Cytotoxicity of Human Macrophages for Tumor Cells

ENHANCEMENT BY HUMAN LYMPHOCYTE MEDIATORS

DEBORAH J. CAMERON and W. HALLOWELL CHURCHILL, *Department of Medicine, Harvard Medical School, Peter B. Brigham and Robert B. Brigham Hospitals, Boston, Massachusetts 02120*

ABSTRACT Human macrophages, derived from peripheral blood monocytes, acquire enhanced cytotoxicity for human target cells after incubation in mediator-rich supernates from antigen-stimulated lymphocytes. Maximum cytotoxicity was observed after 24-h incubation in mediators. In comparison to normal macrophages, mediator-activated macrophages were cytotoxic to five of the six malignant cell lines tested but had no effect on five nonmalignant cell lines. In 20 experiments with one target (SK-BR-3), mean cytotoxicity was 23±2.7% and with another target (MA-160), was 29±3.4%. Macrophages became cytotoxic after 8-h incubation with mediators and the enhanced cytotoxicity persisted for at least 40 h after the lymphocyte mediators were removed. These findings are consistent with the hypothesis that macrophages, activated by antigen-induced lymphocyte mediators, can contribute to the host resistance to tumor growth in man.

INTRODUCTION

There is considerable evidence from animal studies that activated macrophages are an important component in host resistance to tumor growth (1–3). In vitro studies from our own and other laboratories have established that supernates from antigen- or mitogen-stimulated lymphocytes contain a mediator, macrophage-activating factor (MAF),[1] which enhances macrophage mediated cytotoxicity for tumor cells in addition to modifying a number of other macrophage functions (4–7). Much less is known about the mechanisms that control the interaction of human monocytes or monocyte-derived macrophages[2] with human target cells. There is some evidence that normal, unstimulated monocytes may inhibit growth of HeLa and other tissue cell lines (8–9). However, the effect of mediator-activated human macrophages on human target cells is unknown.

The purpose of this study was to develop an in vitro system with which to compare the effect of normal and activated macrophages on both malignant and nonmalignant human target cells. We have found that human macrophages derived from peripheral blood monocytes, after being activated in vitro by mediator-rich supernates, acquire enhanced cytotoxicity for malignant targets. However, these same macrophages do not have enhanced cytotoxicity for nonmalignant targets.

METHODS

Preparation of lymphocyte supernates. Human peripheral blood mononuclear cells were isolated on a Ficoll-Hypaque (Ficoll, Pharmacia Fine Chemicals, Piscataway, N. J.; Hypaque, Winthrop Laboratories, New York) gradient after collecting the buffy coat by dextran sedimentation (10). The cells were washed twice in Eagle's minimal essential medium that contained 100 U penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B/ml (MEM-PSA) and resuspended at a concentration of 2.5×10^6 cells/ml in MEM-PSA supplemented with 10% human AB+ serum and 1% L-glutamine (10% MEM-PSA) (Grand Island Biological Co., Grand Island, N. Y.).

Cells were incubated for 48 h at 37°C in a 5% CO_2 95% air

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[1] *Abbreviations used in this paper*: FCS, fetal calf serum; MAF, macrophage-activating factor; MEM-PSA, Eagle's minimal essential medium that contained 100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B/ml; 10% MEM-PSA, Eagle's minimal essential medium supplemented with 100 U of penicillin, 100 μg of streptomycin, 0.25 μg of amphotericin B/ml, 10% AB+ serum, and 1% L-glutamine; SK-SD, streptokinase-streptodornase; TCM, Eagle's minimal essential medium supplemented with 100 U of penicillin, 100 μg of streptomycin, 0.25 μg of amphotericin B/ml, 15% heat-inactivated fetal calf serum, and 1% L-glutamine.

[2] For purposes of this paper we chose to define macrophages that have acquired enhanced cytotoxicity as a result of MAF treatment, as activated macrophages.

atmosphere with or without 50 U/ml streptokinase-streptodornase varidase (SK-SD) (American Cyanamid Co., Lederle Laboratories Div., Pearl River, N. Y.). The cell-free supernates were obtained by centrifugation and SK-SD in the original amount was added to the control supernate. Supernates used to activate monocytes were used either fresh or after storage at −20°C.

