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Susceptibility of Radiation Chimeras to Experimental *Pseudomonas aeruginosa* Infection. (30597)

JAMES R. WATSON* AND CAROLYN W. HAMMOND
Department of Microbiology, University of Illinois Medical Center, Chicago

It has been demonstrated that mice can be protected against lethal doses of X-irradiation if they are injected shortly after exposure with bone marrow cells of unirradiated mice which may be either syngeneic(1), allogeneic(2), or closely related xenogeneic (3). Such transplanted cells repopulate the hematopoetic tissues of the X-irradiated mice thus enabling them to survive(4). A number of studies have also been made of the immunologic responsiveness of radiation chimeras(5,6,7,8).

The purpose of this investigation was to determine the susceptibility of syngeneic and allogeneic chimeras to experimental infection.

Materials and methods. Mice. CF-1 and (C57Bl/6 × DBA/2)F_1 (hereafter designated B6D2F_1) female mice 9 to 10 weeks old were housed 10-12 animals per cage in an air conditioned, humidity controlled room with food and water available *ad libitum*. Water bottles were changed daily and cages weekly. All mice in each experiment came from the same shipment. *Irradiation.*† X-radiation factors were 400 kv; 5 ma; inherent filtration, 1.5 mm Al; added filtration, 0.4 mm Cu; target distance to midline of the body 95 cm for the CF-1 mice and 100 cm for the B6D2F_1 animals; dose rate was approximately 18 r per minute. The mice were placed in perforated plastic centrifuge tubes inserted between metal clips mounted on a turntable which was centered directly under the X-ray beam. *Bone marrow preparation.* Bone marrow cells were obtained from the femora and humeri of non-irradiated mice by aspiration into a 2 cc syringe having a #26 gauge needle which fitted into the marrow cavity. Aspirated marrow cells were then

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TABLE I. B6D2F$_1$ Mice Challenged with *Pseudomonas aeruginosa* 43-46 Days Post-Irradiation.

Inocula	Mortality			
	Chimeras†		Unirradiated controls	
		%		%
2.5×10^7	24/40*	60	18/40*	45
2×10^7	24/40	60	20/40	50
1.5×10^7	9/20	45	11/20	55
1×10^7	13/40	33	6/40	15
5×10^6	0/20	0	1/20	5

* No. dead/No. challenged.
† P = .5 when compared to unirradiated controls by the method of Chi Square.

suspended in tissue culture medium 199. Each donor mouse provided enough inoculum for intravenous injection of 2 irradiated recipients. *Challenge microorganism.* This was a highly streptomycin resistant strain of *Pseudomonas aeruginosa* which has been used by one of us(9) for many years as a challenge microorganism in irradiated mice. Virulence has been maintained by weekly passage through CF-1 mice. An 18-hour culture was washed off a streptomycin (1000 u/ml) agar plate in 5 ml saline, and any clumps of bacteria were dispersed by vigorous pipetting back and forth in a test tube. The suspension was made up to contain 10^9 bacteria per ml by means of a Coleman spectrophotometer and then diluted to provide the desired inocula. Actual numbers of bacteria inoculated were determined from triplicate platings of appropriate dilutions of the suspension used. All challenge inoculations were made by the intraperitoneal route. *Statistical analysis.* The difference in susceptibility to experimental Pseudomonad infection between chimera and control mice was determined by Chi-square analysis.

Results. Syngeneic chimeras. Preliminary studies established the LD_{100} dose of X-irradiation for B6D2F$_1$ mice to be 950 r. Two hundred B6D2F$_1$ mice were exposed to 950 r total body X-irradiation in groups of fifty. Four hours post-irradiation, they were injected intravenously with approximately 8×10^6 nucleated bone marrow cells suspended in 0.5 ml of tissue culture medium 199. In all experiments non-irradiated control animals received an equal volume of medium 199 without cells.

Forty-three to 46 days post-irradiation surviving chimeras in a given experiment were randomly distributed into 4 groups, each containing 10 mice. An equal number of unirradiated B6D2F$_1$ animals was included.

Chimera and control groups were injected intraperitoneally with 0.5 ml of graded inocula of *Pseudomonas aeruginosa*. Table I shows that the difference in susceptibility to experimental infection between chimeras and control animals is not statistically significant (P = 0.5).

