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MECHANISMS OF TUMOR CELL CAPTURE BY ACTIVATED MACROPHAGES: EVIDENCE FOR INVOLVEMENT OF LYMPHOCYTE FUNCTION-ASSOCIATED (LFA)-1 ANTIGEN[1]

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The lymphocyte function-associated (LFA)-1 molecule is expressed on certain populations of macrophages that have an augmented capacity to capture tumor cells. Accordingly, we analyzed the role of LFA-1 in the establishment of such cell-cell interactions. F(ab')$_2$ fragments of the M17/4, anti-LFA-1 monoclonal antibody (MAb) inhibited the interaction between activated macrophages and tumor cells by up to 80% in a dose-dependent manner. The anti-LFA-1 MAb reduced (between 55 to 79%) the number of P815, LSTRA, or EL-4 tumor cells bound to trypsin-sensitive structures on bacillus Calmette Guerin activated macrophages. The inhibition appeared selective, because a F(ab')$_2$ fragment of anti-Mac-1 did not inhibit such binding. Inhibition of tumor cell capture could be observed as soon as 15 min after the onset of the cell-cell interaction between activated macrophages and tumor cells. Optimal inhibition occurred when both tumor targets and macrophages were precoated with the MAb. Although P815, LSTRA, EL-4, and BW5147 tumor cells all expressed LFA-1, only the first three but not BW5147 cells were bound by activated macrophages. Furthermore, endotoxin-pulsed macrophages elicited by thioglycollate broth expressed the LFA-1 antigen but did not exhibit selective tumor cell capture. Finally, anti-LFA-1 inhibited the development of weak into strong binding. Taken together, the results suggest that LFA-1 molecules can participate in the interaction between activated macrophages and neoplastic cells.

Mononuclear phagocytes, when activated by various stimuli, are endowed with the ability to capture and then to destroy tumor cells selectively (1). The general cell-cell interaction that initiates this important host defense response can be divided into two distinct subtypes (2, 3). The first, termed nonselective binding, is a low-affinity, high-capacity binding that cannot be abrogated by trypsin, is mediated by many macrophage populations, and does not lead to cytolysis (2). The second, termed selective binding, is a high-affinity low-capacity binding that requires the presence of trypsin-sensitive surface structures on the macrophages, is mediated only by primed or activated macrophages, and leads to cytolysis (2, 3). Initially, macrophages bind a variety of cells in which binding can rapidly be disrupted by application of <16 dynes force per cell (4). If the targets are neoplastic, this nonselective and weak binding can be converted by primed and activated macrophages over 60 to 90 min at 37°C to binding requiring >200 dynes per cell to disrupt (4).

The lymphocyte function-associated (LFA)[2]-1 antigen is one member of a glycoprotein family that is important to a variety of cellular adherence reactions (5, 6). Specifically, LFA-1 molecules participate in the adhesion between target cells and cytolytic T lymphocytes (CTL) or natural killer (NK) cells, neutrophils and antibody-coated targets, and homotypic reactions between B lymphocytes (7–17). Evidence for this assertion lies in the observation that monoclonal antibodies (MAb) directed against LFA-1 block adhesion between leukocytes and targets. Interestingly, neutrophils from patients deficient in LFA-1 do not adhere well to glass (18). LFA-1 comprises an α-chain of $M_r \sim 180{,}000$ and a β-chain of $M_r \sim 95{,}000$ (5, 6, 19). The Mac-1 antigen, which is the receptor for C3bi and mediates adhesion between monocytes or granulocytes and C3bi-coated particles, shares a common β-chain with LFA-1 (5, 19–21). The α-chain of LFA-1 however, is distinct from the α-chain of Mac-1 (19). Deficiencies in the Mac-1/LFA-1 glycoprotein family are associated with profound defects in host defense mechanisms (15, 17, 19, 22–24).

It has recently been established that LFA-1 is expressed on some macrophages (25) in addition to B and T lymphocytes, neutrophils, and NK cells (5, 6). Specifically, LFA-1 is present on macrophages activated or primed in vivo (25). Macrophage-activating factor (MAF) or interferon-γ (IFN-γ), which induce competence for selective binding of tumor cells (26, 27), induce the expression of LFA-1 on inflammatory macrophages in vitro (28). Because LFA-1 is expressed by populations of macrophages capable of selectively binding target cells, we asked whether LFA-1 could participate in the capture of these targets. We present evidence here that LFA-1 does par-

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[2] Abbreviations used in this paper: LFA, lymphocyte function associated; TG, thioglycollate broth; MAb, monoclonal antibody; RCF, relative centrifugal force.

ticipate in the selective interaction between tumor cells and activated macrophages.

