Cell Growth-dependent Variation in the Sensitivity of Human and Mouse Tumor Cells to Complement-mediated Killing

S. H. Ohanian, M. Yamazaki, S. I. Schlager, and M. Faibisch

ABSTRACT

Cells removed from asynchronous cultures during lag, log, and stationary phases of growth were found to differ in their sensitivity to antibody/complement-mediated killing. The human lymphoblastoid line, Raji, was relatively more susceptible to killing by human anti-HLA antibody plus rabbit complement during the lag and log phases of growth, while the human lymphoid cell line, PY, was relatively more susceptible to rabbit antilymphocyte serum or human anti-HLA plus rabbit complement during the log and late-log phases of growth. The mouse mastocytoma cell line, P815, was relatively resistant to killing by rabbit anti-P815 plus guinea pig complement during the middle log phase of growth. The variation in sensitivity of the three cell lines was dependent upon the concentration of antibody used to sensitize the cells but not due to differences in antigen expression, antigen density, or net synthesis of DNA, RNA, protein, complex carbohydrate, or lipid-containing macromolecules.

These data suggest that the variability in susceptibility of the cells for complement-mediated killing is due to changes in physiological and/or physicochemical properties of the cells which either affect the ability of the cells to repair complement-mediated damage or nullify the activity of cell-bound complement.

INTRODUCTION

Differences in the sensitivity to killing by antibody plus complement have been reported for nucleated cells from several species. Some reports show that variations correlated with cell cycle-specific populations of the cells. In this regard, mouse tumor cell lines YAC (4), L1210 (11), and YCB (3, 15) were more susceptible in their G1 than in their G2 or S phases of growth. However, human lymphoid cells, RPMI 8866, were more susceptible in their G2 or S phase of growth (21), while Chinese hamster cells were more susceptible in S phase of growth (29). In similar experiments with RPMI 8866 cells, variation in sensitivity to complement-mediated killing was observed with cells from asynchronous cultures but not with cells from synchronous cultures (6). Studies with cultured human cells W1-L2 (8, 22), RPMI 1788, and RPMI 4098 (10) and mouse S 107 myeloma (13) also showed no variation in complement-mediated killing at any stage of synchronous growth. The reasons for these disparate results are not known.

The observations that nucleated cells from the same or different species do not show a similar cell cycle-dependent variation in sensitivity to complement-mediated killing suggests that properties common to the cells in synchrony are not directly related to the variations in sensitivity. In studies where sensitivity or lack of sensitivity of cells to immune attack was observed, no information was presented on the metabolic properties of the cells. Chemical and physical changes as well as stimulation of certain cellular metabolic pathways have been reported as a result of complement attack (for review, see Ref. 19). Using guinea pig hepatoma cells as target cells, we have confirmed these latter observations and, in addition, have shown that modification of specific lipid synthesis of the cells by chemical and physical agents influenced the sensitivity of cells to complement-mediated attack (17, 19, 20, 26, 27). Other investigators have also shown that chemical modification of cells from different animal species influences sensitivity of the cells to immune attack but have not made a detailed analysis of the effects of such treatments on cellular macromolecular synthesis (1, 9, 14). In the present paper, data are presented showing that 2 human cell lines (Raji and PY cells) and one mouse cell line (P815) from asynchronous culture vary in their sensitivity to humoral immune attack at different phases of cell growth. The cells were analyzed for antigen expression, net synthesis of DNA, RNA, protein, complex carbohydrate, and lipid to determine if there was a correlation with the cell variations to the complement killing.

