BINDING OF BACILLUS CALMETTE-GUÉRIN-ACTIVATED
MACROPHAGES TO TUMOR TARGETS

Selective Inhibition by Membrane Preparations from
Homologous and Heterologous Neoplastic Cells*

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The mononuclear phagocyte system constitutes an important element of the immune defenses of the host against neoplasia (reviewed in [1–5]). Mononuclear phagocytes activated for cytolysis by agents such as bacillus Calmette-Guérin (BCG),[1] though not macrophages from most sites of inflammation induced by sterile phlogistic agents, efficiently lyse tumor target cells in vitro (reviewed in [5–8]). The cytolysis is target selective in that activated macrophages usually destroy neoplastically transformed cells to a greater extent than their nontransformed counterparts (5–8). However, the basis of this discriminatory cytolysis is poorly understood. Cytolysis by activated macrophages has generally been found to be contact-dependent (5–8), and cinemicrographic studies have demonstrated that activated macrophages interact extensively and intimately with the surface of neoplastic, but not nonneoplastic, targets (9–11). Recently, observations made with two distinct experimental systems have indicated the intimate contact between macrophages and targets represents firm physical binding of neoplastic targets to the surface of activated macrophages (12, 13). The binding, which has the same selectivity as cytolysis in regard to both type of macrophage and type of target (12, 13), has subsequently been shown to be an initial and necessary event in macrophage-mediated cytolysis (14, 15). The binding of tumor cells to activated macrophages may thus represent a part of the sensory mechanism by which mononuclear phagocytes discriminate between neoplastic and nonneoplastic cells. However, the basis of selective target binding by activated macrophages has not yet been established. This report presents evidence that membrane preparations of three murine, nonadherent neoplastic cells, but not of lymphocytes, contain structures that inhibit the binding of intact homologous and heterologous neoplastic targets to BCG-activated murine macrophages.

Materials and Methods

Mice. Inbred C57BL/6J mice (The Jackson Laboratories, Bar Harbor, Maine) of 8–12 wk of age were used in all experiments.

Tissue Culture. Cell lines were maintained in Eagle's minimum essential medium (Grand

[1] *Abbreviations used in this paper:* BCG, bacillus Calmette-Guérin; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; TG, thioglycollate broth.

Island Biological Co., Grand Island, N. Y.) supplemented with fresh glutamine (2 mM), penicillin (125 U/ml), streptomycin (62.5 μg/ml), plus 10% fetal calf serum (FCS) (Sterile Systems, Inc., Logan, Utah) as previously described (13). Washes of targets and macrophages were performed in Hanks' balanced salt solution (HBSS) (Microbiological Associates, Walkersville, Md.) containing 5% FCS. All tissue culture media, sera, reagents, and membrane preparations were endotoxin-free (defined as containing <0.4 ng/ml of endotoxin, as quantified by the Limulus amebocyte lysate (Cape Cod Associates, Woods Hole, Mass) assay.

Target Cells. The origin of the target cells employed (the EL-4 lymphoma from C57BL/6J mice, the RLσ1 leukemia from BALB/c mice, and the P815 mastocytoma from DBA/2 mice) have been previously described (13). Cell lines were maintained in vitro as described (13). Normal splenic lymphocytes were obtained from unimmunized C57BL/6J mice and prepared as previously described (13). To examine rapidly dividing nonneoplastic targets as well, lymphoblasts were induced in splenic lymphocytes by lipopolysaccharide from *Escherichia coli* 055:B5 (LPS) (10 μg/ml; Difco Laboratories, Inc., Detroit, Mich.) for 48 h of culture in RPMI-1640 supplemented with nonessential amino acids, sodium pyruvate, and 5% FCS. The resultant cultures of lymphoblasts, which contained >80% blast forms as determined by microscopy, were bound to a slightly greater extent and were lysed to the same extent as normal lymphocytes by BCG macrophages (see Table I, footnote § and Table III, footnote ‖). Lymphocytes and lymphocyte-membrane preparations were thus employed as controls in subsequent experiments.

Reagents. Brewers' thioglycollate broth (TG), prepared according to manufacturer's instructions, was obtained from Difco Laboratories, Inc. (catalogue No. B236). BCG, Phipps strain 1029, was purchased from the Trudeau Institute, Saranac Lake, N. Y. $Na_2[^{51}Cr]O_4$ (sp ac: 50–400 μCi/mg) was purchased from Amersham Searle Inc., Chicago, Ill.

