Prevention and Treatment of Spontaneous Leukemia in Germfree AKR Mice (35817)

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Strain AKR mice are destined genetically to develop virus-induced spontaneous lymphatic leukemia (1). At average age of 8 months (range 3 to 13 months), about 95% of them develop with declining frequency, swollen thymuses, lymph nodes, spleens, and visceral organs—all infiltrated with immature lymphoid cells which may eventually appear in the blood. The leukemogenic Gross-type virus is transmitted congenitally to progeny which are asymptomatic until a short time before death, when they develop dyspnea, rough fur, and swollen lymph nodes (2). Strain AKR mice are thus considered experimental models of spontaneous lymphatic leukemia, and as such, have been used for studies on prevention and treatment of the disease (3, 4).

Since germfree (GF), or gnotobiotic mice do not develop bacterial complications following exposures to immunosuppressive agents, they can be given maximum subtoxic doses by which to determined specific therapeutic benefits (4, 5). An initial therapeutic trial with cyclophosphamide (CP) was implemented with GF mice which were infected congenitally with lymphocytic choriomeningitis (LCM) virus (6). In the course of 12 months, LCM mice have developed thymic depletion and then severe generalized immunoproliferative lesions, plasmacytomas, hypergammaglobulinemia, and complicating glomerulonephritis (7). The initial stages of infiltrating lesions could be detected at age 5 months by microscopic examination of organs. The development of lesions was prevented when young LCM mice were injected with CP over a period of 7 months; and established lesions were reversed in older LCM mice by weekly injections of CP. They remained viremic (6).

In an initial trial, young GF AKR mice were treated with 6 I.P. doses of CP, and another group with saline. Half of the CP-treated mice died at 27 weeks after onset of treatment; while among the saline-treated mice, half of them died at 8 weeks after onset of treatment (4). Leukemic GF AKR mice were administered 6 doses of CP at daily intervals, and the average survival was prolonged from 7 days in untreated mice to 47 days in 8 CP-treated mice. Four additional CP-treated mice survived 91, 112, 133, and 140 days.

It was of interest to ascertain the benefits of continuous weekly treatments of GF AKR mice with CP, to determine if spontaneous leukemia could be prevented in younger mice, and reversed in older mice, in a pattern similar to that in the LCM mice referred to above.

Methods. Cyclophosphamide (CP) (Cytoxan, Mead-Johnson Co., Evansville, Ind.) was sealed aseptically in glass ampoules which, with ampoules of saline, were sterilized by peracetic acid spray and passed into the sterile isolator in which the mice were held. The CP was dissolved quantitatively in saline just prior to intraperitoneal (ip) inoculation. Previous assays revealed that, at a weekly ip dose level of 2 mg of CP, most of the GF mice would survive indefinitely (3, 5); whereas conventional mice would die.

Germfree AKR mice were derived from conventional stock by cesarean section, and subsequently propagated as germfree colonies through 20 generations. They developed the same incidence (at av age 8 months, range 3–13 months) and manifestations of spontaneous leukemia as the conventional mice from which they had been derived (7). They were examined at weekly intervals

for bacterial flora, and all tests were negative. They carried leukemia virus and transmitted it prenatally to progeny. Aside from a unique wasting-like fatal disease in an occasional 3–4 months old GF mouse, they appeared healthy until symptoms of acute leukemia appeared in them (9). The symptoms of leukemia in GF mice were readily recognized: dyspnea, kyphosis, a barrel-like thorax, rough fur, disinclination to move, and occasional swollen palpable lymph nodes. When such mice were examined at autopsy, the thymus glands were swollen from 4 to 10×, and less frequently other tissues (spleen, lymph nodes, liver, and kidney) were swollen. The tissues were congested and infiltrated with large anaplastic lymphoid cells, with many mitotic figures among them.

Therapy. Thirty-two randomly selected GF AKR mice (Group I, 15; Group II, 17), at 5 months of age, received 2 mg, ip, of CP at weekly intervals over a period of 8 months. The mice of Group I were observed for 3 months thereafter, and then all survivors were killed and examined for gross and microscopic evidence of disease. The 17 mice of Group II were killed for examinations at 3 days after cessation of treatments. In an additional Group (III), 40 GF AKR mice were observed for symptoms of leukemia; and each was then inoculated ip with 5 mg of CP and thereafter 2 mg of CP at weekly intervals. Mice which became sick, in spite of treatments, were removed from the GF isolator and examined for gross and microscopic evidence of diseases.

Examinations. Mice were killed by ether inhalation, organs were fixed in Bouin's fluid, embedded in paraffin, and sections thereof were stained with hematoxylin and eosin. Organs examined were thymus, lung, spleen, lymph nodes, liver, pancreas, adrenal, kidney, and bone marrow.

Results. Thirty-two GF mice in Groups I and II were treated each week for 8 months with 2 mg of CP. They appeared healthy, except for some depilation. After cessation of CP treatments, 5 mice later developed leukemia on days 3, 65, 66, 75, and 77 (Fig. 1). The rest of them were killed at 80 days after cessation of treatment, and leukemia was noted in one mouse. Leukemia was not observed among any of the 17 mice of Group II when killed at 3 days after cessation of the 8 months of treatment. In the latter group of mice, the thymuses, spleens, and lymph nodes were small. The cortical areas of the thymus glands, and the primary follicles of the spleens and lymph nodes, were also depleted of small lymphocytes. Conventional symptom-free AKR died without evidence of leukemia within 8 weeks after onset of weekly ip injections of 2 mg of CP. They showed extensive weight loss, involution of lymphoreticular tissues and pneumonitis.

TABLE I. Effect of Cyclophosphamide Therapy on Spontaneous Acute Leukemia in Germfree AKR Mice.