MATERIALS AND METHODS

Mice. Inbred female, specific pathogen-free C57BL/6J mice were obtained from The Trudeau Institute, Inc., Saranac Lake, NY. Mice of 8 to 16 wk of age were used. The animals were kept in the Animal and Laboratory Isolation Facility at Duke University.

Reagents. Bacillus Calmette Guerin (BCG), Phipps strain 1029 was purchased from The Trudeau Institute. Brewer's thioglycollate (TG) broth, prepared according to manufacturer's instructions was obtained from Difco Manufacturing Co., Detroit, MI. Pyran co-polymer (MVE-2) was purchased from Hercules, Inc., Wilmington, DE. Endotoxin (Cat. No. 3121) was obtained from Difco Manufacturing Co. Fetal bovine serum (FBS) was obtained from Sterile Systems, Inc. (Logan, UT; endotoxin <0.019 ng/ml). All reagents contained less than 0.25 ng/ml of endotoxin as quantified by the limulus amoebocyte lysate assay (Cape Cod Associates, Woods Hole, MA). Trypsin (T-0134) was obtained from Sigma Chemical Co., St. Louis, MO.

Tissue culture. All cell lines were maintained in Eagle's minimal essential medium (EMEM; Grand Island Biological Co., Grand Island, NY) supplemented with 2 mM glutamine, 6.25 μg streptomycin/ml, 125 U penicillin/ml, and 10% heat-inactivated FBS. Washes of the macrophage monolayers were performed with Hanks' balanced salt solution (HBSS; Microbiological Associates, Walkersville, MD) supplemented with 5% FBS.

Target cells. The tumor cell lines, the P815 mastocytoma, the EL-4 lymphoma, the BW5147 thymoma, and the LSTRA cell lines were maintained in vitro in 5% CO_2 at 37°C and were passaged twice weekly.

Macrophages. The elicitation, identification, and functional analysis of macrophages in various stages of development have been described in detail (29–31) and are routinely assessed in our laboratory. In brief, inflammatory macrophages were obtained 3 days after i.p. injection of 1 ml of sterile TG. Pyran-elicited macrophages were obtained 5 days after the injection of 100 μg of MVE-2. BCG-activated macrophages were obtained from mice primed with i.d. injection of 75 μg of Ribi cell wall vaccine 3 to 6 wk before use. Ten days before harvest, the mice were given 5×10^7 organisms i.p. and then 10×10^7 organisms 3 days before peritoneal lavage. The peritoneal exudate cells (PEC) were obtained by peritoneal lavage with 10 ml of cold HBSS supplemented with 10 U heparin/ml. The PEC were centrifuged at 250 × G for 5 min, were resuspended in EMEM + 10% FBS. Aliquots of 2×10^5 macrophages in 100 μl of medium were plated in flat bottomed microtiter plates (Limbro 7600605). The plates were incubated at 37°C in 5% CO_2 for 2 hr and the monolayers were washed (×3) to remove nonadherent cells.

Treatment of macrophages in vitro. Macrophage monolayers were incubated at 37°C with 0.15 ml of EMEM + 10% FBS containing LPS for 18 hr. After this exposure period, macrophage monolayers were subjected to radioimmunoassay and were assayed for their ability to bind tumor targets.

Monoclonal antibodies (MAb). The MAb used in this study were described (8, 19, 32). The M17/4 (αLFA-1, IgG2a) MAb was partially purified from culture supernatants by ammonium sulfate (2.2 M) precipitation and was dialyzed extensively against 0.1 M Tris HCl buffer, pH 7.8. M3/38 (αMac-2, IgG2a) was used as culture supernatant. $F(ab')_2$ fragments of M17/5 (αLFA-1, IgG2b) and M1/70 (αMac-1, IgG2b) were prepared by pepsin digestion as described (21).

