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A microassay for the rapid and selective binding of cells from solid tumors to mouse macrophages

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Summary. A microassay was developed to study the rapid binding characteristics of murine macrophages activated by gamma interferon and muramyl dipeptide to adherent neoplastic or nonneoplastic target cells. The binding of tumor cells to both activated and nonactivated macrophages was time- and temperature-dependent, and independent of tumor cell type. Activated macrophages bound more tumor cells than nonactivated macrophages. The initial binding of macrophages to target cells did not necessarily lead to lysis. First, primed macrophages bound tumor cells but did not lyse them, and second, nonactivated macrophages bound nontumorigenic cells without subsequent lysis. The rapid binding assay described here could prove useful in investigating the recognition mechanism(s) between macrophages and tumor cells derived from solid primary and metastatic cancers.

Introduction

The importance of macrophages in host defense mechanisms has been extensively documented in recent years [see 2, 8, 15 for review]. It has been shown that macrophages rendered tumoricidal (or activated) by various immunomodulators, such as lymphokines or muramyl dipeptides in either free form or entrapped within liposomes, are capable of destroying neoplastic cells in vitro while leaving nonneoplastic cells unharmed, even under cocultivation conditions [9]. The susceptibility of neoplastic cells to lysis by activated macrophages is independent of such tumor cell characteristics as invasion and metastasis [6, 7, 8], antigenicity and immunogenicity [7], growth rate [7, 10], and resistance to lymphocyte or natural killer cell-mediated lysis [8].

The mechanisms responsible for this effector function are not understood. Studies on the interaction of nonadherent tumor cells with murine macrophages suggested that the binding of activated macrophages to tumor cells serves as the selective recognition of the target cells and is a prerequisite for the subsequent lysis of the tumor cells by secretion of specific tumor cytolytic factors by the macrophages [1, 2]. In addition, the binding process was demonstrated to be necessary but not sufficient for the destruction of tumor target cells. This conclusion was also supported by time-lapse microcinematography [3], which showed that a dynamic interaction between activated macrophages and neoplastic targets is characterized by brief periods of intimate contact that finally culminate in target cell lysis.

To date, all binding studies have used such nonadherent neoplastic cells as mastocytoma [17, 18] lymphoma [12, 17, 18, 21], and leukemic cells [2, 17, 18, 25] which are not subject to binding artifacts such as adherence to plastic, homotypic aggregation, and other nonspecific binding phenomena. We report here the development of a convenient, rapid microassay that is useful for the study of specific interactions between adherent target neoplastic cells and murine macrophages. We show that the binding is selective for tumor cells, and that activated macrophages bind neoplastic cells to a greater extent than unactivated macrophages. The binding characteristics appear to be similar to those observed for nonadherent neoplastic cells and correlate with the subsequent lysis of tumor cells (but not nonneoplastic adherent cells) by macrophages activated to the tumoricidal state.

Materials and methods

Mice. Specific pathogen-free mice of C57BL/6 and B6C3F1 strains, 8 to 10 weeks old, were purchased from the National Cancer Institute-Frederick Cancer Research Facility's Animal Production Area (Frederick, Md.).

Cell lines. The B16 melanoma cell lines, low metastatic B16-F1, high metastatic B16-F10, and highly invasive B16-BL6, were derived from a spontaneous melanoma in a C57BL/6 mouse, and were then adapted to grow in vitro [5, 13]. Tumor cell line UV-2237 is a fibrosarcoma of recent origin induced in C3H/Hen mice by chronic UV irradiation and adapted to in vitro growth after one passage in immunosuppressed syngeneic hosts [16]. The nonneoplastic cell line 10T1/2 of mouse embryo cells was derived from C3H mice [23]. Normal epidermal cells were isolated from the C57BL/6 mouse tail skin by the method of Steinmüller and Wunderlich [26]. All cell lines were cultured in 75 cm^2 flasks (Falcon Plastics, Oxnard, Calif.) and maintained in Eagle's minimal essential medium supplemented with 5% fetal bovine serum (FBS), vitamin solution, sodium pyruvate, nonessential amino acids, and L-glutamine

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(M. A. Bioproducts, Walkersville, Md.). Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. All cultures were free of mycoplasma and pathogenic mouse viruses.

