Role of ICAM-1 and ICAM-2 and alternate CD11/CD18 ligands in neutrophil transendothelial migration

Andrew C. Issekutz,* Derek Rowter,* and Timothy A. Springer†

*Departments of Pediatrics, Pathology and Microbiology/Immunology, Dalhousie University, Halifax, Nova Scotia, Canada; and †Center for Blood Research and Department of Pathology, Harvard Medical School, Boston, Massachusetts

Abstract: We evaluated the relative contribution of ICAM-1 and ICAM-2, known ligands on endothelium for LFA-1 and Mac-1, in spontaneous neutrophil (PMN) transendothelial migration (TEM) across IL-1-activated HUVEC monolayers or TEM induced by C5a or IL-8 across unstimulated HUVEC grown on polycarbonate filters. Adhesion blocking mAb to ICAM-1 [R6.5 F(ab)$_2$] or ICAM-2 [CBR IC2/2 F(ab)$_2$] tended to inhibit TEM under each condition but, in general, inhibition was significant only with both ICAM-1 and ICAM-2 blockade. mAb to LFA-1 partially inhibited migration to C5a or IL-8 across unstimulated HUVEC and inhibition was not altered by additional treatment of HUVEC with mAbs to ICAM-1 and -2. In contrast, with IL-1 HUVEC, mAb to ICAM-1 significantly inhibited this LFA-1-independent TEM. mAb to Mac-1 alone partially inhibited TEM and, when combined with mAb to LFA-1, migration was almost completely blocked with all TEM conditions tested. The contribution of alternate ligands for Mac-1 in mediating Mac-1-dependent but ICAM-1/-2-independent C5a-induced TEM was examined using anti-LFA-1-treated PMN and anti-ICAM-treated resting HUVEC. Addition of RGD peptides, fibronectin, fibrinogen, heparins, collagens alone or in combination, even to heparinase-treated HUVEC, did not inhibit this Mac-1-mediated PMN TEM. The results indicate that: (1) LFA-1 mediates PMN TEM primarily by interaction with ICAM-1 and ICAM-2; (2) ICAM-2 may function in concert with ICAM-1 in this role, especially on unstimulated endothelium, and (3) Mac-1 on PMN also plays a major role in TEM and can utilize yet to be identified ligands distinct from ICAM-1 or -2, especially on unstimulated endothelium. *J. Leukoc. Biol.* 65: 117–126; 1999.

Key Words: *adhesion molecule · leukocyte · endothelium · Mac-1 · CD102 · LFA-1*

INTRODUCTION

A characteristic feature of acute inflammation is the migration of leukocytes, especially polymorphonuclear leukocytes (PMN), from blood into the involved tissues. In part, this migration is believed to be induced by chemotactic factors produced in the inflamed tissue, which bind to specific receptors on PMN and activate intracellular signal transduction leading to adhesion of the leukocyte to vascular endothelium and a motile response [1–4].

It is now also recognized that leukocyte migration has an important endothelial cell-dependent component. Activation of endothelial cells by cytokines, such as interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and bacterial products such as endotoxin (lipopolysaccharide, LPS) leads to increased PMN adhesion to the endothelium and transendothelial migration [5–7]. Adhesion molecules, such as P-selectin, E-selectin, and intercellular adhesion molecule-1 (ICAM-1) are in part responsible for this leukocyte endothelial cell interaction [5–7]. These molecules are expressed on the endothelium and interact with sialyl Lewis^x carbohydrate-containing molecules, and the CD11/CD18 integrins (β_2 integrins) on the PMN in this process.

The transendothelial migration of PMN *in vitro*, via the chemotactic factor-dependent or the endothelial cell-dependent mechanisms, appears to have a nearly absolute requirement for the presence and function of the CD11/CD18 leukocyte adhesion molecule complex [6, 8–10]. Furthermore, *in vivo* assessment of leukocyte migration to sites of infection or inflammation, especially in the skin, have demonstrated failure of leukocyte mobilization in patients with congenital CD18 deficiency or in experimental animals treated with anti-CD18 mAbs [11–14]. Of the β_2 or CD18 complex, CD11a/CD18 and CD11b/CD18, also known as LFA-1 and Mac-1, respectively, each appear to be important and in combination mediate all CD11/CD18-dependent transendothelial migration *in vitro* [5–7] and PMN accumulation in at least some types of inflammatory reactions *in vivo* [12, 15, 16].

The LFA-1 and Mac-1 β_2 integrins are known to bind to ICAM-1 and LFA-1 and, according to one report, Mac-1 also may bind to ICAM-2 [17–21]. Both of these ICAMs are

---

Abbreviations: PMN, polymorphonuclear neutrophils; TEM, transendothelial migration; HUVEC, human umbilical vein endothelial cells; IL-1, interleukin-1; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; HSA, human serum albumin; PBS, phosphate-buffered saline; FCS, fetal calf serum.

Correspondence: Dr. Andrew C. Issekutz, Department of Pediatrics, Dalhousie University

Infection Immunology Research Labs., IWK Grace Health Centre, 5850 University Ave., Halifax, NS B3J 3G9. E-mail: aissekutz@iwkgrace.ns.ca

Received May 6, 1998; revised October 1, 1998; accepted October 3, 1998.

constitutively expressed on endothelial cells, including human umbilical vein endothelium (HUVEC). ICAM-1 on HUVEC has been reported to contribute to PMN transendothelial migration [8, 9], but the role of ICAM-2 in this process and its importance in comparison to ICAM-1 has not been determined. Furthermore, although Mac-1 can bind to ICAM-1, it can also recognize numerous other ligands, including plasma proteins, e.g., Factor X, C3bi, fibrinogen, fibronectin, and other Arg-Gly-Asp (RGD) sequence proteins, other extracellular matrix proteins (collagen, laminin), heparin like glycosaminoglycans, carbohydrate structures related to β-glucan, and several microbial products [6, 22–28]. These Mac-1 ligand interactions have been observed primarily with adhesion studies with PMN or with purified proteins. The importance of the Mac-1/ICAM-1 interaction and of interactions with these other ligands in mediating PMN transendothelial migration has not been defined. This study was aimed to address these questions and reports that ICAM-2 is a major contributor with ICAM-1 to LFA-1-mediated PMN transendothelial migration. Furthermore, Mac-1-mediated transendothelial migration induced by chemotactic factors is ICAM-1 independent and only partly ICAM-1 dependent when endothelium is activated with IL-1.

MATERIALS AND METHODS

Monoclonal antibodies

The mAbs used included mAb 60.3 (IgG2a; a gift from Bristol-Myers Squibb, Seattle, WA) [29, 30], mAb LPM19c (IgG1; anti-CD11b, a gift from K. Pulford, Oxford, UK) [31], and mAb TS1/22 (IgG1, anti-CD11a/CD18, or LFA-1) [32], which are all known to block adhesion functions of their respective CD11/CD18 antigens. The mAbs W6/32 (IgG2a anti-HLA-class I framework), 3C10 (IgG1 anti-CD14), 5-43 (IgG1, anti-CR1), and mAb TS1/22 were obtained from the American Tissue Culture Collection (Bethesda, MD). The mAbs reactive with ICAM-1 were mAb R6.5 [IgG1 as $F(ab)_2$] [13], mAb CBR-IC 1/13, and CBR-IC 1/11, the latter two reacting specifically with the domain-3 binding region for Mac-1 [33]. mAb CBR-IC2/2 [IgG1 as $F(ab)_2$] recognizing ICAM-2 [18] and mAb BB11 (IgG2b, gift from Dr. R. Lobb, Biogen, Cambridge, MA) against E-selectin [34] were also employed.

Reagents

Recombinant human IL-1α, which had a specific activity of 4×10^7 U/mg, was a gift from Dr. D. Urdal (Immunex Corp., Seattle, WA). Recombinant human IL-8 (NAP-1) were kind gifts from Sandoz Pharmaceutical (Vienna, Austria). Each of the cytokines was diluted immediately before use in 0.1% LPS-free human serum albumin (HSA; Connaught Labs, Don Mills, ON) in phosphate-buffered saline (PBS). Recombinant human C5a was a gift from CIBA-Geigy Pharmaceuticals (Summit, NJ). The following were purchased from Sigma Chemical Co. (St. Louis, MO): Factor X, β-glucan, fibrinogen and its γ-peptide, fibronectin, Type II bovine nasal collagen and Type IV human placental collagen, mouse laminin, and heparinase I and III. The GRGDSP peptide was from Bachem Fine Chemicals (Torrance, CA).

