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Studies on Target Cells of Friend Spleen Focus-forming Virus in Mice[1] (38007)

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Susceptibility of mice to infection by Friend spleen focus-forming virus (SFFV) depends on both genetic (1–4) and environmental factors (5), some of which act by controlling the availability of target cells (3, 5–7); another acts by altering the microenvironment (8). Results from recent studies suggest that certain hemopoietic cells might be involved in viral replication and cell transformation (3, 5, 6, 9). However, it is still uncertain which of the hemopoietic cells and how many types of these cells are involved in these processes. In addition, it is still not known whether all SFFV-infected cells that produce new infectious viruses are also transformed to multiply and become leukemic cells.

The purpose of this study is to investigate the nature of the target cells of SFFV for virus replication and cell transformation. Growth of SFFV, SFFV-induced tumor cells, and certain hemopoietic cells was studied in mice that had received different treatments to alter their population of hemopoietic cells.

Materials and Methods. Mice. Inbred C3H/HeJ, C57BL/6J × C3H/HeJ (B6C-3F_1) and its reciprocal hybrid, and random-bred Swiss mice were bred and maintained at the Faculty of Science, Mahidol University. All mice used were 5–10 weeks old.

Mice were irradiated supralethally (900 rads) by a ^{60}Co (Siemens) unit. The average exposure rate of the ^{60}Co unit was 185 rads/min. Dosimetry was made with a 200-rad Victoreen Chamber.

In two experiments, groups of C3H/HeJ mice were given 60 mg of myleran (Burroughs Wellome, London) per kg of body weight by a stomach tube (10). The drug was dissolved in acetone and then diluted with corn oil (Mazola) prior to administration. At intervals after myleran treatment, the treated and control mice were injected with 1,000–5,000 FFU.

Virus. Mirand's strain of Friend spleen focus-forming virus (SFFV) (11) was passed in C3H/HeJ mice for at least 5 cell-free passages. The virus was quantitated by the spleen focus assay method (12) in groups of 6–8 Swiss mice. The titers of the virus were expressed in terms of focus-forming units (FFU).

SFFV-induced tumor cell assays. Spleen cell suspensions were prepared from SFFV-infected C3H/HeJ mice and tumor cells were enumerated by the tumor colony assay method (13) in groups of 6–8 B6C3F_1 and its reciprocal hybrid. A linear relationship was observed between the number of cells injected and the number of tumor colonies produced in the spleens of these hybrid mice (13). The concentration of SFFV-induced tumor cells was expressed in terms of tumor colony-forming units (TCFU).

Normal hemopoietic colony-forming cell assays. Normal hemopoietic colony-forming cells from C3H/HeJ mice were assayed in 12 heavily irradiated C3H/HeJ mice by the spleen colony assay method (15). The con-

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centration of hemopoietic colony-forming cells was expressed in terms of normal colony-forming units (NCFU).

Results. (1) *Supralethal irradiation.* Mice (C3H/HeJ) were supralethally irradiated and divided into two groups. Mice of the first group were infused with 10^7 syngeneic bone marrow cells while mice of the second group were infused with 10^7 syngeneic bone marrow cells and 7,000 FFU and SFFV (bone marrow cells and virus were incubated at 37° for 30 min prior to injection (16)). At intervals after irradiation, normal colony-forming cells, SFFV, and SFFV-induced tumor cells were recovered from the spleens of these mice. More virus and tumor cells were recovered from the spleens of infected mice containing more normal colony-forming cells (Fig. 1). SFFV reached its plateau at Day 7 while SFFV-induced tumor cells increased logarithmically up to Day 11. Normal colony-forming cells increased at a slower rate than tumor colony-forming cells.

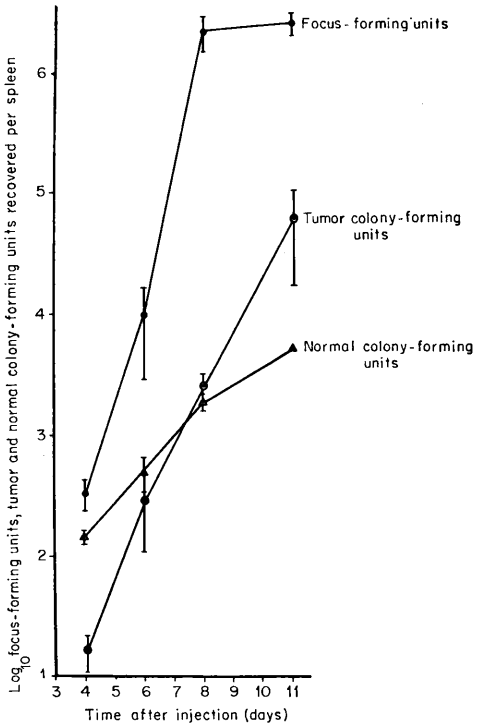

FIG. 1. Growth of SFFV, SFFV-induced tumor cells, and normal colony-forming cells from the spleens of heavily irradiated mice (900 rads) at intervals after the injection of 10^7 bone marrow cells and 7,000 FFU of SFFV. Mean and standard error of the FFU, TCFU, and NCFU recovered per spleen at each interval are shown.

(2) *Myleran.* At intervals after myleran treatment, groups of treated C3H/HeJ and control mice were injected with 1,000–5,000 FFU or SFFV. Six days after the virus injection, SFFV and SFFV-induced tumor cells were recovered from these mice. Normal colony-forming cells were recovered from the spleens of myleran-treated and control mice in a separate experiment conducted in an identical fashion.

