CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 46, 272–283 (1988)

Modulation of Surface CD11/CD18 Glycoproteins (Mo1, LFA-1, p150,95) by Human Mononuclear Phagocytes

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Mo1, LFA-1, and p150,95 are structurally related glycoproteins of the CD11/CD18 complex that are expressed on the membrane of human leukocytes. In the neutrophil, the surface expression of the CD11/CD18 complex is up-modulated (Mo1 > p150,95 ≫ LFA-1) by stimulatory factors that include calcium ionophore A23187, phorbol myristate acetate (PMA), and *N*-L-formyl-L-leucyl-L-phenylalanine (fMLP). Here, in an immunofluorescence analysis, we have examined CD11/CD18 glycoprotein expression by human monocytes, pulmonary alveolar macrophages (PAM, obtained by bronchoalveolar lavage), and breast milk macrophages (BMM) as compared to neutrophils before and after exposure to A23187 (1 μ*M*), fMLP (0.1 μ*M*), or PMA (0.1 μg/ml) for 15 min at 37°C. Unstimulated monocytes within unfractionated blood mononuclear cells kept at 4°C (n = 13) expressed all three CD11/CD18 glycoproteins, and exposure to A23187 resulted in significant increases in the surface expression of Mo1 (median of 5.7-fold), LFA-1 (median of 2.1-fold), and p150,95 (median of 7.2-fold). Exposure to fMLP- or PMA-stimulated increases of lesser magnitude. CD11/CD18 expression by PAM (n = 9) was barely detectable and was unaffected by exposure to A23187. In contrast, BMM (n = 11) expressed all three CD11/CD18 glycoproteins (with considerable variability among specimens), but no increase was stimulated by A23187. These results demonstrate that monocytes, like neutrophils, have the capacity to respond to activating factors with an increase in CD11/CD18 glycoprotein expression; macrophage differentiation is accompanied by a loss (PAM) or retention (BMM) of CD11/CD18 expression that is unmodulated in response to activation.

INTRODUCTION

The CD11/CD18 complex consists of three plasma membrane glycoproteins, Mo1 (CD11b/CD18), LFA-1 (CD11a/CD18), and p150,95 (CD11c/CD18), expressed by murine and human leukocytes (see (1, 2) for reviews). These molecules are heterodimeric glycoproteins, each consisting of a distinct higher molecular weight α subunit (containing the CD11a, b, and c antigenic epitopes) that is noncovalently associated with a structurally identical 95-kDa β subunit (containing the CD18 epitope). The generation of several murine monoclonal antibodies specific for the CD11a, b, c, and CD18 epitopes has made it possible to quantitate the expression of these glycoproteins on the cell surface and to ascertain their functional significance *in vitro*. Mo1 (CD11b/CD18; gp 155,95) is ex-

0090-1229/88 $1.50

pressed by human neutrophils, monocytes and certain macrophages, null cells, and myeloid leukemic blasts (3, 4). It serves as a receptor for C3bi (CR3) (5–7) and plays a role in neutrophil aggregation (8, 9) and neutrophil/monocyte adhesion to substrates (10–12). LFA-1 (CD11a/CD18; gp 177,95) is expressed by both lymphoid and myeloid cells and promotes lymphoid cell-adhesive interactions that include lymphocyte blastogenesis and cell-mediated cytotoxicity (13–16). p150,95 (CD11c/CD18), the third and most recently characterized member of the CD11/CD18 complex, is found on human neutrophils and monocytes (17, 18) and may contribute to the adherence of these cells to endothelial cell monolayers (19). An inherited deficiency in leukocyte expression of CD11/CD18 glycoproteins is associated with impaired immunity against pyogenic microorganisms (see (20, 21) for review).

The identification of the CD11/CD18 glycoprotein complex has led to an investigation of cellular mechanisms that modulate the expression of these adhesion-promoting glycoproteins on the plasma membrane of human leukocytes. It had previously been reported that the surface expression of several leukocyte receptor structures such as the receptors for C3b (CR1) and fMLP were increased as a result of isolation procedures or after exposure of the cells to various factors that mimic the effects of inflammatory or chemotactic stimuli (22, 23). On the basis of these observations, we and others examined human peripheral blood neutrophils and monocytes to determine if the surface expression of the CD11/CD18 glycoproteins were affected in a similar fashion. It was found that exposure of neutrophils to soluble activating factors such as calcium ionophore A23187, *N*-L-formyl-L-leucyl-L-phenylalanine (fMLP), or phorbol myristate acetate (PMA) resulted in a 5- to 10-fold increase in surface Mol (24–26) and p150,95 (17, 18) expression; the surface density of LFA-1 was relatively unaffected by this stimulation (17, 25). Whereas peripheral blood monocytes purified by adherence to plastic substrate failed to exhibit significant change in Mol or LFA-1 expression in response to A23187, PMA, or fMLP (27), it was found that elutriation-purified or unfractionated mononuclear cell monocytes did respond to soluble stimuli (C5a and fMLP) with an increase in Mol (28) and p150,95 (18).

