that other mechanisms are determinants of this markedly enhanced susceptibility.

In other experimental models developed in this laboratory, the effects of immunosuppressive drugs, pregnancy, housing conditions, and sex on the pathogenesis of EMC virus infection in mice are being studied, and it has been consistently demonstrated that an enhanced mortality is correlated with a prolonged viremia and greater target organ involvement. The failure to clear the virus from the blood of the X-irradiated mice is explicable by the following mechanisms: (*a*) an increased production of EMC virus that overloads the normal mechanisms responsible for clearing virus from the blood, (*b*) an equal rate of production of EMC virus but a decrease rate of clearance, or (*c*) a combination of both of these mechanisms. Many viruses appear to be cleared from the serum of nonimmune mice by the reticuloendothelial system (8). Specific antibody has been shown to enhance the rate of clearance of infective virus particles from the blood (9, 10). That nonimmune clearance mechanisms can be altered has been demonstrated by Brunner et al. (11) who showed that an intravenous injection of thorotrast decreases the clearance of Newcastle disease virus (NDV) in nonimmune mice. The present study, however, demonstrates that the rate of clearance of EMC virus in nonimmune animals following intravenous inoculation is unaffected by X-irradiation. Thus, if a decreased clearance of EMC virus from the blood results in the establishment of the persisting viremia, an explanation for the failure to clear the viremia should be found in an altered immune response rather than in a depression of the nonimmune clearance mechanisms. Svehag (12) using poliovirus in rabbits, and Uhr (10), using phage in guinea pigs, have presented evidence that a primary antibody response to a viral antigen may occur as early as 8–12 hr after polio-

virus infection and by 24 hr after exposure to bacteriophage. These results suggest that the antibody response is rapid enough to make a contribution to the host defense against a primary viral infection. The present study presents evidence that (*a*) the administration of exogenous antibody within 72 hr after infection is effective in decreasing the mortality of X-irradiated mice infected with EMC virus; (*b*) low levels of antibody present early in the infection can alter the pathogenesis of the EMC virus in irradiated mice as indicated by a lengthening of the incubation period and a reduction in mortality, thereby reversing the effects of X-irradiation on EMC virus infection in mice; and (*c*) low levels of serum antibody are associated with the clearance of large quantities of virus from the blood stream. It is proposed, therefore, that antibody produced early in the infection contributes to the host defenses by limiting the development of the viremia. Uhr (10) has shown that an enhanced rate of clearance of phage injected by the intravenous route occurs as early as 24 hr after the inoculation and that the clearance of the phage is associated with the appearance of serum antiphage antibody. The enhanced clearance of the phage and the appearance of antibody were eliminated by X-irradiation and this resulted in a prolongation of the viremia. Similarly, the clearance of EMC virus in the nonirradiated host was consistently associated with the appearance of specific neutralizing antibody. In contrast, mice which had received X-irradiation not only failed to clear their viremia, but also failed to develop detectable levels of neutralizing antibody. Taliaferro (13) has demonstrated that 350–650 R of whole body X-irradiation can delay and depress the antibody response to a variety of antigens. It is postulated that such a delay and depression in the immune response of the X-irradiated mouse to EMC virus results in a decreased capacity to neutralize and clear virus from the blood. An increased production of virus resulting from a facilitated spread of the infection in the absence of neutralizing antibody and, possibly, from altered local organ resistance, could also contribute to the establishment and maintenance of the persisting viremia. It is not possible on the basis of the data presented to delineate the relative contribution of the decreased neutralization or clearance and enhanced virus replication to the development of the persistent viremia and the higher levels of virus in the blood. It would seem likely, however, that both mechanisms are operating in the observed failure to clear the virus from the blood.

The persistence of virus in the blood of mice receiving X-irradiation would be expected to result in the seeding of the target organs with greater virus inoculum than the lower titered, transient viremia found in the nonirradiated controls. The higher titers of virus in the heart and brain evidence the greater target organ involvement of the X-irradiated mice in comparison to that of control mice. It is reasonable to postulate, therefore, that this greater target organ involvement in part reflects a larger viral challenge delivered during the course of the prolonged viremia and that the enhanced mortality of the X-

irradiated mice may be a result of the more extensive infection of critical target organs.

The circulating interferon response to EMC virus infection is similar for both the X-irradiated and nonirradiated mice. Evidence that circulating interferon plays a protective role in viral infection has been presented by Baron et al. (14). The data presented in the present study strongly suggest that alteration in interferon production is not responsible for the decreased resistance of the mice following exposure to X-irradiation. During the course of infection with EMC virus, interferon production is of relatively low magnitude with peak levels occurring on the 3rd day with a titer of 150 units/ml, whereas the interferon response of mice to an avirulent virus such as NDV peaks at 8 hr with levels as high as 30,000 units/ml. These data suggest that in infections in which the interferon response is rapid and of high magnitude, interferon may make a major contribution to the resistance of the host to that virus, but in infections in which the response is slower and of low magnitude, its contribution to resistance may not be as significant. It is proposed, therefore, that the present concepts of the relative role of interferon and the immune response to host resistance during the course of certain primary virus infections should be modified to consider the nature of the pathogenesis of the specific virus-host interaction.

SUMMARY

The resistance of mice to encephalomyocarditis (EMC) virus was markedly decreased by prior exposure to whole body X-irradiation. In contrast to nonirradiated controls, the course of EMC virus infection in X-irradiated animals was characterized by (*a*) an enhanced mortality, (*b*) shortening of the incubation period, (*c*) higher levels of virus in the blood during the viremic phase and persistence of the viremia until death, (*d*) failure to develop detectable serum levels of neutralizing antibody, and (*e*) the earlier appearance and higher levels of virus in brain and heart tissue. The level of interferon in the serum during the course of infection was similar in both groups. The administration of relatively small quantities of anti-EMC virus neutralizing antibody to X-irradiated mice during the early phases of the infection with EMC virus restored their resistance to levels comparable to nonirradiated animals. An alteration of local organ defense mechanisms in the central nervous system could not be demonstrated. It is proposed that (*a*) the inability of the X-irradiated animal to elaborate specific neutralizing antibody was a critical determinant in their failure to clear the viremia, (*b*) this increase in the level and duration of the viremic phase resulted in the exposure of target organs to a greater inoculum of virus, and (*c*) the enhanced mortality observed in irradiated mice reflected this greater target organ involvement. The experimental model presented, therefore, suggests that the immunologic response is a critical determinant of host resistance during this primary systemic virus infection.

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