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Immunology

Time-dependent resistance or susceptibility of tumor cells to cytotoxic antibody after exposure to a chemotherapeutic agent

(melphalan/myeloma cells/cell-surface antigens/cell cycle/fluorescence-activated cell sorter)

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ABSTRACT **We report that a chemotherapeutic agent (melphalan) can affect the sensitivity of tumor cells to cytotoxic antibody. Depending on the time interval between drug treatment and subsequent exposure to antibody and complement, the tumor cells can be either more resistant or more susceptible to antibody when compared to control cells. The number of tumor cells surviving the combined treatment was determined by a colony inhibition assay. The two antisera used in this study were directed against either virus-specific or myeloma protein-specific antigens on the surface of S107 murine myeloma cells; identical results were obtained with both sera. Twenty-four hours after exposure to the drug, the number of tumor cells surviving the antibody treatment increased. During this period of increased resistance, the tumor cells were temporarily arrested in the G_2 phase of the cell cycle. After this period of maximal resistance, the effect of cytotoxic antibody on the cells changed such that 4 days after melphalan treatment the cells were significantly more susceptible to the antibody than were the sham-treated control cells. The period of increased susceptibility correlated with an increased density of S107 myeloma protein and viral antigens on the surface of the tumor cells. Eight days after the drug treatment, the susceptibility of the tumor cells and the density of surface antigens both returned to normal levels. This study shows that the correct time interval between exposure to a drug and subsequent treatment with antibody is critical for maximal killing of the tumor cells. The basis for the differential sensitivity of the tumor cells to antibody may be related to the drug-induced changes in the cell cycle and in antigen expression on the cell surface.**

Several antimetabolic or chemotherapeutic agents severely affect macromolecular synthesis in normal and malignant cells and often arrest cycling cells in their progression from one phase of the cell cycle to another (1, 2). The drug concentrations required to reversibly block the progression of the cells in cycle are generally much lower than those doses required to kill tumor cells (3). Tumor cells that are not reached by cytotoxic concentrations of the drug might, therefore, be temporarily inhibited in one phase of the cell cycle. This arrest in a particular stage of the cycle may modify the properties of the cell-surface membrane and could therefore influence the effectiveness of subsequent treatment with cytotoxic antibodies. In this paper we demonstrate that a chemotherapeutic agent can make tumor cells either markedly resistant or very susceptible to specific antibody and complement. The increased resistance initially observed after drug treatment correlated with an arrest of the tumor cells in the G2 phase of the cell cycle, whereas the subsequent period of increased susceptibility correlated with the period in which the density of the surface antigens was significantly elevated.

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MATERIALS AND METHODS

Cells. The S107 myeloma culture line, originally derived from a BALB/c mouse myeloma, produces a phosphorylcholine-binding $\kappa\alpha_1$ myeloma protein with well-defined idiotypic determinants (4). The subclone S107.3.7.1 used in this study has a doubling time of 15.1 hr, secretes large amounts of myeloma protein, and displays a high density of surface immunoglobulin (5). The S107 myeloma cell line was maintained in Dulbecco's modified Eagle's medium containing 20% heat-inactivated horse serum, glutamine, nonessential amino acids, penicillin, and streptomycin.

Consistent with the reports that many murine myeloma lines produce RNA tumor viruses (6) we have found that this subline also releases large amounts of type C RNA tumor viruses by using the following criteria: electron-microscopic visualization of type C particles, [^{3}H]uridine incorporation and positive reverse transcriptase activity in sucrose gradient-purified fractions corresponding to a buoyant density of 1.16–1.18 g/cm^3, and positive cell surface immunofluorescence upon treatment with heterologous antisera specific for the 71,000-dalton glycoprotein (gp71) component of Rauscher murine leukemia virus.