Human PMN purification

Human PMNs were purified as described previously [35, 36] from ACD-heparin-anticoagulated venous blood of healthy donors. Briefly, red cells were sedimented with 6% dextran-saline (Abbott Labs, Montreal, Canada), leukocyte-rich plasma was collected, and leukocytes were labeled with $Na_2{}^{51}CrO_4$ (Amersham, Oakville, Ontario, Canada). PMNs were then purified by discontinuous Percoll gradient centrifugation, washed, and resuspended to 10^6 PMN/mL in RPMI-1640, 0.5% HSA, 10 mM HEPES, pH 7.4. This method yielded PMNs of ≥95% purity with essentially no red cell contamination and ≥98% cell viability.

Endothelial cell cultures

HUVEC were isolated and cultured in flasks as described by Jaffe et al. [37] and grown on filters as previously described by us [35, 36]. Briefly, endothelial cells were isolated from umbilical cords after treatment with 0.5 mg/mL collagenase (Cooper Biomedical, Mississauga, Ontario, Canada) in 0.01 M PBS (pH 7.4) and grown in RMPI-1640 (Sigma) containing 2 mM L-glutamine, 2-mercaptoethanol, sodium pyruvate, and penicillin B/streptomycin and supplemented with 20% fetal calf serum (FCS; Hyclone, Logan, UT), 25 μg/mL endothelial cell growth supplement (Collaborative Research, Lexington, MA), and heparin (45 μg/mL; Sigma). This is referred to as growth medium. Cells were cultured in gelatin-coated culture flasks (NUNC, GIBCO). The HUVEC were detached using 0.025% trypsin, 0.01% EDTA (Sigma) and cultured on PVP-free polycarbonate filters bearing 5-μm pores in Transwell culture plate inserts (6.5-mm diameter, Transwell 3421; Costar, Cambridge, MA). The filters were first prepared by coating with 0.01% gelatin (37°C, 18 h) followed by application of 3 μg of human fibronectin (Collaborative Research) in 50 μL of water at 37°C for 2 h. Fibronectin was then replaced by HUVEC (1.5×10^4 cells), from the first or second passage, added above the filter in 0.1 mL of growth medium and 0.6 mL of growth medium was added to the lower compartment beneath the filter. The HUVEC formed a tight permeability barrier in 5–6 days and were evaluated for confluence before use by ^{125}I-labeled HSA diffusion as previously described [35].

PMN transendothelial migration

Migration assays were performed as described previously [35, 36]. Briefly, HUVEC monolayers on the filters and the lower compartments were washed with RPMI 1640 and they were transferred to a new, clean well (lower compartment). To this well, 0.6 mL of RPMI-1640, 10 mM HEPES, 0.5% HSA was added containing the chemotactic stimulus (C5a, IL-8). Before immersion of the HUVEC-filter unit, 0.1 mL of medium containing 1×10^5 labeled PMNs was added above the HUVEC. After incubation (75 min at 37°C, 5% CO_2) migration was stopped by washing the upper compartment twice with 0.1 mL of RPMI-1640 to remove nonadherent PMNs. The undersurface of the filter was wiped with a cotton swab saturated with ice-cold PBS-0.2% EDTA solution and this was added to the lower compartment. The cells that spontaneously detached from the undersurface of the filter or were removed by the swab were lysed by addition of 0.5% Triton X-100 and all the ^{51}Cr released in the lower compartment and on the swab was quantitated. The results are expressed as the percentage of the total ^{51}Cr PMNs added above the HUVEC that migrated through the HUVEC-filter unit. All the stimulation conditions were performed with triplicate replicates.

Antibody treatments

In some experiments, ^{51}Cr PMNs were treated for 20 min at room temperature with the mAbs indicated at saturating concentrations (20–40 μg/mL), as determined by immunofluorescence flow cytometry, and then tested for migration in the presence of the antibody. None of the mAb treatments caused PMN aggregation or activation as assessed by oxidative burst using luminol dependent chemiluminescence or bipolar shape change. Other reagents were added to the PMN suspension just before adding them to the HUVEC. In some experiments, the HUVEC were treated for 40 min at 37°C with saturating concentrations of mAbs as determined by enzyme-linked immunoabsorbent assay followed by the addition of the ^{51}Cr-labeled PMNs. These mAbs were present throughout the migration period as well. In some cases, the HUVEC was treated with heparinase III (1 U/mL) or I (1 U/mL) as reported previously [26] for 40 min before performing the PMN migration assay.

Statistical analysis

Data were analyzed by analysis of variance followed by the Tukey test of multiple comparisons. P values exceeding 0.05 were not considered significant.

RESULTS

Effect of ICAM-1 and ICAM-2 blockade on chemotactic factor-induced PMN transendothelial migration

The contribution of the CD11/CD18 integrins, LFA-1 and Mac-1 on PMN and of ICAM-1 and ICAM-2 on endothelium to PMN transendothelial migration induced by the potent chemotactic factor, C5a, was investigated as shown in **Figure 1**. C5a at an optimal chemotactic concentration of 2×10^{-9} M, predetermined in previous studies [36], induced 67% of PMN to transmigrate across the HUVEC and filter barriers. Blocking LFA-1 adhesion function with the mAb TS1/22 significantly but only partially inhibited this response. Similarly, blocking Mac-1 with mAb LPM19C inhibited transmigration to a comparable degree. The combination of these two mAbs essentially eliminated any PMN transendothelial migration in response to C5a, as also reported previously using this or similar transendothelial migration systems [9, 10, 36]. To investigate the role of ICAM-1 and ICAM-2 on HUVEC in PMN transmigration, the adhesion function blocking mAbs R6.5 and CBR-IC2/2, respectively, were employed in their $F(ab)_2$ forms. As shown in Figure 1, anti-ICAM-1 or anti-ICAM-2 treatment of the HUVEC had a slight but not significant inhibitory effect on transmigration when the PMN were not mAb treated. Addition of mAb to E-selectin to the anti-ICAM-1 and -2 mAbs had no effect on migration (not shown). However, combination of anti-ICAM-1 and anti-ICAM-2 mAbs had a significant inhibitory effect, but still only decreased the migration from 67 to 48% of PMN transmigrating. Treatment of PMN with anti-LFA-1 mAb did not further inhibit migration when the HUVEC were treated with anti-ICAM-1 + ICAM-2 mAbs. In contrast, treatment of the PMNs with anti-Mac-1 mAb abolished PMN transendothelial migration when ICAM-1 and -2 on the HUVEC were blocked. These findings indicate that the ICAM-1 + ICAM-2 blocking mAbs effectively blocked the LFA-1 pathway and that in the presence of anti-ICAM-1 + anti-ICAM-2 ± anti-LFA-1 blockade, all of the PMN transendothelial migration was mediated by Mac-1.

Figure 2 shows the results with use of the IL-8 chemotactic factor to induce PMN transendothelial migration. IL-8 induced optimal PMN transmigration at a concentration of 5×10^{-9} M), but this response was significantly weaker than with C5a, inducing about 42% of PMN to transmigrate. Anti-LFA-1 treatment of the PMN inhibited this response by approximately 50%. Antibody to Mac-1 inhibited PMN migration to a somewhat greater degree, i.e. by approximately 75%, and the combination of anti-LFA-1 with anti-Mac-1 mAbs decreased migration to virtually the unstimulated level, i.e. to about 4%. Treatment of the HUVEC with anti-ICAM-1 mAb [R6.5 $F(ab)_2$] or anti-ICAM-2 mAb alone tended to inhibit migration, but this was not significant. In the IL-8-induced transmigration, addition of anti-ICAM-1 mAb to anti-ICAM-2 mAb had a statistically significant additive inhibitory effect, blocking migration by more than 50% and to a level comparable with anti-LFA-1 treatment of the PMN. Furthermore, LFA-1 mAb treatment of PMNs in combination with anti-ICAM-1 + ICAM-2 treatment of HUVEC had no further inhibitory effect compared to either anti-ICAM-1 + -2 treatment or anti-LFA-1 treatment alone. In marked contrast, treating the PMNs with antibody to Mac-1 completely blocked their migration when ICAM-1 and ICAM-2 on the HUVEC was also blocked. These observations suggest

