Characterization of Macrophage Adhesion Molecule†

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Received May 19, 1987; Revised Manuscript Received August 10, 1987

ABSTRACT: Macrophage adhesion molecule (MAM), an abundant surface molecule which functions in the adhesion and spreading of guinea pig macrophages on surfaces, is characterized as a heterodimer of the trypsin- and plasmin-sensitive glycopeptide gp160 (MAM-α) and the glycopeptide gp93 (MAM-β). The density of MAM molecules is estimated at 630 000 per macrophage on the basis of quantitative binding of ^{125}I-labeled monoclonal antibody. The glycopeptide subunits display microheterogeneity on isoelectrofocusing; the p*I* is 5.8–6.3 for gp160 (MAM-α) and 6.4–7.0 for gp93 (MAM-β). A neutrophil gp160,gp93 molecule was shown to be indistinguishable from macrophage MAM on the basis of electrophoresis, isoelectrofocusing, and reactivity with 10 monoclonal antibodies. A related heterodimer of gp93 associated with a larger, antigenically different glycopeptide (gp180,gp93) was identified on circulating lymphocytes. Cumulative properties indicate that MAM is the guinea pig analogue of human Mo1 and mouse Mac-1.

Macrophages are vital effector cells in immune defense. Many critical defense functions of macrophages take place at the cell surface. The importance of characterizing macrophage surface components derives from their participation in chemotaxis, adherence, spreading, endocytosis, secretion, recognition of tumor cells, and interaction with lymphocytes and endothelial cells.

Most macrophage surface proteins were found to be resistant to proteinases, as was predicted, since these cells persist at sites rich in proteinases (Pearlstein et al., 1978; Remold-O'Donnell, 1980). Among guinea pig macrophage surface proteins, one abundant glycopeptide is uniquely sensitive to mild trypsin and plasmin treatment (Remold-O'Donnell & Lewandrowski, 1982a). The content and the biosynthesis of that glycopeptide, gp160, were shown to be down-regulated in macrophages activated in vivo (Remold-O'Donnell & Lewandrowski, 1982b).

The present study demonstrates that the trypsin- and plasmin-sensitive glycopeptide gp160 exists in the macrophage membrane in noncovalent association with the glycopeptide gp93. The gp160,gp93 heterodimer molecule was shown to function in the adherence and spreading of macrophages on surfaces[1] and has been called macrophage adhesion molecule (MAM).[2] The present study characterizes the MAM molecule of macrophages and neutrophils. The purification of MAM and the composition of separated MAM-α and MAM-β are presented in the following paper (Remold-O'Donnell & Savage, 1988).

Materials and Methods

Cells. Peritoneal macrophages were obtained from male Hartley guinea pigs (ARI Breeding Labs, East Bridgewater, MA; Elm Hill Breeding Labs, Chelmsford, MA) 5 days after intraperitoneal injection of 30 mL of 1% sodium caseinate (Oren et al., 1963). The cells were washed twice with cold HBSS (300*g* for 5 min); they were ~90% macrophages, ~10% neutrophils, and ≥98% intact (trypan blue exclusion).

Peritoneal neutrophils were collected from guinea pigs 16 h after intraperitoneal injection of 25 mL of 10% sodium caseinate (Oren et al., 1963; ≥98% neutrophils, ≥98% intact).

To obtain peripheral lymphocytes, fresh guinea pig blood anticoagulated with acid–citrate–dextrose was centrifuged at 150*g* for 8 min. The pelleted cells at ~22 °C were diluted with 1 volume of Ca^{2+}/Mg^{2+}-free HBSS with 20 μg/mL leupeptin and were layered onto 17 mL of Ficoll–Hypaque. After centrifugation, the mononuclear interface cells were washed 1–4 times with Ca^{2+}/Mg^{2+}-free HBSS with 2% FCS to deplete platelets; they were cultured at 5×10^6/mL in RPMI 1640 with 2% FCS for 1 h in plastic tissue culture flasks to deplete monocytes. The nonadherent cells (lymphocytes) which were pelleted in HBSS were ≥98% viable.

