INHIBITION OF CTL-LINE LYSIS AFTER GANGLIOSIDES TREATMENT

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INTRODUCTION

Gangliosides, characteristic, nearly ubiquitous, constituents of the plasma membrane, may influence cell interactions, as well as growth and differentiation of normal and neoplastic cells. Moreover, it was demonstrated that exogenous gangliosides can inhibit lymphocyte responses to antigenic and mitogenic stimuli [1,2] and, more recently, that they are able to induce selective modulation of the CD4 molecule in mouse, rat, and human T lymphocytes [3,4].

In this study we observed that target cell lysis by cytotoxic T lymphocytes (CTLs), and cytotoxicity mediated by lymphokine-activated killer cells (LAK), as well as large granular lymphocytes (LGL) were inhibited or strongly reduced following incubation with the monosialoganglioside GM1 and with the chemically modified GM1 molecule, AGF 118.

RESULTS AND DISCUSSION

To investigate GM1 incorporation by the plasma membrane of cytotoxic cells, the following different effector cell populations were used: a) a CTL-line (phenotype: $CD3^+$, $CD8^+$, $CD56^+$, $CD11a^+$) originally obtained by stimulation of peripheral blood lymphocytes (PBLs) with irradiated not matched PBLs, and maintained by restimulation (every 15 days) with irradiated cells in medium containing 10% human T cell growth factor (TCGF) and 100 U/ml recombinant interleukin-2 (rIL-2,Glaxo, Switzerland); b) LAK cells, generated by culturing PBLs in medium containing high rIL-2 doses (200 U/ml), and c) LGL (phenotype: $CD3^-$, $CD16^+$), obtained from PBLs of patients with lymphoproliferative disease of granular lymphocytes, and maintained in medium containing 10% TCGF, 100 U/ml rIL-2 and irradiated PBLs. Cytotoxic cells were incubated for different time intervals (from 1/2-4 hrs) with varying (from 50-500 $\mu g/ml/10^6$ cells) concentrations of GM1 [kindly provided by FIDIA Research Laboratories, Italy]. Following incubation, GM1 incorporation was assessed by evaluating the mean fluorescence intensity (MFI) of cells stained with rabbit anti-GM1 polyclonal antibody (FIDIA) and swine anti-rabbit immunoglobulin fluorescein-conjugated antibody (Dakopatts, Denmark) as a second step. Cytoflorimetric analysis, using an Epics-C flow cytometer (Coulter Electronics), demonstrated high GM1 uptake by cytotoxic cells following incubation with a GM1 concentration of 50 $\mu g/ml/10^6$ cells for 4 h at 37°C. At this dose level, no toxic effects on cell viability were detected by means of a dye exclusion assay.

The cytotoxic activity of GM1-treated cells was then evaluated by means of a ^{51}Cr release assay in serum-free medium. As shown in table 1, GM1 abolished CTL lytic activity against specific PHA-induced blasts. Similarly, LAK and LGL lytic activities against Raji and K562 target cells, respectively, were reduced following GM1 pretreatment, albeit to a lower extent than that observed with CTL. Since serum proteins inactivate GM1 activity, further experiments were carried out using AGF 118 which is still active in medium containing 5% serum [5]. However, inhibition of LAK and LGL cytotoxicity achieved by incubation with 50 $\mu g/ml/10^6$ cells was similar to that obtained following treatment with GM1 (Table 1). The inhibitory effect of GM1 was confirmed even when CTL activity was assayed

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by evaluating N-α-benzyloxylcarbonyl-L-lysin thiobenzyl (BLT)-esterase release, which is considered a specific marker of cytotoxic granule contents exocytosis (data not shown). To determine the mechanisms by which the gangliosides cause inhibition of cytotoxic activity, we studied possible GM1 binding to different surface molecules involved in lymphocyte-target cell interaction. To this end, the expression of CD3, CD8, CD11a, and α/β T cell receptor chains was evaluated on CTL cells stained with fluorescent antibodies. Preliminary results demonstrated that GM1 treatment does not mask these receptors, in the contrast to that observed for CD4 molecule expression [5]. An alternative possibility that gangliosides treatment can interfere with mechanisms mediating cell signal transduction is now under study.

TABLE 1

CYTOTOXIC ACTIVITY AFTER GANGLIOSIDES TREATMENT +

Effector cells *	$LU_{30}/10^6$ cells	Percent of inhibition
CTL	200 ± 18	-
CTL + GM1	0	100
CTL + AGF118	ND	ND
LAK	21 ± 3	-
LAK + GM1	12 ± 3	43
LAK + AGF118	8 ± 2	62
LGL	25 ± 5	-
LGL + GM1	12 ± 3	52
LGL + AGF118	12 ± 7	52

+ Both GM1 and AGF118 were used at 50μg/ml/10^6 cells. GM1 incubation was performed in serum-free medium.
* Cytotoxic activity of effector cells was evaluated using a ^{51}Cr release assay following incubation at 37°C for 4 hrs.

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