Radioimmunoassay. This procedure has been described (25, 28). In brief, macrophage monolayers were incubated for 30 min at 4°C with 100 μl/well of 2% normal mouse serum, 8% normal horse serum, and 0.02% sodium azide in phosphate-buffered saline (PBS). The fluid was aspirated, and 25 μl of MAb diluted in PBS containing 8% horse serum and 0.02% sodium azide (referred to as "diluent") were added for 2 hr in the cold. After three washes with 150 μl of the diluent, 30 μl (1×10^5 cpm) of ^{125}I-labeled $F(ab')_2$ of goat anti-rat IgG_{HL} (Cappel Labs, Malvern, PA) were added per well. ^{125}I (Amersham) labeling of antibodies was performed by the chloramine T technique. Specific activities of several batches that were prepared were 2.8 to 4.2 μCi/μg. After 2 hr in the cold, the plates were washed (150 μl × 3) with diluent, and the monolayers were dissolved by the addition of 100 μl of 0.25% sodium dodecyl sulfate (SDS) per well. The content of each well was transferred into a tube and was counted in Packard gamma scintillation spectrometer. Triplicate wells were used per each point tested. Standard error did not exceed 7%.

Trypsinization of macrophage monolayers. Macrophage monolayers were treated with 1 mg/ml trypsin in Earle's balanced salt solution (EBSS) for 20 min at 37°C (2).

Quantification of selective target binding. The binding of tumor targets to macrophages was performed as described (2, 33). Tumor targets were labeled with $Na_2{}^{51}CrO_4$ as described (29). The tumor cells were washed, and 0.1 ml containing 5×10^5 cells/ml were added to each microtiter well. Maximal input of label was determined by the addition of the same amount of cells to wells without macrophages. The cultures were incubated for 1 hr at 37°C in 5% CO_2. Each well was washed vigorously four times with HBSS + 5% FBS. The remaining adherent cells were lysed with 150 μl of 0.25% SDS per well. The content of each well was transferred into a tube and was counted in a Packard gamma scintillation spectrometer. Triplicate wells were used per each point tested. Selective binding was determined by the formula:

$$\text{No. of selectivity bound targets} = (X - Y) \times 5 \times 10^4$$

$$\text{in which } X = \frac{\text{cpm bound to EBSS treated macrophages}}{\text{Total cpm added}}$$

$$\text{and } Y = \frac{\text{cpm bound to trypsinized macrophages}}{\text{Total cpm added}}$$

Inhibition of selective binding by MAb. Unless otherwise stated, macrophage monolayers after treatment with EBSS or EBSS containing trypsin were preincubated for 20 min at room temperature with 50 μl of various doses of $F(ab')_2$ fragments in EMEM + 10% FBS. After this step, targets were added, and binding was quantified as described above. Percent inhibition was calculated by the formula:

$$\% \text{ Inhibition} = \frac{(X - Y) \text{ in medium} - (X - Y) \text{ in antibody}}{(X - Y) \text{ in medium}} \times 100$$

Quantification of strength of binding. To determine the strength of binding, we made use of a modification of the method of McClay et al. (4, 34). In brief, BCG-activated macrophages were prepared in monolayers at 3×10^5 macrophage/cm^2 as described in 6 mm wells. After nonadherent cells were removed by vigorous washing, EMEM + 10% FBS with or without $F(ab')_2$ fragments (444 ng/well) were added at 22°C for 30 min. The P815 targets, previously labeled with $Na_2{}^{51}CrO_4$, were added directly to the wells at 5×10^4 cells/well, and the plates were incubated at 37°C in 5% CO_2 for 90 min. After the binding period, enough medium was added to each well to yield a positive meniscus. The plates were then sealed carefully with adhesive-backed pressure sensitive film (Falcon, Oxnard, CA). The plates were inverted and were centrifuged at the speeds necessary to generate the desired relative centrifugal force (RCF) for 5 min at 4°C. After centrifugation, the plates were quick-frozen in ethanol/dry ice, and the bottom of each well (i.e., the macrophage monolayer with bound targets) was cut off. Radioactivity was determined, and the number of targets bound were calculated by using the following formula:

$$\text{Targets bound} = \frac{\text{cpm remaining on macrophage monolayer}}{\text{total added cpm}} \times (5 \times 10^4)$$

Each group was performed with four replicates. All results are representative of three separate experiments.