To obtain peripheral blood neutrophils, pelleted blood cells (described above) were incubated with 1 volume of 2% dextran in 0.15 M NaCl at ~22 °C for 30–40 min to sediment erythrocytes. The supernatant was aspirated, and the leukocytes were fractionated by Ficoll–Hypaque centrifugation. Residual erythrocytes were removed from the Ficoll–Hypaque pellet by water lysis. The neutrophils were washed twice in HBSS; they were ≥98% viable.

Radioiodination of Surface Moieties, Cell Lysis, and Lentil Lectin Chromatography. Macrophages, neutrophils, and lymphocytes were labeled with ^{125}I using lactoperoxidase and H_2O_2 as described (Remold-O'Donnell, 1980). The cells were lysed with 0.5% NP-40, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM DFP, and 3 mM iodoacetamide, and, where

† This work was supported by American Cancer Society Grant IM-341, U.S. Public Health Service Grant CA21225, and Research Career Development Award CA00620 (E.R.-O.) from the National Cancer Institute, Department of Health and Human Services.

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[1] E. Remold-O'Donnell, manuscript in preparation.

[2] Abbreviations: HBSS, Hanks' balanced salt solution; mAb, monoclonal antibody; PBS, phosphate-buffered saline; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; SDS, sodium dodecyl sulfate; NP-40, the detergent Nonidet P-40; ramIgG, affinity-purified IgG fraction of rabbit anti-mouse IgG antiserum; Iodogen, 1,3,4,6-tetrachloro-3α,6α-diphenylchloroglycoluril; MAM, macrophage adhesion molecule; FCS, fetal calf serum; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DFP, diisopropyl fluorophosphate; DTSP, dithiobis(succinimidyl propionate); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0006-2960/88/0427-0037$01.50/0

indicated, lentil lectin adherent proteins were purified.

Cross-Linking of gp160–gp93. Macrophages were treated with dithiobis(succinimidyl propionate) (DTSP; Pierce Chemical Co., Rockford, IL) in HBSS for 20 min at ~22 °C. The reaction was terminated with 1 mM lysine, and the cells were pelleted in HBSS. In addition, macrophage extracts dialyzed against 50 mM sodium borate buffer, pH 9.0, and 100 mM NaCl were incubated with or without 25 μM DTSP; excess reagent was removed by dialysis.

SDS Electrophoresis. For SDS electrophoresis (Laemmli, 1970), fractions were heated with an equal volume of 2% SDS in 60 mM Tris–glycine buffer, pH 6.8, with or without 2% mercaptoethanol for 2 min at 100 °C. The resolving gel was 370 mM Tris-HCl buffer, pH 8.8, 7.5% polyacrylamide, 0.2% bis(acrylamide), and 0.1% SDS; the stacking gel was 60 mM Tris-HCl buffer, pH 6.8, 4% polyacrylamide, 0.1% bis-(acrylamide), and 0.1% SDS; and the running buffer was 42 mM Tris–190 mM glycine, pH 8.3, with 0.1% SDS. Molecular weights of 200 000, 130 000, 94 000, 68 000, 40 000, 29 000, and 22 000 were indicated by myosin, β-galactosidase, phosphorylase *a*, albumin, creatine kinase, carbonic anhydrase, and soybean trypsin inhibitor, respectively.

Double-Detergent (NP-40–SDS) Electrophoresis. Cell fractions were prepared for double-detergent electrophoresis by dilution to 0.12% NP-40 and 60 mM Tris–glycine buffer, pH 6.8 at ~22 °C. The resolving gel, stacking gel, and running buffer contained the SDS electrophoresis components plus 0.1% NP-40.

Two-Dimensional Electrophoresis. In a modified procedure (O'Farrell, 1975), the sample was applied to the basic end of a 2.4-mm tube gel containing 3.3% polyacrylamide, 9.2 M urea, 3.2% Triton X-100, 0.3% ampholytes (Ampholine, LKB) of range pH 4–6, 4.2% of range pH 5–7, and 0.5% of range pH 3.5–10 and focused for 20 h at 400 V and for 2 h at 1000 V. pH values are averages of water extracts of duplicate 5-mm slices. The focused gels were incubated in 80 mM Tris-HCl, pH 6.8, 2% SDS, and 5% mercaptoethanol for 50 min at ~22 °C and separated on SDS electrophoresis.

