ROLE OF OXYGEN-DEPENDENT MECHANISMS IN ANTIBODY-INDUCED LYSIS OF TUMOR CELLS BY ACTIVATED MACROPHAGES*

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Leukocytes may lyse tumor cells in vitro in at least five situations. With phagocytes, cytolysis can be triggered by pharmacologic or particulate agents that elicit the respiratory burst (1–4). Such cytotoxicity is oxidative (1–4). Lectins can also initiate cytolysis. With neutrophils as effector cells, the mechanism appears oxidative (5), but the biochemical basis of lectin-dependent cytolysis by lymphocytes is unknown. Third, nonimmune leukocytes, such as activated macrophages and natural killer cells, may kill or inhibit tumor cells spontaneously. Several substances have been proposed to mediate spontaneous cytotoxicity by activated macrophages in vitro (6–10). Finally, leukocytes may lyse tumor cells to which they are specifically immune, and cytotoxicity by nonimmune leukocytes may be elicited by specific antibody against the tumor cells. With regard to mononuclear leukocytes, there is almost no information identifying cytotoxic mechanisms in these last two instances.

In earlier investigations into the basis of pharmacologically induced cytolysis by activated macrophages, we took two general approaches. First, we ascertained that target cells were susceptible to lysis by H_2O_2 in the amounts that the effector cells released in response to phorbol myristate acetate (PMA)[1] under the conditions of the assay (2). Second, we learned that deprivation of either oxygen or glucose prevented PMA-induced H_2O_2 release from the effector cells and abolished cytolysis (3). Scavengers of H_2O_2 prevented cytolysis as well (3). Previous work by other investigators makes it likely that activated macrophages produce reactive metabolites of oxygen during interaction with antibody-coated tumor cells (11–13). Therefore, in the experiments reported here, we used the second approach described above to study alloantiserum-dependent lysis of lymphoma cells by activated macrophages (14). The results suggest that this form of cytolysis is predominantly oxidative.

Materials and Methods

Basic Assay. Peritoneal cells from Bacille Calmette-Guérin (BCG)-treated mice were tested for cytolysis of ^{51}Cr-labeled lymphoma cells sensitized with alloantiserum or in the presence of PMA as detailed in the accompanying report (14). Percent inhibition of alloantiserum-dependent cytolysis was calculated as $100(1 - A/B)$, where A is cytolysis of sensitized cells minus cytolysis of sham-sensitized cells in the experimental set, and B is the corresponding

* Supported by National Cancer Institute grant CA-22090.

‡ Scholar of the Leukemia Society of America and Irma T. Hirschl Career Scientist.

[1] *Abbreviations used in this paper:* BCG, Bacille Calmette-Guérin; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; MEM, Eagle's minimum essential medium; MEM-P-S, MEM modified to omit bicarbonate and to increase phosphate to 6 mM, containing 5% FBS; PMA, phorbol myristate acetate.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/80/07/0198/11 $1.00
Volume 152 July 1980 198–208

difference in the control set. In dose-response curves (Figs. 1 and 2), sham-sensitized cells were not tested at each drug dose, and so values for such cells were not subtracted.

Oxygen Deprivation. Details of the procedure are given elsewhere (3). Lymphoma cells were sensitized as before (14), except for the use of modified Eagle's minimum essential medium (MEM) (Grand Island Biological Co., Grand Island, N. Y.); bicarbonate was replaced with 5 mM additional sodium phosphate, sufficient NaOH to bring the pH to 7.35, and sufficient NaCl to bring the osmolarity to 300 mosmol/liter (Advanced Instrument Osmometer; Advanced Instruments, Inc., Needham Heights, Mass.) (MEM-P). Penicillin (100 U/ml), streptomycin (100 μg/ml), and 5% fetal bovine serum (FBS) were added (MEM-P-S). The tumor cells were washed three times by centrifugation under oil, using extensively deoxygenated MEM-P-S dispensed through a catheter from an N_2-flushed syringe, as described (3). Care was taken to resuspend the pellet after each centrifugation, by repeated aspiration through a catheter attached to an N_2-filled syringe. BCG peritoneal cells were washed in the same way. Effectors and targets were diluted in deoxygenated MEM-P-S, taken up in N_2-flushed syringes, and dispensed, respectively, to the bottom and sidearm compartments of deoxygenated reaction tubes during a brisk efflux of pyrogallol-scrubbed N_2 entering through a stopcock (3). The tubes were then stoppered. Through the stopcock, the tubes were alternately evacuated for 60 s, and filled with N_2, for 10 cycles. Then the stopcocks were closed, and the contents of the tubes were mixed by inversion. The tubes were centrifuged (180 *g* for 5 min) and placed in a water bath at 37°C. The tubes that were to serve as controls were opened and flushed with air from a syringe. 10 min later, they were reclosed to retard evaporation. After 4.5 h, the tubes were centrifuged at 700 *g* for 10 min. 1 ml of the supernate was removed to a tube. The