RESULTS

Dose, specificity, macrophage, and time requirements for inhibition of tumor cell capture by anti-LFA-1. To avoid any participation of the Fc portions of Ig in the cellular interactions under scrutiny, $F(ab')_2$ fragments of the M17/5 anti-LFA-1 MAb were prepared (21). These $F(ab')_2$ fragments consistently inhibited the number of P815 tumor cells bound to BCG-activated macrophages in a dose-dependent manner (Figs. 1 and 2). To conserve the $F(ab')_2$, we used the optimal inhibiting concentration (i.e., 444 ng/well) in most subsequent experiments. The inhibitory activity of anti LFA-1 appeared selective, because $F(ab')_2$ fragments of the M1/70 MAb directed at Mac-1 did not inhibit binding (Fig. 2).

To assess the type of binding inhibited, we examined selective and nonselective binding of tumor cells by primed and activated macrophages. The inhibition of total binding was principally due to decreases in selective binding, although small decrements in nonselective bind-

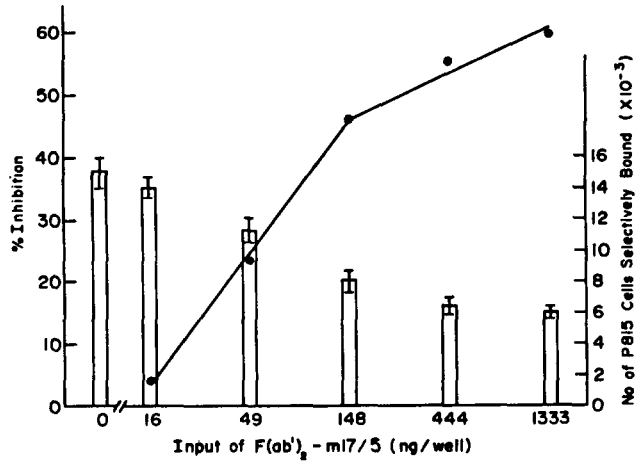

Figure 1. Dose response course of targets binding inhibition. BCG-activated macrophages (2×10^5) treated or untreated with trypsin were incubated with various doses $F(ab')_2$ of anti LFA-1 MAb for 20 min. Target cells (P815) were added, and binding was measured after a 1-hr incubation. The results are expressed as number of targets selectively bound to macrophages (*bars*) and as percent inhibition of binding (*graph*) (see *Materials and Methods*). This experiment was repeated twice, and similar results were obtained.

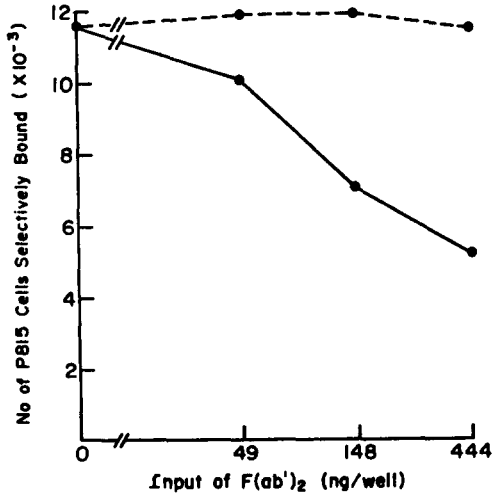

Figure 2. Anti-Mac-1 $F(ab')_2$ fragments do not inhibit selective target capture. BCG macrophages (2×10^5) were treated with 50 μl of various doses of m 1/70 (α-Mac-1 MAb) $F(ab')_2$ fragments (*dashed line*) or with various doses of m17/5 (αLFA-1 MAb) $F(ab')_2$ fragments (*solid line*). The results are expressed as the number of P815 targets selectively bound (see *Materials and Methods*). This experiment was repeated twice, and similar results were obtained.

ing were also observed (Table I). When we examined target binding to inflammatory macrophages elicited by TG broth, which binds targets principally in the nonselective manner (2), their overall binding capacity was slightly reduced by $F(ab')_2$ fragments of M17/4, (i.e., inhibition of total binding by 2 to 4%) (see Fig. 6 below). We next analyzed the kinetics of inhibition. Reduced capture of tumor cells was observed within 15 min after mixing of the cells (Fig. 3). The extent of inhibition then rose progressively until binding was completely established at ~60 min of incubation (Fig. 3).

