

Curative Effect of Split Low Dosage Total-Body Irradiation on Mice Infected with the Polycythemia-inducing Strain of the Friend Virus Complex¹

Rong-Nian Shen,² Ned B. Hornback, Li Lu, Peter Young, Zacharie Brahmi, and Hal E. Broxmeyer

Departments of Radiation Oncology [R-N. S., N. B. H.], Medicine [L. L., Z. B., H. E. B.], (Hematology/Oncology) [L. L., H. E. B.], Obstetrics/Gynecology [P. Y.], Microbiology/Immunology [Z. B., H. E. B.], and the Walther Medical Research Institute [H. E. B.], Indiana University School of Medicine, Indianapolis, Indiana 46223

ABSTRACT

Split low dose total-body irradiation (TBI) with 150 cGy was assessed for its efficacy in modifying the disease induced in DBA/2 mice by the polycythemia-inducing strain of the Friend virus complex (FVC-P, composed of a Friend murine leukemia helper virus and a spleen focus-forming virus). All FVC-P injected mice were dead within 40 days; however, infected mice receiving TBI on days 5 and 12 exhibited long-term survival. FVC-P-injected mice receiving TBI treatment on days 5 and 12 had normal leukocyte counts, normal spleen weights, and no detectable spleen focus-forming virus. Although the FVC-P-infected mice had decreased proportions of L3T4⁺ cells and increased proportions of Lyt-2⁺ cells, these were returned to normal following TBI treatment. Apparently the time sequence of TBI treatments is important since one treatment with TBI on day 5, or two treatments with TBI on days 12 and 18, was not as efficacious. The inability of *in vitro* irradiation doses of up to 1000 cGy to inactivate FVC-P which was subsequently injected into murine hosts suggests that the effectiveness of the TBI treatment *in vivo* is not due to a direct radiation effect on the virus. These results indicate a possible relationship between L3T4⁺ and Lyt-2⁺ numbers or their ratio in the curative efficacy of TBI in FVC-P-infected mice.

INTRODUCTION

The treatment of leukemia continues to be a clinical challenge. Intensive chemotherapy and radiotherapy induce myelosuppression or immunosuppression, or both, which requires substantial supportive care, and some patients die as a result of bone marrow failure or infection resulting from the combined effects of immunological incompetence and chronic neutropenia. Thus, it is important to develop efficient but less toxic methods for treating leukemia.

Animal models are useful for establishing the potential efficacy of various treatment protocols. The pathophysiology of murine retrovirus-induced leukemias is well characterized in terms of hematological parameters as well as cellular and molecular biology (1). Abnormalities in biomolecule-cell interactions, similar to those detected in human leukemia, have been noted in mice infected with a murine retrovirus, FVC-P (2).³ Lactoferrin protected mice from the hematological disease associated with FVC-P but only when the lactoferrin was administered simultaneously or shortly after the inoculation of FVC-P into the mice (3, 4). Since human cancer patients do not usually seek treatment until the disease is well established, we wished to identify a treatment that would be effective at a later stage of infection. We chose to study radiation therapy. In

general, depletion of suppressor T-lymphocytes in tumor-bearing hosts is associated with prolonged survival (5). The generator of T-lymphocyte cells or T-lymphocyte cells themselves have been found to be more sensitive to irradiation than other lymphoid cells (6-9). Based on this information, we determined the efficacy of split low dosage TBI on the progress of the hematological disorder induced in DBA/2 mice by FVC-P.

MATERIALS AND METHODS

Mice. Seven- to 8-wk-old female DBA/2 mice (Cumberland Laboratories, Clinton, TN) were used throughout these experiments.

Friend Virus Complex. The FVC-P contains a replication-helper Friend murine leukemia virus (clone 201) and a replication-defective SFFV. The stock virus contained 10⁵ SFFU/ml and 10⁶ plaque-forming units/ml (2). FVC-P was diluted to a 1:10 ratio in sterile pyrogen-free saline, and 0.5 ml of this medium was injected *i.v.* into each mouse.

