Accelerated Clearance of Exogenously Administered Erythropoietin by Mice with Rauscher Viral Leukemia

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SUMMARY

Both the endogenous plasma erythropoietin levels and the rate of plasma clearance of injected erythropoietin have been investigated in SJL/J mice that had been rendered leukemic by the injection of Rauscher virus. The results show an elevated level of endogenous erythropoietin coupled with an accelerated rate of erythropoietin clearance. These results are inconsistent with the hypothesis previously suggested by other workers that erythropoietin production is impaired by Rauscher leukemia. As an alternative, it has been proposed that as a result of the leukemia both erythropoietin production and clearance are accelerated to such a degree that very little reserve production capacity remains to allow for additional hormone synthesis in response to the stimuli of extreme anemia or bleeding.

INTRODUCTION

It has been previously reported that, compared with normal animals, mice infected with RLV exhibit significantly lowered hematocrits (1, 6, 15), coupled with an impaired ability to respond to a stimulus for the production of EP (2, 4). To account for this reduction in response to stimulation of EP production, it has been suggested that the virus may directly affect EP synthesis, resulting in underproduction (2, 4). On the other hand, the same authors (2, 4) have also reported background levels of EP in Rauscher leukemic mice which are significantly greater than normal. It is also known that mice with Rauscher leukemia exhibit massive increases in the amount of hematopoietically active splenic tissue (1, 15). These observations raise the question as to whether the reported effects of the leukemia on EP levels might be accounted for by other possibilities not previously considered, such as altered clearance and/or utilization of EP in the leukemic mouse. The purpose of this paper is to investigate this question.

MATERIALS AND METHODS

EP. EP for injection was obtained from Connaught Laboratories, Toronto, Canada. The preparation used was Step I sheep plasma EP dissolved in sterile 0.9% NaCl solution at a concentration of 10 units/ml. This was injected into the tail veins of the animals used in the clearance tests in the quantity of 5 units/mouse.

Test Animals and Leukemia Induction. The clearance test animals, both normal and leukemic, were adult 14-week-old female SJL/J mice (The Jackson Laboratories, Bar Harbor Maine). This strain of mice was chosen because our laboratories have previously used it extensively for both Rauscher and Friend leukemia studies (9, 12, 13, 14). Although others (8) reported that the SJL/J mouse, at 1 year of age, developed a spontaneous reticulum cell sarcoma that may be of viral origin, our animals showed no evidence of this defect. Moreover, they were considerably younger than the youngest mouse in which this condition was seen [J. Roths (The Jackson Laboratories), personal communication]. Rauscher leukemia was induced in these mice by the injection of 40 SED50 units of virus in the manner previously described (9, 13, 14). At the same time, a separate set of normal animals from the same group was set aside to serve as controls.

EP Assay. At the appropriate time after EP injection, the clearance test mice were anesthetized with ether and exsanguinated via the vena cava. The blood was centrifuged and the plasma was pooled in appropriate clearance time groupings. The experiment was repeated twice and, in each repeat, approximately 24 normal and 24 leukemic mice were used as EP recipients at each time period studied. These yielded an average of 6 to 8 ml of pooled plasma which was then frozen until used. Assay of the EP content of these plasmas was carried out in a manner similar to that described by Mirand et al. (7), utilizing the exhypoxic polycythemic mouse system and the CF1 assay mouse previously used in our laboratories (10, 11). Starting at 4 days after their removal from hypoxia, each of the assay mice was given 1.2 ml (i.p.) of the clearance test plasma, 0.4 ml/day for 3 consecutive days. Twenty-four hr after the last plasma injection, the mice were given 1 μCi of citrate-buffered \[^{59}\text{Fe} \text{Cl}_3\] by tail vein injection. Forty-eight hr later the assay mice were anesthetized and bled from the vena cava. The red cells were separated by centrifugation, washed once in 0.9% NaCl solution, and the \[^{59}\text{Fe} \text{Cl}_3\] uptake was determined using a gamma counter.

Received July 27, 1973; accepted January 7, 1974.

1 These studies were supported in part by NIH-National Cancer Institute Grant 1P02 Ca 10438-05, and by Atomic Energy Commission Contract AT (11-1)-3097.

2 The abbreviations used are: RLV, Rauscher leukemia virus; EP, erythropoietin.
in the blood of each assay mouse was calculated in the manner previously reported by these laboratories (10, 11). The rate of EP clearance was determined in terms of percentage of $^{59}$Fe incorporation in the assay animals. For a test of the effects of the virus on the CF1 assay mice, similar procedures were also carried out using only the CF1 mouse. Statistical analyses were performed by means of the techniques described by Dixon and Massey (3). All values are given as mean ± 1 S.E.