Collection and cultivation of peritoneal exudate macrophages. Thioglycollate-stimulated peritoneal exudate macrophages (PEM) were collected by peritoneal lavage from B6C3F1 mice given i. p. injections of 1.5 ml of thioglycollate broth (Baltimore Biological Laboratories, Cockeysville, Md.) 4–5 days before harvest [22]. The PEM were centrifuged at 250 *g* for 10 min, resuspended in serum-free medium at a concentration of 5×10^5 cells/ml, and 0.2 ml of cell suspension was plated into 96-well round-bottom Corning No. 25850 Microtest II plastic tissue culture dishes (Corning Glass Works, Corning, NY). After a 60-min incubation, the wells were rinsed with media to remove nonadherent cells and the culture was re-fed with medium containing 5% FBS with or without macrophage activators (see below). The resultant macrophage monolayer was 80%–90% confluent and >98% pure, according to morphologic and phagocytic criteria [15].

Macrophage activating agents. N-acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide, MDP) and its lipophilic derivative, muramyl tripeptide phosphatidylethanolamine (MTP-PE), were the kind gift of Ciba-Geigy Ltd. (Basel, Switzerland). N-acetylmuramyl-D-alanyl-D-isoglutamine (MDP-D) was purchased from Sigma Chemical Co. Ltd. (St. Louis, Mo.). *Escherichia coli*-derived murine recombinant gamma interferon (IFN-γ; specific activity 17×10^6 units/mg) was generously supplied by Genentech, Inc. (South San Francisco, Calif.). Lipopolysaccharide (*E. coli* 055:B5; LPS) was purchased from Difco Laboratories (Detroit, Mich.).

In vitro activation of macrophages by free or liposome-encapsulated activating agents. All reagents and media used in in vitro activation of PEM were endotoxin free (detection limit of <0.125 ng/ml) as determined by the *Limulus* amebocyte lysate assay (Associates of Cape Cod, Inc., Woods Hole, Mass.). Macrophages were incubated in medium alone, in medium containing LPS (10 μg/ml), IFN-γ (10 units/ml), MDP 10 μg/ml), or the combination of IFN-γ (10 units/ml) and MDP (10 g/ml), and in medium containing 100 nmol of multilamellar vesicles (MLV) with entrapped MTP-PE (4 μg/μmol lipid), as described previously [24]. Following a 24-h activation period, the wells were washed with medium to remove the activators, and appropriate concentrations of radiolabeled target cells were added for the binding assay.

Binding assay. Target cells in an exponential growth phase were incubated in medium containing 5% FBS supplemented with ^{125}I IUdR (0.3 μCi/ml; sp. act. 2000 mCi/mmol; New England Nuclear, Boston, Mass.). The target cells were washed twice with warm Ca^{2+} and Mg^{2+}-free Hanks' balanced salt solution to remove unincorporated label. The cells were harvested by a short trypsinization (0.25% trypsin and 0.02% EDTA for 1 min at 37 °C), and resuspended in cold medium containing 5% FBS. The cells were spun at 250 *g* for 5 min and resuspended in cold serum-free medium to form a single suspension of cells. Appropriate numbers of ^{125}I IUdR-labeled target cells in a volume of 0.1 ml/well were added to cultured activated or unactivated macrophages. The cultures were incubated at 4 °C, at 25 °C (room temperature), or at 37 °C for 5–60 min. Then the medium was discarded and 0.3 ml of phosphate-buffered saline (PBS) was added to the wells. The plates were placed on a Mini-Orbital shaker (Bellco Biotechnology, Vineland, NJ) and shaken for 30 s at an instrument setting of 8. The plates were then washed three times with PBS. The remaining macrophage-bound target cells were lysed with 0.1 ml of 0.1 *N* NaOH. The lysate was absorbed on cotton swabs and monitored for radioactivity by gamma scintillation counting. The binding capacity (number of targets bound/well of 10^5 macrophages) was expressed as follows:

$$\text{Binding capacity} = \frac{\text{cpm of targets bound to macrophages}}{\text{cpm of total targets added}} \times \text{total number of targets added}$$

