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Changes in plasma membrane phospholipids inhibit antibody-mediated lysis

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ABSTRACT

A variety of mechanisms have been proposed to explain how tumors evade immune destruction. This work has identified one such mechanism that determines susceptibility to immune lysis; membrane phospholipid composition altered susceptibility to antibody plus complement (Ab + C)-mediated lysis. Effects on antibody plus complement-mediated lysis were correlated with levels of major histocompatibility complex (MHC) molecules but not inherent resistance to complement damage. This cellular mechanism could be a means by which tumor cells escape immune detection and destruction.

1. Introduction

Tumor cells grow progressively in vivo despite the host's immune response; which include tumor-specific cytotoxic T lymphocytes (CTL; 1), tumor-specific antibodies and natural killer (NK) cells [1–14]. Many investigators have tried to identify unique tumor cell characteristics correlated with susceptibility to immune cytolysis, starting with differences in tumor cell composition and metabolism. For example, Mandel and Clark [15] studied the fatty acid composition of tumor cells in an attempt to correlate plasma membrane fluidity with changes in either antibody plus complement (Ab) or CTL-mediated lysis of the cells, with no effect. Shinitzky et al. [16] analyzed tumor cell membrane viscosity, noting that when plasma membrane viscosity increased the cells became better immunogens. Finally, Schlager and Ohanian [17] and Schlager [18] correlated the ability of tumor cells to incorporate fatty acids into complex membrane-bound lipids with changes in susceptibility to Ab- and CTL-mediated lysis. Unfortunately, these studies were not able to specifically and uniquely alter these individual parameters to determine specific effects on cytolysis. More recently, other investigators have found additional roles for phospholipids in inducing and preventing apoptosis, immune regulation, tumor progression and drug resistance [19,20].

In addition, an earlier study examining tumorigenic esophageal cell lines demonstrated a correlation between tumor cell lipids and the ability of a tumor-specific antibody to bind to its tumor antigen [21]. These results implicated tumor cell lipid composition as a potential mechanism for tumor escape from immune destruction. In the current study, we examined the effects of specific alterations in the phospholipid composition of target cells [22–24] on susceptibility to antibody-mediated (Ab + C) cytolysis. Growth of cells in choline analogs significantly changed target cell susceptibility to Ab + C lysis. A correlation of lytic sensitivity with changes in MHC levels was observed. It is proposed that membrane phospholipid composition may significantly alter a cell's susceptibility to immune destruction, allowing tumors to escape immune recognition and lysis.

2. Materials and methods

2.1. Mice

Balb.c and Balb.k inbred mice were used in these studies. All animal studies were reviewed, approved and conducted according to AAALAC guidelines.

2.2. Phospholipid supplementation of LM cells

Cultures of LM cells (fibroblast of C3H origin) were grown at 37C in Higuchi's medium [25] containing 20 mM HEPES buffer, pH 7.4. This medium is a chemically-defined, lipid-free medium that supports growth of LM cells indefinitely in the absence of serum when choline (C) is present. Cells to be grown in the presence of C analogs were harvested, washed and resuspended in the desired medium. The following C analogs were utilized: ethanolamine (E), monomethylethanolamine (MME), dimethylethanolamine (DME), L-2-amino-1-butanol (AB) and 3-aminopropanol (AP). Analogs were added at 40 μg/ml and except for AB, were obtained from Eastman

Abbreviations: Ab + C, antibody + complement; AB, L-2-amino-butanol; AP, 3-aminopropanol; C, choline; CVF, cobra venom factor; DME, dimethylethanolamine; E, ethanolamine; MME, monomethylethanolamine.

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Kodak Company. AB was obtained from Aldrich Chemical Company. The cells were grown for 3 days in the supplemented medium prior to use in the assays [22].

2.3. Phospholipid and fatty acid analyses

Cells grown in choline were incubated for 5d in the presence of 5 μCi/ml of ^{32}P-orthophosphate prior to exposure to C analogs. Cells were then exposed to C analogs for 3d, keeping the ^{32}P concentration constant. Cells were washed 2× in Ca^{2+}, Mg^{2+}-free PBS and whole cell lipids extracted by the method of Bligh and Dyer [26]. The phospholipids were separated by 2-dimensional thin layer chromatography (TLC) on silica gel H (250 μm), made visible by autoradiography [27], and counted in Omnifluor–toluene–Triton X-100–H_2O (4%–2:1:0.2) scintillation fluid in a scintillation counter. Phospholipids were identified by comparison with known standards. The solvent system consisted of chloroform–methanol–H_2O (87:31:5) in the first dimension and *N*-butanol-glacial acetic acid–H_2O (80:26:26) in the second dimension.