Preparation of macrophage monolayers. The mononuclear cells were washed twice in MEM-PSA and resuspended in MEM-PSA supplemented with 10% gamma globulin-free horse serum at a concentration of either 12.5, 6.25, 2.5, or 1.25×10^6 cells/ml. 200-μl aliquots of these mononuclear cell suspensions were added to Falcon microtiter plate wells (No. 3040, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). After an incubation period of 1 h at 37°C, the nonadherent cells were removed by gently washing three times with warm (37°C) MEM-PSA. 200 μl of 10% MEM-PSA was then added to each well, and the monocyte preparations were allowed to develop into macrophages by incubating them in this medium for 5 d at 37°C in a 5% CO_2, 95% air atmosphere (11). For convenience these monocyte-derived macrophages will be referred to as macrophages although they may differ in some respects from tissue macrophages. On the third day, 50 μl of fresh 10% MEM-PSA was added to each well.

After the 5-d incubation period, the medium was removed and macrophages were incubated for an additional 2 d with either 200 μl of mediator-rich supernate or control supernate. The mediator-rich and control supernates were made to contain 10% AB+ serum, 1% L-glutamine, and 1% PSA (100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B) before incubation with the macrophages.

Target cells

Tumor cell lines. Five adherent human cell lines were obtained from Dr. J. Fogh at Sloan Kettering Institute for Cancer Research, New York. Caki-1 was derived from a renal clear cell carcinoma. Calu-1 was derived from a poorly differentiated epidermoid carcinoma of the lung. SK-BR-3 was derived from an adenocarcinoma of the breast and HT-29 was derived from an adenocarinoma of the colon (12). These four cell lines were grown in McCoy's medium supplemented with 15% heat-inactivated fetal calf serum (FCS), 1% L-glutamine, 100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B. The fifth cell line, SK-Mes-1, was derived from a squamous cell carcinoma of the lung and was maintained in MEM-PSA medium that contained 15% heat-inactivated FCS, 1% L-glutamine, and 1% nonessential amino acids.

MA-160, a long-term tissue culture line originating from a prostatic nodule was obtained from Microbiological Associates, Walkersville, Md. and was grown in MEM-PSA supplemented with 15% heat-inactivated FCS and 1% L-glutamine. This cell line now has malignant growth characteristics in that it has lost contact inhibition.

Normal cell lines. WI-38 and M-7, derived from diploid human embryonic lung cells, were grown in MEM-PSA supplemented with 1% L-glutamine and 1% heat-inactivated FCS. MA-112, derived from a whole human embryo was also grown in the above medium.

MRC-5, also obtained from human embryonic lung cells, was grown in MEM-PSA supplemented with 8% heat-inactivated FCS and 1% L-glutamine. MA-349, derived from human embryonic intestinal cells, was grown in MEM-PSA supplemented with 10% heat-inactivated FCS and 1% L-glutamine.

Preparation of target cells for cytotoxic assay. All target cells were cultured as monolayers in 250-ml plastic culture flasks (Falcon Labware). The target cells were labeled by replacing the culture medium with fresh medium that contained 20 μCi [^{3}H]thymidine ([*methyl*-^{3}H]thymidine, 40–60 Ci/mmol sp act, New England Nuclear, Boston, Mass.). After 24-h incubation, the labeled monolayers were washed with MEM-PSA and trypsinized with 4 ml of 0.25% trypsin-EDTA for 10 min at 37°C. The reaction was stopped by the addition of 4 ml of MEM-PSA supplemented with 15% heat-inactivated FCS and 1% L-glutamine (TCM). After washing the cells twice in TCM, the cell suspension was adjusted to a concentration of 25,000 target cells/ml.

