The Integrin \( \alpha 9 \beta 1 \) Mediates Adhesion to Activated Endothelial Cells and Transendothelial Neutrophil Migration through Interaction with Vascular Cell Adhesion Molecule-1

Yasuyuki Taooka,* John Chen,* Ted Yednock,‡ and Dean Sheppard*†

*Lung Biology Center, Center for Occupational and Environmental Health, Cardiovascular Research Institute and the Department of Medicine, University of California, San Francisco, California 94143; and ‡Elan Pharmaceuticals, South San Francisco, California 94080

Abstract. The integrin \( \alpha 9 \beta 1 \) has been shown to be widely expressed on smooth muscle and epithelial cells, and to mediate adhesion to the extracellular matrix proteins osteopontin and tenascin-C. We have found that the peptide sequence this integrin recognizes in tenascin-C is highly homologous to the sequence recognized by the closely related integrin \( \alpha 4 \beta 1 \), in the inducible endothelial ligand, vascular cell adhesion molecule-1 (VCAM-1). We therefore sought to determine whether \( \alpha 9 \beta 1 \) also recognizes VCAM-1, and whether any such interaction would be biologically significant. In this report, we demonstrate that \( \alpha 9 \beta 1 \) mediates stable cell adhesion to recombinant VCAM-1 and to VCAM-1 induced on human umbilical vein endothelial cells by tumor necrosis factor-\( \alpha \). Furthermore, we show that \( \alpha 9 \beta 1 \) is highly and selectively expressed on neutrophils and is critical for neutrophil migration on VCAM-1 and tenasin-C. Finally, \( \alpha 9 \beta 1 \) and \( \alpha 4 \) integrins contribute to neutrophil chemotaxis across activated endothelial monolayers. These observations suggest a possible role for \( \alpha 9 \beta 1 \)/VCAM-1 interactions in extravasation of neutrophils at sites of acute inflammation.

Key words: integrin • \( \alpha 9 \beta 1 \) • \( \alpha 4 \) • neutrophil migration • vascular cell adhesion molecule-1

Integrins are heterodimeric receptors for extracellular matrix and cell surface counter-receptors which play important roles in embryonic development, inflammation, wound healing, and tumorigenesis (Hynes, 1987, 1992; Ruoslahti and Pierschbacher, 1987). Integrin ligand-binding specificity is determined by structural features of each subunit, but there is considerable ligand-binding overlap among integrin heterodimers. One clue to ligand-binding overlap has been the degree of sequence homology among integrin subunits. For example, the integrin \( \alpha \) subunits \( \alpha 5 \), \( \alpha 6 \), \( \alpha I \beta \), and \( \alpha 8 \) are all closely related, and integrin heterodimers containing these \( \alpha \) subunits recognize ligands containing the peptide sequence arginine-glycine-aspartic acid (Hynes, 1992; Schnapp et al., 1995). Similarly, the \( \alpha m \), \( \alpha L \), and \( \alpha x \) subunits are highly homologous to one another and recognize closely related immunoglobulin family members as ligands (Hynes, 1992). We previously cloned and sequenced the integrin \( \alpha 9 \) subunit, and have shown that it forms a single integrin heterodimer, \( \alpha 9 \beta 1 \) (Palmer et al., 1993). The \( \alpha 9 \) subunit cDNA sequence is 41% identical to the integrin \( \alpha 4 \) subunit sequence, but 27% identical to any other integrin subunit, identifying \( \alpha 9 \) and \( \alpha 4 \) as sole members of a subfamily of integrin \( \alpha \) subunits.

