0022-1767/82/1285-2281$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 128, No. 5, May 1982
Printed in U.S.A.

Mac-1 ANTIGEN: QUANTITATIVE EXPRESSION IN MACROPHAGE POPULATIONS AND TISSUES, AND IMMUNOFLUORESCENT LOCALIZATION IN SPLEEN[1]

MAY-KIN HO[2] AND TIMOTHY A. SPRINGER[3]

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115

The extent of Mac-1 expression by different macrophage populations and tissues has been examined and contrasted to that of Ia. Mac-1 is expressed on >86% of unelicited peritoneal macrophages as well as on macrophages elicited by thioglycollate, Con A, LPS, *Listeria monocytogenes*, or peptone, as determined by immunofluorescent flow cytometry analysis. All populations express similar quantities of Mac-1/cell. The number of ^{125}I-M1/70 MAb bound per thioglycollate-elicited macrophage is 1.6×10^5 at saturation. Immunoprecipitation from ^{35}S-methionine labeled cells confirmed that macrophages elicited by diverse agents contain similar amounts of Mac-1 polypeptides of 170,000 and 95,000 M_r. In contrast, the amount of Ia antigen in Con A- or *Listeria*-elicited macrophages is much higher than in unelicited macrophages or macrophages elicited by LPS or thioglycollate. Mac-1 is thus a general marker for macrophages elicited by different means, even though the macrophages differ widely in functional activities and in Ia antigen expression. Tissue absorption studies show that the amount of Mac-1 expressed by spleen and bone marrow is 5 and 7% that of thioglycollate-elicited peritoneal exudate cells (PEC), respectively. Lung and liver weakly absorb ^{125}I-M1/70 whereas heart, kidney, and brain are not absorptive. When used in indirect immunofluorescent staining of frozen spleen sections, M1/70 intensely stains cells with macrophage morphology in splenic red pulp and marginal zone, but not the T-dependent areas of white pulp.

Mac-1 is a mouse macrophage cell surface antigen defined by the M1/70 rat anti-mouse monoclonal antibody (MAb).[4] Immunofluorescent flow cytometry analysis shows that Mac-1 is expressed abundantly on thioglycollate-elicited peritoneal macrophages and in lesser amounts on blood monocytes, granulocytes, 8% of spleen cells, and 50% of bone marrow cells (1). M1/70 also stains murine natural killer (NK) cells (2). Mac-1 is not present on thymocytes or peripheral lymph node cells. Mac-1 is expressed on the P388D$_1$ macrophage-like cell line but not on B or T lymphoid cell lines. Immunoprecipitation of ^{125}I-labeled macrophage cell surface proteins shows that Mac-1 contains polypeptide chains of 170,000 and 95,000 M_r. An antigen defined by the 1.21J MAb has a similar if not identical biochemical structure and cellular distribution (3). In humans, M1/70 cross-reacts with an antigen expressed on blood monocytes and in lesser amounts on granulocytes and NK cells (4). Thus, the distribution in mouse and human is quite similar.

The above studies suggested that Mac-1 might be of general use in distinguishing macrophages from T and B lymphocytes. However, the only type of murine macrophages previously examined for Mac-1 expression were those elicited by thioglycollate. Furthermore, recent studies with monoclonal antibodies have emphasized the diversity of macrophage cell surface phenotypes. Thus, the Mac-2 antigen is expressed on thioglycollate-induced macrophages but not on *L. monocytogenes*-induced or resident macrophages (5). The 54-2 antigen is expressed on thioglycollate-induced and bone marrow-cultured macrophages but not on blood monocytes or resident macrophages (6). For a marker to be of general use for distinguishing macrophages from lymphocytes, it should be present on all macrophages, independent of the differentiative signals to which they have been exposed. Characterization of the distribution of Mac-1 on different types of macrophages is also important in assessing possible relationships to previously described macrophage cell surface receptors, ectoenzymes, and functional activities.

Therefore, in this paper the expression and structure of Mac-1 on peritoneal resident macrophages and on macrophages elicited by differnt agents have been examined by immunofluorescence, quantitative site number determination, and immunoprecipitation. The expression of Mac-1 has been compared to that of Ia, which shows differential expression on macrophages depending on the eliciting agent (7). We have also examined by absorption the tissue distribution of Mac-1 and have used immunofluorescence to localize Mac-1-bearing cells in spleen sections. The results show that Mac-1 is expressed on all types of macrophages examined, validating the use of Mac-1 as a general marker for distinguishing macrophages from lymphocytes and suggesting that it must have a generalized role in macrophage function. Mac-1 expression cannot be detected in lymph node cells or in the periarteriolar lymphoid sheath region of spleen but is found in the marginal zone and red pulp. The results also show that Mac-1 is synthesized by macrophages and is present on the cell surface in sufficient quantity for biochemical purification and characterization.