Cytotoxicity assay. After 5 d of maturation and an additional 2 d of incubation in mediator-rich or control supernates, the macrophage monolayers were washed twice with warm (37°C) MEM-PSA, overlaid with 200 μl of the target cell suspension, and incubated at 37°C in a moist atmosphere of 5% CO_2 in air. After an incubation period of 24 h, the plates were washed twice with saline. (The optimal period of cocultivation was found to be between 6 and 24 h). The number of tumor cells remaining in the wells was then determined by the assessment of residual adhering radioactivity, as has been described previously (4).

Determination of macrophage number. Because the degree of cytotoxicity of activated macrophages was calculated by comparing the residual adherent radioactivity in wells that contained an equal number of activated and control macrophages, it was necessary to count the number of cells in the monolayers at the time of addition of target cells.

The number of macrophages in monolayers prepared in parallel with the experimental monolayers was determined as follows: the macrophage monolayers were washed twice with 200 μl of Eagle's minimal essential medium, 50 μl of 1% xylocaine was added to each well, and the cells were incubated at 37°C for 20 min. 50 μl of MEM-PSA that contained 15% FCS then were added, after which the detached cells were collected and counted on the Coulter counter (Coulter Electronics Inc., Hileah, Fla.). Microscopic examination showed no residual cells in the wells after the treatment. The cell numbers from three monolayers were used to calculate the mean cell number.

Generation of in vitro sensitized lymphocytes. Mononuclear cells were suspended at a concentration of 5×10^6 cells/ml in 10% MEM-PSA. The various target cells utilized in these experiments were treated with 5 ml of mitomycin C (50 μg/ml) for 30 min at 37°C. The flasks were then washed three times with MEM-PSA to remove all remaining mitomycin C. 10 ml of the mononuclear cell suspension was then added to the various treated target cells. As a control, mononuclear cells were incubated in a flask without target cells. After incubation at 37°C for 7 d, the nonadherent cells were removed from each flask, washed twice in MEM-PSA, and their cytotoxic effects determined. To assay for cytotoxicity, control lymphocytes or lymphocytes previously cultured with mitomycin C-treated cells were mixed with [^{3}H]thymidine-labeled target cells at a ratio of 50:1. These mixtures were then plated in microtiter plates and incubated at 37°C. After 24 h of incubation, the residual adherent radioactivity was determined.

Calculation of results. Because all target cells used in this study were adherent, the loss of radioactivity from the microtiter wells was used as a measure of a cell's cytotoxicity. That this was a legitimate assay for cytotoxicity is shown in Results. To use the data derived from the several effector target cell ratios employed in each experiment, residual adherent radioactivity in microtiter wells that contained activated or control macrophages was plotted as a function

of the number of macrophages present at the time of addition of tumor cells. The data was then analyzed by simple linear regression by least squares (13), with the additional requirement that both lines pass through the same intercept on the ordinate. In this case the sum of the errors above and below the lines are not necessarily equal as they would be in simple linear regression without this additional constraint. This point represents target cells in the absence of macrophages and thus this is the same for each line. The one regression line drawn represents the number of target cells left after treatment with mediator-activated macrophages and the other represents the number of target cells remaining after treatment with control macrophages. The degree of cytotoxicity of activated macrophages was then calculated by comparing the residual adherent radioactivity (calculated from the regression analysis) corresponding to 10^5 activated macrophages and the residual radioactivity corresponding to an equal number of control macrophages as follows:

$$\text{Percent kill} = 100 - \left(\frac{\text{mean counts per minute with activated macrophages}}{\text{mean counts per minute with control macrophages}}\right) \times 100.$$

All experiments were done in triplicate. To determine statistical significance, the slopes of the two lines were compared by a one-tailed t test. The calculation of statistical significance and the regression analysis was carried out with a DEC-20 computer (Digital Equipment Corp., Marlboro, Mass.) and a program provided by Dr. Colin Begg (Sidney Farber Cancer Institute; Boston, Mass.). Results with a t value corresponding to a P of 0.05 or less were considered to be significant. In several experiments, P values between 0.10 and 0.05 were obtained. Experiments with P values in this range are designated. In 75 experiments conducted, all but 4 of 53 cytotoxic values of 12% or greater were significant ($P \leq 0.05$) or in a few cases of borderline significance ($P \leq 0.10$). However, cytotoxic values <12% were significant in only 1 of 22 experiments.