Macrophages. Peritoneal macrophages were elicited in C57BL/6J mice by BCG and TG as previously described (16). The peritoneal exudate cells obtained 3 d after inoculation of these phlogistic agents were plated to result in uniform densities of 2.5×10^5 adherent macrophages/cm^2. After 3 h, the wells were washed vigorously four times to result in monolayers of purified macrophages (>99%), as judged morphology, staining for nonspecific esterase, and phagocytic uptake of IgG-coated erythrocytes (17).

Preparation of Membranes. Crude membrane preparations of EL-4, P815, and RLσ1 tumor targets and of normal lymphocytes were prepared as previously described (18). Briefly, 1.0×10^{10} targets were lysed by freezing and thawing in Tris-buffered saline (0.15 M NaCl and 0.01 M Tris, pH 8.3). The resultant suspension was centrifuged at 300 g for 12 min at 4°C to remove nuclei and heavy cellular debris. The crude membrane preparation was pelleted at 100,000 g for 60 min at 4°C, resuspended in the Tris-sodium chloride buffer, and pelleted again at 100,000 g. The precipitate was resuspended in HBSS. Protein concentration of the suspended membrane preparation was determined by the method of Lowry et al. (19). The suspension was divided into aliquots, which were stored at −20°C. After thawing for use, the membrane preparations were sonicated (30 s in a Cole-Parmer ultrasonicator, model 8845-30, Cole-Parmer Instrument Co., Chicago, Ill.) to disperse any large aggregates present. The membranous character of each batch of membranes, after sonication, was verified by electron microscopy.

To obtain membrane preparations enriched in plasma membranes, we employed the method of Crumpton and Snary (20). In brief, 2×10^{10} EL-4 cells were disrupted by brief sonication in a bath-type sonicator, and nuclei were removed from the preparation by centrifugation at 300 g for 10 min at 4°C. The mitochondrial fraction was removed by centrifuging at 4,000 g for 15 min, and the microsomal pellet was obtained by centrifuging the supernate at 20,000 g for 30 min. After one wash, the microsomal fraction was resuspended in 36% sucrose (10 mM Tris-HCl, pH 7.5), overlaid with 25% sucrose and centrifuged at 87,000 g for 18 h. The interfacial band from the gradient was recovered and, after homogenization in a Dounce homogenizer in the buffer, was twice sedimented by centrifugation at 75,000 g for 20 min at 4°C. The resultant pellet was enriched in plasma membranes, as indicated by a 15-fold increase in serologically detectable H-2 activity (18) per microgram protein over the crude membrane preparations described above.

Labeling of Targets. The P815, EL-4, RLσ1 and lymphocytic targets were labeled with $Na_2[^{51}Cr]O_4$ as previously described (13) to yield 6×10^3–10×10^3 cpm/10^5 targets.

Binding Assay. The basic assay for quantification of binding has been described previously

(13). At the time of binding (4 h after explantation of the macrophages), most of the BCG-activated and TG-elicited macrophages are well spread and spread to an equal degree (13). Binding of the neoplastic targets is most consistently observed to those BCG macrophages which are well spread, when observed by either phase-contrast microscopy (13), scanning electron microscopy (13), or cinemicroscopy (C. Stewart, P. Marino, and D. Adams, unpublished observations). To conserve the amounts of membrane preparation employed, the assay was conducted in flat-bottomed, 6-mm microtiter wells (73-003-05; Linbro Chemical Co., Hamden, Conn.). In brief, wells containing a constant density of purified macrophages (2.5 × 10⁵ Mφ/ cm² or a total of 7.2 × 10⁴ macrophages/well) were cocultivated with single-cell suspensions containing 7.5 × 10⁴ targets previously labeled with $Na_2[^{51}Cr]O_4$. The cultures were incubated at 37°C under 5% CO_2 for 1 h, after which, the targets were removed by aspiration and four vigorous, uniform washes with a plunger-type pipette. Under these conditions, the number of residual targets, >99% of which are adherent to macrophages, is uniform from well to well as determined by microscopic examination of the cultures (13). Furthermore, the neoplastic targets are so firmly attached to the BCG-activated macrophages that >10⁻³ dyn of force is required to disrupt the binding, when strength of binding is determined by the method of McClay et al. (21). By contrast, loosely adherent targets, which can be detached by <10⁻⁶ dyn of force, are readily removed by the four washes.

