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SHORT COMMUNICATIONS

Antigens Associated with the Activation of Murine Mononuclear Phagocytes *in Vivo:* Differential Expression of Lymphocyte Function-Associated Antigen in the Several Stages of Development[1]

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Two well-characterized antigens [Mac-1 and lymphocyte-function-associated antigen (LFA-1)], expressed on a variety of leukocytes, are members of a family of surface proteins associated with multiple recognition functions. To analyze expression of these proteins during macrophage development, we utilized both radioimmunoassay and flow cytometry. As previously reported, Mac-1 is expressed on murine macrophages in all stages of development. We found LFA-1 to be present on murine mononuclear phagocytes but only in certain stages of their development. Specifically, we found LFA-1 was expressed on murine tissue macrophages but only on those activated *in vivo* by bacillus Calmett Guerin (BCG) or, to a lesser extent, primed by pyran copolymer. Although LFA-1 was absent on inflammatory (responsive) and resident tissue macrophages it was also present on blood-borne monocytes. Activated macrophages also selectively expressed the H-11 and Ly-6 antigens. Thus, these data indicate that LFA-1 is selectively expressed on mononuclear phagocytes of the tissues but only on those in the primed and activated stages of development.

INTRODUCTION

The functional development of mononuclear phagocytes is accompanied by increased competence for cell–cell interactions such as antimicrobial function, antibody-dependent cellular cytotoxicity, tumor cell binding, and direct cytolysis of tumor cells [for review, see (1)]. Competence for these functions is generally acquired in a cascade which can be divided into four operationally distinct stages, i.e., resident, responsive, primed, and activated macrophage (2). Thus, macrophages elicited by sterile inflammation (*responsive macrophages*), though not resident peritoneal macrophages, can respond to lymphokines by gaining further capability to interact with endotoxin. These latter macrophages, which have been termed

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primed macrophages, can bind tumor cells and also become cytotoxically competent upon interaction with trace amounts of endotoxin (*activated macrophages*) (1, 2). Alternatively, activated or primed macrophage can be obtained *in vivo* following injection of bacillus Calmett Guerin (BCG)[3] or pyran copolymer, respectively.

Recently, a novel family of leukocyte-differentiation antigens, detected by monoclonal antibodies (MAb) and sharing common functional and structural properties, has been described (3). The Mac-1 protein, detected on macrophages, granulocytes, and natural killer cells but not on lymphocytes, comprises two chains: α of 170,000 M_r and β of 95,000 M_r (4, 5). Mac-1 is the type 3 complement receptor (CR3) on macrophages (6). The lymphocyte-function-associated antigen (LFA)-1, present on B cells, T cells, and myeloid cells, is involved in the adhesion of cytotoxic T cells (CTL) to targets [for review see (3)]. LFA-1 comprises a unique α chain of 180,000 M_r and a β chain identical to that of Mac-1 (7, 8). Deficiencies in this family of proteins have profound effects on host defense (9, 10).

We undertook the present study in order to determine whether LFA-1 is expressed by tissue macrophages and, if so, whether the expression and intensity of LFA-1 antigen, as well as other antigens recognized by MAb, varied between macrophages in different stages of activation. We here report evidence that LFA-1 is expressed on tissue macrophages activated or primed *in vivo* but is absent or low on resident peritoneal or inflammatory macrophages. The expression of Mac-1 antigen is conserved through all these stages. Thus, LFA-1 is selectively expressed by tissue macrophages which are primed or activated for lytic competence.

MATERIALS AND METHODS

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

Reagents. BCG, Phipps strain 1029, was purchased from The Trudeau Institute. Pyran copolymer (MVE-2) was purchased from Hercules Inc., Wilmington, Delaware. Protease peptone (PP) broth and Brewer's thioglycollate (TG) broth, prepared according to manufacturer's instructions, were obtained from Difco Manufacturing Co., Detroit, Michigan.