Monoclonal Antibodies. Balb/c or Balb/cByJ female mice (Jackson Laboratory, Bar Harbor, ME, and Cumberland View Farms, Clinton, TN) received intravenous injections of 10^6 metrizamide-purified macrophages (Remold-O'Donnell, 1982) at 0, 2, 6, and 18 weeks (mouse 1; M2, M3, and M4 mAb) or 0, 2, 6, 11, and 14 weeks (mouse 2; M6 mAb) or 0, 2, 8, and 22 weeks (mouse 5; M15, M16, and M17 mAb). Other mice received 3×10^6 macrophages intraperitoneally and at 2, 7, and 12 weeks 10^6 macrophages intravenously (mouse 3; M8 mAb) or at 2 and 8 weeks 10^6 macrophages intravenously and at 28 weeks 20 μg of pure gp93 intravenously (mouse 4; M7, M11, M12, M13, and M14 mAb). Mouse 6 (M25 and M26 mAb) received 20 μg of pure gp160 in CFA subcutaneously, and at 2 weeks 10 μg of gp160 in IFA subcutaneously, and at 5 weeks 5 μg of gp 160 in IFA subcutaneously, 5 μg intraperitoneally, and 5 μg in saline intravenously. All mice were sacrificed 3 days after the final injection; spleen cells were mixed at a 10:1 ratio with NS-1 myeloma cells and 35% poly(ethylene glycol) and hybridized as described (Kennett et al., 1978).

After 2–3 weeks of culture in 480 microtiter wells, supernatants from the largest colonies were assayed by immunoprecipitation of ^{125}I-labeled macrophage lentil lectin adherent fraction, SDS electrophoresis, and autoradiography. Colonies secreting antibodies that immunoprecipitate MAM were cloned at limiting dilution (M2, M4, M7, M8, M11, M12, M13, M15, M17, and M25). Colonies secreting antibodies that immunoprecipitate the precursor glycopeptides (M14 and M26) were selected by immunoprecipitation of macrophage lysates pulse-labeled with [^{35}S]methionine (Remold-O'Donnell, 1987). Balb/c mice received 0.5 mL of pristane intraperitoneally, followed after ~3 weeks by 10^7 hybridoma cells; ascites fluid was collected 1.5–2 weeks thereafter. Homogeneous antibodies were prepared from ascites by adherence at pH 8.0 to protein A–agarose and elution at low pH (Ey et al., 1978).

Antibodies were classified as anti-gp160 (anti-MAM-α) (M2, M4, M17, M25, and M26) or anti-gp93 (anti-MAM-β) (M7, M8, M11, M12, M13, M14, and M15) based on immunoblots and/or immunoprecipitation of the dissociated glycopeptides.[1] Other cloned cell lines from these fusions are M6 (IgG1, anti-gp~200) and M3 and M16 (IgG1, anti-gp~54).

Immunoblots. Cell lysates were separated on 1.5-mm SDS electrophoresis gels and transferred to 0.2-μm nitrocellulose sheets (Towbin et al., 1979) (Schleicher & Schuell, Keene, NH) at constant 60 mA (160 V/1.6 A power supply; Bio-Rad Laboratories, Richmond, CA) with 42 mM Tris–190 mM glycine buffer, pH 8.3, for 18 h at ~22 °C in a Bio-Rad Trans-Blot cell.

