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USE OF MONOSACCHARIDE INHIBITORS TO STUDY THE CYTOSTATIC ACTION OF CYTOTOXIC T LYMPHOCYTES, MACROPHAGES, AND NONADHERENT SPLENOCYTES

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UDC 612.112.94.017.4.014.46:547.455

KEY WORDS: peritoneal lymphocytes; macrophages; splenocytes; cytostasis; monosaccharides.

Experiments have shown [9] that during immunization of mice with tumor cells not containing antigens of the H-2 complex, killer-cell activity cannot be found but cytostatic T-cells with the Lyt $1^{+}23^{-}$ phenotype are generated. Without preliminary sensitization the mouse splenocytes can exert a cytostatic action on tumor cells [5]. This effect is linked with the action of the cells themselves and not of soluble factors. These cells are incapable of phagocytosis but they form E-rosettes and have the Fc-receptor. The inhibitory action of monosaccharides and of phosphorylated monosaccharides on the recognition stage has been described in the literature, especially at the lytic stage of action of cytotoxic cells [13, 14]. However, the effect of monosaccharides on the function of cytostatic effectors has not been investigated, and this served as the motivation for the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out BALB/c mice aged 2-4 months. Cytostatic peritoneal lymphocytes (PL) were obtained in BALB/c mice on the 11th day after intraperitoneal injection of EL-4 cells, as described previously [1]. Macrophages were obtained by washing out the peritoneal cavity of the mice with medium containing heparin (10 U/ml) 3 days after intraperitoneal injection of 1.5 ml of a 10% solution of peptone into the animals, followed by adsorption on plastic Petri dishes and removal of the adherent cell population. The fraction of splenocytes not adherent to plastic was used as the effectors. To assess the functional activity of LP the method in [3] was used. The target cells and cold inhibitors were EL-4 and P-815 tumor cells, subcultured in mice.

TABLE 1. Cytostatic Action of Different Effector Cells on P-815 Cells during Incubation for 18 h (M ± m)

Effectors	Ratio effectors/targets	Synthesis inhibition, %	
		DNA	RNA
Macrophages	20:1	51±6	62±7
Spenocytes	20:1	71±6	78±7
PL	10:1	91±4	92±3

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 480-483, October, 1987. Original article submitted December 22, 1986.

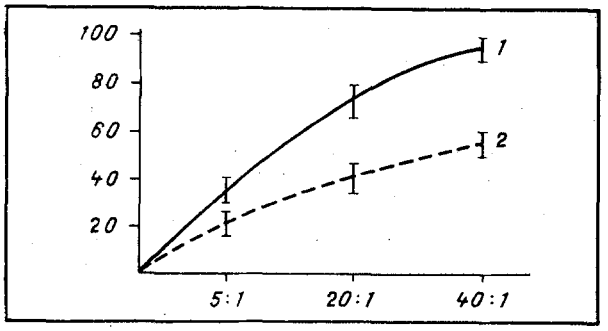

Fig. 1. Effect of unlabeled EL-4 and P-815 cells on cytotoxic activity of LP obtained in BALB/c mice after intraperitoneal injection of EL-4 cells. Abscissa, ratio of cold inhibitor/target cell; ordinate, inhibition (in %). 1) Cold inhibitor of EL-4 cell, 2) cold inhibitor of P-815 cell. Cytotoxicity of PL against EL-4 target cells varied from 35 to 50% with a killer to target cell ratio of 10:1.

To test the cytostatic action, we used a modified method [6], based on recording inhibition of RNA and DNA synthesis in the target cells. The target cells were P-815 cells. The effector cells were treated beforehand with actinomycin D in a concentration of 1 μg/ml per 10^7 cells at 37°C for 1 h. This treatment did not affect effector cell activity [5]. Incubation lasted 8 h at 37°C in air with 5% CO_2. ^{3}H-uridine or ^{3}H-thymidine was added to the wells 4 h before the end of incubation. The cytostatic index (CSI) was calculated by the equation given in [6].

Various sugars, added at the beginning of incubation in a final concentration of 25 mM, nontoxic for cells [7], were used as inhibitors of cytostasis.