Assay for macrophage-mediated tumor cytotoxicity. For macrophage-mediated cytotoxicity assay, flat-bottom Corning No. 25860 Microtest II plastic tissue culture plates were used. Cytotoxicity was assessed by the radioactive release assay described in detail elsewhere [24]. In brief, ^{125}I IUdR target cells (10^4) were added to 38-mm^2 wells to obtain an initial macrophage-to-target cell ratio of 10:1. No significant differences were detected in plating ^{125}I IUdR-labeled target cells to either the plastic or macrophage monolayers. Radiolabeled target cells were plated alone as an additional control. All cultures were re-fed with medium 24 h after the addition of the target cells to remove any nonplated targets, and then incubated for an additional 48 h at 37 °C. The assay was terminated by washing the cultures twice with PBS to remove nonadherent cells and harvesting the remaining viable, adherent cells by lysis with 0.1 ml of 0.1 *N* NaOH. The lysate was absorbed on cotton swabs and monitored for radioactivity in a gamma counter. The percentage of generated macrophage cytotoxicity was calculated as follows:

$$\text{Percent cytotoxicity} = \frac{\text{cpm of target cells with normal macrophages} - \text{cpm of target cells with activated macrophages}}{\text{cpm of target cells with normal macrophages}} \times 100$$

Fluorescence microscopy. Since it is difficult to distinguish adherent macrophages unambiguously from tumor cells in some experiments, the tumor cells were labeled with rhodamine 123 (Eastman Kodak, Rochester, NY), a vital fluorescent mitochondrial dye [14]. B16-F10 cells were incubated at 37 °C for 15 min with 6 μg/ml rhodamine 123 in medium containing 5% FBS. The culture was rinsed three times with medium and incubated for 5 min with media containing 5% FBS. This washing procedure was repeated twice more. Target tumor cells then labeled with rhodamine 123 were harvested and resuspended in serum-free medium as described for the binding assay.

The PEM were plated onto coverslips (12 mm^2 in 24-well plates to a confluent monolayer and subsequently cultured in medium containing 5% FBS alone or in the presence of IFN-γ (10 units/ml) and MDP (10 μg/ml) for 24 h. Rhodamine 123-labeled tumor cells (2.5×10^5) were added to the culture wells and incubated for 10 min at room temperature. The coverslips were then washed three times and observed by fluorescence microscopy.

Statistical analysis. The statistical significance of differences between groups was determined by Student's two-tailed *t*-test.

Results

Conditions for binding

In the first set of experiments, we determined the optimal conditions for binding by investigating the dose response, kinetics, and temperature dependence of tumor cells binding to normal macrophages and macrophages activated with 10 units/ml recombinant mouse IFN-γ admixed with 10 μg/ml MDP. We have shown previously that either IFN-γ (10 units/ml) or MDP (10 μg/ml) alone could not activate the macrophages to the tumoricidal state. In contrast, the combination of IFN-γ and MDP is highly synergistic in activating macrophages to become tumoricidal [24]. Different concentrations of ^{125}I IUdR-labeled target tumor cells ($1-5\times10^4$) suspended in cold serum-free medium were added to the wells in a total volume of 0.1 ml. After 10 min at room temperature, the wells were washed extensively. The washing procedure did not disturb the macrophage monolayer, as determined by light microscopic examination. The results of one experiment of five are shown in Fig. 1. The addition of 1 to 5×10^4 B16-F10 target cells to activated and unactivated macrophages (10^5) led to a linear increase in binding capacity. The binding of B16-F10 to the activated macrophages was significantly higher than to the unactivated macrophages at all tested concentrations of target tumor cells.

The kinetics of binding was followed for 60 min with the addition of 2×10^4 and 5×10^4 cells/10^5 macrophages at room temperature. This experiment was carried out more than six times. The results were very reproducible, and thus one representative experiment is shown in Table 1. Significant differences in tumor cell binding to activated macrophages were observed after a 10-min incubation

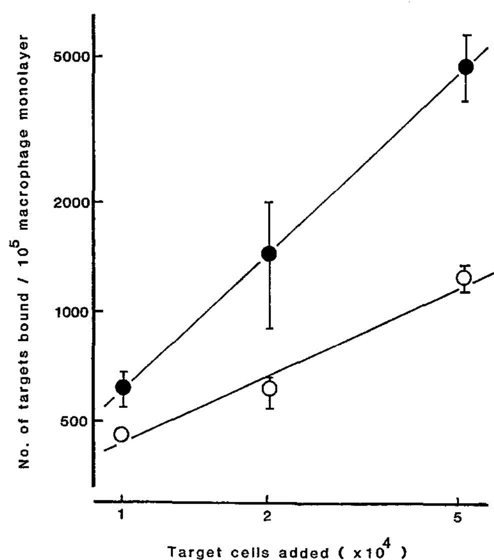

Fig. 1. Effect of various numbers of B16-F10 cells on binding to macrophages at 10 min. Macrophages were activated with (●——●) or without (○——○) the combination of 10 units/ml of IFN-γ and 10 μg/ml of MDP for 24 h before the addition of ^{125}I IUdR-labeled B16-F10 tumor cells

