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DIFFERENCES BETWEEN PROPERTIES OF SPECIFIC T SUPPRESSORS AND CYTOTOXIC T LYMPHOCYTES IMMUNE TO ANTIGENS OF THE H-2 COMPLEX

I. F. Abronina, A. V. Karaulov, and B. D. Brondz

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During immunization of mice with allogeneic spleen or tumor cells a spectrum of subpopulations of T lymphocytes differing in their properties is formed [6]. The possibility of induction of T cells blocking activation of DNA synthesis and generation of cytotoxic T lymphocytes (CTL) in a unidirectional mixed culture of normal lymphocytes (mixed lymphocyte culture – MLC), and their concentration by the adsorption-elution method on a monolayer of allogeneic target cells (TC), was demonstrated by the writers previously [2]. Inhibition of the immune response in MLC may be due to the activity either of CTL eliminating stimulators from MLC [10], or of a special category of T suppressor cells, inactivating reacting lymphocytes [8, 9].

The object of this investigation was to make a comparative study of the properties of T suppressors and CTL formed after immunization of mice with allogeneic spleen or tumor cells. For this purpose specific T lymphocytes were enriched by the adsorption-elution method, by means of which T suppressors can be concentrated 30 times [2] and CTL 6 to 8 times [1].

EXPERIMENTAL METHOD

Mice of congeneic B10.D2, abbreviated to D2(H-2^d), and C57BL/10, abbreviated to B10(H-2^b), lines were obtained from the Department of Genetics, Research Laboratory of Experimental Biology and Medicine, "Svetlye Gory." D2 mice were immunized by a single intravenous injection of 9×10^7 spleen cells, irradiated with γ-rays in a dose of 1500 rads (^{137}Cs, 740 rads/min), or intraperitoneally with 25×10^6 cells of E14 ascites leukemia induced in C57BL/6 (H-2^b) mice. In some experiments, 2 days before immunization, D2 mice were given an intraperitoneal injection of cyclophosphamide (CP) in doses of 25-200 mg/kg body weight, dissolved immediately before injection, and hydrocortisone (HC) in a dose of 2.5 mg per mouse. Four days after intravenous immunization, 1×10^7 D2 spleen cells were treated with mitomycin C (Calbiochem), in a dose of 50 μg/ml, in 1 ml Eagle's medium for 30 min at 37°C, washed 3 times, and added to a unidirectional MLC inorder to block activation of DNA synthesis [11] and CTL generation [13]. To suppress DNA synthesis, immune lymphocytes treated with mitomycin C in the ratio of 1.5:1 were added to the reacting lymphocytes, and normal lymphocytes, similarly treated, were added to the control lymphocytes. Suppressor activity was determined by calculating the index of inhibition (II) of DNA synthesis and CTL generation by the formula $[(a-b)/a]\times100$, where a and b stand for incorporation of ^{3}H-thymidine or specific liberation of ^{51}Cr respectively in the control and

Laboratory of Immunology and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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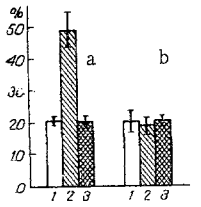

Fig. 1. Cytotoxic effect of D2 spleen cells obtained after intravenous immunization with B10 cells: a) B10 TC; b) D2 Tc. 1) Intact lymphocytes; 2) eluted from B10 monolayer; 3) eluted from B10 monolayer and then treated with anti-Thy-1,2 serum and complement. Dose of lymphocytes $1.6 \cdot 10^6$ per well. Vertical axis – specific liberation of ^{51}Cr.

experimental cultures. In some experiments, before addition to the MLC, the immune lymphocytes were adsorbed on a monolayer of allogeneic macrophages; the adherent lymphocytes were eluted with pronase [1], washed, and treated with anti-Thy-1,2 serum in a dilution of 1:4 with rabbit complement (Cedarlane) 1:20. Cytotoxic activity of the lymphocytes was determined by the microtest [7] in D2 spleen cells, obtained on the 4th day after intravenous immunization, as described above, or on the 11th day after intraperitoneal immunization with EL4 leukemia cells. The cytotoxic index was calculated by the formula:

$$\frac{\text{IL} - \text{NL}}{\text{ML} - \text{NL}},$$

where IL, NL, and ML represent immune, normal, and maximal liberation of ^{51}Cr into the medium after incubation of TC with immune and normal lymphocytes and with a 2% solution of sodium dodecylsulfate respectively [7].