To determine the nature of the cytostatic effects anti-Thy 1.2, anti-Lyt 1.2, and anti-Lyt 2.2 antisera were used.

EXPERIMENTAL RESULTS

Analysis of the cytotoxic activity of LP showed that they produce lysis of EL-4 cells but not of P-815 cells. However, as is clear from Fig. 1, the use of P-815 cells as cold inhibitors led to inhibition of the cytotoxicity of PL. The writers showed previously [1] that intraperitoneal injection of a lethal dose of L-1210 cells (leukemia cells with the same genotype as P-815 cells) into BALB/c mice at the peak of rejection of an allogeneic EL-4 tumor did not cause death of the animals. On the basis of these results the existence of cytostatic effectors was postulated in the population of alloreactive cells obtained on rejection of the EL-4 cells.

Results in support of this hypothesis are given in Table 1. Alloreactive lymphocytes had a powerful cytostatic action on P-815 cells. Splenocytes and macrophages also produced cytostasis, although it was weaker. The degree of inhibition of DNA synthesis in the target cells depended on the duration of incubation with effector cells and reached a maximum (Fig. 2) on the 3rd day; however, further incubation led to a decrease in CSI.

The results of inhibition of the cytostatic effect of different effector cells by sugars are given in Fig. 3. L-fucose inhibited the cytostatic action of all the effector cells used; inactivation to cytostasis, tested with respect to RNA synthesis in target cells was characteristic of macrophages and splenocytes, whereas that tested with respect to DNA synthesis was characteristic of alloreactive cells. L-mannose blocked the cytostatic action of both alloreactive cells and macrophages but had little effect on splenocyte function. D-ribose, L-ribose, and D-galactosamine inhibited the cytostatic effect only of splenocytes. D-galactose inhibited cytostasis only of alloreactive cells; cytostasis tested with respect to RNA synthesis, moreover, was blocked more strongly. D-mannose, D-fucose, and D-glucose did not inhibit cytostasis in general.

During the use of antisera to analyze the cytostatic effector cells it was found that anti-Thy 1.2 antibodies depressed CSI of the alloreactive cells by 39% and of splenocytes by 21%; anti-Lyt 1.2-antibodies depressed CSI of the alloreactive cells by 27% but did not affect

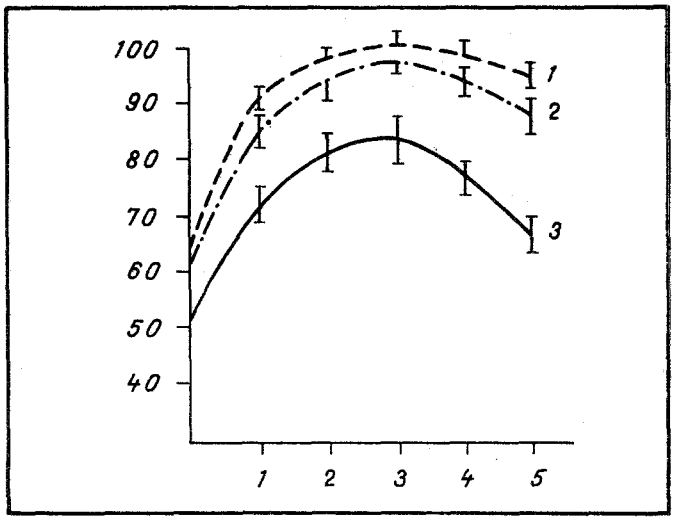

Fig. 2. Effect of duration of combined incubation on inhibition of DNA synthesis in P-815 target cells. Abscissa, time (in days); ordinate, CSI. 1) Effector cells — PL, 2) effector cells — splenocytes, 3) effector cells — macrophages.