Table 1. Kinetics of B16-F10 binding to macrophage monolayer

No. of targets	Time after addition (min)	Binding capacity to:	
		Control macrophages	Activated macrophages[a]
B16-F10[b]	10	2190 ± 445[c]	9588 ± 813[d]
5×10^4	20	22990 ± 1104	26300 ± 2251
	40	29920 ± 1817	28421 ± 2889
	60	36034 ± 1117	37067 ± 977
2×10^4	10	277 ± 19	398 ± 27[d]
	20	3146 ± 196	5445 ± 274[d]
	40	5694 ± 1020	6905 ± 1582
	60	7413 ± 773	9045 ± 1044

[a] Control macrophages were incubated in medium. Activated macrophages were incubated in medium containing 10 units/ml of IFN-γ and 10 μg/ml of MDP for 24 h
[b] ^{125}I IUdR-labeled B16-F10 cells were added in a 0.1-ml volume
[c] Number of target cells bound well containing macrophage monolayer ± SD of quadruplicate cultures
[d] Significant increase in binding, $P<0.001$

with an input of 5×10^4 tumor cells and after a 20-min incubation with an input of 2×10^4 tumor cells. After 40–60 min, almost equal numbers of cells were bound to unactivated and activated macrophages.

In the next set of experiments, we investigated whether the tumor cell binding to activated macrophages was preferential over binding to other monolayer cultures. The degree of binding of the B16-F10 cells to tumor cell monolayers and to monolayers of unactivated or activated macrophages were carried out with an input of 2×10^4 B16-F10 cells for 20 min at 4 °C, 25 °C, and 37 °C. The results shown in Table 2 demonstrate that tumor cell binding to tumor cell monolayers or to nonactivated macrophages was very similar. Tumor cell binding to activated macrophages was, however, significantly higher (at 25 °C). This binding was temperature-dependent, since it was inhibited at 4 °C (Table 2). Similar results have been observed in experiments in which nonadherent tumor cells were used [17]. At 37 °C, there was a significant increase in tumor cell binding mainly due to binding artifacts such as adherence to plastic (results not shown here) and homotypic tumor cell interaction. Although activated macrophages still bound to tumor cells to a greater extent than to normal macrophages, the increase in binding was not as dramatic as that observed at 25 °C.

Table 2. Various influences on binding of target cells

Plated onto monolayer of:	Binding capacity at:		
	4 °C	25 °C	37 °C
B16-F10[a]	311 ± 24[c]	517 ± 126	4574 ± 1311
Macrophages[a]			
Control	354 ± 20	421 ± 51	8321 ± 642
Activated[b]	472 ± 83	1299 ± 106	11813 ± 480

[a] B16-F10 cell and macrophages were preplated onto the wells 24 h before addition of ^{125}I IUdR-labeled B16-F10 cells
[b] Same as [a] in Table 1
[b] Same as [c] in Table 1