EXPERIMENTAL RESULTS

Suppressor activity of D2 anti-B10 T lymphocytes in MLC was discovered on the 1st or 2nd day, reached a maximum by the 3rd-6th day, and gradually decreased until the 12th-20th day after intravenous immunization with irradiated allogeneic cells [4]. Conversely, the cytotoxic effect of immune D2 anti-B10 lymphocytes was weak between the 2nd and 20th days after the same immunization (about 20%) and nonspecific: it was the same on incubation with allogeneic (B10) and syngeneic (D2) TC (Fig. 1). After elution of lymphocytes adherent to the monolayer of B10 macrophages, a two-fourfold increase in cytotoxic activity was observed. This increase

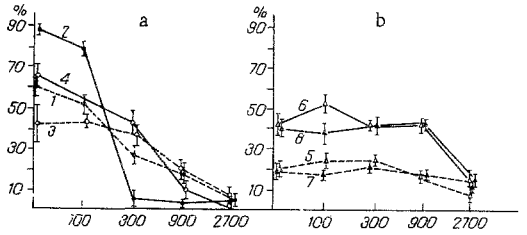

Fig. 2. Radioactivity of T suppressors and D2 anti-B10 CTL. Abscissa, dose of irradiation (in rads); ordinate, II (a) and specific liberation of ^{51}Cr (b). Inhibition of DNA synthesis (1 and 2) and CTL generation (3 and 4) by intact (1 and 3) suppressors and by suppressors eluted from B10 monolayer (2 and 4). Cytotoxic effect on B10 CTL TC induced by intravenous injection of B10 spleen cells (5 and 6) and intraperitoneal injection of EL4 cells (7 and 8). 5 and 6) Intact CTL, 6 and 8) eluted CTL. Dose of CTL $0.8 \cdot 10^6$ per well.

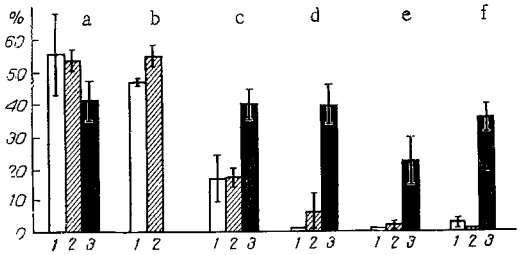

Fig. 3. Sensitivity of precursors of suppressors and CTL to cyclophosphamide and hydrocortisone. Vertical axis: II of DNA synthesis (1) and CTL generation (2), specific liberation of ^{51}CR (3) with a dose of CTL of $1.6 \cdot 10^6$ per well. Intact lymphocytes (a), and lymphocytes treated with CP in doses of 25 (b), 50 (c), 100 (d), and 200 (e) mg/kg body weight, and also with hydrocortisone (f) in a dose of 2.5 mg per mouse.

was immunologically specific, for it was not manifested against D2 TC and was due to the action of T lymphocytes: treatment with anti-Thy-1,2 serum in the presence of complement completely abolished the specific increase in the cytotoxic effect (Fig. 1).