MATERIALS AND METHODS

Cells. Human Burkitt lymphoma cells (Raji) were supplied by Dr. S. Ferrone (Columbia University). The human peripheral blood lymphoid cell (PY) was supplied by Dr. D. Mann (National Cancer Institute, NIH), and the mouse mastocytoma cell (P815-X2) was supplied by Dr. W. Farrar, Jr. (National Institute of Dental Research, NIH). Suspension cultures of Raji and PY were grown in RPMI 1640 \(^{6}\) (Grand Island Biological Co., No. 320-1875) supplemented with 10 and 20% FCS, respectively, and 1% antibiotic/antimycotic (Grand Island Biological Co., No. 600-5240). Suspension cultures of P815 were grown in Eagle’s minimal essential medium with Earle’s salts plus glutamine (Grand Island Biological Co.; No. 320-1095), 10% FCS, and 2% antibiotic/antimycotic. The cells were incubated at 37\(^{\circ}\) under 5% CO\(_2\)/air, and cell passage was performed by suspending stationary-phase cells (approximately 1 to 2 \(\times\) 10\(^6\) cells/ml) to a final concentration of 2 to 5 \(\times\) 10\(^6\) cells/ml fresh medium.

Antisera. Human BC (anti-HLA 3, 12) alloantiserum was obtained from Dr. D. B. Amos (Duke University). Human multispecific Schwarz alloantiserum were obtained from the tissue-typing laboratory at the Laboratory of Immunobiology, National Cancer Institute, NIH, Bethesda, Maryland 20205.

\(^{6}\) The abbreviations used are: RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640; FCS, fetal calf serum; HBSS, Hanks’ balanced salt solution; TCA, trichloroacetic acid.
Medical College of Virginia, Richmond, Va. Rabbit anti-human lymphocyte serum was obtained from Microbiological Associates, Inc., Bethesda, Md. Rabbit anti-P815 was prepared as described previously (25). All sera were heated at 56°C for 30 min, distributed in small volumes, and stored at -20°C until used.

Complement. Young rabbit (NIH) and guinea pig (JEM Research, Inc., Kensington, Md.) sera were used as the source of complement. All sera were absorbed at least 4 times with 5 x 10^7 cells/ml of serum at 0°C. The serum was filtered (0.45-μm Millipore HA filter; Millipore, Bedford, Mass.) and stored at -20°C.

Cytotoxicity. Twenty μl of appropriately diluted antibody were added to an equal volume of target cell suspension (10^5/ml) in HBSS/10% FCS in U-bottomed microtest plates (Linbro Scientific Co., New Haven, Conn.). After incubation for 30 min at ambient temperature, the cells were washed, 20 μl of the rabbit complement, in excess, were added, and the suspension was incubated at 37°C for 60 min. At this time, 20 μl of 0.4% trypan blue were added, and the percentage of cells taking up the dye was determined. Controls included cells plus complement alone and cells plus buffer alone. The cell and complement control values were ≤10%. Cytotoxicity experiments with P815 cells were performed with 100 μl of reagents, and guinea pig complement was used in place of rabbit complement. Rabbit and guinea pig complements were diluted 1/4 and 1/3, respectively, with HBSS/10% FCS.

Measurement of Antigen Expression. On various days after initiation of culture, Raji, P815, and PY cells were washed with 10 ml of HBSS/10% FCS, and 50 μl of appropriately diluted antibody were added to tubes containing different numbers of the absorbing cells. After incubation at 0°C for 30 min, the cells were removed by centrifugation, and the residual antibody activity was determined with cells which were most susceptible to killing by the antibody plus complement. The cytotoxicity test was performed as described above. The dilutions of antibody tested were: human BC alloantiserum (1/50); rabbit anti-human lymphocyte serum (1/50); and rabbit anti-P815 (1/800). Appropriate cells, complement, and buffer controls were included in the experiments.

Cell surface area of the cells used for absorption was determined by measuring cell diameters with a calibrated eyepiece micrometer. Measurements were made on at least 50 cells in samples applied to a hemocytometer. The measurements were made 3 times with cells from 3 separate experiments. Surface area was calculated using the formula

\[ A = \pi D^2 \]

where \( A \) is surface area, and \( D \) is cell diameter.

