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Rauscher Leukemia as a Model for Cancer Therapy Studies. II. Variation in Response of Splenic CFU-S between Normal and Rauscher Leukemic Mice following Exposure to Hydroxyurea[1] (40734)

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Murine viral leukemias have been previously proposed as models for human leukemia by several authors in addition to ourselves (1–3). In previous studies it has been demonstrated that infection of susceptible mice with Rauscher murine leukemia virus (RLV) leads to large increases in peripheral WBC, spleen size, differentiating hematopoietic cells in the spleen, and the number of total CFU-C and CFU-S per spleen (4, 5). These changes first become evident about 1 week after infection, dependent on virus dose, with the peak increase being found about 2 weeks after initial infection (6, 7). After that time, the rate of overall increase in spleen mass exceeds that for CFU-S and CFU-C, and thus the relative proportions of these stem cells to spleen mass decreases, although their total numbers remain higher by several factors than that for normal spleen (8). Related to this there is also an increase in the total number of G_0 cells among the splenic CFU-S of the Rauscher leukemic mouse. Calculations made from previous *in vivo* studies (8, 9) reveal that by 2 weeks after RLV administration the total number of G_0-phase CFU-S per spleen is approximately 15× higher than that for normal mice. Other studies directed at determining the effect of Rauscher leukemia development on S-phase CFU-S have shown an initial increase in the number of stem cells in DNA synthesis shortly after infection, and then a decrease to extremely low levels as the disease reaches terminal phase (10).

The studies described below were designed to test the effect of the chemotherapeutic drug, hydroxyurea (HU), on splenic CFU-S in advanced Rauscher leukemia, comparing the response to that of splenic CFU-S in normal mice given the same exposure to HU. For this purpose the time point of 27 days after leukemia initiation was chosen, a time at which the clinical signs of advanced disease are readily evident with approximately 30% of the mice having already succumbed to the disease. At this point total CFU-S levels are still more than 7× normal but their number in cycle is greatly reduced, with the proportion in DNA synthesis demonstrable by in vitro ^{3}HTdR suicide technique having dropped from the normal of 10% to less than 1%

Materials and methods. Mice. Female SJL/J mice were obtained at 6–8 weeks of age from Jackson Laboratories (Bar Harbor, Maine). These were quarantined five per cage before use, maintained in autoclaved cages and bedding, and given autoclaved mouse chow and autoclaved-acidified water *ad lib.*

Virus. The Rauscher virus utilized in these studies was originally obtained from NIH in 1969, passaged one time in the SJL/J mouse, and harvested from the serum. This serum preparation was diluted with Na-citrate buffer and stored at −72°C until use. Assay for virus activity was carried out by the $SED_{50/14}$ (spleen enlargement dose) as described by Chirigos (11) and used previously in our laboratories (4–10). Mice receiving virus were given 50

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$SED_{50/14}$ units at 12 weeks of age by ip injection.

Hydroxyurea. Virus infected SJL/J mice and age-matched normal SJL/J mice were given 0.5 mg HU/g of body wt by ip injection when the leukemia reached advanced state (27 days after virus administration) and when the leukemic mice were all showing clear clinical signs of advanced disease.

CFU-S assay. Assay for numbers of pluripotent stem cells (CFU-S) were carried out using the Till and McCulloch technique (12) as previously employed by us (4–10). Briefly, for each item of data reported a minimum of four donor animals were used. These were sacrificed by cervical dislocation, their spleens removed and a single cell suspension of the pooled cells prepared in Ca- and Mg-free Hanks' solution. A minimum of 20 recipient mice were used in each assay. These were exposed to 950 R of X irradiation 2 hr before injection of the spleen cell suspension, using a G.E. Maxitron 300 therapeutic X-ray unit. Eight days later the CFU-S recipients were sacrificed and the number of colonies per spleen counted. For both normal and leukemic mice exposed to HU the assays were run at the same time in parallel with their non-HU-treated controls, as indicated in the tables. Results of each experiment are given in terms of the mean number of colonies formed and the standard error, calculated by Student's t test. Cumulative means for all experiments combined were also calculated in the same fashion.

Determination of S-phase CFU-S. Determination of S-phase CFU-S was achieved utilizing the tritiated thymidine suicide technique (13), as also employed earlier by us (10). In this the spleen cells were briefly exposed *in vitro* to high levels (250 Ci/ml) of ^{3}HTdR for 20 min between extraction from the donors and injection into the recipients. Previous studies have shown that this level of ^{3}HTdR is sufficient to kill essentially all cells in S. Determination of the percentage in S was then made by comparing the number of surviving CFU-S with that obtained from assay of a second aliquot of the same cell preparation not exposed to ^{3}HTdR.

