Response to Erythropoietin in Germfree Mice

The germfree animal possesses hematological properties that differ from normal. Thus, among other features, its leukocyte counts are lower[1] and its lymph nodes, spleen, liver, and other organs comprising the reticuloendothelial system are smaller[1,2]. The present study reports an augmented erythropoietic response to erythropoietin (ESF) in germfree mice. Similar results have been observed by KEIGHLEY[3] in the fasted specific pathogen-free rat.

Adult male germfree mice (HAUSCHKA-MIRAND/ICR Swiss strain) were obtained from the Charles River Breeding Laboratories, Boston, Massachusetts. They were kept in flexible plastic isolators of the Trexler type[4] to maintain the axenic environment. The conventional mice were of the same strain but raised under ordinary laboratory conditions. These were also placed in Trexler isolators to preclude the possibility that any effects occurring in the germfree animals were due to confinement in the isolators. The axenic mice were given sterilized food and water[5] while the conventional mice had access to ordinary non-sterile food and water. For the germfree animals, samples of food, water and stools were cultured daily as a check on the axenic environment of the isolators. Routine microbiological tests were made for bacteria, fungus, PPLO and other parasites. At the termination of the experiments, the blood, fecal contents, lung, liver and spleen from the germfree mice were cultured as a final check on the maintenance of the germfree state.

Half of the germfree animals were transfused i.p. on 2 successive days with 1 ml of homologous germfree packed red cells[6]. These cells had been washed in sterile saline under aseptic conditions prior to injection. Half of the conventional mice were similarly transfused but with washed red cells from non-axenic mice. Experimental treatment was begun 3 days after the last injection of red cells. In these plethorized groups, mice with hematocrit values less than 60% at the termination of the experiments were not included in the data.

Four groups of mice were established: (A) axenic hypertransfused, (B) axenic non-transfused, (C) conventional hypertransfused, and (D) conventional non-transfused. Half of the animals in each of these 4 groups were injected s.c. with Step III sheep ESF (lot No. K147137 supplied by the National Heart Institute, NIH) at a dose level of 1 U/day in 0.2 ml sterile saline for 3 days. Prior to use, the ESF was passed through a Millipore filter (HA –.45 μ) to ensure sterility. The remaining 50% of mice in each of the 4 groups received s.c. injections of 0.2 ml sterile saline, the vehicle for the hormone, daily for 3 days. All mice were injected i.m. with 2 mg iron dextran (Imferon, Lakeside laboratory, Wisconsin) on the day that ESF or vehicle injections were begun to preclude changes in circulating iron pool that could falsify interpretation of the RBC Fe^{59} incorporation values. At 24 h after the last injection of ESF or the vehicle, the mice received i.v. injections of 0.5 μc Fe^{59} in 0.2 ml sterile saline; 48 h later blood was drawn from the dorsal aorta and hematocrit and % RBC Fe^{59} incorporation values determined[6]. Peripheral red, reticulocyte and white cell counts, and hemoglobin concentrations were also recorded at this time.

The Table indicates that ESF stimulated per cent RBC Fe^{59} incorporation to a considerably greater extent in the germfree than in the conventional mice. The peripheral reticulocyte levels in these 2 groups of mice paralleled closely the radioiron incorporation values. Although this augmented response was noted in both the plethorized and non-plethoric germfree mice, it occurred much more strikingly in the former group. No significant effects were produced in the 4 groups of ESF on hemoglobin levels and red cell counts in these short-term experiments. White cell counts were significantly lower in the axenic than in the conventional mice but these values were not significantly altered by ESF.

The explanation for the increased erythropoietic response to ESF in the germfree as compared to the conventional animal remains obscure. One possibility is that greater numbers of responsive stem cells are present or that an increased sensitivity of these elements to ESF

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Response to erythropoietin (ESF) of axenic and conventional hypertransfused and non-hypertransfused mice (means ± S.E.m.)