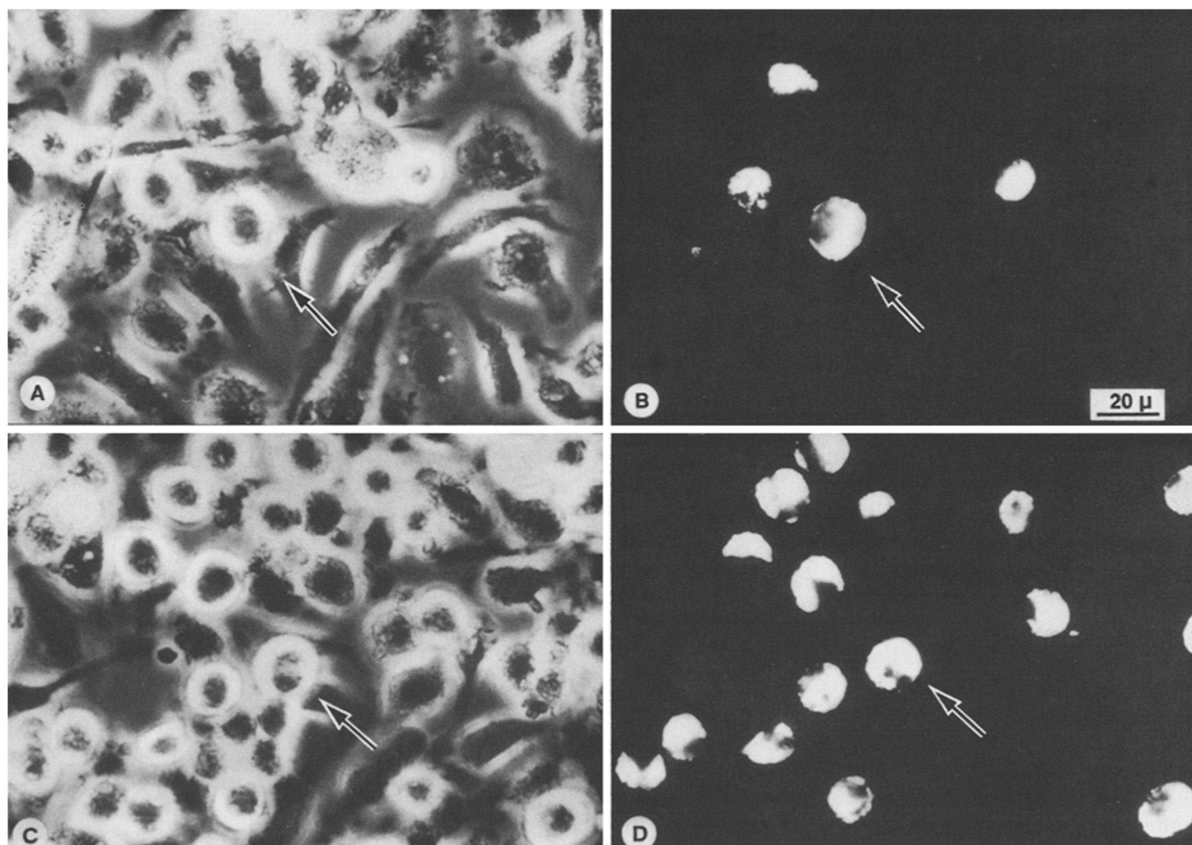

Fig. 2. Binding of rhodamine 123-labeled B16-F10 cells to macrophages treated with (C, phase; D, fluorescence) or without (A, phase; B, fluorescence) 10 units/ml of IFN-γ and 10 μg/ml of MDP. Arrows point to corresponding cells

Cultures of control, nonactivated, and activated macrophages with bound tumor cells were examined under phase and fluorescence microscopy. To distinguish bound tumor cells from macrophages, the B16-F10 tumor cells were prelabeled with rhodamine 123, a vital stain specific for the mitochondria [14]. As seen in Fig. 2, after a 10-min incubation at 25 °C, more fluorescent tumor cells bound to activated macrophages (Fig. 2C and D) than to nonactivated macrophages (Fig. 2A and B).

Collectively, the results indicate that optimum tumor cell-macrophage binding can be investigated by adding 2 or 5×10^4 target cells to macrophage monolayers for 20 or 10 min respectively, at room temperature.

Binding of tumorigenic and nontumorigenic cells to control and activated macrophages

In the next set of experiments, various tumorigenic and nontumorigenic cells were added to control and activated macrophage monolayers. The data of one experiment of three are shown in Table 3. Only tumorigenic cells (B16-F10, B16-F1, B16-BL6, UV 2237) demonstrated an increased binding affinity to activated macrophages. In contrast, nontumorigenic murine cells (10T1/2 or fresh epidermal tail skin cells) exhibited a similar or even lower binding affinity to activated macrophages as compared with control macrophages.

Table 3. Binding of tumorigenic and nontumorigenic cells to macrophages

Target cells[a]	Binding capacity to:	
	Control macrophages	Activated macrophages[b]
Tumorigenic:		
B16-F10 melanoma	2165 ± 69[c]	4798 ± 157[d]
B16-F1 melanoma	457 ± 39	986 ± 146[d]
B16-BL6 melanoma	2698 ± 1561	7130 ± 726[e]
UV 2237 fibrosarcoma	1660 ± 39	2697 ± 304[d]
Nontumorigenic:		
10T1/2 embryonic fibroblasts	3755 ± 681	1445 ± 234
Normal epidermal tail skin	580 ± 79	540 ± 59