Spleen Focus-forming Units. SFFV were assayed by the SFFU assay (3). At autopsy the spleen from each primary mouse was weighed, homogenized, and diluted into four concentrations. Each spleen extract dilution was then injected into normal secondary mice. Nine days later, the mice were sacrificed and the spleens were removed, weighed, and fixed in Bouin's solution for the SFFU assay.

Source of T-Lymphocyte. Spleen single-cell suspensions were prepared by mincing the spleens individually in RPMI and filtering the crude suspension through a fine steel screen mesh.

Monoclonal Antibodies. Anti-L3T4 (which recognizes T-helper cells) and anti-Lyt-2 (which recognizes T-suppressor cells) were purchased from Becton-Dickinson (Mountain View, CA).

Analysis of T-Lymphocyte Subsets. Mouse spleen cells were analyzed by indirect immunofluorescence, using a FACS IV flow cytometry system (Becton-Dickinson, Sunnyvale, CA). Incubation of 1 million cells with monoclonal antibodies Anti-L3T4 (4 μ l phycoerythrin conjugate) or Anti-Lyt-2 (1 μ g fluorescein conjugate) or mouse IgG 2a+b, (5 μ l fluorescein conjugate as the background; Becton-Dickinson, Mountain View, CA) took place for 30 min at 4°C. After washing 3 times, cells were diluted to 1.0 ml with phosphate-buffered saline for FACS analysis. The cells were kept at 4°C until they were analyzed.

TBI. Mice were placed in a plexiglass box. A dose of 150 cGy TBI was delivered from a Siemens 250 kVp X-ray therapy machine, filtered with 1.0 mm Cu, giving a half-value layer of 2.1 mm Cu at an SSD of 50 cm, and with a dose rate of 78.13 cGy/min. The sham-treated mice were placed in the plexiglass box for the same length of time as the treated mice, but they were not irradiated.

Statistical Method. The results are reported as the mean \pm SE. A two-tailed Student's *t* test was used to determine whether two sample means were significantly different.

RESULTS

Survival and Virus Analysis. In order to determine the effects of irradiation on the survival of mice infected with FVC-P, mice were irradiated with 150 cGy either on day 5, days 5 and 12, or days 12 and 18, and their survival was compared with sham TBI-treated mice (Fig. 1). The survival of TBI-treated mice was extended when they were irradiated on day 5 or on days 12 and 18, with 100% survival noted when they were irradiated on days 5 and 12. In our first study, all 35 mice receiving sham TBI treatment were dead 40 days after virus

Received 10/23/87; revised 1/18/88; accepted 1/27/88.

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¹ Supported by Public Health Service Grants CA 36464 and CA 36740 (H. E. B.) from the National Cancer Institute, grants from the Little Red Door, Marion County Cancer Society, Inc. (R-N. S. and L. L.), and a research grant from Phi Beta Psi sorority (L. L.).

² To whom requests for reprints should be addressed, at Department of Radiation Oncology, Indiana University School of Medicine, Radiation Therapy Building, Room 053, 535 Barnhill Drive, Indianapolis, IN 46223.

³ The abbreviations used are: FVC-P, polycythemia-inducing strain of the Friend virus complex; TBI, total-body irradiation; SFFU, spleen focus-forming units; SFFV, spleen focus-forming virus; MHC, major histocompatibility.