In an effort to understand the structural basis of \( \alpha 9 \beta 1 \) ligand-binding in more detail, we recently mapped the \( \alpha 9 \beta 1 \) ligand-binding site in the extracellular matrix protein tenasin-C (Yokosaki et al., 1994). \( \alpha 9 \beta 1 \) binds to a single exposed loop in the third fibronectin type III repeat of tenasin-C (B-C loop) to a minimal sequence "EIDGIEL" (Schneider et al., 1998; Yokosaki et al., 1998). We noticed that a critical portion of this sequence (IDG) is homologous to the tripeptide sequence IDS present in the previously mapped ligand-binding site for the \( \alpha 4 \beta 1 \) ligand, vascular cell adhesion molecule-1 (VCAM-1; Clements et al., 1994; Yokosaki et al., 1998). Therefore, we undertook

‡ Address correspondence to Dean Sheppard, Lung Biology Center, UCSF Box 1854, San Francisco, CA 94143. Tel.: (415) 206-5901. Fax: (415) 206-4123. E-mail: deans@itsa.ucsf.edu

I Abbreviations used in this paper: EGM, endothelial cell growth media; FMLP, formyl-methionylleucylphenylalanine; HUVE, human umbilical vein endothelial; ICAM, intercellular adhesion molecule; IFN, interferon; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1.
the current study to determine whether α9β1 recognizes VCA M-1 as a ligand and whether or not any such interaction is biologically significant.

Materials and Methods

Reagents

BSA, formyl-methionylleucylphenylalanine (FMLP), and dextran were purchased from Sigma Chemical Co. Recombinant human tumor necrosis factor (TNF-α), recombinant human interferon (IFN-γ; specific activity of 10^5 U/mg), and recombinant interleukin 8 (IL-8) were obtained from R&D Systems, Inc. Fluorescent reagent, 2′,7′-bis(carboxyethyl)-5,6-carboxy-fluorescein acetoxymethyl ester (BCECF-AM) was purchased from Molecular Probes, Inc. A recombinant form of the third fibronectin type III repeat of chicken tenasin-C (PRIETO AND KATRYN CROSSIN) (Scraps Research Institute, La Jolla, CA) and prepared in E. coli was used according to the manufacturer’s specifications.

Antibodies, Cells, and Cell Culture

Mouse mAbs, Y9A2 against human α9β1 (WANG ET AL., 1996) and a N100266M (100266) against α4 (KENT ET AL., 1995), were prepared as previously described. Mouse mAbs, W6/32 against human MHC and IB4 against the integrin β2 subunit, were prepared from hybridomas obtained from American Type Tissue Collection. Mouse monoclonal antihuman VCAM-1 (CD106) was purchased from R&D Systems. FITC-labeled mouse monoclonal anti-CD16 antibody was purchased from Caltag. Human umbilical vein endothelial (HUVE) cells were purchased from Clonetics and grown in endothelial cell growth media (EGM) containing 2% FBS, 10 ng/ml human recombinant EGF, 50 μg/ml gentamycin, 2 subunit, were prepared from hybridomas obtained from Escherichia coli (Jolla, CA) and prepared in E. coli. A recombinant VCAM-1/IgG chimera (YEDNOCK ET AL., 1995), was produced in baculovirus as previously described. Recombinant intercellular adhesion molecule-1 (ICAM-1-C) fusion protein was a gift from B. I. Mihok (Centre Medecale U niversitaire, Geneva, Switzerland) to D. E. Re (University of California, San Francisco, CA). Ficol-hypaque plus for isolation of neutrophils from venous blood was purchased from Pharmacia Biotech, Inc. and used according to the manufacturer’s specifications.

Flow Cytometry

Cultured cells were harvested by trypsinization and rinsed with PBS. Non-specific binding was blocked with normal goat serum at 4°C for 10 min. Cells were then incubated with primary antibodies (unconjugated or conjugated with FITC) for 20 min at 4°C, followed by secondary antibodies conjugated with phycoerythrin (Chemicon International, Inc.). Between incubations, cells were washed twice with PBS. The stained cells were resuspended in 100 μl of PBS and fluorescence was quantified on 5,000 cells with a FACSCalibur (Becton Dickinson and Co.).