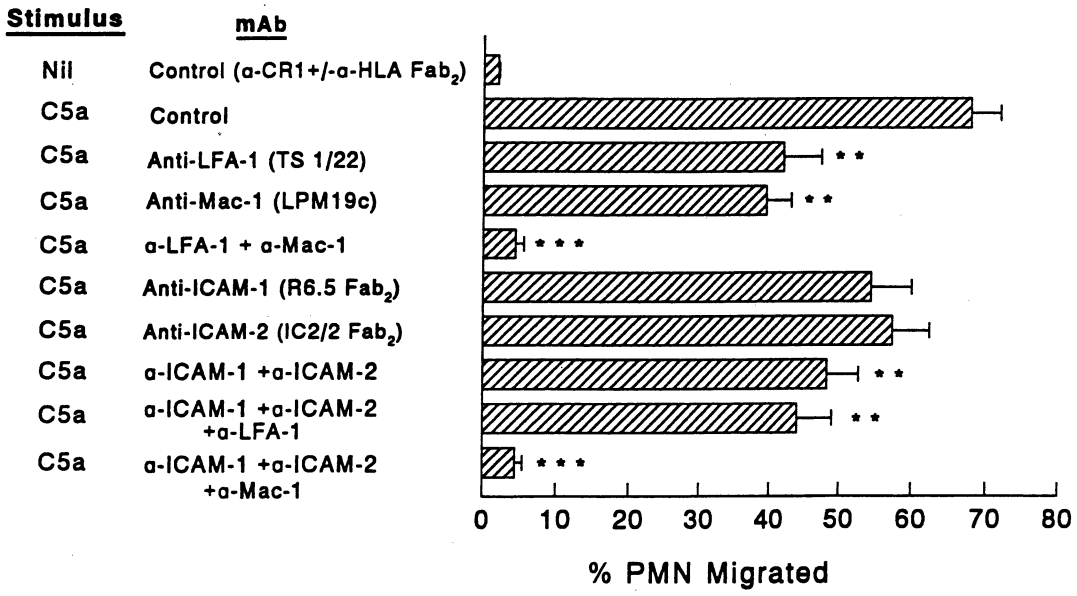

Fig. 1. The effect of antibody to LFA-1, Mac-1, ICAM-1, and ICAM-2 on PMN transendothelial migration. The migration of ^{51}Cr-labeled PMN across unstimulated HUVEC monolayers was quantitated as described in Materials and Methods. Migration was induced by C5a (2×10^{-9} M) added to the lower compartment beneath resting HUVEC monolayers. PMN were either treated with mAb to LFA-1 (TS1/22) or to Mac-1 (LPM19c) or in combination for 20 min (22°C) before addition above the HUVEC monolayers. Control mAb was an anti-CR-1 mAb (543). Where indicated, the HUVEC were pretreated with anti-ICAM-1 mAb [R6.5 $F(ab)_2$] or anti-ICAM-2 [CBR-IC2/2 $F(ab)_2$, 30 μg/mL] for 30 min before addition of PMN. Control mAb for the HUVEC treatments was an anti-HLA Class I [mAb W6/32 $F(ab)_2$]. PMN migration time was 75 min. Values are mean ± SEM of five to eight experiments. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to control mAb treatments of the PMN and/or HUVEC.

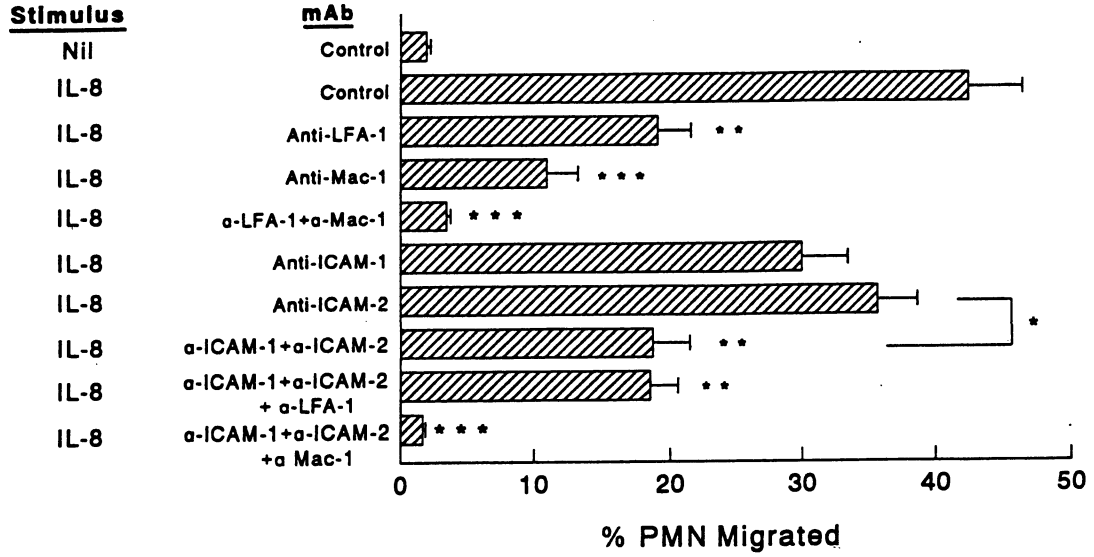

Fig. 2. Effect of antibody to LFA-1, Mac-1, ICAM-1, and ICAM-2 on PMN transendothelial migration induced by IL-8. Migration of PMN across unstimulated HUVEC monolayers was induced by IL-8 (5×10^{-9} M) added to the lower compartment beneath the HUVEC monolayer. Migration time and mAb treatments were as in Figure 1 using the same mAbs. Values are mean ± SEM of four to six experiments. *P* values are as in Figure 1.

that different mechanisms for transendothelial migration function in concert and these involve an LFA-1-mediated pathway on the PMN engaging an ICAM-1/ICAM-2 pathway on the endothelium and a Mac-1 pathway on the PMN, which may utilize ligands other than ICAM-1 and -2.

Investigation of Mac-1 ligands contributing to the Mac-1-mediated PMN transendothelial migration

The results in Figures 1 and 2, using C5a and IL-8, indicate that Mac-1 on PMN can mediate 50–60% of the transendothelial migration. Therefore, we investigated, using C5a as the chemoattractant, the potential involvement of some of the putative ligands for Mac-1. Mac-1 has been reported to bind to ICAM-2 [17], to domain-3 of ICAM-1, which is distinct from the domain-1 binding region for LFA-1 [6, 19, 20], to RGD peptides and similar sequences in fibrinogen and fibronectin, as well as other extracellular matrix proteins such as collagens, laminin, as well as to glycosaminoglycans related to heparan sulfates [22–28]. Also Mac-1 has a lectin binding region that recognizes carbohydrate structures such as β-glucan [38]. To determine which of these interactions alone or in combination may be mediating PMN transendothelial migration via the Mac-1 pathway, PMN that were treated with mAb to LFA-1 were added to HUVEC monolayers, which were untreated, treated with anti-ICAM-1 mAb R6.5 $F(ab)_2$, or with other antibodies to HUVEC adhesion molecules, as shown in **Figure 3.** As expected, anti-LFA-1-treated PMN were partially inhibited in their transmigration across the HUVEC in response to C5a. Treating the endothelium with anti-ICAM-1 mAb R6.5 $F(ab)_2$, which is known to block the interaction of LFA-1 with ICAM-1 and also the interaction of Mac-1 with ICAM-1 [13, 25], did not significantly inhibit further compared with anti-LFA-1 alone. Adding treatment with mAb CBR-IC2/2 of the HUVEC, which is reported to at least partially inhibit Mac-1 binding to ICAM-2 [17], did not inhibit transmigration.

Because Mac-1 recognizes a different domain on ICAM-1 from LFA-1, the possibility existed that the R6.5 mAb may be more effective at blocking the LFA-1/ICAM-1 interaction than the Mac-1/ICAM-1 interaction at domain 3 of ICAM-1. Therefore, two other mAbs to ICAM-1 (clones CBR-IC1/13 and CBR-IC1/11), which are known to recognize epitopes in domain 3 of ICAM-1 and block Mac-1 binding [33], were also used. However, these antibodies alone (not shown) or in combination with the R6.5 mAb, had no additional inhibitory effect on the Mac-1-mediated transmigration, as shown in Figure 3.

