EFFECT OF LEUKOCYTIC SERUM PREPARATION ON HEMATOPOIETIC CELLS IN VITRO

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UDC 612.419.014.2-06:615.365.018.53.015.44/-085.23

KEY WORDS: leukocytic serum, bone marrow, short-living suspension cultures, clonogenic precursor cells of granulocytes and macrophages, GI-3S_2 granulopoiesis inhibitor.

Incubation of leukocytes in blood serum for 48-72 h leads to accumulation of substances stimulating regeneration in the serum. The most important of the biological effects of leukocytic serum (LS) is stimulation of cell proliferation and phagocytosis. LS, simulating conversion of blood lymphocytes into an inflammatory (wound) focus in a simplified manner, has been used for the treatment of indolent wounds and trophic ulcers [4]. Numerous investigations [1-3, 5] aimed at elucidating the chemical nature of stimulating factors of LS, comparison of its action with that of other known substances, and testing LS on various test systems have not yet led to any satisfactory conclusion regarding the nature of the biological activity of LS. Processes lying at the basis of accumulation of the stimulating factors of LS, their chemical identification, and relations between general and tissue-specific factors are still being studied. In addition, there are as yet no biological models whereby the action of LS can be recorded objectively. There is reason to suppose that acccumulation of stimulating substances in LS is connected with breakdown and metabolic activity of leukocytes: monocyte-macrophages, granulocytes, and lymphocytes. It is thus interesting to study the effect of the factors of LS on the corresponding precurosr cells of related tissue nature. In the investigation described below the effect of LS was studied on the DNA-synthesizing and clonogenic activity of cells of myeloid and lymphocytic nature: rat bone marrow and thymus cells, precursor cells of granulocytes and macrophages (CFU-GM) of mouse bone marrow, and human myeloid leukemia HL-60 cells.

EXPERIMENTAL METHOD

Leukocytic and native (for the control experiments) sera were prepared from horse blood by the method in [5]. Freshly prepared or lyophilized serum was used. Horse serum produced by the firms Flow Laboratories (England), Gibco (USA), and Serva (West Germany), and embryonic calf serum (ECS; from Gibco) also were used. An inhibitor of granulopoiesis (purified low-molecular-weight fraction GI-3S_2) was obtained from calf spleen in the Laboratory of Regulation of Cell Proliferation, Institute of Experimental Medicine, Hungarian Academy of Sciences. The DNA-synthesizing activity of the cells was investigated in short-living suspension cultures [9]. Bone marrow (3×10^6/ml) and thymus (5×10^6/ml) cells from male Wistar rats weighing 40 g were cultured in medium 199 with the addition of the test substances at 37°C for 4 h. ^{3}H-thymidine (1 μCi/ml, specific activity 19.5 Ci/mmole, from UWVR, Czechoslovakia) was added to the medium 1 h before fixation and the radioactivity of DNA was determined on a scintillation counter. CFU-GM were assayed by the agar cultures method [13]. Bone marrow cells from male (CBA × C57BL/6)F_1 mice, in a concentration of 0.5×10^5 nucleated cells in 1 ml, were cultured in plastic Petri dishes (50 × 13 mm, Flow Laboratories) in a mixture of McCoy 5A medium, 25% horse serum (with different ratios of native to leukocytic serum), 10% colony-stimulating factor (CSF), and 0.3% agar (Koch-Light, England) in a CO_2 incubator for 7 days. Colonies were identified morphologically in permanent preparations of agar gels fixed in absolute methanol and stained by the Giemsa method. A supernatant of Fisher's medium, conditioned by lung tissue of mice into which endotoxin (Difco, USA), in a dose of 10 μg/mouse, had been injected intraperitoneally for 30 min, was used as CSF.

Laboratory of Histogenesis, N. K. Kol'tsov Institute of Developmental Biology Academy of Sciences of the USSR, Moscow. Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol 102, No. 9, pp. 337-339, September, 1986. Original article submitted July 18, 1985.