Antisera. Rabbit antisera to S107 myeloma protein were prepared by subcutaneous immunization with approximately 1 mg of purified (7) myeloma protein emulsified in complete Freund's adjuvant followed by monthly subcutaneous injections of the protein in incomplete Freund's adjuvant. Guinea pig anti-S107 idiotypic antisera were prepared by first "tolerizing" the animals by intracardiac injection of 5 mg of soluble MOPC-47A (BALB/c $\kappa\alpha_1$) and then immediate injection into footpads of S107 myeloma protein in complete Freund's adjuvant (8). The sera were collected 3 weeks later. The idiotypic specificity of the antisera was demonstrated by using a sensitive hemagglutination inhibition technique (9). The guinea pig antisera used in the present experiments bound to the S107 idiotype 4000- to 5000-fold more effectively than to the phosphorylcholine-binding MOPC-167 myeloma protein of a non-S107 idiotype.

Goat antiserum was raised against sucrose gradient-purified Kirsten murine leukemia–sarcoma virus (MuLV). The virus was isolated from the cell-free supernatants of infected normal rat kidney (NRK) cells. The goat received three intravenous injections of NP-40 disrupted virus at 20-day intervals and then was bled at weekly intervals for 2 months. Indirect immunofluorescence showed that the anti-MuLV antiserum reacted with infected NRK cells but not with uninfected NRK cells. Preimmune serum did not react with either virus-infected or control cells. Fluorescein-labeled goat anti-rabbit IgG and rabbit anti-goat IgG were obtained from Miles.

Melphalan Treatment. The phenylalanine mustard mel-

Abbreviation: MuLV, Kirsten murine leukemia–sarcoma virus.

phalan [*p*-di(2-chloroethyl)amino-L-phenylalanine], provided as a powder by the Burroughs Wellcome Co. (Research Triangle Park, NC), was dissolved in an ethanol solution containing 2% (wt/vol) HCl. After the appropriate dilution in culture medium, this solution was added to exponentially growing S107 cultures that were at a concentration of 2–3 × 10^5 cells per ml. The cells were incubated with the drug for 24 hr and then washed and resuspended in fresh medium.

Colony Inhibition Assay. Myeloma cells were cloned in soft agar by using a modification of a method described by Coffino *et al.* (10). Feeder layers prepared from F344 rat embryo fibroblasts (Microbiological Associates, Bethesda, MD) were grown to approximately 75% confluency in 24-well Falcon culture dishes. The culture fluid was removed and the fibroblasts were overlaid with 0.4 ml of culture medium containing 0.21% agarose (supplemented with glutamine, nonessential amino acids, penicillin, streptomycin, NCTC-109, and 20% horse serum). This base layer was then allowed to gel for 20 min at 24°C.

Myeloma cells were treated for 3 hr with complement and either a 1:96, 1:144, 1:192, or 1:288 dilution of heat-inactivated anti-MuLV antiserum or a 1:18, 1:24, 1:36, or 1:48 dilution of heat-inactivated anti-idiotypic antiserum. Growth medium/agarose (0.8 ml) was added to 0.2 ml of the treated myeloma cells and 0.08-ml aliquots of this mixture were then distributed over the base layers. The dishes were incubated at 37°C and 7.5% CO_2 in 100% humidity for 10 days. At this time, the number of colonies per well was determined. The complement used in these experiments was serum from selected rabbits with low natural cytotoxicity. This complement was subsequently absorbed with 80 mg of agar per ml of serum (11).

Quantitation of Surface Antigen and Cell Size. Fluorescent staining of the myeloma cells was done at 0°C in culture medium containing 0.1% sodium azide. One-tenth milliliter of a dilution of the primary antiserum that gave maximal staining (either a 1:240 dilution of rabbit anti-S107 myeloma protein or a 1:50 dilution of goat anti-MuLV antiserum) was mixed with 1 × 10^6 pelleted S107 myeloma cells and incubated for 20 min. The cells were then centrifuged through a 10% bovine serum albumin cushion and the pellet was resuspended in 0.1 ml of fluorescein-labeled secondary antibody (either a 1:20 dilution of goat anti-rabbit IgG or a 1:20 dilution of rabbit anti-goat IgG) for 20 min. The cells were again pelleted through a 10% albumin cushion and resuspended at 2 × 10^6 cells per ml. Negative control staining in which the cells were incubated only with the secondary antibody was performed for each experiment. Ten thousand myeloma cells were then individually analyzed on a fluorescence-activated cell sorter, FACS-2, (Becton–Dickinson, Mountain View, CA) by using the 488-nm laser line, and histograms were collected in which the number of cells was plotted as a function of fluorescence intensity or as a function of forward angle light scattering. Although any increase in cell size is sensitively detected by an increase in light scatter, factors other than increased cell size can also increase the amount of light scattered by a cell (12). Therefore, at times when light scatter was increased, the cell diameter was also directly measured by microscopic examination with an ocular micrometer.

