SUPPRESSIVE EFFECT OF AKR MOUSE BLAST CELLS ON ANTIBODY FORMATION IN VIVO AND LYMPHOCYTE PROLIFERATION IN VITRO

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The study of antibody formation in mice with low predisposition to leukemia during exposure to erythropoiesis-stimulating factors has demonstrated the presence of competitive relations between cells taking part in erythroid and lymphoid branches of hematopoiesis, and evidently connected with the suppressor action of cells of the erythroid series on immunocompetent lymphocytes [1]. Injection of phenylhydrazine (PH) into such animals activates splenic erythropoiesis, increases the number of early erythroid precursors (CFUs-5) [6] and erythroblasts [9], and leads to the accumulation of cells of erythroid nature, with a suppressive action relative to the humoral immune response [1]. Adoptive transfer of splenocytes from donors exposed to the action of PH into syngeneic, immunized recipients inhibits accumulation of antibody-forming cells (AFC), whereas splenocytes from intact donors have no such action [1]. The number of early erythroid precursors, identified by the number of colony-forming cells on the 5th day after transplantation of hematopoietic cells (CFUs-5) in the spleens of young AKR mice was increased a little, and after the animals were given phenylhydrazine it increased further compared with the number in mice of lines with low predisposition to leukemia [3]. In AKR mice in the preleukemic stage, the number of CFUs-5 was greater than in healthy young animals, but under these circumstances injection of PH caused no further increase in the number of erythroid colonies [6]. The erythroid origin of blast cells accumulating in the spleen during this period of development of the disease is demonstrated by the results of recent investigations in which these cells were found to be sensitive to treatment with anti-Er-suppressor serum [8]. These findings suggests that in the early stages of leukemogenesis immature cells of the erythroid series possessing an immunosuppressor function, accumulate in the spleen of AKR mice, in the same way as they accumulate in mice of lines with low predisposition to leukemia, in a model of the "erythropoietic" spleen induced by injection of PH [1].

To test this hypothesis we studied the effect of splenocytes of AKR mice in the preleukemic period on development of the humoral immune response in vivo and on proliferation of lymphocytes of healthy syngeneic donors in vitro, compared with the suppressor activity of splenocytes from young AKR mice treated with PH.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1 and AKR mice obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. The AKR mice were divided into two groups: young healthy animals (2-3 months) and animals in the stage of preleukemia. The features of the latter stage were enlargement of the peripheral lymph nodes, neutrophilic leukocytosis ($9 \cdot 10^9$-$12 \cdot 10^9$) and lowering of the hematocrit index to 40-42% [2]. The donors of the splenocytes used as effectors in the in vitro system for transfer to syngeneic recipients in vivo were young mice, previously injected with PH by the scheme in [9], and mice in the preleukemic stage. Cell suspensions were prepared by the usual method [5]. To enrich the population with blast cells, splenocytes were separated in a bovine serum albumin (BSA)

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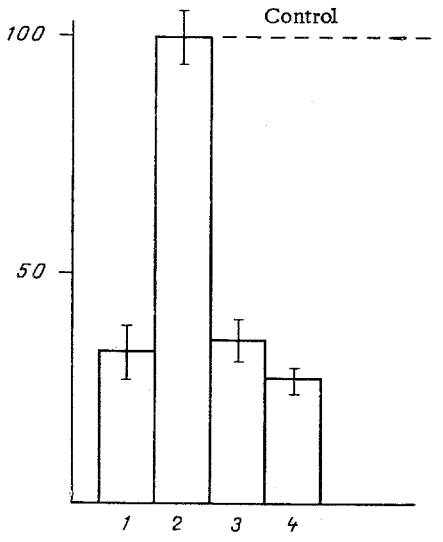

Fig. 1. Effect of transfer of blast cells isolated from spleens of (CBA × C57BL)F_1 and AKR mice on formation of IgM AFC (in % of control) in syngeneic recipients immunized with SRBC. 1) Transfer of 10^7 splenic blast cells from (CBA × C57BL)F_1 donors stimulated by PH; 2) transfer of 10^7 splenic blast cells from intact (CBA × C57BL)F_1 donors; 3) transfer of 10^7 splenic blast cells from young AKR mice stimulated by PH; 4) transfer of 10^7 blast cells isolated from the spleen of intact AKR mice in the preleukemic stage. Relative number of AFC per 10^6 nucleated splenic cells of immunized recipients, not receiving cells from syngeneic donors, was taken as 100%.

gradient by the method in [12], and, as was shown previously, the suppressor cells were concentrated in fractions 2 and 3 of BSA. The purity of separation was verified morphologically by staining films by the May–Gruenwald–Giemsa method. The cell composition was characterized by a high (70%) content of immature forms of the erythroid series, with admixture of other undifferentiated cells. Before addition of the effector cells to culture in vitro, they were treated with mitomycin to prevent proliferation. Splenocytes from intact young mice served as the target cells. Cells numbering 200,000 per well were cultured for 72 h in the presence of mitogens: concanavalin A (con A, from Pharmacia, Sweden) and lipopolysaccharide (LPS, from Difco, USA), in a dose of 100 μg/ml. The proliferative activity of the cells was estimated by measuring incorporation of ^{3}H-thymidine, using an Intertechnique SL-30 scintillation counter (France). Effector cells (10^7) were transferred in vivo into syngeneic recipients, immunized with sheep's red blood cells (SRBC, $2 \cdot 10^8$) by the method described previously [1]. The level of IgM AFC in the recipient's spleens was determined on the 4th day after immunization by the method in [11].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that transfer of suppressor-enriched splenocytes from PH-stimulated donors of a line [(CBA × C57BL)F_1] with low predisposition to leukemia, immunized with SRBC, into syngeneic recipients led to a threefold fall of the IgM AFC level in the spleens of those recipients. This effect was clearly defined and statistically significant. Similar phenomena also are observed if healthy AKR mice in the stage of preleukemia are used in the experiments. In other words, this effect can be reproduced in mice with different genotypes, whether with low or with high predisposition to leukemia. Similar results also were produced by transfer of splenocytes from intact donors in the preleukemic stage to syngeneic AKR recipients (Fig. 1).

