Effect of Inhibiting DNA, RNA, and Protein Synthesis of Tumor Cells on Their Susceptibility to Killing by Antibody and Complement

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SUMMARY

A number of metabolic inhibitors and chemotherapeutic agents have been found to increase the sensitivity of a chemically induced guinea pig hepatoma (line 1) to killing by antibody and complement. We have investigated whether the mechanism whereby these drugs increase sensitivity to killing is attributable to their primary action of inhibiting DNA, RNA, or protein synthesis. Line 1 cells incubated for 1, 4, or 17 hr with actinomycin D (25 μg/ml), adriamycin (40 μg/ml), or puromycin (5 μg/ml) or with 5-fold lower concentrations of these drugs were maximally inhibited (>90%) in their ability to synthesize DNA, RNA, and protein within 1 hr. However, only cells incubated for 17 hr with the high concentrations of drugs showed increased sensitivity to killing by antibody and complement. Line 1 cells incubated with high concentrations of these drugs for 17 hr, washed, and resuspended in drug-free medium recovered their resistance to killing by antibody and complement within 4 hr. These cells even after culture for 24 hr in drug-free medium did not regain their ability to synthesize DNA, RNA, or protein. A similar lack of correlation between synthesis of these macromolecules and sensitivity to antibody-complement-mediated killing was observed after the cells were treated with physical agents that inhibit macromolecular synthesis. Both heat-treated and X-irradiated cells were inhibited in their ability to synthesize DNA, RNA, and protein immediately after treatment; however, only X-irradiated cells (6 and 16 hr postirradiation) were increased in their sensitivity to antibody-complement-mediated killing. Our data show that the ability of line 1 tumor cells to resist humoral immune attack does not depend solely on their ability to synthesize DNA, RNA, or protein.

INTRODUCTION

A number of metabolic inhibitors or chemotherapeutic agents used in the treatment of neoplasia have been found to increase the sensitivity of the cells to killing by antibody and complement (4, 18-20). These effects required relatively high drug concentrations and a long incubation time of the cells with drugs. In addition, it has been shown that culture of guinea pig tumor cells in media deficient in essential nutrients or with certain inhibitors of cellular energy metabolism failed to increase the sensitivity of the cells to killing by antibody and complement (2). These studies raised the question of whether the appearance of drug-induced sensitivity to humoral immune attack could be correlated with the inhibition of macromolecular synthesis. This prompted us to investigate whether the mechanism of action of the effective drugs in increasing sensitivity to antibody-complement-mediated killing could be attributed to their primary action of inhibiting macromolecular synthesis.

In this paper we have compared the ability of line 1 tumor cells to synthesize DNA, RNA, and protein to the susceptibility of these cells to killing by antibody and GPC. We now report the existence of conditions for inhibition of the metabolism of line 1 cells by chemical agents (i.e., actinomycin D, puromycin, or adriamycin) or by physical treatment (i.e., heat or X-irradiation) that do not affect the sensitivity of the cells to antibody-GPC-mediated killing.

MATERIALS AND METHODS

Animals. Inbred Sewall-Wright strain 2 male guinea pigs were obtained from the Frederick Cancer Research Center, Frederick, Md. The animals weighed approximately 250 g.

Cells and Reagents. Line 1, a diethylnitrosamine-induced hepatic tumor passed in strain 2 guinea pigs, was collected as described in Refs. 13, 24, and 25 and suspended in RPMI 1640-20% FCS (Grand Island Biological Co., Grand Island, N. Y.). All cytotoxicity assays were performed in isotonic Veronal-buffered saline containing 0.1% gelatin, 0.001 M Mg2+, and 0.00015 M Ca2+.

Treatment of Line 1 Tumor Cells with Drugs. The metabolic inhibitors used in this study, actinomycin D (NSC 3053) and adriamycin (NSC 123127), were obtained from the National Cancer Institute Cancer Therapy Evaluation Branch, Bethesda, Md. Puromycin dihydrochloride (Lot 43C-0430) was obtained from Sigma Chemical Co., St. Louis, Mo.