[a] ^{125}I IUdR-labeled target cells were added to macrophage monolayer in a 0.1-ml volume
[b] Same as [a] in Table 1
[c] Same as [c] in Table 1
[d] Significant increase in binding, $P < 0.001$
[e] $P < 0.005$

Binding of B16-F10 cells to macrophages pretreated with various immunomodulators

The increased binding of tumorigenic cells to activated macrophages was not related to the method of activation

Table 4. Binding of B16-F10 targets to macrophages treated with vaious activators

Macrophages incubated with:[a]	Dose/ml	Binding capacity (No. of targets bound/10^5 macrophages monolayer)
Medium alone		1736 ± 53[c]
IFN-γ + MDP	10 units + 10 μg	5327 ± 102[d]
LPS	10 μg	4013 ± 115[d]
MLV MTP-PE	4 μg/μmol lipid	3130 ± 448[d]
MDP	10 μg	2770 ± 587[e]
MDP-D	10 μg	1764 ± 53
IFN-γ	10 units	4005 ± 182[d]
IFN-γ + MDP[b]	10 units + 10 μg	1644 ± 268

[a] Macrophages were incubated with or without activators for 24 h before the addition of ^{125}I IUdR-labeled B16-F10 cells
[b] Macrophages were incubated with IFN-γ and MDP for 1 h
[b] Same as [c] in Table 1
[d] Significant increase in binding, $P<0.001$
[e] $P<0.02$

used, i.e., IFN-γ and MDP. We base this conclusion on the data shown in Table 4. Increased binding of B16-F10 melanoma cells was observed with macrophages activated to the tumoricidal state by a 24-h preincubation with IFN-γ and MDP, LPS, or with liposomes containing MTP-PE [11, 24]. Increased binding of tumor cells was observed with all activated macrophages. No enhancement in tumor cell binding was observed when macrophages were incubated with the steroisomer of MDP, MDP-D, which has no immunopotentiating activities including macrophage activation [4, 19, 24]. To rule out that IFN-γ and MDP could bring about nonspecific "sticking" of tumor cells, macrophages were treated with these agents for 1 h, and then the binding assay was carried out. No increase in tumor binding was observed.

Correlation of binding with macrophage-mediated cytotoxicity

The above data suggested that increased initial tumor cell binding to macrophages might be associated with the development of tumoricidal capacity in the macrophages. Therefore, we carried out the binding assay with primed (but not tumoricidal) and tumoricidal macrophages. Normal macrophages, macrophages primed with free MDP (10 μg/ml), a dose insufficient for development of tumoricidal capacity [25], and activated macrophages (IFN-γ and MDP or LPS) were used in parallel studies of binding (10 min) and antitumor cytotoxicity (72 h). The B16-F10 melanoma and the nontumorigenic 10T1/2 cells were used as targets. The data are shown in Table 5. Macrophages activated with either IFN-γ and MDP or with LPS effectively bound and lysed the B16-F10 tumor target cells (69% specific lysis). The binding capacity of MDP-primed macrophages to B16-F10 tumor cells was also increased threefold as compared with that of untreated macrophages. However, no tumor lysis occurred. Identical results were observed with incubation of macrophages with 10 units/ml of IFN-γ alone (results not shown here). These data suggest that the initial binding of macrophages to tumor cells and subsequent lysis of tumor cells are probably independently regulated functions of macrophages.

Macrophage interaction with the nontumorigenic 10T1/2 cells differed from that observed for the tumorigenic B16-F10 cells. Activated macrophages bound to 10T1/2 cells to a lesser extent than did control macrophages. Neither control nor activated macrophages lysed the 10T1/2 cells in the 3-day cytotoxicity assay.

Discussion

The exact mechanism by which activated macrophages recognize susceptible tumor cells is not understood [2, 6, 8, 15]. Most recent studies suggest, however, that macrophage binding to target cells must precede target cell lysis [3]. To date, much of our knowledge regarding the binding of macrophages to tumor cells is based on studies that have used suspension tumor cells such as mastocytoma [17, 18], lymphoma [12, 17, 18, 21], leukemia [2, 17, 18, 25], and hepatocytomas growing as ascites [20]. Relatively little information is available on the binding of macrophages to cells isolated from solid tumors. The present work concerns the development of a rapid binding assay that could be applied to study in vitro cell-cell interaction of tumor cells derived from solid primary tumors or from metastatic lesions.