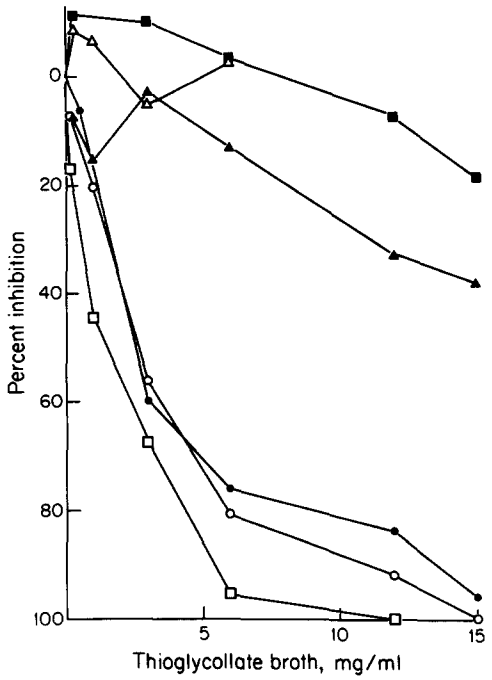

FIG. 1. Effect of thioglycollate broth on six functions of BCG-activated macrophages. ●, cytolysis of sensitized TLX9 cells (control lysis is 50.0%). ○, PMA-induced cytolysis (control is 60.0%). □, PMA-induced H_2O_2 release (control is 6.0 nmol in 90 min from adherent cells on a 13-mm cover slip). ■, adherent cell protein after 60 min (control is 23 μg/cover slip). △, phagocytosis of ^{14}C-acetylated starch granules (control is 2.1 mg starch/mg cell protein in 20 min). ▲, binding and ingestion of sheep erythrocytes (RBC) opsonized with rabbit antibody (control is 3.3 RBC/phagocyte).

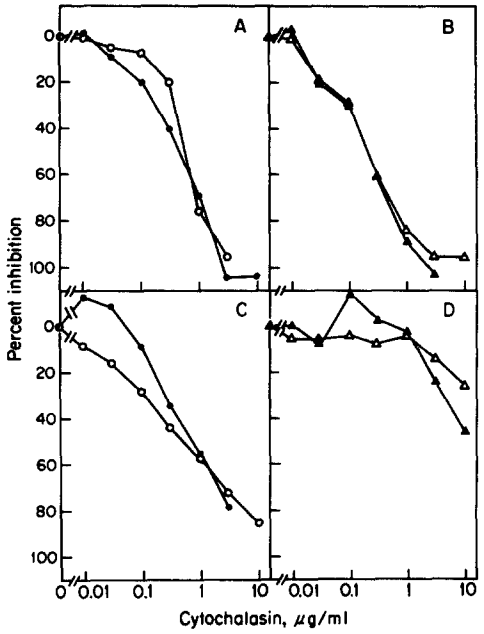

FIG. 2. Effect of cytochalasins on cytotoxicity, hydrogen peroxide release, and glucose uptake by BCG peritoneal cells. (A and B) cytochalasin B. (C and D) dihydrocytochalasin B. 0 dose represents DMSO control (0.167% vol/vol). ●, alloantiserum-dependent cytolysis (control is 41.5% in A, 32.0% in C). ○, PMA-induced cytolysis (control is 75.4% in A, 65.0% in C). ▲, PMA-induced H_2O_2 release (control is 0.42 nmol/μg cell protein in C, 0.61 nmol/μg cell protein in D, measured over 2 h). △, glucose uptake (control is 18.4 pmol/μg cell protein in B, 24.7 pmol/μg cell protein in D, measured over 6 min). Glucose uptake at each concentration of cytochalasins was the same with and without 20 ng/ml PMA; mean values are presented.

remaining volume was measured, and transferred, along with three rinses of distilled water, to another tube. Both tubes were counted, and specific release was determined as before (2).

