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ABILITY OF TUMOR CELLS TO RESIST HUMORAL vs. CELL-MEDIATED IMMUNE ATTACK IS CONTROLLED BY DIFFERENT MEMBRANE PHYSICAL PROPERTIES

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SUMMARY: Adriamycin-treated P815 tumor cells are increased in their killing by antibody plus complement (Ab-C) but not cytotoxic T lymphocytes (CTL). Conversely, mitomycin C-treated cells show increased killing by CTL but not by Ab-C. Hydrocortisone treatment decreases the killing of P815 cells by both Ab-C and CTL; epinephrine is ineffective in doing so. The drug and hormone effects are reversible. Adriamycin-treated P815 cells showed markedly increased plasma membrane fluidity. Mitomycin C-treated P815 cells showed markedly decreased net negative cell surface charge. Hydrocortisone-treated P815 cells showed decreased membrane fluidity and increased negative cell surface charge. There was no effect by mitomycin C on membrane fluidity, by adriamycin on surface charge, or by epinephrine on either membrane property. Reverted cells returned to control values of fluidity and cell surface charge. The results indicate that plasma membrane fluidity and cell surface charge are unique membrane physical properties that control tumor cell susceptibility to Ab-C and CTL killing, respectively.

INTRODUCTION

The concept that the metabolic properties and chemical composition of tumor cells can influence their susceptibility to killing by humoral (antibody-complement) and cellular (cytotoxic T-lymphocytes) immunologic factors has been advanced by several investigators (1-6). In this regard, it was shown that metabolic inhibitors and hormones that increase or decrease, respectively, the susceptibility of tumor cells to killing by antibody plus complement (Ab-C) or by cytotoxic T-lymphocytes cause characteristic changes in the ability of the cells to synthesize specific lipid-containing molecules that result in discrete changes in cellular lipid composition (1-6). Increased tumor cell sensitivity to Ab-C attack correlated with a decreased

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cholesterol:phospholipid mole ratio (CHOL:PL) and an increased content of unsaturated fatty acid (UFA) (3-5, 7). Tumor cells increased in sensitivity to CTL attack were unchanged in their CHOL:PL or UFA content, but displayed an increase in highly polar positively charged phospholipid content (1-6). These results suggested that the lipid content of tumor cell membranes may be fundamental for the mechanism by which they resist immune killing, and that different membrane lipid constituents are involved in the interaction of the cells with Ab-C vs. CTL attack systems. Because the cellular CHOL:PL and percent UFA are known to affect membrane fluidity (8-11), and the appearance of positively-charged molecules in the membrane will affect cell surface charge, the previous results prompted the present study to determine whether cell surface membrane physical properties might be involved in tumor cell defense against different forms of immunologic attack.

MATERIALS AND METHODS

P815 murine mastocytoma cells were maintained in vitro in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 2% of a penicillin/Streptomycin/fungizone solution (Grand Island Biological Co.).

P815 tumor cells were incubated for 24 hrs. at 37°C in medium alone or in medium containing 20 μg adriamycin/ml, 50 μg mitomycin C/ml, $5x10^{-4}$M L-epinephrine methyl ether HCl, or 10^{-3}M hydrocortisone sodium succinate as described in (1, 6). The cells were then tested for their susceptibility to Ab-C killing by rabbit anti-P815 antibody plus guinea pig C in a trypan blue exclusion assay and to CTL killing by allogeneic (C57Bl/6), P815-sensitized splenic T-lymphocytes in a 4 hr ^{51}Cr release assay. Details of these assays are described fully in (1, 6). In addition, after 24 hrs. of incubation with drug or hormone, the cells were washed thoroughly and reincubated for 24 hrs. at 37°C in tissue culture medium alone. These reverted cells were then similarly tested for their susceptibility to Ab-C and CTL killing.

The drug- or hormone-treated cells and the reverted cells were also tested for their membrane fluidity and cell surface charge properties. Isolated plasma membranes were prepared by the method of Rethy et al (12) and tested for purity by enzymic analysis and distribution of ^{125}I-iodosulfanilic acid as described by Schlager and Ohanian (13). Fluorescence polarization measurements on the isolated membranes labeled with 1,6-diphenyl-1,3,5-hexatriene (DPH) were carried out as described by Shinitzky and Barenholz (14) and Van Hoeven et al (15).