The nitrocellulose membranes were treated at ~22 °C with 0.05% Tween-20 (Pierce Chemical Co.) in PBS containing 5% w/v milk solids (Carnation Co., Los Angeles, CA) (Tween–PBS–5% milk) for 30 min, with five changes of Tween–PBS over 25 min, with mAb (ascites diluted 1 to 1000 or culture supernatant at 1 to 10) in Tween–PBS–0.1% milk for 60 min, with five changes of Tween–PBS, and with 0.5–1.0 μg/mL ^{125}I-labeled, affinity-purified rabbit antibodies to mouse IgG (ramIgG; 0.5 μCi/μg) in Tween–0.1% milk–20 mM sodium phosphate buffer, pH 7.2, and 150 mM NaI for 60 min. The membrane was washed with Tween–PBS (five changes), PBS, and water and was subjected to autoradiography.

ramIgG was prepared from the IgG fraction of rabbit anti-mouse IgG (H and L chains) antiserum (Cappell Laboratories Inc., Cochransville, PA) by chromatography on normal mouse IgG (Cappell) coupled at 2 mg/mL to CNBr-activated Sepharose 4B as described (Mellman et al., 1980). The IgG (200 μg in 100 μL) was reacted with 1 mCi of Na^{125}I and 10 μg of Iodogen for 50 min at 4 °C; it was purified as described (Remold-O'Donnell et al., 1986) and stored at −20 °C at 100 μg/mL in PBS–0.5% milk.

Binding of ^{125}I-Labeled M2 mAb to Macrophages and Neutrophils. M2 IgG (200 μg) was reacted with 1 mCi of Na^{125}I and 10 μg of Iodogen, and the ^{125}I-labeled IgG was purified and characterized (Remold-O'Donnell, 1986). Macrophages or neutrophils at 10^7/mL were preincubated in Eagle's minimum essential medium with 5 mg/mL BSA and 100 μg/mL nonimmune guinea pig IgG (Cappel Labs) at 4 °C for 20 min. Duplicate sets of 1.3×10^6 cells were incubated in 130 μL of the same medium with four to six concentrations (4–120 nM) of ^{125}I-labeled M2 mAb for 60 min at 4 °C in the absence or presence (nonspecific binding) of 0.6 or 1.0 μM nonradiolabeled M2 mAb; 10^6 cells were layered onto 200 μL of 10% metrizamide and 1 mg/mL BSA in PBS in a 4 × 40 mm conical polyethylene tube which was centrifuged at 4000*g* for 2.5 min (Fisher Model 59 centrifuge). The tips of the tubes were cut off, and cell-bound ^{125}I-mAb was quantified by γ counting.

Immunoprecipitation. Fixed *Staphylococcus aureus* (The Enzyme Center, Boston, MA) or protein A–agarose was incubated at ~22 °C for 30 min with rabbit antiserum to mouse IgG (Miles Laboratories, Elkhart, IN) (20 μL of antiserum/6 mg of bacteria or resin). The resulting complexes were washed

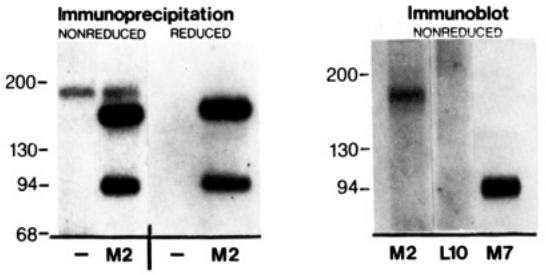

FIGURE 1: (Left panel) Immunoprecipitation of ^{125}I-labeled glycoproteins from macrophage lysates. Shown is an autoradiograph of M2 mAb immunoprecipitate (M2) and control precipitate (–) separated by SDS electrophoresis under nonreducing and reducing conditions. The molecular weights of marker proteins are indicated on the left. (Right panel) Immunoblot of macrophage lysate probed with various mAbs. Macrophage lysate was fractionated by nonreducing SDS electrophoresis, transferred to nitrocellulose, and treated with the indicated antibodies followed by ^{125}I-labeled ramIgG. Autoradiography exposures were 36 h for M2 mAb and the control mAb L10 (Remold-O'Donnell et al., 1984) and 4 h for M7, an anti-gp93 mAb.