Macrophages. The elicitation, identification, and functional analysis of macrophages in all four stages of development have now been described in detail (1) and routinely assessed in our laboratory. In brief, inflammatory macrophages were obtained 3 days after ip injection of 1 ml of sterile TG or PP. BCG-activated macrophages (11) were obtained from mice primed with id injection of 75 μg of Ribi cell wall vaccine 3 to 6 weeks before use. Ten days before harvest the mice were given 5×10^7 organisms ip and then 10×10^7 organisms 3 days before peritoneal lavage. Pyran-elicited macrophages were obtained 5 days after injection of 100 μg of MVE-2. Resident macrophages were obtained by peritoneal lavage of untreated animals.

[3] Abbreviations used: BCG, bacillus Calmett Guerin; macrophage, murine peritoneal macrophages; CTL, cytotoxic T lymphocyte; MAb, monoclonal antibodies; LFA, lymphocyte function associated; TG, thioglycollate; PEC, peritoneal exudate cells; PP, proteose peptone; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline.

The peritoneal exudate cells (PEC) were obtained by peritoneal lavage with 10 ml of cold Hanks' balanced salt solution supplemented with 10 units of heparin/ml. The PEC were centrifuged at 250*g* for 5 min, resuspended in Eagle minimum essential medium + 10% fetal bovine serum (Sterile Systems, Inc., Salt Lake City, Utah). Aliquots of 2×10^5 macrophages in 100 μl of medium were plated in flat-bottom 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. We routinely found the cell monolayers to contain >95% macrophages as determined by morphology by Wright's stain or a histochemical assay for nonspecific esterase.

Monocytes. Monocytes were obtained by a modification of the technique described by Pawlowski *et al.* (12). In brief, mice were injected with 50 units of heparin 30 min before use and were bled to death into 50-ml polypropylene tubes. Cells were pelleted, washed twice with $Ca^{2+}Mg^{2+}$-free phosphate-buffered saline (CMF-PBS). The cells were resuspended in CMF-PBS containing 0.3 m*M* EDTA and carefully layered into tubes containing lymphocyte separation medium (Bionetics) according to the manufacturer's instructions and centrifuged at 800*g* for 35 min at 20°C. The mononuclear cell fraction was removed from the gradient and washed 2× with CMF-PBS. To remove platelets adherent to monocytes, mononuclear cells were resuspended in FBS containing 5 m*M* EDTA and incubated for 15 min at 37°C. Following two more washes, mononuclear cells were stained and subjected to flow cytometry.

Monoclonal antibodies. The characteristics of the MAb used in this paper are summarized in Table I. M17/4 (αLFA-1) MAb was partially purified from culture supernatant by ammonium sulfate (2.2 *M*) precipitation and dialyzed extensively against 0.1 *M* Tris–HCl buffer, pH 7.8. All other MAb were used as culture supernatants.

Radioimmunoassay. Monolayers of macrophages 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 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") was added for 2 hr in the cold. Following two washes with 150 μl of cold diluent, 25 μl (1.5×10^5 cpm) of ^{125}I-labeled $F(ab')_2$ of goat anti-rat IgG_{HL} (Cappel Labs, Malvern, Pa.) was added per well. ^{125}I (Amersham) labeling of antibodies was performed by the chloramine-T technique. Specific activity of several batches

TABLE 1

MAb Used in this Paper

Antibody	Subclass	Antigen[a]	Refs.
M 1/70. 15.1	IgG_{2b}	Mac-1 (170, 95)	(3, 4)
M 3/38. 1.2	IgG_{2a}	Mac-2 (32)	(12)
M 5/114	IgG_{2b}	$I\text{-}A^b$ (35, 28)	(3)
M 17/4.4	IgG_{2a}	LFA-1 (180, 95)	(3, 5)
M 5/106	IgM	Ly-6 (?)	(16)
30 H-11	IgG_{2b}	H-11 (?)	(17)

[a] M_r of polypeptide chain(s) $\times 10^{-3}$.

prepared was 2.8–4.2 μCi/μg. Following 2 hr in the cold, the plates were washed (150 μl $\times$ 3) and the monolayers were dissolved by the addition of 100 μl of 0.25% sodium dodecyl sulfate per well. The content of each well was transferred into a tube and counted in a Packard gamma scintilation spectrometer. Triplicate wells were used per each point tested; standard error of the mean for the triplicates did not exceed $\pm$7%.

