

the control level. The efficiencies of transfection of both the above types of recipients under these circumstances were the same. Consequently, the increase in total absorption of infectious DNA molecules was evidently not the decisive factor in the formation of competence for these types of recipients and cannot in general be used as a prognostic criterion of the possibility of realization of the subsequent stages of transfection.

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CHANGES IN PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS AFTER ADRENALECTOMY

G. I. Bezin and O. O. Romashko

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In the early stages after adrenalectomy a decrease in the total cell population and in the number of colony-forming units (CFU) in the bone marrow is found in (CBA×C57BL)_F₁ mice and is accompanied by a significant increase in the number of proliferating stem cells in the bone marrow and spleen, determined by the "thymidine suicide" method. After normalization of the total number of CFU, followed by the total cell population of the bone marrow, the level of dividing stem cells returns to its initial value.

KEY WORDS: adrenalectomy; hematopoietic stem cells; proliferation; migration; bone marrow; spleen.

The level of endogenous glucocorticoids is an important component of the system controlling migration and recirculation of hematopoietic stem cells (colony-forming units, CFU_s) at the whole-body level [2]. Experiments on mice have shown a sharp decrease in the number of CFU_s in the bone marrow 24 h after adrenalectomy, with a return to their normal number by the end of the first week after the operation, whereas increased liberation of stem cells into the circulating blood and spleen was observed throughout this period [5]. Such rapid recovery of the numerical composition of CFU_s in the bone marrow could have been the result of stimulation of their proliferation [3]. The object of the present investigation was to test this hypothesis experimentally.

EXPERIMENTAL METHOD

At various times after bilateral adrenalectomy on (CBA×C57BL)_F₁ mice the number of CFU_s in the bone marrow and spleen and their total cell population were determined by the method of exogenous colony formation. At the same time the proportion of proliferating CFU_s was determined as in [6]: Suspensions of hematopoietic cells were incubated at 37°C for 30 min and the specific activity of thymidine-³H added was 200 μCi/ml. The results of the investigation were assessed by Student's t-test.

EXPERIMENTAL RESULTS

The number of CFU_s in the bone marrow 24 h after adrenalectomy was reduced almost by half

Institute of Biophysics, Ministry of Public Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 89, No. 3, pp. 326-327, March, 1980. Original article submitted March 6, 1979.

TABLE 1. Changes in Numerical Composition of CFU_s and Cell Population of Bone Marrow and Spleen of Mice After Adrenalectomy (M±m)

Time after operation, days	Mock adrenalectomy				Adrenalectomy			
	bone marrow		spleen		bone marrow		spleen	
	cell popula. millions	No. of CFU _s in femur	cell popula. millions	No. of CFU _s in spleen	cell popula. millions	No. of CFU _s in femur	cell population millions	No. of CFU _s in spleen
1	17.4±0.6 (23)	4559±348 (20)	238±27 (24)	2378±302 (22)	11.4±1.1* (22)	2317±232* (22)	256±16 (22)	4496±421* (21)
3	18.6±1.1 (15)	5078±447 (21)	173±13 (15)	2688±195 (23)	13.7±0.9* (13)	3238±462* (19)	228±19* (14)	4724±322* (19)
5	19.6±1.0 (12)	4760±325 (25)	225±11 (13)	2186±208 (25)	16.1±1.2* (17)	4880±472 (26)	261±18 (17)	6174±276* (27)
7	20.1±1.2 (17)	5268±615 (27)	215±14 (17)	2724±149 (28)	18.9±1.4 (20)	4956±382 (30)	297±47 (21)	3621±234* (32)
14	19.3±0.8 (22)	4086±266 (18)	269±17 (22)	2362±310 (19)	21.0±0.7 (23)	4559±348 (17)	315±12* (23)	2518±187 (17)
21	19.7±1.2 (10)	3878±310 (8)	330±23 (11)	1986±228 (8)	19.7±1.7 (12)	4094±446 (9)	398±17* (12)	2269±193 (9)

*Differences from control significant (P<0.05).

Legend. Number of mice given in parentheses.

