and 3rd days in cultures of lymph lymphocytes may be associated with mobilization of reserves from the circulating lymphocytes and also from cells of lymphoid tissue.

Lymph drainage thus acts as a stimulus for the mobilization of antigen-sensitive lymphocytes from lymphoid tissue. It is evidently these cells which, in ordinary situations, exist in the body in a "resting" state and enter the circulation only when maximal strain is placed on the immune system [11]. It should also be noted that this reaction is of short duration and may be followed by weakening of the immune response; for that reason, when therapeutic drainage of the thoracic duct is carried out, immunologic control is essential.

LITERATURE CITED

1. D. K. Bashirova, Zh. Mikrobiol., No. 1, 50 (1973).
2. D. K. Bashirova and A. A. Sorokin, Zh. Mikrobiol., No. 3, 140 (1977).
3. N. I. Vylegzhanin and D. K. Bashirova, Byull. Éksp. Biol. Med., No. 8, 52 (1972).
4. N. R. Ling, Lymphocyte Stimulation, Am. Elsevier (1968).
5. E. A. Luriya, Hematopoietic and Lymphoid Tissue in Culture, Consultants Bureau, New York (1977).
6. G. Nossal, Antibodies and Immunity, Basic Books (1969).
7. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
8. V. I. Pokrovskii, Zh. Mikrobiol., p. 124 (1975).
9. J. J. Oppenheim and S. Perry, Proc. Soc. Exp. Biol. (New York), 118, 1014 (1965).
10. M. J. Tong et al., New Engl. J. Med., 293, 318 (1975).

EFFECT OF α_1-PROTEASE INHIBITOR (α_1-ANTITRYPSIN) ON THE INTENSITY OF TRANSFORMATION OF PHYTOHEMAGGLUTININ-STIMULATED LYMPHOCYTES

F. S. Baranova, A. A. Berman, and Yu. M. Zaretskaya

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α_1-Antitrypsin (α_1-AT) reduces the intensity of transformation of human peripheral blood lymphocytes stimulated by phytohemagglutinin. The degree of inhibition is determined by the antiprotease activity of the α_1-AT. Maximal inhibition of transformation was shown to be 50%. Participation of α_1-AT in the control of activity of lymphoid tissue cells is postulated.

KEY WORDS: antiproteases; lymphocyte transformation.

Natural and synthetic inhibitors of proteinases have been shown to modify the development of several immunologic phenomena [5, 6, 9]. A special place among them is occupied by blood serum proteins with antiprotease activity, in connection with their possible role in the regulation of the biological activity of lymphoid tissue cells in vivo [1, 4].

The object of this investigation was to study the effect of α_1-antitrypsin (α_1-AT) on the intensity of transformation of human peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA).

EXPERIMENTAL METHOD

α_1-AT was isolated from citrated donor's plasma by Liener's method [7] without modification. The method included salting out with $(NH_4)_2SO_4$ at 50-70% saturation, chromatography on DEAE-Sephadex A-50 and concanavalin (Con) A-Sepharose. The antiprotease activity of the serum proteins and individual fractions was determined from inhibition of hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE) by trypsin [1] and was

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Laboratory of Immunogenetics, Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow.

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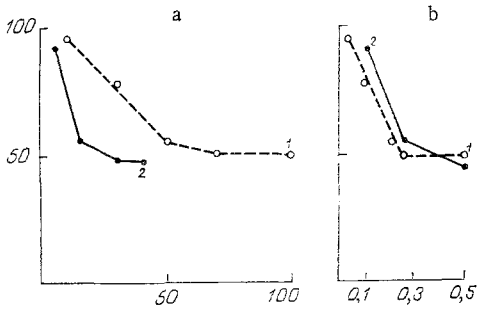

Fig. 1. Intensity of transformation of lymphocytes stimulated by PHA in the presence of α_1-AT. Abscissa: in (a): concentration of α_1-AT (in μg/ml), in (b): α_1-AT activity in culture medium (in IU/ml); ordinate, intensity of transformation (in % of control). α_1-AT with specific antiprotease activity of: 1) 3.5 IU/mg, 2) 14.6 IU/mg.

expressed in inhibitory units (IU).* Manchini's method was used for the quantitative determination of α_1-AT, using M-partigen obtained from "Boehringer."

Lymphocytes were isolated from the suspension of leukocytes by Boyum's method [3]. Culture of the lymphocytes and preparation of the radioactive specimens were carried out by methods described previously.

EXPERIMENTAL RESULTS

Two preparations of α_1-AT, with different levels of specific antiprotease activity (14.6 and 3.5 IU/mg) were used. The α_1-AT concentration in the first preparation, determined by Manchini's method, was 85% as protein.

As the results given in Fig. 1 show, α_1-AT, if added to the lymphocyte culture simultaneously with PHA, reduced the intensity of transformation of the stimulated lymphocytes. The preparation with a specific activity of 14.6 IU/mg was active in a concentration of 15 μg/ml, whereas the preparation with a specific activity of 3.5 IU/mg had no effect on the intensity of transformation in that concentration. Hence it follows that inhibition of lymphocyte transformation was due to the antiprotease activity of the preparations. This conclusion was confirmed by the results shown in Fig. 1. The curves of intensity of transformation as a function of antiprotease activity in the culture medium were virtually identical for α_1-AT preparations differing in their specific activity.

The degree of inhibition of transformation, incidentally, depended on antiprotease activity in the culture medium over a narrow range of that activity up to 0.25 IU/ml. A further increase in α_1-AT activity did not lead to stronger inhibition of transformation. This rules out lymphocytotoxicity of α_1-AT as the possible mechanism of inhibition of transformation. Moreover, in the test with trypan blue, no differences were found in the number of dead cells in the control and experimental cultures. Consequently, α_1-AT reduced the intensity of transformation through its influence on stages depending on manifestation of proteinase activity.

As regards the incomplete inhibition of transformation of stimulated lymphocytes by α_1-AT, this could be due to the reversibility of the action of α_1-AT, interaction between the inhibitor and a particular subpopulation of cells, or control of the proteolytic activity of accessory cells, strengthening the intensity of transformation, by α_1-AT.

It has recently been shown that α_1-AT controls the response of macrophages to mediators produced by sensitized lymphocytes [10], inhibits the development of the primary immune response in vitro [2], and is present on the membrane of blast cells from a culture of lymphocytes stimulated by concanavalin A [8]. Other proteinase inhibitors from the blood serum, namely α_2-macroglobulin and thermostable and acid-stable inhibitor of serine proteinases, also reduce the intensity of transformation of lymphocytes stimulated by mitogens in vitro [1, 4]. Meanwhile virtually nothing is known of the role of these inhibitors in the control of biological activity of lymphoid tissue cells in vivo.

*One inhibitory unit corresponds to the quantity of inhibitor inhibiting hydrolysis of 1 μmole BAEE under standard conditions.

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

I. G. Sidorovich, S. Yu. Pchelintsev, and R. M. Khaitov

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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].

Institute of Biophysics, Ministry of Health of the USSR, Moscow.

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