Glucose Deprivation. BCG peritoneal cells were dispensed to 12- × 75-mm glass tubes and incubated at 37°C in 5% CO_2 in air for 2 h in MEM that contained no glucose, with 2% FBS that had been dialyzed for 14 h against a 60-fold excess of normal saline. Sensitized or sham-sensitized TLX9 cells were washed to remove glucose-containing mouse serum and then added to the effectors. The medium was made 5.5 mM in galactose (Sigma Chemical Co., St. Louis, Mo.). The tubes were centrifuged, incubated, and harvested at 4.5 h as before (2).

Effects of Thioglycollate Broth. 10 g of Brewer's thioglycollate broth (Difco Laboratories, Detroit, Mich.) was boiled in 100 ml water, autoclaved, stored in the dark at room temperature in a flask with a cotton plug until green, and refrigerated until use. The broth was then mixed in the indicated proportions with MEM that contained 1 or 5% FBS, and added to cultures of BCG peritoneal cells in tubes for cytotoxicity assays, or adherent to 35-mm plastic culture dishes (Nunc, Roskilde, Denmark) or 13-mm glass cover slips (Clay-Adams Inc., Parsippany, N. J.) for assays of adherent cell protein (15), phagocytosis of ^{14}C-acetylated starch granules (15), rosetting and phagocytosis of sheep erythrocytes opsonized with rabbit anti-sheep IgG (16), or measurement of H_2O_2 release in response to PMA (17), using the cited methods.

Glucose Uptake. Approximately 2 × 10^6 BCG peritoneal cells in 0.6 ml of MEM-10% FBS were plated on 13- × 27-mm glass coverslips (Bellco Glass, Inc., Vineland, N. J.) in 35-mm carrier dishes. After 1 h, 2 ml of additional MEM-10% FBS was added. 2–3 h later, the cover slips were rinsed in five beakers of normal saline, then placed in a 35-mm dish maintained at 37°C with 1.5 ml of Krebs-Ringer phosphate buffer (14) that contained 0.3 mM glucose, pH 7.35, with or without various doses of cytochalasin B (Aldrich Chemical Co., Milwaukee, Wis.) or dihydrocytochalasin B (the gift of Dr. John Loike, The Rockefeller University, New York)

in dimethylsulfoxide (DMSO) (Sigma Chemical Co.) (final concentration of DMSO, 0.167%, vol/vol). After 20 min, the dishes were given 0.5 μCi of 2-[^{14}C(U)]-deoxy-D-glucose, 337 mCi/mmol (New England Nuclear, Boston, Mass.). 6 min later, the cover slips were removed, drained on gauze, rinsed in four beakers of ice-cold normal saline, and again drained on gauze (total rinsing time, 30 s). The slips were dried and their radioactivity determined by liquid scintillation counting in 0.1 ml water and 10 ml Aquasol II (New England Nuclear) in an LKB Ultrabeta 1210 (LKB Produkter, Bromma, Sweden).

Other Reagents. The following were from Sigma Chemical Co.: catalase (thymol-free), superoxide dismutase, horseradish peroxidase, ferricytochrome *c*, sodium benzoate, diethyldithiocarbamate, and sodium azide. Diazabicyclooctane and dinitrophenol were from Eastman Kodak Co. (Rochester, N. Y.). Mannitol and sodium iodide were from Matheson, Coleman & Bell (East Rutherford, N. J.). Lactoperoxidase was from Calbiochem-Behring Corp., American Hoechst Corp. (San Diego, Calif.).

Results

Oxygen Deprivation. When the partial pressure of oxygen was severely reduced, alloantiserum-dependent lysis of TLX9 lymphoma cells by BCG peritoneal cells was inhibited by an average of 62% in three experiments (Table I). Deoxygenation was not toxic to the effector cells, because readmission of air to the tubes at the end of the deoxygenation procedure permitted the full expression of cytotoxicity (Table I). Inhibition of cytolysis by anaerobiosis did not appear to be a consequence of reduced mitochondrial respiration, because three inhibitors of mitochondrial respiration (cyanide, azide, and dinitrophenol) were without significant effect (Table II).

