EFFECT OF HYDROCORTISONE ON THE STAGES OF IMMUNOGENESIS

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UDC 612.017.1.014.46: 615.357.453

The effect of hydrocortisone was studied on the following stages of immunogenesis: migration of hematopoietic stem cells from the bone marrow; migration of B lymphocyte precursors of antibody-forming cells; cooperative interaction of T and B lymphocytes in the primary immune response to antigenic stimulation by sheep's red cells. Experiments were carried out on F_1(CBA × C57BL) mice. Hydrocortisone, in doses not inhibiting proliferation of hematopoietic cells, had a marked immunodepressive effect which was the resultant of inhibition between different stages of immunogenesis: migration of stem cells and B lymphocytes and cooperation between T and B lymphocytes.

KEY WORDS: hydrocortisone; T and B lymphocytes; migration; cellular interaction; immune response.

Corticosteroids are known to inhibit both the humoral and the cellular immune response [3, 9, 14]. However, the mechanisms of the immunodepressive action of these substances have not been adequately studied.

Recent research has revealed the basic stages of immunopoiesis: migration of stem cells from the bone marrow into the central lymphoid organs – the thymus and the bursa of Fabricius or its analog, differentiation of these cells into T and B lymphocytes, further dissemination of lymphocytes into peripheral lymphoid organs, and cooperative interaction between T and B lymphocytes during the immune response to antigenic stimulation [2, 4, 7, 10, 12, 15]. In the light of these findings it is important to study the effect of corticosteroids on the concrete stages of immunopoiesis.

The most interesting problem is the study of doses of a preparation that do not inhibit proliferative activity of cells of the hematopoietic system and, in particular, the stem cells. The writers showed previously that hydrocortisone acetate, in doses of 20-50 mg/kg, had no effect or only a very slight mitostatic action on proliferation of stem cells [3, 14]. Meanwhile the drug has a clear selective lymphotoxic action (prevents T lymphocytes from inducing a graft versus host reaction) in a dose of 5 mg/kg.

In the present investigation the action of the above doses of hydrocortisone acetate was assessed with the aid of appropriate models on the following stages: 1) migration of colony-forming stem cells (CFCs) from the bone marrow; migration of B lymphocytes which are precursors of antibody-forming cells (AFCs); 3) cooperative interaction between T and B lymphocytes in the course of the primary immune response.

EXPERIMENTAL METHOD

F_1(CBA × C57BL) mice aged 3-4 months and weighing 22-24 g were used. The animals were irradiated on the RUM-17 apparatus at a dose rate of 215 R/min and on the ÉGO-2 apparatus with Co^{60} γ rays at a dose rate of 300 R/min. The effect of the preparation on migration of the stem cells was studied on an experimental model devised previously [5]. Mice were irradiated in a dose of 800 R but with part of their hind limbs up to the midcalf level screened. On the seventh to eighth day after irradiation the number of colonies formed from stem cells migrating from the protected area of bone marrow was counted in the spleen. Spe-

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Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 11, pp. 63-66, November, 1975. Original article submitted June 25, 1974.

TABLE 1. Effect of Hydrocortisone on Migration of Stem Cells from Bone Marrow

Dose of hydrocortisone (in mg/kg)	Number of animals	Number of AFCs migrating from bone marrow (M ± m)	P
—	10	9,4±0,6	—
5	10	9,0±0,8	>0,5
20	12	7,1±0,9	<0,05
50	15	5,8±0,8	<0,05

cial investigations showed that the growing colonies developed only from stem cells migrating from the screened area [7]. To study the effect of hydrocortisone on migration of the stem cells the drug was injected intraperitoneally in doses of 20 and 50 mg/kg during the 2 days after irradiation, once a day.

Migration of the B lymphocytes of the bone marrow into peripheral lymphoid organs [6, 8] also was assessed in lethally irradiated animals with part of their bone marrow screened. For this purpose, 2 days after irradiation the animals were given an intravenous injection of a suspension of $2 \cdot 10^7$ syngeneic thymocytes mixed with $2 \cdot 10^8$ sheep's red cells. Control mice received an injection of sheep's red cells only after irradiation. On the eighth day after immunization of the mice the number of AFCs was determined by Jerne's method [11]. The number of AFCs in this model was determined entirely by the number of B cells migrating to the spleen from the screened bone marrow, for the number of transplated thymocytes interacting cooperatively with these cells was the same in all groups compared; this was shown by previous investigations [4]. Changes in migration of B lymphocytes in one way or the other produced by the drug led to a corresponding change in the number of AFCs. To study the effect of hydrocortisone on migration of the B lymphocytes the drug was injected intraperitoneally in a dose of 20 mg/kg during the first 2 days after irradiation and before transplantation of the thymus cells.

To study the activity of hydrocortisone on cooperation between B and T lymphocytes the following model was used [13]. Three hours after whole-body irradiation of the mice in a dose of 800 R they received an intraperitoneal injection of hydrocortisone (20 mg/kg), and 30 min later bone marrow cells (B lymphocytes) in a dose of $1 \cdot 10^7$, thymus cells (T lymphocytes) in a dose of $2 \cdot 10^7$, or a mixture of these cells were transplanted into them. At the same time, $2 \cdot 10^8$ sheep's red cells were injected into the animals. On the eighth day after injection of cells and antigen, the number of AFCs in the spleen of the recipient mice was determined by Jerne's method [11].