Cell surface charge measurements were carried out on whole unfractionated P815 cells using the method of cell partition into immiscible phases of

Table 1: Effect of Drug Treatment on the Susceptibility of P815 Tumor Cells to Killing by Antibody plus C and by Cytotoxic T-Lymphocytes.[a]

Treatment	(A) Antibody-Complement[b] % Cytotoxicity +24 hr[c]	+ 24 hr/-24 hr[d]
Adriamycin	84 ± 2	11 ± 4
Mitomycin C	2 ± 1	6 ± 1
Untreated	4 ± 1	4 ± 1

Treatment	(B) Cytotoxic T-lymphocytes[e] % Cytotoxicity +24 hr[c]	+ 24 hr/-24 hr[d]
Adriamycin	42 ± 1	43 ± 2
Mitomycin C	71 ± 2	38 ± 3
Untreated	40 ± 2	42 ± 3

[a]Means ± SE of four experiments.
[b]P815 cells were treated with rabbit anti-P815 antibody (diluted 1:500) plus guinea pig C (diluted 1:4). Cytotoxicity was quantified by trypan blue dye exclusion.
[c]P815 cells incubated for 24 hrs. at 37°C with or without drug.
[d]P815 cells incubated for 24 hrs. at 37°C with or without drug, washed, and reincubated for 24 hrs. at 37°C in drug-free media.
[e]P815 cells were mixed with allogeneic P815-sensitized spleen cells at an effector:target ratio of 100:1. Cytotoxicity was quantified by specific ^{51}Cr release after 4 hrs. incubation at 37°C.

critical mixtures of aqueous polymer solutions developed by Ballard et al (16). This method correlates very well with whole cell electrophoresis in measuring net negative cell surface charge (17).

RESULTS

Effect of Drug and Hormone Treatment on the Susceptibility of P815 Cells to Ab-C and CTL Killing. P815 tumor cells incubated for 24 hrs. at 37°C with adriamycin were increased, compared to untreated controls, in their susceptibility to Ab-C killing; mitomycin C treatment did not have this effect (Table 1A). Cells washed free of drug and recultured for 24 hrs. at 37°C in drug-free medium regained control levels of susceptibility to Ab-C attack (Table 1A). P815 tumor cells incubated for 24 hrs. at 37°C with mitomycin C were increased, compared to untreated controls, in their susceptibility to CTL killing; adriamycin treatment did not have this effect (Table 1B).

Table 2: Effect of Hormone Treatment on the Susceptibility of P815 Tumor Cells to Killing by Antibody plus C and by Cytotoxic T-lymphocytes.[a]

Treatment	(A) Antibody-Complement[b] % Cytotoxicity +24 hr[c]	+ 24 hr/-24 hr[d]
Epinephrine	52 ± 5	62 ± 2
Hydrocortisone	8 ± 2	58 ± 3
Untreated	48 ± 3	52 ± 2

Treatment	(B) Cytotoxic T-Lymphocytes[e] % Cytotoxicity +24 hr[c]	+ 24 hr/-24 hr[d]
Epinephrine	58 ± 3	50 ± 2
Hydrocortisone	18 ± 3	58 ± 2
Untreated	60 ± 3	59 ± 3

[a]Means ± SE of four experiments.
[b]P815 cells were treated with rabbit anti-P815 antibody (diluted 1:200) plus guinea pig C (diluted 1:4). Cytotoxicity was quantified by trypan blue dye exclusion.
[c]P815 cells incubated for 24 hrs. at 37°C with or without hormone.
[d]P815 cells incubated for 24 hrs. at 37°C with or without hormone, washed, and reincubated for 24 hrs. at 37°C in hormone-free media.
[e]P815 cells were mixed with allogeneic P815-sensitized spleen cells at an effector:target ratio of 200:1. Cytotoxicity was quantified by specific ^{51}Cr release after 4 hrs. incubation at 37°C.

Again, cells washed free of drug and reincubated in drug-free medium for 24 hrs. at 37°C reverted to control levels of susceptibility to CTL attack (Table 1B). P815 cells cultured for 24 hrs. at 37°C with hydrocortisone were rendered more resistant, compared to untreated controls, to Ab-C (Table 2A) or CTL (Table 2B) killing; epinephrine treatment had no effect on the cells' susceptibility to either form of immune attack (Table 2). Cells washed free of hormone and reincubated in medium alone for 24 hrs. at 37°C reverted to control levels of susceptibility to Ab-C and CTL attack (Table 2).