Immunoprecipitation and Western Blotting

Cells were lysed in immunoprecipitation buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM CaCl2, 1% Triton X-100, 0.1% SDS, and 0.1% NP-40) supplemented with 10 μg/ml pepstatin (Sigma Chemical Co.), 10 μg/ml leupeptin, 5 μg/ml aprotonin (Calbiochem-Novabiochem Corp.), and 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.). Human neutrophils (10^7) were incubated with 1 mM disopropyl fluorophosphate (Sigma Chemical Co.) for 15 min before cell lysis. After pre-clearing with protein G-Sepharose, the supernatant was incubated with primary antibody for 2 h at 4°C and immune complexes were captured by protein G-Sepharose for 45 min at 4°C. The beads were washed five times, and boiled in 2.5× nonreducing Laemmli sample buffer, and samples were separated by SDS-PAGE on 7.5% gels under reducing conditions and transferred to Immobilon membranes. Membranes were blocked with 4% casein, incubated with affinity-purified anti-α9 cytoplasmic domain antiserum 1057 (PALMER ET AL., 1993), and developed with luminol.

Cell Adhesion Assays

Wells of nontissue culture treated polystyrene 96-well flat bottomed microtiter plates (Nunc Inc.) were coated with incubation with 100 μg VCA M-1 or TNFα3RA A for 1 h at 37°C. A fet receptor, was washed with PBS, then blocked with 1% BSA in DMEM at 37°C for 30 min. Control wells were filled with 1% BSA in DMEM. SW480 or CHO cells were detached using trypsin/EDTA and resuspended in serum-free DMEM. For blocking experiments, cells were incubated with 10 μg/ml Y9A2 and/or 100 ng/ml IFN-γ for 15 min at 4°C before plating. The plates were centrifuged (top side up) at 10 g for 5 min before incubation for 1 h at 37°C in humidified 5% CO₂. Nonadherent cells were removed by centrifugation (top down) at 48 g for 30 min. A detached wells were fixed with 1% formaldehyde and stained with 0.5% crystal violet, and the washed wells were washed with PBS. The relative number of cells in each well was evaluated after solubilization in 40 μl of 2% Triton X-100 by measuring the absorbance at 959 nm in a microplate reader (Bio-Rad Laboratories). Three determinations were carried out in triplicate.

For adhesion assays on HUVE cells, confluent monolayers of HUVE cells were prepared in 96-well plates in 250 μl of EGM with 2% FBS. Plates were washed twice with serum-free DMEM, then stimulated for 24 h at 37°C with TNF-α (3 ng/ml) or IFN-γ (3 ng/ml) in serum-free DMEM. SW480 cells were detached using trypsin/EDTA and labeled with 2 μM BCECF-AM at room temperature for 30 min. Then cells were washed three times with serum-free DMEM and incubated with blocking antibody, Y9A2 (10 μg/ml), 100226 (10 μg/ml), or combinations of these antibodies for 15 min on ice. In some experiments, HUVE cells were incubated with CD106 (5 μg/ml) for 15 min at 37°C. 50,000 cells in 200 μl of serum-free DMEM were added to each well, and plates were centrifuged at 20 g for 5 min, and covered with aluminum foil to prevent photobleaching. Plates were then incubated for 60 min at 37°C in 5% CO₂. A fet receptor, nonadherent cells were removed by washing twice with serum-free DMEM. Finally, 200 μl of the same medium was added to each well, and fluorescence was quantified with a fluorometer (Fluoroskan II; Labsystems) at excitation wavelength 485 nm and emission wavelength 538 nm. The adherent ratio (%) was calculated as follows: (fluorescence from experimental sample – fluorescence from negative control sample) + total fluorescence added to chamber. Three determinations were carried out in triplicate.