While these observations suggest that CD11/CD18 glycoprotein expression by circulating monocytes may be under regulatory control, little is known about the expression of the CD11/CD18 complex by members of the human mononuclear phagocyte system that exist outside of the intravascular compartment, i.e., tissue macrophages. These cells contribute to the "front line" defense against pathogenic microorganisms and could rely on the CD11/CD18 surface glycoproteins to facilitate adhesion-dependent interactions. In order to determine if representative tissue macrophages respond to inflammatory factors with an up-modulation of surface Mol, LFA-1, and p150,95 expression, we have made a comparison of CD11/CD18 glycoprotein expression by peripheral blood monocytes, pulmonary alveolar macrophages (PAM), and breast milk macrophages (BMM) before and after stimulation of these cells by exposure to soluble factors (calcium ionophore A23187, PMA, and fMLP) that up-modulate CD11/CD18 surface density by peripheral blood neutrophils. By surface immunofluorescence analysis, we have

found that the expression of Mo1, p150,95, and, to a lesser extent, LFA-1, is up-regulated by brief exposure of monocytes to soluble activating factors at 37°C, when compared to baseline antigen expression by monocytes subjected to minimal isolation processing and maintained at 4°C. In contrast to the capacity of monocytes and neutrophils to respond to activating stimuli with an increase in CD11/CD18 expression, differentiated macrophages, as represented by PAM and BMM, do not exhibit significant alteration in surface antigen density. Baseline antigen expression by macrophages is heterogenous, ranging from negligible to barely detectable CD11/CD18 expression by PAM, and variable degrees of CD11/CD18 expression by BMM. The potential physiological significance of these findings is discussed.

MATERIALS AND METHODS

Reagents. The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): calcium ionophore A23187 (C-7522), fMLP (F-3506), PMA (P-8139), and dimethylsulfoxide (DMSO, D-5879). A23187, fMLP, and PMA were dissolved in DMSO and stored as stock solutions at −80°C until used. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG + IgM antibodies were obtained from Tago, Inc. (Burlingame, CA).

Solutions. Phosphate-buffered saline (PBS, without calcium or magnesium) and Dulbecco's phosphate-buffered saline (DPBS) were mixed according to standard formulations using reagent grade chemicals. Hanks' balanced salt solution (HBSS) was obtained from KC Biologicals (LM-218, Lenexa, KS).

Monoclonal antibodies. The generation and characterization of murine monoclonal antibodies anti-Mo1 (anti-CD11b, IgG2a; clone 44) (5), anti-LFA-1 (anti-CD11a, IgG1; clone L11) (15), anti-p150,95 (anti-CD11c, IgG1; clone L29) (M. A. Arnaout and L. L. Lanier, unpublished results, July 1986), anti-β (anti-CD18, IgG2a; clone IB4) (7), anti-Ia (IgG2a; clone 9-49) (29), anti-Mb1 (IgG1; clone U42) (30), anti-J5 (anti-CD10, IgG2a) (31), and anti-Mo2 (anti-CD14, IgG2b) (32) have been described. Antibodies L11, L29, and IB4 were gifts from Drs. Hergen Spits (Netherlands Cancer Institute, Amsterdam), Lewis L. Lanier (Becton–Dickinson Monoclonal Center, Mountain View, CA), and Samuel D. Wright (Rockefeller University, New York, NY), respectively. Antibody-containing ascites was used in all immunofluorescence assays (with the exception of antibody IB4 which was purified immunoglobulin).