To determine the saturating amount of ^{125}I-F(ab')$_2$ of goat anti-rat Ig, various doses of this reagent ranging from 1.25×10^4 to 4×10^5 cpm were added either to TG macrophages coated with maximum amounts of Mac-1 (i.e., a dilution of 1:10) or to BCG macrophages coated with a minimal dilution of anti-LFA-1 (i.e., a dilution of 1:100). Full saturation of antibody-coated macrophages could be achieved using $\geq 1 \times 10^5$ cpm/well, even when the macrophages were coated with saturating concentrations of MAb directed against an antigen which is abundantly expressed on macrophages (i.e., Mac-1). Specifically, the amount of radioactivity bound to MAb-coated macrophages did not increase further over a fourfold range of the radioactive reagent (i.e., from 1×10^5 to 4×10^5 cpm). Thus, we routinely used 1.5×10^5 cpm/well of the secondary antibody in the radioimmunoassay.

To reduce nonspecific binding of MAb, we used a solution composed of 2% normal mouse serum and 8% normal horse serum for preincubation of macrophages prior to the application of MAb. This procedure resulted in binding of 100 to 400 cpm when 2% normal rat serum was used as a negative control. The radioimmunoassay utilized here does not measure binding via Fc receptors. No correlation between expression of a certain antigen and MAb isotype could be made (see Fig. 1 for example).

Flow cytometry. Mononuclear cells and BCG-activated, pyran copolymer-primed, or TG-induced PEC were washed twice and 1×10^6 cells were placed into plastic tubes. Diluted MAb [100 μl (1:100 for LFA-1 and 1:10 for all the other MAb)] were added for 30 min on ice and then washed three times with cold diluent. Control groups received P3 $\times$ 63 Ag8653 myeloma cells supernatant. A saturating amount of fluorescein isothiocyanate (FITC)-conjugated F(ab')$_2$ of goat anti-rat IgG (Cappel Labs) was then added for 30 min on ice (100 μl of 1:15 dilution). Following three washes in cold diluent the labeled cells were filtered to remove clumps and subjected to flow cytometry analysis. Samples were analyzed by a System 50 cytofluorograph of Ortho Diagnostic Systems. The instrument was operated in dual-beam mode with axial light loss measurements as an indication of cell size coming from the 514-nm line and 90′ scatter from the 488-nm line. All data analysis was carried out on an Ortho Model 2150 computer system. Appropriate windows for fluorescent gating were employed to distinguish each major population present in the PEC, including lymphocytes, granulocytes, small inflammatory macrophages, and a continuum of larger macrophages (S. Haskill, unpublished observation). FITC fluorescent values were simultaneously determined from the 488-nm beam for each of the cell populations as required.

RESULTS

Expression of LFA-1 Assessed by Radioimmunoassay

To assess the expression of antigens detected by MAb, we utilized a solid-phase radioimmunoassay on different populations of macrophages. Pyran copolymer-