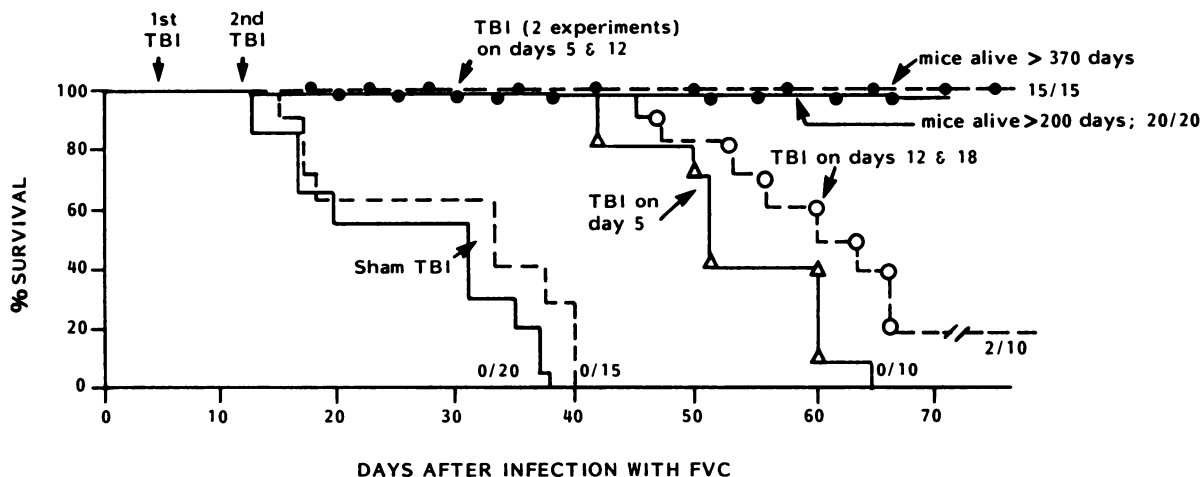


Fig. 1. Comparative survival curves of DBA/2 mice injected i.v. with FVC-P. Mice were either treated with TBI on day 5 only (Δ , 1 experiment), on days 12 and 18 (\circ , 1 experiment), or on days 5 and 12 (\bullet , first experiment with 15 of 15 mice still alive after 370 days; \bullet , second experiment with 20 of 20 mice still alive after 200 days). Control mice were sham treated ($-$, first experiment; $-$, second experiment). The ratio of the number of mice surviving to the total number of mice in each group is given next to each curve.

administration; however, 15 or 15 mice treated with TBI on days 5 and 12 were still alive when the experiment was terminated past 370 days after virus infection. Similar results were obtained in a second study in which 20 of 20 mice were still alive when that experiment was ended past 200 days after virus infection. Autopsies of the surviving mice which had received TBI on days 5 and 12 demonstrated normal spleen size while all of the mice receiving sham TBI or TBI on day 5 or days 12 and 18 had enlarged spleens.

An analysis of SFFV in the spleens of mice receiving FVC-P (Table 1, A) was consistent with the survival studies shown in Fig. 1. No SFFU, a measure of SFFV, were found in spleens of FVC-P-infected mice treated with one dose of TBI on day 5 and sacrificed on day 12, or treated with TBI on days 5 and 12 and sacrificed on day 26. This was in comparison to the high titers of SFFV noted in the spleens of FVC-P-infected mice that were sham treated. The spleen weights of secondary assay mice (Table 1, B) mimicked the relative numbers of SFFU in these mice.

That these doses of irradiation did not directly influence the

virus preparations themselves is suggested by the survival curves shown in Fig. 2. FVC-P was irradiated *in vitro* with 150, 300, or 1000 cGy prior to administration of these preparations into the mice. All mice receiving these preparations were dead by day 38 with autopsies noting enlarged spleens, in comparison to mice that received untreated FVC-P but were irradiated on days 5 and 12 and are still alive at greater than 60 days post-viral inoculation.

Hematological Analysis. The nucleated leukocyte counts (Fig. 3A) and spleen weights (Fig. 3B) of FVC-P-infected mice revealed that the increases associated with this infection were not apparent in mice receiving TBI on days 5 and 12. The counts in these latter mice were similar to those of mice not inoculated with FVC-P, whether or not they were irradiated.