The remaining label was solubilized and the amount of residual radioactivity determined. Binding was calculated by the formula: total number of targets bound

$$= \frac{\text{counts per minute bound to monolayers}}{\text{total counts per minute present}} \times 7.5 \times 10^4$$

Binding to plastic alone in the absence of macrophages (<7.5 × 10² tumor targets or <1% of total label/well or <20 × 10² lymphocytes or <3% of total label/well) was determined in each experiment and then subtracted from each value to give binding to macrophages (number of targets bound to macrophages).

Assay for Macrophage-mediated Cytotoxicity. Macrophage-mediated cytotoxicity was determined as previously described (14), except that experiments were conducted in microtiter wells (7.5 × 10⁴ targets plus 7.2 × 10⁴ purified adherent macrophages cultured for 18 h).

Results

Effect of Membrane Preparations on Binding. To examine the possibility that the structure(s) mediating binding was located in the membranes of neoplastic targets, we prepared suspensions of membranes from various targets. Binding of P815 targets to BCG-activated macrophages was inhibited in a dose-dependent fashion by membrane preparations from EL-4, RLσ1, or P815 neoplastic targets, but not by membranes from lymphocytes (Fig. 1 A). At a concentration of 125 μg of membrane/well, binding of P815 targets was inhibited 72–85% by membrane preparations from the three tumor cells (Fig. 1 A). Inhibition of ≤93% by this concentration of membrane preparation was observed in other experiments (data not shown). Similar results were obtained when the same membrane preparations were tested against binding of either RLσ1 or EL-4 targets (data not shown). A concentration of 50 μg of membrane/well, representing a midpoint on the initial linear portion of the inhibition curve (Fig. 1 A), was chosen for use in subsequent experiments.

The binding of neoplastic targets to BCG-activated macrophages is saturable (5). Exposure of BCG-activated macrophages to membrane preparations from the EL-4 targets reduced the number of EL-4 targets bound at saturation but did not prevent saturation of binding (Fig. 1 B). Similar observations were made when binding of RLσ1 targets to BCG-activated macrophages was examined (data not shown).

Selectivity of Membrane Preparations in Inhibition of Binding. Binding of RLσ1 targets
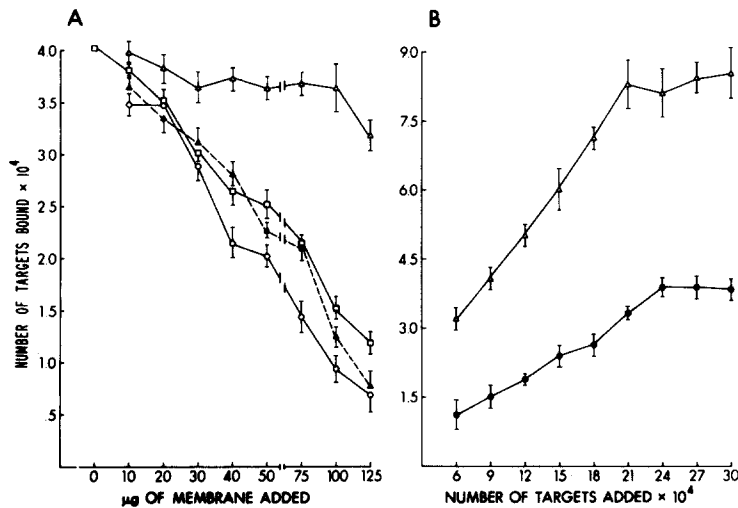
Fig. 1.   (A) Dose-response of inhibitory activity of crude membrane preparations from lymphocyte (△) P815 (□), EL-4 (▲), and RL♂1 (○) targets on the binding of ^{51}Cr-labeled P815 targets to BCG-activated macrophages (2.5 × 10^{6} macrophages/cm^{2}). The membrane preparations of tumor targets or splenic lymphocytes from normal C57BL/6J mice were added to the macrophage cultures over a range of concentrations (10–125 μg/well). After 1 h, the cultures were washed to remove all nonadherent membrane fragments. Control wells, to which no membranes were added, were similarly washed. The cultures were then challenged with 7.5 × 10^{4} ^{51}Cr-labeled P815 tumor targets. The number of P815 targets bound after 1 h of cocultivation was then quantified. Similar data were obtained in an additional experiment. (B) Effect of EL-4 membrane preparation on saturation of binding of ^{51}Cr-labeled EL-4 tumor targets to BCG-activated macrophages. EL-4 membrane preparations (50 μg/well) were added to established cultures of BCG-activated macrophages. After 1 h, the cultures were washed to remove the nonadherent membranes; control wells were similarly washed. The macrophage cultures were then challenged with various numbers of ^{51}Cr EL-4 tumor cells (6.0 × 10^{4}–30.0 × 10^{4}). After 1 h of cocultivation, the number of targets bound to the BCG-activated macrophages were quantified. Binding in EL-4-treated cultures (●) was compared with untreated controls (△). Addition of 50 μg of lymphocyte membranes to similar culture did not result in any inhibition of binding of EL-4 targets to BCG-activated macrophages (data not shown).