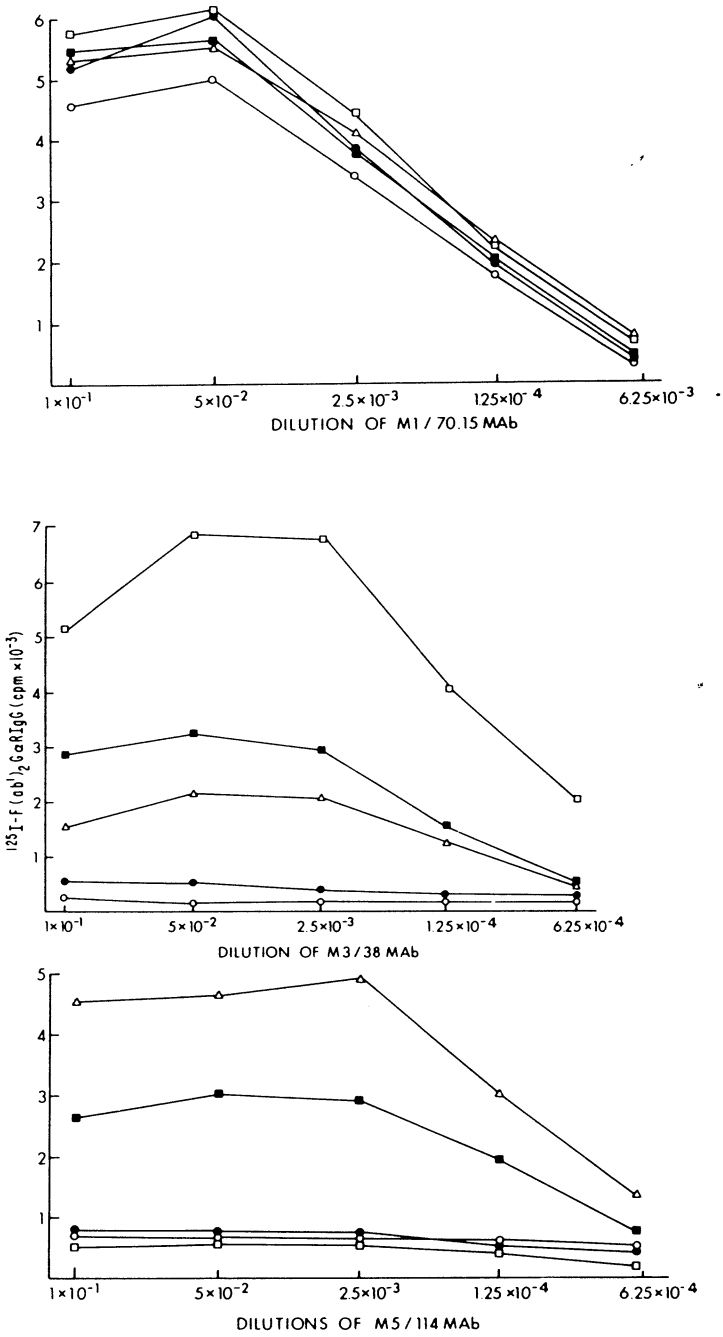

FIG. 1. Macrophages (2×10^5/well) derived from BCG-activated (△), Pyran-primed (■), TG-induced (□), PP-induced (○), and resident (●) mice were plated. Various dilutions of M1/70 (αMac-1) MAb (upper) were applied on the cells followed by the addition of ^{125}I-F(ab')$_2$ GαRat IgG (see Materials and Methods). Results are expressed as the mean of cpm of radioactive reagent bound to macrophages in triplicate wells. This experiment was repeated twice. (Middle) MAb used was αMAC-2 (M3/38). This experiment was repeated four times. (Lower) MAb used was αI-A^b (M5/114). This experiment was repeated three times with similar results.