Glucose Deprivation. We found earlier that several hours of incubation in glucose-free medium markedly decreased the ability of BCG-activated macrophages to secrete hydrogen peroxide or lyse lymphoma cells when exposed to PMA (3). Glucose deprivation reduced the cytolysis of sensitized TLX9 cells by BCG peritoneal cells by 62% in three experiments (Table III). The effect on PMA-induced cytolysis, tested concurrently, was more marked (91% inhibition, Table III). Results were similar with P388 lymphoma cells (not shown).

Thioglycollate Broth as a Scavenger. Because thioglycollate broth-elicited macrophages were unable to mediate substantial PMA-induced (2) or alloantiserum-dependent

TABLE I

Effect of Oxygen Deprivation

TLX9 cells*	Percent specific release of ^{51}Cr	
	In air‡	In N_2§
Sensitized‖	68.2 ± 1.9	29.9 ± 7.4
Sham sensitized¶	10.7	7.7
Percent inhibition	—	61.5 ± 2.6

* 4×10^4 ^{51}Cr-labeled TLX9 cells were incubated for 4.5 h with 2×10^6 peritoneal cells from BCG-treated mice. Spontaneous release from TLX9 cells without effector cells averaged 23.9 ± 7.4% in air and 18.4 ± 2.6% in N_2.

‡ The tubes were prepared as in §, then opened to air for 10 min at the beginning of the incubation.

§ The tubes were deoxygenated as described in Materials and Methods.

‖ Exposed to alloantiserum and fresh mouse serum before washing into deoxygenated medium. Means ± SEM for three experiments.

¶ Data from one experiment with nonimmune mouse serum in place of alloantiserum.

TABLE II
Effect of Inhibitors of Mitochondrial Respiration

Agent	Tumor cells (number of experiments)	Percent specific release of ^{51}Cr*	
		Without agent	With agent
Cyanide‡	Sensitized (2)§	74.8 ± 7.1	69.4 ± 13.1
	Sham sensitized plus PMA (1)‖	66.7	69.4
Azide¶	Sensitized (2)	52.0 ± 15.8	42.8 ± 19.0
	Sham sensitized plus PMA (2)	67.6 ± 17.8	79.9 ± 10.1
Dinitrophenol**	Sensitized (2)	74.8 ± 7.1	68.6 ± 6.1
	Sham sensitized plus PMA (1)	66.7	68.9

* Means ± SEM for the number of experiments indicated.
‡ 1 mM.
§ 2×10^4–4×10^4 TLX9 cells sensitized with alloantiserum and fresh mouse serum.
‖ TLX9 cells treated with nonimmune mouse serum and incubated with 100 ng/ml PMA.
¶ 0.1 or 1.0 mM.
** 10 μM.

TABLE III
Effect of Glucose Deprivation

Tumor cells*	Percent specific release with glucose‡	Percent inhibition without glucose§
Sensitized	40.4 ± 9.7	61.6 ± 7.8
Sham sensitized plus PMA¶	41.3 ± 14.6	90.9 ± 8.0

* 3.5×10^4 ^{51}Cr-labeled TLX9 cells were incubated for 4.5 h with 2.0×10^6 BCG peritoneal cells in MEM that contained 2% dialyzed FBS. Means ± SEM of three experiments.
‡ BCG cells were preincubated for 2 h with 5.5 mM glucose and cultured with TLX9 cells in 5.5 mM glucose.
§ BCG cells were preincubated for 2 h without hexose and cultured with TLX9 cells in 5.5 mM galactose.
¶ PMA, 100 ng/ml.

cytotoxicity (14), despite being activated by other criteria (12, 18, 19), we speculated that components of thioglycollate broth might themselves scavenge reactive species of oxygen important in cytotoxicity.

When thioglycollate broth was added to BCG-activated macrophages, it was a potent suppressor of their release of detectable H_2O_2 in response to PMA (Fig. 1). When BCG-activated macrophages were exposed to 2 mg/ml of thioglycollate broth for 45 min, followed by extensive washing, H_2O_2 release was still reduced by 50% (not shown). Thus, thioglycollate broth appeared to become rapidly cell associated.

Thioglycollate broth suppressed both antiserum-dependent and PMA-induced cytotoxicity with a dose-response curve nearly the same as that for H_2O_2 release (Fig. 1). At 6 mg/ml, both forms of cytotoxicity were suppressed to the level seen in the absence of alloantiserum or PMA (Fig. 1; Table IV).

