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Sequential Response of Pluripotent and Committed Hematopoietic Colony Forming Cells to Rauscher Leukemia Virus[1] (39812)

JAMES P. OKUNEWICK[2] AND PAUL A. CHERVENICK

Viral Oncogenesis Section, Cancer Research Unit Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, and Division of Hematology, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

Introduction. Evidence from several laboratories has indicated an involvement of both the pluripotent colony-forming cell (CGU-S) (1-5) and the myeloid committed colony-forming cell (CFU-C) (6-8) in the development of leukemia following the injection of Rauscher leukemia virus (RLV). Studies of these cells have shown that both increase in number as a result of the disease, and it has been suggested that they, as well as erythroid progenitors (9, 10), may be virus target cells. Conversely, it has also been suggested that the CFU-S is not itself an oncornavirus target cell, but responds indirectly to the disease, providing replacement cells for the more differentiated hematopoietic progenitor compartments, after the kinetics of the latter have been affected by direct action of the virus (11). The present studies were designed as an attempt to shed additional light on this controversy through a determination of the exact temporal relationship of the changes in the CFU-S, the CFU-C, and other related hematopoietic parameters after injection of a low (50 $SED_{50/14}$ units) leukemogenic dose of RLV.

Materials and methods. Sufficient female SJL/J mice to cover the time period illustrated in the results were injected on Day 0 of each experimental run with 50 $SED_{50/14}$ (50% splenomegalies at 14 days) units of Rauscher virus, titered according to the method of Chirigos and March (12). On each of the various days, as indicated in the results, four of these were sacrificed for analysis of CFU-S and CFU-C content in the spleen. For CFU-S assay, the Till and McCulloch (13) technique, as previously utilized by us (1, 2), was employed. CFU-C were assayed by a modification of the soft gel culture system described by Pluznik and Sachs (14) and Bradley and Metcalf (15), utilizing methyl cellulose in place of agar (16). The experiments were repeated twice and virtually identical results were obtained. Therefore, the data from the two repeats were combined into Fig. 1. To maximize the validity of the relationship between the two types of cells being assayed, the same animals were used on each day as the source of both the CFU-S and CFU-C, except for the few instances where data for only one parameter are given on a particular day. For the purpose of these assays, spleen cells from four or more animals were pooled in Hank's solution and, from these, dilutions of the individual cell suspensions were made for injection into lethally irradiated syngeneic mice (CFU-S assay) or plating on methyl cellulose (CFU-C assay). At the same time, spleen weights were determined and samples of tail blood were taken for total leukocyte and differential counts. All cell counts were carried out on a Coulter counter Model Fn. Differential counts were made on between 500 and 1000 white cells after staining with Giemsa.

Results. Figure 1 illustrates the change in CFU-S and CFU-C as a function of time after RLV injection. The data indicate a significant drop in the proportion of CFU-S after Day 2, with maximum decrease on Day 4. A similar, though not statistically significant, drop in CFU-C is suggested at the same time. Subsequently, a significant increase in both cell types occurs with the peak in CFU-S occurring at 14 days, pre-

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[2] Send reprint requests to James P. OKunewick, Ph.D., Head, Viral Oncogenesis Section, Cancer Research Unit, Allegheny General Hospital, Pittsburg, Pa. 15212.

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ceding the 15-day peak of the CFU-C, and then a subsequent decline occurs in both. The initial decrease in CFU-S and CFU-C also precedes those clinical symptoms of the disease, splenomegaly and elevation in white blood count, which reflect massive changes in the kinetic balance of hematopoiesis. Splenomegaly development begins roughly concurrently with the rise in the two CFU compartments, while the statistically significant increase in WBC, and in particular granulocytes, does not occur until after the peak change occurs in the CFU compartments.

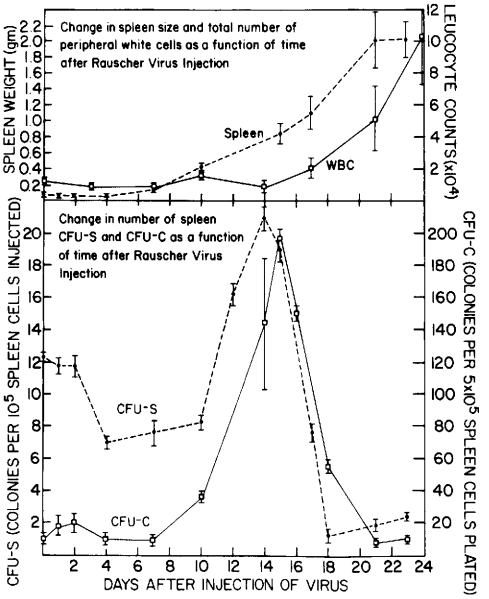

FIG. 1. Changes in CFU-S, CFU-C, spleen size, and peripheral white count as a function of time after Rauscher virus injection. The data are compiled from two separate experiments, in each of which a minimum of four animals was studied at each point represented. The error bars reflect one standard error for the combined data of the two experiments.