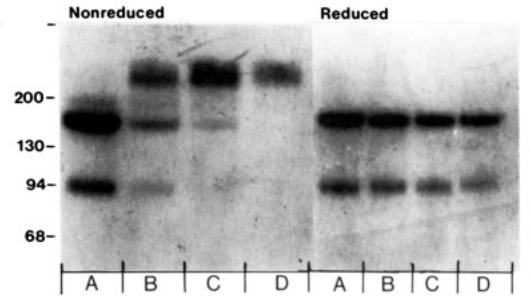

FIGURE 2: Cross-linking of macrophage surface glycoproteins by DTSP. Shown is an autoradiograph of an SDS electrophoresis gel of M2 immunoprecipitates of lysates of ^{125}I-labeled macrophages treated with (A) 0, (B) 50, (C) 150, and (D) 500 μM DTSP examined under nonreducing conditions and after treatment with mercaptoethanol to cleave the cross-linking agent.

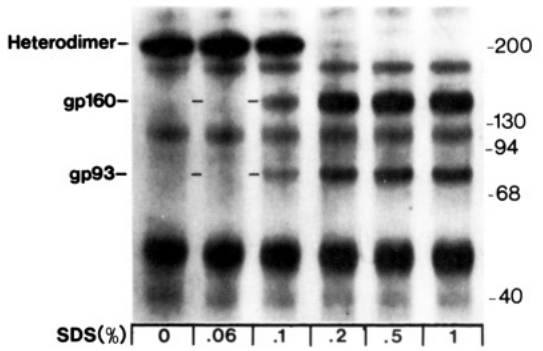

FIGURE 3: Double-detergent (NP-40–SDS) electrophoresis of macrophage glycoproteins. ^{125}I-Labeled glycoproteins purified by lentil lectin chromatography were prepared for electrophoresis using double-detergent conditions (0.12% NP-40, left lane) or by addition of SDS (concentrations indicated) at ~22 °C to samples containing 0.12% NP-40. The positions of marker proteins (solubilized with 1% SDS) are indicated on the right; this technique is not appropriate for molecular weight determination (see text). The identities of gp160 and gp93 (indicated on the left) were verified when the pure glycopeptides became available. Note the disappearance of the heterodimer and the appearance of the glycopeptides gp160 and gp93 as the SDS concentration is increased.

once in buffer B (10 mM Tris-HCl, pH 8.6, 0.1% SDS, 0.05% NP-40, and 300 mM NaCl) and once in buffer A (12 mM sodium phosphate buffer, pH 7.4, and 200 mM NaCl). The complexes were incubated with hybridoma culture supernatant (200 μL/6 mg of bacteria or resin) at ~22 °C for 1.5 h, and the washing procedure was repeated. The ternary complexes were incubated for 1–3 h with 50–200 μL of ^{125}I-labeled cell lysate or purified fractions. The complexes were washed twice and extracted for electrophoresis at 100 °C with 2% SDS in Tris–glycine buffer, pH 6.8. For isoelectrofocusing, directly conjugated M2–Sepharose (Remold-O'Donnell & Savage, 1988) was used as the precipitiating agent, and the washed complexes were extracted twice at 50 °C for 20 min with 9.5 M urea, 0.5% NP-40, and 60 mM dithiothreitol. To screen hybridomas, 75 μL of culture supernatant was incubated with 75 μL of ^{125}I-labeled macrophage lentil lectin adherent fraction or with lysates of macrophages pulse-labeled with [^{35}S]-methionine (Remold-O'Donnell, 1987) for 2 h and then combined with 6 mg of fixed *S. aureus* containing bound rabbit antibodies to mouse IgG.

RESULTS

Glycopeptides gp160 and gp93 Exist as a Heterodimer in the Macrophage Surface Membrane: Coprecipitation. The monoclonal antibody M2 was found to immunoprecipitate two ^{125}I-labeled macrophage components, gp160 and gp93 (Figure 1, left panel); these were previously shown to be glycopeptides (Remold-O'Donnell, 1980). When examined by immunoblotting, M2 antibody reacts with gp160 and not with gp93 (Figure 1, right panel), demonstrating that the M2 epitope is expressed only on gp160 and suggesting that gp93 coprecipitates via its binding to gp160.

