Effect of Cortisol on Rauscher Virus Infection

Isadore Brodsky, Evelyn M. Ross, S. Benham Kahn, and Shirley D. Braverman

Department of Medicine, Section of Hematology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102

SUMMARY

The effect of cortisol therapy on the hematopoietic response to Rauscher virus infection was evaluated in nonsplenectomized and splenectomized BALB/c mice. In nonsplenectomized mice, cortisol caused a significant reduction in leukocytosis, lymphocytosis, monocytosis, granulocytosis, and splenomegaly. Splenectomy modified the white blood cell response to infection in a manner similar to the effect of cortisol. There was a distinct difference between the effect of splenectomy or cortisol therapy on erythropoiesis and thrombopoiesis. Splenectomy increased the severity of the thrombocytopenia and anemia following infection with Rauscher virus, whereas cortisol therapy had no effect on the thrombocytopenia but did improve the anemia in nonsplenectomized mice. The implications of these results in evaluating the chemotherapy of virus-induced murine and human leukemia are discussed.

INTRODUCTION

The virus-induced murine leukemias have been used as experimental models in the evaluation of the antileukemic effect of drugs (5-8, 18, 21, 22). Chirigos and his coworkers (5-8) have used experimental models in the evaluation of the antileukemic effect of drugs for their antiviral activity, their capacity to influence neoplasia, and its ability to retard the growth of virus-induced neoplasms. The adrenal corticosteroids and chemotherapeutic agents, such as 6-mercaptopurine, triethylene melamine, and methotrexate, can objectively alter the course of Rauscher leukemia using the criteria of decrease in splenic weight and prolongation of life span (5-8). Similar results were obtained in the treatment of Friend leukemia with either corticosteroids, X-ray therapy, or chemotherapeutic drugs (15, 16, 18, 21, 22). The corticosteroids can also delay or decrease the incidence of spontaneous lymphocytic leukemia in AKR mice (23, 24). All of the above studies were performed in nonsplenectomized mice, with little or no emphasis on the stage of leukemia at presentation or the effect of therapy on the hemoglobin concentration, platelet count, leukocyte, lymphocyte, monocyte, and granulocyte counts. The implications of these studies in evaluating the chemotherapy of virus-induced murine and human leukemia are discussed.

MATERIALS AND METHODS

Virus. Rauscher virus was obtained from Dr. Frank Rauscher, National Cancer Institute, Bethesda, Maryland. The source of virus was BALB/c spleen and the virus suspension was prepared by the Hazleton Laboratory, Falls Church, Va. The virus was maintained by serial passage of clarified spleen suspension into BALB/c mice at weekly intervals. The spleens were homogenized with a mortar and pestle in a cold room and suspended in Hanks’ balanced salt solution containing 125 units of penicillin and 125 μg streptomycin per ml. This suspension was clarified by centrifugation at 1300 × g for 20 minutes and the supernatant centrifuged at 2000 × g for 10 minutes. The supernatant was then divided into small portions and frozen at −60°C. The virus used in these experiments was in its sixth passage. The virus titer was PDD 500,000 3.2 (4). In this bioassay, the titer was arbitrarily defined as the dilution of virus which caused a depression in platelet count (PDD) to 500,000 per cu mm.

Virus Inoculum. Preparation of virus inoculum was carried out as follows: for each experiment a frozen aliquot of a 20% extract of leukemic spleens was thawed and either 0.2 ml undiluted (10⁰) or ten-fold diluted in 0.9% saline solution (10⁻¹) was inoculated intravenously into the tail veins of
I. Brodsky, E. M. Ross, S. B. Kahn, and S. D. Braverman

nonsplenectomized or splenectomized mice. Uninfected control mice were inoculated with 0.2 ml of 0.9% saline solution.

Mice. BALB/c weanlings (16-18 gm) 4 to 6 weeks of age obtained from Dr. Rauscher were maintained in plastic cages, 10 per cage, and fed Purina laboratory chow, micro-mix pellets and water ad libitum. Mice were splenectomized by previously described procedures (4). The splenectomized mice were allowed to recover for 4 weeks before being used in experiments.

Hematologic Studies. Blood for determination of the hematologic data was obtained by puncture of the retroorbital venous plexus with a heparinized capillary tube. The hemoglobin concentration, white blood count, and platelet count were determined by methods previously published (2, 3). Peripheral smears were made on glass slides and stained with Wright's mixture; two hundred cells were counted and the differential was obtained. The absolute lymphocyte, monocyte, and granulocyte counts were calculated from the total white blood count and differential (4).