For fatty acids analysis, cells were harvested, phospholipids extracted and separated from neutral lipids on a silicic acid column (0.3 g Unisil in a disposable Pasteur pipette plugged with glass wool). The neutral lipids and phospholipids were eluted with 5 ml of chloroform and 4 ml of methanol, respectively. Fatty acid methyl esters were prepared from the phospholipid fraction by the addition of 2.5 ml of pure methanol and 0.2 ml of H_2SO_4 to samples in Teflon-lined screw-capped tubes. The tubes were flushed with nitrogen, sealed and heated at 80 °C for 2.5 h after which tubes were cooled, 2.0 ml of H_2O and 2.5 ml of pentane was added, and the pentane layer removed for analysis of fatty acid content via gas layer chromatography (OV351 capillary column, 62 m in length, 1 ml/min Helium flow rate, 155–200 °C at 8 °C/min and 201–245 °C at 5 °C/min).

2.4. Antibodies

H-2^d–anti-H-2^k antisera was produced in Balb.c mice following intraperitoneal injection of 2×10^7 Balb.k splenocytes, every other week for 10 weeks. The mice were bled by cardiac puncture and the sera were heat-inactivated at 56 °C, 30 min. The sera was absorbed with Balb.c splenocytes (1×10^7, 2×) prior to use.

The hybrodoma 15.3.1 (monoclonal anti-H-2K^k, [28]) was obtained from the Salk Institute (LaJolla, CA). Culture supernatants were purified by affinity chromatography on protein A columns and adjusted to 1.5 mg/ml prior to use [29].

2.5. Antibody plus complement (Ab + C)-mediated cytotoxicity assays

Target cells were labeled with ^{51}Cr (sodium chromate) and incubated at 4 °C, 30 min with serial dilutions of antisera in microtitre plates. The cells were washed 3× and a 1:10 dilution of rabbit complement (Pel-Freez) was added. The mixture was incubated at 37 °C, 30 min before washing (3×). Residual radioactivity in the cell pellets was determined using a gamma counter. Controls included media, complement and antibodies alone.

2.6. Measurement of complement

Experiments examining the ability of antibody when bound to target cells to deplete complement were performed as follows. Control and lipid altered cells were incubated with anti-H-2K^k monoclonal antibody and complement (rabbit) as described above. The cells were centrifuged (500*g*, 5 min) and the supernatant fluid (containing residual complement) were removed. The supernatant was added to hemolysin-sensitized sheep red blood cells (SRBC) and incubated for 45 min, 37 °C. The mixtures were centrifuged (500*g*, 10 min) and the absorbance of the supernatants was measured by spectrometry (541 nm). The optical density was compared to a standard curve to determine the levels of residual complement. Controls consisted of (a) cells to which no complement was added, (b) cells to which was added complement that had been incubated with antibody at 37 °C without the presence of cells, and (c) cells to which was added unabsorbed complement.

2.7. Antibody-independent complement activation

The ability of complement to induce lesions in target cell plasma membranes (independent of antibody) was examined as follows. ^{51}Cr-labeled cells were placed in 96 well microtitre plates (5×10^4 cells in 50 μl). Rabbit complement (50 μl, 1:5 dilution, Pel-Freez) and cobra venom factor (CVF; 50 μl, 1:5 dilution, Cordis Laboratories) were added, in that order, and the plates incubated at 37 °C. Controls consisted of cells incubated with (a) media alone, (b) complement alone, or (c) CVF alone. Plates were processed and ^{51}Cr release was calculated as described previously.

2.8. FACS analysis

H-2 antigen levels were examined by FACS analysis. The anti-H-2K^k mAb and a FITC-labeled rabbit-anti-mouse IgG antibody (Cappel Laboratories) were used. Samples were analyzed using an Ortho 50H Cytofluorgraph equipped with a 4 W argon laser.

2.9. Statistics

All statistical analyses were performed using Student's *t*-tests ($p < 0.05$) as found in SPSS.

3. Results

3.1. Specific alteration of cell membrane phospholipid composition by choline analogs

Target cells were grown in the presence of choline or one of five analogs (E, MME, DME, AB or AP) to specifically change membrane phospholipid composition. Choline-supplemented media served as the control media and was used for long term maintenance of the cells. As shown in Table 1, these culture conditions resulted in significant and specific alterations in the phospholipid profile of the cells. For AB and AP supplementation, it was possible to significantly introduce an unnatural phospholipid component into the cellular phospholipid profile. Cells grown in E-supplemented media poorly incorporate the analog as previously noted [19]. No significant differences were found in any of the minor phospholipids (e.g., sphingomyelin, phosphatidylserine, phosphatidylinositol, and cardiolipin).

Analyses were also performed to determine if these modifications affected the fatty acid composition of the cells, as this variable has been shown to alter target cell lysis [30]. As shown in Table 2, no significant changes in fatty acid composition due to phospholipid alterations were observed. Fatty acids of the 18:1 variety were the major constituent (>50%) in each one of the analog-supplemented cells. Only minor variations in other fatty acids (16:0, 16:1, 18:0) were observed.

3.2. Membrane phospholipid alterations inhibit Ab + C cytolysis

As shown in Fig. 1, changes in cellular phospholipid composition resulted in significant changes in susceptibility to Ab-mediated lysis using two different antibodies; an H-2^d–anti-H-2^k