Neutrophil Migration Assays

Neutrophils were purified from human peripheral venous blood containing 20 U/ml of heparin. Neutrophils were isolated by ficoll-hypaque density gradient centrifugation, followed by 3% dextran sedimentation (GRESHAM ET AL., 1986). Erythrocytes were subjected to hypotonic lysis, remaining neutrophils were washed and resuspended in PBS. The isolated neutrophils were >95% pure and >95% viable as assessed by Wright-Giemsa staining and trypan blue exclusion, respectively. Cell migration was analyzed essentially as described by MARCKS ET AL. (1991). In brief, glass coverslips were placed in 35-mm culture dishes and incubated with 100 μl serum-free media containing 10 μg/ml VCA M-1/g, 10 μg/ml TNFα3RA A, and 5 μg/ml of ICA M-1 or 1% BSA for 60 min at 37°C, washed, and then incubated with 1% BSA for 30 min. Neutrophils were incubated with blocking antibody, Y9A2 (30 μg/ml), 100226 (10 μg/ml), IB4 (20 μg/ml), or combinations of antibodies for 15 min at 4°C, and were then incubated for 10 min at 37°C with or without 10 nM FMLP. 106 cells were plated onto the coverslip area of each well and allowed to attach at 37°C for 5 min. Dishes were then placed on a video microscopy stage and individual fields (200×) were recorded for 3 min. Three different fields were examined in each chamber. To count the number of migrating cells in a given field, outlines were made of each cell. Cells were considered to have migrated when both the leading edge and tail of the cell moved >7 μm from their initial position. At least 40 neutrophils were analyzed per field and the ratio of migrating to total cells was calculated.

Neutrophil Transmigration Assays

Transendothelial neutrophil migration was assessed as described by COOKE ET AL. (1997).
per et al. (1995). HUVE cells were plated onto polycarbonate inserts (Transwell, 6.5-mm diameter, 8-μm pore for 24-well plate; Costar Corp.) in 200 μl of serum-containing EGM, and allowed to grow to confluence over 72 h. 500 μl serum-free DMEM was added to the lower chamber of each well. 24 h before addition of neutrophils, upper chambers were washed twice with serum-free media and new medium with or without 3 ng/ml of TNF-α. Immediately before the addition of neutrophils, the upper chambers were washed twice with serum-free DMEM and medium in the lower chamber was replaced with 500 μl serum-free DMEM with 10 nM FMLP or 50 ng/ml IL-8. In some experiments HUVE cells were incubated with CD106 (5 μg/ml) at 37°C for 15 min. Purified neutrophils were incubated with no antibody, Y9A2 (10 μg/ml), 1H4 (10 μg/ml), W6/32 (10 μg/ml), or combinations of antibodies for 15 min at 4°C, and 2 × 10^5 cells in 200 μl of media were added to each upper chamber. After 3 h at 37°C in 5% CO_2, nonadherent cells in the upper chamber were removed. Medium, including migrated neutrophils from the lower chamber, was collected, collected all the neutrophils that had transmigrated, and the absence of additional adherent neutrophils was confirmed microscopically. The medium and all washes were pooled and resuspended, and cells were counted with a hemocytometer. All determinations were carried out in duplicate and repeated at least twice.

Results

αβ1 Mediates Static Adhesion of Resting α9-transfected SW480 Cells and CHO Cells to VCAM-1

To determine whether VCAM-1 could function as a ligand for α9β1, we performed cell adhesion assays with two different cell lines, SW480 and CHO, that had been stably transfected with either an α9-expression plasmid or empty vector. Both cell lines stably expressed α9β1 on the cell surface as demonstrated by flow cytometry with the anti-α9β1 antibody Y9A2 (Fig. 1, A and B). Adhesion assays were performed on plates coated with either the known α9β1 ligand, recombinant TNFα3RAA (Fig. 1, C and D), or recombinant VCA M-1/Ig (Fig. 1, E and F). For both cell lines, α9-transfected cells adhered to both TNFα3 and to VCAM-1 in a concentration-dependent manner, whereas...
mock-transfectants did not adhere to either substrate. Adherence of each α9-transfected cell line was completely inhibited by the anti-α9β1 antibody, Y9A2, demonstrating that this effect was mediated by α9β1.