Cell Cycle Analysis. The myeloma cells were stained for DNA content by using the fluorochrome mithramycin as described (13, 14). Mithramycin fluoresces after binding to DNA and the intensity of fluorescence is directly proportional to the quantity of DNA present in the cell. One million myeloma cells were suspended in 1 ml of a mithramycin solution (200 μg of mithramycin per ml in 25% aqueous ethanol/15 mM $MgCl_2$) and incubated for 20 min at 25°C. We analyzed 10,000 myeloma cells with the cell sorter by using the 457.9-nm laser line in order to obtain the DNA histograms. Metaphase spreads of the S107 cells were used to distinguish between cells in either G_2 or M.

RESULTS

Inhibition of Myeloma Cell Growth by Virus-Specific and Idiotype-Specific Antibody. S107 myeloma cells were incubated for 3 hr with complement and either anti-MuLV antiserum or anti-idiotypic antiserum. The treated cells were then cloned in soft agar (200 cells per culture well) in order to determine the level of inhibition of colony formation due to the antibody treatment. Cells that were exposed to medium alone rather than antibody and complement consistently grew with 80–100% cloning efficiency. Exposure to complement without antibody reduced the colony number by less than 10% (Table 1). Similarly, treatment with either preimmune sera and complement or immune sera without complement was as ineffective as treatment with complement alone (data not shown). However, treatment of the myeloma cells with complement and either a 1:144 dilution of anti-MuLV antiserum or a 1:18 dilution of anti-idiotypic antiserum reduced the number of colonies that formed by 99% or 98%, respectively.

Resistance of Myeloma Cells to Antibody 24 Hr after Melphalan Treatment. Before treatment with antibody and complement, S107 myeloma cells were exposed to the chemotherapeutic agent melphalan at a final drug concentration of 200 ng/ml. After 24 hr, the cells were washed, incubated for 3 hr with antibody and complement in fresh medium, and then cloned in order to determine the level of inhibition of colony

Table 1. Resistance of S107 tumor cells to idiotype-specific and MuLV-specific antibodies 24 hr after melphalan treatment

Antibody treatment	No. of colonies per culture* From sham-treated cells	No. of colonies per culture* From melphalan-treated cells	Relative resistance to antibody†
Medium alone	160 ± 5	185 ± 8	—
Medium and complement	146 ± 5	179 ± 9	—
1:144 anti-MuLV and complement	0.8 ± 0.3	37 ± 1	38
1:192 anti-MuLV and complement	20 ± 2	145 ± 3	6
1:288 anti-MuLV and complement	96 ± 2	176 ± 4	1
1:18 anti-idiotype and complement	1.8 ± 0.3	41 ± 3	19
1:24 anti-idiotype and complement	7 ± 1	65 ± 6	8
1:36 anti-idiotype and complement	79 ± 2	141 ± 6	1

S107 myeloma cells were treated with melphalan (200 ng/ml) or with the drug diluent alone as described in the text. After 24 hr melphalan-treated and sham-treated cells were washed twice and incubated for 3 hr with either of the two antisera at the designated concentrations and a 1:15 dilution of absorbed rabbit complement.

* The cells were diluted in culture medium/agarose so that 200 myeloma cells were in each of 4 replicate wells of Falcon 24-well dishes. The number of colonies in each well was counted 10 days later and the results were recorded as mean number of colonies per well of four replicate cultures ±SEM.