Effect of Drug and Hormone Treatment on the Physical Properties of P815 Tumor Cell Membranes. Plasma membranes isolated from adriamycin-treated P815 tumor cells were nearly doubled in their fluidity compared to untreat-

Table 3: Effect of Drug and Hormone Treatment on P815 Cell Membrane Physical Characteristics[a]

	(A) Fluidity (ϕ)[b]		(B) Surface Charge (σ)[c]	
Treatment	+24 hr[d]	+24 hr/-24 hr[e]	+24 hr[d]	+24 hr/-24 hr[e]
Adriamycin	0.50 ± .04	0.24 ± .05	1.16 ± 0.29	1.34 ± 0.29
Mitomycin C	0.25 ± .03	0.23 ± .04	0.86 ± 0.19	1.42 ± 0.22
Epinephrine	0.30 ± .05	0.26 ± .06	1.24 ± 0.02	1.20 ± 0.10
Hydrocortisone	0.12 ± .06	0.20 ± .04	1.47 ± 0.19	1.21 ± 0.13
Untreated	0.27 ± .06	0.21 ± .03	1.22 ± 0.22	1.36 ± 0.12

[a]Means ± SE of five experiments.
[b] ϕ = Fluidity (poise^{-1}). This is the reciprocal of microviscosity and was determined by a fluorescence polarization method employing diphenylhexatriene as a probe.
[c] σ = Surface charge density. This was determined by a cell partition method employing a dextran/polyethyleneglycol partition system.
[d]See Tables 1 and 2, footnote c.
[e]See Tables 1 and 2, footnote d.

ed controls; mitomycin C treatment had no such effect (Table 3A). In contrast, membranes isolated from hydrocortisone-treated cells were reduced by 55% in their fluidity compared to untreated cells; membranes from epinephrine-treated cells were the same as controls (Table 3A). Membranes isolated from drug- or hormone-treated reverted cells were indistinguishable from controls in their fluidity (Table 3A). Partition cell surface charge density measurements of normal P815 cells in culture alone for 24 hrs. at 37°C showed a σ value of 1.22; cells treated with mitomycin C were reduced by 30% in this value whereas hydrocortisone-treated cells were increased by 20% in their σ value compared to the controls (Table 3B). Treatment of the P815 cells with adriamycin or epinephrine had no effect on the cells' surface charge density (Table 3B). Drug- or hormone-treated reverted cells showed surface charge density values indistinguishable from those of control, untreated cells (Table 3B)

DISCUSSION

In this report, evidence is presented that suggests that certain unique physical properties of tumor cells enable them to resist C-mediated vs CTL-med-

iated killing. Previous work had shown that drugs, hormones, or other metabolic modulating agents that affect a tumor cell's susceptibility to immune attack also affect the lipid metabolism and composition of the cells; from these studies, a correlation was established between the metabolic state and chemical composition of a tumor cell and its susceptibility to Ab-C or CTL killing (1-7, 13). The present data serve to clarify this correlation. The ability of a tumor cell to resist Ab-C attack is correlated with biochemical changes in the cell that affect its cell surface membrane fluidity: i.e., increased fluidity correlates with increased susceptibility to Ab-C killing. This is consistent with reports from other laboratories showing that the efficiency of C in lysing erythrocytes, bacteria, liposomes, artificial black lipid membranes, and nucleated cells is related directly to the fluidity of the target membrane (reviewed in 4, 5).

In contrast, P815 tumor cells that were modulated in their susceptibility to CTL killing were unaffected in membrane fluidity, but were instead markedly affected in their surface charge density. Increased susceptibility to CTL killing correlated with decreased net negative surface charge density. In tumor-CTL interactions, evidence has been presented that intimate contact between the lymphocyte and its target occurs and may (18) or may not be (19) antigen specific for target cell killing to occur. The present data indicate that the ability of the CTL to approach and interact with its target is as important in determining the outcome of CTL attack as antigen recognition. Lymphocytes have a relatively high σ value (16, 17); thus, their ability to enter into the intimate contact with the target should depend on the σ of the target. A high-σ target cell (e.g., hydrocortisone-treated) will be more repulsive to the CTL than a low-σ (e.g., mitomycin C-treated) target. In addition, the cytotoxic principle that the CTL exerts on the tumor target may

be dependent on the surface membrane physical properties of the target. Further experiments are necessary to investigate these possibilities.

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