Macromolecular Synthesis. Cells were tested for their ability to synthesize DNA, RNA, protein, complex carbohydrate, and lipid. Cells (125,000 to 1.5 x 10^5) were suspended in 0.5 ml RPMI 1640 containing 1 μCi [H]thymidine per ml (Amersham/Searle Corp., Arlington Heights, Ill.; 28 Ci/mmol), 2 μCi [3H]uridine per ml (Amersham/Searle; 43 Ci/mmol), 0.2 μCi [1^-C]palmitic acid per ml (New England Nuclear; 50.2 mCi/mmol), or 2 μCi [1^-C]glucosamine hydrochloride per ml (Amersham/Searle; 38.6 Ci/mmol). Protein synthesis was determined in RPMI 1640 deficient in essential amino acids but containing 1 μCi of 14C-labeled L-amino acid-labeling mixture (Schwarz/Mann, Orangburg, N. Y.; L-arginine, 460 mCi/mmol; L-leucine, 312 mCi/mmol; L-lysine, 300 mCi/mmol; L-valine, 260 mCi/mmol). After 1 hr of incubation at 37°C, the cells were washed 3 times with ice-cold HBSS and 3 times with ice-cold 20% TCA. The TCA-precipitable radioactivity was dissolved in 10 ml Aquasol (New England Nuclear, Boston, Mass.) and quantified in a Beckman Model LS-333 scintillation counter. Controls included addition of the radiolabeled compounds to tubes containing medium alone (these values were less than 200 cpm).

RESULTS

Sensitivity to Humoral Killing of Cells Taken from Various Cell Growth Phases. Typical growth curves for Raji, PY, and P815 cells are shown in Chart 1. After initiation of culture, Raji and P815 cells are in lag-phase growth for approximately 1 day, log phase for 3 days, and stationary-phase growth by Days 5 to 7. PY cells, on the other hand, are in lag-phase growth for 1 to 2 days, log phase for 5 days, and stationary phase at approximately 9 days after initiation of culture. The doubling times for Raji, P815, and PY cells are approximately 17, 12, and 24 hr, respectively.

Cells taken from lag, log, and stationary phases of growth were examined for their sensitivity to killing by antibody plus complement. The cells were sensitized with equal volumes of serial 2-fold dilutions of the appropriate antibody, complement was added, and cytotoxicity was measured as described in "Materials and Methods." The results of representative experiments with Raji, PY, and P815 are presented in Charts 2, 3,
and 4, respectively. Raji cells sensitized with near excess or limiting dilutions (1/20 or 1/40, respectively) of antibody were more susceptible to killing by rabbit complement on Days 1 to 4 of culture (lag and log phase) than on Days 5 and 7 (stationary phase) (Chart 2). Results of antibody dose-response experiments presented in Chart 2B show that the differences in susceptibility to complement-mediated killing were not observed when the cells were sensitized with excess concentrations of antibody (1/10 dilution).

Variations in sensitivity to antibody/complement killing were also seen with the human lymphoid cells, PY (Chart 3). Cells sensitized with near excess (1/20) or limiting (1/80) concentrations of human anti-HLA antibody were more susceptible to killing by rabbit complement on Days 4 and 7 (log phase) than on Day 2 (lag phase) or Days 9 and 11 (stationary phase) (Chart 3, A and B). Similar results were observed with PY cells sensitized with rabbit anti-human lymphocyte serum and tested with rabbit complement (data not shown). Antibody dose-response experiments presented in Chart 3B show that the relatively resistant stationary-phase cells remained resistant when sensitized with excess antibody (1/10).

Chart 4, A and B, presents the results with P815 mouse mastocytoma cells. These cells when sensitized with limiting (1/1600) rabbit anti-P815 antisera were more susceptible to killing by guinea pig complement on Days 1, 2 (lag, early log), 4 (late log), and 7 (stationary) than on Day 3 (midlog phase) of culture. Chart 4B shows that growth phase-dependent variation in susceptibility to killing by guinea pig complement was not observed when the cells were sensitized with excess antibody (diluted 1/400).

We next wished to determine if the variation in sensitivity of the cells was due to differences in antigen expression.