Having observed no requirement for ICAM-1 or -2 in the Mac-1-mediated PMN migration, the role of other putative ligands was investigated. In these experiments, the addition of the RGD peptide GRGDNP to the PMN suspension before and during the PMN transmigration assay, at concentrations (0.1–1 mM) known to inhibit Mac-1 binding to RGD peptides [24, 28, 39] had no effect on migration. Fibrinogen is readily bound by activated Mac-1, but the presence of free fibrinogen or the fibrinogen gamma peptide, which contains a Mac-1 recognition region [40], with or without blockade of ICAM-2 in the presence of anti-ICAM-1, did not inhibit Mac-1-mediated transmigration either. Similarly, a range of concentrations of two different forms of heparin, which are reported to inhibit Mac-1-heparin and glycosaminoglycan binding [22, 26], did not inhibit Mac-1-mediated PMN transmigration. Inclusion of type 2 and type 4 collagens (or laminin, not shown), which are known to be ligands for Mac-1-mediated PMN adhesion [24, 28], had no effect on the PMN migration response. The lectin binding domain of Mac-1 has been reported to be involved in phagocytic recognition of β-glucan on yeast particles and may also be involved in intra-membrane molecular associations between Mac-1 and some GPI linked proteins [38, 41–43]. To evaluate the contribution of this interaction, soluble β-glucan was included in the PMN suspension during the migration. However, β-glucan did not inhibit PMN transendothelial migration via the Mac-1 pathway.

Recent studies by Diamond et al. [22] and Coombs et al. [26]

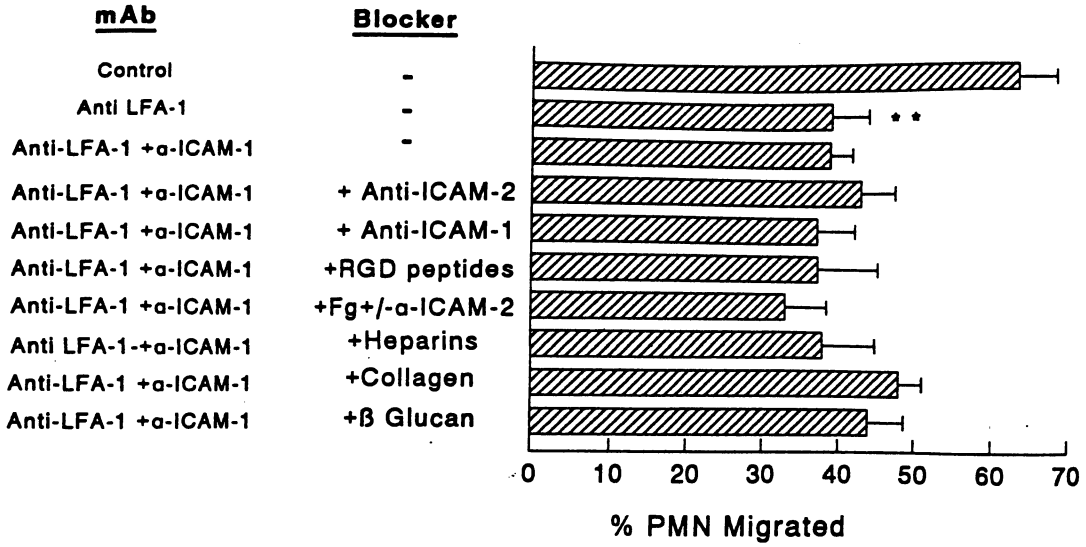

Fig. 3. Effect of blocking putative Mac-1 ligand interactions on PMN transendothelial migration induced by C5a. PMN migration was induced as in Figure 1 using C5a. PMN were treated with control mAb or with anti-LFA-1 (TS1/22) and the HUVEC were treated with anti-ICAM-1 mAb clone R6.5 $F(ab)_2$ as indicated in the column at left, as in Figure 1. The column labeled blocker shows additional treatments of either the HUVEC using anti-ICAM-2 mAb [CBR-IC2/2 $F(ab)_2$] or additional anti-ICAM-1 mAbs reactive with domain-3 (CBR-IC1/13 or IC1/11). Other blocking treatments included GRGDSP peptide (0.1–1 mM) human fibrinogen (Fg), or Fgγ peptide (100–500 μg/mL, results pooled), heparins (porcine intestinal mucosa, bovine lung, or low molecular weight heparin at 10–1000 μg/mL, results pooled), or bovine nasal (Type II) or human placental (Type IV) collagen (50–500 μg/mL), or β-glucan (50–200 μg/mL) added to the PMN suspension 20 min before addition to the HUVEC. All mAbs and agents were present throughout the migration assay. Values are mean ± SEM of four to eight experiments. $^{**}P < 0.01$.

have shown that heparin and heparan sulfate glycosaminoglycans may be important ligands for Mac-1. Glycosaminoglycans are abundant on vascular endothelium. Therefore, we investigated the potential role of these structures in Mac-1-mediated transendothelial migration. For these experiments, heparinase III treatment of the HUVEC was employed, using conditions reported previously to block Mac-1-glycosaminoglycan-mediated adhesion to stromal cells *in vitro* [26]. Heparinase III treatment of the HUVEC had no deleterious effect on the permeability of the monolayer and did not alter baseline or C5a-induced PMN transmigration (not shown). However, when the system was designed to quantitate PMN transmigration via the Mac-1 pathway, i.e. by using anti-LFA-1-treated PMN and anti-ICAM-1 [R6.5 $F(ab)_2$] -treated HUVEC, heparinase III treatment of the HUVEC had no effect on transmigration. Furthermore, adding fibrinogen γ peptide alone or in combination with anti-ICAM-2 mAb or with fibronectin ± β-glucan did not significantly inhibit transmigration. In two experiments, inclusion of Factor X in the blocking treatment of the PMN also did not inhibit PMN transmigration via the Mac-1 pathway (**Figure 4**). In all of these experiments, the permeability of the endothelial monolayer remained comparable to monolayers in which no blocking treatments were used, i.e. there was no adverse effect on the monolayer integrity before and during the assay (not shown).

Finally, we considered that some combination of plasma constituents such as fibrinogen, fibronectin, Factor X, and haptoglobulin, all of which are reported to be ligands for Mac-1 [6, 7, 44, 45], might interact with and regulate Mac-1 recognition of ligands on endothelium by binding to Mac-1 from the soluble phase. To assess this, IL-8 was used as the chemotactic factor, since plasma rapidly inactivated the C5a chemotactic agent as expected. Inclusion of up to 40% plasma in the upper and lower compartment of the chemotactic chamber had no effect on IL-8-induced transendothelial migration (not shown). Furthermore, treating PMNs with antibody to LFA-1 and the HUVEC with anti-ICAM-1 partially inhibited the transmigration, as shown in Figure 2, and in the presence of 40% plasma there was no further inhibition (no plasma control = 18 ± 2.5%; with plasma = 14.2 ± 2.5% of PMN transmigrated; n = 3). In the case of both IL-8 and C5a, the degree of LFA-1 versus Mac-1-mediated transmigration was not dependent on the kinetics of migration because terminating the incubations at earlier time points to achieve 50% maximal response, i.e., at 40-min incubation, did not alter the degree of inhibition by anti-LFA-1 or anti-Mac-1 mAb treatment of PMNs. Furthermore, the contribution of ICAM-1 versus ICAM-2 to the migration response was also not affected (not shown).

