Relation of Disease Stage to Humoral and Cellular Impairment of Spleen Reticuloendothelial System Depression in a Rat Leukemia

Burton S. Dornfest

Department of Anatomy, Downstate Medical Center, State University of New York, Brooklyn, New York 11203

SUMMARY

In the rat leukemia studied there is an early reversible depression of reticuloendothelial system (RES) function followed by a late-stage secondary depression, culminating in death, associated with an infiltration of leukemic cells into the bone marrow and spleen. In one set of experiments to assess RES function, isolated spleens of nonleukemic rats were perfused with blood from rats in progressively advanced stages of the leukemia. In a reciprocal set of experiments, isolated spleens of rats in comparable stages of the leukemia were perfused with blood from nonleukemic rats. The clearance of $^{99m}$Tc-sulfur colloid from the blood perfusate was used as a test of RES function. In the first set, clearance was depressed in nonleukemic spleens perfused with blood collected from rats 1 hr after they had been inoculated with tumor cells, and it was more markedly depressed in spleens perfused with blood collected from rats in a late stage of the leukemia 6 days later. Perfusion of nonleukemic spleens with blood from rats in intermediate stages of the leukemia did not depress clearance. In the reciprocal study, clearance was depressed in spleens of rats that were inoculated with tumor cells 1 hr before perfusion with nonleukemic blood. Clearance values were at the control level in spleens of rats in all other stages of the leukemia when calculated on the basis of total spleen weight. When calculated on a unit weight basis, clearance values were lower in the enlarged spleens of rats in advanced stages of the leukemia. The results indicate that impairment of RES clearance of $^{99m}$Tc-sulfur colloid is associated with alterations in the humoral content of the blood of this leukemic rat. There is indirect evidence to suggest that in the terminal stage of the leukemia an impairment of splenic RES cells to clear $^{99m}$Tc-sulfur colloid may develop secondarily.

INTRODUCTION

There is relatively little information associating functional changes of the RES to the severity of leukemia. There is even less information that relates RES functional changes to alterations in RES cellular activity and/or plasma humoral factors. Least is known about the respective stages in leukemia during which such changes develop. Recent studies in this laboratory, using an acute myelogenous leukemic rat (Shay chloroleukemia) (8, 18), have shown that there is an impairment of RES function in the terminal stage of this experimental leukemia. Impairment was evidenced by a decreased ability in the leukemic condition of the splenic RES to clear inert colloidal gold and colloidal sulfur from blood perfused through isolated rat spleens (6, 7). It was further observed in these studies that the impairment is probably associated with both an RES cellular defect and a defect or alteration in the humoral content of the blood of the leukemic animal (7). In addition, the concentration of leukemic cells in the blood was found to be associated with the depression of splenic clearance of colloidal gold (6). This study was undertaken for the dual purpose of (a) relating the impairment of splenic clearance to the stage in the development of this leukemia and (b) determining whether RES cellular and plasma humoral changes develop concurrently or at different times during the pathogenesis.

MATERIALS AND METHODS

Animals and Tumor Characteristics. An acute myelogenous leukemia (18) was transferred to nonleukemic (normal) recipient 200- to 225-g random-bred male Long-Evans rats through i.v. inoculation of $2 \times 10^6$ tumor cells obtained from leukemic donor rats. The procedure used for maintaining and transferring the tumor has been described in previous reports (3, 6, 7, 9). In recent studies (6, 7), rats inoculated with this tumor were in an advanced stage of the leukemia by the 6th to 7th day after transfer. The leukemia at that time had been characterized by a marrow myeloblast count in excess of 80% of the total marrow cellularity, splenomegaly, and a blood leukocyte count ranging from $50 \times 10^3$ to $15 \times 10^4$ cells/μm. Rats inoculated with tumor cells did not survive beyond 7 days.

Spleen Perfusion. The procedure for perfusion of the isolated rat spleen has been described in detail previously (3). Spleens used for perfusion were isolated from ether-anesthetized donor rats, placed within a 37° moist chamber, and perfused for 2 hr with whole blood through cannulas inserted into the splenic artery and vein. Whole blood used as perfusate was collected through the abdominal aorta of blood donor rats into syringes containing 0.2 mg heparin.
Altered RES Function in a Rat Leukemia

The buffy coat layer of leukocytes, and retaining the supernatant plasma to reconstitute the hematocrit. Once passed through the spleen, blood was not recirculated.