Isolation of human cells. After Human Protection Committee approval and informed consent, specimens of human cells were obtained either from normal volunteers or from patients undergoing diagnostic procedures. Peripheral blood leukocytes were obtained by venipuncture of volunteer adult donors. Heparinized whole blood specimens were diluted 1:1 with HBSS, underlaid with Ficoll–Hypaque (Histopaque-1077, Sigma Diagnostics), and subjected to density gradient centrifugation (33) for 25 min at 25°C. All subsequent steps were carried out at 4°C. The granulocyte–erythrocyte pellet was washed once with PBS and subjected to distilled water lysis for 45 sec. Isotonicity was restored, and granulo-

cytes were washed in PBS and resuspended in DPBS. Viability was consistently >90% by trypan blue staining. Mononuclear cells from the Ficoll–Hypaque interface layer were washed twice in PBS and resuspended in DPBS. Viability was consistently >85%.

Pulmonary alveolar macrophages (PAM) were recovered from the bronchoalveolar lavage fluid either of normal volunteers or of patients undergoing diagnostic bronchoscopy (for evaluation of isolated pulmonary nodules). Donors included both smokers and nonsmokers. Specimens were filtered through loose gauze to remove mucous strands, diluted in PBS, washed once, and resuspended in DPBS (all steps at 4°C). Specimens were 94% ± 8 macrophages (as assessed in 7/16 specimens) by morphologic criteria (Wright–Geimsa staining).

Breast milk macrophages (BMM) were isolated from breast milk (15–150 ml) donated by normal lactating volunteers who were generally less than 1 week postpartum (range 1 day to 9 months). Milk (or colostrum) was diluted 1:1 with HBSS and cells were pelleted, washed twice with PBS, and resuspended in DPBS. Specimens were 78% ± 11 macrophages (as assessed in 6/11 specimens) by morphologic criteria and were kept at 4°C throughout.

In vitro stimulation of leukocytes. Leukocytes were suspended in DPBS at a concentration of 2–10 × 10^6 cells/ml and placed in polypropylene culture tubes (Falcon Plastics, Oxnard, CA). The cells were stimulated by the addition of A23187 (1 μ*M*), fMLP (0.1 μ*M*), or PMA (0.1 μg/ml) and incubated for 15 min at 37°C. Control cells received an equivalent amount of DMSO diluent (0.1% v/v) and were incubated for 15 min at either 4 or 37°C. Cells were then diluted in ice-cold PBS, pelleted, and resuspended in immunofluorescence wash buffer (DPBS containing 0.1% (w/v) glucose and 1 mg/ml Pentex human gamma globulins (82-310-2, ICN Immunobiologicals, Lisle, IL)).

Immunofluorescence flow cytometric analysis. Aliquots (100 μl) containing 0.2–2.0 × 10^6 cells were subjected to indirect immunofluorescence staining for the expression of CD11/CD18 determinants, Mo2 (CD14), and Ia relative to background staining using isotype-identical negative controls (anti-Ia, J5, or Mb1), as described (34). All antibodies were used in excess to assure saturation of all antigenic sites. Intact, viable cells were selected by bit map gating (log forward vs log 90° light scatter). Positive staining for Mo1, Mo2, and Ia plus Mo2 was used to select for neutrophils, monocytes, and macrophages, respectively. Immunofluorescence intensity as a measure of relative antigen expression was analyzed on a Coulter Electronics (Hialeah, FL) EPICS C flow cytometer with the use of a logarithmic amplifier. The channel number (log scale) representing the mean fluorescence intensity (major fluorescence peak) of 5000 cells exposed to either experimental or isotype-identical negative control antibodies was determined. The corresponding linear fluorescence intensity channel was calculated from a logarithmic-linear calibration formula (35). Specific fluorescence intensity represents the computed mean channel number (0–230 channels, linear scale) of cells stained with the experimental monoclonal antibody minus the mean channel of cells stained with the negative control reagent. For each experiment shown in Tables 1, 2, and 3, the mean fluorescence intensity (linear channel number) of cells stained with the negative control antibody is indicated in parentheses.

