Since the formation of the immune response takes place on account of cooperative interaction between different populations and subpopulations of cells in different lymphoid organs, the role of several proteinase inhibitors in the regulation of different stages of immunogenesis can be postulated.

The results thus confirm existing views on the importance of proteolytic reactions in the activation and transformation of lymphocytes and they indicate the possibility of their control by blood serum α_1-antiproteinase inhibitor.

LITERATURE CITED

1. F. S. Baranova, O. G. Ogloblina, L. K. Popova, et al., Byull. Éksp. Biol. Med., No. 5, 569 (1978).
2. R. K. Arora, H. C. Miller, et al., Nature, 274, 589 (1978).
3. A. Böyum, Tissue Antigens, 4, 269 (1974).
4. P. S. Chase, Cell. Immunol., 5, 544 (1972).
5. D. A. Hart, Cell. Immunol., 32, 146 (1977).
6. R. Hirschhorn, J. Grossman, W. Troll, et al., J. Clin. Invest., 50, 1206 (1971).
7. I. E. Liener, O. R. Garrison, and Z. Pravda, Biochem. Biophys. Res. Commun., 51, 436 (1973).
8. J. J. Lipsky, R. W. Berninger, L. R. Hymon, et al., Fed. Proc., 37, 1336 (1978).
9. S. Nakamura, M. Joshinaga, and H. Hayashi, J. Immunol., 117, 1 (1976).
10. H. G. Remold and R. P. Rosenberg, J. Biol. Chem., 250, 6608 (1975).

SUPPRESSIVE EFFECT OF BONE MARROW CELLS OF NORMAL AND LEUKEMIC MICE ON ANTIBODY PRODUCTION BY SPLEEN CELL CULTURES In Vitro

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The suppressive activity of bone marrow cells from AKR and (CBA × C57BL)F_1 mice aged 2 and 10 months in relation to the primary immune response of spleen cells to sheep's red blood cells in vitro was investigated. In leukemic AKR mice the suppressive activity of the bone marrow was shown to rise considerably until the 9th-10th month compared with that at the age of 2 months. In (CBA × C57BL)F_1 mice the suppressive activity of bone marrow at these same times was unchanged.

KEY WORDS: leukemia; bone marrow; suppression.

Recent investigations have demonstrated the regulatory role of bone marrow in the immune response. On the one hand, it stimulates antibody production in both the inductive [6] and productive [1, 12] phases of antibody synthesis. On the other hand, bone marrow cells can also depress the immune response of spleen cells [2-4].

The object of this investigation was to analyze the ability of bone marrow cells of (CBA × C57BL)F_1 mice and mice of the leukemic strain AKR, of different ages, to suppress the immune response of spleen cells cultured in vitro. AKR mice constitute a unique model with which to study the pathology of the immune system during growth of tumors. The oncogenic RNA-containing Gross virus, which is transmitted vertically, is maintained in these mice. This virus is present in carriers for several months in a latent form and it induces the formation of a thymoma, followed by disseminated lymphatic leukemia [9].

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TABLE 1. Suppressive Action on Antibody Production in Culture of Bone Marrow Cells from AKR Mice (averaged results of 3 experiments)

Group	Number of cells in culture, × 10^6				Spleen: bone marrow ratio	Survival rate of cells on 4th day in culture, %	Number of AFC per 10^6 living cells	Suppression of immune response, %
	spleen		bone marrow					
	2 months	10 months	2 months	10 months				
1	2,5	—	—	—	—	60	967,8±89,6	—
2	4	—	—	—	—	64	659,7±47	—
3	4,5	—	—	—	—	51	595,4±43	—
4	—	2,5	—	—	—	72	36±16,2	—
5	—	4	—	—	—	46	60±26,8	—
6	—	4,5	—	—	—	55	11,3±6	—
7	2,5	—	2,5	—	1:1	65	0	100
8	4	—	1	—	4:1	49	56±16,3	91
9	4,5	—	0,5	—	9:1	49	210±45,6	63
10	4,5	—	0,3	—	15:1	46	440±37,2	25
11	2,5	—	—	2,5	1:1	44	0	100
12	4	—	—	1	4:1	41	0	100
13	4,5	—	—	0,5	9:1	41	0	100
14	4,5	—	—	0,3	15:1	48	160±24,1	73

TABLE 2. Suppressive Action on Antibody Production in Culture of Bone Marrow Cells from (CBA × C57BL)F_1 Mice

Group	Number of cells in culture, × 10^6				Spleen: bone marrow ratio	Survival rate of cells on 4th day in culture, %	Number of AFC per 10^6 living cells	Suppression of immune response, %
	spleen		bone marrow					
	2 months	10 months	2 months	10 months				
1	2,5	—	—	—	—	30	639,7±130,2	—
2	4	—	—	—	—	60	496,3±51,7	—
3	4,5	—	—	—	—	40	763,3±128,1	—
4	—	2,5	—	—	—	70	548,7±16,2	—
5	—	4	—	—	—	63	503,3±14,5	—
6	—	4,5	—	—	—	65	671,3±16,2	—
7	2,5	—	2,5	—	1:1	38	44,7±17,3	92
8	4	—	1	—	4:1	30	260±10,2	48
9	4,5	—	0,5	—	9:1	46	296,3±23,2	62
10	4,5	—	0,3	—	15:1	44	580±46,5	24
11	2,5	—	—	2,5	1:1	62	48,7±7,8	93
12	4	—	—	1	4:1	53	195,7±18,6	74
13	4,5	—	—	0,5	9:1	54	249,5±9,5	65
14	4,5	—	—	0,3	15:1	42	592±4,2	22

EXPERIMENTAL METHOD

AKR and (CBA × C57BL)F_1 mice aged 2 and 9-10 months were used in the experiments. The development of leukemia in AKR mice was judged from the presence of blast forms in the peripheral blood and bone marrow.