Inhibition of binding of various tumor lines. We next wished to determine whether binding of other tumor cells could be inhibited. $F(ab')_2$ fragments of M17/4 blocked capture of EL-4 and of LSTRA tumor cells significantly (e.g., 66% and 79% inhibition, respectively) (Fig. 4). $F(ab')_2$ fragments of the M1/70 did not significantly affect binding of any of the three targets (see legend, Fig. 4).

Localization of cellular site of inhibition. In the experiments described above, binding was assessed after preincubation of the macrophages for 20 min with MAb, followed by addition of targets without removal of unbound antibody. To determine whether inhibition required precoating of LFA-1 on targets, as well as on macrophages, the macrophages or targets were treated separately with antibody, were washed extensively, and were then mixed. Effective inhibition of binding by anti-LFA-1 could be obtained only when both targets and activated macrophages were treated with $F(ab')_2$ fragments of αLFA-1 (Table II).

Relationship between expression of LFA-1 on tumor cells and macrophages and their potential for macrophage-target interaction. Various tumor lines were then analyzed for their expression of LFA-1 and the ability to be captured by activated macrophages. Tumor cells of the P815, BW5147, EL-4, and LSTRA lines expressed comparable levels of LFA-1 (Fig. 5*A*). A MAb of the same isotype as the M17/4 (e.g., the M3/38 αMac-2 MAb) failed to bind to tumor cells significantly but could bind to TG-elicited macrophages (data not shown). When we examined binding of these targets, the BW5147 cells were not efficiently captured by BCG-activated macrophages as compared with P815 EL-4, and LSTRA targets (Fig. 5*B*). The BW5147 could not be effectively lysed by the same macrophages (data not shown).

TG-elicited macrophages can be induced to express the LFA-1 antigen after overnight incubation with endotoxin (28), conditions that do not lead to induction of competence for selective binding (1). When we analyzed the ability of such macrophages to bind tumor cells, either 2 ng/ml or 10 ng/ml of lipopolysaccharide (LPS) induced LFA-1 expression on TG-elicited macrophages but not augmented capacity for P815 capture (Fig. 6). The low level of trypsin-sensitive binding mediated by LPS-treated TG macrophages could not be significantly inhibited by $F(ab')_2$ fragments of the M17/5 MAb (Fig. 6). In the same experiment, the interaction mediated by BCG macrophages was inhibited by 52% by the anti-LFA-1 MAb.

Effect of anti-LFA-1 $F(ab')_2$ fragments on development of strong target binding. Previous experiments have demonstrated that the number of tumor targets bound by BCG-activated macrophages over a range of applied RCF yields a predictable pattern (4). Basically, the great majority of added targets (i.e., 90%) remain bound to the macrophage monolayer from 1 to 100 × G of applied RCF. The number of targets then gradually declines with increasing RCF until only 20 to 35% of the targets remain bound at >1200 × G of RCF. Targets remaining bound at RCF less than 100 × G includes targets that are strongly bound. Thus weak binding may be calculated by subtracting the number of strongly bound targets from the total number of targets bound at 100 × G (4). Additional experiments have demonstrated that weakly bound targets can be converted to strongly bound if the targets are tumor cells and if the macrophages are activated (4).

When we preincubated macrophages with the M17/4 anti-LFA-1 MAb and then added P815 tumor cells, the

TABLE I

Inhibition of selective binding by primed and activated macrophages

Macrophage population[a]	Treatment[b]	Total No. of Targets Bound ($\times 10^{-3}$)[c] ± SD	No. of Targets Nonselectively Bound ($\times 10^{-3}$)[d] ± SD	Contribution to Inhibition[e]	No. of Targets Selectively Bound ($\times 10^{-3}$)[f]	Contribution to Inhibition[e]
Pyran-primed macrophages	Medium only	12.11 ± 1.1	3.94 ± 0.3	21%	8.17	79%
	M17/5	5.70 ± 0.8	2.59 ± 0.5		3.11	
BCG-activated macrophages	Medium only	15.73 ± 1.2	3.50 ± 0.4	13%	12.23	87%
	M17/5	7.12 ± 0.65	2.35 ± 0.5		4.72	

[a] Pyran-primed or BCG-activated macrophages (2×10^5) were plated per well.
[b] Macrophage monolayers were pretreated with 50 μl/well of medium or 444 ng/well of F(ab')$_2$ M17/5 (αLFA-1).
[c] Data are given as the total amount of P815 targets bound per culture.
[d] Data are given as the amount of P815 targets bound to trypsinized macrophages (see *Materials and Methods*).
[e] Percentage of the total degree of inhibition when decrease in total number of targets bound is 100%.
[f] The results are given as the difference between the number of targets bound to trypsinized macrophages and the total targets bound (see *Materials and Methods*).
[g] Similar data obtained in two experiments.