For analysis of L3T4⁺ and Lyt-2⁺ spleen lymphocyte subsets, mice were divided into 4 groups: (a) uninfected untreated control mice, (b) mice not given FVC-P but exposed to 150 cGy TBI on days 5 and 12 from the start of the experiment, (c) untreated FVC-P-infected mice, and (d) FVC-P-infected mice exposed to 150 cGy TBI on days 5 and 12. Mice were sacrificed on days 8, 12, 19, and 26 after infection with FVC-P, and days

Table 1 Effect of TBI on spleen focus-forming viruses in DBA/2 mice infected with FVC-P

DBA/2 mice were infected i.v. with the FVC on day 0. The mice infected with FVC were treated first with TBI on day 5 and then were sacrificed on day 12 (postirradiation 1 wk). The other mice received the second TBI on day 12 and were then sacrificed on day 26 (after first irradiation 3 wk). Results are the averages of 3 primary mice per group. The spleen extract from each dilution of each primary mouse was assessed in 3 secondary mice.

Dilution of extract	SFFU and spleen weights in 2 DBA/2 mice per dilution of spleen extract			
	FVC + sham TBI (days 0-12)	FVC + first TBI (days 0-12)	FVC + sham TBI (days 0-26)	FVC + second TBI (days 0-26)
A) SFFU				
10 ⁻¹	TNTC ^a	0 ^b	TNTC	0 ^b
10 ⁻²	TNTC	0 ^b	TNTC	0 ^b
10 ⁻³	43.0 ± 5	0 ^b	TNTC	0 ^b
10 ⁻⁴	17.1 ± 2	0 ^b	42.0 ± 7	0 ^b
B) Spleen weight (GMS)				
10 ⁻¹	0.42 ± 0.02	0.11 ± 0.01	0.82 ± 0.1	0.09 ± 0.02 ^b
10 ⁻²	0.20 ± 0.02	0.10 ± 0.01	0.34 ± 0.06	0.08 ± 0.02 ^b
10 ⁻³	0.13 ± 0.01	0.10 ± 0.03	0.17 ± 0.02	0.07 ± 0.01 ^b
10 ⁻⁴	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.03	0.07 ± 0.01

^a TNTC, numbers of SFFU were too numerous to count; GMS, —.

^b Significant difference (P, at least <0.001) from mice who did not receive TBI when using same dilution of spleen extract.

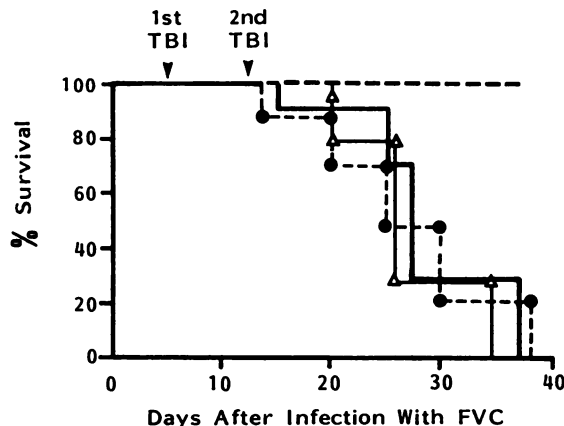


Fig. 2. Comparative survival curves of DBA/2 mice receiving i.v. injections of either nonirradiated FVC-P or FVC-P that were previously irradiated *in vitro* with 150 cGy ($-$), 300 cGy (\bullet), or 1000 cGy (Δ). These mice were not irradiated before or after virus administration. As a comparison, nonirradiated FVC-P was given to mice receiving 150 cGy TBI treatment on days 5 and 12 after virus inoculation ($-$). These latter mice are still all alive more than 60 days after virus inoculation. Each group is composed of 10 mice.

Fig. 3. Comparison of white blood cell counts (A) and spleen weight (B) of uninfected untreated mice, untreated FVC-P-infected mice, FVC-P-infected mice receiving TBI treatment on days 5 and 12, and uninfected mice receiving TBI treatment on days 5 and 12.