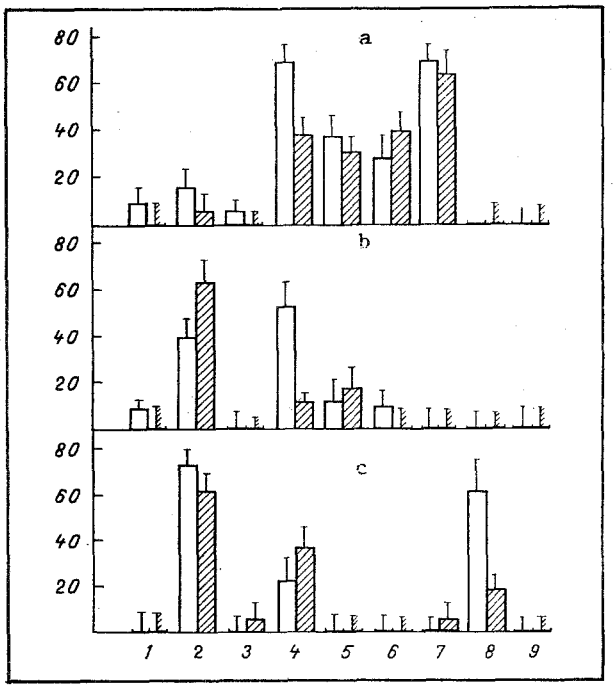

Fig. 3. Inhibition (in %) of cytostatic action of various effector cells on P-815 cells by sugars: a) effector cells — splenocytes, b) effector cells — macrophages, c) effector cells — PL. Shaded columns indicate inhibition of cytostasis in test with ^{3}H-thymidine, unshaded columns — inhibition of cytostasis in test with ^{3}H-uridine. 1) D-mannose, 2) L-mannose, 3) D-fucose, 4) L-fucose, 5) D-ribose, 6) L-ribose, 7) D-galactosamine, 8) D-galactose, 9) D-glucose.

cytostasis of splenocytes; anti-Lyt 2.2-antibodies depressed CSI neither of alloreactive cells nor of splenocytes. The antisera depressed CSI only in the presence of complement. Consequently, in a population of nonadherent splenocytes and alloreactive cells not only T-cells possessed a cytostatic action.

Compared with data in the literature [13], the distribution of monosaccharides by their effectiveness on inhibition of cytotoxic cells differed in the present study. The use of

monosaccharides and of larger carbohydrate-containing molecules for inhibition by some workers, and the results of the use of the glycosylation inhibitor tunicamycin, are in agreement with the hypothesis [9] that recognition of carbohydrates on the target-cell membrane is an old mechanism, used by cells of the immune system for recognition and lysis. We previously [2] demonstrated the effect of a group of gangliosides on the sensitivity of tumor cells to the membranotoxic action of splenocytes and on the membranotoxic activity of the splenocytes themselves. The role of membrane gangliosides GM_1 and GD_3 in the cytostatic action of macrophages on P-815 cells was established recently [10]. It is evident that until recently no attempt has been made to examine the problem of what mechanisms are responsible for the adaptive reaction of phylogenetically old mechanisms of individual cells of the immune system relative to the tumor cell. However, this problem must be tackled in connection with publication of research [4] which, in particular, demonstrated the appearance of tumor-specific cytostatic macrophages in mice immunized with tumor cells.

After the appearance of V-genes in phylogeny, the response of the immune system to an antigen was provided mainly by proliferation of antigen-specific clones of lymphocytes. Several adaptive events, leading to the development of immunity, take place on the surface of the individual cell. Data published in this paper and, in particular, on the special anticytostatic activity of L-fucose, may be related to this problem. It has recently been shown that α-L-fucose [11] can induce not only a sharp increase in normal killer-cell (NKC) activity in mixed culture for 24-48 h, but can also widen the spectrum of the target cells, i.e., can affect specificity of NKC. The same monosaccharide inhibits cytotoxic cells when they are tested with target cells.

It is generally accepted that endogenous lectins on lymphocytes and macrophages are glycoprotein molecules that participate in interaction of this type. The general term "endogenous lactins" may include, in particular, enzymes of carbohydrate metabolism. For example, incomplete biosynthesis of blood group antigen leads to the appearance of terminal galactose residues, which may act as acceptors of α-2,3-sialyltransferase, which participates in biosynthesis of a known tumor antigen 19.9.

It can be postulated on the basis of the facts described above that the molecular equivalents of changes leading to the appearance of tumor-specific cells of natural resistance are adaptive synthesis of ectoenzymes of carbohydrate metabolism of lymphocytes and macrophages, and the associated changes in the surface glycoconjugates of effector cells. The conditions have now been created for direct experimental analysis of this possibility.

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