Results. To determine the baseline response of normal CFU-S to HU, a curve of the number of surviving splenic CFU-S in normal mice and their proportion in DNA synthesis as a function of time after the drug was constructed. The data are given in Fig. 1. The proportion of CFU-S in DNA synthesis, as determined by ^{3}HTdR suicide technique (10, 13), was negligible at 4 hr but increased thereafter. At 16 hr it was at maximum, but the total number of CFU-S still had not appreciably changed from that at 4 hr. These two time periods were then selected for the points of comparison of the response of normal and leukemic mouse CFU-S to HU.

Table I gives the comparison of the data for the effects of HU on CFU-S/10^5 spleen cells from normal and Rauscher leukemic mice at 4 and 16 hr. It is apparent from that data that HU exposure suppresses CFU-S numbers in both normal and leukemic mice by 4 hr after injection. However, while for the normal mouse one sees what appears to be a still further suppression at 16 hr after HU, such is not the case for the leukemic mouse. Rather, there is a significant increase in the leukemic mouse in the number of CFU-S/10^5 spleen cells at 16 hr after HU.

Table II illustrates the effect of HU on total splenic mass in these same mice. Here, one sees a somewhat different response. As is characteristic of Rauscher

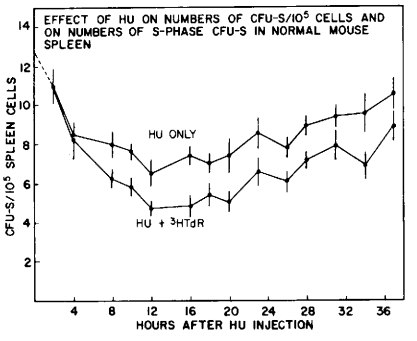

FIG. 1. Upper curve: CFU-S repression and recovery in normal SJL/J mice exposed to a single sublethal dose of hydroxyurea *in vivo*. Lower curve: Additional repression of the same CFU-S population upon subsequent exposure to tritiated thymidine *in vitro*. The difference between the two curves reflects the proportion of cells in DNA synthesis. The error bars reflect 2 SE of the mean.

TABLE I. EFFECT OF HU ON SPLENIC CFU-S IN NORMAL AND RAUSCHER LEUKEMIC SJL/J MICE AT 4 AND 10 HR AFTER DRUG INJECTION

Expt No.	Colonies per 10^5 spleen cells		
	Control	4 hr Post-HU	16 hr Post-HU
		Normal mice	
1	13.7 ± 0.7	7.0 ± 0.7	8.9 ± 0.7
2	12.1 ± 1.0	8.6 ± 0.6	7.3 ± 0.5
3	11.6 ± 0.6	10.2 ± 1.0	7.3 ± 0.6
Cumulative mean	12.8 ± 0.6	8.6 ± 0.9	7.5 ± 0.5
% of Control	—	66.9	58.1
		Leukemic mice	
1	4.6 ± 0.3	—	6.2 ± 0.2
2	4.9 ± 0.2	—	6.7 ± 0.2
3	4.6 ± 0.2	—	7.6 ± 0.2
4	3.1 ± 0.3	—	6.5 ± 1.3
5	4.3 ± 0.2	2.5 ± 0.2	—
6	3.8 ± 0.3	2.3 ± 0.1	—
Cumulative mean	4.2 ± 0.3	2.4 ± 0.1	6.7 ± 0.2
% of Control	—	57.1	159.3

leukemia, the leukemic mice have pronounced splenomegaly leading to spleens more than 20 times the normal size. However, while in the Rauscher leukemic mouse HU produced a reduction in spleen size that was evident at 4 hr and highly significant at 16 hr, a different response was seen in the normal mouse. In the case of the normal mouse, although there was also a reduction in size at 4 hr, there was a slight, but statistically significant, increase in spleen size at 16 hr.

These data were then taken into account and a corrected total estimate for CFU-S per whole spleen was calculated to give the total colony former potential (CFP) per whole spleen in the normal and leukemic mouse, using the method of Markoe *et al.* (5). Table III gives this corrected estimate for total potential colony formers per spleen. In both the normal and leukemic mice HU exposure reduced CFP to 50% of control levels by 4 hr. For the normal mouse this CFP level remained unchanged at 16 hr, although as can be seen from Fig. 1 recovery probably occurs at later times. In contrast to this, even correcting for the change in overall spleen mass, for the leukemic mouse there was a rapid rebound, approaching 100% of pretreatment levels by 16 hr, confirming the difference in recovery potential in the CFU-S compartment of leukemic versus normal mice after HU exposure.