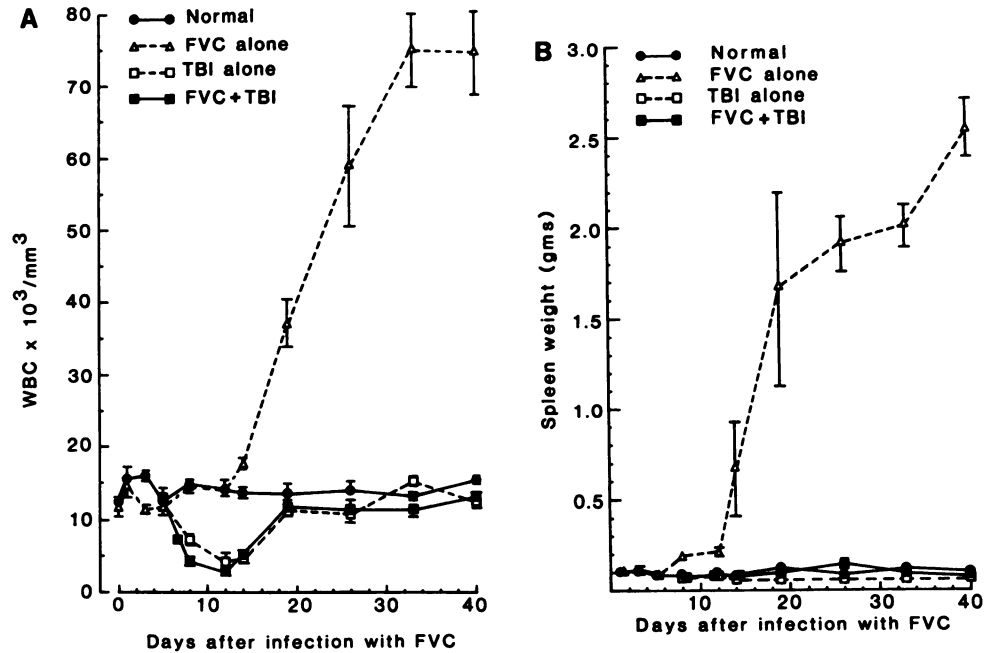


Table 2 Effect of 150 cGy TBI on T-lymphocyte subsets in DBA/2 mice with and without infection of FVC-P

Group	Treatment	Lymphocyte subset	Day (% positive)						
			1	3	5	8	12	19	26
1	None	L3T4 ⁺	24.2 ± 0.4 ^a	25.8 ± 1.3	27.3 ± 0.8	24.4 ± 2.3	26.0 ± 2.8	24.7 ± 2.6	24.6 ± 2.7
		Lyt-2 ⁺	13.8 ± 0.2	12.6 ± 0.1	13.8 ± 0.1	15.6 ± 0.6	15.7 ± 0.2	12.5 ± 1.4	11.9 ± 2.4
		L3T4 ⁺ /Lyt-2 ⁺	1.8 ± 0.01	2.1 ± 0.1	1.9 ± 0.04	1.6 ± 0.2	1.6 ± 0.2	2.0 ± 0.3	2.2 ± 0.5
2	TBI alone	L3T4 ⁺				29.2 ± 0.5	31.8 ± 0.7	29.5 ± 2.0	23.0 ± 0.6
		Lyt-2 ⁺				13.4 ± 0.2	13.0 ± 0.3	8.4 ± 1.6	6.9 ± 0.3
		L3T4 ⁺ /Lyt-2 ⁺				2.2 ± 0.03	2.4 ± 0.003	3.7 ± 0.6	3.3 ± 0.1
3	FVC-P infection alone	L3T4 ⁺	23.8 ± 1.1	23.5 ± 0.8	26.3 ± 0.9	25.2 ± 2.3	29.7 ± 1.1	24.2 ± 2.3	15.0 ± 0.4
		Lyt-2 ⁺	13.1 ± 0.2	12.5 ± 0.5	14.3 ± 0.2	15.6 ± 0.3	16.4 ± 0.4	45.7 ± 4.3	30.0 ± 4.9
		L3T4 ⁺ /Lyt-2 ⁺	1.8 ± 0.07	1.9 ± 0.04	1.8 ± 0.08	1.6 ± 0.02	1.8 ± 0.04	0.6 ± 0.09	0.5 ± 0.07
4	FVC-P infection + TBI	L3T4 ⁺				31.1 ± 0.4	36.3 ± 1.2 ^b	35.0 ± 0.7 ^b	36.9 ± 0.9 ^b
		Lyt-2 ⁺				13.6 ± 0.8	14.5 ± 0.2 ^b	14.5 ± 1.2 ^b	10.9 ± 0.3 ^b
		L3T4 ⁺ /Lyt-2 ⁺				2.3 ± 0.1 ^b	2.5 ± 0.1 ^b	2.4 ± 0.02 ^b	3.4 ± 0.02 ^b