**Antigen Expression during Cell Growth.** Cells collected at various times after initiation of culture were used to absorb appropriately diluted antibody, and the residual antibody activity was determined using Raji d3, P815 d7, and PY d7 cells as described in "Materials and Methods."

The results presented in Table 1 show that approximately 4 to 5 x 10^5 Raji cells removed from lag (d1), log (d3), and stationary phase (d5 and d7) of growth were required to absorb 50% of the cytotoxic activity from BC antisera. For PY cells removed from culture at Days 2, 4, 7, 9, and 11, approximately 0.5 to 0.6 x 10^5 cells were required to absorb 50% of the activity from rabbit anti-human lymphocyte serum. Similar results were seen with anti-HLA-antibody (data not shown). With P815 cells, 1, 2, and 2 x 10^5 log (d3), late log (d4), and stationary phase (d7) cells, respectively, absorbed equivalent amounts of anti-P815 activity.

The cell surface area measured as described in "Materials and Methods" decreased as the cells aged in culture (Table 1). The surface area of Raji and P815 in lag and log phases of growth was approximately 618 ± 50 sq µm, whereas with stationary-phase cells, the area decreased to approximately 290 ± 23 sq µm. The surface area of PY cells in log and stationary phases of growth was approximately 2400 ± 200 sq µm and decreased to approximately 1050 ± 90 sq µm.
Table 1 shows that antigen density on Raji and PY increased approximately 1.4- to 2-fold as the cells progressed from log to stationary phases of growth. With log-phase P815 cells, antigen density was the same for log- and stationary-phase cells (Table 1). No correlation between antigen density and sensitivity was seen. The antigen density of resistant Raji and PY cells was 1.4- to 2-fold greater than on sensitive cells, while no change in antigen density was observed with the P815 cells.

**Macromolecular Synthesis of Cells from Different Growth Phases.** Since no apparent correlation between antigen expression and sensitivity to antibody/complement killing was observed, the cells at lag, log, and stationary phases of growth were tested for their ability to synthesize DNA, RNA, protein, carbohydrate, and complex lipid. Cells were labeled as described in "Materials and Methods." Since the results obtained with Raji, PY, and P815 were similar, only the results for Raji will be presented (Chart 5). The cell lines showed maximum incorporation of thymidine, uridine, and amino acids into TCA-precipitable macromolecules during the lag and log phases of growth and decreased incorporation during the stationary phase. Incorporation of glucosamine by all 3 cell lines was low and did not vary at different phases of the growth of the cells. The cells showed variations in their ability to incorporate $[^{14}\text{C}]$ palmitate into TCA-precipitable macromolecules. Raji cells tested at Days 5 and 7 showed a decrease in incorporation of $[^{14}\text{C}]$palmitate compared to cells tested at Days 1 and 3 (Chart 5). In contrast, PY cells showed a 50% decrease in palmitate incorporation on Day 7, compared to all other time points (data not shown). P815 cells tested on Days 1, 2, and 7 incorporated $[^{14}\text{C}]$palmitate to a lesser degree (25 to 50%) than cells tested at Days 3 and 5 (data not shown).

**DISCUSSION**

In this paper, we have shown that 2 human lymphoblastoid cells (Raji and PY) and mouse mastocytoma cells (P815) vary in their sensitivity to killing by antibody plus complement during asynchronous cell growth in vitro. Raji cells were relatively more susceptible to killing by human anti-HLA antiserum plus complement during their lag and log phases of growth. The human lymphoid cells, PY, were relatively more susceptible to killing by rabbit anti-human lymphocyte serum or human anti-HLA antibody plus complement during their log and late-log phases of growth. In contrast to the human cell lines, P815 mouse mastocytoma cells were relatively resistant to antibody/complement killing for approximately 24 hr during their log phase of growth. The differences in sensitivity of the 3 cell lines were not correlated with changes in antigen expression or density as measured by quantitative absorption tests. Antigen expression remained constant on the human cell lines, while on the mouse cell line, the expression decreased as the cells aged in culture. The cell surface area of the cell lines in stationary phase was approximately 50% of that determined for lag-phase cells. Thus, antigen density at the times the cells were relatively sensitive to complement-mediated killing ranged from no detectable changes (P815) to approximately 20 to 50% less (Raji and PY) than that determined for the resistant cells. A similar lack of correlation between antigen density or expression and sensitivity to humoral immune attack has been reported for several cell lines including guinea pig hepatoma cells (15, 19, 28), human lymphoid cells (6, 21), and YCAB murine tumor cells (5, 11). While the results do not rule out the necessity for antigen in antibody-dependent, complement-mediated killing of cells, they do direct one to consider properties of the target cell which can influence the effectiveness of complement attack. It has been reported that during cell growth, changes occur in fluidity (2, 16), fragility, and turnover of membrane components (7, 12, 30, 31). Physicochemical and metabolic properties of cells have been shown to influence humoral immune killing (20).