Contribution of ICAM-1 and ICAM-2 to PMN migration across IL-1-activated HUVEC

Stimulation of HUVEC with IL-1 or TNF-α is known to result in PMN transendothelial migration. As shown in **Figure 5**, activation of HUVEC with IL-1α for 4 h increased PMN transmigration from a background level of 2.5% up to 28%. To investigate the role of ICAM-1 and ICAM-2 on HUVEC, the HUVEC was pretreated with mAb R6.5 or CBR-IC2/2, respectively, as in the previous experiments. Control mAb [anti-HLA-class I, W6.32 $F(ab)_2$] had no effect on migration, as shown in Figure 5. Anti-ICAM-1 mAb significantly inhibited PMN transendothelial migration. Although anti-ICAM-2 mAb did not have a significant effect, the addition of mAb to ICAM-1 had a significant additive inhibitory effect, decreasing the PMN migration from 28 to 11%. Blocking LFA-1 on PMNs with mAb TS1/22 also inhibited PMN transmigration, but not quite as

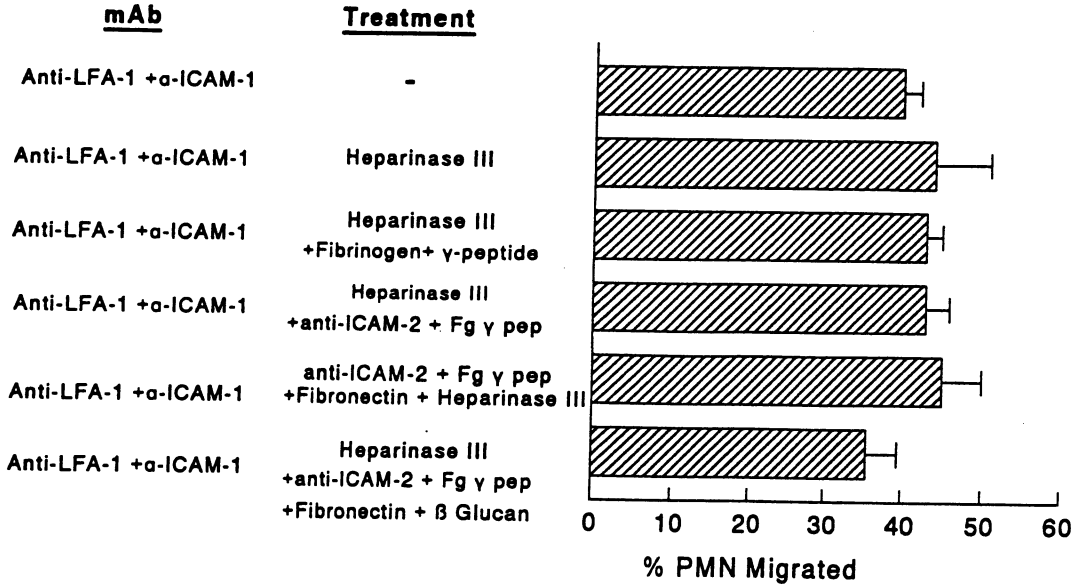

Fig. 4. Effect of heparinase treatment of endothelium on PMN transendothelial migration. The PMN were treated as in Figure 3 with anti-LFA-1 mAb (TS1/22) and HUVEC was treated with anti-ICAM-1 mAb [R6.5 F(ab)$_2$]. Other treatments were as in Figure 2, except that the HUVEC was pretreated with heparinase I and/or heparinase III at 1 U/mL (45 min at 37°C) before addition of antibody-treated PMN. C5a was added to the lower compartment to initiate the migration. Fibrinogen γ peptide and fibronectin were applied to the PMN suspension at concentrations of 200 and 300 μg/mL, respectively. Values are mean ± SEM of three to four experiments.

effectively as blocking ICAM-1 and ICAM-2 on the HUVEC. These results were in contrast to the observations with C5a- or IL-8-induced PMN migration (Figs. 1 and 2) across unstimulated HUVEC. Furthermore, the migration of anti-LFA-1-treated PMN across IL-1-stimulated HUVEC was inhibited significantly further by anti-ICAM-1 mAb [R6.5 F(ab)$_2$] treatment of the HUVEC, suggesting that on IL-1-activated HUVEC, the R6.5 F(ab)$_2$ mAb was blocking an LFA-1-independent migration pathway. This too was in contrast to IL-8 or C5a-induced migration through unstimulated HUVEC where this effect was not observed (Figs. 1 and 2). As shown in Figure 5, antibody to Mac-1 partially inhibited transendothelial migration by about 50% and the combination of anti-LFA-1 and anti-Mac-1 mAbs completely inhibited IL-1-stimulated PMN migration, indicating that this was an LFA-1/Mac-1-mediated migration response as expected from previous reports [8, 9]. It

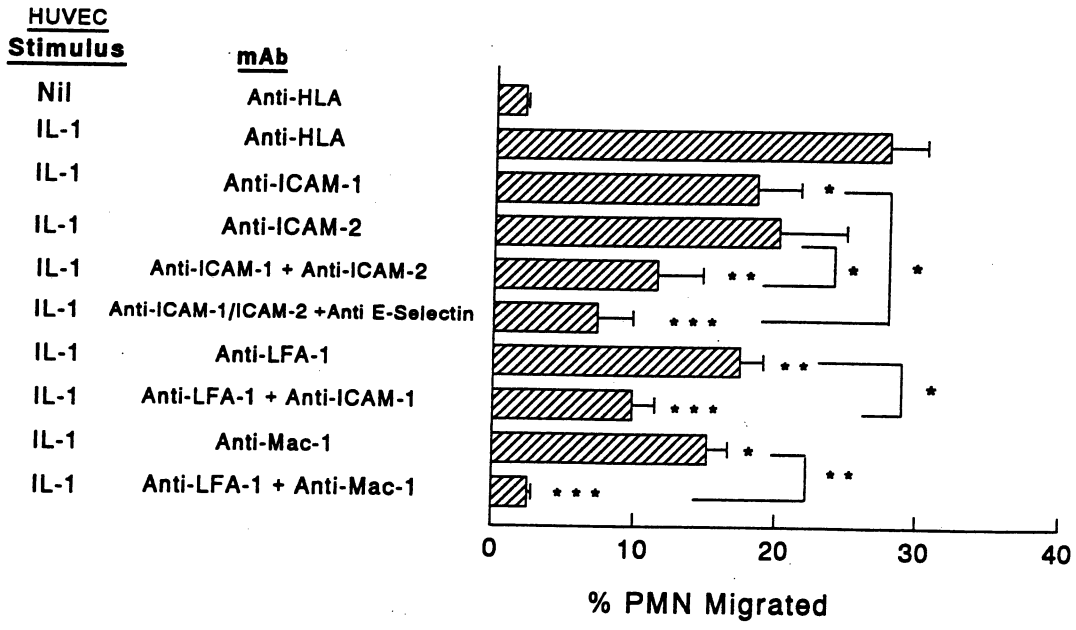

Fig. 5. Effect of antibody to LFA-1, Mac-1, and ICAM-1 and -2 on PMN migration across IL-1-activated endothelium. The HUVEC were stimulated with IL-1α (0.5 ng/mL for 4 h) followed by washing and addition of PMN treated with anti-LFA-1 mAb (TS1/22) or anti-Mac-1 (LPM19c) as in Figure 1. The HUVEC were pretreated for 30 min with anti-ICAM-1 [R6.5 F(ab)$_2$], anti-ICAM-2 (CBR-IC2/2), anti-E-selectin (BB11, 20 μg/mL) mAb alone or in combination as indicated. Control mAb was anti-HLA Class I mAb [W6/32 F(ab)$_2$]. PMN migration was quantitated after 75 min. Values are mean ± SEM of five to six experiments. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

should be indicated that neither anti-LFA-1 + anti-ICAM-1 nor anti-ICAM-1 + anti-ICAM-2 treatments decreased absolute PMN migration below 10%, i.e. approximately one-third of the response remained. Adding anti-E-selectin mAb to treat the HUVEC along with the anti-ICAM-1 and anti-ICAM-2 tended to further inhibit PMN transmigration. These results would indicate that in the PMN transmigration response across IL-1 activated endothelium ICAM-1, ICAM-2, and E-selectin each contribute to the total transmigration. At least part of the Mac-1-mediated transendothelial migration appears to be due to interactions with ICAM-1 on the HUVEC under these conditions.

DISCUSSION

The transendothelial migration of PMN is known to be mediated by CD11/CD18 integrins and in particular by LFA-1 and Mac-1, which function in concert, as shown previously [8–10] and in the PMN migration system used here with C5a- or IL-8-induced migration through unstimulated HUVEC or across IL-1-stimulated HUVEC. It is worth noting that the TEM response is certainly preceded by PMN adhesion. However, on resting HUVEC, even during C5a- or IL-8-induced TEM, the adhesion accounts for only 4–7% of added PMN at any given time. The effect of mAb treatments on this low level of adhesion was not reliably measurable. In contrast, the substantial adhesion on IL-1-activated HUVEC (20–30% of PMN), was inhibited by approximately 50% by LFA-1 plus Mac-1 blockade (not shown) [8, 9], whereas migration was blocked by >90%. This indicates that although adhesion must be a prerequisite for migration, there is not a quantitative correlation, probably because migration involves cellular processes in addition to static adhesion.

