CHARACTERISTICS OF T-SUPPRESSORS CONCENTRATED BY ELUTION FROM AN ALLOGENEIC TARGET CELL MONOLAYER

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Quantitative enrichment of the fraction of immune lymphocytes eluted from a monolayer of corresponding allogeneic target cells with suppressors specific for H-2 antigen is associated with a 2-3-fold increase in the number of T-lymphocytes synthesizing the DNA of the cells and in the total uptake of thymidine-^{3}H on account of an increase in the relative proportions of medium-sized and large lymphocytes in the population, and also in the percentage of small and medium-sized DNA-synthesizing lymphocytes. Complete abolition of the suppressor effect by anti-Thy-1 and anti-T-antibodies, but not by anti-B-serum and the resistance of this effect to carrageenan and iron carbonyl point to the T-cell nature of the eluted suppressors.

KEY WORDS: suppressors; T-lymphocytes; DNA synthesis.

Specific T-suppressors, blocking the immune response to the antigen used for their induction, play a very important role in the regulation of the immune response [3]. An essential step in the study of the properties of these cells and their receptors is the development of methods of their isolation from other cell populations. One such method is by concentration of T-suppressors specific for heterologous protein antigens [12], by means of their adsorption of antigenic immunosorbent. T-suppressors specific for H-2 antigens, induced in mice with irradiated allogeneic spleen cells, and blocking DNA synthesis and killer generation in a mixed lymphocyte culture (MLC), were selectively adsorbed on a monolayer of the corresponding target cells, and by subsequent elution of the cells with pronase they could be concentrated 30-fold [4].

The object of the present investigation was to characterize the fraction of eluted suppressors.

EXPERIMENTAL METHOD

To induce suppressors in B10.D_2 (H-2^d) mice, hereafter abbreviated to D_2, they were immunized by a single intravenous injection of 9×10^7 spleen cells from C57BL/10 (H-2^b) mice, hereafter abbreviated to B10, irradiated with γ-rays in a dose of 1500 rad (^{137}Cs, 740 rad/min). Four days later the D_2 spleen cells were treated with mitomycin C and added to a unidirectional MLC [9]. The methods of determining incorporation of thymidine-^{3}H in a microvariant of MLC [2], of its blocking by suppressors [5], absorption of the suppressors on a monolayer of target cells, and their elution with pronase [4] were described previously. For the autoradiographic investigation 10^7 lymphocytes were incubated for 1 h at 37°C with periodic shaking in 1 ml of medium RPMI-1640 containing 10% embryonic calf serum and 1 μCi thymidine-^{3}H. After fixation, treatment with emulsion, and staining with methyl green–pyronine [1], 600 lymphocytes were counted in each film, distinguishing (by the diameter of the nucleus) small (under 7 μ), medium-sized (7-10 μ), and large (over 10 μ) lymphocytes. Total DNA synthesis was determined in a β-spectrometer after incubation of 10^6 lymphocytes in a volume of 0.2 ml of the above-mentioned medium for 1.5 h at 37°C. The cytotoxic test with antibodies was carried out by the method in [6] in the presence of 1:10 rabbit complement (Cederlane). Mouse antibodies against T-cells, anti-Thy-12 [11], and against B-cells, anti-Mls* [13], and also exhausted rabbit antisera against mouse T-cells, antithymocytic (anti-Tt) and antimarrow (anti-Tm) (Cederlane) and rabbit anti-T-

*Minor lymphocyte stimulation locus.

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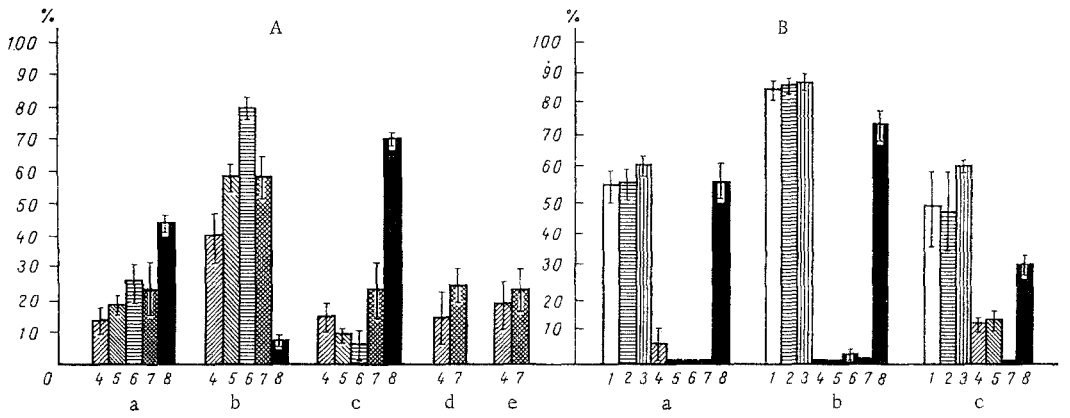