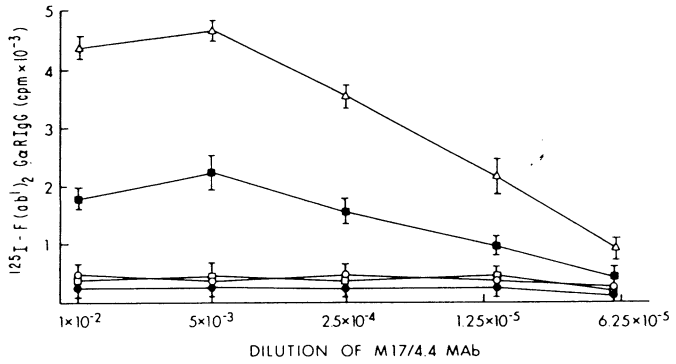

FIG. 2. The same as in Fig. 1. MAb used was M17/4 αLFA-1. This experiment was repeated eight times with similar results.

induced and BCG-elicited macrophages were used to represent primed and activated macrophages, respectively; TG- and PP-induced macrophages were used as inflammatory (responsive) macrophages.

To test whether our experimental system yielded data compatible with previous reports, we first examined the expression of Mac-1, Mac-2, and I-A on various populations of macrophages. No significant changes in the levels of Mac-1 expression could be observed on different macrophage populations, indicating that Mac-1 expression is conserved throughout the macrophage activation cascade (Fig. 1, upper). Mac-2 antigen is not present on resident or PP-elicited macrophages but is highly expressed on TG-elicited macrophages (Fig. 1, middle), in accordance with previous results (13). In one out of four experiments, PP-elicited macrophages did have some reactivity with the Mac-2 MAb (i.e., 1500 cpm bound at 1:10 dilution; data not shown). Relative to TG-elicited macrophages, Mac-2 was expressed in lower amounts on pyran-primed macrophages and BCG-activated macrophages (Fig. 1, middle). I-A molecules were extensively expressed on primed and activated macrophages, but not on inflammatory or resident macrophages (Fig. 1, lower), which data are in agreement with previous studies (14, 15).

When we experienced expression of LFA-1, the antigen was detected at high levels of BCG-activated macrophages and to a lesser extent on pyran-primed macrophages (Fig. 2). Resident, PP-induced, and TG-induced macrophages did not express this antigen (Fig. 2). These data suggest that activated macrophages (and to a lesser degree primed macrophages) are LFA-1$^+$. As noted above, other markers such as Mac-1 and Mac-2 did not distinguish between activated or primed macrophages and other type of macrophage populations. Two other MAb were found to distinguish activated from inflammatory macrophages. MAb directed at Ly-6 and H-11 showed reactivity with BCG-activated but not TG-induced macrophages (Fig. 3).

Flow Cytometry of Macrophage Populations Developing in Vivo

To verify the results obtained utilizing radioimmunoassay, flow cytometric analysis was performed. The macrophages were labeled with saturating amounts of MAb followed by FITC-conjugated F(ab')$_2$ anti-rat IgG and then analyzed by flow

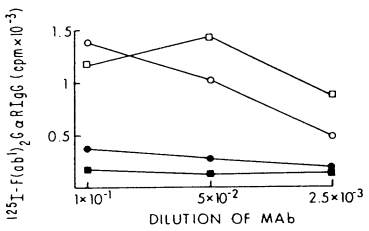

FIG. 3. Various dilutions of αLy-6 MAb were assayed on BCG (□) and TG (■) macrophage. Also shown is the reactivity of H-11 MAb with BCG (○) and TG (●) macrophage. This experiment was repeated twice with similar results.

cytometry. By this technique, we verified the previous observations. Specifically, the amount of LFA-1 expression on activated and primed macrophages was far greater than inflammatory macrophages (Table 2). Of note, the percentage of LFA-1-positive macrophages was greater in the population of BCG-activated cells than in the population of pyran-primed cells (Table 2). Of further note, 29% of either large or small TG macrophages expressed low levels of fluorescence for I-A (mean fluorescence channel 22). These results indicate that flow cytometry may be more sensitive than the radioimmunoassay used here, since utilizing the latter technique we found that TG macrophages appeared to be negative for expression of I-A (Fig. 1). Granulocytes and lymphocytes within the BCG-elicited PEC were also positive for LFA-1, but had a lower fluorescent intensity (mean fluorescent channels 37 and 65, respectively; data not shown).