RESULTS

When human monocytes within the mononuclear cell fraction obtained by Ficoll–Hypaque density gradient centrifugation were exposed to A23187 (1 μM) for 15 min at 37°C and subsequently examined for the expression of the CD11/CD18 epitopes of Mo1, LFA-1, and p150,95, a reproducible increase in all three glycoproteins was observed, as compared to mononuclear cells kept at 4°C prior to immunofluorescence analysis (Table 1). As previously observed for the neutrophil (17, 24, 25), the most consistent increases were seen in the surface density of Mo1 and p150,95 (5.7- and 7.2-fold, respectively (median of 13 determinations), while the increase in LFA-1 was less dramatic (2.1-fold). Exposure of the mononuclear cells to fMLP (0.1 μM) or PMA (0.1 μg/ml) under the same conditions stimulated increases of lesser magnitude (2.0-, 1.6-, and 2.2-; 1.8-, 1.5-, and 1.9-fold median increase (n = 4) for Mo1, LFA-1, and p150,95 after fMLP and PMA stimulation, respectively). Warming of the mononuclear fraction (37°C for 15 min in DPBS containing DMSO diluent alone) resulted in -fold median increases in monocyte Mo1, LFA-1, and p150,95 glycoprotein expression of 1.6, 1.3, and 1.5, respectively (n = 4). It is therefore evident that the monocyte, like the neutrophil, can respond to a stimulus (the contribution of warming in the presence of an inflammatory agent) with an increase in the surface expression of the CD11/CD18 glycoprotein complex.

Since bone marrow-derived circulating monocytes may give rise to tissue macrophages during a process of differentiation (36), we next sought to determine whether the modulated expression of the CD11/CD18 glycoprotein complex is a consistent feature of the mononuclear phagocyte lineage. As representative examples of tissue macrophages, we chose to examine pulmonary alveolar macrophages (PAM) and breast milk macrophages (BMM) since these cells exist in fluid phase and require no purification processing prior to analysis. As shown in Table 2, PAM demonstrated very little baseline expression of Mo1 or p150,95 (as compared to background staining by isotypic negative control monoclonal reagents). Barely detectable expression of LFA-1 (as indicated by anti-CD11a and anti-CD18 immunofluorescent staining) was observed in several specimens. Ia antigen expression was a consistent finding confirming the immunological identification of PAM. Exposure of PAM to A23187 under conditions that stimulated maximal increases in monocyte or neutrophil CD11/CD18 glycoprotein expression failed to up-modulate the surface density of these determinants by PAM (Table 2). In experiments not shown, there was little detectable expression of CD11b or CD18 found in whole cell lysates of PAM subjected to SDS gel electrophoresis and Western blotting. Thus, in the case of PAM, the CD11/CD18 glycoprotein complex is minimally expressed and the magnitude of baseline expression is unaffected by exposure to A23187. In contrast, BMM (as shown in Table 3) generally expressed the constituents of the CD11/CD18 complex, but with considerable individual variation in baseline density, ranging from strongly positive (Experiment 1, Table 3) to barely detectable levels of expression (Experiment 3, Table 3). Despite this individual variability in baseline CD11/CD18 expression, the failure of A23187 (or PMA or fMLP in data not shown) to stimulate an increase in an-

TABLE 1

INCREASED SURFACE EXPRESSION OF CD11/CD18 GLYCOPROTEINS BY MONOCYTES STIMULATED BY CALCIUM IONOPHORE A23187[a]

		Specific immunofluorescence intensity							
		Expt 1		Expt 2		Expt 3		Expt 4	
Epitope	Glycoprotein	Control	A23187	Control	A23187	Control	A23187	Control	A23187
CD11b	Mo1α	12.72 (0.25)	44.85 (0.59)	2.73 (0.23)	17.38 (0.24)	9.57 (0.25)	54.96 (0.26)	2.99 (0.23)	25.01 (0.30)
CD11a	LFA-1α	5.49 (0.29)	16.25 (0.42)	4.13 (0.25)	9.84 (0.25)	10.42 (0.25)	20.60 (0.23)	4.28 (0.22)	24.33 (0.29)
CD11c	p150,95α	1.03 (0.29)	7.22 (0.42)	0.34 (0.25)	3.56 (0.25)	0.29 (0.25)	10.44 (0.23)	0.58 (0.22)	8.99 (0.29)
CD18	Common β	18.91 (0.25)	44.85 (0.59)	6.60 (0.23)	25.07 (0.24)	21.16 (0.25)	58.13 (0.26)	6.99 (0.23)	30.46 (0.30)
CDw14	Mo2	24.37 (0.25)	34.77 (0.59)	10.44 (0.23)	18.92 (0.24)	33.19 (0.25)	61.47 (0.26)	9.86 (0.23)	22.98 (0.30)

[a] Human peripheral blood mononuclear cells ($5-10 \times 10^6$ cells/ml of DPBS) were either stimulated with A23187 (1 μM) and incubated at 37°C for 15 min or exposed to DMSO diluent and kept at 4°C (control). Cells were then stained by indirect immunofluorescence for the expression of the CD11b, CD11a, CD11c, CD18, and CDw14 epitopes. PMT settings for Experiments 1–4 were 1125, 1150, 1200, and 1100 mV, respectively. Monocytes were selectively analyzed by flow cytometric gating using the monocyte-specific marker Mo2 (CD14). Numbers indicate the specific fluorescence intensities as calculated under Materials and Methods. Numbers in parentheses indicate the mean channel fluorescences of cells stained with an isotype-identical negative control antibody. These data are representative of those observed in an analysis of nine additional specimens.