Mice treated with myleran 4 and 8 days prior to SFFV infection had a great reduction (500–2,000-fold) in the recoverable SFFV and SFFV-induced tumor cells (Table I). Mice treated with myleran at 12 and 15 days before SFFV infection did not show any reduction in the amounts of SFFV while SFFV-induced tumor cells were decreased about tenfold. Myleran treatment at any of these times before SFFV infection abolished the presence of the normal colony-forming cells.

Discussion. Destruction of hemopoietic cells by irradiation or by myleran (10, 15, 17) resulted in a great reduction in the susceptibility of mice to SFFV infection (Fig. 1 and Table I). Such treated mice became more susceptible to SFFV when more hemopoietic cells were available by an infusion of bone marrow cells. Normal colony-forming cells as well as other hemopoietic cells were stimulated to proliferate in these mice (15). Thus, a change in the susceptibility to SFFV in the treated mice was probably due to an alteration in the number of target cells for SFFV.

Growth kinetics of SFFV, SFFV-induced tumor cells, and normal colony-forming cells were compared (Fig. 1 and Table I). A fixed period of time had elapsed between the time of infection and that of harvesting SFFV and SFFV-induced tumor cells from the spleens of the treated and control mice. Thus, the increase in the virus and tumor cells should be directly proportional to the increase in the target cells.

The role of normal colony-forming cells

TABLE I. Effects of Myleran on Normal Colony-Forming Cells and Target Cells of SFFV.

Days after myleran treatment	Recovery from the spleens of myleran-treated and control mice		
	FFU/spleen ± SE[a]	TCFU/spleen ± SE[b]	NCFU/spleen ± SE[c]
no treatment	$1.9 \times 10^4 \pm 7.9 \times 10^3$	$6.5 \times 10^3 \pm 1.6 \times 10^3$	$0.4 \times 10^3 \pm 3.9 \times 10^2$
4	60 ± 46	<10	<10
8	40 ± 40	<10	<10
no treatment	$7.2 \times 10^4 \pm 1.9 \times 10^3$	$7.7 \times 10^3 \pm 1.7 \times 10^3$	$2.9 \times 10^3 \pm 4.0 \times 10^2$
12	$8.4 \times 10^4 \pm 3.3 \times 10^4$	$9.3 \times 10^2 \pm 2.8 \times 10^2$	<10
15	$1.2 \times 10^5 \pm 4.6 \times 10^4$	$7.6 \times 10^2 \pm 2.0 \times 10^2$	<10

[a,b] SFFV (1,000–5,000 FFU) was inoculated into groups of C3H/HeJ mice previously treated with myleran at various intervals. Six days after SFFV injection, the spleens of these mice were assayed for SFFV and for SFFV-induced tumor cells.

[c] At intervals after myleran treatment, the treated and control C3H/HeJ mice were assayed for normal colony-forming cells.

as the target cells was implicated in some of the earlier studies (3, 6) but not from this study. Although more virus and tumor cells were recovered from the spleens of mice containing more colony-forming cells, the growth rate of SFFV and SFFV-induced tumor cells was somewhat different from the normal colony-forming cells (Fig. 1). More importantly, at 12 and 15 days after myleran treatment, normal colony-forming cells were undetectable while appreciable amounts of both SFFV and SFFV-induced tumor cells were obtained. Thus, it is *not* likely that normal colony-forming cells were the target cells. Bone marrow myelocytes and blood neutrophils were probably *not* the target cells either; high levels of these cells (10) were found in myleran-treated mice at the period when a low level of target cells was observed (Table I).

Which cells, then, are likely to be the target cells for SFFV? At 4 days after myleran treatment, iron-incorporating cells, i.e., pronormoblasts, normoblasts, and blood reticulocytes (10) and Srivatanakul, unpublished results) as well as target cells for SFFV, decrease to a level below normal. At 12 and 15 days after the treatment, a significant number of these cells appeared while high levels of both SFFV and SFFV-induced tumor cells reappeared (Table I). Thus, cells in the erythrocytic pathway, probably erythropoietin-sensitive cells (5, 9), might be involved in SFFV replication and SFFV-induced tumor cells.

The results from this study support the view that there are at least two and possibly three types of target cells: a cell that is infected by SFFV and supports its replication and a cell that is transformed by SFFV into a tumor colony-forming cell, and/or a cell that is transformed by SFFV into a tumor colony-forming cell which is capable of supporting virus growth as well. The amounts of SFFV recovered from myleran-treated and control mice 12 and 15 days later were the *same,* while SFFV-induced tumor cells in myleran-treated mice were about 10 times lower than that of the control mice (Table I). Whether *all* the cells capable of being transformed by SFFV can also support virus replication was not elucidated by the present study.

Summary. Growth kinetics of certain hemopoietic cells and target cells of Friend spleen focus-forming virus (SFFV) were studied in mice treated with supralethal irradiation (900 rads) and infusion of bone marrow cells, or with myleran. In supralethally irradiated mice, the presence of SFFV and SFFV-induced tumor cells corresponded with that of normal colony-forming cells but with somewhat different growth rates. In myleran-treated mice, however, SFFV and SFFV-induced tumor cells were present when normal colony-forming cells were not observed. Thus, the results indicate that normal colony-forming cells were probably not the target cells for SFFV. The results are consistent with the view that

at least two or possibly three types of target cells were present; the target cells for SFFV replication might be different from those for SFFV-induced tumor cells.

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