MATERIALS AND METHODS

Hybridoma lines and monoclonal antibodies. M1/70 is a subcloned rat spleen cell × NSI hybridoma line that secretes an IgG2b MAb containing only specific heavy and light chains (8). Purified MAb from spinner culture

Received for publication November 30, 1981.
Accepted for publication February 9, 1982.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
This work was supported by Grant CA27547 from the United States Public Health Service and by Council for Tobacco Research Grant 1307.
[2] Recipient of postdoctoral fellowships from the Anna Fuller Fund and USPHS Grant 1 F32 AI 06190.
[3] Recipient of an American Cancer Society Faculty Award.
[4] Abbreviations used in this paper: HEPES, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid; MAb, monoclonal antibody; M_r, molecular mass; PEC, peritoneal exudate cells; TG, thioglycollate; NK, natural killer; DME, Dulbecco's modified Eagle's medium; FITC, fluorescein isothiocyanate.

supernatant was prepared by $(NH_4)_2SO_4$ precipitation and DEAE and Sephadex G-200 chromatography as previously described (4).

Cell preparation. Male C57/BL6 mice (Jackson Laboratory, Bar Harbor, ME), 8 to 16 wk of age, were used in most experiments. Peritoneal exudates were obtained after i.p. injection of one of the following reagents: 1.5 ml Brewer's thioglycollate; 1.5 ml 10% protease peptone; 40 μg *Salmonella typhosa* LPS (Westphal) (all 3 reagents were from Difco Laboratories, Detroit, MI); 15 μg concanavalin A (Sigma Chemicals, St. Louis, MO); or 2 × 10^4 live *Listeria monocytogenes* organisms (the kind gift of Dr. Emil Unanue). The time of treatment was usually 4 days for thioglycollate and 3 days for the others. Cells were harvested in PBS containing 10 U/ml heparin (Sigma). For immunofluorescent studies of glass-adherent macrophages, cells were resuspended to 2 × 10^5/ml in RPMI 1640 supplemented with 10% FCS and 10 mM HEPES.[4] Cells (1 ml) were incubated in 16-mm culture wells (Costar, Cambridge, MA) containing a 12-mm round cover slip (Bellco Glass, Vineland, NJ) for 2 hr at 37°C. They were then processed according to the procedures of Beller *et al.* (7).

Tissue absorption studies. Lung, liver, brain, heart, and kidney from freshly sacrificed animals were homogenized between the frosted ends of 2 glass slides. Large fragments were removed by 1 × *G* sedimentation for 3 min, and the homogenate was washed twice in PBS by centrifugation at 2000 × G for 15 min. Lymphoid organs, such as spleen, thymus, peripheral lymph node, and bone marrow from femurs were teased with forceps. The resulting cells and tissue clumps were washed twice in PBS before use. All procedures were carried out at 4°C. Aliquots of tissues were assayed for protein by the method of Lowry.

Limiting concentrations of ^{125}I M1/70 MAb (determined by titrating a constant number of cells with increasing concentrations of antibody) were mixed with equal volumes of 5-fold diluted tissues in 96-well microtiter plates. PBS was used as the negative tissue control. After shaking at 4°C for 1 hr, and centrifuging at 2000 × G for 15 min, the residual antibodies were recovered from the supernatant. Ten microliters of the absorbed antibody were added to 2 × 10^5 thioglycollate-elicited PEC in 50 μl PBS, 10% BSA, and 4 mg/ml human IgG. After being shaken for 1 hr at 4°C, the cells were washed 4 times with 0.01 M Tris HCl, pH 7.4, 0.14 M NaCl, and 1% horse serum and were gamma counted.

Site number estimation. Purified M1/70 MAb (0.1 mg/ml) was iodinated with 0.5 to 1.0 mol carrier-free ^{125}I per mol protein, using 1,3,4,6-tetrachloro-3,6-diphenylglycoluril and the methods of Fraker and Speck (9), except that the reaction was terminated by transfer to fresh tubes containing 20 μl of 0.4 mg/ml L-tyrosine.

Thioglycollate-elicited peritoneal exudate cells (PEC; 20 μl, 10^7/ml) were mixed with 80 μl of ^{125}I M1/70 at varying concentrations. The amount of binding was determined after shaking 1 hr at 4°C as described in tissue absorption studies. Binding of ^{125}I M1/70 to PEC is specific because it is inhibitable by the presence of unlabeled M1/70.

Immunofluorescent flow cytometry. Cells (50 μl, 2 × 10^7/ml) were incubated with equal volumes of MAb-containing spent culture supernatants for 30 min, or with control M1/69 HK supernatant or NSI supernatants plus 50 μg/ml normal rat IgG. The cells were washed 3 times and resuspended in 50 μl of affinity-purified FITC-rabbit $(Fab')_2$ anti-rat IgG, absorbed with mouse IgG (diluted to 60 μg/ml in L15 medium; fluorescein/protein, 19 μg/mg), for 30 min. After another 3 washes, the cells were analyzed on a Becton Dickinson FACS-II using glutaraldehyde-fixed sheep red cells as standards. All procedures were carried out in L15 medium + 0.5% BSA + 10 mM HEPES + 11 mM glucose at 4°C. During the second-stage incubation and subsequent washes, 0.01 M sodium azide was included in the medium to prevent patching.

Determination of fluorescence intensity and its linear relation to antigen sites/cell has previously been described in detail (9A). The mean channel number of positive cells and control, unlabeled, cells was determined by integration and was converted to fluorescence intensity with a calibration curve of glutaraldehyde-fixed sheep red blood cells at different gain settings. Control fluorescence intensity was subtracted to obtain the mean specific fluorescence intensity.