TABLE 2

EXPOSURE OF PULMONARY ALVEOLAR MACROPHAGES TO CALCIUM IONOPHORE A23187 FAILS TO STIMULATE AN INCREASE IN CD11/CD18 GLYCOPROTEIN EXPRESSION[a]

		Specific immunofluorescence intensity							
		Expt 1		Expt 2		Expt 3		Expt 4	
Epitope	Glycoprotein	Control	A23187	Control	A23187	Control	A23187	Control	A23187
CD11b	Mo1α	0.01 (0.56)	0.01 (0.53)	0.04 (0.39)	0.08 (0.41)	0.07 (0.47)	0.12 (0.41)	1.53 (2.18)	0.68 (2.72)
CD11a	LFA-1α	0.21 (0.47)	0.11 (0.48)	0.42 (0.28)	0.40 (0.28)	0.57 (0.30)	1.60 (0.30)	1.78 (2.24)	1.01 (1.95)
CD11c	p150,95α	0.23 (0.47)	0.09 (0.48)	0.18 (0.28)	0.18 (0.28)	0.91 (0.30)	0.57 (0.30)	0.07 (2.24)	0.00 (1.95)
CD18	Common β	0.18 (0.56)	0.11 (0.53)	0.53 (0.39)	0.29 (0.41)	0.59 (0.47)	0.56 (0.41)	0.95 (2.18)	0.16 (2.72)
Ia		7.52 (0.56)	4.50 (0.53)	4.50 (0.39)	3.73 (0.41)	12.87 (0.47)	10.56 (0.41)	10.79 (2.18)	13.49 (2.72)

[a] Human pulmonary alveolar macrophages ($2-10 \times 10^6$ cells/ml in DPBS) were either stimulated with A23187 (1 μM) and incubated at 37°C for 15 min or exposed to DMSO diluent and kept at 4°C (control). Cells were then stained by indirect immunofluorescence for the expression of the CD11b, CD11a, CD11c, CD18, and Ia epitopes. PMT setting was 800 mV for all four experiments shown. Numbers indicate the specific fluorescence intensity as calculated under Materials and Methods. Numbers in parentheses indicate mean channel fluorescence of cells stained with an isotype-identical negative control antibody. These data are representative of those observed in an analysis of five additional specimens. PAM were obtained from a smoker with a pulmonary nodule (Experiment 1), a nonsmoker with hemoptysis (Experiment 2), a nonsmoker healthy volunteer (Experiment 3), and a smoker healthy volunteer (Experiment 4).

TABLE 3

EXPOSURE OF BREAST MILK MACROPHAGES TO CALCIUM IONOPHORE A23187 FAILS TO STIMULATE AN INCREASE IN CD11/CD18 GLYCOPROTEIN EXPRESSION[a]

		Specific immunofluorescence intensity							
		Expt 1		Expt 2		Expt 3		Expt 4	
Epitope	Glycoprotein	Control	A23187	Control	A23187	Control	A23187	Control	A23187
CD11b	Molα	19.33 (0.92)	19.51 (0.74)	1.13 (1.18)	1.52 (1.28)	0.12 (1.03)	0.31 (1.25)	10.32 (2.65)	12.53 (2.80)
CD11a	LFA-1α	18.69 (0.47)	16.96 (0.66)	1.15 (1.09)	1.37 (1.28)	0.20 (0.89)	0.00 (1.36)	3.10 (2.37)	3.87 (2.96)
CD11c	p150,95α	16.67 (0.47)	14.25 (0.66)	3.29 (1.09)	5.55 (1.28)	0.17 (0.89)	0.20 (1.36)	5.06 (2.37)	5.58 (2.96)
CD18	common β	26.60 (0.92)	28.35 (0.74)	2.53 (1.18)	3.22 (1.28)	0.09 (1.03)	0.27 (1.25)	13.11 (2.65)	11.70 (2.80)
Ia		4.86 (0.92)	5.20 (0.74)	30.45 (1.18)	34.08 (1.28)	6.82 (1.03)	7.29 (1.25)	12.68 (2.65)	10.54 (2.80)
CD14	Mo2	11.35 (0.92)	12.97 (0.74)	3.20 (1.18)	3.61 (1.28)	25.73 (1.03)	22.69 (1.25)	8.63 (2.65)	7.87 (2.80)