† Changes in the sensitivity of the melphalan-treated tumor cells to antibody and complement was calculated by Eq. 1. The *P* values as determined by Student's *t* test for the 1:144 and 1:192 dilutions of anti-MuLV and for the 1:18 and 1:24 dilutions of anti-idiotype were all <0.001.

formation resulting from the combination of drug treatment and subsequent antibody treatment. At the drug concentration used, melphalan alone did not significantly affect cloning efficiency (Table 1). Similarly, the cloning efficiency of melphalan- and sham-treated cells was not significantly affected by complement alone. The melphalan-treated cells, however, exhibited significantly increased resistance to subsequent treatment with specific antibodies and complement when compared to sham-treated control cells. The relative sensitivity of the cells to antibody (Ab) and complement (C) was calculated by Eq. 1:

$$\text{Relative sensitivity} = \frac{\dfrac{\text{No. clones surviving melphalan and (Ab + C)}}{\text{No. clones surviving melphalan and C}}}{\dfrac{\text{No. clones surviving sham and (Ab + C)}}{\text{No. clones surviving sham and C}}} \quad [1]$$

A relative sensitivity greater than one indicated the cells were more resistant to antibody treatment whereas a relative sensitivity less than one indicated the melphalan-treated cells were more susceptible to cytotoxic antibody. The increased resistance 24 hr after melphalan treatment was most apparent when the highest antiserum concentrations were used; at these concentrations there was a 38-fold increased resistance to anti-MuLV antibody and a 19-fold increased resistance to anti-idiotypic antibody (Table 1).

At lower concentrations of the antisera, the relative drug-induced resistance was less evident because the tumor cells showed significant ability to survive the antisera treatment even in the absence of melphalan pretreatment. Clones of melphalan-treated cells that had resisted high concentrations of specific antibody were isolated and grown in liquid suspension culture. Retesting of the resulting sublines to inhibition by antibody 14 days after isolation showed that all sublines tested possessed the same susceptibility as control cells. This indicated that the increase in resistance to antibody 24 hr after exposure to melphalan was due to reversible drug-induced metabolic effects rather than to a drug-induced heritable alteration.

Changes in the Sensitivity of Myeloma Cells to Antibody after Melphalan Treatment. At various times after melphalan treatment, myeloma cells were exposed to antibody and complement and then cloned in order to quantitate the degree of inhibition of colony formation by the antibody. In repeated experiments we found that after an initial period of increased resistance to specific antibody, melphalan-treated cells exhibited a period of increased susceptibility to both anti-MuLV antibody and anti-idiotypic antibody. The biphasic effect of the drug treatment is illustrated in Fig. 1. The increased resistance of the melphalan-treated cells was best demonstrated when higher concentrations of antibody were used (Table 1), whereas during the period of increased susceptibility, the increased effectiveness of antibody and complement was best demonstrated by using lower concentrations of antibody, which had only slightly inhibitory effects on cloning. In order to determine when the increased resistance could first be seen, we evaluated resistance to antibody 3, 6, 12, and 24 hr after the addition of melphalan (not shown). The resistance to the two antisera first rose slightly above control levels 12 hr after melphalan treatment ($P < 0.005$ for both sera). After maximal resistance 1 day after melphalan treatment, the level of resistance declined during days 2 and 3 until day 4 when there was a 34-fold increased susceptibility to inhibition by anti-MuLV antibody and a 28-fold increased susceptibility to inhibition by anti-idiotypic antibody. At days 5 and 6, the relative increase in susceptibility was less and finally, at day 8, the cells exhibited