The treatment of tumor cells with drugs has been described previously (18-20). Briefly, line 1 tumor cells (5 × 10⁶ cells/ml) in RPMI 1640-20% FCS with added drugs (see

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2 The abbreviations used are: GPC, guinea pig complement; RPMI 1640-20% FCS, Roswell Park Memorial Institute Tissue Culture Medium 1640 containing 20% fetal calf serum and 1% antibiotic-antimycotic mixture; RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640 alone.
legend to Chart 1 for concentrations) were incubated at 37° in 5% CO₂-air. At the appropriate time intervals, aliquots of the suspensions were drawn off, and the cells were washed and tested for their ability to synthesize DNA, RNA, and protein and for their susceptibility to killing by antibody and complement. Controls consisted of tumor cells cultured in RPMI 1640-20% FCS without added drugs.

**X-irradiation of Line 1 Tumor Cells.** Line 1 cells (10⁶ cells/ml) suspended in 1 ml RPMI 1640 were irradiated in air in 12- x 75-mm polypropylene-capped tubes with a Westinghouse 250 KVCP Dual Quadrocondex X-ray unit (200 kV, 15 ma, 0.9 mm Cu). Cells were irradiated at a distance of 25 cm from the X-ray portals. After irradiation with 3000, 6000, and 9000 R, the cells were suspended at 5 × 10⁶/ml in RPMI 1640-20% FCS and incubated at 37° in 5% CO₂-air. Control unirradiated cells were prepared concurrently.

**Heat Treatment of Line 1 Tumor Cells.** Line 1 cells (10⁶ cells/ml) suspended in RPMI 1640 were heated for 30 min at 43°, 44°, or 45°. Controls consisted of cells maintained at ambient temperature (23°) in RPMI 1640-20% FCS. After treatment an equal volume of RPMI 1640-20% FCS was added, and the cell suspensions were incubated at 37° in 5% CO₂-air.

**Measurement of Macromolecular Synthesis.** Line 1 cells were tested for their ability to incorporate precursors of DNA, RNA, and protein. Two hundred fifty thousand cells were suspended in 0.5 ml RPMI 1640-20% FCS containing actinomycin D (25 μg/ml), adriamycin (40 μg/ml), or puromycin (5 μg/ml). One, 4, and 17 hr later, the cells were tested for their ability to incorporate [³H]thymidine, [³H]uridine, and [¹⁴C]-labeled amino acids and for their susceptibility to killing by anti-Forsmann IgM or specific antitumor antibody plus GPC.

**RESULTS**

Effect of Metabolic Inhibitors on Macromolecular Synthesis and Susceptibility to Antibody-Complement-mediated Killing of Line 1 Tumor Cells. Five million line 1 tumor cells were cultured at 37° in 10 ml RPMI 1640-20% FCS containing actinomycin D (25 μg/ml), adriamycin (40 μg/ml), or puromycin (5 μg/ml). One, 4, and 17 hr later, the cells were tested for their ability to incorporate [³H]thymidine, [³H]uridine, and [¹⁴C]-labeled amino acids and for their susceptibility to killing by anti-Forsmann IgM or specific antitumor antibody plus GPC.

Chart 1 illustrates the results of a representative experiment. Line 1 cells cultured with actinomycin D, adriamycin, or puromycin were inhibited 85 to 99% in their incorporation of labeled precursors after incubation for 1, 4, or 17 hr. These cells were resistant to killing by anti-Forsmann or specific antitumor antibody plus GPC after incubation with the drugs for 1 and 4 hr. A marked increase in susceptibility to killing by either antibody plus GPC, compared to control cells, was observed only after 17 hr of exposure to the drugs (Chart 1).

We next chose conditions (as suggested from the data in Chart 1) that would result in maximal inhibition of macromolecular synthesis of the cells to determine whether these cells would at any time become susceptible to antibody-complement-mediated killing. Line 1 cells (5 × 10⁶/ml) were cultured for 1 hr at 37° in RPMI 1640-20% FCS containing the appropriate concentrations of drugs (Chart 2). The cells were then washed free of drug, resuspended at 5 × 10⁶ cells/ml in RPMI 1640-20% FCS without added drugs, and reincubated at 37°. Immediately after treatment and 4 and 17 hr later, aliquots of the cells were tested for their ability to incorporate the radiolabels and for their susceptibility to killing by antibody plus GPC. After 1 hr in culture with actinomycin D or puromycin, the tumor cells were inhibited 85 to 95% in their ability to incorporate [³H]thymidine, [³H]uridine, or [¹⁴C]-labeled amino acids (Chart 2); this inhibition persisted after incubation for 4 and 17 hr in drug-free medium. At no time during the course of the experiments were the cells rendered susceptible to killing by either anti-Forsmann or specific antitumor antibody plus GPC (Chart 2). Similar results were obtained with adriamycin (data not shown).