RESULTS

Cytotoxicity of mediator-activated macrophages. In these experiments normal monocyte-derived macrophages activated in vitro with lymphocyte mediator-rich supernates were consistently cytotoxic for the several target cells tested. Dose-response curves for several experiments are shown in Fig. 1. The residual radioactivity adhering to macrophage monolayers is plotted on a logarithmic scale on the ordinate and the number of macrophages remaining at the time of addition of labeled tumor cells is plotted on the abscissa. Although there was a linear decrease in the survival of tumor cells with increasing numbers of normal macrophages, the wells that contained activated macrophages consistently had less residual radioactivity than did wells that contained macrophages treated with control supernates. After establishing the line of best fit for both activated and control macrophages, the percentage of cytotoxicity was calculated from the adherent radioactivity at a macrophage target cell ratio of 20:1. The data from ten of these experiments is shown in Table I.

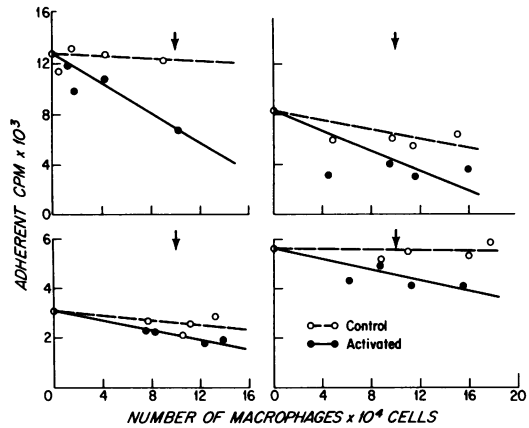

FIGURE 1 Cytotoxicity of activated macrophages. Radioactivity adhering to macrophage monolayers after 24 h is plotted as a function of the number of macrophages present at the time of addition of tumor cells. Macrophages were preincubated for 2 d with MAF-rich (activated) or control lymphocyte supernates before addition of tumor cells. Each point represents the mean of three replicates, all of which were used in calculating the regression lines. ↓, represents 10 × 10^4 macrophages or an effector:target ratio of 20:1.

As can be seen from this table, in most experiments there was little killing by macrophages treated with control supernates in comparison to wells that contained tumor with no added macrophages. Furthermore, macrophages treated with control supernate had no more effect than macrophages treated with tissue culture media. In a total of 20 experiments with one

TABLE I
Cytotoxicity of In Vitro Activated Macrophages for SK-BR-3 Tumor Cells

Experiment	Residual adhering radioactivity: Tumor alone	Tumor + control macrophages	Tumor + activated macrophages	Percentage kill
		cpm		%
1	3,150	3,013	2,111	30
2	10,589	8,583	4,723	45
3	2,400	2,530	2,100	17
4	7,100	5,002	4,237	16
5	4,764	1,430	1,025	28
6	8,166	6,366	4,232	34
7	5,619	5,209	4,543	13
8	3,316	2,645	1,713	36
9	6,290	6,000	4,200	30
10	7,530	6,100	4,700	23

Residual adherent radioactivity was determined after 24 h of cocultivation of macrophages with [^{3}H]thymidine-labeled SK-BR-3 cells at the effector:target cell ratio of 20:1, which corresponds to 10^5 macrophages. Percent kill is significant ($P \leq 0.05$) in all experiments except Nos. 1 and 4, in which $P \leq 0.10$.

target (SK-BR-3), significant cytotoxicity was obtained in 18. The mean was 23±2.7%. With a second target (MA-160), the mean of 20 experiments was 29±3.4%.