Internal labeling and immunoprecipitation. For biosynthetic labeling, 10^7 peritoneal exudate cells were plated on tissue culture flasks and incubated 18 hr at 37°C unless otherwise specified. Nonadherent cells were washed off, and adherent cells were labeled with 200 μCi L-^{35}S-methionine (New England Nuclear) in 1 ml of methionine-free DME supplemented with 10% dialyzed FCS for 6 hr. Subsequently, the adherent monolayer was washed 3 times in PBS and was detergent solubilized. After centrifugation at 100,000 × G for 1 hr, the supernatant was recovered. Immunoprecipitation and SDS-PAGE were carried out as described (10).

Immunofluorescent staining of spleen sections. Fresh spleens from male BALB/c mice were frozen in OCT compound (Ames Co., Div. of Miles Laboratories, Elkhart, IN). Sections of 3 μm were cut and stored at −35°C for up to 2 wk. Sections were thawed, fixed in acetone for 15 min, and washed twice in PBS. Subsequently, they were stained with 40 μl of anti-Mac-1 (M1/70, 5 μg/ml) or anti-Thy-1 (M5/49, 5 μg/ml) supernatants for 30 min, washed twice in PBS, and stained for another 30 min with 40 μl of FITC-$F(ab')_2$ rabbit anti-rat IgG previously absorbed with mouse IgG. All staining was performed at room temperature in a moist chamber. After being washed twice in PBS, the sections were mounted in Aqua-mount (Lerner Laboratories, Stamford, CT). Controls include sections stained with anti-Mac-1 or anti-Thy-1 MAb only, as well as with NSI clonal supernatants plus 5 μg/ml normal rat IgG followed by the second-stage FITC reagent.

RESULTS

Expression of Mac-1 by peritoneal macrophages elicited by different means. Unelicited peritoneal macrophages and macrophages elicited by sterile inflammatory agents and by bacteria differ widely in function, ectoenzyme profiles, and expression of Ia, Mac-2, and 54-2 antigens (6, 7, 11–15). Therefore, Mac-1 was also examined for expression on different types of peritoneal macrophages. Cells were labeled with M1/70 MAb, a second step FITC-conjugated reagent, and were analyzed by immunofluorescent flow cytometry (Fig. 1). Scatter gates were set so that the analysis included all macrophage-sized cells but excluded most lymphocytes, polymorphs, and smaller cells present in the peritoneal exudates. As previously described, thioglycollate-induced macrophages are Mac-1^+ (Fig. 1*A*). In addition, macrophages elicited by Con A, LPS, *L. monocytogenes*, and peptone, and unelicited resident macrophages were also found to be 86 to 92% strongly Mac-1^+ (Fig. 1*B–F*). The mean fluorescence intensity of the different macrophage preparations did not vary by more than a factor of 2.2 (Table I). Thioglycollate-elicited cells showed the highest fluorescence intensity. Because of some overlap in scatter distributions, a small percentage of non-macrophages were included in these analyses. Parallel experiments with the same scatter gates showed that the Con A-, LPS-, and *Listeria*-elicited cells were 4 to 11% Thy-1^+. Since normal T cells and Con A-activated T blasts do not synthesize Mac-1, 86 to 97% labeling in these epxeriments may have represented 100% labeling of macrophages.

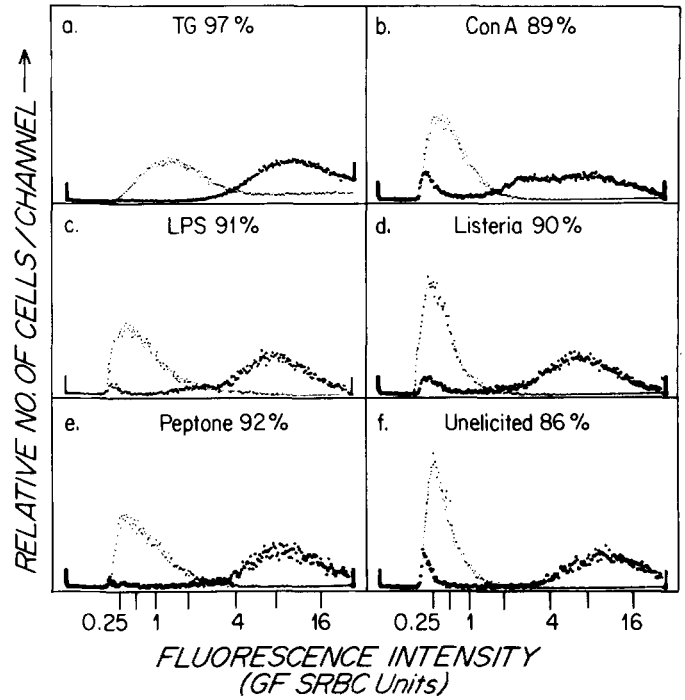

Figure 1. Immunofluorescent flow cytometry analysis of peritoneal macrophages elicited by different agents. PEC were labeled with M1/70 (*dark curve*) or NSI control supernatant plus 50 μg/ml normal rat IgG (*light curve*) as described in *Materials and Methods*. The scatter gates were set to exclude RBC, dead cells, and most lymphocytes and polymorphonuclear leukocytes. The histograms shown are plotted with the relative cell number on a linear scale and fluorescence on a logarithmic scale.