Interpeptide disulfide bonds are not responsible for the gp160–gp93 association since the glycopeptides migrate separately under nonreducing conditions (Figure 1). The chelating agents EDTA and EGTA (2 mM) did not prevent the coprecipitation of gp93 and gp160 by M2 antibody (not shown), suggesting that gp160–gp93 association is not exclusively due to divalent cations.

Cross-Linking of gp160,gp93. The reversible cross-linking reagent dithiobis(succinimidyl propionate) (DTSP; Lomant 1976) was used to determine whether gp160 and gp93 are associated prior to interaction with antibody. Treatment of macrophages with DTSP yielded a cross-linked component of M_r ~250 000 revealed by immunoprecipitation of lysates with M2 mAb (Figure 2A). Cleavage of the cross-linking moiety with mercaptoethanol converted the M_r ~250 000 component to M_r 160 000 and 93 000 (Figure 2A). Identical results were obtained when lysates were cross-linked with DTSP (not shown). Thus, gp160 and gp93 exist in close proximity on the macrophage surface and in cell lysates.

Analysis of gp160,gp93 by Double-Detergent Electrophoresis. To examine gp160–gp93 association, we developed the technique of double-detergent electrophoresis, which employs the nonionic detergent NP-40 together with low concentrations of SDS. On double-detergent electrophoresis, (a) most macrophage surface polypeptides migrate at positions close to, but not necessarily identical with, their positions on SDS electrophoresis (not shown), (b) gp160,gp93 in cell lysates migrates as a heterodimer of approximate M_r >200 000 (Figure 3, left lane), and (c) gp160,gp93 which has been treated with SDS migrates as separate glycopeptides (Figure 3). Thus, double-detergent electrophoresis directly demonstrates the association of gp160 with gp93.

Cumulatively, coprecipitation by monoclonal antibodies, cross-linking on the cell surface, and migration as a complex on double-detergent electrophoresis demonstrate that gp160 and gp93 are subunits of a heterodimer molecule. The gp160,gp93 molecule has been given the alternative name MAM (macrophage adhesion molecule; see Discussion).

MAM-α Is Trypsin Sensitive. The α-chain of MAM, gp160, is identical with the surface glycopeptide previously

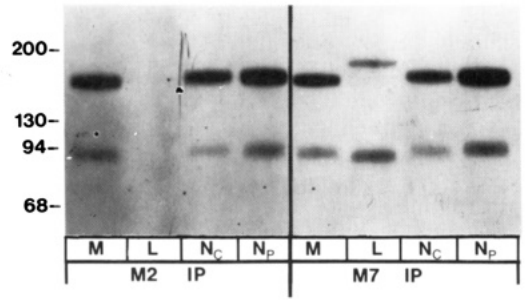

FIGURE 4: Immunoprecipitation of ^{125}I-labeled surface glycoproteins from lysates of macrophages, lymphocytes, and neutrophils. Shown is an autoradiograph of a reducing SDS electrophoresis gel of (M) macrophages, (L) lymphocytes, (Nc) circulating blood neutrophils, and (Np) peritoneal neutrophils immunoprecipitated as indicated with the anti-gp160 antibody M2 or the anti-gp93 antibody M7.

designated as particularly trypsin and plasmin sensitive (Remold-O'Donnell, 1980). M2 immunoprecipitates prepared from macrophages, which were treated with 6 μg/mL trypsin, migrated on SDS electrophoresis at M_r 160 000 and 93 000 without reduction and after reduction at M_r 93 000, 85 000, and 71 000 as previously established for the trypsin-sensitive glycopeptide (not shown).

Quantifying MAM on Macrophages. MAM molecules on intact macrophages were quantified by dose-dependent binding of ^{125}I-labeled M2 antibody. Scatchard analysis of the binding data indicated 630 000 binding sites (mean of eight experiments), corresponding to 630 000 surface MAM molecules per macrophage, assuming 1:1 binding of antigen and antibody. The amount of intracellular MAM in macrophages was shown to be negligible compared to the amount of surface MAM based on quantitative cleavage of the α-subunit (gp160) by trypsin treatment of intact macrophages (Remold-O'Donnell, 1982).