Data obtained in vitro were in agreement with those given above. As will be seen in Fig. 2, spontaneous proliferation of spleen cells, like the proliferative response to LPS in AKR mice at the preleukemic stage, were clearly depressed compared with young animals of the same line. Meanwhile, on combined culture of con A-stimulated splenocytes from healthy animals with blast cells from preleukemic AKR mice, inhibition of proliferation by 43% was

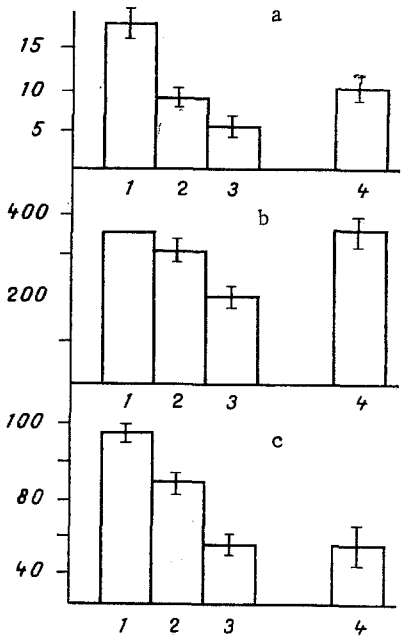

Fig. 2. Effect of blast cells isolated from spleens of AKR mice on spontaneous and mitogen-induced proliferation of syngeneic lymphocytes. Ordinate, radioactivity (cpm•10^3). 1) Proliferative activity of splenocytes from young AKR mice; 2) proliferative activity of splenocytes from young AKR mice after addition of blast cells isolated from spleens of young AKR mice receiving PH; 3) proliferative activity of splenocytes of young AKR mice after addition of blast cells isolated from spleens of AKR mice in the preleukemic stage, 4) proliferative activity of splenocytes of AKR mice in the preleukemic stage. a) Spontaneous proliferation; b) in presence of con A; c) in presence of LPS.

observed, whereas LPS-induced proliferation was inhibited under these circumstances by 63%. During culture of spleen cells from healthy mice with erythroblasts obtained from young animals previously injected with PH, a more marked inhibitory response was observed. Con A-induced proliferation was inhibited by 19%, LPS-stimulated proliferation by 26%. Spontaneous proliferation of splenocytes from healthy mice also was inhibited much more strongly (by 73%) by blast cells from preleukemic mice than those from mice receiving an injection of PH (55%).

Blast cells isolated from the "erythropoietic" spleen of PH-stimulated young animals, and also blast cells accumulating in the spleens of preleukemic AKR mice, possess a similar suppressive action against spontaneous and mitogen-induced proliferation of young mouse splenocytes in vitro and inhibit the development of a humoral immune response in immunized recipients during syngeneic transfer in vivo. These results suggest that disturbances in the erythroid system in the preleukemic period in AKR mice, manifested as the accumulation of immature erythroid precursors, possessing a suppressor action against immunocompetent lymphocytes, may be a pathogenetic stage in the development of leukemia.

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FUNCTIONAL CHARACTERISTICS OF THE ANTIOXIDATIVE SYSTEM OF MYCOBACTERIA GROWN ON MEDIA MODIFIED BY PERFLUORODECALIN

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One trend in the improvement of culturing of mycobacteria is the production of artificial nutrient media corresponding most fully to the physiological needs of various representatives of the genus *Mycobacterium*. One factor leading to optimization of biomass accumulation by strains of mycobacteria during periodic culture is additional aeration of the nutrient medium with the aid of gas-carrying perfluorocarbon liquids, such as perfluorodecalin (PFD), whose oxygen capacity is over 40 vols. %. PFD was used for the first time as a support medium for growth of tissue culture cells [1] and during the development of methods of culture of *Mycobacterium leprae* [4].

An essential condition for the existence of oxygen-metabolizing microorganisms is integrity of their antioxidative protection system [8, 15]. This accounts for the interest in the study of the response of the antioxidative enzyme system of the medically important strains of mycobacteria *M. bovis* BCG and *M. lufu* to culture in liquid medium with the addition of oxygenated PFD.

EXPERIMENTAL METHOD

Cultures (12-14-day) of *M. bovis* BCG and *M. lufu* were obtained on Shkol'nikova's medium with PFD (see the paper by A. A. Yushchenko et al. in this issue*). Oxygen was passed through the PFD beforehand for 20 min. A culture of mycobacteria grown on Shkol'nikova's medium without PFD served as the control.

The mycobacteria were sedimented from the nutrient medium by centrifugation (in the experimental version the PFD was first removed) at 5000 g for 15 min and the supernatant was discarded; the residue was washed with 1 ml of K-phosphate buffer, pH 7.4, and recentrifuged under the same conditions. The resulting residue was resuspended in 1.5 ml of the above-mentioned buffer and frozen in liquid nitrogen and thawed 4 times. The disintegrated mycobacteria were sedimented at 5000 g (15 min) and the supernatant was drawn off and kept for 2 weeks at −40°C. Superoxide dismutase (SOD) activity was determined by the method in [5] at 30°C, glutathione transferase (GT) activity was determined relative to 1-Cl-2,4-dinitro-

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