To further examine this question, macrophages were purified by adherence on glass coverslips, stained, and examined microscopically. Adherent preparations from all exudates were 93 to 99% macrophages as determined by Wright-Giemsa staining and were >95% Mac-1$^+$ by immunofluorescence. Mac-1 was also present on all macrophages in 1-day thioglycollate-elicited exudates. Therefore, Mac-1 is expressed by essentially all unelicited macrophages and macrophages elicited by sterile inflammatory agents, bacteria, or other immune stimuli.

Biosynthesis of Mac-1 and Ia antigens: comparison of macrophages elicited by various means and spleen and lymph node cells. To study the biosynthesis of Mac-1 and Ia by various macrophage populations, peritoneal macrophages elicited by various agents were purified by plastic adherence and internally labeled with ^{35}S-methionine. Spleen cell and lymph node cell suspensions were also labeled. Immunoprecipitates prepared from cell lysates containing equal quantities of incorporated radioactivity were then analyzed by SDS-PAGE. Unelicited macrophages as well as macrophages elicited by thioglycollate, Con A, LPS, *Listeria,* and peptone incorporated similar amounts of ^{35}S-methionine into Mac-1 antigens (Fig. 2*A*, lanes 1–6). After the same fluorogram exposure, no Mac-1 biosynthesis by spleen or lymph node cells was detected (Fig. 2*A*, lanes 7 and 8). However, Mac-1 could be seen in immunoprecipitates of spleen cell lysates but not of lymph node lysates after prolonged fluorogram exposure (result not shown). This is consistent with previous findings that a small subpopulation of spleen cells but not lymph node cells are Mac-1$^+$.

In contrast to Mac-1, the amount of ^{35}S-methionine incorporation into Ia was found to vary markedly with the eliciting agent. The level of Ia syntheisis by Con A- and *Listeria*-elicited macrophages (Fig. 2*B*, lanes 3 and 5) was much higher than that by unelicited macrophages (Fig. 2*B* lane 2) or by macrophages elicited by thioglycollate or LPS (lanes 1 and 4). Peptone-elicited cells showed an intermediate level of Ia synthesis (Fig. 2*B*, lane 6). By comparing fluorograms exposed for different lengths of time, the amounts of ^{35}S-methionine incorporated into Ia by Con A- and *Listeria*-elicited cells were estimated to be 20 times and 16 times more than that by thioglycollate-elicited macrophages. High levels of Ia antigen synthesis were also detected in cells from spleen and lymph node (Fig. 2*B*, lanes 7 and 8). This was probably due to the presence of large numbers of B lymphocytes in these tissues.

Estimation of M1/70 binding sites on thioglycollate-elicited macrophages. To determine the number of Mac-1 cell surface sites per macrophage, saturation labeling of PEC with increasing concentrations of ^{125}I-M1/70 MAb was carried out (Fig. 3). Results from 5 independent experiments showed that 1.6 ± 0.3 × 10^5 molecules of M1/70 IgG were bound per thioglycollate-elicited macrophage. Based on this value, the average number of M1/70 binding sites on macrophages elicited by other agents was estimated by comparing their mean fluorescent intensity after M1/70 staining to that of thioglycollate-induced cells (Table I). The affinity of M1/70 was 1.1 × 10^9 M^{-1} as determined by Scatchard analysis of the data from the same binding assays.

TABLE I

Estimation of M1/70 binding sites on macrophages elicited by different agents

Eliciting Agent	Mean Specific Fluorescence Intensity[a]	M1/70 Binding Sites/Cell × 10^{-4}
Thioglycollate	12.5	16.0[b]
Con A	5.6	(7.2)[c]
LPS	8.0	(10.2)
Listeria	6.4	(8.2)
Peptone	10.2	(13.1)
None	11.2	(14.3)

[a] See *Materials and Methods.*

[b] Based on saturation binding of ^{125}I-M1/70 to thioglycollate-elicited PEC.

[c] ( ) estimated by comparing the mean specific fluorescence intensity of the test macrophage population to that of thioglycollate-elicited macrophages.