Experimental Groups. Rats given injections of tumor cells were sacrificed at intervals of 1 hr and 1, 2, 4, and 6 days after inoculation to obtain the spleens and blood for perfusion. Two sets of experiments were performed. In the 1st set, blood collected from the tumor cell-inoculated rats in progressively advanced stages of the leukemia was used to perfuse isolated spleens obtained from nonleukemic rats. Three spleens of nonleukemic rats were perfused with blood from 6-day tumor cell-postinoculated rats in which the concentration of leukocytes was reduced (referred to as WBC-reduced as compared to WBC-intact blood). In the 2nd set, the isolated spleens of the tumor cell-inoculated rats in comparable stages of the leukemia were perfused with blood of nonleukemic rats. In addition, a group of experiments was performed in which rats were given an equivalent number of normal WBC and sacrificed 1 hr after injection to obtain the spleens for perfusion. A reciprocal group of experiments to use blood of rats given injections of normal WBC for perfusing nonleukemic spleens was not conducted because of the technical problem of obtaining the large number of normal WBC required. The controls for all experiments were perfusions in which spleens of nonleukemic rats were perfused with blood of nonleukemic rats.

99mTc-S Clearance. The 99mTc-S colloid used to test RES clearance was prepared from a commercial kit obtained from E. R. Squibb, Inc., Princeton, N. J. (Tesuloid). A Mal- linckrodt 99mTc generator was the source of the 99mTc used for labeling the sulfur colloid particles. Ten μl of sulfur colloid labeled with 50 to 100 μCi of 99mTc (40 to 80 μCi/μg) were added to each 50-ml volume of blood perfusate. The labeled sulfur colloid was used on the day of preparation. The median and mean sizes of the colloid particles as determined by filtration through Nucleopore filters were 0.37 and 0.52 μm, respectively (Nucleopore Corp., Pleasanton, Calif.). The median was determined from an equation for percentage of radioactivity retained (γ) as a linear function of pore size (x), with the y's in logs: log y = 2.058606 — 0.9708819x. The mean was obtained by calculation after estimating the frequency distribution that would result from the application of this equation to the decrease of radioactivity from the total radioactive count. In sizing the particles, it was assumed that the percentage of radioactivity passing through the filter reflected the particle size. The amount of colloid introduced into the blood was a function of the volume of colloid suspension and was independent of radioactivity. One-ml samples of the arterial inflow and venous outflow of blood were collected from the splenic artery and vein at 15-min intervals and counted for radioactivity in a γ scintillation counter. The percentage of 99mTc-S that was cleared from the blood perfusate during passage through the spleen was calculated on the basis of both total and unit weight (per 0.1 g) of splenic tissue by dividing the radioactivity of the blood by the initial radioactivity of the total volume of blood perfused, as shown by the formula:

\[ \text{%} \text{99mTc-S cleared} = \frac{\text{cpm/spleen} \cdot \text{cpm}/0.1 \text{g}}{\text{initial cpm/total vol blood perfused}} \times 100 \]

The initial radioactivity of the total volume of blood perfused was obtained by multiplying the radioactivity of 1 ml of arterial blood by the total volume of blood collected from the splenic vein at the end of 2 hr. The radioactivity and wet weight of the spleen was determined after each experiment. Calculations to determine \( p \) values involving figures based on percentage were calculated by using arc sine transformation. All values were compared against the appropriate controls and were considered significant at a level where \( p < 0.05 \) using Fisher's t test.

Hematological and Histological Procedure. Spleens were fixed in 10% formalin and stained with hematoxylin and eosin. Femoral bone marrow, nucleated cell counts × 10⁶/mg femoral bone marrow, and WBC counts of arterial and venous blood samples collected from the splenic artery and vein were made as has been previously described using a Coulter electronic cell counter (3). The percentages and types of nucleated cells were determined from Wright-stained blood and marrow smears. Leukocyte counts and packed RBC volume were determined for blood and spleen donor rats before each experiment.