When line 1 cells were incubated for 17 hr with 5-fold-lower concentrations of puromycin (1 μg/ml) or actinomycin D (5 μg/ml), they were inhibited 90 to 95% in their incorporation of the radiolabeled precursors but showed no increase in susceptibility to killing by antibody plus GPC (data not shown).

It has been previously reported (19, 20) that tumor cells cultured for 17 hr with metabolic inhibitors, washed free of
drugs, and reincubated in drug-free medium will revert to the resistant state within 4 hr. Experiments were next performed to determine whether the reversion of drug-treated cells to a state of resistance to antibody-complement-mediated killing was related temporally with the recovery of the ability of the cells to synthesize DNA, RNA, or protein. Line 1 tumor cells \(5 \times 10^6\) cells/ml were cultured for 17 hr at 37°C in RPMI 1640-20% FCS containing the appropriate concentrations of drugs (Chart 3). At this time the cells were washed free of drug, resuspended at 5 \(\times 10^6\) cells/ml in RPMI 1640-20% FCS, and reincubated at 37°C. Immediately and 4 and 17 hr later, aliquots of the cells were tested for their ability to incorporate \(^{3}H\) thymidine, \(^{3}H\) uridine, and \(^{14}C\)-labeled amino acids and for their susceptibility to killing by antibody plus GPC. As expected (19, 20), cells exposed to drug for 17 hr, washed, and tested immediately (0 hr) had been rendered susceptible to killing by anti-Forssman and specific antitumor antibody plus GPC. Reversion to the resistant state occurred within 4 to 17 hr of culture in medium free of inhibitors (Chart 3). No recovery in the ability of the cells to incorporate the labeled precursors was observed at any of the time intervals tested (Chart 3). These results suggested that the effect of metabolic inhibitors on the susceptibility of the line 1 cells to killing by antibody and complement was not due to the primary action of inhibiting the synthesis of DNA, RNA, and protein. We next examined two purely physical agents reported to inhibit macromolecular synthesis of nucleated cells, X-irradiation (21) and heat (3, 5, 9, 11, 12, 15), to determine whether a nonchemical treatment could increase the susceptibility of line 1 cells to killing by antibody and GPC. These treatments were used to obviate the need for the continued incubation of the cells...
with chemical agents and to determine the exact time of insult on the cells.

**Effect of Physical Agents on Macromolecular Synthesis and Susceptibility to Antibody-Complement-mediated Killing of Line 1 Tumor Cells.** Line 1 cells were exposed to 3000, 6000, and 9000 R of X-irradiation as described in "Materials and Methods." Immediately postirradiation and 1, 6, and 16 hr later, the cells were tested for their ability to incorporate [³H]thymidine, [³H]uridine, and [¹⁴C]-labeled amino acids and for their susceptibility to killing by anti-Forssman or specific antitumor antibody plus GPC. With cells exposed to 9000 R, maximal inhibition (60 to 80%) of incorporation of radiolabels was observed immediately postirradiation and persisted after 16 hr in culture at 37° (Chart 4). Cells exposed to 3000 or 6000 R were not maximally inhibited in their incorporation until after 16 hr in culture at 37°. None of these cells demonstrated increased susceptibility to killing by either antibody plus GPC until after 6 hr in culture postirradiation; the increase in susceptibility of the cells to killing was most marked 16 hr after irradiation (Chart 4).