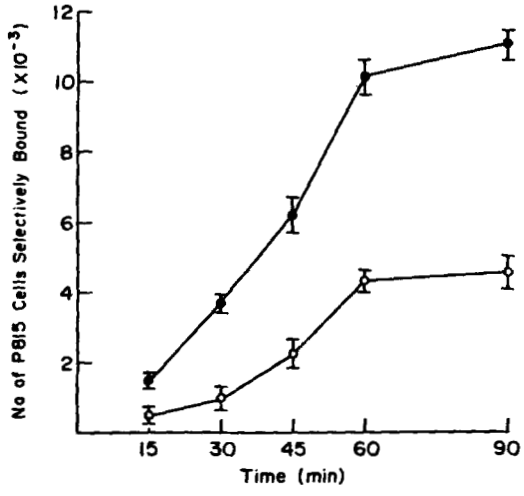

Figure 3. Time requirements for inhibition of tumor binding. BCG macrophages (2×10^5) treated with medium (*closed symbols*) or 444 ng/well of anti LFA-1 F(ab')$_2$ fragments (*open symbols*). Radiolabeled P815 cells were added, and the plates were incubated for various times. The results are expressed as number of P815 targets selectively bound. This experiment was done once.

TABLE II

Inhibition requires coating of both targets and activated macrophages with anti-LFA-1 MAb

Group	F(ab')$_2$ Added (ng/well)	Pretreatment of Macrophages	Pretreatment of P815	Number of Selectively Bound Targets ($\times 10^{-3}$)	Percent Inhibition
1	—	—	—	13.7	—
2[b]	444	—	—	6.6	52
3[b]	148	—	—	8.4	39
4[c]	—	444	—	12.7	7
5[c]	—	148	—	13.1	4
6[d]	—	—	125	13.6	1
7[e]	—	444	125	8.2	40
8[e]	—	148	125	9.8	28

[a] BCG-activated macrophages (2×10^5) were treated with F(ab')$_2$ of anti LFA-1 MAb and selective binding radiolabeled P815 cells was determined (See *Materials and Methods*).
[b] Macrophages were treated with 50 μl containing 444 ng or 148 ng antibody for 20 min room temperature. Antibody excess was not removed.
[c] Macrophages were treated as in group C. Antibody was aspirated, and the cultures were washed three times with medium.
[d] Radiolabeled P815 cells (4×10^6) were treated with 10 μg of F(ab')$_2$ fragments (in Table: 125/ng/5 $\times 10^4$ targets added per well) for 20 min on ice, were washed three times, and were added to untreated macrophages.
[e] Targets were treated as in group D and were added to macrophages treated as in group C.
[f] Groups 1 through 5 were repeated four times, and groups 6 through 8 were repeated twice. Similar results were obtained in these additional experiments.

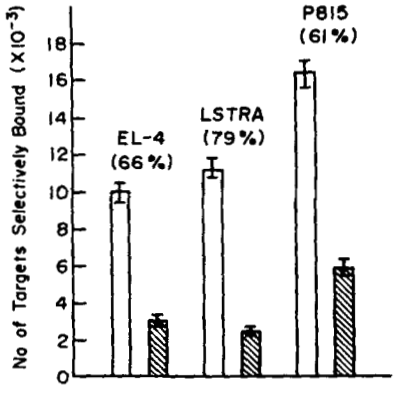

Figure 4. Anti-LFA-1 F(ab')$_2$ fragments inhibit binding of various targets. BCG macrophages (2×10^5) were treated with 50 μl of medium or 444 ng/well of F(ab')$_2$ fragments and target cells (EL-4, LSTRA, and P815) were added for 1 hr. *Closed bars* represent the number of targets selectively bound in the presence of F(ab')$_2$ and *open bars* represent the number of targets selectively bound in presence of medium. *Number in brackets* show percent inhibition. In such experiments, F(ab')$_2$ fragments of M1/70 inhibited binding of EL-4 targets 6 to 8%, binding of LSTRA targets 8 to 10%, and binding of P815 targets 6 to 7%. Similar results were obtained in four experiments.

estimated number of weakly bound targets was not significantly different between treated and untreated groups (Table III). The number of strongly bound targets (i.e., targets remaining adherent at 1300 × G) was significantly inhibited (Table III). The overall number of targets bound at 100 × G, however, decreased; this decrement could be attributed to the loss in the number of targets that were strongly bound (Table III).