^a Each data point is expressed as the mean ± SE for 3 mice assayed individually. TBI was administered on days 5 and 12 after injection of FVC-P mice or after start of experiment in noninoculated mice.

^b Significant difference (*P* at least <0.05) from mice who did not receive TBI when injected with same lethal doses of FVC-P.

3, 7, 14, and 21 after the first exposure to TBI. Mice in groups 1 and 2 were also sacrificed on days 1, 3, and 5 (Table 2). FVC-P-infected mice manifested a decrease in the percentage of L3T4⁺ cells and an increase in the percentage of Lyt-2⁺ cells, resulting in a decreased L3T4⁺/Lyt-2⁺ ratio. FVC-P-infected mice receiving TBI treatment on days 5 and 12 demonstrated an increased percentage of L3T4⁺ cells and levels of Lyt-2⁺ cells similar to those from untreated mice as well as a L3T4⁺/Lyt-2⁺ ratio equal to or greater than that in untreated mice, and equal to that of uninfected mice receiving TBI treatment. When the density expression of the L3T4⁺ antigen (Fig. 4A) and the Lyt-2⁺ antigen (Fig. 4B) were assessed, it became apparent that not only were the percentage of L3T4⁺ cells decreased and the percentage of Lyt-2⁺ cells increased after viral inoculation (Table 2), but the density expression of L3T4 was decreased (Fig. 4A) and the density expression of Lyt-2 (Fig. 4B) was increased. The density expression of L3T4 and Lyt-2 on cells from infected mice treated with TBI was similar to that of untreated uninfected mice and of uninfected mice treated with TBI.

DISCUSSION

In addition to our studies measuring the effectiveness of lactoferrin (2-4) to modify the hematological disease induced by the Friend virus, other forms of therapy are used including viral and nonviral inducers of interferon, interferon itself, and combinations of chemo- and immunotherapy (10-17). Supralethal irradiation (900-950 cGy) did not cure the mice infected with Friend virus (18, 19). North has demonstrated that sublethal doses (400-500 cGy) of TBI can cure or suppress some solid tumors, because X-irradiation preferentially eliminates suppressor T-cells. It was pointed out that sublethal irradiation fails to result routinely in tumor regression because it also destroys effector T-cells (20, 21). To these studies are now added the effects of split low dose irradiation which, when given at days 5 and 12 after viral inoculation with FVC-P, results in 100% long-term survival of the virus-infected mice. The effectiveness of the split dose treatment with TBI was intimately dependent on the timing of the irradiation, since doses given only on day 5 or on days 12 and 18 were not nearly as effective