to BCG-activated macrophages was inhibited by membrane preparations from the heterologous EL-4 and P815 targets as well as by membrane preparations from the homologous targets (Table I). Likewise, binding of EL-4 and P815 targets was inhibited by membrane preparations from heterologous and homologous targets (Table I). However, membrane preparations of lymphocytes did not inhibit binding of any of the three neoplastic targets to BCG-activated macrophages (Table I). The low level of binding of lymphocytes to BCG-activated macrophages was not inhibited by any of the four membrane preparations, including membrane preparation from lymphocytes (Table I). Finally, the low level binding of RL♂1, EL-4, P815, or lymphocyte targets to TG-elicited macrophages was not significantly inhibited by membrane preparations from any of the four targets (Table I).

Adsorption of Inhibitory Activity.   Target binding to macrophages is selective not only in regard to type of target bound but in regard to type of macrophage that binds the targets (13). To examine the selectivity of macrophages in interacting with membrane preparations, we incubated limiting concentrations of membrane preparations from P815, EL-4, and RL♂1 targets with monolayers of TG-elicited or BCG-activated macrophages for 1 h and then removed the membrane preparations. Inhibitory

TABLE I

Effect of Membrane Preparations on Binding of Homologous and Heterologous Targets by BCG-activated Macrophages

Added to wells*	Number of targets bound to macrophages (percent inhibition)‡			
	Lympho-cytes§	RLσ1	EL-4	P815
			× 10³	
BCG Mφ + medium	5.0 ± 0.6	15.5 ± 1.1	21.5 ± 2.1	28.5 ± 2.5
BCG Mφ + membranes of lymphocytes	6.5 ± 1.1	14.5 ± 0.7 (−6%)	19.5 ± 1.7 (−9%)	28.0 ± 1.2 (−2%)
BCG Mφ + membranes of RLσ1 targets	6.5 ± 0.8	6.5 ± 1.4 (−58%)	12.5 ± 1.1 (−42%)	18.5 ± 1.0 (−35%)
BCG Mφ + membranes of EL-4 targets	5.0 ± 1.3	8.0 ± 1.3 (−48%)	10.5 ± 0.7 (−49%)	18.0 ± 1.5 (−37%)
BCG Mφ + membranes of P815 targets	6.5 ± 0.9	9.5 ± 0.9 (−39%)	14.0 ± 1.4 (−35%)	17.0 ± 1.4 (−40%)
TG Mφ + medium	3.0 ± 0.8	6.0 ± 0.8	7.0 ± 2.0	9.5 ± 1.8
TG Mφ + membranes of lymphocytes	3.0 ± 1.1	5.0 ± 1.0	4.5 ± 1.8	7.5 ± 1.1
TG Mφ + membranes of RLσ1	5.0 ± 1.0	4.0 ± 1.2	3.5 ± 0.9	7.5 ± 0.7
TG Mφ + membranes of EL-4	4.0 ± 1.1	4.0 ± 1.0	4.5 ± 1.0	9.0 ± 1.4
TG Mφ + membranes of P815	4.0 ± 1.2	6.5 ± 1.8	5.5 ± 0.5	8.5 ± 1.1

* Cultures of BCG-activated or TG-elicited macrophages (BCG Mφ and TG Mφ, respectively) (2.5 × 10⁵ macrophages/cm²) were established. Medium or membrane preparation (50 μg/well) from various targets were added to the cultures. After 1 h, the cultures were washed to remove nonadherent membranes and then challenged with 7.5 × 10⁴ ⁵¹Cr-labeled lymphocytes, RLφ1, EL-4, or P815 targets. After 1 h of cocultivation, the number of targets bound to the BCG Mφ was quantified.