Related Molecules on Neutrophils and Lymphocytes. We questioned whether MAM is present on other cells. The anti-gp160 mAb M2 precipitates two glycopeptides from ^{125}I-labeled peritoneal neutrophils and circulating blood neutrophils; these comigrate with gp160 and gp93 of macrophages (Figure 4, first, third, and fourth lanes), suggesting identity. M2 mAb does not precipitate ^{125}I-labeled glycoproteins of blood lymphocytes (Figure 4, second lane), indicating that blood lymphocytes express no detectable surface gp160. M7, an anti-gp93 mAb (Figure 1, immunoblot), precipitates gp160,gp93 from macrophages and neutrophils and, in addition, an apparent gp180,gp93 heterodimer from lymphocytes (Figure 4). These findings suggest that macrophages and neutrophils, on the one hand, and lymphocytes, on the other hand, express different surface heterodimers consisting of a common smaller glycopeptide (gp93) associated with different larger glycopeptides (gp160 and gp180). The gp180,gp93 molecule of lymphocytes was not further examined. Very low levels of a gp180,gp93 heterodimer precipitable by M7 mAb, but not by M2, were detected in some lysates of peritoneal neutrophils (not shown).

Comparison of gp160,gp93 on Macrophages and Neutrophils. The relatedness of macrophage gp160,gp93 and neutrophil gp160,gp93 was examined by using multiple mAb. Both gp160,gp93 heterodimers are precipitated by M7 (Figure 4), M8, M11, M12, M13, and M15 antibodies (not shown), all of which are anti-gp93 mAb, indicating that the smaller subunits have multiple shared epitopes and are probably identical. The macrophage and neutrophil heterodimers are also precipitated by the anti-gp160 antibodies M2 (Figure 4), M4, M17, and M25 (not shown), indicating that the larger

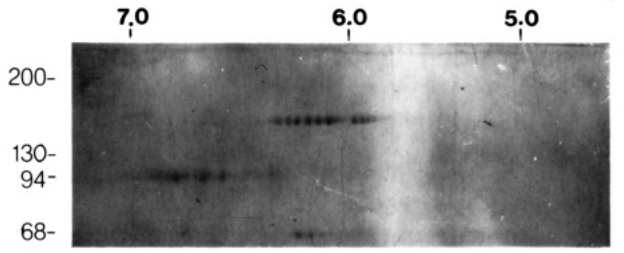

FIGURE 5: Two-dimensional analysis of macrophage gp160 and gp93. Shown is a Coomassie blue stained gel of polypeptides immunoprecipitated with M2–Sepharose. Isoelectrofocusing is on the horizontal axis and SDS electrophoresis (reducing conditions) on the vertical axis.

subunits also have multiple shared epitopes.

Isoelectrofocusing of gp160,gp93. On isoelectrofocusing, macrophage gp160 was found at pH 5.8–6.3; macrophage gp93 focused at pH 6.4–7.0 (Figure 5). Both gp160 and gp93 focused as a series of defined spots, indicating microheterogeneity. Neutrophil gp160 and gp93 were indistinguishable from macrophage gp160 and gp93 on isoelectrofocusing (not shown), providing further evidence that the neutrophil and macrophage heterodimers are identical.

Quantifying MAM on Neutrophils. The number of binding sites for ^{125}I-labeled M2 mAb on peritoneal neutrophils averaged 210 000 (two experiments), indicating that there are approximately 210 000 MAM molecules on the neutrophil surface. Trypsin treatment of ^{125}I-labeled neutrophils, which distinguishes surface molecules (trypsin sensitive) from intracellular molecules (trypsin insensitive), indicated that neutrophils, unlike macrophages, contain a significant intracellular pool of MAM (not shown).