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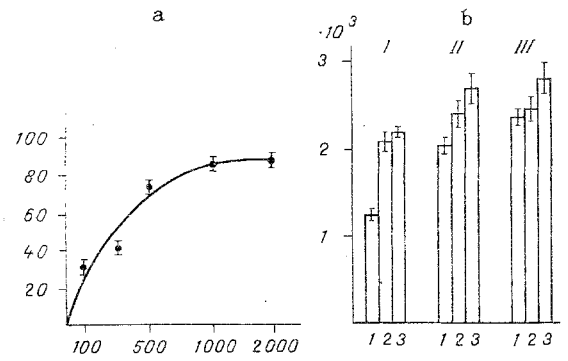

Fig. 1. Action of LS on ^{3}H-thymidine incorporation into rat bone marrow cells in suspension cultures: a) dependence of stimulating effect of LS on dose of preparation. Abscissa, LS concentration (in μg/ml); ordinate, incorporation of ^{3}H-thymidine (in per cent of control); b) action of LS and ECS on ^{3}H-thymidine incorporation into bone marrow cells. Ordinate, number of ^{3}H-thymidine incorporation (in cpm). I: 1) medium 199, 2) LS 500 μg/ml, 3) LS 1000 μg/ml; II: 1) ECS 3%, 2) ECS 3% + LS 500 μg/ml, 3) ECS 3% + LS 1000 μg/ml; III: 1) ECS 10%, 2) ECS 10% + LS 50 μg/ml, 3) ECS 10% + LS 1000 μg/ml.

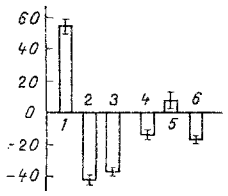

Fig. 2. Combined action of LS and granulopoiesis inhibitor GI-3S$_2$ on rat bone marrow cells in suspension cultures. Ordinate, incorporation of ^{3}H-thymidine (in percent of control): 1) LS (500 μg/ml, 2) GI-3S$_2$ (500 μg/ml), 3) GI-3S$_2$ (300 μg/ml), 4) LS (500 μg/ml) + GI-3S$_2$ (500 μg/ml), 5) LS (500 μg/ml) + GI-3S$_2$ (300 μg/ml), 6) LS (300 μg/ml) + GI-3S$_2$ (500 μg/ml).

To determine the clonogenic activity of the HL-60 cells the method of culture in capillary tubes was used [12]. HL-60 cells, grown in medium RPMI-1640 with the addition of 10% inactivated ECS, were suspended in modified McCoy 5A medium with the addition of 25% horse serum (with native and leukocytic sera in differnt proportions), and 10% CSF, and 1.5×10^4 cells in 1 ml were cultured in 100 μl of agar mixture containing 0.18% agar in glass capillary tubes in a CO_2 incubator for 7 days. Colonies and clusters were counted under magnification of 40.

EXPERIMENTAL RESULTS

Addition of LS to the medium led to stimulation of ^{3}H-thymidine incorporation into rat bone marrow cells during short-term culture. The stimulating effect of LS increased with an

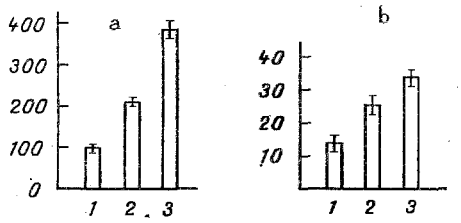

Fig. 3. Stimulating action of LS on CFU-GM of mouse bone marrow cells and colony-formation of human myeloid leukemia HL-60 cells in agar. Ordinate: a) number of colonies on Petri dish; b) number of colonies per 1.5×10^3 cells. 1) control, 25% native horse serum (NHS); 2) 25% LS; 3) 10% LS + 15% NHS.

increase dose, to reach a maximum with a concentration of LS of 1000 μg/ml, and it remained unchanged despite further increase in concentration of the LS preparation (Fig. 1a). LS had no marked stimulating action on ^{3}H-thymidine incorporation by rat thymus cells. Comparison of LS and ECS showed that both sera have stimulating effects on ^{3}H-thymidine incorporation by bone marrow cells (Fig. 1b). Concentrations of ECS usually used in such cases (3-10%) were several times higher than LS concentrations giving the same effect (1% serum corresponds approximately to a concentration of 800 μg/ml of the freeze-dried product). Addition of LS to medium containing ECS (Fig. 1b) caused no significant increase of the stimulating effect. It can therefore be tentatively suggested that both LS and ECS contain the same stimulating factors. It is not yet clear whether incorporation of ^{3}H-thymidine was stimulated by an increase in the number of DNA-synthesizing cells or by intensification of DNA synthesis (shortening of the S period).

