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Allogeneic Bone Marrow Chimerism in Germ-free Mice. II. Prevention of Reticulum Cell Sarcomas in SJL/J Mice[1] (37837)

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Murphy described a unique mouse strain (SJL/J) in which reticulum cell sarcomas appeared spontaneously at average age 10 months (range 8–12 months) (1). The etiology of this complex neoplastic disease has not been identified, but it is likely that genetic factors play decisive roles in susceptibility to the disease. The appearance of the tumor was usually preceded by a decline in immunological competence, as observed in conventional and in germ-free (GF) subjects (2, 3). The tumors involved infiltrations of the lymphoreticular tissue (lymph nodes, spleens, Peyer's patches) and the visceral organs by a variety of cells, which included reticulum cells, lymphocytes, plasma cells, and giant cells—all interspersed with varying amounts of connective tissue (4, 5).

It has been the fate of conventional mice, in which allogeneic bone marrow chimeras have been established, to die of so-called graft-versus-host (GVH) disease (6, 7). This disease is characterized by a clinical syndrome which includes "wasting," diarrhea, and dermatitis. At autopsy, the lymphatic organs are small and depopulated of cells, and there is histological evidence of septicemia in organs such as liver, lung, kidney, and spleen. Germ-free mice with allogeneic bone marrow chimerism survived without the clinical manifestations listed above; they lived without disease symptoms for months, and they manifested immunological reactivities to antigens (8–10). At autopsy, the lymph nodes, spleens, Peyer's patches, and thymus glands were small but repopulated by lymphocytes.

There has been considerable interest in testing the efficacy of transplanted allogeneic bone marrow cells in animals with spontaneously developing neoplastic diseases, but most trials in conventional disease-free mice have failed because of fatal GVH disease (11, 12). In contrast, GF allogeneic chimeric mice do not develop fatal GVH disease (8). If bone marrow transplantation has any merit for the immunoprophylactic and therapeutic treatment of neoplasms, then trials with GF SJL/J mice should test it under optimal conditions. The procedure of bone marrow chimerism has prevented spontaneous leukemia in GF AKR mice with DBA/2 bone marrow cells (13). With this in mind, GF SJL/J mice were irradiated and inoculated intravenously with viable bone marrow cells from GF C3H mice. Under such conditions, reticulum cell sarcomas did not develop in the chimeric GF SJL/J mice, while conventional counterpart mice died of GVH disease.

Methods. Two GF strains of inbred mice were involved in this investigation: SJL/J (H^{-2S}) and C3H (H^{-2K}). The former was derived from a breeding nucleus provided by Dr. Ira H. Pilgrim, The University of Utah, and the C3H/He mice were obtained from the A. R. Schmidt Co., Madison, WI. They were propagated by brother–sister mating under GF conditions for over 30 generations. Mice of each strain were conventionalized by moving them from the GF isolator system to the clean quarters of the animal house where they were further propagated by

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brother–sister matings.

Five groups of 17, 18, 17, 20, and 15 GF male and female SJL/J mice, at 11 weeks of age, were irradiated (whole body) using a procedure that has been described elsewhere (8). The conditions for irradiation of both GF and conventional mice were as follows: The source was a 260-kVp therapy X-ray machine operated at 250 kV and 15 mA with a filtration of 1.0 mm Al and 0.25 mm Cu (HLV, 1.05 mm Cu). Mice were irradiated dorsoventrally with a skin–target distance of approximately 50 cm. The animals were placed in a circular polyethylene restrainer separated into 20-wedge-shaped compartments equidistant from the center. Exposure was made at noon in order to avoid possible diurnal variation in radiosensitivity. Dosimetry, measured in air, was determined in the restrainer under experimental conditions with a 250-R thimble ionization chamber read in a Model 570 Victoreen condenser R-meter. Conventional mice were given 850 rads and GF mice were given 1000 rads in single whole-body exposure at a rate of 39 rads/min. The two dose schedules exceed the LD_{100} for C3H/He strain mice.

Twenty-four hours later, adult donor GF C3H mice were killed by cervical dislocation. The femurs were excised, and their contents were expelled by repeated flushing through the amputated ends by syringe and needle with Medium 199 solution. The pool of C3H bone marrow cells were refrigerated on crushed ice until inoculated. Each irradiated SJL/J mouse was injected intravenously with 10^7 pooled viable bone marrow cells or the contents of two femurs from mature GF C3H mice.

The chimeric SJL/J mice were maintained in GF plastic isolators on Sani-cell bedding for periods up to age 15 months and examined weekly for microbial status (14). They were fed steam-sterilized Teklad diet and water ad lib. Individual mice were removed periodically for gross and histopathological examinations, and their spleen cells were examined by cytotoxicity tests to determine if they were of donor or host type (15). Each chimeric mouse was killed by ether anesthesia and exsanguinated from the orbital plexus. Representative specimens of all visceral organs and the lymphoreticular tissues were fixed in Bouin's solution, embedded in paraffin, and sections thereof were stained with hematoxylin and eosin.