Discussion

MAM, a surface molecule of guinea pig peritoneal macrophages, is characterized as a heterodimer of the protease-sensitive glycopeptide gp160 and the glycopeptide gp93. gp160 and gp93, also called MAM-α and MAM-β, are coprecipitated by mAb directed against either glycopeptide and can be cross-linked in intact macrophages. Their association can be directly demonstrated by a new technique, double-detergent electrophoresis. The name MAM (macrophage adhesion molecule) acknowledges the role of this molecule in mediating adhesion and spreading of macrophages on surfaces, in that mAbs directed against certain epitopes of MAM-α and MAM-β specifically and significantly inhibit adhesion.[1]

The MAM molecule appears to be the guinea pig analogue of the human molecule Mo1 and the mouse molecule Mac-1 for the following reasons. Mo1, Mac-1, and MAM are noncovalently linked surface heterodimer molecules; the apparent molecular weights of their component glycopeptides are similar (Ho & Springer, 1982; Todd et al., 1982). Mac-1, like MAM, is a particularly prevalent molecule on macrophages (Ho & Springer, 1982). Mo1 and Mac-1 were each demonstrated to be members of a family of heterodimer molecules with identical β-subunits and different α-subunits. There are three known members of the human family, Mo1, LFA-1, and p150/95 (Lanier et al., 1985), all of which are adhesive molecules (Bullock & Wright, 1987). The presence of gp180,gp93 on guinea pig lymphocytes suggests that MAM is part of a family of heterodimer molecules with identical β-subunits and different α-subunits. Mo1 and Mac-1 are expressed on monocytes/macrophages, neutrophils, and natural killer cells (Springer et al., 1979; Todd et al., 1981). MAM was identified on macrophage and neutrophils. Finally, with the exception of two conservative substitutions, the N-terminal

amino acid sequence of MAM-α (gp160) is identical with those of Mol-α (15 residues compared) and Mac-1-α (18 residues compared) (Pierce et al., 1986).

MAM shares with Mol and Mac-1 the function of mediating the adhesion and spreading of macrophages on surfaces. This function was clearly demonstrated by inhibition of macrophage adherence by four anti-MAM monoclonal antibodies.[1]

In addition to mediating adhesion, Mac-1 and Mol display receptor activity for particles coated with the complement component C3bi (CR3 activity; Beller et al., 1982; Arnaout et al., 1983; Wright et al., 1983). In the case of Mol, CR3 activity and cell adhesion function were shown to residue in different regions of the molecule defined by different mAbs (Dana et al., 1986; Ross et al., 1985). Attempts thus far to demonstrate CR3 activity of MAM by inhibiting the binding of C3bi-coated particles to macrophages with anti-MAM mAb have been unsuccessful (not shown). It is, therefore, not presently known whether MAM possesses CR3 activity.

Some conclusions can be made about the association of the two glycopeptides in the MAM heterodimer. Interpeptide disulfide bonds are not involved (Figure 1). Neither the nonionic detergent NP-40 nor the chelating agents EDTA/EGTA dissociate the heterodimer, indicating that hydrophobic bonds and Ca^{2+} bonds, respectively, are not sufficient to account for the association. In its insensitivity to EDTA/EGTA, MAM differs from another adhesive molecule, GPIIb/IIIa, the fibrinogen receptor of human platelets, which is a Ca^{2+}-dependent heterodimer (Kunicki et al., 1981). Cumulatively, these negative findings suggest that the gp160–gp93 association is maintained by ionic interactions, a hypothesis consistent with the dissociation of the heterodimer by acid (Remold-O'Donnell & Savage, 1988).

Whereas MAM is strictly a surface molecule in macrophages, the neutrophil molecule is found both on the cell surface and in intracellular pools. In biochemical properties, the MAM molecule of neutrophils is indistinguishable from MAM of macrophages on the basis of electrophoresis, isoelectrofocusing, and reactivity with 10 monoclonal antibodies. This latter finding is of practical as well as theoretical importance since it sets the stage for purification of MAM from the more prevalent source, the peritoneal neutrophil (Remold-O'Donnell & Savage, 1988).

Acknowledgments

We thank Drs. Dianne Kenney and Chester Alper for critical reading of the manuscript and Pam DiBona and Anandi Mehta for skillful assistance.

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