[a] Human breast milk macrophages ($2-10 \times 10^6$ cells/ml in DPBS) were either stimulated with A23187 (1 μM) and incubated at 37°C for 15 min or exposed to DMSO diluent and kept at 4°C (Control). Cells were then stained by indirect immunofluorescence for the expression of the CD11b, CD11a, CD11c, CD18, CD14, and Ia epitopes. PMT settings for Experiments 1–4 were 1050, 900, 1300, and 1100 mV, respectively. Numbers indicate the specific fluorescence intensities as calculated under Materials and Methods. Numbers in parentheses indicate mean channel fluorescence of cells stained with an isotype-identical negative control antibody. These data are representative of those observed in an analysis of seven additional specimens. BMM were obtained from healthy lactating volunteers at 6 weeks (Experiment 1), 4 days (Experiment 2), 8 days (Experiment 3), and 5 days (Experiment 4) postpartum.

tigen density was a consistent observation seen in an analysis of 11 specimens. Therefore, BMM, like PAM, do not respond to the A23187 stimulus with an up-modulation in surface CD11/CD18 glycoprotein expression. This lack of responsiveness serves to distinguish these macrophages from the circulating monocytes.

DISCUSSION

In this immunofluorescence analysis, we have examined CD11/CD18 epitope expression by human mononuclear phagocytes from both intra- and extravascular compartments to determine if these cells, like the neutrophil, can up-modulate the surface density of Mol (CD11b/CD18), p150,95 (CD11c/CD18), and LFA-1 (CD11a/CD18). We have found that peripheral blood monocytes do express all four CD11/CD18 epitopes, and after brief exposure to calcium ionophore A23187 at 37°C, the surface expressions of monocyte Mol, p150,95, and LFA-1 increase by factors of 5.7, 7.2, and 2.1, respectively (as compared to surface antigen expression of unstimulated monocytes kept at 4°C). Exposure of monocytes to PMA or to fMLP under the same conditions results in increases in epitope density of lesser magnitude. Our observations are thus in general agreement with those of Yancey *et al.* (28) who demonstrated a 1.5-fold increase in Mol (CD11b/CD18) expression by monocytes after exposure of these cells to C5a, and those of Springer *et al.* (18) who reported a 4- to 6-fold increase in monocyte p150,95 (CD11c/CD18) after stimulation by fMLP.

Since monocytes are immature members of the mononuclear phagocyte system that also includes extravascular tissue macrophages (36), we examined pulmonary alveolar and breast milk macrophages (PAM and BMM, respectively) to determine if these cells, as examples of differentiated mononuclear phagocytes, express CD11/CD18 glycoproteins, and, if so, whether surface glycoprotein expression is under regulatory control. On the basis of an analysis of nine specimens of PAM (isolated from normal or patient volunteers, both smokers and nonsmokers), there was negligible CD11b and CD11c epitope expression above background staining; CD11a and CD18 epitope expression was generally detectable at low antigen density. PAM therefore exhibit little, if any, surface Mol and p150,95, but do express some LFA-1 glycoprotein. Exposure of these cells to A23187 (the stimulus inducing the greatest increase in the neutrophil and monocyte glycoprotein expression) failed to stimulate a detectable rise in CD11/CD18 antigen density. To exclude the possibility that our failure to detect significant surface Mol and p150,95 expression was due to either a "masking" or degradation of surface antigenic sites by blocking factors or proteases in the lavage fluid (37), we subjected whole cell lysates of PAM to SDS–polyacrylamide gel electrophoresis and Western blotting using polyclonal antisera specific for CD11b and CD18. As compared to parallel Western blots of neutrophil lysates, there was neglible expression of CD11b and barely detectable expression of CD18. These results are consistent with the notion that there is little cytoplasmic or surface expression of Mol and p150,95 (and relatively little expression of LFA-1) and that expression is not up-modulated by exposure to A23187. In contrast, macrophages isolated from human breast milk do express detectable levels of surface Mol, p150,95, and LFA-1 glycoproteins although the baseline (unstimulated) level of expression