Evidence that residual adherent radioactivity reflects true cytotoxicity. Because adherent radioactivity is a measure of remaining viable target cells, it was important to establish that loss of radioactivity was non-cell-associated and consequently a valid measure of cell death. Therefore, we examined the distribution of the [^{3}H]thymidine from SK-BR-3 cells after 24 h of interaction with activated macrophages. After cocultivation of macrophages with tumor, the supernate from the wells that contained tumor cells alone or tumor cells plus activated or control macrophages were collected and centrifuged to remove any intact cells. The results of these experiments are shown in Table II. In each experiment, the cell pellet spun from the supernates that contained mixtures of either activated or control macrophages with tumor had the same amount of radioactivity. In the mixtures of tumor with either activated or control macrophages, the loss of adherent radioactivity was associated with a corresponding increase in the radioactivity of the cell-free supernate. Thus, the relatively greater radioactivity in supernates from the mixture that contained activated macrophages is a measure of the macrophage-induced cytotoxicity. This finding was confirmed by viability studies with trypan blue. Greater than 90% of the adherent cells were viable, whereas none of the cells in the pellet collected from the supernate were viable.

TABLE II

Distribution of [^{3}H]Thymidine Label After 24 h of Incubation of Macrophages with Labeled Target Cells

Experiment	Cell mixture plated	Percentage of total label		
		Adhering	Cell pellet	Cell-free supernate
		%		
1	Tumor	46	5	49
	Tumor and control macrophages	38	19	43
	Tumor and activated macrophages	17	23	60
2	Tumor	67	13	20
	Tumor and control macrophages	42	22	36
	Tumor and activated macrophages	34	24	42
3	Tumor	54	3	43
	Tumor and control macrophages	34	10	56
	Tumor and activated macrophages	26	10	64

The target cell used in these experiments was SK-BR-3.

Kinetics of induction of cytotoxic macrophages. The cytotoxic capacity of human macrophages incubated in mediator-rich supernates for 4, 8, 24, or 48 h was compared with that of human macrophages incubated in control supernates for the same period of time. Fig. 2 summarizes the results of five experiments. In all experiments maximum cytotoxicity was obtained with cells activated for 24 h. Macrophages activated for 4 h were not cytotoxic for the target cells. By 8-h activation, cytotoxicity was observed but was less pronounced than at 24 h. After 48-h activation, cytotoxicity was in the same range observed at 8-h activation.

Duration of mediator-induced macrophage cytotoxicity. It is known that mediator-activated macrophages in the mouse lose their cytotoxic capacity for tumor cells in vivo or in vitro within a few hours after removal of mediator-rich supernates (6, 14). Therefore, we attempted to establish whether continued exposure to MAF was required to maintain the cytotoxic capacity of human macrophages. In several experiments, the macrophages were activated for 8 h with mediator-rich supernates, washed, and cultured for another 40 h in fresh tissue culture medium to determine if human macrophages retain the ability to kill tumor cells once the activation factor has been removed.

As shown in Table III, mediator-activated human macrophages can still kill tumor cells effectively even if the MAF is removed 40 h before assay. The extent of kill after 8 h of activation is in the same range as when the macrophages are exposed to MAF for 8 h and then maintained in TCM for an additional 40 h before testing.

Because maximal cytotoxicity occurs after 24-h activation (Fig. 2), an additional experiment was carried

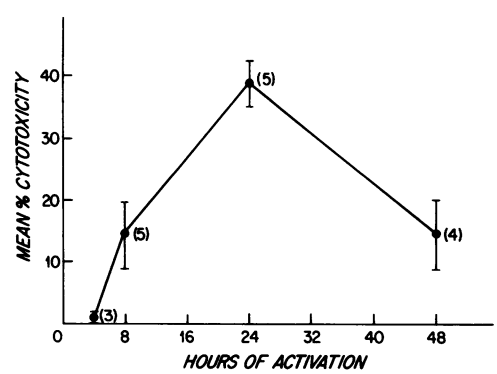

FIGURE 2 Kinetics of enhancement of macrophage-mediated cytotoxicity by lymphocyte mediators. Macrophages were activated for varying time intervals and then cocultivated with labeled target cells. Numbers in parenthesis indicate number of experiments, and brackets indicate 1 SE (SEM).

TABLE III
Duration of Macrophage-Mediated Cytotoxicity

Experiment	Cytotoxicity		
	MAF		
	8 h	48 h	mediator + 40 h fresh medium
	%		
1	—*	14‡	14
2	—	23	15
3	—	16	12.5§
4	8‡	12	15

Macrophages were activated for various time periods and then cocultivated with target cells for 24 h. All results are significant ($P \leq 0.05$) except as noted.
* Indicates not done.
‡ Not significant.
§ Borderline significance $P \leq 0.10$.