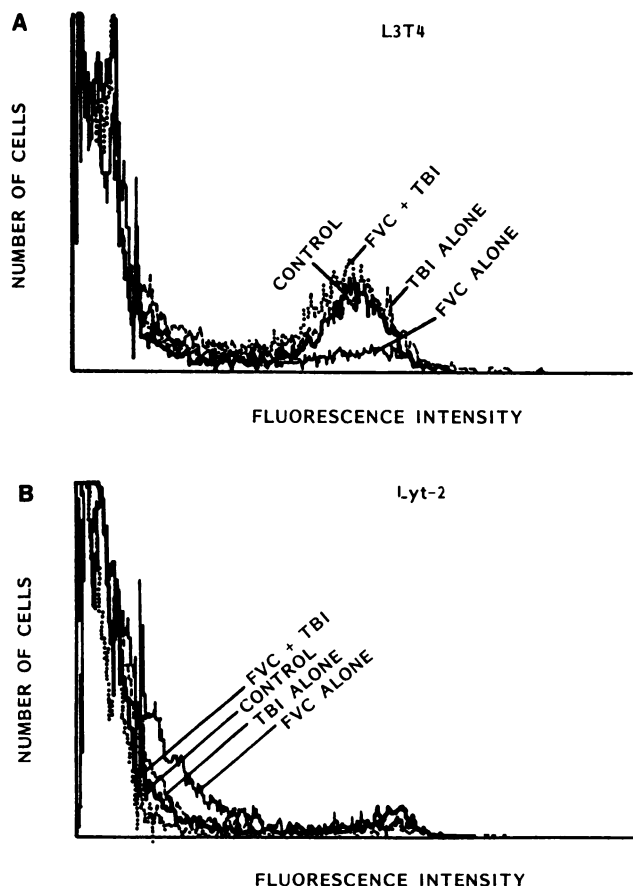


Fig. 4. Profile of L3T4 (A) and Lyt-2 (B) antigen expression on spleen cells of mice. The profiles shown represent reproducible results from the spleens of 1 of 3 mice in each of the following groups: uninfected untreated mice, untreated FVC-P-infected mice, uninfected mice treated with TBI, and FVC-P-infected mice receiving TBI treatment. Cells used were the same as those described in the 26-day point of Table 2.

as those given on days 5 and 12 in prolonging the life span of the FVC-P-injected mice.

The mechanism underlying the efficacy of split low dose TBI in curing the hematopoietic disease induced by the FVC-P is not known. However, our results demonstrated that irradiation of the virus *in vitro* does not change the lethal infectivity of the virus when subsequently administered *in vivo*. This suggests that the irradiation is not directly inactivating the virus or its replication, but that it may be mediating its effect through cells in the mice. In this context, it has been shown by others that the fatal disease induced by the Friend virus is accompanied by a profound immunosuppression which affects T-cell, B-cell, and macrophage function (22–24). In our study, mice infected with FVC-P had an increased proportion of spleen cells with the Lyt-2⁺ phenotype as well as an increased density distribution of Lyt-2 antigens on these cells. Additionally, there was a decreased proportion of spleen cells with the L3T4⁺ phenotype and a decreased density distribution of the L3T4 antigen on these cells. Others have demonstrated that depletion of T_H lymphocyte cells in tumor-bearing hosts is associated with prolonged survival. Levels of Lyt-2⁺ cells in FVC-P-infected mice receiving TBI were equal to those of mice not given the FVC-P. Also, the mice that had received curative doses of TBI on days 5 and 12 demonstrated levels of L3T4⁺ cells that were above those of mice not given the FVC-P but equal to those of mice not given virus but treated with TBI. Moreover, FVC-P-infected mice receiving TBI demonstrated density distributions of antigens similar to those of untreated control mice that did

not receive the virus. L3T4 is considered to be the murine analogue of the human Leu-3/T4 (CD4) molecule and expression of this antigen correlates primarily with MHC class II antigen reactivity (25). Lyt-2 appears to be similar to the human Leu-2/T8 (CD8) molecule and expression of this antigen correlates mainly with MHC class I antigen reactivity (26–28). It is intriguing to speculate that the curative effects of TBI were mediated through L3T4⁺ or Lyt-2⁺ cells or their combination or were based on the ratio of these cell types and that the effects noted may relate to L3T4 or Lyt-2 antigen expression in the context of MHC class I or II antigen reactivity. However, our experiments, while establishing an association between T-cells and virus replication *in vivo*, do not allow us to establish that there was a cause-effect relationship. It would be of interest to see if this type of treatment, which was well tolerated by the mice, would be beneficial in other types of virally induced disease.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Mary Eaton for reviewing the manuscript and Patti Kivett for her secretarial assistance.

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