Table I indicates the changes in total WBC as well as total lymphocytes and granulocytes in the blood following RLV infection. The data clearly indicate that, although the major cause for the increase in WBC levels may be found in the lymphoid component, as originally observed by Rauscher (17), there is also a smaller clear increase in the total number of myeloid cells noted by Day 21. The failure to see a still larger increase in peripheral myeloid cells may be related to a partial differentiation block of these cells in the blood-forming organs caused by Rauscher leukemia, as reported by Seidel (18) and van Griensven *et al.* (19). As might be expected, the increase in number of blood granulocytes occurs after the change in the CFU-C compartment. Relative to this, it should also be pointed out that, although the proportion of splenic CFU-C have returned to normal by 3 weeks, the total number of CFU-C per spleen is actually still elevated by a factor of between 10 and 20 times due to the splenomegaly of these mice. Hence, a continued increased output into the peripheral blood, as observed, in total number of granulocytic cells might be expected.

Discussion. Although earlier workers (9, 17) have shown that Rauscher disease is primarily an erythroleukemia with target cells among the erythroid-committed progenitors, later studies (5, 7, 20, 21) have established that other hematopoietic cell types are also involved. These include both the granulocytic progenitors and, espe-

TABLE I. INCREASE IN NUCLEATED BLOOD CELLS DURING RAUSCHER LEUKEMIA DEVELOPMENT.

Days after virus	WBC/mm³	Lymphoid cells/mm³	Myeloid cells/mm³	All other[a] cell types/mm³
Control	11,630 ± 1,320	10,560	590	480
3	8,800 ± 1,390	7,900	470	430
7	7,400 ± 800	6,630	560	210
10	15,740 ± 2,250	14,520	610	610
17	21,520 ± 5,720	20,460	610	450
21	51,160 ± 20,010	48,960	1,610	590
28	133,340 ± 36,790	131,240	1,470	630
31	82,290 ± 51,260	78,380	2,260	1,650
38	151,090 ± 39,410	146,860	1,810	2,420

[a] Includes monocytes, nucleated erythroid cells, and cells in which the exact type could not be accurately determined.

cially, the pluripotent stem cell. Thus the possibility exists that more than one target cell for the virus may be found among the hematopoietic primitive progenitor cells (22). Relative to this is the question of what role is played by the pluripotent stem cell (CFU-S) with respect to committed stem cells derived from it. If targets for the virus are to be found only among the committed stem cells, with the changes in the CFU-S compartment occurring as a secondary effect resulting from the disturbances occurring among the committed cells, then a study of the temporal relationship of events should clearly show the effects on the CFU-S compartment following those on the other compartments. Conversely, if the CFU-S is also directly affected by the virus, then events in that compartment should be evident at the same time or earlier than those in the committed stem cell compartment.

The results of the present study show that the changes seen in the CFU-S clearly occur before those seen in the spleen mass or in the peripheral blood. Relative to the committed CFU-C, the initial decrease in number is more pronounced in the CFU-S than in the more differentiated CFU-C, and the point of peak numbers occurs 1 day earlier in the CFU-S. Hence, the data would not seem to support the hypothesis that the response of the CFU-S to Rauscher virus is a secondary effect dependent on earlier disturbances occurring in the more differentiated compartments.

From separate studies, it is also clear that evidence of the disease is most readily found among developing, differentiating cells (5, 9, 10, 20, 21). Thus it has been suggested that complete expression of the disease may require some amount of cell differentiation (23). In conjunction with this, it is also possible that one direct effect of the virus on the CFU-S might be to force it along a differentiation pathway. Indeed, the early dip seen in the CFU-S might suggest this.

Thus, the present results are neither inconsistent nor in contradiction with the earlier evidence that target cells for the murine leukemia viruses exist among other committed progenitor cells (9, 10). Rather they support the contention that the pluripotent stem cells also may be direct targets, in addition to the committed stem cells, for the Rauscher virus (1–5). Hence they are in agreement with previous reports from our laboratory (24) and others (7, 8), showing a very early effect of Rauscher virus on the CFU-S, as well as an action of the virus on many other hematopoietic cell types (5, 18), and suggest that response of the CFU-S is not dependent on previous events occurring in the more differentiated compartments.

Summary. The effects of Rauscher leukemia virus injection on the development of response in the CFU-S and CFU-C compartments and on the development of the clinical responses of splenomegaly, total leukocyte, lymphocyte, and granulocyte count have been measured with respect to each other as a function of time. The results indicate an action of Rauscher disease on both the granulocytic and the pluripotent stem cell compartments, with the effects on the pluripotent compartment generally preceding or occurring simultaneously with those seen on the committed granulocytic progenitors, the peripheral white blood compartment, and on spleen size. These findings are consistent with the hypothesis that the response of the CFU-S in the disease reflects a direct effect of the virus on that cell, rather than an indirect effect brought about by earlier disturbances occurring among the committed and differentiating stem cells and in the blood-forming organs and the peripheral blood.

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