RESULTS

Nonleukemic Rat Spleens Perfused with Blood of Tumor Cell-inoculated Rats. The data for the 1st set of experiments are in Table 1. The results show that the clearance values for 99mTc-S are markedly lower than are the control values in spleens of nonleukemic rats that are perfused with blood from rats that had been inoculated with tumor cells 1 hr or 6 days prior to exsanguination. This is true whether the calculations are based on total spleen weight or unit weight (per 0.1 g) of splenic tissue. Clearance was more markedly depressed when blood of 6-day-, as compared to 1-hr-, postinoculated rats was used as perfusate regardless of whether or not the WBC of the blood was reduced. The \( p \) value \(( p = 0.054 \) for percentage clearance per spleen, as compared to the \( p \) value \(( p = 0.033 \) for percentage clearance per unit weight of splenic tissue, in experiments in which nonleukemic spleens were perfused with blood of rats inoculated 1 hr previously is just short of the level of significance. No significant differences from control clearance values were observed for spleens perfused with blood of 1-, 2-, or 4-day-postinoculated rats. The weights of spleens of nonleukemic rats perfused with WBC-intact or WBC-reduced blood of 6-day postinoculated donors were significantly greater than the weights of nonleukemic spleens perfused with nonleukemic blood (control spleens). Perfusion of 6-day WBC-intact blood resulted in the sequestration of from 20 to 45 × 10⁷ leukocytes in the nonleukemic spleens, as evidenced by the difference between the WBC of the arterial inflow and venous outflow blood. Blood smear counts showed that about 90% of the sequestered cells were leukemic. In blood collected from 6-day-postinoculated leukemic rats, 20 to 65% of the leukocytes were...
leukemic blast-type cells, whereas, in blood of 1-, 2-, and 4-day-postinoculated rats, the percentage of leukemic cells ranged from 2 to 4%, 2 to 7%, and 5 to 10%, respectively. There was no net sequestration of cells in nonleukemic spleens that were perfused with other than 6-day WBC-intact leukemic blood. In experiments in which WBC-reduced leukemic blood was used as perfusate, the WBC could not be reduced below \( 4 \times 10^9 \) cells/cu mm (range, 4 to \( 25 \times 10^9 \) cells/cu mm) by the centrifugation method used to reduce the count because of differences in leukocyte densities.

**Spleens of Tumor Cell-inoculated Rats Perfused with Nonleukemic Rat Blood.** The data in Table 2 for the 2nd set of experiments indicate that the clearance of \(^{99m}\)Tc-S was significantly lower when calculated on the basis of either total or unit weight in spleens of rats inoculated with leukemic cells 1 hr before perfusion with blood of nonleukemic rats. Splenic clearance of \(^{99m}\)Tc-S remained unchanged from control values on the basis of total spleen weight in 1-, 2-, 4-, and 6-day tumor cell-postinoculated rats. However, clearance values were significantly lower when calculated on the basis of unit spleen weight in the 4- and 6-day-postinoculated rats in which the spleens were enlarged and weighed significantly more than those of the control group. The decrease in clearance values on a unit weight basis is accounted for entirely by the weight increases of the spleens. In the 4- and 6-day-postinoculated rats, clearance values per unit weight decreased 61 and 246%, respectively, while corresponding spleen weights increased by 55 and 244%. A significant decrease in femoral bone marrow cellularity was evident in the 1-hr, 4-day, and 6-day-postinoculated rats. In the 6-day-postinoculated rat, the hematocrit was normal and the marrow myeloblast count ranged from 25 to 67% of the nucleated cell counts \( \times 10^9 \) mg femoral bone marrow. Examination of histological sections showed the red pulp of spleens of 6-day-postinoculated rats and spleens of noninoculated rats perfused with WBC-intact 6-day leukemic blood to be infiltrated with leukemic blast-type cells. The spleens of the 6-day leukemic animals, however, were not as heavily infiltrated by leukemic cells as had been observed in earlier studies (4-7).

In 1 group of experiments, not included in the tables, spleens of 4 rats treated 1 hr previously with normal leukocyte injections were perfused with blood of nonleukemic rats. In this study, there was no significant difference found from control values in the mean percentage values for clearance. In any 1 experiment, regardless of the test conditions imposed, the percentage of \(^{99m}\)Tc-S removed from the blood remained relatively constant during the 2 hr of perfusion as shown by periodic comparison of arterial and venous blood radioactivity.

Examination of the data shows that the WBC of blood perfusate collected by aortic exsanguination from leukemic or control noninoculated blood donor rats (Table 1) is lower than the tail WBC of comparable spleen donor rats (Table 2). This is because the WBC of aortic blood is significantly less than that of rat tail blood (3).