**α9β1 Mediates Adhesion to TNF-α-activated, but not to IFN-γ-activated HUVE Cells, Via Interaction with Induced VCAM-1**

To determine whether α9β1-mediated adhesion to VCAM-1 was biologically significant, we next examined the role of this integrin in adhesion of cells to resting HUVE cells, and to HUVE cells that had been activated by incubation with TNF-α (3 ng/ml), a well characterized inducer of VCAM-1 expression, or IFN-γ (3 ng/ml), a cytokine that does not induce VCAM-1 expression. The effects of each cytokine on VCAM-1 expression under the conditions used in these experiments were examined by flow cytometry with anti–VCAM-1 antibody CD106 (Fig. 2, B–D). As expected, resting HUVE cells (Fig. 2 B) and HUVE cells stimulated with IFN-γ (Fig. 2 D) did not express detectable levels of VCAM-1, but VCAM-1 was dramatically induced by TNF-α (Fig. 2 C). All cell lines examined demonstrated baseline adhesion to resting HUVE cells, and demonstrated a similar level of adhesion to HUVE activated by IFN-γ, and this baseline adhesion was unaffected by anti-α9β1 antibody (Fig. 2 A). However, only α9-transfected cells demonstrated enhanced adhesion to TNF-α-treated HUVE. This enhanced adhesion was reversed completely to basal levels by antibody to either α9β1(Y9A2) or to VCAM-1 (CD106), demonstrating that it was due to an interaction between α9β1 and VCAM-1.

**α9β1 Is Expressed on Neutrophils**

We have previously demonstrated that α9β1 is widely expressed on epithelial and smooth muscle cells (Palmer et al., 1993), but expression on leukocytes has not been reported. To determine whether α9β1 is expressed on cells likely to encounter activated endothelial cells, we performed flow cytometry on whole blood leukocytes with the α9β1 antibody Y9A2. We evaluated expression on neutrophils, monocytes, and lymphocytes by gating on each population separately, based on differential light scattering. From a separate atopic donor we evaluated expression on eosinophils, which were separated from other leukocytes based on light scattering and the absence of surface expression of CD16. In parallel, we examined expression of the structurally related integrin subunit, α4. α9β1 was not detected on lymphocytes or eosinophils and was expressed at low levels on monocytes (Fig. 3 A). In contrast, α9β1 was highly and uniformly expressed on human neutrophils. As expected, α4 was highly expressed on lymphocytes, monocytes, and eosinophils, but was also detected on neutrophils, albeit at considerably lower levels.

Expression of α9 on neutrophils was further confirmed by immunoprecipitation with Y9A2 followed by Western blotting with an affinity-purified antiserum raised against

![Figure 2](https://jcb.rupress.org/content/jcb/145/4/416/F2.large.jpg)
a unique portion of the α9 cytoplasmic domain. A band of 160 kD (appropriate molecular mass for α9) was detected in lysate of human neutrophils after immunoprecipitation with Y9A2, but not after immunoprecipitation with the control antibody R6G9 (Fig. 3B).

**α9β1 Mediates Migration of FMLP-activated Neutrophils on TNfα3 or VCAM-1**

To determine whether α9β1 expression on neutrophils was biologically significant, we initially sought to examine static adhesion of neutrophils to dishes coated with either TNfα3 or VCAM-1. However, in the absence of antibodies against β2 integrins, neutrophils avidly adhered to all surfaces examined, and in the presence of β2 integrin blocking antibodies, neutrophils could not be induced to adhere to either VCAM-1 or TNfα3 by incubation with MnCl₂, FMLP, phorbol esters, or the β1 activating antibody TS2/16 (data not shown). Therefore, we examined the possible role of α9β1 in another important neu-