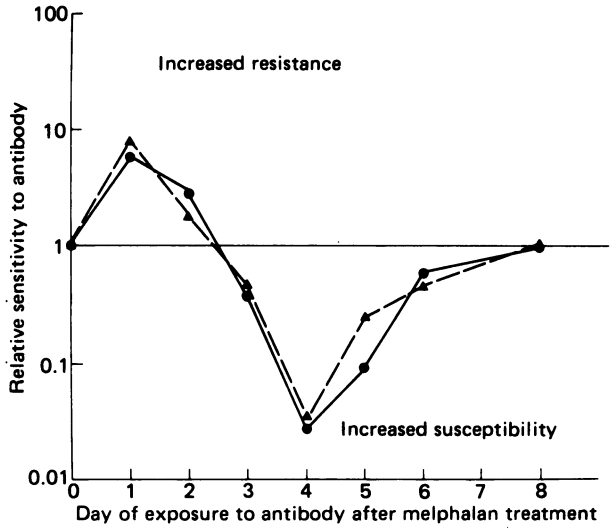

FIG. 1. Relative sensitivity of melphalan-treated S107 cells to antibody-mediated inhibition of colony formation at various times after drug treatment. S107 myeloma cells were treated with melphalan (200 ng/ml) at 37°C in 7.5% CO_2. Control cells were sham-treated with a similar concentration of the drug diluent. After 24 hr, the cells were washed and incubated in fresh medium. At different times thereafter, aliquots of cells were withdrawn, washed, diluted to 2×10^4 cells per ml, and then incubated for 3 hr at 37°C with a 1:15 dilution of rabbit complement and either a 1:192 dilution of anti-MuLV antiserum (● — ●) or a 1:24 dilution of anti-idiotypic antiserum (▲ - - ▲). This mixture was then diluted in culture medium/agarose and in 24-well dishes. The number of colonies in each well was counted 10 days later and the results were recorded as means of four replicate cultures. The relative sensitivity of the drug-treated cells to the antisera and complement at a particular time point was calculated by using Eq. 1. The P values of the change in resistance to anti-MuLV and anti-idiotype as determined by Student's t test were <0.001 for both sera on day 1, <0.1 and <0.15 on day 2, <0.05 and <0.025 on day 3, <0.001 for both sera on day 4, <0.001 and <0.005 on day 5, <0.05 and <0.025 on day 6, and <0.48 and <0.3 on day 8 after melphalan treatment.

the same sensitivity to cytotoxic antibodies as tumor cells not previously exposed to the chemotherapeutic agent. A similar biphasic change in the sensitivity of the drug-treated cells to inhibition by antibody as described above was also observed when we used increased doses of melphalan (400–1600 ng/ml) which decreased cloning efficiency up to 90%.

Effects of Melphalan Treatment on the Cell Cycle of Myeloma Cells. At different times after melphalan treatment we measured the relative amounts of DNA per cell within the population of melphalan-treated and sham-treated S107 cells by using flow cytofluorometric analysis of mithramycin-stained cells. The DNA distribution histogram of untreated myeloma cells in exponential growth (day 0 in Fig. 2) showed most of the tumor cells in either the G1 or S phase of the cell cycle and only approximately 10% of the cells in the G2 and M phases. In contrast, the majority of the tumor cells had accumulated in the G2 and M phases of the cell cycle 24 hr after melphalan treatment. By phase microscopy of cell smears none of 1000 myeloma cells examined during this time were in prophase or metaphase, indicating that most myeloma cells were arrested in the G2 rather than in the M phase of the cell cycle. The time at which there was the maximal accumulation of the tumor cells in G2 correlated with the time at which the myeloma cells exhibited their maximal increased resistance to the antibody and complement. Subsequent to 1 day after melphalan treatment, the tumor cells rapidly began to leave the G2 phase and exhibited an almost normal cell cycling pattern 3 days after melphalan treatment. The period during which the cells were

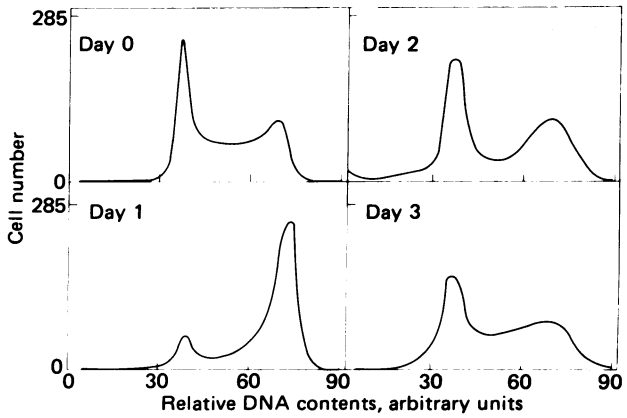

FIG. 2. DNA distribution histograms for exponentially growing untreated S107 myeloma cells (day 0) and for S107 cells 1, 2, and 3 days after melphalan treatment (200 ng/ml). Ten thousand tumor cells stained with mithramycin were analyzed by the fluorescence-activated cell sorter.

returning to a normal cycling pattern corresponded with the time at which the initially increased resistance to antibody declined toward control levels.