Fig. 1. Changes in numbers of T- and B-lymphocytes (A) and activity of suppressors in MLC (B) in spleen of D_2 anti-B10 mice after treatment with anti-T and anti-B antibodies. Intact immune lymphocytes (a) and immune lymphocytes eluted from monolayer of B10 (b) or D_2 (c) macrophages and not adherent to monolayer of B10 (d) or D_2 (e) macrophages, not treated (1) or treated in the presence of complement with the following sera: normal mouse 1:10 (2), normal rabbit 1:10 (3), anti-Thy-1, 2 1:10 (4), anti-Tt (5), anti-Tm (6), and ATG (7) in dilutions of 1:40 (A) or 1:10 (B), and anti-Mls 1:2 (8). In Fig. 1B, immune lymphocytes, after treatment, were added to syngeneic reacting cells in the ratio of 1:1.5. Ordinate: A) cytotoxic index of antibodies (in %), B) index of inhibition of incorporation of thymidine-^{3}H into MLC (in %).

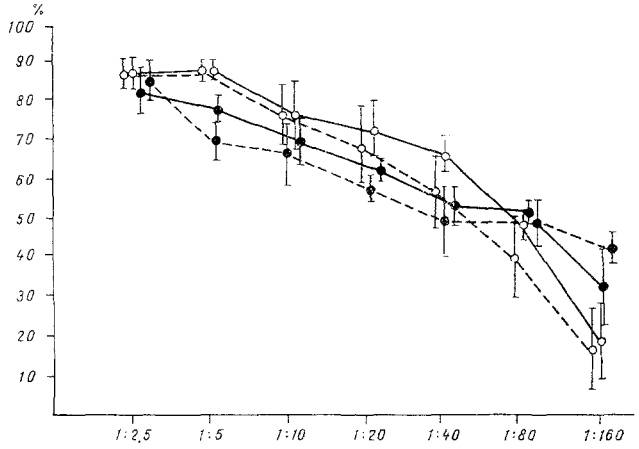

Fig. 2. Resistance of D_2 anti-B10 suppressors eluted from monolayer of B10 cells to carrageenan and iron carbonyl. Abscissa, ratio of suppressors and reacting lymphocytes in MLC; ordinate, index of inhibition of thymidine-^{3}H incorporation. Continuous line with empty circles – lymphocytes untreated with carrageenan; continous line with filled circles – lymphocytes not treated with iron carbonyl; broken line with empty circles – lymphocytes treated with carrageenan; broken line with filled circles – lymphocytes treated with iron carbonyl.

globulin (ATG), were used. Data on the high activity and specificity of these antisera were described previously [5]. To remove macrophages the cell suspensions were treated in vitro with carrageenan [10] or iron carbonyl [7], obtained from Marine Colloids and GAF Corp. (USA) respectively. To verify completeness of removal of the macrophages, films stained by Giemsa's method were examined under the microscope and a test for nonspecific esterase also was used [9]. Judging from these criteria, contamination of the suspension of peritoneal exudate cells treated with iron carbonyl by macrophages did not exceed 1-3%.

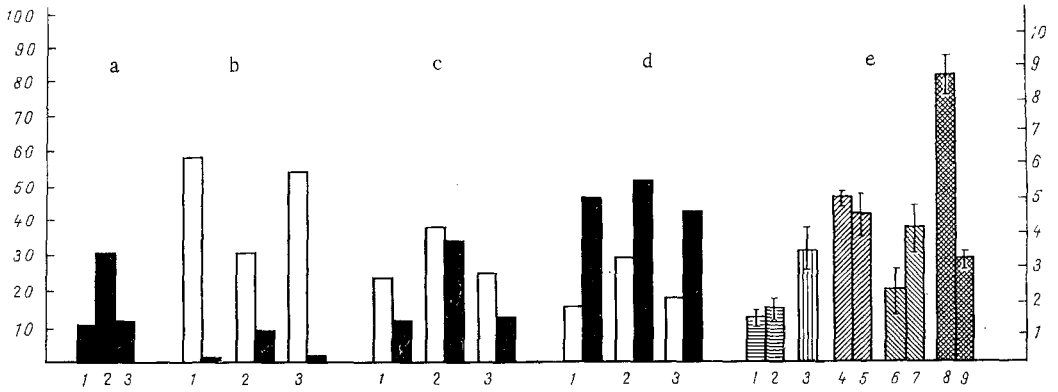

Fig. 3. Morphological composition and DNA synthesis in intact and eluted D_2 anti-B10 spleen cell populations. Proportion of thymidine-^{3}H-labeled cells (black columns) among all lymphocytes (a), small (b), medium-sized (c), and large (d) lymphocytes (white columns) – intact (1), and eluted from monolayer of B10 cells (2) and D_2 cells (3); e) total incorporation of thymidine-^{3}H into D_2 lymphocytes – normal (1 and 2), immunized with D_2 spleen cells (3) and with B10 spleen cells (4-9). Intact immune lymphocytes (4 and 5), cells not adherent to monolayer of B10 cells (6) and D_2 cells (7), and eluted from monolayer of B10 cells (8) and D_2 cells (9). 2 and 5) Lymphocytes treated with pronase, 25 μg/ml, for 30 min at 37°C. Ordinate: on left – % of cells, on right – incorporation of thymidine-^{3}H (in cpm · 10^3).