varied considerably among specimens (n = 11, ranging from barely detectable expression to high antigen density). The source of this individual variability is not clear since all specimens were maintained at 4°C prior to a single wash step and subsequent immunofluorescence analysis, and antigen density did not correlate with other variables such as the number of days postpartum (generally 1–7 days; range 1 day to 9 months) that the sample was collected. What was consistent among all specimens was a failure to exhibit up-modulation of the CD11/CD18 epitopes after exposure to activating stimuli.[1] Therefore, on the basis of this immunofluorescence analysis of CD11/CD18 glycoprotein expression by mononuclear phagocytes, we conclude that monocytes, like neutrophils, can respond to activating stimuli with an increase in surface CD11/CD18 glycoprotein expression, but that CD11/CD18 expression by differentiated phagocytes may vary (depending upon anatomical location) and that control of surface expression is lost. These differences in CD11/CD18 expression may represent yet another example of the broad heterogeneity exhibited by members of the mononuclear phagocyte system (reviewed in Ref. (38)).

The physiological significance of the capacity to modulate surface CD11/CD18 expression as shown by neutrophils and monocytes but not by pulmonary alveolar macrophages and breast milk macrophages is unclear. In the neutrophil, we and others have shown that exposure of cells to inflammatory stimuli that increase surface expression of Mo1 and p150,95 also results in enhanced CR3 activity (8) and promotes adhesion-dependent phenomena such as aggregation (8, 9) and adherence to endothelial cell monolayers (11, 12). Since these activities are CD11/CD18 dependent (abrogated by certain anti-CD11/CD18-specific reagents), one could reasonably conclude that the heightened functional response is related to up-modulation in surface Mo1 and p150,95 expression (24). However, we have not excluded the possibility that an increase in functional capacity resulting from exposure of the cells to an inflammatory stimulus is instead due to a qualitative change in receptor structure (rather than simply to an increase in receptor number). Precedent for this possibility was reported by Wright *et al.* (39, 40) who observed the "activation" of phagocytosis-promoting function of monocyte or neutrophil CR3 (Mo1) by fibronectin or PMA out of proportion to changes in receptor number. The reported "clustering" of CR3 receptors on the plasma membrane (41) or glycoprotein phosphorylation (40, 42) represents at least two qualitative changes that could result in enhanced functional competence. Thus, the physiological significance of CD11/CD18 up-modulation stimulated by activating factors has not been unequivocally demonstrated. The fact that increases in neutrophil Mo1 density can occur *in vivo* (in the setting of inflammatory conditions such as hemodialysis leukopenia (8) and systemic lupus erythematosis (43)) does, however, suggest that the phenomenon is biologically relevant. In response to an inflammatory stimulus, monocytes and neutrophils may depend upon the enhanced expression of CD11/CD18 glycoproteins to facilitate contact with vas-

[1] The possibility that up-modulation of BMM CD11/CD18 glycoprotein occurs but is undetectable because of epitope modification is unlikely (monoclonal reagents detecting four distinct epitodes were employed) but represents an alternative explanation of these results.

cular endothelium, thus allowing emigration and diapedesis to inflammatory foci within affected tissue compartments. One could speculate that tissue macrophages, having already breached the endothelial barrier during ontogeny, have no need to express and/or regulate the surface density of the CD11/CD18 class of adhesion-promoting molecules.

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of Mr. Matthew D. Adams and Ms. Lucinda R. Tripp, and the secretarial support of Mr. Dennis C. Dicks and Ms. Tracy Lockhart. Appreciation is extended to the nurses of the University of Michigan Newborn Services and Obstetrics Department for their assistance in obtaining breast milk specimens. The authors also thank Drs. Hergen Spits, Lewis Lanier, and Samuel Wright for their generous gifts of monoclonal reagents. The work was supported by NIH Grants CA39064-02 (R.F.T.), HL07622 (D.R.F.), and AI21964 (M.A.A.), and by March of Dimes Grant 1011 (M.A.A.). M.A.A. is an Established Investigator of the American Heart Association.

Note added in proof. In the interim since the submission of this paper, similar observations relating to the modulation of monocyte CD11/CD18 glycoprotein expression were reported by Miller *et al.* (44).

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Received June 17, 1987; accepted with revision September 21, 1987