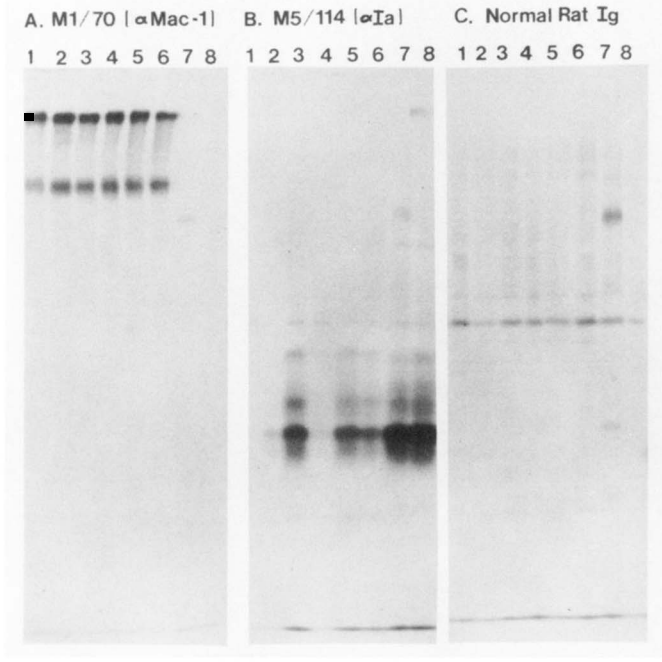

Figure 2. SDS-PAGE of [^{35}S]-methionine-labeled Mac-1 and Ia antigens from macrophages elicited by various means and from spleen and lymph node cells. Resident or exudate macrophages were purified by 2 hr adherence on tissue culture flasks, and labeled with [^{35}S]-methionine as described in *Materials and Methods.* Single cell suspensions from spleen and lymph node were processed similarly except that the adherence step was omitted. Equal quantities of Triton X-100 lysates (1.5 × 10^6 cpm) were shaken with MAb coupled to Sepharose CL-4B for 90 min at 4°C: *A*, 8.7 μg M1/70 MAb; *B*, 20 μg M5/114 MAb; *C*, 8 μg normal rat IgG as control. Beads were washed, boiled in SDS-sample buffer containing 5% 2-mercaptoethanol, and the eluates were subjected to SDS-10% PAGE and fluorography (20). The macrophage lysates used for immunoprecipitation were elicited as follows: *lane 1*, 4-day thioglycollate; *2*, unelicited; *3*, Con A; *4*, LPS; *5*, *Listeria monocytogenes; 6*, protease peptone. Lysates of spleen and lymph node cells were used in *lanes 7* and *8*, respectively.

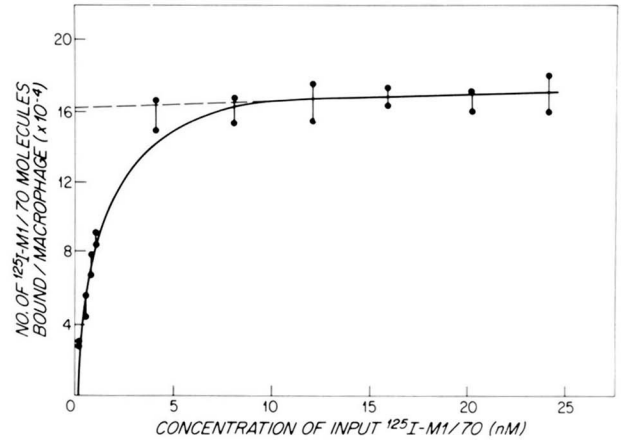

Figure 3. Saturation binding of ^{125}I-M1/70 to thioglycollate-elicited macrophages. Increasing concentrations of ^{125}I-M1/70 were incubated with 2 × 10^5 4-day thioglycollate-elicited PEC and binding was determined as described in *Materials and Methods.* Differential counts of Wright-Giemsa-stained PEC showed that approximately 85% were macrophages. Specific activity of ^{125}I-M1/70 MAb was 3.8 × 10^6 cpm/μg. The number of sites was determined by extrapolation to the *y* axis, which corrects for nonsaturable, nonspecific binding.

Absorption studies on lymphoid and nonlymphoid tissues. To study the expression of Mac-1 on various tissues, a limiting amount of ^{125}I-M1/70 MAb was incubated with various tissues,

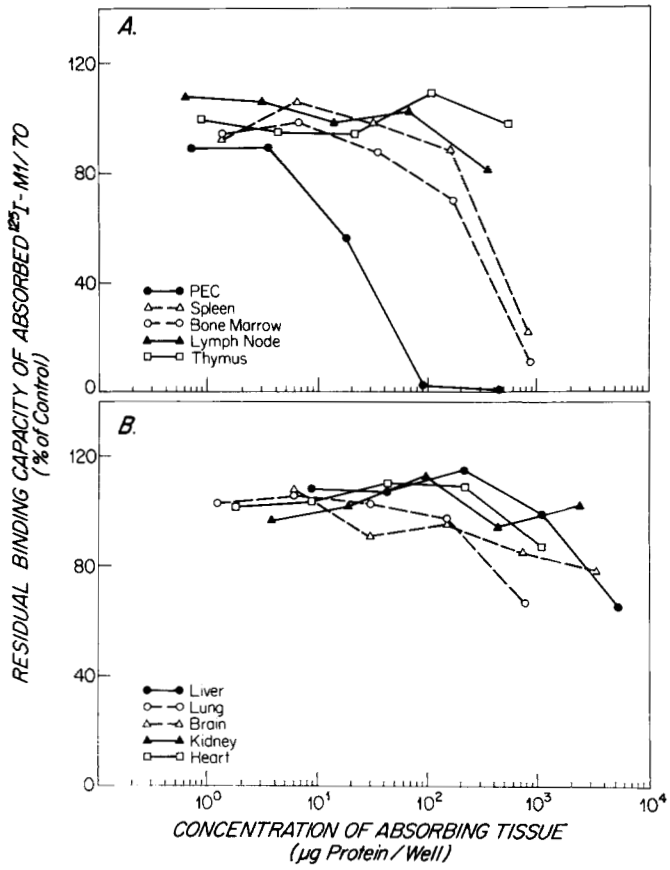

Figure 4. Absorption of ^{125}I-M1/70 with various tissues. Limiting amounts of ^{125}I-M1/70 were incubated with increasing quantities of mouse lymphoid (*A*) or nonlymphoid (*B*) tissues. Residual binding activity to PEC was determined in a direct binding assay as described in *Materials and Methods*. Binding capacity was calculated as the percentage of ^{125}I-M1/70 bound after absorption with PBS.

and the residual binding capacity to thioglycollate-elicited PEC was measured. ^{125}I-M1/70 MAb was absorbed by spleen, PEC, and bone marrow, but not by thymus or peripheral lymph node (Fig. 4*A*). By comparing the amount of tissue protein required for 50% inhibition, the absorption by spleen and bone marrow was estimated to be 5 and 7% that of PEC, respectively. Liver and lung showed a suggestion of absorbing capacity (Fig. 4*B*). Heart, brain, and kidney were not absorptive (Fig. 4*B*).