Adrenal Corticosteroid Treatment. Nonsplenectomized and splenectomized BALB/c mice infected with either 10^0 or 10^-1 virus dilution were treated for two weeks with a daily 1.0 mg i.p. dose of cortisol (hydrocortisone) suspended in 0.1 ml of physiologic saline solution. Cortisol therapy was started on the day of infection. Similarly infected mice were not treated with cortisol but were injected daily with a 0.1 ml of physiologic saline for 2 weeks. Uninfected nonsplenectomized and splenectomized BALB/c mice were injected daily for 2 weeks with either 1.0 mg cortisol or 0.1 ml of physiologic saline (i.p.).

In all groups hematologic parameters were measured at the end of 2 weeks. Logarithmic transformations were introduced where necessary to stabilize the variances. P value ≤ 0.05 was considered significant.

The effect of cortisol on virus-infected and uninfected mice was evaluated by t tests for each of the hematologic parameters.

RESULTS

Charts 1 and 2 present the observed means and standard deviations of the hematologic parameters for the nonsplenectomized and splenectomized groups respectively. Tables 1 and 2 present these data in summary form.

Effect of Cortisol on Uninfected Nonsplenectomized Mice (Chart 1, Table 1). Cortisol significantly increased the leukocyte count and granulocyte count and decreased splenic weight. There was no significant change in hemoglobin concentration or platelet, lymphocyte, and monocyte counts.

Effect of Cortisol on Uninfected Splenectomized Mice (Chart 2, Table 1). Splenectomy alone causes significant thrombocytosis, leukocytosis, lymphocytosis, monocytes, and granulocytosis (4). Cortisol therapy caused a significant decrease in the leukocyte count primarily because of a significant decrease in lymphocytes and monocytes. There was no significant change in the hemoglobin concentration, granulocyte, or platelet count.

Effect of Cortisol on Infected Nonsplenectomized Mice (Chart 1, Table 2). Rauscher virus infection at titers of 10^0 and 10^-1 caused thrombocytopenia, leukocytosis, lymphocytosis, monocytes, granulocytosis, anemia, and splenomegaly. Cortisol-treated mice showed a significant decrease in leukocytosis, lymphocytosis, monocytes, granulocytosis, and splenomegaly. Cortisol therapy had no effect on the thrombocytopenia but did cause a significant improvement in the anemia.

Effect of Cortisol on Infected Splenectomized Mice (Chart 2, Table 2). When mice were infected with 10^0 titer prior splenectomy alone modified the effect of subsequent Rauscher virus infection. Thrombocytopenia was more severe. A rise in leukocyte, lymphocyte, and monocyte counts occurred but this rise was not as great as in similarly infected nonsplenectomized mice (Chart 1, 2). In the infected splenectomized group the granulocyte count fell following infection. Hemoglobin concentration decreased in the splenectomized infected mice and was similar to the decrease that occurred in the nonsplenectomized group.

Cortisol therapy caused a significant decrease in leukocyte and lymphocyte counts in the infected splenectomized mice, but it had no significant effect on the granulocyte or monocyte counts. There was a significant increase in hemoglobin concentration and platelet count.

When mice were infected with 10^-1 titer splenectomy alone modified the effect of subsequent Rauscher virus infection. Severe anemia and thrombocytopenia developed, but instead of a rise in the white blood count, as noted with 10^0 virus dilution, a fall in leukocyte, lymphocyte, monocyte, and granulocyte counts occurred.

Cortisol therapy caused a significant rise in the hemoglobin concentration, platelet count, and monocyte count. A significant fall occurred in the lymphocyte count, but there was no significant change in the granulocyte count. The total leukocyte count did not change significantly because the fall in lymphocytes and rise in monocytes cancelled each other's effect.

DISCUSSION

The current study illustrates the effect of cortisol on the prelymphoid leukemic phase of Rauscher Virus infection. In nonsplenectomized mice the hormone causes a significant reduction in the virus-induced leukocytosis, lymphocytosis, monocytes, granulocytosis, and splenomegaly. With therapy the hemoglobin concentration rises but the thrombocytopenia is unchanged.

Prior splenectomy alone modifies the preleukemic phase of subsequent Rauscher virus infection, particularly with the higher dilutions of virus (10^-1--10^-3) (4). Anemia and thrombocytopenia are more severe and a depression in the white blood cell elements, rather than the characteristic elevation noted in nonsplenectomized mice, occurs. In the present study the 10^0 dilution of virus did cause an elevation in the white blood cell elements in splenectomized mice, but this rise was substantially less than in similarly infected nonsplenectomized mice. The results with the 10^-1 dilution of virus were similar to those previously published (4).