Table 5. Binding activity and tumor cytotoxicity mediated by activated macrophages

Macrophages incubated with:[a]	Dose/ml	Binding capacity to: (No. of targets bound/10^5 macrophages monolayer)		Radioactivity in viable cells on day 3 (cpm ± SD)	
		B16-F10	10T1/2	B16-F10	10T1/2
Medium alone		1386 ± 221[b]	3298 ± 174	3309 ± 199[c]	2785 ± 147
IFN-γ + MDP	10 units + 10 μg	8557 ± 361[d]	1982 ± 69	1019 ± 185 (69%)[e]	3212 ± 584
LPS	10 μg	5563 ± 572[d]	1823 ± 36	1029 ± 232 (69%)[e]	3223 ± 319
MDP	10 μg	5354 ± 651[d]	2280 ± 377	3593 ± 503	2964 ± 190
Target cell alone				3590 ± 199	2994 ± 252

[a] Macrophages were incubated with or without activators for 24 h before the addition of ^{125}I IUdR-labeled target cells
[b] Same as [c] in Table 1
[c] Mean cpm ± SD of triplicate cultures
[d] Significant increase in binding, $P<0.001$
[e] Number in parentheses is the percentage of cytotoxicity as compared with that of untreated macrophages at corresponding ratio (density) to target cells. $P<0.001$

The initial binding of target cells to macrophage monolayers depended on the nature of the macrophages (normal or activated), the nature of the target cells (nontumorigenic or tumorigenic), and the assay conditions (cell number, length of incubation, and temperature). With a short 10 to 20 min incubation period, more tumorigenic cells bound to activated macrophages than to control, nonactivated macrophages or to monolayers of tumor cells. Such nontumorigenic cells as tail skin epidermal cells and 10T1/2 cells did not, however, show preferential rapid binding to activated macrophages. In fact, the nontumorigenic cells used here actually exhibited identical or greater initial binding to the control than the activated macrophages.

To maintain reproducibility of results, the macrophage-labeled target cell cultures were washed thoroughly. The use of round-bottom wells was therefore important in minimizing nonspecific binding, since this type of well could be washed more effectively than a flat-bottom well. Despite vigorous washing, the plated monolayers of macrophages were not disrupted, as assessed by microscopy and crystal-violet staining techniques.

We decided to terminate the assay after 10 to 20 min of cocultivation, because this initial binding period best distinguished the interaction of tumorigenic cells with control and activated macrophages. When tumorigenic cells were allowed to interact with control or tumoricidal macrophages for a long time (4 h or more), no differences in binding could be observed. These results are consistent with those of long-duration cytotoxicity assays, in which identical plating efficiencies are observed for adherent tumor cells added to wells cultured with unactivated or activated macrophages [22].

Macrophages activated by lymphokines or Bacillus Calmette-Guérin have a higher affinity for leukemia and lymphoma cells with respect to binding and cytolysis [2, 25]. Our present results with tumor cells from solid tumors are consistent with these observations. In our activation protocols, we used such defined immunomodulators, as IFN-γ, MDP, LPS, and liposomes containing MTP-PE [24]. We have shown previously that the incubation of macrophages with low doses of MDP (10 μg/ml) or with a low dose of IFN-γ (10 units/ml) does not generate tumoricidal properties in the cells [24]. The combination of IFN-γ and MDP, however, is highly synergistic in activating macrophages to become tumor-cytotoxic [24]. The present results show that a marked increase in binding of tumorigenic cells occurs to both tumoricidal and primed macrophages. These data suggest that the capacity of macrophages to bind tumorigenic cells rapidly and their capacity to destroy such cells subsequently are not synonymous, they are likely to be independently regulated macrophage functions. Indeed, the binding event appears to occur in minutes, whereas the lysis event can take up to 3 days.

The immediate tumor cell binding to activated macrophages involved a dynamic process. We base this conclusion on the data from experiments in which binding was inhibited at 4 °C. Moreover, binding experiments carried out with glutaraldehyde-fixed macrophages were unsuccessful (data not shown), implying that a metabolically active macrophage is necessary for tumor cell binding to occur.

The development of a rapid assay that measures the initial interaction of activated macrophages with tumorigenic cells could be instrumental in advancing our knowledge about this process. Studies on the manipulation of either tumor cell or macrophage membranes or both, and their influence on macrophage tumor recognition can now be done.

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