Localization of M1/70$^+$ cells in spleen sections. To determine the morphology and the localization Mac-1$^+$ cells in spleen, M1/70 MAb was used to stain frozen spleen sections by indirect immunofluorescence. Areas with Mac-1$^+$ cells were aligned with T-dependent areas of the spleen by staining adjacent sections with an anti-Thy-1 MAb. T lymphocytes in the periarteriolar lymphatic sheath were intensely stained by anti-Thy-1 MAb (Fig. 5*A*). In contrast, few if any Mac-1$^+$ cells were seen in T-dependent areas (Fig. 5*B*). However, numerous brightly Mac-1$^+$ cells were found localized in the marginal zone (Fig. 5*B*) and scattered in the red pulp (Fig. 5*C*). Two-color immunofluorescence staining with anti-Mac-1 and anti-Thy-1 reagents gave similar results (not shown). Since the morphology of cells in frozen sections was not well-preserved, we also stained adjacent sections with hematoxylin and eosin. Most of the cells stained strongly by M1/70 had the morphologic and staining characteristics of macrophages. In addition, some smaller cells were stained weakly for Mac-1. Most of these had segmented nuclei and resided in the red pulp. They probably represent granulocytes, which were previously shown to express only small quantities of Mac-1 (1). The other small cells in the red pulp may be monocytes and natural killer cells, which are known to be weakly Mac-1$^+$ (1).

DISCUSSION

A previous report from this laboratory described the expression of Mac-1 on thioglycollate-induced macrophages, granulocytes, blood monocytes, and 50% of bone marrow cells, but not on lymphocytes or thymocytes (1). The P388D, macrophage-like cell line, but not B or T lymphoid lines, was also found to express Mac-1. However, the expression of Mac-1 by macrophages elicited by other agents was not explored. Recent studies have shown that peritoneal macrophage surface antigen expression, in addition to function, ectoenzyme profile, and physiology, is markedly influenced by the eliciting agent (6, 7, 11–15). For example, the number of Ia$^+$ macrophages in peritoneal exudates elicited by Con A and *Listeria monocytogenes* is much higher than that in the normal peritoneal cavity or in exudates induced by thioglycollate, oil, peptone, and LPS (7). Two other structurally distinct macrophage antigens, Mac-2 and 54-2, are also differentially expressed on macrophage populations. Mac-2 is found in abundance on thioglycollate-elicited macrophages but not on unelicited macrophages or macrophages elicited by Con A, LPS, or *Listeria* (5). 54-2 antigen is expressed by thioglycollate-elicited macrophages, cultured bone marrow macrophages, and mast cells but not by resident peritoneal macrophages or monocytes (6). Its presence on macrophages elicited by other agents is not known. A sense is emerging from these studies that macrophages can be divided into antigenically distinct subsets, as is the case for lymphocytes. This prompted us to examine the distribution of Mac-1 on macrophages elicited by agents other than thioglycollate.

In contrast to Ia, Mac-2, and 54-2 antigens, the Mac-1 antigen appears to be a general macrophage marker. Peritoneal resident and exudate macrophages elicited by thioglycollate, Con A, LPS, *Listeria monocytogenes,* or peptone were found to be >86% Mac-1$^+$ by immunofluorescent flow cytometry, and >95% Mac-1$^+$ by direct microscopic examination of glass-adherent, stained macrophages. The average amount of Mac-1 expressed by the different populations varied only 2-fold, with the highest mean fluorescence intensity detected on macrophages elicited by thioglycollate or peptone.

The number of M1/70 binding sites per thioglycollate-elicited macrophage was found to be 1.6×10^5 at saturation. Based on this value, resident macrophages were estimated to bind 1.4×10^5 M1/70 MAb/cell, in good agreement with values independently obtained for the 1.21J MAb to Mac-1 (3). Thioglycollate-elicted macrophages have 1.3×10^5 Mac-2 and 4×10^4 Mac-3 sites/cell (15). Resident macrophages express close to 10^5 H-2, Fc receptor II, and F4/80 antigen binding sites/cell (3). Of all these antigens, Mac-1 is expressed in the highest quantity. It is a major macrophage surface component, and is present in sufficient quantities for isolation and characterization of unlabeled material.

The expression of Mac-1 by diverse macrophage populations was confirmed by biosynthetic labeling experiments. Unelicited peritoneal macrophages as well as macrophages elicited by thioglycollate, Con A, LPS, *Listeria,* and peptone incorporated a similar proportion of ^{35}S-methionine into Mac-1. Ia antigen synthesis, however, varied markedly. The M_r of the Mac-1 polypeptides was the same for all macrophage populations. In contrast, the M_r of Mac-3, a distinct macrophage cell surface