The results of this study demonstrate that ICAM-1 and ICAM-2 on endothelium both are functionally important counterligands for PMN transendothelial migration. This appears to be the case whether the endothelium is unstimulated or IL-1-activated because combined ICAM-1 plus ICAM-2 blockade had additive inhibitory effect on migration (Figs. 1, 2, and 5). This finding is likely related to the fact that ICAM-2 is expressed at relatively high levels on resting HUVEC, in fact, considerably greater than ICAM-1 [unpublished observations and ref. 18]. However, even after IL-1 activation and increased ICAM-1 expression [5–9], ICAM-2 still appears to have a role as a ligand on HUVEC (Fig. 5). This role appears to be as a ligand for LFA-1, since blockade of LFA-1 on PMN resulted in the same degree of inhibition of PMN transendothelial as blocking of ICAM-1 and ICAM-2 on the HUVEC, at least in the case of unstimulated HUVEC (Figs. 1 and 2). Adding anti-LFA-1-treated PMN to anti-ICAM-1- and anti-ICAM-2-treated HUVEC did not further inhibit migration (Figs. 1 and 2). This is most likely due to blocking of a common pathway or counter-receptor on the PMN and on the HUVEC. The results also demonstrate that the mAb treatments were effective in functionally blocking all LFA-1 on PMN or ICAM-1 or ICAM-2 on the HUVEC.

The studies designed to assess the Mac-1 components of transendothelial migration strongly indicate that, in the case of unstimulated endothelium, ICAM-1 is not an important counter-receptor for Mac-1-mediated migration, even though the Mac-1 mechanism accounts for at least 50% of the total PMN transmigration (Figs. 1, 2, and 3). This is supported by the fact that mAb R6.5 to ICAM-1 had no effect on Mac-1-mediated PMN migration, although it is known to block Mac-1 binding to ICAM-1 [13, 25]. Furthermore, two other mAbs (CBR-IC1/13 and IC1/11) recognizing epitopes in the Mac-1 recognition region [33] also did not inhibit Mac-1-mediated transendothelial migration in response to C5a or IL-8 across unstimulated HUVEC (Fig. 3). Thus, on resting endothelium, the form or level of constitutively expressed ICAM-1 does not appear to participate significantly as a counter-receptor for Mac-1-mediated PMN transendothelial migration in response to chemotactic factors. Recently, similar observations with regard to ICAM-1-independent but Mac-1-mediated PMN migration across platelet monolayers were made [46], confirming that Mac-1 utilizes alternate ligands during PMN migration. In accordance with this, Diamond et al. [47] also presented evidence that unstimulated endothelium expresses a novel ligand for Mac-1-mediated PMN migration.

The experimentation directed at defining the alternate Mac-1-ligand interactions on HUVEC responsible for PMN transendothelial migration indicates that probably none of the well-recognized Mac-1 ligand interactions function as primary counter-receptors on HUVEC mediating this process. An interaction of Mac-1 with either HUVEC bound or synthesized plasma proteins, such as fibrinogen, fibronectin, or other RGD-containing proteins or with other plasma constituents such as Factor X (see text and Fig. 3) or haptoglobulin [6, 27, 40, 44, 45] appears unlikely because none of these components at high concentrations in soluble form altered the Mac-1-mediated transendothelial migration when LFA-1 and ICAM-1 and/or -2 were blocked (Fig. 3 and Fig. 4). Even in the presence of up to 40% human heparinized plasma, IL-8-induced migration was unaffected when LFA-1 and ICAM-1 were blocked (see text). In addition, ICAM-2 also does not appear to contribute to Mac-1-mediated migration, since mAb CBR-IC2/2, which is known to block Mac-1/ICAM-2-mediated adhesion of monocytic cell lines [17], had no effect on Mac-1-mediated PMN transmigration (Fig. 3). It also appears that extracellular matrix protein recognized to mediate PMN adhesion via a Mac-1-dependent mechanism [23, 24, 28], proteins such as fibronectin, collagen (Fig. 3), or laminin (see text) are not the major counterligands in Mac-1-mediated transendothelial migration. Recently, Mac-1 has been recognized as a heparin and glycosaminoglycan (GAG) binding integrin [22, 26]. Because such structures are prominent on endothelium, we investigated their involvement by two approaches. First of all, we added various forms of soluble heparin under conditions shown previously by Diamond et al. to inhibit Mac-1-heparin and heparan sulfate adhesion interactions [22]. Second, GAGs capable of binding to Mac-1 were enzymatically cleaved from HUVEC with the use of heparinase III (or I alone and in combination, not shown) employing conditions shown previously to abolish Mac-1/GAG adhesion [26]. Neither of these treatments altered PMN transendothelial migration (Figs. 3 and 4).

A lectin-binding region of Mac-1, known to recognize yeast β-glucan [38], has received increasing attention because it not only mediates PMN or monocyte activation by phagocytosis of yeast particles or yeast cell walls, but also appears to function in intramembrane association with and possibly signaling for GPI linked membrane proteins including CD16 and CD87 [41–43]. This raised the possibility that the carbohydrate structures on HUVEC might be presented and engaged by Mac-1 on PMN. However, attempts to saturate and compete out such a putative interaction with high concentrations of soluble β-glucan reported to block the β-glucan binding function of Mac-1 on PMN [38], also did not modify PMN transendothelial migration (Figs. 3 and 4). Finally, because Mac-1 has so many putative ligands, which may be expressed on HUVEC or on extracellular matrix (ECM) proteins associated with endothelium, multiple combinations of blockers and antagonists were employed simultaneously such as heparinase III treatment combined with mAb to ICAM-1 and -2, fibrinogen γ-peptide, fibronectin, and β-glucan (Fig. 4) ± soluble laminin and collagen (not shown). However, even these combinations did not modulate Mac-1-mediated transendothelial migration, suggesting that Mac-1 probably engages a yet to be identified ligand on HUVEC during transendothelial migration, rather than utilizing these known ligands as alternates during PMN migration.

Our results indicate that IL-1 activation of HUVEC modifies the ligand(s) available for interacting with Mac-1 for mediating transendothelial migration. Under these conditions, ICAM-1 does appear to contribute to Mac-1-dependent PMN transmigration, as well as serving as a ligand for LFA-1, since mAb R6.5 (anti-ICAM-1) significantly inhibited migration relative to anti-LFA-1 mAb treatment alone (Fig. 5). The shift in the relative role for ICAM-1 under these conditions for Mac-1 engagement may be related to the marked increase in expression of ICAM-1 known to be induced by IL-1 [5, 6, 8, 9, 20, and unpublished observations], but alternative glycosylation of this induced ICAM-1 may also modify Mac-1 recognition as previously proposed [19]. A major role for ICAM-1 in PMN interaction with cytokine-activated HUVEC is in accordance with previous reports [8, 9], although in those studies the contribution of ICAM-2 to the overall transendothelial migration response was not examined. In general, the findings indicate that for transendothelial migration on IL-1-activated HUVEC, the alternative and undefined Mac-1 ligand(s) involved in migration across resting endothelium in response to chemotactic factors are relatively less important. The reason for this might be the up-regulation of ICAM-1 by IL-1, thus providing a sufficient foothold and/or down-modulation of the putative ligand(s) on endothelium by cytokine activation. However, it is also possible that a gradient of a chemotactic factor such as C5a or IL-8 may activate Mac-1 on PMN to a state recognizing a broader range of ligands than occurs on IL-1-activated HUVEC. Evidence for varying affinity states for different ligands has been observed in the case of VLA-4 [48], another integrin capable of recognizing multiple ligands [6, 7]. This is the more likely mechanism because blocking experiments, conducted as in Figures 3 and 4, of Mac-1-mediated PMN migration in response to C5a across IL-1-activated HUVEC yielded results comparable to migration across unstimulated HUVEC (Figs. 3 and 4 and not shown).