Control groups of mice were (a) untreated, (b) given X-ray treatment only, and (c) conventional SJL/J mice with bone marrow cells from conventional C3H mice. Conditions of husbandry for the conventional mice were the same, except that they carried an undefined microbial flora from the environment.

Results. Fourteen GF SJL/J mice, subjected only to the X-irradiation procedure, died at average 8.7 days (range 7–10 days) with symptoms of "wasting," diarrhea, and pneumonia. At age 11 months, 90% of 86 untreated conventional and 136 GF SJL/J mice developed gross evidence of a mixed-type reticulum cell sarcoma (3). Evidence of disease was detected beyond age 8 months in the form of swollen lymph nodes and Peyer's patches and focal accumulations of abnormal cells in the visceral organs (livers, lungs, kidneys). In the early stages of disease, the large lymph nodes and Peyer's patches had prominent germinal zones, and as these expanded, they were no longer identifiable as such. The tissues were engorged irregularly with some or all of the following cell types: reticulum cells, lymphocytes, plasma cells, eosinophilic polymorphonuclear leukocytes, and multinucleated giant cells. As the mice aged, the lesions became more prominent, and the lymph nodes and Peyer's patches attained up to 10× normal size. In the swollen spleens, the white pulp had frequently displaced the red pulp with combinations of the cell types noted above. The organs appeared smaller in GF mice than in conventional mice. In GF mice, the cecums were greatly enlarged, thin walled, and the lamina propria were thin with few cells. These are characteristics of the axenic mouse.

Twenty irradiated conventional SJL/J mice, inoculated with bone marrow cells from adult conventional C3H mice, appeared healthy for several days, then showed clinical evidence of GVH disease. They developed a hunched-up, wasted, and emaciated ap-

pearance, scaly dermatitis and diarrhea, and they died soon thereafter at average age 21 days (range 7–49 days). Autopsy examinations revealed that the organ components of the lymphoreticular system were small: The lymph nodes, spleens, Peyer's patches, and thymus glands were depleted of cells, and the mice showed evidence of pneumonia and hepatitis. The lungs, livers, spleens, and some of the lymph nodes were infiltrated with polymorphonuclear leukocytes.

Of the 11 GF chimeric SJL/J mice in Group I which were subjected to complete necropsy examinations, all appeared symptom free between ages 10 and 15 months, at which time the experiment was terminated (Table I). The four that were found dead and degenerated earlier in the experimental period showed no gross evidence of tumors. The male mice were aggressive fighters and inflicted wounds on the backs of each other. All of the GF chimeric mice had cataracts of the optic lens and some loss of hair. The body weights were relatively low. The average white blood cell counts were within normal limits (average 5613/mm^3, range 1320–11,500), although the hematocrit levels were slightly lower than usual (average 37.6%, range 28–46%). None of them showed evidence of reticulum cell sarcomas. Their spleens were populated by C3H cells, as determined by cytotoxicity tests. The lymph nodes, Peyer's patches, spleens, and thymus glands were small and within normal size limits observed in GF mice of other disease-free strains. The thymus glands had clearly defined cortical zones with small lymphocytes and occasional pyknotic cells. The medullary areas of the thymus were distinct but less thickly populated than expected. The lymph nodes contained distinct cortical follicles of lymphocytes, free of germinal zones; the subcortical areas were somewhat less thickly populated with cells, and the medullary areas were free of plasma cells. The Peyer's patches consisted of small distinct follicles of small lymphocytes without germinal zones. The spleens contained defined primary follicles of small lymphocytes, without germinal zones; the red pulp was made up predominantly of scattered lymphocytes and very rare leukocytes and megakaryocytes.

Two mice had fluid in the chest cavity. In 8 of 11 mice, the kidneys showed occasional areas of fibrotic replacement of tubules, but the glomeruli appeared intact. This was in sharp contrast to the severe kidney lesions

TABLE I. Characteristics of Chimeric Germfree SJL/J Mice with C3H Bone Marrow Cells.

Number[a]	Age (months)	Sex	Weight (g)	WBC (mm^3)	Hematocrit (%)	Lesions[b]			
						Glomerulonephritis	Hydrothorax	Lymphocyte infiltration[c]	Fibrosis of pancreas
D-1	7.5					Degenerated—No gross lesions			
D-2	8.5					Degenerated—No gross lesions			
D-3	9	F				Degenerated—No gross lesions			
D-4	10	M				Degenerated—No gross lesions			
K-5	10	M	21.3	3,600	46	—	—	—	—
K-6	10	M	22.3	6,800	38	+	—	—	+
K-7	12	F	17	11,000	40	+	—	Liver	—
K-8	12	F	19.2	11,000	35	—	—	Liver	—
K-9	13	M	20.7	7,800	38	+	+	Lung	+
K-10	13	M	19.5	11,500	38	+	+	Liver	—
K-11	13	M	20	1,320	37	—	—	Liver	—
K-12	14	M	21.4	6,710	41	+	—	Lung	+
K-13	15	M	22.2	1,320	35	+	—	—	+
K-14	15	F	18.9	1,540	38	+	—	—	+
K-15	15	F	19	6,160	28	+	—	Lung	+

[a] Two were unaccounted for. D = found dead; K = killed.