To learn whether thioglycollate broth might be toxic to macrophages, or inhibit binding to their Fc receptors, three other macrophage functions were measured: ability to adhere to a plastic surface during vigorous washing, as measured by residual cell protein; phagocytosis of ^{14}C-acetylated starch granules under conditions optimized to detect differences in the rate of uptake (15); and binding and ingestion of sheep erythrocytes opsonized with specific antibody. As shown in Fig. 1, these three functions

TABLE IV
Effect of Thioglycollate Broth

Experiment	Tumor cells	Percent specific release of ^{51}Cr*	
		Without thioglycollate	With thioglycollate‡
1	Sensitized§	81.8 ± 2.1	2.9 ± 1.0
	Sham sensitized plus PMA¶	66.7 ± 2.0	11.5 ± 1.3
2	Sensitized	69.3 ± 2.2	5.3 ± 1.9
	Sham sensitized plus PMA	45.5 ± 2.5	8.6 ± 1.8
3	Sensitized	49.9 ± 1.3	9.5 ± 7.3
	Sham sensitized plus PMA	60.0 ± 3.1	14.2 ± 1.1

* Means ± SEM of triplicates.
‡ Thioglycollate broth, 6 mg/ml.
§ TLX9 cells sensitized with alloantiserum and fresh mouse serum.
¶ TLX9 cells treated with nonimmune mouse serum and incubated with PMA (100 ng/ml).

TABLE V
Effect of Additional Scavengers of Reactive Metabolites of Oxygen

Agent	Dose	Number of experiments	Percent lysis of sensitized TLX9*	
			Agent absent	Agent present
Catalase	1–10 mg/ml	7	26.2 ± 7.9	31.1 ± 8.0
Superoxide dismutase	0.1–2.5 mg/ml	9	35.0 ± 6.7	38.0 ± 7.3
Horseradish peroxidase	0.02–2.5 mg/ml	9	39.2 ± 7.1	33.9 ± 4.7
Mannitol	50 mM	2	33.3 ± 17.5	31.8 ± 6.9
Ethanol	50–100 mM	2	33.3 ± 17.5	26.4 ± 4.7

* Means ± SEM for the indicated number of experiments.

were not suppressed at 6 mg/ml, the dose that inhibited H_2O_2 release and cytolysis almost completely.

Other Scavengers. A number of other scavengers of reactive metabolites of oxygen were without consistent effect under the conditions tested. These included catalase, superoxide dismutase, horseradish peroxidase, mannitol, and ethanol (Table V). In smaller numbers of experiments, the following agents were also ineffective at the concentrations tested: lactoperoxidase (2.5 mg/ml) and iodide (0.2 mM), ferricytochrome *c* (130 μM), sodium benzoate (10 mM), diazabicyclooctane (5 mM), and diethyldithiocarbamate (1 mM) (data not shown).

Effect of Cytochalasins. Because of the inhibitory effect of glucose deprivation on alloantiserum-dependent cytolysis, we speculated that cytochalasin B might inhibit cytolysis by interfering with glucose transport, rather than through its disruptive effect on contractile elements (20). As shown in Fig. 2A and B, cytochalasin B did suppress glucose transport, PMA-induced H_2O_2 release, PMA-induced cytotoxicity, and alloantiserum-dependent cytotoxicity by BCG peritoneal cells; all with the same dose-response curve (ID_{50} = 0.5 μg/ml). However, dihydrocytochalasin B (21) had only a weak inhibitory effect on glucose transport and H_2O_2 release (ID_{50} not reached

at 10 μg/ml) (Fig. 2D). The suppressive effect of dihydrocytochalasin B on PMA-induced and alloantiserum-dependent cytolysis was the same as that of cytochalasin B (Fig. 2C). Thus, the inhibitory effect of dihydrocytochalasin B on cytotoxicity was independent of its effect on glucose uptake. Cytochalasin B had no effect on the susceptibility of TLX9 cells to lysis by reagent H_2O_2 or nascent H_2O_2 generated by glucose oxidase (not shown), and thus was not likely to suppress cytotoxicity by acting on the target cells.

Discussion

Alloantiserum-dependent cytolysis of lymphoma cells by BCG peritoneal cells appeared from these studies to have a predominantly oxidative basis. This conclusion was based on three lines of evidence: 62% inhibition of cytolysis by oxygen deprivation, which could not be attributed to an effect on mitochondrial respiration; a similar degree of inhibition by depriving the effector cells of glucose, which markedly reduces their ability to release H_2O_2 (3); and complete inhibition of cytolysis by thioglycollate broth, at concentrations that appeared to scavenge, or to prevent the release of hydrogen peroxide, without exerting toxic effects on other effector cell functions.