The importance of Mac-1 in PMN emigration *in vivo* has recently been questioned, especially since the finding that Mac-1-deficient mice have normal PMN accumulation in the inflamed peritoneum [49]. This suggests that LFA-1 plays a major role in this PMN migration. However, mice genetically deficient in LFA-1 developed 40–50% of the PMN infiltration response in the peritoneum, as compared to wild-type mice [50], suggesting that other CD11/CD18 integrins may also be involved in the LFA-1 knockout mice. Furthermore, in the Mac-1-deficient animals, PMN accumulation in the peritoneum was inhibited substantially more by mAb to LFA-1 (by 78%) than in wild-type mice, where the same antibody treatment inhibited PMN accumulation by only 58%. This may be an indication that in Mac-1-deficient animals LFA-1 plays a greater role in mediating a normal PMN infiltration response than in normal mice. Other *in vivo* studies, based on mAb inhibition of PMN migration, have suggested that Mac-1 is an effective alternate in mediating migration into tissues, although this role was only apparent when the function of LFA-1 was blocked. This was observed in the mouse peritoneum, in the rat in dermal inflammation and arthritis, and in dermal and peritoneal inflammation in the rabbit [12, 15, 16, 51]. In most studies, blocking LFA-1 or Mac-1 alone with mAb had little or marginal inhibitory effect, but blocking both of these integrins resulted in dramatic and synergistic inhibition of PMN accumulation in the tissues. It is interesting to note that, in the rabbit, qualitative differences were observed in anti-Mac-1 mAb-treated animals, manifest primarily as diminished PMN migration into the connective tissue with persistent PMN association with the postcapillary venules [16]. Resolution in that study was not sufficient to assess whether the PMN had migrated through the vascular wall or remained trapped in the wall. Thus, overall the weight of evidence would suggest that Mac-1 can function as an effective alternate to the LFA-1 mechanism during *in vivo* PMN emigration and that this is demonstrable *in vitro* by PMN transendothelial migration.

There are some quantitative, rather than qualitative differences between the degree of inhibition *in vitro* by mAbs to LFA-1 and to Mac-1 of human PMN transendothelial and the *in vivo* models of PMN infiltration. These could be species differences or true *in vivo/in vitro* system differences. Our results of the degree of inhibition by mAb LPM19C to Mac-1 of PMN transendothelial migration in response to chemotactic factors is somewhat greater than reported by Furie et al. [10]. However, this quantitative difference may be related to differences in endothelial cell culture systems used or, more likely, in the anti-Mac-1 mAb used for blocking the multiple functional interactions of Mac-1 with its ligands. We have screened a large panel of mAbs to human Mac-1 and have observed major differences in inhibition of PMN transendothelial migration, ranging from no inhibition to the degree of inhibition reported here with mAb LPM19C. This mAb has been shown to be particularly effective in blocking at least four different Mac-1 ligand adhesive interactions [25]. We selected this mAb for these reasons and because we have not found it to induce any PMN aggregation or activation as measured by PMN shape

change or oxidative burst induction [unpublished observations], effects that could influence PMN migration.

In conclusion, this study shows that ICAM-2 and ICAM-1 both contribute to PMN transendothelial migration on both resting and cytokine-activated endothelium and that these two ICAMs function in concert as counterligands primarily for LFA-1 in this process. In addition, Mac-1 can mediate PMN transendothelial migration *in vitro* by engaging yet to be defined counter-receptors on endothelium or secreted by endothelium. These are apparently distinct from many of the recognized Mac-1 ligands present in plasma, on ECM proteins, GAGs, and on HUVEC (ICAMs). These results may provide a partial explanation of why inflammatory responses and leukocyte recruitment still can occur in the ICAM-1-deficient transgenic mouse [52, 53]. These findings also would predict that strategies for regulating leukocyte migration *in vivo*, designed to block the ligands on vascular endothelium for CD11/CD18 integrins, will likely be very difficult to develop due to the multiple and redundant interactions.

ACKNOWLEDGMENTS

The authors are grateful to the colleagues mentioned in Materials and Methods who have provided valuable important reagents for these studies. We also gratefully acknowledge the technical help of K. MacLeod and the excellent secretarial assistance of M. Hopkins. This work was supported by grants MT-7684 from the Medical Research Council of Canada and CA31799 from the National Institutes of Health.

REFERENCES

1. Movat, H. Z. (1985) Chemotaxis—Inflammation induced by immune complexes: A model for complement-chemotaxis-and PMN-leukocyte-mediated process. In *The Inflammatory Reaction* Amsterdam: Elsevier, 235–244.
2. Goldstein, I. M. (1992) Complement: Biologically active products. In *Inflammation. Basic Principles and Clinical Correlates* (J. I. Gallin, I. M. Goldstein, and R. Snyderman, eds.) New York: Raven Press, 63–83.
3. Buettner, H. M., Lauffenburger, D. A., Zigmond, S. H. (1989) Measurement of leukocyte motility and chemotaxis parameters with the Millipore filter assay. J. Immunol. Meth. 123, 25–37.
4. Snyderman, R., Uhing, R. J. (1988) Phagocytic cells: Stimulus-response coupling mechanisms. In *Inflammation. Basic Principles and Clinical Correlates* (J. I. Gallin, I. M. Goldstein, and R. Snyderman, eds.) New York: Raven Press, 309–324.
5. Bevilacqua, M. P. (1993) Endothelial-leukocyte adhesion molecules. Annu. Rev. Immunol. 11, 767–804.
6. Springer, T. A. (1995) Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. Annu. Rev. Physiol. 57, 827–872.
7. Carlos, T. M., Harlan, J. M. (1994) Leukocyte-endothelial adhesion molecules. Blood 84, 2068–2101.
8. Luscinskas, F. W., Cybulsky, M. I., Kiely, J.-M., Peckins, C. S., Davis, V. M., Gimbrone, M. A., Jr. (1991) Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J. Immunol. 146, 1617–1625.
9. Smith, C. W., Marlin, S. D., Rothlein, R., Toman, C., Anderson, D. C. (1989) Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. J. Clin. Invest. 83, 2008–2017.
10. Furie, M. B., Tancinco, M. C. A., Smith, C. W. (1991) Monoclonal antibodies to leukocyte integrins CD11a/CD18 and CD11b/CD18 or intercellular adhesion molecule-1 inhibit chemoattractant-stimulated neutrophil transendothelial migration in vitro. Blood 78, 2089–2097.
11. Hawkins, H. K., Maffelfinger, S. C., Anderson, D. C. (1992) Leukocyte adhesion deficiency: clinical and postmortem observations. Ped. Pathol. 12, 119–130.
12. Issekutz, A. C., Issekutz, T. B. (1992) The contribution of LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) to the in vivo migration of polymorphonuclear leucocytes to inflammatory reactions in the rat. Immunol. 76, 655–661.
13. Argenbright, L. W., Letts, L. G., Rothlein, R. (1991) Monoclonal antibodies to the leukocyte membrane CD18 glycoprotein complex and to intercellular adhesion molecule-1 (ICAM-1) inhibit leukocyte-endothelial adhesion in rabbits. J. Leukoc. Biol. 49, 253–257.
14. Doerschuk, C. M., Winn, R. K., Coxson, H. O., Harlan, J. M. (1990) CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. J. Immunol. 144, 2327–2333.
15. Jutila, M. A., Rott, L., Berg, E. L., Butcher, E. C. (1989) Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and MAC-1. J. Immunol. 143, 3318–3324.
16. Rutter, J., James, T. J., Howat, D., Shock, A., Andrew, D., De Baetselier, P., Blackford, J., Wilkinson, J. M., Higgs, G., Hughes, B., Robinson, M. K. (1994) The in vivo and in vitro effects of antibodies against rabbit β2-integrins. J. Immunol. 153, 3724–3733.
17. Xie, J. L., Li, R., Kotovuori, P., Vermot-Desroches, C., Wijdenes, J., Arnaout, M. A., Nortamo, P., Gahmberg, C. G. (1995) Intercellular adhesion molecule-2 (CD102) binds to the leukocyte integrin CD11b/CD18 through the A domain. J. Immunol. 155, 3619–3628.
18. Staunton, D. E., Dustin, M. L., Springer, T. A. (1989) Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. Nature 339, 61–65.
19. Diamond, M. S., Staunton, D. E., Marlin, S. D., Springer, T. A. (1991) Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell 65, 961–971.
20. Makgoba, M. W., Sanders, M. E., Luce, G. E., Dustin, M., Springer, T. A., Clark, E. A., Mannoni, P., Shaw, S. (1988) ICAM-1, a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature 331, 86–90.
21. Marlin, D., Springer, T. A. (1987) Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 51, 813–819.
22. Diamond, M. S., Alon, R., Parkos, C. A., Quinn, M. T., Springer, T. A. (1995) Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD18). J. Cell Biol. 130, 1473–1482.
23. Davis, G. E. (1992) The Mac-1 and p150,95 β_2 integrins bind denatured proteins to mediate leukocyte cell-substrate adhesion. Exp. Cell Res. 200, 242–252.
24. Lundgren-Akerlund, E., Olofsson, A. M., Berger, E., Arfors, K. E. (1993) CD11b/CD18-dependent polymorphonuclear leucocyte interaction with matrix proteins in adhesion and migration. Scand. J. Immunol. 37, 569–574.
25. Diamond, M. S., Garcia-Aguilar, J., Bickford, J. K., Corbi, A. L., Springer, T. A. (1993) The I domain is a major recognition site on the leukocyte integrin Mac-1 (CD11b/CD18) for four distinct adhesion ligands. J. Cell Biol. 120, 1031–1043.
26. Coombe, D. R., Watt, S. M., Parish, C. R. (1994) Mac-1 (CD11b/CD18) and CD45 mediate the adhesion of hematopoietic progenitor cells to stromal cell elements via recognition of stromal heparan sulfate. Blood 84, 739–752.
27. Zhou, L., Lee, D. H. S., Plescia, J., Lau, C. Y., Altieri, D. C. (1994) Differential ligand binding specificities of recombinant CD11b/CD18 integrin I-domain. J. Biol. Chem. 269, 17075–17079.
28. Walzog, B., Schuppan, D., Heimpel, C., Hafezi-Moghadam, A., Gaehtgens, P., Ley, K. (1995) The leukocyte integrin Mac-1 (CD11b/CD18) contributes to binding of human granulocytes to collagen. Exp. Cell Res. 218, 28–38.
29. Arfors, K-E., Lundberg, C., Lindbom, L., Lundberk, K., Beatty, P. G., Harlan, J. M. (1987) A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. Blood 69, 338–340.
30. Beatty, P. G., Ledbetter, J. A., Martin, P. J., Price, T. H., Hansen, J. A. (1983) Definition of a common leukocyte cell-surface antigen (GP95-150) associated with diverse cell-mediated immune functions. J. Immunol. 131, 2913–2919.
31. Uciechowski, P., Schmidt, R. (1989) Cluster report: CD11. In *Leucocyte Typing IV: White Cell Differentiation Antigens* (W. Knapp, B. Dorken, W. Gilks, E. Rieber, R. Schmidt, H. Stein, and A. von dem Borne, eds.) Oxford: Oxford University Press, 543–551.
32. Anderson, D. C., Miller, L. J., Schmalstieg, F. C., Rothlein, R., Springer, T. A. (1986) Contributions of the Mac-1 glycoprotein family to adherence-