[b] All mice developed cataracts of the optic lens. None had tumors.

[c] All lesions in lung and liver showed minimal infiltration by lymphocytes.

in chimeric AKR mice in which the fibrotic replacement of tubules was more extensive and many of their glomeruli were degenerated and hyalinized (13). In six of the chimeric mice, the exocrine areas of the pancreas glands were replaced by fibrotic tissue to varying extent. Small discrete perivascular aggregations of small lymphocytes were observed occasionally in the livers and lungs (Table I).

At present, four additional groups of 18, 17, 20, and 15 GF chimeric SJL/J mice are under observation at age 14, 14, 12, and 11 months, respectively. One mouse had an open hyperplastic wound in the lumbar region which was a localized fibrosarcomas. A second mouse developed a carcinoma in the perirectal area. None of the others have shown clinical evidence of disease. Eight of these were killed for examinations at age range 7–12 months, and they showed no evidence of reticulum cell sarcoma. Sixty mice remain under observation.

Discussion. Mice of several genetic strains develop neoplastic disease spontaneously, in high incidence, and under GF conditions: They include leukemia in AKR mice (16), reticulum cell sarcoma in SJL/J mice (1), and an immunoproliferative disease in Haas mice associated with persistent lymphocytic choriomeningitis (LCM) virus infection (17). The lesions have been prevented in the AKR and Haas strains by repeated administrations of immunosuppressive cyclophosphamide (18, 19). Leukemia has also been prevented by allogeneic chimerism in GF AKR mice (12), and this report describes the prevention of reticulum cell sarcoma in GF SJL/J mice by the same procedure. In both instances, conventional counterpart chimeric mice died of GVH disease. Explanations are speculative, but it appears that new regulatory mechanisms were introduced through the induction of chimerism—either through the damaging effects of X-irradiation, through new transplanted allogeneic cell lines which replaced those destroyed by the irradiation, or through incomplete restoration of specific cells in the transplanted inoculum. The latter may have resulted in some reduction in thymic function (9).

The visceral organs of normal GF mice (strains CFW, DBA/2, C3H, Swiss-Webster, C57 B1, ICR) show no accumulations of lymphoid cells in the perivascular and periductal areas of visceral organs. Also, their lymph nodes, spleens, and Peyer's patches are small and relatively inactive, and the serum globulin levels and white blood cell counts are relatively low. The thymus glands show the same pattern of age-related proliferation and involution as the conventional stock from which they had been derived. Changes from this uniform baseline pattern are decisive and readily detected histologically. The tissue changes in untreated GF SJL/J mice are extensive when compared with disease-free GF mice of other strains. The chimeric SJL/J mice resemble the latter, except for radiation-induced degenerative lesions in the optic lens, the kidneys, and the pancreases and the very small aggregations of lymphocytes in the perivascular areas of the liver and lung. These changes are not diagnostic of reticulum cell sarcoma.

An important control segment of this project is the effect of syngeneic bone marrow cells in lethally irradiated SJL/J mice. In the report on AKR mice (13), inoculation of syngeneic bone marrow cells did restore the leukemogenic process. Thus far, two groups of 19 and 18 irradiated SJL/J mice restored with SJL/J bone marrow cells have been observed for 5 months. No evidence of disease has been observed in them; however, we would not expect changes until they reach 8–12 months of age. Therefore, they remain under observation.

Despite the successful prevention of leukemia in AKR mice and reticulum cell sarcomas in SJL/J mice by bone marrow transplantation, two areas must be investigated before the procedure can be considered for the treatment of neoplastic diseases: Will bone marrow transplantations reverse the neoplastic process after its appearance? What is the role of the microbial flora in its success or failure? Answers to these questions will improve the prospects for application of data from the animal model to the treatment of cancer in man.

Summary. Germ-free and conventional

SJL/J mice develop reticulum cell sarcomas spontaneously, whereas C3H mice do not. Chimerism was established in lethally irradiated SJL/J mice by transplanted bone marrow cells from C3H mice. Conventional chimeric mice died of graft-versus-host disease, but germ-free chimeric mice survived indefinitely. At age 12 months, 95% of the germ-free SJL/J mice had reticulum cell sarcomas, while up to the age of 15 months, germ-free chimeric SJL/J mice had none.

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