Since labeling of cells was done under saturating conditions for all reagents, the linear fluorescent intensity expressed as mean fluorescent channel should be pro-

TABLE 2

Flow Cytometric Analysis of MAC-1, MAC-2, I-A, and LFA-1 on TG-Elicited, Pyran-Induced, and BCG-Activated Macrophages

	Fluorescent intensity[a]				Percentage positive Mϕ[b,c]			
Cells	Mac-1	Mac-2	I-A	LFA-1	Mac-1	Mac-2	I-A	LFA-1
TG	183	351	22	−1	82	93	28	—
Pyran	NT	NT	181	40	NT	NT	82	48
BCG	190	77	253	81	78	48	75	70

[a] Linear fluorescence intensity is expressed as mean fluorescence channel. Background fluorescence has been subtracted.

[b] The figures indicates % positivity of cells within large and small Mϕ gated population. Positivity of cells was determined by cumulative subtraction of number of cells in background from cells in sample, while the mean fluorescence intensity of the control served as the lower limit. NT = not tested.

[c] Sites/cell were calculated using an estimate of 1.6×10^5 Mac-1 molecules/TG-elicited Mϕ (5, 18). The ratio of FITC-anti-rat IgG bound to IgG_{2b} has been found to be 0.54 of that bound to IgG_{2a} as determined with MAb binding to the same site on the same cell surface antigen (5). The values for Mac-2 and LFA-1 were adjusted accordingly. Sites/cell estimates: (i) TG macrophage, 1.65×10^5 Mac-2 and 1.9×10^4 I-A. (ii) Pyran macrophage, 1.58×10^5 I-A and 1.9×10^4 LFA-1. (iii) BCG macrophage, 1.66×10^5 Mac-1; 3.6×10^4 Mac-2; 2.2×10^5 I-A; and 3.8×10^4 LFA-1.

This experiment was repeated twice with similar results.

portional to the number of antigen sites/macrophage. Using published data for the number of Mac-1 sites per TG-induced macrophage (5, 18) and correcting for the amount of fluorescence label bound to different subclasses of IgG (5), we estimated the number of sites/macrophage. Although such estimates may not completely reflect the absolute number of antigen molecules/cell because some binding of MAb is bivalent (19), such data are useful in comparing one cell population with another. We estimated the expression of Mac-2 to be $\sim 1.65 \times 10^5$ sites per TG macrophage, which is in excellent agreement with a previous report indicating that TG macrophages express $\sim 1.7 \times 10^5$ Mac-2 molecules/cell (13). In contrast, only 3.6×10^4 Mac-2 sites were estimated on BCG macrophages (Table 2). BCG macrophages were estimated to express $\sim 3.8 \times 10^4$ LFA-1 molecules/macrophage, while pyran-induced macrophages expressed only 50% of this amount (i.e., $\sim 1.9 \times 10^4$).

Since calculations of antibody binding sites/cell are average estimate of sites on macrophage population, the mean fluorescence figures were further analyzed for changes as related to cell size. For both I-A and LFA-1, the number of sites/BCG macrophage was proportional to cell size (Fig. 4).

Expression of LFA-1 on Monocytes

Since marrow elements have been found to be LFA-1 positive (20) while monocyte-derived inflammatory macrophages are LFA-1 negative (Fig. 2), it became of interest to determine whether monocytes express LFA-1. Monocytes were selected from mononuclear cells isolated from blood by using axial light loss and 90° scatter, which characteristics resemble those of small macrophages activated by BCG. The correctness of this gating was confirmed by the fact that only this subpopulation of the mononuclear cells was Mac-1^+. Analysis of monocytes with two different photomultiplier settings showed that ~80% of these cells express ~56% of the intensity expressed by BCG macrophages (i.e., an estimate of 2.1×10^4 sites/cell;

