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SUPPRESSOR T CELLS OF TUMOR-BEARING MICE INHIBIT ANTITUMOR CYTOTOXIC T LYMPHOCYTE MATURATION IN MONOCULTURE

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UDC 616-006.04-092:612.112.94.017.4]-092.9-07

KEY WORDS: antitumor cytotoxic lymphocytes; suppressor cells; lymphocyte monocultures.

Limitation of the immune response by suppressor cells is one of the principal pathogenetic mechanisms formed during tumor growth. Suppression of T-cell type is observed in patients with various forms of cancer [1, 9, 11], suggesting that it may be one cause of the ineffectiveness of antitumor immunity. If transplantable tumors are used as the experimental model, the formation of two types of suppressor T cells (TS) may be discovered [7]. TS of the first type ($Lyt2^+$, $L3T4^-$) appear in the early stage of tumor growth and soon disappear. Their action is connected with inhibition of production of interleukin-2 (IL-2) by helper T cells [8]. In the later stages of tumor growth TS of the second type appear ($L3T4^+$, $Lyt2^-$), and are present until death of the tumor-bearing animal. They can suppress the manifestations of adoptive immunity [5] and inhibit the formation of cytotoxic T lymphocytes (CTL) in secondary mixed culture of lymphocytes and tumor cells [4]. The mechanism of action of suppressors of this type has so far received little study.

Previously the writers described a model whereby secondary antitumor CTL (CTL-2) can be generated during in vivo immunization and subsequent in vitro maturation, in the absence of stimulating cells (in monoculture) [3]. By using this model, new opportunities are presented for the study of the mechanism of suppression.

EXPERIMENTAL METHOD

Male inbred C57BL/6 (B6) and C57BL/10 (B10) mice were used. The animals were obtained from the nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and used at the age of 5-10 months. Sarcoma MCh-11 and lymphoma EL-4 were maintained in ascites form by daily passage through syngeneic mice (B10 and

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 2, pp. 186-187, February, 1992. Original article submitted June 21, 1991.

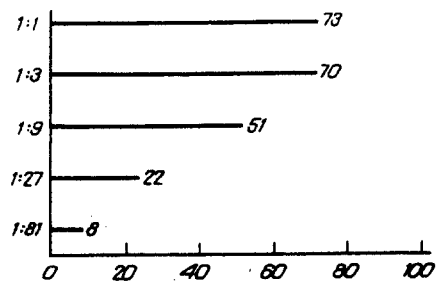

Fig. 1

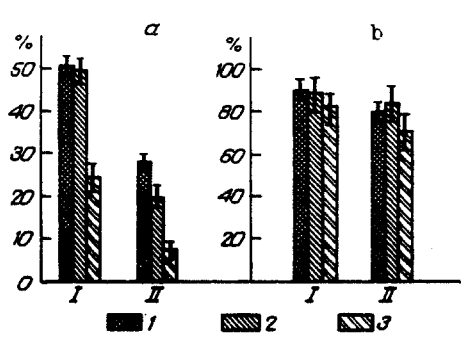

Fig. 2

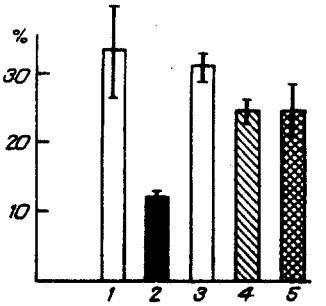

Fig. 3

Fig. 1. Effectiveness of suppression of maturation of antitumor CTL-2 by suppressor cells. Abscissa, suppression index (%); ordinate, ratio suppressor/pCTL-2.

Fig. 2. Specificity of suppression. Ordinate, cytotoxic index (%); sc obtained from animals with MCh-11 tumors (3), introduced into system generating CTL-2 against MCh-11 (a) or against EL-4 (b). Suppressor/pCTL-2 ratio 1:9. Monoculture did not contain sc (1) or contained sc from intact animals (2). Effector/target ratio 90:1 (1), 30:1 (2).