The effect of cortisol therapy on the white blood cell elements of infected nonsplenectomized mice is similar to the effects of infection with the 10^-1 dilution of virus in non-cortisol-treated splenectomized animals. Actually, the response of the splenectomized animals to infection results in a more pronounced fall
Chart 1. Means and standard deviations of the hematologic parameters for virus-infected and uninfected nonsplenectomized mice treated with and without cortisol. The open bars indicate those mice that did not receive cortisol; the checked bars indicate the cortisol-treated animals. Two virus dilutions, $10^0$ and $10^{-1}$, were tested. Normal indicates the group that was not infected with virus. The $P$ values presented beneath the bars represent the statistical analyses for the cortisol-treated versus non-cortisol-treated mice in each of the 3 groups. N.S. indicates not significant. The data shown on the semilogarithmic scales are the geometric means $\times$ antilog (S.D. of log). The data shown on the arithmetic scale (hemoglobin concentration) are the arithmetic means $\pm$ S.D.
Chart 2. Means and standard deviations of the hematologic parameters for virus-infected and uninfected splenectomized mice treated with and without cortisol. The open bars indicate those mice that did not receive cortisol; the checked bars indicate the cortisol-treated animals. Two virus dilutions, $10^0$ and $10^{-1}$ were tested. Normal indicates the group of mice that was not infected with virus. The $P$ values presented beneath the bars represent the statistical analyses for the cortisol-treated versus non-cortisol-treated mice in each of the 3 groups. N.S. indicates not significant. The data shown on the semilogarithmic scales are the geometric means ± antilog (S.D. of log). The data shown on the arithmetic scale (hemoglobin concentration) are the arithmetic means ± S.D.
in white blood cell elements than does the response of non-splenectomized infected animals to cortisol therapy.

Infected splenectomized mice were also treated with cortisol in an attempt to further modify the early phase of Rauscher infection. At both the $10^0$ or $10^{-1}$ virus titer the major effect of cortisol therapy on the white blood cell elements was to cause a decrease in the lymphocyte count. However, the importance of this decrease is difficult to evaluate since cortisol therapy causes a sharp decrease in lymphocyte count in uninfected splenectomized mice. Both the hemoglobin concentration and platelet count rose with cortisol therapy. In the non-splenectomized mice cortisol therapy improved the anemia but had no effect on the thrombocytopenia.

Cortisol is most effective in the treatment of infected non-splenectomized mice. Splenectomy and cortisol therapy have similar effects on the white blood cell response to Rauscher virus infection. Since the early white blood cell response to Rauscher infection appears to be spleen-mediated, the effectiveness of cortisol therapy is probably related to its ability to cause a significant reduction in splenic size. This effect can be considered a medical splenectomy. In Rauscher infection cortisol causes a decrease in splenic size, probably by the process of lymphocytolysis (11, 17). It is also possible that cortisol causes a reduction in splenic size by inhibiting viral replication. Chirigos et al. (6) have shown that immune serum therapy of Rauscher disease caused a temporary reduction in virus titer and spleen weight, presumably on the basis of a direct antiviral effect. The immune serum was obtained from mice inoculated with formalin-inactivated spleen extract (6, 12). Unfortunately, a control immune serum against normal mouse spleen was not used and therefore a direct cytologic effect of the immune serum on the spleen cannot be ruled out. In our data we demonstrate a failure of cortisol to alter thrombocytopenia in the non-splenectomized mice. Since thrombocytopenia is one of the most sensitive indices of Rauscher infection (4), we conclude that cortisol does not alter virus replication. Chirigos (5) also noted that spleen weights in mice treated with the corticosteroid, prednisone, were lower than those in untreated control animals, but prednisone was not shown to have antiviral activity following bioassay of donor plasma in recipient mice. Actually, the results indicated an increase in viral yield of 0.2 logs (5).

There is a distinct difference between the effect of splenectomy or cortisol therapy on erythropoiesis and thrombopoiesis. Splenectomy increases the severity of the thrombocytopenia and anemia following infection, whereas cortisol therapy has no effect on the thrombocytopenia and improves the anemia in non-splenectomized mice. In infected splenectomized mice cortisol improves both the anemia and thrombocytopenia.