DISCUSSION

We present evidence here that LFA-1 molecules participate in the cell-cell interaction between activated macrophages and neoplastic targets. F(ab')$_2$ fragments of the M17/5 anti-LFA-1 MAb reduced the number of three neoplastic cells that were captured by BCG-activated or by pyran-primed macrophages (Table I and Fig. 4). The extent of inhibition, which varied from 55 to 79%, depended upon the dose of antibody (Fig. 1) and the target that was used (Fig. 3). The inhibition appeared to be selective, because F(ab')$_2$ fragments of an isotype-matched MAb (the M3/38 anti Mac-1 MAb) did not block

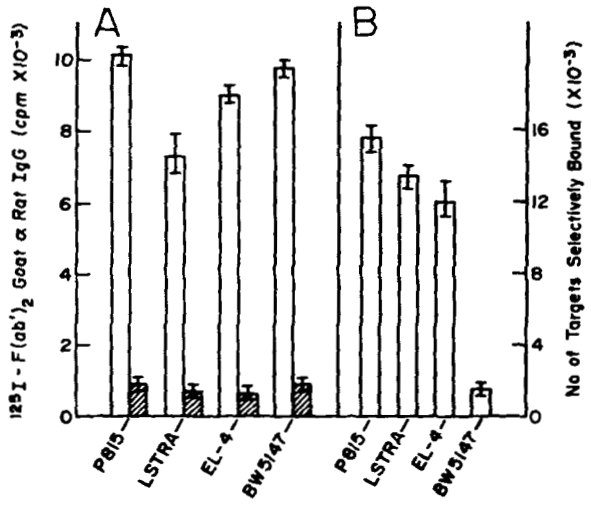

Figure 5. BW5147 tumor cells express LFA-1 but cannot be captured by activated macrophages. In *Panel A*, tumor targets (1×10^6) were incubated on ice with 1/500 dilution of M17/4 (LFA-1) MAb or 1/50 dilution of M3/38 (αMac-2), were washed, and were subjected to ^{125}I-F(ab')$_2$ of goat anti-rat IgG. The results expressed are cpm bound/1×10^6 tumor cells. In *Panel B*, 2×10^5 BCG macrophages were incubated with 5×10^4 tumor cells. The results are expressed as number of targets selectively bound. This experiment was repeated three times, and similar results were observed.

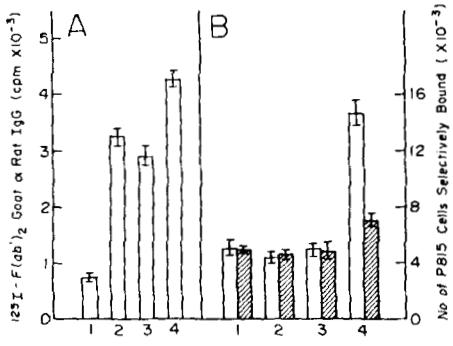

Figure 6. LPS-stimulated TG macrophages express LFA-1 but do not exhibit augmented capture of targets. In *Panel A*, 2×10^5 TG macrophages incubated for 18 hr with medium (*Column 1*), LPS 2 ng/ml (*Column 2*), LPS 10 ng/ml (*Column 3*), and BCG-activated macrophages (*Column 4*), were subjected to radioimmunoassay. The results expressed are cpm bound/2×10^5 macrophages by using 1/500 dilution of M17/4 (αLFA-1). *Panel B* shows equivalent cultures subjected to P815 cells binding after treatment with medium (*open bars*) or 444 ng/well of F(ab')$_2$ of anti-LFA-1 (*closed bars*). This experiment was repeated twice with similar results.

tumor binding (Fig. 2). Selective rather than nonselective binding was inhibited (Table I and Fig. 6*B*). The presence of LFA-1 on both the tumor cells and activated macrophages appeared to be important in the interaction (Table II). This conclusion does not imply, however, that the interaction of LFA-1 molecules is necessarily homologous (i.e., LFA-1 binding to LFA-1); the heterologous binding of LFA-1 to other molecules on both macrophages and tumor cells could also participate.