‡ Target used for quantification of binding. Number of targets bound in thousands (mean ± SEM). Percent inhibition as compared with medium control. Similar results obtained in four additional experiments.

§ The binding of lymphoblasts (Materials and Methods) to BCG Mφ under these conditions was 5.2 ± 0.5 × 10³ and 7.0 ± 0.6 × 10³ targets bound in two similar experiments (compare to binding of lymphocytes of 5.0 ± 0.6 × 10³ (see above) and 6.5 ± 0.7 × 10³).

activity of membrane preparations from P815, EL-4, and RLσ1 cells was removed by incubation of the membrane preparations with BCG macrophages (Table II). Incubation of membrane preparations with monolayers of TG macrophages, however, removed little of the inhibitory activity (Table II).

Effect of Inhibition of Binding on Cytotoxicity.   To determine the effect of membrane preparations on macrophage-mediated tumor cytotoxicity, we incubated monolayers of BCG-activated macrophages with various membrane preparations for 1 h, removed the excess membranes, and challenged the macrophages with intact targets. Exposure of the BCG-activated macrophages to membrane preparations from RLσ1, EL-4, or P815 targets inhibited subsequent cytolysis of all three of the targets (Table III). Exposure of BCG-macrophages to membrane preparations from lymphocytes did not significantly alter the degree of subsequent cytolysis (Table III).

Effect of Plasma Membranes on Binding.   To ascertain the locus of inhibitory activity in the crude membrane preparations, we examined the effects of preparations enriched ≈15-fold in plasma membranes (Fig. 2). Preparations enriched in plasma membranes from EL-4 targets inhibited binding of homologous EL-4 targets and of heterologous P815 targets more effectively than did crude membrane preparation from the EL-4 targets (i.e., the amount of membrane preparation in μg of protein required to inhibit binding 50% was ≈10-fold greater in the crude membrane preparation than in the plasma membrane preparation when the two were tested against EL-4 targets and ≈8-fold greater when the two were tested against P815 targets).

Discussion

Activated mononuclear phagocytes have been observed to lyse neoplastic targets in preference to their normal counterparts in many laboratories (reviewed in [5, 6, 14, 16]). The basis of selective cytolysis by activated macrophages is still poorly understood

TABLE II

Effect of Adsorption of Membrane Preparations on Monolayers of BCG-activated or TG-elicited Macrophages

Added to wells*	Number of targets bound to macrophages‡			
	Lympho-cytes	EL-4	RLσ1	P815
	$\times 10^3$			
BCG Mφ + medium	4.5 ± 0.5	23.8 ± 2.8	24.3 ± 3.1	27.8 ± 1.8
BCG Mφ + membranes of lymphocytes	5.3 ± 1.2	24.0 ± 2.1	24.3 ± 1.5	27.0 ± 2.1
BCG Mφ + membranes of lymphocytes adsorbed to TG Mφ	NT§	25.3 ± 3.2	25.8 ± 1.0	26.3 ± 2.0
BCG Mφ + membranes of lymphocytes adsorbed to BCG Mφ	NT	25.0 ± 1.8	26.5 ± 1.9	23.5 ± 2.4
BCG Mφ + membranes of EL-4 targets	5.3 ± 0.5	12.0 ± 1.1	8.3 ± 2.1	13.5 ± 1.8
BCG Mφ + membranes of EL-4 targets adsorbed to TG Mφ	NT	12.8 ± 0.9	11.3 ± 2.9	15.0 ± 1.1
BCG Mφ + membranes of EL-4 targets adsorbed to BCG Mφ	NT	21.8 ± 3.1	20.8 ± 1.4	24.0 ± 2.1
BCG Mφ + membranes of RLσ1 targets	5.3 ± 0.7	11.3 ± 0.4	11.3 ± 0.3	16.5 ± 0.5
BCG Mφ + membranes of RLσ1 targets adsorbed to TG Mφ	NT	12.0 ± 0.9	12.8 ± 1.2	19.5 ± 1.2
BCG Mφ + membranes of RLσ1 targets absorbed to BCG Mφ	NT	19.8 ± 1.3	20.3 ± 2.1	25.0 ± 1.4
BCG Mφ + membranes of P815 targets	4.5 ± 0.4	14.3 ± 1.5	10.5 ± 1.2	12.0 ± 1.2
BCG Mφ + membranes of P815 targets absorbed to TG Mφ	NT	13.5 ± 2.0	15.0 ± 0.5	15.0 ± 1.0
BCG Mφ + membranes of P815 targets absorbed to BCG Mφ	NT	20.8 ± 1.2	21.3 ± 1.4	23.5 ± 1.9