TABLE 2. Changes in Number of Proliferating CFU_s (in %) in Bone Marrow and Spleen of Adrenalectomized Mice

Time after operation, days	Mock adrenalectomy		Adrenalectomy	
	bone marrow	spleen	bone marrow	spleen
1	0; 0; 8; 5; (3, 2)	0; 3; (1, 5)	26.6; 30.8; 21.7; 20.0. (24.8)	28.6; 33.3. (30.9)
3	0; 4.6; 6.0. (2,6)	0; 5.6. (2,8)	30.2; 70.8; 41.2. (47.4)	8.2; 41.3. (24.7)
5	9.3; 11.2. (10,2)	4; 6.3. (5,1)	25.1. 29.5. (27,3)	16.4; 11.1* (13,7)
7	2.6; 9.3. (5,9)	0; 2.0. (1,0)	11.7; 25.0. (18,3)	10.2; 5.3* (7,7)
14	7.1; 0. (3,5)	8.0; 0. (4,0)	22.3; 16.8. (19,5)	19.0*; 0. (9,5)
21	7.8	9.0	13.1*	11.5*

*Difference from control not significant (P>0.05).

Legend. Mean values given in parentheses.

and the total cell population also fell (Table 1). Very significant stimulation of proliferation of bone-marrow stem cells was observed at this time (Table 2). After 3 days the total number of myelokaryocytes and CFU_s began to increase, but was still significantly below normal. There were more dividing CFU_s than at the previous time. On the 5th day after adrenalectomy the number of stem cells in the bone marrow was back to normal, the cell population was still below the control level, and proliferation of CFU_s remained increased. Later (7th-21st days) the number of nucleated bone marrow cells and of CFU_s was close to the number in the control mice, and after 7 and 14 days the number of stem cells in the S-phase was still increased. Changes in these indices in the spleen were of a different character. The number of CFU_s 1-7 days after adrenalectomy was significantly increased, and there was also some increase in the total cell population of the spleen. A marked increase in the intensity of proliferation of splenic CFU_s was not observed until after 1-3 days, and later this change was ill-defined or absent altogether.

Stimulation of migration of stem cells from the bone marrow in the hypocorticoid state [3] leads to a marked decrease in the number of nucleated cells in the marrow, including the number of stem cells, as the present investigation showed. On the 1st-3rd days after adrenalectomy proliferation of CFU_s was stimulated. Evidently on account of this the number of bone-marrow stem cells was restored to normal as early as after 5 days, although increased migration of CFU_s from the bone marrow continued even later after removal of the adrenals. Evidence of this was given by observations showing that 7 and 14 days after adrenalectomy the number of CFU_s in 1 ml peripheral blood was 81±6 and 39±4 compared with 25±3 and 23±3 respectively in the control mice (P<0.05). The considerable increase in the number of CFU_s in the spleen after 1-7 days could have been the result both of the increased supply of stem cells from the bone marrow and of activation of proliferation of CFU_s present in the spleen.

It was shown previously in experiments involving transplantation of cells from intact syngeneic bone marrow into adrenalectomized, lethally irradiated recipients, that the hypocorticoid state does not affect repopulation of the spleen by exogenous CFU_s and is not accompanied by stimulation of their proliferation in the spleen [3]. It may be that the dividing CFU_s which we detected in the spleen of the adrenalectomized mice had received the stimulus for proliferation earlier — before their arrival in the spleen.

The increased emigration of CFU_s from the bone marrow, induced by the hypocorticoid state, was thus accompanied by activation of stem cell proliferation. This was possibly a reaction to the sharp decrease in the number of myelokaryocytes, on the "feedback" principle. However, the possibility cannot be ruled out that lowered concentrations of endogenous glucocorticoids in the blood initially induce stimulation of CFU_s proliferation, as has been shown in the case of thymus cells [8]. The problem of the role of the T-cell population in this phenomenon also requires explanation, for on the one hand it is functionally closely dependent on the glucocorticoid level in the body [1, 7] and, on the other hand, it has itself a significant influence on the state of migration of stem cells [4].

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