Days after onset of treatment[a]	Condition of mice (no.)		
	Dead		
	Negative leukemia	Positive leukemia	Alive
10–20	1		
20–30		3	
30–40		3	
40–50		2	
50–60		3	
60–70		1	
70–80		1	3
80–90	1	3	
90–100	3	1	2
110–120	1	1	2
120–130		1	2
140–150			3
150–160		1	2

[a] Intraperitoneal inoculations of 5 mg of cyclophosphamide at first sign of illness and 2 mg at weekly intervals thereafter.

Among the 40 leukemic GF AKR mice which were given 5 mg of CP and then 2 mg of CP at weekly intervals, 20 died of leukemia at intervals ranging from 20 to 160 days after onset of symptoms (average survival period 66 days), 6 died without evidence of leukemia, and 14 now survive at periods ranging from 70 to 150 days (Table I). Untreated GF AKR mice died within 7 days after appearance of leukemic symptoms. In AKR mice which developed leukemia, in

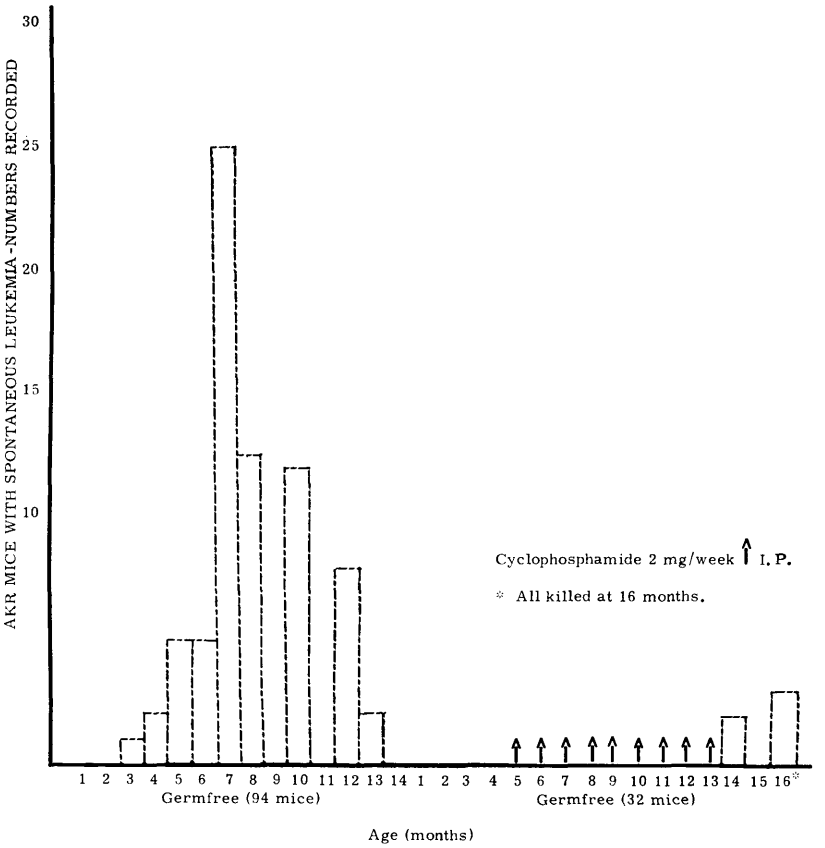

FIG. 1. The development of spontaneous leukemia in untreated germfree AKR mice, compared with mice which had been treated with cyclophosphamide from ages 5 to 13 months.

spite of CP therapy, the lesions resembled those of untreated AKR mice with spontaneous leukemia.

Discussion. The present studies indicate that spontaneous AKR leukemia was prevented, so long as the mice were given CP on a frequent schedule and maintained under GF conditions. If the mice had already developed leukemia, and then received repeated and prolonged treatments with CP, death could be deferred for significant periods, but eventually most of them would die of leukemia. It is likely that continuous treatment of asymptomatic preleukemic AKR mice with CP suppressed the development of a "critical mass" of leukemic cells which would have exceeded the cytotoxic capabilities of the drug. This may explain the limited therapeutic benefits of CP compared to the preventive benefits. Under these circumstances, leukemia is controlled and not cured because the dynamic disease process continues following cessation of treatment. CP therapy seems to be directed more at the lesion than at the causative virus, as has been shown in the immunoproliferative lesions of mice congenitally infected with LCM virus (6, 10).

The incidence of spontaneous leukemia has been altered in AKR mice by neonatal thymectomy (11), by daily administrations of preformed interferon (12), by antithymocyte serum (13), and by immunization procedures (14). Immunosuppressive therapy is effective in modifying the course of malignant diseases, but many of the treated subjects die of secondary infections (15). This emphasized the need for microbiological control as a prerequisite for adequate therapy. Human patients with leukemia have been subjected to immunosuppressive therapies in

a bacteria-controlled environment, such as the "life island" (16). Under such conditions, treated patients have experienced fewer episodes of bacterial infection, but they continue to suffer the effects of endogenous antibiotic-resistant bacteria, of fungi, and of viruses—which limit the efficacy of therapy. The therapeutic test system used in this study is ideal for neoplastic disease, since it provides test animals with predictable spontaneous leukemia under axenic conditions, in which secondary infections, and other factors in the environment do not complicate the interpretation of results.

Summary. Germfree AKR mice were inoculated with cyclophosphamide at weekly intervals for long periods. Leukemia was prevented for 13 months so long as treatment was continued, but the disease appeared after the treatment was stopped. Similar treatments of leukemic AKR mice deferred the fatal issue significantly. However, the leukemic process was suppressed and not cured. Germfree AKR mice offer distinct experimental advantages for studies on leukemia.

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