* Cultures of purified cultures of BCG-activated or TG-elicited macrophages (BCG Mφ and TG Mφ, respectively) were established at a density of 2.5 × 10⁵ macrophages/cm² as described in Materials and Methods. A limiting amount of membrane preparation (50 μg of membrane) was then added to each well. After 1 h, the macrophage cultures were washed to remove the nonadherent portions of the membrane preparations. These portions were then pooled, pelleted by centrifugation, resuspended in fresh medium, sonicated, and finally added to additional cultures of BCG Mφ as described in Materials and Methods. The resuspensions were performed so that the residual portion from one adsorption well (i.e., the residuum of 50 μg of adsorbed membrane) was added to one well of macrophages. After 1 h, the macrophage cultures were washed and then challenged with 7.5 × 10⁴ ⁵¹Cr-labeled lymphocytes, EL-4, RLσ1, or P815 targets. The number of targets bound to the BCG Mφ was quantified 1 h later.

‡ Targets used for challenge. Number of targets bound in thousands (mean ± SEM). Percent inhibition as compared with medium control. Similar results obtained in two additional experiments.

§ Not tested.

and currently appears to be best correlated with neoplastic transformation of the targets and, specifically, with acquisition of the capacity to form tumors in vivo in compromised hosts (22, 23). Also, activated macrophages move to, cluster about, and interact vigorously with the surfaces of neoplastic targets when observed by cinemicrography (9–11). These observations have led to the speculation that susceptibility to lysis by activated macrophages relates to changes in the surface of neoplastic cells (5, 24).

Table III

Effect of Membrane Preparations on Target Cytolysis by BCG-activated Macrophages*

Added to wells‡	Cytolysis of bound targets (percent inhibiton of cytolysis)§			
	Lym-pho-cytes‖	RLσ1	EL4	P815
BCG Mφ + medium	0	36 ± 1%	35 ± 1%	55 ± 3%
BCG Mφ + membranes of lymphocyte targets	0	34 ± 2% (−6)	37 ± 2% (+6)	54 ± 2% (−2)
BCG Mφ + membranes of RLσ1 targets	3 ± 1%	15 ± 1% (−58)	20 ± 1% (−43)	27 ± .4% (−51)
BCG Mφ + membranes of EL-4 targets	0	8 ± 1% (−78)	18 ± 1% (−49)	30 ± 1% (−45)
BCG Mφ + membranes of P815 targets	3 ± 1%	20 ± 1% (−44)	16 ± 1% (−54)	24 ± 1% (−56)

* The degree of target cytolysis cannot be compared with that previously described (14), because the present experiments were conducted under endotoxin-depleted conditions (see Materials and Methods).

‡ BCG-activated macrophages (BCG Mφ) (2.5 × 10^5 macrophages/cm^2) were incubated with 50 μg of membrane preparations from lymphocytes, RLσ1, El-4, or P815 targets. After 1 h, the nonadherent membranes were removed and the cultures washed. 7.5 × 10^4 labeled targets were added to the cultures. 1 h later, unbound targets were removed. After 18 h, the net cytolysis of the bound targets was determined as described in Materials and Methods.

§ Percent net cytolysis (mean ± SEM). Percent inhibition as compared with medium control. The data on cytolysis in this Table were obtained in the same experiment presented in Table I. Similar results obtained in three additional experiments.