dependent granulocyte functions: Structure-function assessments employing subunit-specific monoclonal antibodies. J. Immunol. 137, 15–27.
33. Parkos, C. A., Colgan, S. P., Diamond, M. S., Nusrat, A., Liang, T. W., Springer, T. A., Madara, J. L. (1996) Expression and polarization of intercellular adhesion molecule-1 on human intestinal epithelia: consequences for CD11b/CD18-mediated intractions with neutrophils. Mol. Med. 2, 489–505.
34. Benjamin, C., Dougas, I., Chi-Rosso, G., Luhowskyj, S., Rosa, M., Newman, B., Osborn, L., Vassallo, C., Hession, C., Goelz, S., McCarthy, K., Lobb, R. (1990) A blocking monoclonal antibody to endothelial-leukocyte adhesion molecule-1 (ELAM1). Biochem. Biophys. Res. Commun. 171, 348–353.
35. Drake, W. T., Lopes, N. N., Fenton, J. W., II, Issekutz, A. C. (1992) Thrombin enhancement of interleukin-1 and tumor necrosis factor-α induced polymorphonuclear leukocyte migration. Lab. Invest. 67, 617–627.
36. Issekutz, A. C., Chuluyan, H. E., Lopes, N. (1995) CD11/CD18 independent transendothelial migration of human polymorphonuclear leukocytes (PMN) and monocytes: Involvement of distinct and unique mechanisms. J. Leukoc. Biol. 57, 553–561.
37. Jaffe, E. A., Nachman, R. L., Becker, C. G., Minick, C. R. (1973) Culture of human endothelial cells derived from umbilical veins. Identification by morphological and immunologic criteria. J. Clin. Invest. 52, 2745–2752.
38. Thornton, B. P., Vetvicka, V., Pitman, M., Goldman, R. C., Ross, G. D. (1996) Analysis of the sugar specificity and molecular location of the β-glucan-binding lectin site of complement receptor type 3 (CD11b/CD18). J. Immunol. 156, 1235–1246.
39. Van Strijp, J. A. G., Russell, D. G., Tuomanen, E., Brown, E. J., Wright, S. D. (1993) Ligand specificity of purified complement receptor type three (CD11b/CD18, $\alpha_M\beta_2$, Mac-1): Indirect effects of an ARG-GLY-ASP (RGD) sequence. J. Immunol. 151, 3324–3336.
40. Altieri, D. C., Plescia, J., Plow, E. F. (1993) The structural motif glycine 190-valine 202 of the fibrinogen gamma chain interacts with CD11b/CD18 integrin ($\alpha_M\beta_2$, Mac-1) and promotes leukocyte adhesion. J. Biol. Chem. 268, 1847–1853.
41. Todd, R. F. (1996) The continuing saga of complement receptor type 3 (CR3). J. Clin. Invest. 98, 1–2.
42. Sitrin, R. G., Todd, R. F., III, Petty, H. R., Brock, T. G., Shollenberger, S. B., Albrecht, E., Gyetko, M. R. (1996) The urokinase receptor (CD87) facilitates CD11b/CD18-mediated adhesion of human monocytes. J. Clin. Invest. 97, 1942–1951.
43. Stockinger, H. (1997) Interaction of GPI-anchored cell surface proteins and complement receptor type 3. Exp. Clin. Immunogenet. 14, 5–10.
44. El Ghmati, S. M., Van Hoeyveld, E. M., Van Strijp, J. A. G., Ceuppens, J. L., Stevens, E. A. M. (1996) Identification of haptoglobin as an alternative ligand for CD11b/CD18. J. Immunol. 156, 2542–2552.
45. Wachtfogel, Y. T., DeLa Cadena, R. A., Kunapuli, S. P., Rick, L., Miller, M., Schultze, R. L., Altieri, D. C., Edgington, T. S., Colman, R. W. (1994) High molecular weight kininogen binds to Mac-1 on neutrophils by its heavy chain (domain 3) and its light chain (domain 5). J. Biol. Chem. 269, 19307–19312.
46. Diacovo, T. G., Roth, S. J., Buccola, J. M., Bainton, D. F., Springer, T. A. (1996) Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the β_2-integrin CD11b/CD18. Blood 88, 146–157.
47. Diamond, M. S., Staunton, D. E., deFougerolles, A. R., Stacker, S. A., Garcia-Aguilar, J., Hibbs, M. L., Springer, T. A. (1990) ICAM-1 (CD54): A counter-receptor for Mac-1 (CD11b/CD18). J. Cell. Biol. 111, 3129–3139.
48. Masumoto, A., Hemler, M. E. (1993) Multiple activation states of VLA-4: Mechanistic differences between adhesion to CS1/fibronectin and to vascular cell adhesion molecule. J. Biol. Chem. 268, 228–234.
49. Lu, H. F., Smith, C. W., Perrard, J., Bullard, D., Tang, L. P., Shappell, S. B., Entman, M. L., Beaudet, A. L., Ballantyne, C. M. (1997) LFA-1 is sufficient in mediating neutrophil emigration in Mac-1-deficient mice. J. Clin. Invest. 99, 1340–1350.
50. Schmits, R., Kündig, T. M., Baker, D. M., Shumaker, G., Simard, J. J. L., Duncan, G., Wakeham, A., Shahinian, A., Van der Heiden, A., Bachmann, M. F., Ohashi, P. S., Mak, T. W., Hickstein, D. D. (1996) LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. J. Exp. Med. 183, 1415–1426.
51. Issekutz, A. C., Issekutz, T. B. (1993) A major portion of polymorphonuclear leukocyte and T lymphocyte migration to arthritic joints in the rat is via LFA-1/MAC-1-independent mechanisms. Clin. Immunol. Immunopathol. 67, 257–263.
52. Sligh, J. E., Jr., Ballantyne, C. M., Rich, S. S., Hawkins, H. K., Smith, C. W., Bradley, A., Beaudet, A. L. (1993) Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA 90, 8529–8533.
53. Xu, H., Gonzalo, J. A., St.Pierre, Y., Williams, I. R., Kupper, T. S., Cotran, R. S., Springer, T. A., Gutierrez-Ramos, J.-C. (1994) Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. J. Exp. Med. 180, 95–109.