Figure 3. Expression of α4 or α9 integrins on leukocytes. (A) Whole blood leukocytes were stained with control antibody E7P6 that recognizes αvβ6, an integrin not expressed on leukocytes (unshaded peaks), Y9A2 against α9β1, or 100226 against α4 (shaded peaks). Fluorescence of lymphocytes, neutrophils, eosinophils, and monocytes were analyzed separately by gating on each population on the basis of a plot of forward versus side scattering of light. Fluorescence of eosinophils was analyzed from separate atopic donor by gating on eosinophils based on light scattering and the absence of expression CD16. (B) Western blot with anti-α9 antiseraum 1057 of lysates of human neutrophils that had been immunoprecipitated with anti-α9β1 antibody Y9A2 or the control antibody R6G9 against the irrelevant integrin αvβ6 (lane 2). The expected molecular mass of the α9 subunit (160 kD) is shown by the left hand arrow. The position of molecular size marker (kD) is shown to the right.
neutrophil function, cell migration. Migration was examined by counting the numbers of individual neutrophils that migrated on chambers coated with either TNf3RAA or V C A M - 1 in the presence or absence of the activating agonist F M L P (10 nM). In the absence of F M L P , very few neutrophils migrated on either substrate (Fig. 4 A ), and antibodies against α 9 β 1 , α 4 , or β 2 integrins had no effect. In the presence of F M L P , neutrophil migration was significantly enhanced on TNf3RAA, an effect that was abolished by antibody against α 9 β 1 . F M L P also enhanced neutrophil migration on V C A M - 1 , and this effect was partially inhibited by antibodies against α 9 β 1 or α 4 , and completely inhibited by the combination of both antibodies. These data demonstrate a significant role for α 9 β 1 in mediating neutrophil migration on both substrates. A antibody against β 2 integrins had no effect on neutrophil migration on F M L P - induced neutrophil migration on TNf3RAA or V C A M - 1 . However, as expected, antibody against β 2 inhibited F M L P - induced migration on the β 2 integrin ligand ICAM-1, whereas antibodies against α 9 β 1 or α 4 had no effect (Fig. 4 B ).

\[ \text{Figure 4. Neutrophil migration on V C A M - 1 or TNf3RAA. Neutrophils were allowed to migrate on glass coverslips coated with: (A) 1% BSA or TNf3RAA (10 μg/ml); or (B) V C A M - 1 (10 μg/ml) or ICAM-1 (5 μg/ml), for 3 min in the presence or absence of F M L P (10 nM), and in the presence of: no antibody; α 9 β 1 antibody, Y 9 A 2 (10 μg/ml); α 4 antibody, 100226 (10 μg/ml); antibody 1B 4 (20 μg/ml); or the combination of these antibodies. The percentage of migrating cells was determined by analyzing \( \geq 40 \) cells from each of three microscopic fields, and is expressed as the mean (± SD) of triplicate values from two separate experiments.} \]

We next sought to determine whether the effect of α 9 β 1 and α 4 integrins described above was relevant to an in vitro model of neutrophil extravasation–migration across endothelial monolayers. H U V E cells were grown to confluence on the top side of permeable filter supports and incubated in the presence or absence of TNF-α (3 ng/ml). Purified neutrophils were added to the apical compartment in the presence or absence of F M L P added to the basal compartment. These studies were performed in the absence of blocking antibodies, or in the presence of antibodies against α 9 β 1 , α 4 , β 2 , V C A M - 1 , control antibody against M H C , or combinations of these antibodies. As expected, in the absence of blocking antibodies, F M L P greatly increased neutrophil migration into the bottom compartment, and this effect was augmented by pretreatment of H U V E cells with TNF-α (Fig. 5 A ). No antibody affected basal migration across unstimulated H U V E cells or F M L P - induced migration across unstimulated H U V E cells (Fig. 5 B ). However, antibody against either α 9 β 1 or α 4 inhibited the augmented migration induced by TNF-α. A antibody against V C A M - 1 was equally effective in inhibiting migration across TNF-α–treated H U V E cells, suggesting that TNF-α augmented transmigration was mediated by an interaction between α 9 β 1 and α 4 integrins and V C A M - 1 . As previously reported, antibody against β 2 integrins also partially inhibited transmigration in response to F M L P , but this effect was surprisingly small. Essentially identical results were obtained when IL-8 was used as a chemoattractant in place of F M L P (data not shown).