The fact that inhibition of cytotoxicity was incomplete after deprivation of oxygen or glucose is open to several interpretations. The effector cells may employ a nonoxidative mechanism of cytolysis in addition to a predominant oxidative mechanism. There may be more than one class of effector cells, with the minor class employing a nonoxidative means of cytolysis. Finally, the techniques we used to deprive effector cells of substrates for the generation of H_2O_2 may not have been completely effective.

According to this last view, it is not surprising that the same techniques suppressed PMA-induced cytolysis more markedly than cytolysis induced by antibody. PMA-induced target cell injury is mediated by diffusion of H_2O_2 through serum-containing medium between effector and target cells (2, 3). In contrast, antibody-induced cytolysis is contact dependent (14). It is not known whether reactive metabolites of oxygen are secreted by macrophages predominantly at the zone of contact during their interaction with antibody-coated tumor cells. However, it is likely that secretion of O_2^- (superoxide anion) and H_2O_2 is greatest at the region of the cell surface receiving the stimulus, as documented by cytochemical studies of granulocytes (22) and macrophages (M. L. Karnovsky. Personal communication.) during phagocytosis. Thus, the volume of distribution of H_2O_2, and the quantity of serum-associated reducing substances, might be much smaller in antibody-dependent cytolysis than in cytotoxicity induced by PMA. A given reduction in H_2O_2-releasing capacity of macrophages, such as by deprivation of oxygen or glucose, should bring the effective concentration of H_2O_2 below a cytotoxic level more readily in the case of PMA-induced cytotoxicity.

The contact dependence of antibody-induced cytolysis makes investigation of its oxidative basis technically difficult for two further reasons. First, indicators used in assays for reactive metabolites of oxygen may gain limited access to the sites of secretion. Other workers have demonstrated superoxide release from granulocytes during extracellular lysis of antibody-coated carcinoma cells (5 nmol/10^6 cells in 1 h) (13), and from monocytes during contact with aggregated IgG on a porous, non-phagocytizable surface (34.2 nmol/2.5 × 10^6 cells in 30 min) (11). Amounts of H_2O_2

in the same range would be adequate to injure many target cells, even if distributed throughout the assay volume (2). We do not report measurements of H_2O_2 release during interaction of macrophages with sensitized tumor cells because the assay currently available to us (17) is inhibited by the target cells (C. Nathan. Unpublished observations.). Other assays would be expected to be less susceptible to such inhibition (23), and hopefully will be available for future studies. Second, the contact dependence of antibody-induced cytolysis may account for the inability to inhibit it with enzymatic scavengers. Antibody-dependent lysis of tumor cells by granulocytes had an oxidative basis in the studies of Clark and Klebanoff (24) and Hafemann and Lucas (13). Yet, catalase and superoxide dismutase were without effect (13, 24), as we also noted. J. Michl and S. Silverstein (Manuscript in preparation.) have provided direct evidence for the exclusion of exogenous enzymes from the zone of contact between macrophages and a surface coated with immune complexes. By using electron microscopy, they found that horseradish peroxidase was readily detected on the undersurface of macrophages adherent to antigen-coated glass, but was unable to enter the same region after addition of specific antibody directed against the antigen.

In the present study, one scavenger of H_2O_2 did completely abolish both antibody-dependent and PMA-induced cytolysis, namely, thioglycollate broth. Components of thioglycollate broth that might react with H_2O_2 include sodium thioglycollate, methylene blue, proteose peptone, beef infusion, and agar. The active components in this complex mixture have not been identified, nor have we established whether, or how, they may gain access to the zone of contact. Either endocytosis of the thioglycollate broth or its rapid adherence to the cell surface may be involved.