Discussion. The data indicate marked differences between the response of normal and of Rauscher leukemic mice to HU, both with regard to the effect on the total splenic mass and the hematopoietic stem cell compartment. In the normal animal significant recovery in spleen mass occurs by 16 hr after the drug, while the size of the splenic

TABLE II. EFFECT OF HU ON SPLEEN SIZE AT 4 AND 16 HR AFTER INJECTION

	Control	4 hr	16 hr
		Normal mice	
Mean spleen weight (g)	0.1002 ± 0.0045	0.0758 ± 0.0056	0.0865 ± 0.0045
% of Control	—	75.6	86.3
		Leukemic mice	
Mean spleen weight (g)	2.2720 ± 0.0869	1.9793 ± 0.1408	1.3703 ± 0.0858
% of Control	—	87.1	60.3

TABLE III. COLONY FORMING POTENTIAL PER TOTAL SPLEEN 4 AND 16 HR AFTER HU EXPOSURE

	Control	4 hr	16 hr
		Normal mice	
CFP/spleen	18,012	9,116	9,034
% of Control	—	50.6	50.2
		Leukemic mice	
CFP/spleen	134,548	67,997	129,300
% of Control	—	50.5	96.1

CFU-S compartment (CFP/spleen) remains comparatively unchanged. In the leukemic animal the opposite occurs, with a continued decrease in spleen size but almost total recovery in CFP/spleen by 16 hr. Inasmuch as we have previously demonstrated that transplanted CFU-S from Rauscher leukemic mice exhibit essentially the same cellular proliferative rate under optimal growth conditions as normal CFU-S (14), it is doubtful that this accelerated recovery of CFP/spleen in the leukemic mouse after HU can be explained solely on the basis of a shortened doubling time for CFU-S. While some shortening in doubling time may exist, it is equally possible that the expanded population of G_0-phase stem cells in the leukemic mouse (8, 9) may be providing a larger base of residual stem cells from which recovery can occur. Residual G_0 stem cells may also be an important factor in human leukemia as Gavasto has pointed out (15), inhibiting leukemia eradication by conventional chemotherapy and acting as a source for proliferative leukemic clones following drug treatment.

In many earlier studies of chemotherapeutics on normal and leukemic stem cells it has often been the case that only one specific time point was selected to evaluate the comparative effects of various drugs or of various drug dosages (16–19). The present data emphasize the importance in evaluating the effects of chemotherapeutic drugs on normal and leukemic stem cell populations of determining the comparative response at more than one time point after exposure (20–22). For example, looking *only* at the data for 16 hr after HU one could erroneously conclude that the drug either had no effect on the CFU-S population per whole spleen in the leukemic mouse (and thus that all cells were in G_0) as it would seem from only looking at the control and 16-hr columns of Table III, or that production of CFU-S/10^5 leukemic spleen cells was hyperstimulated as it would seem from Table I. Both conclusions would be incorrect. Conversely, looking only at the 4-hr data one would miss entirely the differences in rebound kinetics between the normal and leukemic CFU-S. This also could lead to erroneous conclusions regarding the response to the drug.

On the basis of these results and those of earlier (1–3, 23) studies we would like to suggest that, properly employed, the Rauscher leukemia model might prove useful in evaluating the comparative response of normal and leukemic cell populations to various chemotherapeutic drugs. For this purpose it has at least two important characteristics not already found in the present popular models, L1210 and P-388. First, it arises as a result of a transformation of the host's own cells. Thus, as is the case in man, it is a primary leukemia, not a transplanted one. Second, as is also the case in man (24), stem cell involvement exists but the disease is manifested in its clinical form in changes most readily seen in the differentiating population and at several stages of differentiation (1–3, 23). Third, there is evidence from clinical attempts at treating human leukemia by total marrow ablation and transplantation of marrow from normal donors for a human host etiological factor that can cause the engrafted normal cells to become leukemic (25–27). Thomas has suggested that this host factor could be a virus (25), but as yet a replicative human leukemia virus has not been isolated in any of the above cases (25–27). It is possible that a nonreplicative virus or one capable of limited replication may have been involved. Thus studies on such viruses might yield improved understanding of human leukemogenesis. Rauscher leukemia, does differ from human leukemia, of course, in the fact that involves a replicative virus. Nevertheless, the resultant disease does show similarity to human leukemia in its response to various therapeutic approaches. We have previously shown the applicability of this

leukemia model to radiotherapy (28, 29) and marrow transplantation therapy (30, 31). The present study demonstrates that Rauscher leukemia also responds to the chemotherapeutic drug, hydroxyurea, and may also be useful in other chemotherapeutic studies.

Summary. Normal mice and mice with advanced Rauscher leukemia were given a single dose of HU (0.5 mg/g body wt) and evaluated for the effect of the drug on splenic CFU-S 4 and 16 hr later. Exposure to HU reduced the total number of CFU-S for both normal and leukemic mice by 50% at 4 hr. At 16 hr there was no recovery in CFU-S evident in the normal mouse. However, CFU-S levels in the spleens of the leukemic mice had recovered to nearly that existent before exposure to HU. Effects of HU on total splenic mass differed from that on the CFU-S. Recovery of total splenic mass was seen to have begun in normal animals by 16 hr after HU, while in the leukemic animals spleen size had receded further by 16 hr. The data suggest that, as in man, the hematopoietic recovery responses following exposure to chemotherapeutic drugs may be significantly different in Rauscher viral leukemic mice as compared to normal mice. The possible applicability of Rauscher leukemia as a model for leukemia therapy studies is briefly discussed.

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