Effects of Melphalan Treatment on Cell Size and Amounts of Cell Surface Antigens. At different times after melphalan treatment we measured the amount of light scatter of the myeloma cells by using the cell sorter in order to identify changes in cell size due to this drug treatment. A significant increase in the light scatter signal during the first 2 days after melphalan treatment was observed (Fig. 3). Direct microscopic measurements of the diameter of 100 myeloma cells 24 hr after melphalan treatment also indicated a marked increase in the diameter of the cells (controls, 16.1 μm ± 0.2 SEM; melphalan-treated, 19.6 μm ± 0.2 SEM). Light scatter as analyzed by the cell sorter and cell size as determined by microscopy returned to normal levels 4 days after melphalan treatment.

The amounts of myeloma protein or viral surface antigens were quantitated by indirect immunofluorescent labeling using the cell sorter. Fig. 3 demonstrates the increased amounts of both viral and myeloma protein antigens on the cell surface 24 hr after melphalan treatment. The amounts of the surface antigens remained significantly increased through day 6 as indicated by the differences in the median values of the fluorescence intensity of the melphalan-treated and control cell populations. The fluorescence histograms at 1, 2, and 4 days after melphalan treatment only partially illustrate the observed differences because significant portions (up to 40%) of the analyzed melphalan-treated tumor cells were very brightly fluorescent and therefore off-scale in the histogram. These highly fluorescent cells are however counted and then taken into account when the median of the curve is determined. The density of the antigens on the melphalan-treated tumor cells returned to normal by day 8. The relative differences seen between control and drug-treated cells on each day were reconfirmed in two independent repeats of the entire kinetics experiment.

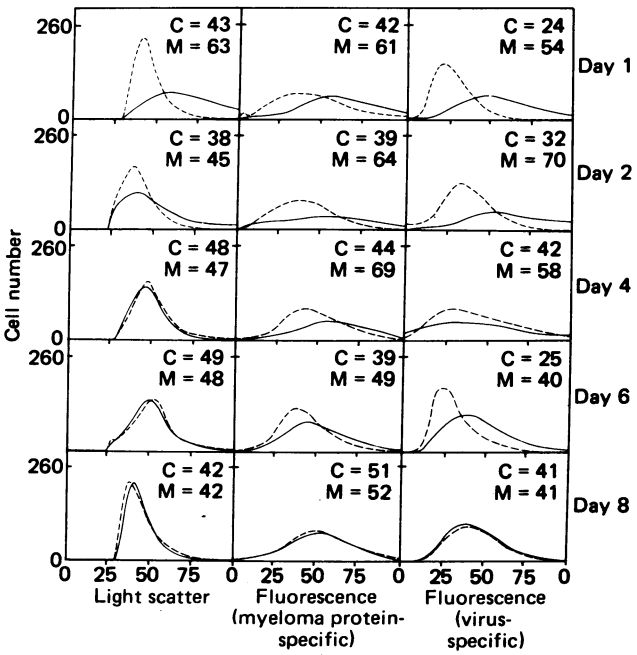

FIG. 3. Amount of light scatter and the intensity of myeloma protein-specific or MuLV-specific fluorescence on melphalan-treated (solid lines) and sham-treated (dashed lines) S107 cells. Myeloma cells were treated with melphalan (200 ng/ml) or with the drug diluent alone and 10,000 myeloma cells were analyzed with the cell sorter 1, 2, 4, 6, and 8 days thereafter as described in the text. Curves obtained on separate days cannot be compared because the voltage output of the cell sorter is recalibrated each day and this readjustment will affect the position of the curves. Therefore, control curves were analyzed each day so that relative comparisons with the drug-treated population could be made. The median value for each control (C) and melphalan-treated (M) curve is designated in the upper right corner of each histogram. Only live cells (based on light scatter) were analyzed for surface fluorescence.

DISCUSSION

These results demonstrate that a chemotherapeutic agent can induce markedly increased resistance of tumor cells to antibody and complement followed by strikingly increased susceptibility to the same reagents. These two opposite effects of the drug on the sensitivity of the tumor cells to cytotoxic antibody were followed by a return of the myeloma cells to normal sensitivity. Therefore, the observed resistance of the tumor cells to antibody and complement appeared to be related to metabolic, reversible drug effects on the tumor cell population rather than to stable, heritable changes which can also be induced in mouse myeloma cells by melphalan at a high frequency (15). The observation that clones of myeloma cells that were initially resistant to antibody subsequently regained normal sensitivity when retested 20 days later further confirmed the conclusion that the drug was producing a metabolic rather than genetic effect.