TABLE IV
Cytotoxicity of In Vitro Activated Macrophage Monolayers for Various Tumor Cells

Experiment	Cytotoxicity					
	SK-BR-3	HT-29	Caki-1	Calu-1	SK-Mes-1	MA-160
	%					
1	35	33	23	—	25	—
2	48	—	30‡	—	−4§	—
3	16	—	5§	—	13	—
4	—*	26	18	—	−22§	—
5	13	−20§	6§	—	23	33
6	—	32	5§	4§	11§	50
7	—	—	—	13‡	—	16
8	—	—	—	6§	13‡	21

These experiments were carried out with a macrophage tumor target cell ratio of 20:1. All results are significant at $P \leq 0.05$ except as noted.
* Indicates not done.
‡ $P \leq 0.10$.
§ Not significant.

out to determine if macrophages activated for 8 h with MAF would still have maximum cytotoxicity after either 24- or 48-h activation even though MAF was removed after the initial 8 h. The results of this experiment show that macrophages activated for 8 h with MAF and cultured for 16 h in fresh medium had a greater cytotoxic effect (42%) than did macrophages that were cultured for 40 h after removal of the MAF (18% cytotoxicity). This finding is consistent with the observation that maximum cytotoxicity occurs after 24-h activation (Fig. 2) but it also indicates that MAF is not required for the entire period. Furthermore, there seems to be some decay in the cytotoxic capacity between 24- and 48-h activation.

Cytotoxicity of activated macrophages for malignant cell lines. Mediator-activated macrophages are cytotoxic for a variety of target cell lines. Table IV shows the effect of mediator-activated macrophages on six tumor targets. Among these targets SK-BR-3 and MA-160 showed the most susceptibility to macrophage mediated cytotoxicity. These cells were killed in all four experiments. The degree of cytotoxicity ranged from 13 to 50%. HT-29 was also quite susceptible to macrophage-mediated killing. HT-29 cells were killed in three of four experiments. However, Caki-1 and SK-Mes-1 cells were less consistently killed by activated macrophages. SK-Mes-1 cells were killed in four of seven experiments and Caki-1 cells were killed in only three of six experiments. Calu-1 was found to be most resistant to the effects of activated macrophages with insignificant cytotoxicity ranging from 4 to 13% in all experiments.

Lack of macrophage-mediated cytotoxicity for normal human target cells. To determine whether macrophage-mediated cytotoxicity was nonspecifically directed against any type of target cell, or restricted to cells with malignant growth potential, several experiments were carried out with normal human target cells of embryonic origin.

Macrophages incubated with mediator-rich supernates were not cytotoxic for the five normal cell lines tested (Table V). In contrast, these macrophages were cytotoxic for the SK-BR-3 or MA-160 tumor cells that were tested in parallel.

Because these targets were not sensitive to the effects of cytotoxic macrophages, it was important to demonstrate that these same targets could be killed by another immunological mechanism. Human mononuclear cells were incubated with mitomycin C-treated WI-38, MRC-5, or M-7 cells. As a control, another aliquot of mononuclear cells was incubated in a flask without added target cells. After 7-d incubation, the nonadherent cells from stimulated and control cultures were washed and then cocultivated with each of their respective radiolabeled target cells. The results are shown in Table VI. All target cells tested were killed by the in vitro sensitized lymphocytes although mediator-activated macrophages had little or no effect on the same targets.