It will be clear from Fig. 2 that T suppressors and CTL differed in their radiosensitivity. Irradiation in a dose of 100 rads did not affect the activity of the T suppressors, but in a dose of 300 rads it reduced the activity of the intact population of suppressors inhibiting DNA synthesis considerably, and practically completely inactivated the same suppressors eluted from allogeneic TC. Suppressors inhibiting CTL generation were inactivated by a dose of 900 rads. Conversely, the cytotoxic effect of the immune lymphocytes was resistant to irradiation by doses of 300 and 900 rads and was reduced after irradiation with a dose of 2700 rads (Fig. 2). Differences in the sensitivity of precursors of suppressors and CTL to CP and HC also were observed (Fig. 3). Precursors of T suppressors, in tests of inhibition of DNA synthesis and of CTL generation, were insensitive to CP in a dose of 25 mg/kg, were partially inactivated by CP in a dose of 50 mg/kg body weight, and were completely inactivated by CP in doses of 100 and 200 mg/kg and also by HC. Precursors of CTL were partially inhibited by CP only in a dose of 200 mg/kg and were resistant to the action of HC (Fig. 3). Similar data showing a difference in sensitivity of precursors of CTL and T suppressors to CP and HC were obtained on induction of these cells in allogeneic MLC [9].

Intravenous immunization with irradiated allogeneic spleen cells in most cases thus induced both T suppressors and CTL. Whereas these conditions were optimal for induction of T suppressors [4], CTL were weakly active and were discovered only after enrichment by elution from a TC monolayer. Unlike CTL, specific T suppressors were highly sensitive to the action of irradiation, and their precursors differed from precursors of CTL in their greater sensitivity to CP and HC.

The results confirmed previous findings indicating high radiosensitivity of T suppressors [16] and the sensitivity of their precursors to the action of CP [15] and HC [9, 12]. The similar radiosensitivity of T suppressors, inhibiting DNA synthesis and CTL generation, the similar sensitivity of their precursors to CP and HC, and also the similarity of the kinetics of their formation [4] and ability to adhere specifically to a TC monolayer [2], suggest that the two reactions are blocked by the same suppressors, inhibiting cell proliferation. Differences in the properties of the T suppressors and CTL, and also of their precursors indicate that the suppressor effect was attributable, not to the cytotoxic action of CTL on stimulator cells in MLC, the elimination of which could lead to inhibition of the response, but to direct inactiviation of reacting lymphocytes by T suppressors. A similar conclusion was drawn during a study of nonspecific T suppressors generated in MLC [14]. Other data also support interaction of specific T suppressors with reacting lymphocytes, and according to them, for suppression to develop it is essential that the two above-mentioned types of cells are identical with respect to the IC subregion of the H-2 complex [3] and antigens of the H-2 complex against which the suppressors and reacting lymphocytes are aimed are located on the same stimulator cell [5].

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INTERACTION OF THE C1q COMPONENT OF COMPLEMENT AND COLLAGEN WITH NUCLEIC ACIDS AND POLYANIONS

D. V. Korogodin and A. M. Poverennyi

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The appearance of proteins which interact with nucleic acids (NA) in solutions with low ionic strength in the blood serum of patients with chronic inflammatory conditions was reported previously [3]. One such protein could be serum collagen-like glycoprotein C1q, reacting with NA [4, 6, 7, 11]. Like collagen, C1q is synthesized by fibroblasts and has a molecular weight of 400,000-500,000; its collagen-like region consists of 78 proline-hydroxyproline (hydroxylysine)-glycine triplets [5, 12, 13]. Interaction between C1q and the antigen–antibody complex or with a polyanion activates the I component of complement, including factors C1r and C1s [8].

The writers showed previously that collagen can interact with NA at neutral pH, and the hypothesis was put forward that these complexes play a role in the pathogenesis of diffuse connective tissue diseases and, in particular, of systemic lupus erythematosus [2].

In the present investigation interaction of the C1q component of complement and acid-soluble collagen with NA, and also the structures of NA which take part in interaction with these proteins, were studied.

EXPERIMENTAL METHOD

C1q was isolated from human blood [14] and collagen from rat skin [1]. The protein concentration was determined by Lowry's micromethod [9]. Labeled DNA was obtained by Marmur's method [10] with additional treatment with pronase from Escherichia coli strain W3110 cells, grown in the presence of ^{3}H-thymidine. The specific activity of the DNA preparations was 25,000-100,000 cpm/μg. Poly I and poly C were from Calbiochem, USA, polyU and polyA from Reanal, Hungary, Dextran sulfate (DS) from Ferak, Berlin; heparin was dissolved

Laboratory of Radiation Biochemistry, Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk.

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