‖ The cytolysis of lymphoblasts by BCG Mφ under these conditions, in two similar experiments, was 1.0% and 0%. The extent of binding of these lymphoblasts is given in Table I, footnote §.

Activated macrophages have recently been found to bind neoplastic target cells with a selectivity that mimics that observed in macrophage-mediated cytolysis (12, 13). Observations from this laboratory showed that activated murine macrophages extensively and selectively bound any of six neoplastic murine targets but not any of three nonneoplastic targets (13). The binding to the surface of the activated macrophages requires living, metabolically active macrophages and the presence of trypsin-sensitive structures on the macrophages (12, 13). Of interest, H-2 differences between murine macrophages and targets were not necessary for such binding and did not prevent it (13). Subsequent observations showed that binding of neoplastic targets to BCG-activated macrophages is an initial and necessary step in cytolysis (14, 15). The binding of neoplastic targets to activated macrophages may thus be a part of the recognition system by which activated macrophages distinguish between neoplastic and nonneoplastic cells for subsequent cytolysis.

The present observations demonstrate that membrane preparations from three disparate murine tumors (i.e., a lymphoma, a leukemia, and a mastocytoma) inhibit binding of the homologous targets to BCG-activated macrophages (Table I). The inhibition produced by membrane preparations was dose-dependent, and >80% of target binding could be abrogated by increasing the amount of membrane preparation added to the macrophages (Fig. 1 A). By contrast, comparable amounts of membrane preparations of lymphocytes did not appreciably inhibit binding of any of the three neoplastic targets to BCG-activated macrophages (Table I). This implies some degree of selectivity in the inhibitory action of membrane preparations, and would appear to exclude trivial explanations of inhibition such as toxicity to the macrophages because of the technique of preparing the membranes or nonspecific inhibition because of the
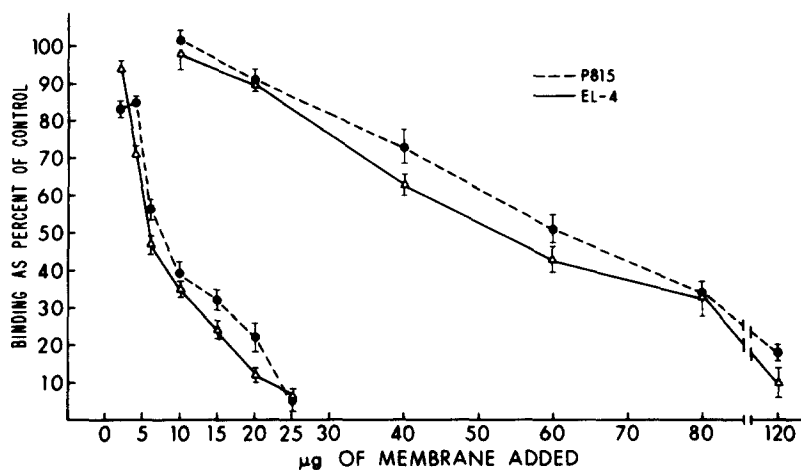
FIG. 2.   Dose-response of inhibitory activity of preparations enriched in plasma membranes from EL-4 targets on binding of P815 and of EL-4 targets to BCG-activated macrophages. Enriched and crude membrane preparations from EL-4 targets were added to the macrophage cultures $(2.5 \times 10^5$ macrophages/cm^2) over a range of concentrations (2-25 μg/well and 10-120 μg/well, respectively). After 1 h, the cultures were washed to remove all nonadherent membrane fragments. Control wells, to which no membranes were added, were similarly washed. The cultures were then challenged with 7.5×10^4 ^{51}Cr-labeled P815 or EL-4 targets. The number of P815 or EL-4 targets bound after 1 h of cocultivation was then quantified. In control wells, $30.8 \pm 2.8 \times 10^3$ and $25.5 \pm 3.1 \times 10^3$ P815 and EL-4 targets, respectively, were bound to the macrophages; data were plotted as a percentage of the binding to those control wells. Binding in cultures treated with plasma membranes is shown by the two responses at the extreme left, whereas binding in cultures treated with crude membrane preparations is shown by the two responses which extend to the extreme right. Similar data were obtained in an additional experiment.