The increased resistance to antibody 24 hr after drug treatment was not due to a loss of antigenic determinants because quantitative immunofluorescence showed an increase rather than a decrease of antigen per cell at this time. However, the time of highest resistance to cytotoxic antibody did correlate with the maximal accumulation of the tumor cells in the G2 phase of the cell cycle. Many other chemotherapeutic agents induce mammalian cell populations to accumulate in G2 (16); however, the effect of these drugs on subsequent sensitivity to specific antibody and complement has not been tested. Other studies suggest that the observations made with melphalan may well apply to a large class of drugs. For example, it has been shown that synchronized cultures of mouse lymphoma lines can be resistant to the cytotoxic effects of antibodies during the S, G2, and M phases of the cell cycle (17, 18). It is, therefore, quite possible that the increased resistance to growth inhibition by antibody is generally related to the arrest of the tumor cells in the G2 phase of the cell cycle. However, data elucidating the mechanism for the increased resistance of nucleated cells during the G2 and M phases are contradictory at present (17–24). As possible mechanisms for the increased resistance, phase-specific

changes in the function, composition, or structure of the surface membrane have been suggested (18). Such changes may cause an increased repair potential or resistance to insertion of terminal complement components into the phospholipid bilayer of the cell membrane (18, 22, 24).

After the tumor cells had reached maximal resistance to the specific antibodies and complement at 24 hr after melphalan treatment, their resistance quickly decreased in the following days so that by 4 days after drug treatment tumor cells showed a significantly increased susceptibility to antibody and complement. At this time, the cell cycle and the size of the tumor cells had returned to normal levels but the amounts of both viral and myeloma protein antigens on the tumor cell surface were significantly increased. In studies using myeloma cell variants differing in the density of surface myeloma protein, we have demonstrated that doubling the amount of idiotypic determinants on the tumor cell surface can cause increased susceptibility to the antibody (5). It is therefore tempting to suggest that the increased sensitivity of the tumor cells that have been released from G2 block was due to the increased density of surface antigen after melphalan treatment. Additional support for such an explanation is the fact that the time of return of the susceptibility of the tumor cells to normal levels 8 days after melphalan treatment correlated with a decrease of the surface antigens to a normal level. Nevertheless another mechanism such as a temporary decrease in the capability of the melphalan-treated tumor cells to repair complement damage of the membrane could also explain the observed findings (25, 26).

It is difficult to relate our findings to those of an earlier report in which guinea pig hepatoma cells, anti-Forssman antibody, and complement were used (27). Within 17 hr after exposure to relatively high concentrations of chemotherapeutic drugs or metabolic inhibitors, guinea pig hepatoma cells, which were normally resistant to anti-Forssman antibody and complement, became more susceptible to trypan blue uptake. The capacity of the drug-treated cells to grow was not tested. Because metabolic inhibitors, chemotherapeutic agents, and radiation often completely and irreversibly arrest cell growth without affecting the trypan blue exclusion, it is conceivable that all the effects observed were on hepatoma cells that had lost their proliferative potential. The cloning assay used in our study avoided measuring the effects of antibodies on drug-treated nonclonogenic cells. The measurement of colony formation has the distinct advantage of measuring the effects sought in treatment—i.e., the inhibition of the capacity of the tumor cells to grow and proliferate.

Studies have shown that antisera directed against tumor antigens can suppress the growth of that tumor *in vivo* (28, 29). However, when the tumor cell number in the host exceeds certain limits, disproportionately larger amounts of tumor-specific antisera are needed or the antisera become noneffective (30). Therefore, the original tumor cell mass must be reduced by chemotherapy before tumor-specific antibodies can be used effectively. Our studies indicate that choosing the correct time interval between application of the chemotherapeutic agent and the antibody is very important, because, depending on the time after drug treatment, chemotherapeutic agents may have either synergistic or antagonistic effects on the susceptibility of tumor cells to tumor-specific antibody.

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