Allogeneic chimeras challenged 43-46 days post-irradiation. The LD_{100} for CF-1 mice was previously determined as 925 r. CF-1 mice which had received 925 r whole-body X-irradiation were inoculated intravenously 24 hours after exposure with approximately 9×10^6 nucleated bone marrow cells suspended in 0.5 ml of tissue culture medium 199. Donors were CF-1 mice. Although the recipient and donor animals are members of the same strain, the resulting chimeras are considered allogeneic, for the genotypes of CF-1 mice are not identical. Control animals were injected intravenously with 0.5 ml tissue culture medium 199.

Each week 2 groups of 50 animals were irradiated and treated with allogeneic bone marrow. This procedure was repeated 4 times so that a total of 400 chimeras resulted. Approximately 43-46 days post-irradiation the surviving mice which had been irradiated and treated in a given week, were randomly divided into 4 lots of 12 mice each. Only those animals which were in excellent condition as indicated by gross appearance were used for *Pseudomonas* challenge. Four groups of 10 non-irradiated mice served as controls in each run. Five-tenths ml of graded inocula of *Pseudomonas aeruginosa* was injected intraperitoneally into both chimeras and control groups. Table II shows that radiation chimeras treated with allogeneic bone marrow cells 43-46 days before were definitely more susceptible to *Pseudomonas aeruginosa* than non-irradiated control animals (P = 0.01).

Allogeneic chimeras challenged 175-177 days post-irradiation. To establish the temporary or permanent nature of the greater susceptibility to experimental infection among

TABLE II. CF-1 Mice Challenged with *Pseudomonas aeruginosa* 43-46 Days Post-Irradiation.

Inocula	Mortality			
	Chimeras†		Unirradiated controls	
		%		%
2.5×10^8	12/12*	100	40/40*	100
5×10^7	30/36	83	19/40	48
2.5×10^7	31/36	86	15/40	38
5×10^6	14/48	29	1/40	3
2.5×10^6	2/36	6	—	—
5×10^5	0/12	0	—	—
2.5×10^5	0/12	0	—	—

* No. dead/No. challenged.
† P=.01 when compared to unirradiated controls by the method of Chi Square.

allogeneic chimeras, a study of the susceptibility of long term chimeras was undertaken. Three hundred sixty CF-1 mice were irradiated and injected with CF-1 bone marrow as previously described.

One hundred seventy-five to 177 days post-irradiation, the surviving chimeras were grouped and challenged with graded inocula of *Pseudomonas aeruginosa*. An equal number of control animals was challenged with the same numbers of bacteria.

Results of a statistical comparison of the overall mortalities of chimera and control mice shown in Table III indicate there was no significant difference (P = 0.15). Over twice as many chimeras died, however, following challenge with the lowest number of microorganisms (1.5×10^7) as did controls. Results of this challenge dose suggest the long time chimeras were somewhat more susceptible to experimental infection than control animals of the same age.

Discussion. Gengozian(5,6) and Makinodan(7) examined the immune status of syngeneic and allogeneic bone marrow chimeras by determining the titer of antibodies produced in response to foreign erythrocytic antigens. They found that irradiated mice injected with syngeneic marrow responded to the antigens in a normal manner when challenged 60 days after treatment, whereas allogeneic chimeras demonstrated a subnormal immune response when challenged 160 to 300 days post-irradiation. The results of experiments reported here on susceptibility to experimental infection in syngeneic and allogeneic chimeras are in agreement with those of Gengozian and Makinodan on the immunologic response. No increased susceptibility was found among mice receiving syngeneic bone marrow while a definite increase was observed 43-46 days post-irradiation among mice receiving allogeneic marrow and was present to only a slight degree 175-177 days post-irradiation. Physically, the mice appeared to be in excellent condition. They were active and their coats were smooth.

TABLE III. CF-1 Mice Challenged with *Pseudomonas aeruginosa* 175-177 Days Post-Irradiation.

Inocula	Mortality			
	Chimeras†		Unirradiated controls	
		%		%
1×10^8	32/32*	100	31/32*	97
3.5×10^7	22/24	92	21/24	88
1.5×10^7	20/32	63	8/32	25

* No. dead/No. challenged.
† P=.15 when compared to unirradiated controls by the method of Chi Square.

Summary. Lethally X-irradiated $B6D2F_1$ mice treated with syngeneic bone marrow cells were found to be no more susceptible to *Pseudomonas aeruginosa* challenge 43 days post-irradiation than unirradiated control animals. Allogeneic CF-1 chimeras proved to be substantially more susceptible to experimental *Pseudomonas aeruginosa* infection 43-46 days post-irradiation but at 175-177 days post-irradiation only a slight increase in susceptibility over control animals of the same age was observed.

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