EXPERIMENTAL RESULTS

Specific suppressors, induced in mice by irradiated allogeneic spleen cells, are T-lymphocytes. Since the number of suppressors increases sharply after elution from an allogeneic monolayer [4], it was interesting to study their nature in this case. It will be clear from Fig. 1A that the proportion of T-cells in the fraction of spleen cells eluted from the allogeneic monolayer (Fig. 1A, b) was 2.5 times higher than in the intact suspension (Fig. 1A, a), and reached 80% when the most active anti-T-serum was used. Conversely, the number of B-lymphocytes in this same fraction was reduced by more than four-fifths. Meanwhile, the number of T-cells in the fraction of cells eluted from a syngeneic monolayer (Fig. 1A, c) was substantially unchanged, whereas the number of B-cells increased. Quantitative enrichment with specific suppressors thus correlates with an increase in the number of T-cells, which is unconnected with the action of pronase, used to elute them from both types of monolayer, on the lymphocytes. The increase in the proportion of the cells carrying T-markers likewise was not due to the action of the culture medium in the lymphocyte population not adherent to the allogeneic (Fig. 1A, d) or the syngeneic monolayer (Fig. 1A, e), and no increase in the number of T-cells was observed.

Treatment of the intact (Fig. 1B, a) immune lymphocytes or those eluted from the allogeneic monolayer (Fig. 1B, b) with antibodies against T-cell markers led to abolition of the action of the suppressors by 97-100%. Conversely, anti-Mls antibodies did not inactivate the suppressors. Meanwhile, suppressors eluted from the syngeneic monolayer were only partially inactivated by anti-T-sera, and also by anti-Mls-serum (Fig. 1B, c). This evidently means that about 25-40% of suppressors, nonspecifically attached to the syngeneic monolayer, are not T-lymphocytes.

It can be tentatively suggested that nonspecific suppressors, constituting about one-fifth of the population of unfractionated suppressors [4], and which are not T-cells, are adherent to any monolayer and can later be eluted. Since, however, the population of lymphocytes eluted from the allogeneic monolayer was enriched 30-fold with specific T-suppressors, the fraction of nonspecific suppressors in it could not exceed 3-5% [4]. For that reason, evidently, suppressors eluted from the allogeneic monolayer were hardly inactivated by carrageenan or iron carbonyl (Fig. 2), which eliminate macrophages but not T-cells.

It will be clear from Fig. 3 that in their morphological composition and the fraction of their cells synthesizing DNA, populations of intact immune lymphocytes and of those eluted from the syngeneic monolayer were very similar. They contained 55-60% of small, 25-60% of medium-sized, and 16-18% of large lymphocytes and only 10% of DNA-synthesizing cells. Conversely, the proportion of small lymphocytes in the lymphocyte fraction eluted from the allogeneic monolayer was reduced to 33%, and the proportions of medium-sized and large lymphocytes were increased to 40 and 27% respectively. The threefold increase in the number of

cells synthesizing DNA in this fraction (Fig. 3a) was connected with an increase in: a) the proportion of cells synthesizing DNA, among small (Fig. 3b) and medium-sized lymphocytes (Fig. 3c); b) the proportion of medium-sized (Fig. 3c) and large lymphocytes (Fig. 3d) in the population. The results of autoradiography are in agreement with those of determination of total DNA synthesis (Fig. 3e): This value was increased by 2 and 3 times after immunization of the mice with syngeneic and allogeneic cells respectively, but it again fell by half in the fraction of immune lymphocytes not adherent to the allogeneic (but not to the syngeneic) monolayer. This points to specific adsorption of a considerable proportion of the DNA-synthesizing immune lymphocytes After their subsequent elution it was found that they synthesized DNA twice and eight times more intensively than intact and nonadherent immune lymphocytes respectively. No increase in DNA synthesis was observed in the fraction of lymphocytes eluted from the syngeneic monolayer, and the increase was not due to treatment of the cells with pronase (Fig. 3e).

The increase in the proportion of DNA-synthesizing cells among all small and medium-sized lymphocytes, and also in the total proportion of medium-sized and large lymphocytes in the fraction of concentrated specific T-suppressors indicates that the latter were in a mitotic cycle, although DNA synthesis is not necessary for their functions. These properties bring the T-suppressors close to T-killers [2], although in many other biological and immunological respects [1] they differ cardinally. These results are in agreement with those of fractionation of T-suppressors, which as a rule, irrespective of the factors inducing them, are large lymphocytes, settling quickly in linear gradients of albumin and Ficoll [5, 14].

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