Group	No. mice	Body wt. (g)	WBC/mm³	Hb (g)	RBC (mi 11/mm³)	Hct %	% RBC Fe^{59} incorporation
(A) Axenic hypertransfused							
1. ESF	18	29.0 ± 0.57	4,405 ± 320	18.7 ± 1.16	11.1 ± 0.71	63.6 ± 1.24	61.6 ± 2.42
2. Saline	18	28.9 ± 0.32	3,759 ± 287	20.2 ± 0.92	11.4 ± 0.62	65.3 ± 1.46	0.78 ± 0.041
(B) Axenic non-transfused							
1. ESF	18	27.8 ± 0.29	3,299 ± 184	14.3 ± 0.84	8.7 ± 0.54	48.2 ± 0.93	50.9 ± 1.85
2. Saline	18	27.6 ± 0.22	2,821 ± 112	14.1 ± 0.72	8.5 ± 0.66	44.8 ± 0.88	35.5 ± 2.23
(C) Conventional hypertransfused							
1. ESF	17	29.2 ± 0.41	6,275 ± 330	22.9 ± 1.03	12.6 ± 0.75	70.5 ± 2.32	30.3 ± 0.824
2. Saline	17	27.2 ± 0.39	5,288 ± 294	20.8 ± 1.17	12.2 ± 0.68	65.5 ± 1.83	0.63 ± 0.117
(D) Conventional non-transfused							
1. ESF	18	27.0 ± 0.52	4,065 ± 277	14.6 ± 0.96	8.7 ± 0.48	47.7 ± 1.19	34.0 ± 1.54
2. Saline	18	26.0 ± 0.43	3,582 ± 117	14.1 ± 0.78	8.6 ± 0.53	43.5 ± 0.98	30.2 ± 1.86

exists in the germfree animal. Another possibility is that the axenic animal provides an environment conducive to augmented activity of the ESF. This could occur if the level of circulating erythropoiesis-inhibiting factor[7,8] was lower in the germfree animal. Finally, it is conceivable that a decreased rate of hormone destruction occurs in the axenic mouse. In this regard, the liver has been implicated as a site of inactivation of the ESF[9,10]. The finding that the liver is less developed, both morphologically and functionally, in germfree than in conventional animals[11,12] supports the possibility that a diminished rate of destruction of ESF occurs in the axenic animal. This could be tested by determining the sojourn time of exogenous and endogenously-produced ESF in the circulation of germfree animals[13].

Zusammenfassung. Unsere gegenwärtigen Ergebnisse zeigen, dass keimfreie Mäuse dem Erythropoietin (ESF) gegenüber einen verstärkten erythrocytenerzeugenden Faktor aufweisen als normale Mäuse.

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The Activity of Erythrocyte Glucose-6-phosphate Dehydrogenase as an Indicator of the Rate of Erythropoiesis

The differences of glucose-6-phosphate dehydrogenase activity (G-6-PD) in red cells of different age have been used for the estimation of red cell mean age[1-4] and for the evaluation of the rate of erythropoiesis[3-5]. Nevertheless, direct comparison between erythrocyte G-6-PD activity and the rate of erythropoiesis, investigated by one of the more accurate methods has not been reported, except a preliminary observation on a small number of patients[3]. In this paper, the correlation between erythrocyte G-6-PD activity and between the rate of erythropoiesis, estimated by the radioiron method is described and compared to that of G-6-PD and reticulocytes.

In a group of 29 subjects with various blood disorders, determination of erythrocyte G-6-PD activity (performed by the method of Kornberg and Horecker[6], with some modifications, described in [3]), ferrocinetic studies (carried out as given in [7]) and current blood examinations were made at the same time. Results and main clinical data on investigated individuals are summarized in the Table. When erythrocyte G-6-PD activity was plotted against red blood cell iron turnover rate (RBCITR), good, statistically significant correlation in both parameters was found. On the other hand, no correlation was stated between RBCITR and reticulocytes both in absolute or in relative values (Figure). Thus, these results suggest that the determination of red cell G-6-PD activity could provide more accurate information of the rate of erythropoiesis than reticulocyte counts, though approximate information can be obtained by the latter[8].

One of the most likely explanations of this difference in G-6-PD and reticulocyte sensitivity for an evaluation of erythropoiesis is probably a short period during which young erythrocytes of peripheral blood are present as reticulocytes, being on the average 39 h[9], while G-6-PD activity decreases gradually during the ageing of red cell[10] and thus better reflects the presence of increased number of young red cells in the peripheral blood. This explanation is also supported by the fact that, in decompensated pernicious anaemia, reticulocytosis after B_{12} treatment disappears essentially sooner than the elevation of erythrocyte G-6-PD activity, the latter change being more sensitive and demonstrable even at that time, when other current hematological features of erythropoietic stimulation disappear[3,11].

Nevertheless, the above given conclusions on the rate of erythropoiesis, based on erythrocyte G-6-PD determination, can be made only in subjects having normal pituitary and thyroid function, or not being treated by larger doses of corticoids, since all these conditions have

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