The results of this study indicate that the measurement of spleen weight alone is an inadequate criterion for evaluating the effect of chemotherapeutic drugs or hormones on Rauscher leukemia. Even prolongation in survival time may be misleading since splenectomy abolishes the early mortality peak that is secondary to splenic rupture and bleeding (4, 25). Therefore, in Rauscher leukemia, any form of therapy that causes a significant decrease in splenic size, either on the basis of inhibition of virus replication or inhibition of virus-induced cellular proliferation, will reduce the risk of splenic rupture and cause an increase in survival.

The early anemia following Rauscher virus infection is due to a decreased red blood cell survival, presumably secondary to metabolic effects of the virus on the erythroid precursors leading to the production of altered red blood cells (2, 4). The mechanism by which cortisol therapy improves the anemia in Rauscher infection has not been established. It may be related to the ability of the adrenal corticosteroids to block the destructive action of the reticuloendothelial system or perhaps to a direct effect of cortisol on red blood cell metabolism leading to a decrease in hemolysis (14, 19). Since shortened red blood cell survival and thrombocytopenia occur rapidly following infection (4, 10), it seems unlikely that the cortisol effect on the anemia and its failure to correct the thrombocytopenia is related to suppression of immune mechanisms.

The mechanism by which splenectomy modifies the course of Rauscher virus infection is not clear. Since the normal mouse spleen is analogous to bone marrow, it is possible that

| Platelet count | Unchanged | Unchanged |
| Leukocyte count | Increased | Decreased |
| Lymphocyte count | Unchanged | Decreased |
| Monocyte count | Unchanged | Decreased |
| Granulocyte count | Increased | Unchanged |
| Hemoglobin concentration | Unchanged | Unchanged |
| Spleen weight | Decreased | Decreased |

Table 1

Effect of cortisol on hematologic parameters in noninfected BALB/c mice.

<table>
<thead>
<tr>
<th>Virus titer</th>
<th>Non-splenectomized</th>
<th>Splenectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^0$</td>
<td>Unchanged</td>
<td>Unchanged</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Table 2

Effect of cortisol on hematologic parameters in Rauscher virus-infected mice.
enlarged spleen can compensate in part for the anemia and thrombocytopenia (2–4). Certainly the initial leukocytosis appears to be spleen mediated (4, 25). Another explanation is that the intact spleen is the primary site of infection and can absorb enough virus particles to partially protect the bone marrow. In nonsplenectomized animals the first histopathologic signs of infection occur in the spleen, whereas in splenectomized mice the first histopathologic changes occur in the bone marrow (25).

The adrenal corticosteroids are effective palliative agents in the murine viral-induced leukemias and spontaneous leukemias (23, 24), and in acute human lymphoblastic leukemia, chronic lymphatic leukemia, and lymphosarcoma (13, 14). The therapeutic efficacy of the hormone is probably related to its ability to cause involution of lymphatic tissue by means of lymphocytokaryorrhexis, inhibition of lymphocyte mitosis at the metaphase stage, and inhibition of DNA synthesis (11). In normal mice the adrenal corticosteroids are the major factor in dampening lymphocyte production in the thymus (11, 17). In our experiments in normal nonsplenectomized mice, granulocytosis, leukocytosis, and decrease in splenic weight occurred after cortisol administration, but there was no change in the lymphocyte count. In the splenectomized group a sharp drop in leukocytes occurred because of a fall in lymphocytes. We have no explanation for the apparent increased susceptibility of the circulating lymphocytes in splenectomized mice to the action of cortisol.

It is apparent that Rauscher virus infection can cause broad hematologic effects. Therefore, any chemotherapeutic agent aimed at eradicating only one cell type, such as the presumably neoplastic lymphocyte, may produce remission, but relapse will occur because of persistence of virus in nonneoplastic reservoirs such as erythroid precursors and megakaryocytes (9). Theoretically, one would have to destroy all hematopoietic and lymphocytic tissue in order to eradicate the virus infection. The therapy of human leukemia is based on the principle of destroying as many neoplastic cells as possible in order to achieve a prolonged remission and, if possible, a cure (13). If human leukemia is caused by a virus, then treatment with chemotherapy must consider not only the destruction of the neoplastic cells but also eradication of the virus in nonleukemic reservoir cells in order to prevent reinfection.

The results of the present study suggest that effective therapy of Rauscher virus leukemia will depend on the development of specific and effective antiviral agents. Greater emphasis should be placed on the antiviral chemotherapy of the murine virus-induced leukemias in order to eradicate the virus in both neoplastic and nonneoplastic cells.

REFERENCES


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