Reduced Erythrocyte Deformability in Anemic Rats with Adjuvant Arthritis

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Accepted May 24, 1990

Abstract—Erythrocytes in anemic rats with adjuvant arthritis (AA) were less deformable compared with normal rats. Swelling of the spleen was noticed in anemic rats with AA. The treatment of anemic rats with recombinant human erythropoietin (r-HuEPO; 30 and 100 U/kg, i.v., for 5 days) resulted in a normalization of both the anemia and erythrocyte deformability. It is suggested that erythrocytes with reduced deformability may be sequestered by endothelial slits of the spleen, which may play a causative role in the anemia in rats with AA.

Erythropoietin (EPO) is a circulating glycoprotein hormone that regulates erythropoiesis, and the efficacy of recombinant human (r-Hu) EPO on chronic renal failure has been established in human clinical studies (1, 2) and animal experiments (3). We recently found that r-HuEPO was also effective against the anemia in rats with adjuvant arthritis (AA, 4). Previous results show, and our results confirm, that the anemia in rats with AA shares many characteristics with anemia in human RA (4, 5), although the mechanisms of these anemias are not clear. Because human or rat erythrocytes are 7–8 \( \mu \)m in diameter (6), they must deform to pass through small vessels, including extremely narrow (0.5–1 \( \mu \)m wide) endothelial slits in the spleen. It has been shown that reduced deformability results in a shortened life span of human erythrocytes (7, 8). The anemia in RA patients is characterized by a modest shortening of erythrocyte survival time (9, 10), although little is known about erythrocyte deformability in this anemia. The present study was undertaken to determine a) whether erythrocyte deformability is reduced in anemic rats with AA, and if so, b) whether the reduction of erythrocyte deformability in this model is modified by erythropoiesis with the administration of r-HuEPO.

Female Lewis rats from Charles River, weighing approximately 170 g (8 weeks old) at the beginning of the experiments, were used. All rats were given normal rat chow and water ad libitum and housed at a constant temperature, humidity, and light-dark cycle in animal quarters. A single intradermal administration of adjuvant was used to induce anemia and arthritis in rats as previously described (4). On day 0, the rats were injected in the right hind footpad with 0.05 ml of Freund's complete adjuvant: 2 mg/ml solution of heat-killed Mycobacterium butyricum (Difco) suspended in liquid paraffin oil. Blood (0.4 ml) was collected from the tail vein before and after adjuvant injection at various intervals. Blood samples were anticoagulated with EDTA and analyzed by auto blood cell analyzers (ELT-8, Ortho and HEG-120A, Omron). The criterion for anemia and arthritis was a 10% reduction of hemoglobin (Hb) concentration and a 2-fold increase in hind footpad thickness, respectively, as compared with normal (noninjected) rats. Rats found to be anemic and arthritic on day 20 were divided into three groups at random: 1) control (vehicle), 2) r-HuEPO (30 U/kg), and 3) r-HuEPO (100 U/kg). r-HuEPO or vehicle (20 mM citrate buffer containing 0.25% human serum albumin and 100 mM sodium salt) was intravenously injected into the tail vein daily for 5 days from day 21. On day 28, rats were anesthetized with sodium pen-
tobarbital (30 mg/kg, i.p.), and blood was collected from the abdominal aorta into a plastic syringe containing heparin as an anticoagulant.

Erythrocyte filterability was determined according to the method of Reid et al. (11). The erythrocyte count was adjusted to $5 \times 10^5$/ml after washing and floating with isotonic phosphate-buffered saline (90 mM NaCl, 5 mM KCl, 50 mM sodium phosphate, pH 7.4) containing 0.1% glucose. The erythrocyte suspension was filtered with a nucleopore membrane (3 µm in diameter) at negative pressure and 23°C. The time required to filter 0.5 ml of the erythrocyte suspension (filtration time) was used as an index of the ery-

Fig. 1. Comparison of hematologic data in normal rats and adjuvant-induced anemic rats with or without r-HuEPO treatment. r-HuEPO (®, 30, 100 U/kg, i.v.) or vehicle (control) was injected daily for 5 days from day 21. Data are expressed as the mean±S.E.M. of 8 rats/each group. *: P<0.05, **: P<0.01 vs. normal group (N, □□). #: P<0.05, ##: P<0.01 vs. control group (C, ▨).
throcyte deformability. Measurement of filtration time was done in duplicate, and the mean time was used for statistical analysis. ATP content in erythrocytes was determined in perchloric acid extracts with phosphocreatine kinase, as described by Lowry and Passoneau (12).

r-HuEPO used in the present study was produced by Chinese hamster ovarian cells in the laboratory of Kirin Brewery Co. (Tokyo, Japan), and its activity was assessed to be identical to natural EPO (3). The doses of r-HuEPO administered and the experimental protocol used in this study were according to our previous data (4). The results are expressed as the mean±S.E.M. of n rats. Statistical significance was determined by one-way analysis of variance. A P value of less than 0.05 was considered statistically significant.