The mere presence of LFA-1 on macrophages or tumor cells does not appear sufficient to initiate selective binding. The BW5147 tumor cells, which express levels of LFA-1 simlar to those expressed by other tumor cells (e.g., P815, LSTRA, and EL-4) (Fig. 5*A*), are not captured (Fig. 5*B*) or lysed (data not shown) by BCG-activated macrophages. Conversely, inflammatory macrophages induced to express LFA-1 antigen by culture with LPS (28) do not acquire augmented capacity for selective capture of tumor cells (29, 30). These results suggest that the presence of LFA-1 molecules on macrophages or tumor cells is in itself not sufficient to produce competence for extensive capture of tumor cells by macrophages. At present, we have no evidence as to whether LFA-1 is necessary for the establishment of selective binding. We are unaware of the existence of either LFA-1 negative macrophage populations endowed with selective-binding capacity or of LFA-1 negative, nonadherent tumor cells that can be selectively bound to activated macrophages, although we have searched extensively for such cells.

LFA-1 has been suggested to participate in the establishment of firm cell-cell adherence between various leukocytes and targets once recognition has occurred (7, 9–12, 15). Many cell pairs involving macrophages, including macrophages that do not express surface LFA-1 (25, 28), establish weak cell-cell attachments (4). When a specific recognition system is put into play, the weak binding can be actively converted into strong (4, 34); such weak and strong binding can be readily distinguished from one another by quantifying the strength of cell-cell attachments (4). We have shown that MAb directed against LFA-1 do not block the establishment of either nonselective binding (Table I) or weak binding (Table III). We additionally show that the M17/5 MAb does inhibit the development of selective binding (Table I) and of strong binding (Table III). Finally, certain macrophages that express LFA-1 can establish only weak, nonselective binding (see Figure 6) (2, 4, 33). Taken together, these data suggest that LFA-1 molecules may participate in the conversion of weak, nonselective binding into strong, selective binding by macrophages.

The precise molecule to molecule interactions that occur in this and in other systems of cell-cell attachment remain to be established (35). For example, one direct route of examining this question (i.e., specific reconstitution LFA-1 negative cells of cells with LFA-1) awaits molecular cloning of the LFA-1 molecule. By use of MAb, LFA-1 has been postulated to stabilize binding after rec-

TABLE III

Effect of F(ab')$_2$ fragments of anti-LFA-1 on the development of strong tumor cell binding by activated macrophages[a]

	Targets Bound (× 10^{-3}) ± SD		Percent Inhibition
	Medium	M17/5	
Total number of targets bound at 100 × G of RCF	39.3 ± 1.7	31.7 ± 1.5	19%
Total number of targets bound at 1300 × G of RCF (number of targets strongly bound)	12.0 ± 1.3	6.5 ± 0.9	46%
Estimated number of targets weakly bound	27.3	25.2	9%

[a] Monolayers of BCG-activated macrophages were prepared and were treated with 444 ng/well M17/5 F(ab')$_2$ as before. The details of the reverse centrifugation assay are described in *Materials and Methods*. Each experimental data point was obtained with four replicates per determination. The results shown are representative of three separate experiments.

ognition of other structures by the T cell receptor (7, 9–11). The presence of LFA-1 on B cells, T cells, myeloid cells, NK cells (6), and primed/activated macrophages (25, 28) suggests that this molecule may well play a general role in adhesion reactions. LFA-1 has been found to function in T cell-dependent kill, natural killing, and antibody-dependent cellular cytotoxicity by neutrophils (11, 15). We present evidence in this report that LFA-1 plays a role in the adhesion between activated macrophages and tumor cells. The data presented here furthermore are consistent with the possibility that LFA-1 may function as a stabilizing molecule after an initial recognition step has taken place. In any case, LFA-1 now appears to participate in one important cell-cell interaction of activated macrophages; it will be of interest to determine whether this molecule participates in other recognition functions mediated by activated macrophages as well.

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