**DISCUSSION**

The results show that there is a depression of clearance of colloidal sulfur in isolated spleens of nonleukemic rats that are perfused with blood of rats in which a myeloblastic leukemia is induced by i.v. inoculation of tumor cells. This response is biphasic and reversible in the early stage of the leukemia. Sulfur colloid clearance was depressed when the blood used to perfuse the spleens was collected from rats 1 hr after inoculation with tumor cells and from rats in a late stage of the leukemia. Blood of rats in intermediate stages of the leukemia, when used as perfusate, did not depress clearance. The high leukocyte count in blood collected from late-stage leukemic rats was not a factor in itself in depressing clearance since reducing the leukocyte count did not restore clearance to the control values. The inability to restore clearance to control levels with leukemic WBC-reduced blood may conceivably be due to the presence in the blood of inhibitory factors secreted by the leukemic cells prior to their removal. The finding that blood of leukemic rats can depress sulfur colloid clearance in perfused nonleukemic spleens indicates that RES depression in this rat leukemia may be associated with either a deficiency of RES-stimulatory factors (opsonins) or the presence of inhibitory factors in the blood. In studies on the Shay rat leukemia using an *in vitro* system, Di Luzio et al. (2) found that i.v. inoculation of tumor cells is associated with an

---

**Table 1**

Clearance of \(^{99m}\)Tc-S in spleens of nonleukemic rats perfused for 2 hr with blood of tumor cell-inoculated leukemic rats

<table>
<thead>
<tr>
<th>Postinoculation time</th>
<th>No. of experiments</th>
<th>WBC count of blood perfusate ( \times 10^9/\text{cu mm} )</th>
<th>Spleen wt (g)</th>
<th>% of (^{99m})Tc-S cleared per 0.1 g</th>
<th>% of (^{99m})Tc-S cleared per spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (noninoculated)</td>
<td>7</td>
<td>6.0 ± 0.4a</td>
<td>0.775 ± 0.04</td>
<td>4.5 ± 0.01</td>
<td>34.1 ± 0.06</td>
</tr>
<tr>
<td>1 hr</td>
<td>4</td>
<td>5.5 ± 1.9</td>
<td>0.757 ± 0.05</td>
<td>3.4 ± 0.01</td>
<td>25.6 ± 0.13</td>
</tr>
<tr>
<td>1 day</td>
<td>3</td>
<td>10.8 ± 0.9b</td>
<td>0.683 ± 0.06</td>
<td>4.1 ± 0.01</td>
<td>28.6 ± 0.06</td>
</tr>
<tr>
<td>2 day</td>
<td>3</td>
<td>12.5 ± 1.6b</td>
<td>0.821 ± 0.03</td>
<td>3.6 ± 0.01</td>
<td>31.3 ± 0.01</td>
</tr>
<tr>
<td>4 day</td>
<td>3</td>
<td>11.7 ± 0.2b</td>
<td>0.754 ± 0.04</td>
<td>4.4 ± 0.02</td>
<td>30.1 ± 0.08</td>
</tr>
<tr>
<td>6 day</td>
<td>5</td>
<td>78.3 ± 20.8b</td>
<td>1.129 ± 0.08</td>
<td>1.8 ± 0.01</td>
<td>19.7 ± 0.04</td>
</tr>
<tr>
<td>12 day</td>
<td>3</td>
<td>14.4 ± 7.1</td>
<td>0.947 ± 0.05</td>
<td>1.5 ± 0.01</td>
<td>15.0 ± 0.10</td>
</tr>
</tbody>
</table>

Note: * Control group in Table 1 and 2 is the same.

* Mean ± S.E.

* Significantly different from control; \( P < 0.05 \).
early depletion of humoral recognition factors necessary for liver Kupffer cells to phagocytize a gelatinized RE test lipid emulsion. An alternate possibility may be that malignant cells depress RES activity through the production and secretion of inhibitory factors. In in vitro studies it has been shown that leukocytes of leukemic patients can adversely affect the metabolism of RBC (17). Healthy or dying malignant cells may release immunosuppressive, cytostatic, or cytotoxic factors into the blood (1, 19), and bacteria residing in a necrotic tumor may also produce suppressive factors (12). In rats in the terminal stage of leukemia, hepatomegaly is evident and nephropathy associated with elevated serum lysozyme levels is a characteristic finding (14, 16). Thus, in the late stage of the disease, interpretation of the results is further complicated because of the possibility that nonspecific factors, associated with changes in the animals' metabolism, that are toxic may adversely alter RES function.