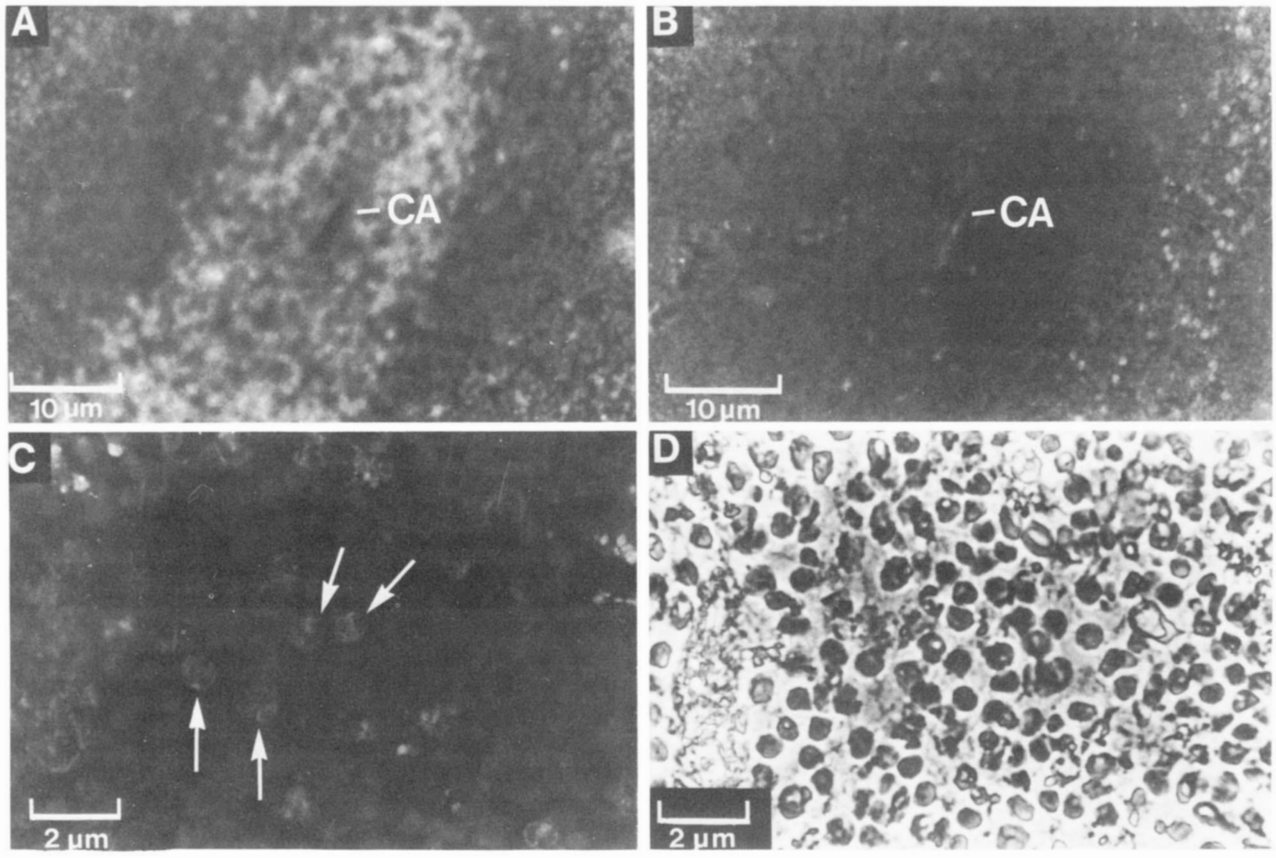

Figure 5. Immunofluorescent staining of spleen sections with anti-Thy-1 and anti-Mac-1 MAb. Frozen spleen sections were stained with anti-Thy-1 (*A*) or anti-Mac-1 (*B* and *C*) followed by FITC-F(ab')$_2$ rabbit anti-rat IgG. Thy 1$^+$ cells are located in the periarteriolar lymphatic sheath (*A*) whereas Mac-1$^+$ cells are scattered in the marginal zone (*B*) (adjacent sections). A branch of the central artery (CA) can be seen in both sections. Mac-1$^+$ cells (*arrows*) are also found in the red pulp (*C*). *D* is the same fold as in *C* viewed under phase contrast.

glycoprotein, can vary from 108,000 to 130,000, depending on the eliciting agent used (manuscript in preparation).

In addition to peritoneal macrophages, spleen and bone marrow cells also express Mac-1. Absorption experiments showed that spleen and bone marrow express 5 and 7%, respectively, as much Mac-1 as PEC, on a protein weight basis. Previous studies showed that 50% of bone marrow cells are Mac-1$^+$ (1), but from relative fluorescence intensities (15) it can be estimated that positive bone marrow cells express only 10% as much Mac-1 as thioglycollate-elicited PEC. Based on these results, the absorption by bone marrow should be about 5% that of PEC, in good agreement with the experimental findings.

Immunofluorescent staining of spleen sections showed that almost all Mac-1 cells reside in the splenic red pulp and marginal zone, areas where other investigators have observed an abundance of macrophage (16–18). Both brightly staining Mac-1$^+$ cells with the morphology of macrophages as well as other, weakly staining cells were found in spleen sections, whereas cells adherent to coverslips isolated from spleen single cell suspensions (results not shown) were only weakly Mac-1$^+$. This is in agreement with other reports that a high proportion of spleen macrophages remain associated with stroma after teasing (19). Few M1/70$^+$ cells were found in the white pulp, where dendritic cells are localized, which suggests that dendritic cells are Mac-1$^-$. These results obtained by *in situ* staining are consistent with recent findings by Nussenzweig *et al.* on isolated spleen cells (3). They showed that Mac-1, Fc receptor II, and a 160,000 M_r antigen identified by the F4/80 MAb were all expressed by splenic macrophages but not by dendritic cells.