Suspensions of bone marrow and spleen cells were cultured by a modified method of Mishell and Dutton in serumless medium with the addition of 10% embryonic calf serum, glutamine, and 2-mercaptoethanol. Sheep's red blood cells (SRBC) were used as antigen. On the 4th day of culture the number of antibody-forming cells (AFC) was estimated by Jerne's method [10]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that spleen cells of normal (before the onset of leukemia) AKR mice aged 2 months, cultured in vitro, led to the development of a marked immune response to SRBC (groups 1, 2, and 3). Spleen cells of AKR mice aged 10 months, in which marked leukemia developed, did not react to this antigen in culture (groups 4, 5, and 6). As regards the immune response to SRBC in cultures of spleen cells from nonleukemic (CBA × C57BL)F_1 mice, no difference was found in the number of AFC in animals aged 2 and 10 months (Table 2, groups 1-3 and 4-6). Spleen cells of (CBA × C57BL)F_1 mice aged both 2 and 10 months produced about equally large numbers of AFC in culture. The number of AFC in cultures of spleen cells stimulated by antigen was 5-8 times higher than the initial value. The addition of bone marrow cells from AKR mice aged 2 or 10 months to cultures of spleen cells from AKR mice aged 2 months in the ratio of 1:1 and 1:4 respectively (Table 1, groups 7, 8, 11, and 12) led to identical suppression of AFC accumulation. However, addition of bone marrow in the ratios of 9:1 and 15:1 led to a marked increase in the ability of bone marrow cells from 10-month-old AKR mice to suppress the primary immune response in vitro compared with animals

aged 2 months (Table 1, groups 9, 11, 13, and 14). The suppressive activity of the bone marrow cells of (CBA × C57BL)F_1 mice aged 2 and 10 months was the same (Table 2, groups 7-14). In other words, the ability of bone marrow cells of normal (CBA × C57BL)F_1 mice was unchanged in the course of the period investigated.

It is stated in the literature that spontaneous lymphoma develops in the thymus of 80-90% of AKR mice aged 6-12 months [5]. During the development of leukemia blast forms appear in the peripheral blood and bone marrow, as also was observed in the 10-month-old AKR mice used in the present experiments. In these same mice, against the background of developing leukemia, a sharp increase was found in the ability of their bone marrow cells to suppress the immune response of spleen cells in vitro. These changes were evidently attributable to the development of leukemia, for no changes in the suppressive properties of the bone marrow cells were observed in (CBA × C57BL)F_1 mice of the same age groups.

The writers showed previously that the suppressive effect of bone marrow is due to cells of the B lymphocyte series [2-4]. The possibility cannot be ruled out that the development of leukemia is accompanied by an increase in the activity of these same cells. Suppressor cells with characteristics of B lymphocytes, inhibiting the T cell response to phytohemagglutinin [8, 11] and to allogeneic target cells [7], are known to appear in the spleen of mice with developing solid tumors. In the present investigation enhancement of the suppressor function, which is a normal feature of bone marrow cells, was demonstrated during leukemia.

LITERATURE CITED

1. L. A. Zakharova and N. S. Galkina, Byull. Éksp. Biol. Med., No. 3, 67 (1974).
2. R. V. Petrov, R. M. Khaitov, R. I. Ataullakhanov, et al., Dokl. Akad. Nauk SSSR, 233, No. 4, 745 (1977).
3. R. V. Petrov, B. B. Fuks, R. M. Khaitov, et al., Zh. Mikrobiol., No. 3, 121 (1978).
4. R. V. Petrov, R. M. Khaitov, R. I. Ataullakhanov, et al., Dokl. Akad. Nauk SSSR, 237, 990 (1977).
5. M. M. Bortin and R. L. Truitt, Biomedicine, 26, 309 (1977).
6. A. K. Duwe and S. K. Singhal, in: Immune Reactivity of Lymphocytes: Development, Expression and Control, New York (1976), p. 607.
7. A. E. Eggers and J. R. Wunderlich, J. Immunol., 114, 1554 (1975).
8. R. Gorczynski, J. Immunol., 112, 1826 (1974).
9. M. F. Greaves, J. J. Owen, and M. C. Raff, T and B Lymphocytes, Amsterdam (1973), p. 15.
10. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
11. D. G. Kilburn, J. B. Smith, and K. M. Gorczynski, Eur. J. Immunol., 4, 784 (1974).
12. R. V. Petrov, A. A. Mikhajlova, R. N. Stepanenko, et al., Cell. Immunol., 17, 342 (1975).