The numerical results were subjected to statistical analysis with calculation of the standard error (m), the arithmetic mean (M), and the level of significance of the differences (P) by the Student–Fisher method or with the aid of the 95% ($P \leq 0.05$) confidence limits (1_p) as described in [1].

EXPERIMENTAL RESULTS

As Table 1 shows, after irradiation of mice, with part of their bone marrow screened, 9.4 ±0.6 colonies were formed in their spleen on account of stem cells migrating from the protected area. After administration of hydrocortisone in doses of 20 and 50 mg/kg, 7.1 ±0.9 and 5.8 ±0.8 colonies respectively appeared in their spleen. Hydrocortisone, in the above doses, does not affect proliferation of hematopoietic stem cells and does not alter the number of endogenous hematopoietic colonies growing after irradiation in sublethal doses [3, 14]. Consequently, in a dose of 20-50 mg/kg, hydrocortisone reduces the intensity of migration of stem cells from the bone marrow.

The study of the effect of hydrocortisone on migration of the B cells gave the following results (Table 2). In the group of mice irradiated with part of the bone marrow screened, and receiving an injection of syngeneic thymocytes and sheep's red cells 2 days after irradiation, 625.0 ±47.8 AFCs were found in the spleen as the result of migration of B lymphocytes, cooperating with the transplanted thymocytes, from the bone marrow into the spleen. However, only 69.0 ±17.6 AFCs were formed in the spleen of mice undergoing similar treatment but receiving hydrocortisone in a dose of 20 mg/kg during the first day after irradiation. These results are evidence of marked inhibition by hydrocortisone of the migration of B lymphocytes from the bone marrow.

The results of experiments to study the effect of hydrocortisone on cooperation between T and B lymphocytes in the immune response (Table 3) show that 18.1 ±3.0 AFCs were formed in the spleen of the immunized recipients of bone marrow cells (B lymphocytes), 17.6 ±2.9 were formed in the recipients of thymus cells (T lymphocytes), and 191.1 ±21.2 AFCs were formed in recipients of a mixture of these two types of cells. This sharp increase in the intensity of the immune response in the last group of mice is the result of the well-known phenomenon of cooperation between T and B lymphocytes [12, 13], leading to a considerable increase in the number of antibody producers.

In recipients of bone marrow cells receiving hydrocortisone, 13.0 ±1.2 AFCs were formed in the spleen. After administration of hydrocortisone to recipients of thymocytes 13.7 ±2.4 AFCs were formed,

TABLE 2. Effect of Hydrocortisone on Migration of B-Lymphocytes from Bone Marrow

Scheme of experiment	Dose of hydrocortisone (in mg/kg)	Number of animals	Number of AFCs in spleen after screening of bone marrow and injection of $2 \cdot 10^7$ thymocytes and $2 \cdot 10^8$ sheep's red cells (M ± m)	lp
Screening of BM + SRBC	—	8	259,3±22,4	206,4—312,2
Screening of BM + T + SRBC	—	8	625,3±47,8	509,5—741,1
Screening of BM + T + SRBC	20	8	69,0±17,6	27,5—110,5

Legend. BM) Bone marrow, SRBC) sheep's red cells, T) thymocytes.

TABLE 3. Effect of Hydrocortisone (20 mg/kg) on Cooperation between T and B Lymphocytes after Transplantation of Mixture of Bone Marrow and Thymus Cells into Irradiated Animals

Number of transplanted cells ($\cdot 10^7$)		Hydrocortisone given	Number of animals	Number of AFCs in spleen	
bone marrow	thymus			M±m	lp
10	—	—	15	18,1±3,0	11,7—24,5
10	—	+	15	13,0±1,2	10,4—15,6
—	20	—	15	17,6±2,9	11,4—23,8
—	20	+	15	13,7±2,4	8,6—18,8
10	20	—	15	191,5±21,2	145,8—236,5
10	20	+	15	46,2±11,0	22,8—69,7

whereas in recipients of a mixture of these two types of cells, treated with hydrocortisone, the number of AFCs in the spleen was 46.2 ±11.0. These results are clear evidence that hydrocortisone has virtually no effect on the immune response in isolated populations of bone marrow or thymus cells but it sharply inhibits the immune response of a mixture of these cells, the result of their cooperative interaction.

It can be concluded from the results of these experiments as a whole that hydrocortisone, in doses without any inhibitory action on proliferation of stem cells, possesses a well-marked immunodepressive action. This action is the combined result of inhibition of the various stages of immunogenesis: migration of stem cells, migration of B cells, and cooperative interaction of T and B lymphocytes. The action of this preparation and, evidently, of the whole group of corticosteroids on immunogenesis is complex in character and takes place at different stages of immunogenesis. These experiments demonstrate that it is possible, in principle, to act on the immune response through factors influencing its separate stages.

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