Previously there has been no general marker for macrophages analogous to Thy-1 for T lymphocytes or surface immunoglobulin for B lymphocytes. Lymphocytes are Mac-1 negative, but blood monocytes, normal splenic and peritoneal macrophages, and peritoneal macrophages elicited by diverse agents all express Mac-1. Mac-1 is present on exudate macrophages at early (1-day) and later (4-day) time points. It therefore appears to be continuously expressed during maturation of monocytes to macrophages. Furthermore, all 8 of 8 different macrophage-like cell lines thus far examined have also been found to be Mac-1$^+$ (unpublished results). Quantitation of its surface expression shows Mac-1 is a major cell surface component. Therefore, despite its weak expression on granulocytes (1) and natural killer cells (2), the presence of Mac-1 on all macrophage populations examined thus far renders it a useful marker for differentiating macrophages from lymphocytes and dendritic cells in cell suspensions, adherent monolayers, and tissue sections. Its ubiquitous and abundant expression on macrophages at different stages of differentiation also suggests that Mac-1 plays an important role in macrophage physiology and function.

REFERENCES

1. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. Eur. J. Immunol. 9:301.
2. Holmberg, L. A., T. A. Springer, and K. A. Ault. 1981. Natural killer activity in the peritoneal exudates of mice infected with *Listeria monocytogenes*: Characterization of the natural killer cells by using a monoclonal rat anti-murine macrophage antibody (M1/70). J. Immunol. 127:1792.
3. Nussenzweig, M. C., R. M. Steinman, J. C. Unkeless, M. D. Witmer, B. Gutchinov, and Z. A. Cohn. 1981. Studies of the cell surface of mouse dendritic cells and other leukocytes. J. Exp. Med. 154:168.

4. Ault, K. A., and T. A. Springer. 1981. Cross reaction of a rat-anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. J. Immunol. 126:359.
5. Ho, M. K., and T. Springer. 1982. Mac-2, a novel 32,000 Mr macrophage subpopulation-specific antigen defined by monoclonal antibodies. J. Immunol. 128:1221.
6. Leblanc, P. A., H. R. Katz, and S. W. Russell. 1980. A discrete population of mononuclear phagocytes detected by monoclonal antibody. Infect. Immun. 8:520.
7. Beller, D. I., J.-M. Kiely, and E. R. Unanue. 1980. Regulation of macrophage populations. I. Preferential induction of Ia-rich peritoneal exudates by immunologic stimuli. J. Immunol. 124:1426.
8. Springer, T. A. 1980. Cell-surface differentiation in the mouse. Characterization of "jumping" and "lineage" antigens using xenogeneic rat monoclonal antibodies. *In* Monoclonal Antibodies, Edited by R. H. Kennett, T. J. McKearn, and K. B. Bechtol. Plenum, New York. Pp. 185–217.
9. Fraker, P. J., and J. C. Speck. 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3α,6α-diphenyl glycoluril. Biochem. Biophys. Res. Commun. 80:849.
9a. Kürzinger, K., T. Reynolds, R. N. Germain, D. Davignon, E. Martz, and T. A. Springer. 1981. A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. J. Immunol. 127:596.
10. Springer, T., G. Galfre, D. S. Secher, and C. Milstein. 1978. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur. J. Immunol. 8:539.
11. Bianco, C., and P. J. Edelson. 1978. Characteristics of the activated macrophage. *In* Immune Effector Mechanisms in Disease. Edited by Weksler *et al.* Grune and Stratton, New York. Pp. 1–9.
12. Morahan, P. S., P. J. Edelson, and K. Gass. 1980. Changes in macrophage ectoenzymes associated with anti-tumor activity. J. Immunol. 125:1312.
13. Kaplan, A. M., P. S. Morahan, and W. Regelson. 1974. Induction of macrophage-mediated tumor-cell cytotoxicity by pyran copolymer. J. Natl. Cancer Inst. 52:1919.
14. Karnovsky, M. L., and J. K. Lazdins. 1978. Biochemical criteria for activated macrophages. J. Immunol. 121:809.
15. Springer, T. A., and M-K. Ho. 1982. Macrophage differentiation antigens: markers for macrophage subpopulations and tissue localization. *In* Hybridomas in Cancer Diagnosis and Treatment. Edited by M. S. Mitchell and H. F. Oettgen. Raven Press, New York. Pp. 53–61.
16. Weiss, L. 1972. *In* The Cells and Tissues of the Immune Response. Structure, Functions, Interactions. Edited by E. G. Osler and L. Weiss. Prentice Hall, Englewood Cliffs, N. J.
17. Hoefsmit, E. C. M. 1973. Mononuclear phagocytes, reticulum cells, and dendritic cells in lymphoid tissues. *In* Mononuclear Phagocytes in Immunity, Infection and Pathology. Edited by R. van Furth. Blackwell Scientific Publications, Oxford. Pp. 129–146.
18. Steinman, R. M., J. C. Adams, and Z. A. Cohn. 1975. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in spleen. J. Exp. Med. 141:804.
19. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J. Exp. Med. 137:1142.
20. Bonner, W. M., and R. A. Laskey. 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83.