The results also show that there is a depression of clearance of colloidal sulfur in isolated spleens of rats inoculated i.v. with tumor cells 1 hr prior to perfusion with nonleukemic rat blood. An alteration in blood humoral factors could not be responsible for the depressed clearance in this group of experiments since the blood of normal rats was used as perfusate. Conceivably, this early depression may be due to the removal by the spleen of the inoculated tumor cells causing a temporary blockade of the RES. Cellular blockade may also be a possible contributing factor in the depressed clearance observed when nonleukemic spleens were perfused with blood of late-stage leukemic rats, since large numbers of leukemic cells become sequestered within the spleens. Clearance remained depressed, however, when the leukemic cell concentration of the blood was reduced, indicating that depression in the late stage of the leukemia may be primarily associated with blood humoral alterations. The sequestration of leukemic cells would explain the increase in the weights of the nonleukemic spleens resulting from perfusion with late-stage leukemic blood. The spleens of nonleukemic rats perfused with late-stage leukemic blood with reduced numbers of leukemic cells also showed a weight increase, although less than the weight increase found if the leukocytes were not reduced in number. This latter weight increase is more difficult to explain. Following the early initial depression of clearance observed in spleens of rats inoculated with tumor cells, clearance of sulfur colloid in spleens of rats in progressively advanced stages of the leukemia that were perfused with nonleukemic blood was found to be at the control level up to the death of the animal. These findings indicate that in the late stage of the leukemia there was no intrinsic impairment in splenic RES clearance of sulfur colloid.

In this study, the spleens of rats in the late stage of the leukemia had a more normal-appearance histology and the bone marrow a lower myeloblast percentage than in previously reported studies (4-7). This tumor over the years had displayed variability in biological characteristics. In initial studies by Shay (8, 18), it was necessary to use rats 7 days old or younger as recipients to assure successful transfer of the tumor. Since then, adult rats have been successful recipients and the tumor has lost its original green hue due to high verodoperoxidase concentrations (9). The survival time of rats inoculated with successive generations of the original tumor cells has decreased from 3 to 8 weeks (8, 9) to 7 to 10 days, and fewer cells are now required to induce leukemia (4-7). The pathogenesis of the tumor in the present study seems similar to that reported by Hoelzer and Harriss (10), in which rats inoculated with 10^7 leukemic cells develop a myelomonocytic leukemia and die by the 7th day with a 50% leukemic bone marrow cell population. Shortening of the latent period and changes in biological characteristics of transplantable tumors during serial animal passages are not uncommon events (8, 11, 13, 15). Sublines of the original cells with more virulent characteristics may develop in the host by selective proliferation. Such selectivity may be associated with changes in host resistance to different cell populations. In previous studies, animals with counts in excess of 70 to 80% marrow myeloblasts have been classified as being in a late stage of this leukemia (4-7), and sulfur colloid clearance was found to be depressed in the spleens of late-stage leukemic rats perfused with blood of nonleukemic rats (7). An inverse relation existed between the marrow myeloblast percentage and splenic RES function, and clearance values were depressed only in spleens of rats in which the marrow myeloblast percentage...
B. S. Dornfest

exceeded 70% of the total bone marrow cellularity. These findings are consistent with the present results, in that the myeloblast count did not exceed 67% of the total marrow cellularity in the late stage of the leukemia and clearance of sulfur colloid was not depressed in the spleen. In the present as compared to former studies, the bone marrow and spleen were not as heavily infiltrated with leukemic cells in the terminal stage of the leukemia. In comparing past data with present, it seems probable that impairment of RES function in this rat leukemia may be associated with both blood humoral and qualitative and/or quantitative RES cellular changes with the latter occurring subsequent to the humoral changes.

ACKNOWLEDGMENTS

I wish to acknowledge the technical assistance of Claire Lipp and to thank Dr. Nathan Solomon and Dr. Joseph Steigman who generously supplied the Tc-S. I also wish to thank Gordon Mestler, our statistician, for his assistance with the statistics.

REFERENCES

Relation of Disease Stage to Humoral and Cellular Impairment of Spleen Reticuloendothelial System Depression in a Rat Leukemia

Burton S. Dornfest


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/36/11_Part_1/4052

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.