Relationship between target cell replication rate and susceptibility to the effects of mediator-activated macrophages. It has been previously suggested that sensitivity to macrophage-mediated cytotoxicity is related to rate of cell division (15). Table VII summarizes the doubling times for these cell lines and shows no correlation between doubling time and sensitivity to macrophage-mediated cytotoxicity. In the first experiment, SK-BR-3, one of the two most sensitive targets, had one of the shortest doubling times,

TABLE V
Effect of In Vitro Activated Macrophages on Normal Target Cells

	Cytotoxicity						
	Nonmalignant target cells					Malignant target cells	
Experiment	WI-38	MRC-5	M-7	MA-112	MA-349	SK-BR-3	MA-160
	%						
1	−8	—	—	—	—	30‡	—
2	−5	—	—	—	—	23	—
3	0	—	—	—	—	28	—
4	0	—	—	—	—	13	33
5	—*	−7	4	0	0	—	31
6	—	−8	−12	2	5	—	35

These experiments were carried out with a macrophage tumor target cell ratio of 20:1. Percent cytotoxicity of normal targets was not significant. Percent cytotoxicity of tumor targets was significant at $P \leq 0.05$ except as noted.
* Not done.
‡ $P \leq 0.10$.

whereas the other sensitive target (MA-160) had one of the longest (43 h). On the other hand the doubling time of the resistant targets derived from nonmalignant cells was in the same range as the susceptible malignant targets. In a second experiment, similar results were obtained (Table VII) in that the doubling time of two sensitive tumor targets was the same as two resistant nonmalignant targets.

To further evaluate the relationship between cell doubling time and sensitivity to macrophage-mediated cytotoxicity, we studied the effect of stopping replication by treatment with mitomycin C. SK-BR-3, a sensitive target originating from malignant tissue and WI-38, a resistant target derived from an embryonic cell line were treated with mitomycin C (50 μg/ml). The dose of mitomycin used caused 94% inhibition of uptake of [^{3}H]thymidine. From the results of these experiments (shown in Table VIII), it can be seen that SK-BR-3 cells could be killed whether or not they were pretreated with mitomycin C. On the other hand, WI-38 cells were not killed, and mitomycin C did not change their susceptibility to the effects of activated macrophages. Therefore, the rate at which a target cell replicates is not directly related to susceptibility to macrophage-mediated cytotoxicity in the system used here.

TABLE VI
Cytotoxicity of In Vitro Sensitized Human Lymphocytes

	Residual adherent radioactivity		
Target cell	Immune lymphocytes*	Control lymphocytes‡	Cytotoxicity
			%
M-7	500	7,100	93
MRC-5	210	1,000	79
WI-38	6,200	15,000	60

* The mononuclear cells were incubated with the various target cells for 7 d. The nonadherent cells were then collected, washed twice, and cocultivated with [^{3}H]thymidine-labeled target cells for 24 h.
‡ Cells were incubated for 7 d in a flask that contained no target cells.

DISCUSSION

In vitro studies have shown that mediators released by antigen- or mitogen-stimulated lymphocytes include MAF that enhances macrophage cytotoxicity for tumor

TABLE VII
Target Cell Doubling Time

	Doubling time	
Target cells	Exp. 1	Exp. 2
	h	
Malignant		
Caki-1	21	—*
SK-BR-3	29	32
SK-Mes-1	30	—
MA-160	43	34
Calu-1	48	—
Nonmalignant		
WI-38	40	35
MRC-5	48	—
M-7	43	38

2×10^4 cells were plated in Linbro dishes (Linbro Chemical Co., Hamden, Conn.). 18, 24, 48, 72, or 96 h of incubation, the cells were detached from the wells and counted.
* Not done.

TABLE VIII
Effect of Target Cell Treatment with Mitomycin on Susceptibility to Macrophage-Mediated Cytotoxicity

Experiment	Treatment	Cytotoxicity SK-BR-3	Cytotoxicity WI-38
		%	
1	None	31*	0
	Mitomycin	50*	0
2	None	18*	2
	Mitomycin	10	0
3	None	42*	−1
	Mitomycin	53*	−10

Before cocultivating with macrophages, target cells were treated with 50 μg/ml of mitomycin for 30 min, and washed.
* Percent kill is significant ($P \leq 0.05$).

cells (4–7). In the guinea pig and mouse, mediator-activated macrophages become cytotoxic for syngeneic tumors and have little or no cytotoxicity for normal target cells (4, 5, 16).

