SHORT COMMUNICATION

Red blood cell phagocytosis in the reticuloendothelial system in rats with carcinomatous anemia

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Introduction

Reduced hematopoiesis, decreased lifetime of red blood cells, enhanced destruction of red blood cells and malnutrition have been noted as causes of anemia associated with malignant tumors. However, the nature of this anemia differs with various types of carcinoma, and many aspects remain to be elucidated. In particular, there is little information on red blood cell phagocytosis of the reticuloendothelial system in animals with oral carcinoma.

Red blood cell phagocytosis in the reticuloendothelial system was evaluated in order to examine red blood cell destruction, a cause of anemia in animals with cancer of the oral cavity.

Methods

This study consisted of two groups of 20 (Wistar, 8-week-old, male) rats each. In the experimental group, cancer was induced by applying a 0.5% 9, 10-dimethyl-1, 2-benzanthracene (DMBA), solution in acetone over the cheek skin three times a week for 32 weeks. In the control group, acetone alone was applied in the same manner as above. Hematologic parameters (red blood cell count, hemoglobin level, and hematocrit value) were measured 32 weeks following initiation of the experiment for each rat in both groups.

$^{51}$Cr-labeled heat damaged red blood cells ($^{51}$Cr-HDRBCs) were then prepared by adding $\text{Na}_2^{51}\text{CrO}_4$ to the blood of normal rats and incubating the mixture at 49.5°C for 30 minutes. These $^{51}$Cr-HDRBCs were administered via the cervical vein to the rats in both groups at a dose of $500 \times 10^3$ cpm per 100 g body weight 32 weeks after initiation of the carcinogenesis experiment. Blood samples were thereafter collected via the tail vein at specified intervals. The $^{51}$Cr radioactivity was determined for both the blood cell fraction and the plasma fraction. Also, the rate of disappearance of $^{51}$Cr-HDRBCs was calculated assuming a value of 100% for the $^{51}$Cr radioactivity obtained immediately after administration of the $^{51}$Cr-HDRBCs. The $^{51}$Cr radioactivity per mg of organ tissue was also determined in the cheek skin (tumor), spleen and femur 120 minutes after administration of the $^{51}$Cr-HDRBCs. The histopathology of the tumors and spleen were examined.

Results

Tumors, designated histologically as squamous cell carcinoma, appeared at the DMBA...
application site within 32 weeks following initiation of the carcinogenesis experiment (Fig. 1). The red blood cell counts, hemoglobin levels and hematocrit values in the experimental group were lower than for the control group. Anemia also occurred in the former (Table 1).

The disappearance of $^{51}$Cr-HDRBCs was delayed in the experimental group (Fig. 2). At 120 minutes after administration of the $^{51}$Cr-HDRBCs, the $^{51}$Cr per mg in the cheek skin was $11.7 \pm 0.7 \text{ cpm/mg}$ in the experimental group, and $7.2 \pm 3.1 \text{ cpm/mg}$ in the controls. These values for the spleen were $574.2 \pm 251.6 \text{ cpm/mg}$ and $1287.4 \pm 548.1 \text{ cpm/mg}$ respectively. Therefore the $^{51}$Cr radioactivity per mg organ tissue in the experimental group was higher for the cheek skin and lower for the spleen.

Spleen weight in the experimental group was $1223.5 \pm 504.9 \text{ mg}$ while that of the controls was $831.4 \pm 134.4 \text{ mg}$. A nincrease in spleen weight and splenomegaly were noted in the experimental group (Fig. 3). Examination of the histopathology revealed that there was a decrease in the number of red blood cells in the red pulp, particularly in the marginal zone around the white pulp, together with reduced phagocytosis by macrophages. An increased number of nucleated cells, mainly white blood cells, was observed (Figs. 4, 5).

**Discussion and Conclusion**

Okamoto$^6$ of this department examined hematologically the relationship between local histological changes and anemia. He observed severe anemia in rats with carcinoma, but found that DMBA had no effect on the anemia.

Marsh et al.$^{1,5}$ reported an attempt to use the rate of disappearance of $^{51}$Cr-HDRBCs as a criterion for red blood cell phagocytosis in the reticuloendothelial system. If phagocytosis accelerates, the disappearance of $^{51}$Cr-HDRBCs will be increased, and vice versa. In cancerous rats, we evaluated red blood cell phagocytosis in the reticuloendothelial system by administration of $^{51}$Cr-HDRBCs. Its disappearance was delayed in comparison with normal rats. The heat damaged red blood cell phagocytosis seems to be reduced in cancerous rats.

The heat damaged red blood cells used in the present study were denatured by heating at $49.5\degree C$ for 30 minutes. Red blood cells damaged by such an experiment are known to show a reduced deformability of the cell membrane. They are mainly broken down in the spleen, as is the case with aged red blood cells$^{7-9}$. Incorporation of the administered $^{51}$Cr-HDRBCs showed decreased values in the spleens of cancerous rats as well as other organs, suggesting a reduction of heat

<table>
<thead>
<tr>
<th>Table 1 Hematological values in cancerous rats.</th>
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<tr>
<td><strong>Anemia occurred in cancerous rats.</strong></td>
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<tr>
<td><strong>Control group</strong></td>
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<tr>
<td>Red blood cells ($10^6/\mu l$)</td>
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<tr>
<td>6.56 ± 0.86</td>
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<tr>
<td><strong>Experimental group</strong></td>
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<tr>
<td>Red blood cells ($10^6/\mu l$)</td>
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<td>4.43 ± 1.14</td>
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mean ± S.D.
Fig. 2 A disappearance of $^{51}$Cr-labeled heat damaged red blood cells
The disappearance rate was low in the experimental group.

Fig. 3 The upper picture is from a spleen in the experimental group and lower is from the control group.
In the experimental group, the weight of the spleen was increased, and splenomegaly was noted.

Fig. 4 Histological image of the spleen in the experimental group
Only a few red blood cells are found around the white pulp. ×44, H-E.

Fig. 5 Histological image of the spleen in the control group
Many red blood cells are found around the white pulp. ×44, H-E.

damaged red blood cell phagocytosis in the reticuloendothelial system. With regard to the histopathology findings, there was a decreased number of red blood cells in the red pulp in the spleen of cancerous rats. Reduced red blood cell phagocytosis by macrophages was also observed. These findings suggest spleen dysfunction.

There have been reports of red blood cell phagocytosis in the reticuloendothelial system in cancerous animals. Ultmann$^{10}$ described hyperfunction of the reticuloendothelial system for red blood cells destruction in cancer patients, and Old et al.$^{11}$ reported that in mice with transplanted sarcoma, phagocytosis in the reticuloendothelial system accelerated during the first 12 days following sarcoma transplantation, decreased with the expansion of the tumor, and then returned to a normal level immediately before the host's death. These reported results are slightly different from those of this study. It should be noted, however, that Sato$^{12}$ reported a decrease in the capability of hemoglobin utilization in the reticuloendothelial system due to a reduction of the $^{59}$Fe re-utilization efficiency of red blood cells in $^{59}$Fe-labeled heat damaged red blood cells administered to cancerous rats. In this study red blood cell phagocytosis in the reticuloendothelial system did not accelerate in cancerous rats with anemia; on the contrary, our results suggested a reduction in the capacity for red blood cell destruction.

From the viewpoint of red blood cell metabolism, re-utilization of free hemoglobin lib-
erated through red blood cell phagocytosis is also an important function of the reticulo-endothelial system\textsuperscript{13,14}. Further investigations are needed to clarify the entire mechanism of red blood cell metabolism in the reticuloendothelial system.

References