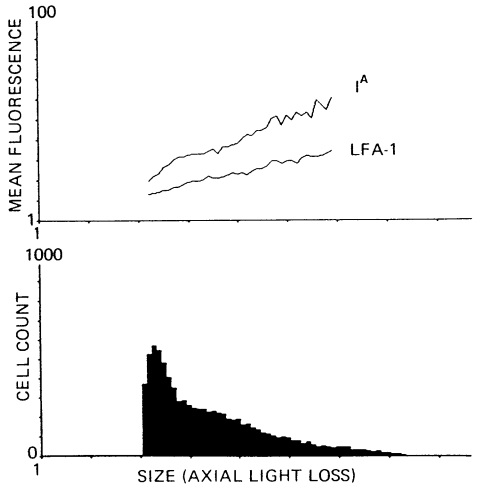

FIG. 4. The fluorescence (upper) and frequency (lower) of LFA-1 and I-A antigens on BCG macrophage, analyzed per increase in cell size (axial light loss).

TABLE 3

Flow Cytometric Analysis LFA-1 Expression by Monocytes

Cells	Fluorescent intensity[a]	Percentage positive
BCG	84	76
Monocytes	47	74

[a] Fluorescent intensity in photomultiplier setting of 4.0 is expressed as mean fluorescence channel. Background fluorescence has been subtracted. Sites/cells for monocytes (2.1×10^4) were calculated using an estimate of 3.8×10^4 LFA-1 molecules/BCG Mϕ (Table 2).

This experiment was repeated twice with similar results.

see Table 3 and Fig. 5). The majority of the remaining mononuclear population (i.e., lymphocytes) were Mac-1^- and LFA-1^+ but had a low fluorescent intensity (data not shown). These results were reproduced utilizing monocytes in radioimmunoassay (data not shown).

DISCUSSION

Changes in the surface proteins of macrophages in different stages of activation are well established events [for review, see (1)]. For example, modulations in the levels of 5′-nucleotidase (21), transferrin receptor (22), receptors for Fc portion of various Ig subclasses (23, 24), and mannose/fucose receptors (25, 26) have been clearly characterized during macrophage development. MAb which distinguish cell surface antigens on macrophages in different activation stages also have been described (26–28). The expression of class II histocompatibility antigen (Ia molecules) was previously demonstrated on macrophages activated or primed *in vivo* or exposed to lymphokine *in vitro* (14, 15). In fact, Ia was found on a small percentage of macrophages in the early stages of development (14). While we could not detect significant levels of Ia on resident or inflammatory macrophages by a radioimmunoassay, (Fig. 1), a modest proportion (i.e., 28%) of TG-induced macrophages expressed low levels of I-A molecules when examined by flow cytometry (Table 2).

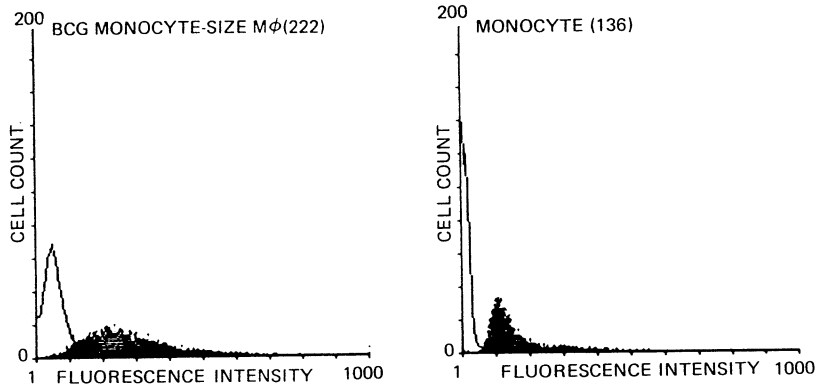

FIG. 5. Expression of LFA-1 antigen on monocytes (right) and BCG monocyte-size macrophages (left). Figures in parentheses indicate mean fluorescence intensity following subtraction of background fluorescence at photomultiplier setting of 4.5. This experiment was repeated twice with similar results.