In the next experiments the action of LS was compared with the effect of a granulopoiesis inhibitor (GI), whose biological action has been sufficiently well studied [6-11]. In our experiments GI-$3S_2$ in doses of 300 and 500 μg/ml reduced ^{3}H-thymidine incorporation into bone marrow cells in culture (Fig. 2). Addition of LS together with GI-$3S_2$ in doses of 300 and 500 μg/ml to the culture medium simultaneously either abolished or substantially reduced the effect of the GI. This action is evidently specific for LS, because it has been shown that GI-$3S_2$ exhibits its action independently of the serum present in this culture medium [7]. Further investigation of interaction (competition) between the GI and factors of LS will yield additional information on the causes of the biological activity of the latter.

LS had a stimulating effect on CFU-GM (Fig. 3a). During culture of mouse bone marrow cells in medium with 25% LS the number of CFU-GM was doubled compared with the control (25% native horse serum – NHS). It may be possible to choose a ratio of LS to NHS at which the stimulating effect would be exhibited more strongly still. In these experiments the greatest effect was observed in medium containing 10% LS and 15% NHS. The evident explanation of this fact is that during preparation of LS, not only do stimulators accumulate, but certain nutrients essential for cells in culture also are lost. Incidentally, the stimulating effect of LS on CFU-GM was manifested only in the presence of CSF. No significant change in the ratio between the types of colonies was found under the influence of LS. LS also had a similar stimulating effect on the clonogenic activity of human myeloid leukemia HL-60 cells (Fig. 3b). Just as in the experiments with clonogenic mouse bone marrow cells, the greatest effect was obtained with medium containing 10% LS and 15% NHS.

The data given above are thus evidence that LS has a distinct effect on important parameters of proliferation of myeloid cells. Further research in this direction will be interesting both for the identification of the active factors of LS, and also for elucidation of the mechanism controlling proliferation and differentiation of cells of the macrophage-granulocyte series.

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STIMULATION AND INHIBITION OF GROWTH OF MICE BEARING THE HUMAN GROWTH HORMONE GENE

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UDC 612.6.052:612.433.65.
018/-06:575.222.75

KEY WORDS: growth hormone, growth hormone gene, oocytes, transgenic animals.

Microinjection of cloned genes into the male pronucleus (nucleus) of a fertilized mouse oocyte [6] is an effective method of introducing foreign genes into the genome [3, 6], where they not only are integrated and transmitted to the progeny [7, 15], but in some cases they may also be expressed [2, 5, 10]. It has been shown that in mice, in whose genome the rat [11] or human [12] growth hormone gene has been integrated with mouse metallothionein I gene promotor, the size and body weight were considerably increased, and a high concentraton of foreign growth hormones was determined in the blood. Meanwhile, after introduction of human growth hormone gene, with its own promotor, into the mouse genome this gene was not expressed, but in some mice signs of a mutagenic action of the foreign DNA, built into the genome [15], were found. Thus the question of the phenotypic action of the foreign growth hormone gene requires further study.

The investigation described below is part of a series devoted to the introduction of foreign genes through the oocyte into the mammalian genome [1, 2]. It gives the results of experiments with human growth hormone gene, under mouse metallothionein I gene promotor, showing that expression of this gene can induce not only stimulation of growth, but also a paradoxical effect: drastic inhibition of growth of the transformed mice.

EXPERIMENTAL METHOD

Plasmid pMThGH 137 [12] was obtained from R. Palmiter (USA). Its EcoRI fragment with human growth hormone gene were isolated by electrophoresis in agarose gel and electroelution. Experiments were carried out on (CBZ × C57BL)F_1 hybrid mice.

Laboratory of Genetic Engineering, N. I. Vavilov Institute of General Genetics Academy of Sciences of the USSR, Moscow. Department of Embryology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 339-342, September, 1986. Original article submitted April 19, 1985.