**Discussion**

The results of the current study demonstrate that the inducible endothelial cell immunoglobulin family member, V C A M - 1 , is an effective ligand for the integrin α 9 β 1 . This receptor–ligand interaction is sufficient to support adhesion of α 9 -transfected cell lines to V C A M - 1 and to TNF-α–activated H U V E cells, an effect that is mediated by the binding of α 9 β 1 to V C A M - 1 . Furthermore, α 9 β 1 is uniformly and specifically expressed on normal resting human neutrophils, and mediates both neutrophil migration on a fragment of tenasin-C or V C A M - 1 and transmigration of neutrophils across TNF-α–activated endothelial monolayers. Together, these data suggest a previously unsuspected role for α 9 β 1 and V C A M - 1 in extravasation of neutrophils at sites of acute inflammation.

In addition to α 9 β 1 , we found detectable, albeit low, levels of the structurally related integrin α 4 subunit on resting human neutrophils. This finding is consistent with several previous reports of α 4 expression on neutrophils from a variety of species (Issekutz et al., 1996; Gao and Issekutz, 1997; Davenpeck et al., 1998). Although the level of expression of α 4 we detected on human neutrophils was one to two orders of magnitude lower than expression on eosinophils, monocytes, and lymphocytes, this low level expression appeared to be biologically significant, since antibody against α 4 partially inhibited migration of neutrophils on V C A M - 1 and migration across TNF-activated endothelial monolayers. Recently, α 4 β 1 has been shown...
to mediate both neutrophil adhesion to VCA M-1 (Daven-
peck et al., 1998) and neutrophil transmigration across fi-
broblast monolayers (Gao and Issakutz, 1997). As ex-
pected, α4 integrins did not contribute to migration on 
TNF-α (9 ng/ml; B) for 24 h. DMEM con-
taining FMLP (10 nM) or DMEM alone was added to the bottom 
chamber. After 3 h at 37°C in 5% CO2, neutrophils that had mi-
gated across the monolayer were collected from the bottom 
chamber and counted. In additional chambers, untreated neutro-
phils were added to HUVE cells that had been preincubated with 
(A) or without TNF-α (3 ng/ml; B) for 15 min with antibody to VCA M-1 (CD 106, 5 μg/ml). Data are 
expressed as the mean (± SD) of quadruplicate measurements 
from two separate experiments.

Figure 5. Transmigration of neutrophils across activated HUVE cell monolayers. Purified human neutrophils that had been incu-
bated with no antibody or antibody to α9β1 (Y9A2, 10 μg/ml), 
α4β1 (100226, 10 μg/ml), β2 (IB4, 20 μg/ml), a combination of 
these antibodies, or human MHC (W6/32, 10 μg/ml) were added to 
the top chambers above microporous chambers containing 
confluent monolayers of HUVE cells that had been incubated with 
(A) or without TNF-α (3 ng/ml; B) for 24 h. DMEM con-
taining FMLP (10 nM) or DMEM alone was added to the bottom 
chamber. After 3 h at 37°C in 5% CO2, neutrophils that had mi-
gated across the monolayer were collected from the bottom 
chamber and counted. In additional chambers, untreated neutro-
phils were added to HUVE cells that had been preincubated for 
15 min with antibody to VCA M-1 (CD 106, 5 μg/ml). Data are 
expressed as the mean (± SD) of quadruplicate measurements 
from two separate experiments.

In summary, we have identified VCA M-1 as a novel and 
biologically significant ligand for the integrin α9β1, have 

demonstrated that this integrin is expressed on neutrophils 
and mediates neutrophil migration on two relevant ligands 
and neutrophil transmigration across activated endothelial 
monolayers. These findings support a role for α9β1/VCA M-1 interactions in extravasation of neutrophils at sites of inflammation.
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