LFA-1 is estimated to be present on T cells, B cells, myeloid cells, and approximately 75% of bone marrow cells (20). We here present evidence that LFA-1 is also present on tissue macrophages but is selectively confined to macrophages in either the primed or the activated stages of development (Figs. 2 and 5 and Tables 2 and 3). The antigen was absent on populations of inflammatory (responsive) or resident tissue macrophages (Fig. 2, Table 2). Similarly, though to a lower extent, activated but not inflammatory macrophages also express the H-11 and Ly-6 antigens (Fig. 3). On the other hand, Mac-1 is present almost to the same extent on all macrophage populations tested (Fig. 1), indicating that Mac-1 is conserved throughout macrophage development as previously shown (18). Both murine (29) and human (4) monocytes express Mac-1. Human monocytes have been described to be LFA-1^+ (30), and we show here that murine monocytes also express this antigen (Table 3; Fig. 5). Since inflammatory macrophages are derived from blood monocytes (31), it is interesting to note that these cells apparently lose or stop synthesizing LFA-1 in the tissues and then reexpress this molecule when developing from the responsive to the primed state. Recently, we have shown that we could reproduce the results shown here *in vitro* (32). Macrophage-activating factor or recombinant γ-interferon, which primes macrophages for lytic function, induces LFA-1 expression on TG-elicited macrophages (32). By contrast, the development from blood monocyte to inflammatory tissue macrophage, at least when induced by TG broth, is accompanied by a large increase in expression of Mac-1 (29). Data presented here further document that Mac-2 is highly expressed on TG-elicited macrophages (13) but is also expressed to a lesser degree on BCG- or pyran copolymer-induced macrophages (Fig. 1, Table 2). These results thus support the concept that macrophage in various stages of development *in vivo* differentially express many surface antigens [for review, see (1 and 33)].

The results obtained with radioimmunoassay were confirmed by flow cytometry. We estimated that the anti-LFA-1 MAb binds to $\sim 3.8 \times 10^4$ sites/BCG macrophage (Table 2). Almost twice this number of sites/cell (7.2×10^4) was detected on a secondary CTL population (20). Though not directed at the CTL alloantigen receptor, MAb to LFA-1 can strongly inhibit the Mg^{2+}-dependent adhesion step of CTL–target cell interaction, so it has been hypothesized that LFA-1 contributes to the overall avidity between CTL and target cells (3). The distribution of LFA-1 on various cell types suggests that it may play a more general role in cellular adhesion [for review, see (3)]. Mac-1, the LFA-1 homolog which carries the β chain of LFA-1 (8), is clearly associated with function of the C_{3b} receptor (6); and deficiencies in expression of LFA-1 can profoundly depress leukocyte-recognition functions (9, 10). The results presented here indicate the α chain LFA-1 is preferentially expressed by primed and activated tissue macrophages, cells which participate extensively in recognition of microbes, tumor cells, and even host cells (i.e., T lymphocytes (1, 14). Experiments are currently under way to analyze the function(s) of LFA-1 in the effector roles of macrophage in the primed and activated stages of development.

To date, one other MAb has been described to react exclusively with structures on activated macrophages. AcM1, a macrophage-specific MAb, reacts with polypeptides on activated macrophages of 70,000 and 45,000 M_r (28). Expression of AcM1 was, however, assayed under conditions which did not distinguish populations of primed from populations of activated macrophages (28). Since αLFA-1 and αAcM1 precipitate glycoprotein chains of different M_r (20, 28), the two antigens do appear

to be distinct entities. We here report that three additional MAb (i.e., LFA-1, H-11, αLy-6) can distinguish primed/activated macrophages from either resident or responsive tissue macrophages. The development of MAb which distinguish various populations of functionally distinct macrophages should be of use in analyzing macrophage development *in vitro* and *in vivo*.

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