Figure 1 shows the hematological data of normal rats and anemic rats treated or not treated with r-HuEPO. The anemia in rats with AA observed on day 20 persisted still on day 28 only in the vehicle-treated group (Fig. 1, A and B). As can be seen in Fig. 1, C and D, this anemia was characterized as microcytic (decreased MCV) and hypochromic (decreased MCH). The filtration time was significantly (P<0.05) prolonged in anemic rats compared with that of normal rats (60.9±0.4 sec, N=8, for anemic rats vs. 59.2±0.6 sec, N=8, for normal rats). r-HuEPO caused a dose-dependent improvement of the anemia, which can be seen in the Ht and Hb (Fig. 1, A and B). Prolonged filtration time was also normalized in r-HuEPO-treated rats, and there was no statistically significant difference (P>0.05) between r-HuEPO-treated and the normal groups (Fig. 1E). ATP content in erythrocytes from r-HuEPO-treated rats was increased in a dose-dependent fashion (Fig. 1F). The number of reticulocytes that reflects the erythropoietic state was significantly and dose-dependently increased in r-HuEPO-treated rats (Fig. 1G).

Body and tissue weights in normal and anemic rats are summarized in Table 1. Body weight was significantly (P<0.05) decreased in anemic rats. A significant (P<0.05) and marked increase (78.0%) was observed in the spleen, whereas a modest increase (4.8%) and a decrease (-5.8%) was observed in the kidney and the liver, respectively. Treatment with r-HuEPO had no obvious effects on the spleen weight (0.73±0.06 for the control, 0.76±0.05 for 30 U/kg, 0.70±0.03 for 100 U/kg).

The present study has shown that erythrocyte deformability was slightly but statistically significantly reduced in anemic rats with AA. The filtration method commonly used to evaluate erythrocyte deformability is affected by the erythrocyte volume. MCV in anemic rats with AA was found to be significantly reduced, which might enable erythrocytes to pass through the nucleopore membrane more easily. Thus, the overall prolongation of filtration time might be partially masked by a reduced MCV.

r-HuEPO administration resulted in a normalization of erythrocyte deformability, in addition to the improvement of anemia. EPO works via its own specific receptors on progenitor cells of erythrocytes to stimulate the proliferation of these cells, whereas EPO receptors are absent in mature erythrocytes. In the present study, r-HuEPO incubated with the erythrocyte suspension was without effect on erythrocyte deformability (data not shown). Thus, it is evident that the normalization of erythrocyte deformability by r-HuEPO was due to an increased population of newly

| Table 1. Comparison of tissue and body weights between adjuvant-induced anemic and normal rats |
|---------------------------------|----------|----------|-------------------|
| **Normal** | **Anemia** | **Anemia/Normal ( % difference)** |
| Whole body | 210±4 | 184±3** | -12.5 |
| Spleen | 0.41±0.01 | 0.73±0.06** | 78.0 |
| Kidney | 1.47±0.04 | 1.54±0.04 | 4.8 |
| Liver | 7.20±0.18 | 6.78±0.19 | -5.8 |

Values of body and tissue weights determined on day 28 are expressed as the mean±S.E.M. (g) of 8 rats. **P<0.01 vs. normal rats.
generated erythrocytes, namely, erythrocytes with reduced deformability were replaced by the deformable erythrocytes as a consequence of r-HuEPO-induced erythropoiesis.

Reduced erythrocyte deformability is considered to be due to a low surface-to-volume ratio, low ATP content, or high Hb content (13, 14). The last factor seems unlikely to be the cause of the reduced deformability in the present study, because MCH was decreased in the present anemia. There was no statistical difference in ATP content between normal and anemic rats. Thus, low ATP also seems to be unlikely as the cause of reduced deformability. r-HuEPO treatment induced a dose-dependent increase in ATP indicative of an increased population of newly generated erythrocytes. The possibility of a low surface-to-volume ratio was not excluded in the present study. However, morphological determination is clearly required to evaluate the mechanism of low erythrocyte deformability.

Anemia is actually a consequence of unbalanced generation and destruction of erythrocytes. The spleen is the main organ that destroys aged or injured erythrocytes, with its extremely narrow endothelial cells. In the present study, a significant increase in the spleen weight, as well as a reduced deformability, was observed in the anemic rat with AA, which is indicative of sequestration of less deformable erythrocytes by the spleen. The mild reduction of erythrocyte deformability in the present model might be explained by the sequestration of erythrocytes with markedly reduced deformability from circulating blood. Indeed, erythrocytes from the intra-splenic circulation are less deformable in rats with phenylhydrazine-induced hemolytic anemia, and reduction of erythrocyte deformability is enhanced after splenectomy in rats with iron deficiency (15). It is provocative to speculate that the sequestration of less deformable erythrocytes by endothelial slits in the spleen plays a causative role in the shortened half life of erythrocytes and subsequently the anemia in human RA.

In summary, our present data have first shown that anemic rats with AA have reduced erythrocyte deformability. It is also indicated that the reduction of erythrocyte deformability in this model is modified by erythropoiesis with the administration of r-HuEPO. The effects of r-HuEPO on the anemia associated with arthritis warrant a further study.

Acknowledgments: The authors wish to thank Mrs. T. Shimoji for excellent technical assistance and Miss T. Nagasawa for preparing the manuscript.

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