Variations in sensitivity of nucleated cells to immune attack have not been directed at determining the composition and synthesis of cellular macromolecules. While chemical, physical, and metabolic changes have been detected in cells from several species as a result of complement attack, the emphasis has been directed mainly at the mechanisms of cell killing. In the present study, the synthesis of specific cellular macromolecules (DNA, RNA, protein, complex carbohydrate, lipid, or lipid-containing macromolecules) was determined with cells at the times they differed in their sensitivity to complement killing. No correlation was found between the net synthesis of these cellular macromolecules and sensitivity to antibody/complement killing. These results confirm and extend our previous observations that the sensitivity of guinea pig hepatoma cells was related to the synthesis and fatty acid composition of specific lipids (23, 24). The methods we have used in the present paper only detect changes in net lipid synthesis. It is possible that subtle differences in the synthesis and/or fatty acid composition of specific lipids and/or lipid-containing macromolecules may occur and influence the cytotoxic activity of complement or enable the cell to repair potentially cytotoxic damage to the membrane.

The sensitivity of the cells to complement killing does not appear to be related to a lack of sufficient nutrients in the medium. This is most obvious with the human lymphoid cell, PY, and the mouse mastocytoma cell, P815. PY cells show a relative increase in sensitivity, whereas P815 cells show a
relative decrease in their sensitivity to humoral immune attack during their midlog phase of growth. At the time when "crowding" or lack of nutrients would be expected (e.g., stationary or late-stationary phases of growth), PY and P815 cells are as sensitive as lag-phase cells (Days 1 and 2 after the cell cycle or asynchronous cell growth add a level of stationary or late-stationary phases of growth), PY and P815 ability of cell lines which show natural variations in sensitivity cells (19), human (6), and mouse (10) cell lines is that differences in sensitivity to complement attack were seen only when more than 60 to 70% of the cells). The observations that cells attack. However, when cultured at high density (1.5 x 10^6/ml) under similar conditions, the cells did not grow but did become sensitive after culture in fresh or conditioned medium from log-phase cultures. Taken together, the results suggest that the physiological state of the cells may also play a role in the ability of the cells to resist immune killing.

In general, the susceptibility of nucleated cells to immune attack may be looked upon as the net result of qualitative and quantitative properties of the attack mechanism versus the total capacity of the cells to nullify the cytotoxic activity which is generated (16, 20). Partial support for this concept in the present study and in previous studies with guinea pig hepatoma cells (19), human (6), and mouse (10) cell lines is that differences in sensitivity to complement attack were seen only when the cells were sensitized with less than excess amounts of antibody (i.e., cells sensitized with enough antibody to kill not more than 60 to 70% of the cells). The observations that cells are relatively sensitive to immune attack at selected parts of the cell cycle or asynchronous cell growth add a level of complexity to many studies directed at understanding the mechanism(s) of immune attack or cancer treatment. The availability of cell lines which show natural variations in sensitivity to complement-mediated killing allows the study of the mechanism(s) by which tumor cells influence the outcome of immune attack.

REFERENCES


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Cancer Res 1983;43:491-495.

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