Fig. 3. Characteristics of antigenic phenotype of suppressor cells. Ordinate, cytotoxic index (%). To system generating CTL-2 specific for MCh-11 were added sc from intact animals, treated with complement (1); sc from tumor-bearing animals, treated with anti-Thy 1.2 McAB (3), anti-Lyt 2.2 McAB (4), anti-L3T4 McAB (5) with complement, or with complement alone (2). Cytotoxic activity was tested with an effector/target ratio of 50:1.

B6 respectively). To obtain memory cells (pCTL-2) syngeneic animals were immunized twice by injection of $1 \cdot 10^7$ irradiated tumor cells into the hind footpads. Secondary immunization was given on the 12th day, and 4 days later cells of the regional lymph nodes were added to a monoculture [3], containing recombinant interleukin-2 (rIL-2) in a concentration of 20 U/ml. After 3 days of maturation the cells were tested for the presence of cytotoxic activity against target tumor cells labeled with chromium-51 [3]. To obtain cells with suppressor activity, B10 mice were injected intradermally with 10^6 living MCh-11 cells. On the 16th day of growth of the tumor, when it measured 15-20 mm in diameter, the spleen was removed from the tumor-bearing animals and a cell suspension prepared from it. Spleen cells (sc) were treated with mitomycin C ("Sigma") 25 μg/ml for 30 min at 37°C, washed 3 times, and added in different relative amounts to the monoculture. As the control sc from intact mice, treated with mitomycin C, were used. The suppression index (SI) was determined by the equation:

$$\frac{\text{CTI control} - \text{CTI expt.}}{\text{CTI control}} \times 100\ \%,$$

where CTI denotes the cytotoxic index. Phenotypic markers of TS were assessed by treating them with monoclonal antibodies (McAB) to Thy 1.2 (hybridoma G4 [2]), Lyt 2.2 (hybridoma H35-17.2 [6]), and L3T4 (hybridoma L172.4 [10]) antigens for 60 min at 4°C, followed by treatment with nontoxic rabbit complement in a dilution of 1:20 for 40 min at 37°C.

EXPERIMENTAL RESULTS

On the 16th day of growth of fibrosarcoma MCh-11 in B10 mice, sc of the tumor-bearing animals were tested for their ability to inhibit generation of CTL-2 specific for MCh-11 in monoculture. As a control a monoculture containing sc from intact animals was used. Different relative amounts of TS and pCTL-2 were tested (Fig. 1). Suppression was carried

out in the presence of exogenous IL-2, evidence of a mechanism of suppression different from inhibition of the production of this lymphokine.

To determine the specificity of action of the suppressor cells, sc from B10 mice with a growing MCh-11 tumor were added to a system generating CTL-2 (B6) against EL-4 (Fig. 2b). The suppressors exhibited weak nonspecific activity (IS 6-15%), whereas their activity in an autologous system was significantly higher (IS 51-58%) (Fig. 2a). The results of evaluation of the phenotypic markers of the suppressors are shown in Fig. 3. Clearly cells suppressing CTL-2 generation are T lymphocytes, for treatment with McAB to Thy 1.2 antigen and complement abolishes suppression virtually completely (Fig. 3, 3). Treatment of the cells with antibodies to antigens L3T4 and Lyt 2.2 (Fig. 3, 4 and 3, 5) also reduces suppressor activity significantly. This fact is evidence that the T-cell population involved in suppression is heterogeneous and consists of Ts: Lyt-2 and L3T4.

The distinguishing feature of maturation of CTL-2 in monoculture is that the process of antigen-recognition takes place in vivo, whereas differentiation into effector CTL-2 takes place in vitro [3]. Thus, Ts prevent maturation of cytotoxic cells after pCTL-2 have interacted with antigen of a syngeneic tumor. The specificity of suppression in this case may be due to the presence of an anti-idiotypic determinant specific for the pCTL-2 idiotype in the effector Ts. Such a TC may either interact directly with pCTL-2, when activated by antigen, or secrete a specific suppressor factor, binding the pCTL-2 idiotype. This hypothesis must clearly await direct experimental confirmation.

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