amount of protein added. Of particular interest, membrane preparations from RLσ1, EL-4, P815 targets also inhibited binding of heterologous targets (Table I). The membrane-associated property responsible for inhibition of binding may thus be shared between the RLσ1, EL-4, and P815 tumor cells. Membrane preparations from these three targets also inhibited cytolysis of both the homologous and heterologous targets (Table III). Previous observations have shown that intact P815 and EL-4 targets inhibit subsequent binding and cytolysis of the homologous and heterologous targets (14). This finding is in contrast to observations on the interaction of target membranes or isolated portions thereof with cytolytic T lymphocytes; these preparations have been observed, with one exception, to inhibit lymphocyte-target binding, but not to inhibit subsequent target cytolysis (20, 25-27). The inhibitory activity of the membranes was enhanced $\approx$8 to 10-fold in preparations enriched in plasma membranes (Fig. 2). The data suggest that the binding of three nonadherent murine tumor cells to BCG-activated macrophages is mediated, in part, by plasma membrane-associated recognition structures that are apparently common to the three neoplastic targets examined but not to normal murine lymphocytes and lymphoblasts.

The binding of various cells to macrophages is of two general sorts. The first, a selective and saturable binding of high degree, is observed between activated macrophages and tumor cells (13). This binding has two functional consequences: cytolysis of the bound targets (14) and enhanced secretion of a cytolytic proteinase.[2] The

second, a nonselective and nonsaturable binding of low degree, is observed between lymphocytes and activated macrophages or between tumor cells and resident macrophages, inflammatory macrophages, other tumor cells, and embryonic fibroblasts (13). To date, a functional consequence of this type of binding has not been identified (14). We here report that binding of the first type was inhibited by membranes of tumor cells, whereas binding of the second type was not (Table I). Furthermore, inhibitory activity of membranes from tumor cells could be adsorbed by passage over BCG-activated macrophages, but not over TG-elicited macrophages (Table II). Taken together, these observations raise the possibility that macrophage-target binding, like ligand-receptor binding, may be divided into specific and nonspecific components (28). This possibility is strengthened by the observation that membrane preparations of tumor cells reduced the number of targets required for saturation but did not abrogate saturation (Fig. 1 B).

The basis of binding between activated macrophages and neoplastic targets is clearly open to multiple possible explanations. The molecular constituents of membranes of neoplastic cells differ both quantitatively and qualitatively from their normal counterparts in many regards (reviewed in [29–32]). As just one type of such change, membranes of tumor cells have been reported to differ in the expression of several glycopeptides and of specific glycoproteins (33, 34). The increased lateral mobility of membrane constituents such as receptors and antibody-binding sites in neoplastic cells (29, 31) might further complicate the matter. Furthermore, recognition might not be confined solely to perception of differences in molecular constituents between neoplastic and nonneoplastic membranes; rather, recognition might involve perception of differences in the spatial arrangement of such constituents as well. Our observations suggest that membranes of three murine tumor cells share membrane-associated recognition structures that mediate the binding of those targets to BCG-activated macrophages. This observation and the experimental system described here may provide tools for analyzing further the basis of binding between activated macrophages and neoplastic cells.

Summary

The binding of tumor cells by activated macrophages is an initial and necessary event in the cytolysis of these targets. The data here indicate that membrane preparations from RLσ1 leukemia targets, EL-4 lymphoma targets, and P815 mastocytoma targets each inhibited binding of its homologous target to bacillus Calmette-Guérin (BCG)-activated murine macrophages in a dose-dependent fashion. Similar amounts of membrane from lymphocytes did not alter binding of the three neoplastic targets to BCG-macrophages. Membranes of the three targets also inhibited binding of the heterologous neoplastic targets. Inhibitory activity of membrane preparations from P815, EL-4, and RLσ1 targets could be adsorbed by incubation of limiting concentrations of the membrane preparations with BCG-activated macrophages but not with thioglycollate broth-elicited macrophages. Exposure of BCG macrophages to membrane preparations from RLσ1, EL-4, or P815 targets inhibited subsequent cytolysis of the three targets. Inhibitory activity was increased in preparations enriched for plasma membrane. The data suggest that binding of three murine, nonadherent

neoplastic targets to BCG-activated murine macrophages is mediated, in part, by recognition structures present within the plasma membranes of the three targets.

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