Human macrophages are known to be important in resistance to intracellular parasites (17–19) and to bacterial infections (20). However, before the present study it was not known whether treatment of human macrophages with MAF-rich supernates from antigen-stimulated lymphocytes would enhance their cytotoxicity for human target cells.

Enhancement of monocyte-derived macrophage cytotoxicity by MAF is observed after 8 h of incubation, is maximum after 24-h activation, and lasts for at least 40 h after removal of MAF from the macrophages. The kinetics of induction of macrophage-mediated cytotoxicity for guinea pig and mouse macrophages is similar in that it is first observed after 8-h activation (6).[3] However, animal macrophages are unlike human macrophages in that the cytotoxic capacity is not detectable within a few hours after removal of mediator-rich supernates (6, 14).

The time required for activated macrophages to kill target cells is similar to that previously found for guinea pig macrophages (4, 5, 21) and is shorter than that needed for mouse macrophages (22, 23).

By now it has been shown in several animal systems that activated macrophages have a quantitatively greater effect on malignant targets than on normal targets (4, 16). We now have evidence that human macrophages exhibit a similar specificity. The basis for the preferential killing of malignant as opposed to normal targets is not clearly understood. Cell-cell contact is presumably important in the process of macrophage-mediated cytotoxicity (21, 24). Activated guinea pig macrophages have enhanced binding to malignant as compared to normal target cells (25). It is possible that the increased binding to malignant targets is in some way related to the greater susceptibility of these targets to the effects of mediator-activated macrophages. Alternatively, the increased susceptibility of malignant targets may relate to a more rapid cell division. However, we did not find any correlation between target cell sensitivity to macrophages and the rate of replication.

Because monocytes are precursors of macrophages, it is possible that the capacity to respond to MAF may be one of the functions developed during the period of in vitro culture. When peripheral blood monocytes were tested immediately after explantation and activation, enhanced cytotoxicity was not observed. How long monocytes must be incubated in vitro before they acquire the capacity to respond to MAF remains to be established.

Several other groups have reported related findings. Gougerot et al. (26) have shown that human peritoneal macrophages, incubated with cell-free supernates prepared from human mixed-lymphocyte cultures, render macrophages cytotoxic for Cr^{51}-labeled KB tumor cells. Human peripheral blood monocytes were also found to be cytotoxic for the KB cells; however, this cytotoxicity was neither consistent nor as strong as that with the peritoneal macrophages.

Normal unstimulated macrophages have been shown to be cytotoxic to HeLa cells (9). In addition, Hersch et al. (27) have found that macrophages activated by concanavalin A-induced mediators cause growth inhibition but not cytotoxicity of the HeLa cells (27).

Rinehart et al. (8), with a postlabeling technique, have studied the effect of unstimulated blood monocytes on several tissue culture lines, among which is one we have studied (Sk-Mes-1). They found that addition of monocytes to targets caused cytostasis and(or) cytotoxicity. The effect correlated with the monocyte target cell ratio, was maximum at 24 h and was observed with four different targets. Our studies examined the effect of monocyte-derived macrophages on prelabeled target cells and thus are not analogous to their findings. These methodologic differences may explain the different results obtained in our studies. We observed (Table I) some cytotoxicity by control macrophages but not to the same extent as found by Rinehart et al. (8).

The role of macrophages in host resistance to tumor growth in man is still largely undefined. Animal studies that suggest a role for macrophages include histologic observation of regressing tumors, studies of delayed hypersensitivity to tumor extracts, and a variety of in vitro studies showing evidence of cell-mediated immunity to tumor targets (1–3). Much less is known about the interaction of human macrophages with

[3] Churchill, W. H. Unpublished observation.

tumor. Macrophages from patients with cancer are known to have impaired chemotaxis (28), possibly a result of an inhibitor produced by the tumor (29). Aside from these observations, relatively little is known about whether macrophages from patients with cancer have different responses to tumor targets than normal macrophages.

In patients with cancer, defective macrophage cytotoxicity may be caused by abnormalities in base-line cytotoxicity or in the enhancement of cytotoxicity induced by lymphocyte mediators. With this assay it should now be possible to distinguish between these alternatives.

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