Previous investigations into a possible oxidative basis of antibody-dependent, cell-mediated cytotoxicity by phagocytic leukocytes have yielded divergent results. The discrepancies may be related to the use of different classes of target cells. As noted, two groups studying the antibody-dependent lysis of tumor cells by granulocytes have presented compelling evidence for the role of oxidative processes (13, 25). In contrast, normal antibody-dependent cytotoxicity by leukocytes from patients with chronic granulomatous disease was observed in experiments using erythrocytes (25, 26) or a herpes simplex virus-infected cell line (27) as targets. The above observations might be reconciled if it is considered that erythrocytes and cell lines infected with herpes virus may be more susceptible than tumor cells to antibody-dependent, cell-mediated cytotoxicity by either oxidative or nonoxidative means. Consistent with this possibility is the observation that nonactivated macrophages can lyse both sensitized erythrocytes (28, 29) and sensitized cell lines infected with herpes virus (30–32), even though antibody-dependent lysis of tumor cells usually requires that the macrophages be activated (14).

Studies of the effects of cytochalasins on antibody-dependent, cell-mediated cytotoxicity have also given divergent results, depending upon the cells employed. Cytotoxicity by guinea pig macrophages (33) and human monocytes (34) against erythroid targets was increased by cytochalasin B. However, cytotoxicity by human blood lymphocytes against Chang cells (35) or mouse lymphoma cells (36) was decreased by cytochalasins. Interpretation of these differences is complex, in part because cytochalasins may have interfered with target cell binding (36, 37) to different degrees. We found that dihydrocytochalasin B abrogated both PMA-induced and antibody-dependent cytotoxicity by BCG peritoneal cells at concentrations that had

no effect on glucose transport, probably indicating a requirement for the contractile network in the effector cells. The use of cytochalasin B alone would not have permitted this interpretation, because the doses that inhibited cytotoxicity were equally inhibitory to glucose transport and H_2O_2 release.

Several substances have been proposed to mediate the cytotoxicity of macrophages for tumor cells under various conditions in vitro, including a nucleoside (thymidine) (6), a deaminase (arginase) (7), a complement cleavage product (C3a) (8), a protease (9, 10), and hydrogen peroxide (3). The cytotoxic effect of C3a has not been confirmed (38). Interpretation of inhibition of cytotoxicity by protease inhibitors is complicated by their numerous effects, including suppression of superoxide release from mononuclear phagocytes (39). Nonetheless, it is of considerable interest that a cytolytic factor was detected in the supernates of activated macrophages (10).

An oxidative mechanism now appears to be involved in the cytotoxicity of activated macrophages under at least the following two conditions: in the presence of pharmacologic agents that trigger the respiratory burst (2, 3), and in the presence of antibodies directed against the tumor cells. It is now appropriate to ask, what defense mechanisms tumor cells may employ to escape oxidative attack, and how such defenses may be countervened. These questions are under study.

Summary

The alloantiserum-dependent lysis of TLX9 lymphoma cells by peritoneal cells from Bacille Calmette-Guérin (BCG)-treated mice was inhibited 62% by depletion of oxygen. This effect did not appear to be a result of interference with mitochondrial respiration because cyanide, azide, and dinitrophenol did not inhibit cytotoxicity. Preincubating the effector cells for 2 h without glucose, which markedly reduces their ability to release hydrogen peroxide, likewise suppressed antibody-dependent cytolysis by 62%. Lysis of sensitized lymphoma cells was virtually abolished by 6 mg/ml of thioglycollate broth, a concentration that also abrogated the detectable release of hydrogen peroxide and the lysis of lymphoma cells by BCG-activated macrophages in response to phorbol myristate acetate (PMA). This concentration of thioglycollate broth was not toxic to the effector cells, as judged by adherence to plastic, binding of opsonized erythrocytes, and phagocytosis of radiolabeled starch granules. Catalase, superoxide dismutase, horseradish peroxidase, mannitol, ethanol, benzoate, and diazabicyclooctane were without consistent effects. Cytochalasin B and dihydrocytochalasin B both markedly suppressed cytolysis, whether induced by antibody or by PMA (ID_{50}, 0.5 μg/ml). Cytochalasin B was an equally potent suppressor of glucose uptake and PMA-induced hydrogen peroxide release by BCG-activated macrophages (ID_{50}, 0.5 μg/ml). However, dihydrocytochalasin B lacked these latter effects, which suggests that cytotoxicity required intact contractile elements. The extracellular lysis of antibody-coated lymphoma cells by BCG-activated macrophages appears to have a predominantly oxidative basis.

We are grateful to M. L. Karnovsky, J. Michl, and S. Silverstein for communication of results before publication, and to Ms. Linda Brukner for excellent technical assistance.

Received for publication 7 March 1980.

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