RESULTS

Sheep EP was injected into normal and Rauscher leukemia SJL/J mice, and the relative pattern of clearance of this exogenous EP from the serum was measured. The results are given in Chart 1. The experiments were repeated twice and data for the mean of each trial is given, as well as for the mean ± S.E. of the pooled data from the 2 experiments. Two effects of the Rauscher leukemia are immediately evident from the chart. First, the background level of endogenous EP was much higher in the Rauscher leukemic mice than in the normal ones. This was true even though in the leukemic mice, there was a spleen mass more than 5 times (531.5 ± 31.4 mg) higher than the normal value of 80 to 90 mg and therefore, theoretically, a much greater potential for EP absorption and utilization. Second, the clearance rate for exogenous EP was much higher in leukemic animals than in normal animals, reaching the background level within 4 hr, compared with that in normal mice which, at 10 hr, had still not reached background level.

EP clearance half-times were calculated for both groups by regression line technique, using the data for the 1st 4 hr for the leukemic mice (up to the point at which the background level was reached) and the entire 10-hr period for the normal mice. The relative clearance half-times thus calculated were 4.13 hr for normal mice and 1.33 hr for Rauscher leukemic mice. Therefore, it is apparent that, given equal amounts of exogenous EP, the leukemic mice initially cleared the hormone at a rate on the order of 3 times faster than normal mice.

The relative zero-time intercepts were calculated from the regression analysis to be 24.6% (1 S.E., 19.2 to 31.6) for the serum from normal mice and 25.9% (1 S.E., 20.4 to 32.8) for the leukemic mice. The leukemic mouse serum showed an initially higher level of EP-stimulating ability, consistent with the observed higher background level of endogenous EP-stimulating factor in these mice when compared to normals. However, from a statistical standpoint, this difference in the calculated zero-time intercepts was not large enough to be considered significant.

Since the serum from the leukemic mice also contained active virus, it was therefore important to verify that none of the above results were adversely affected by the presence of that virus in the assay animals over the assay period. To test this point, normal CF1 mice similar to those used in the assay were given i.v. injections of 40 units of RLV and, 20 min later, were sacrificed and bled. The serum obtained from these mice was assayed for EP-stimulating ability in the same manner as that utilized above for the SJL/J mice. In addition, a separate group of polycythemic CF1 assay mice received direct injections of virus in the same manner as was used for the injection of the sera. The results of these experiments, given in Table 1, are compared with the data for the sera from normal and leukemic SJL/J mice. From these results it can be seen that it is unlikely that any direct effect of the virus on the CF1 assay mouse occurred in the course of the assay which would influence the data given in Chart 1.

Hematocrits of the leukemic SJL/J mice used in these experiments were also measured. As reported by us earlier (5), these fell in a consistent manner from the normal of approximately $52 ± 2$ to the value of $34 ± 3$ at 14 days after injection of the virus. Hence, the virus-infected serum donors were significantly anemic at the time the serum was taken for testing.
DISCUSSION

In their previous reports, both Ebert et al. (4) and Camiscoli et al. (2) cited evidence indicative of a failure in EP production by Rauscher leukemic mice. Specifically, they could find no relationship between the degree of disease-caused anemia in these mice and the level of endogenous EP (2, 4). Moreover, the ability of the leukemic mice to increase their EP production after bleeding was found to be inappropriately low, compared with that of bled normal mice (4). These observations led them to conclude that Rauscher leukemia might somehow directly interfere with EP production.

On the other hand, both laboratories also reported that in all cases the plasma EP levels in their leukemic mice were elevated above that found in their normal control plasma, with some values ranging as high as 6 to 9 times normal (2, 4). This observation, which is confirmed in the findings of this paper, is inconsistent with a hypothesis of impaired EP production. If anything, it would suggest that mice with Rauscher leukemia are producing more rather than less EP. Inasmuch as Mirand et al. (7) have shown that in normal, germfree, and irradiated mice the clearance rate of this hormone can be greatly altered, one obvious choice of an alternative mechanism to explain these higher levels is a change in the clearance rate of the hormone (clearance rate being defined as the rate of loss of the hormonal activity in vivo in the serum). Using the same technique as described by Mirand, our results show that Rauscher leukemia does alter the clearance rate for exogenous EP. However, the clearance half-time is shortened rather than lengthened. Thus the data indicate that it is doubtful that the elevated endogenous EP levels could be due to a slower clearance rate for the hormone.

However, the results are consistent with other factors involved in the hematopoietic changes occurring in the leukemic mouse. For instance, reticulocyte production in Rauscher leukemic mice is much greater than normal (4, 5). Second, massive increases in spleen size occur after RLV infection (5, 16), and it is reasonable to infer that this might result in greater utilization of the hormone. Finally, the observed anemias are certainly of a degree that would result in greater EP production (2, 4, 5). Considering these factors and the present results, we would suggest that the data indicate that EP is probably being both produced and cleared in the Rauscher leukemic mouse much more rapidly than in the normal mouse. Indeed, it is possible that the entire process may be occurring at a rate which is at or near the maximum at which the hormone can be produced. If such were the case, it could explain why there is no apparent relationship between the degree of anemia and the plasma levels of the hormone and, also, why bleeding does not raise the EP levels in the Rauscher leukemic mouse to the same degree as seen in the normal mouse.

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