Similar experiments were performed in which line 1 cells were heated for 30 min at 43°, 44°, or 45°. Immediately and 1, 3, 6, and 24 hr after heating, the cells were tested for their ability to incorporate [³H]thymidine, [³H]uridine, and [¹⁴C]-labeled amino acids and for their susceptibility to killing by...
anti-Foressman or specific antitumor antibody plus GPC. Line 1 cells heated for 30 min at any of these temperatures were consistently inhibited 80 to 90% in their ability to incorporate the radiolabels immediately after heating; this effect persisted for 6 hr after heating. After 24 hr incubation at 37°C, heated cells were inhibited only 30% in their ability to synthesize DNA, RNA, and protein. At no time up to 24 hr after heating were these cells rendered susceptible to killing by either antibody plus GPC. Once out of a total of 9 experiments, an increase in susceptibility to killing was observed; this occurred with anti-Foressman antibody plus GPC immediately and 1 hr after heating at 45°C. After 3 hr incubation at 37°C, the cells were as resistant to killing as were control cells.

**DISCUSSION**

Segerling et al. (18–20) have reported that pretreatment of guinea pig hepatoma cells with certain metabolic inhibitors or chemotherapeutic agents increased the sensitivity of the cells to killing by antibody and complement. These observations suggested that some intrinsic property(s) of the cell under metabolic control may contribute to the ability of the tumor cells to resist humoral immune attack. However, not all inhibitors of cellular metabolism were effective in increasing the sensitivity of the cells to killing (2, 19, 20). We therefore examined the relationship of the ability of the cell to synthesize DNA, RNA, and protein and its susceptibility to killing by antibody and complement. For this purpose we used 3 drugs, adriamycin, actinomycin D, and puromycin, which are known to have their major inhibitory effect on DNA (8, 22, 23), RNA (10), and protein synthesis (16), respectively.

In this paper we have shown that the susceptibility of line 1 tumor cells to killing by antibody and GPC does not correlate with the ability of the cells to synthesize DNA, RNA, or protein. We have demonstrated conditions under which the cells are inhibited in their capacity to synthesize these macromolecules without being rendered sensitive to humoral immune attack. For example, line 1 cells treated with high concentrations of puromycin (5 μg/ml), actinomycin D (25 μg/ml), or adriamycin (40 μg/ml) were inhibited in their capacity to synthesize DNA, RNA, or protein by greater than 90% within 1 hr, and they remained inhibited for a further 16 hr even in the absence of the drugs. However, the increased sensitivity of these cells to killing by antibody and complement was observed only after 17 hr in the continued presence of the drugs. Line 1 cells incubated with 5-fold lower concentrations of any of these drugs showed similar levels of inhibition of DNA, RNA, and protein synthesis, but an increase in sensitivity to antibody-complement killing was never observed.

Furthermore, as previously reported (19, 20), cells treated with these drugs for 17 hr, washed, and resuspended in drug-free medium reverted to a state of resistance to killing by antibody and complement within 4 hr of culture at 37°C. However, these cells never regained their capacity to synthesize DNA, RNA, or protein. All these results suggest that the increased sensitivity of line 1 tumor cells to humoral immune attack following treatment with metabolic inhibitors is not due to the basic property of the cells in inhibiting DNA (adriamycin), RNA (actinomycin D), or protein (puromycin) synthesis.

Two physical means of inhibiting cellular metabolism (heat and X-irradiation) were also tested to determine whether a nonchemical treatment could result in increased sensitivity of line 1 cells to killing by antibody and GPC. The advantage of such physical agents is in knowing the exact time of insult and in having no need for the continued presence of the agent. Our results indicated that inhibition of macromolecular synthesis of the tumor cells following X-irradiation preceded by at least 6 hr the appearance of increased sensitivity of the cells to antibody-complement-mediated killing. In addition, line 1 cells exposed to hyperthermic conditions (43–45°C) were uniformly inhibited immediately after heating in their ability to synthesize DNA, RNA, or protein. However, at no time interval up to 17 hr post-treatment were the cells rendered susceptible to antibody-GPC-mediated killing.

The observations presented here do not exclude the possibility that increased sensitivity to antibody-complement-mediated killing by tumor cells following chemical or physical treatment is associated with an effect by these agents on pathways of cellular metabolism other than DNA, RNA, or protein synthesis. Since the action of antibody and complement occurs on or in the cell membrane (6, 7, 14), we are now studying whether treatments that increase the sensitivity of tumor cells to immune attack can be correlated with a change in the properties of the cell membrane or with changes in other metabolic pathways associated with membrane structure and function.

**REFERENCES**


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