Expression and Polarization of Intercellular Adhesion Molecule–1 on Human Intestinal Epithelia: Consequences for CD11b/CD18–Mediated Interactions with Neutrophils

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ABSTRACT

Background: Epithelial dysfunction and patient symptoms in inflammatory intestinal diseases such as ulcerative colitis and Crohn's disease correlate with migration of neutrophils (PMN) across the intestinal epithelium. In vitro modeling of PMN transepithelial migration has revealed distinct differences from transendothelial migration. By using polarized monolayers of human intestinal epithelia (T84), PMN transepithelial migration has been shown to be dependent on the leukocyte integrin CD11b/CD18 (Mac-1), but not on CD11a/CD18 (LFA-1). Since intercellular adhesion molecule–1 (ICAM-1) is an important endothelial counterreceptor for these integrins, its expression in intestinal epithelia and role in PMN-intestinal epithelial interactions was investigated.

Materials and Methods: A panel of antibodies against different domains of ICAM-1, polarized monolayers of human intestinal epithelia (T84), and natural human colonic epithelia were used to examine the polarity of epithelial ICAM-1 surface expression and the functional role of ICAM-1 in neutrophil-intestinal epithelial adhesive interactions.

Results: While no surface expression of ICAM-1 was detected on unstimulated T84 cells, interferon-γ (IFNγ) elicited a marked expression of ICAM-1 that selectively polarized to the apical epithelial membrane. Similarly, apically restricted surface expression of ICAM-1 was detected in natural human colonic epithelium only in association with active inflammation. With or without IFNγ pre-exposure, physiologically directed (basolateral-to-apical) transepithelial migration of PMN was unaffected by blocking monoclonal antibodies (mAbs) to ICAM-1. In contrast, PMN migration across IFNγ-stimulated monolayers in the reverse (apical-to-basolateral) direction was inhibited by anti–ICAM-1 antibodies. Adhesion studies revealed that T84 cells adhered selectively to purified CD11b/CD18 and such adherence, with or without IFNγ pre-exposure, was unaffected by ICAM-1 mAb. Similarly, freshly isolated epithelial cells from inflamed human intestine bound to CD11b/CD18 in an ICAM-1–independent fashion.

Conclusions: These data indicate that ICAM-1 is strictly polarized in intestinal epithelia and does not represent a counterreceptor for neutrophil CD11b/CD18 during physiologically directed transmigration, but may facilitate apical membrane-PMN interactions after the arrival of PMN in the intestinal lumen.

INTRODUCTION

Many inflammatory diseases of mucosal surfaces are characterized by the transepithelial migration of neutrophils (PMN). For example, in active ulcerative colitis and Crohn's disease, migration of PMN across the intestinal epithelial lining results in the classic histological feature of the crypt abscess (1,2). Using model intestinal epithelia, we have previously shown that specific interactions occurring between PMN and intestinal epithelia during transmigration can influence crucial epithelial functions ranging from barrier maintenance to electrolyte secretion (3–8). Sim-

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ilar relationships between PMN-epithelial interactions and epithelial dysfunction likely also occur in the human intestine, since transepithelial PMN migration correlates well with barrier perturbation and symptomatology in patients with inflammatory bowel disease (9).

Perhaps the best-characterized paradigm for migration of PMN across cell monolayers is migration across vascular endothelium. However, it is likely that migration across endothelia differs substantially from that across polarized columnar epithelia. For example, the initial adhesive interaction between PMN and endothelium occurs under conditions of vascular shear and is regulated by lectin-like molecules termed selectins (10). Shear flow is not encountered during transepithelial migration. Moreover, the geometry of PMN interactions with endothelia and columnar epithelia differs considerably. In the vasculature, initial interactions with PMN occur at the endothelial apical surface, while initial interactions of PMN with epithelia occur at the epithelial basolateral surface. In contrast to relatively flat endothelial cells, columnar epithelial cells are often $>20\ \mu$ in height, likely dictating extensive interactions with the lateral membrane during the process of PMN transepithelial migration. For example, lateral epithelial membrane interactions with PMN are mediated by CD47 (11), events which occur after CD11b/CD18–mediated adhesion. PMN also appear to use distinct repertoires of leukocyte integrins to cross endothelial and epithelial barriers. PMN adhesion to endothelial cells can be inhibited by monoclonal antibodies (mAbs) against CD11a/CD18 (leukocyte function–associated antigen; LFA-1) (12,13), and against CD11b/CD18 (Mac-1, Mol) (12,14,15). In contrast, physiologically directed (basolateral-to-apical) transepithelial migration appears to be mediated exclusively by CD11b/CD18 (16). Additionally, while lectin-like adhesive interactions occur between PMN and epithelia, they are distinct from the selectin-mediated adhesive events characterizing PMN-endothelial interactions (17). Lastly, whereas transendothelial migration of PMN is modulated by the cytokines interleukin 1 (IL-1) and tumor necrosis factor (TNF) and by lipopolysaccharide (LPS) (15), these agents have no effect on PMN migration into, or across, epithelial monolayers (18). However, stimulation of epithelia with interferon-γ (IFNγ), a dominant cytokine of the intestinal mucosa (19,20), modulates *n*-formylated peptide (fMLP)–driven transepithelial migration of PMN via CD11b/CD18–dependent mechanisms (18). Enhanced intercellular adhesion molecule–1 (ICAM-1) expression at the vascular surface of endothelial cells in states of inflammation is important for both CD11b/CD18– and CD11a/CD18–mediated firm adhesion. The regions on ICAM-1 that interact with the CD11a/CD18 and CD11b/CD18 integrins have been characterized and mapped to domains 1 and 3, respectively (21,22). However, the potential role of ICAM-1 in PMN interactions with columnar epithelia is much less clear. Numerous studies report epithelial cell ICAM-1 expression (23–29) and thus, it is often assumed that ICAM-1 plays a crucial role in epithelial-PMN interactions. However, the distribution of ICAM-1 on the surface of polarized epithelial cells has not been unequivocally defined. In addition, there are no reports detailing the role of ICAM-1 in mediating PMN-epithelial interactions during physiologically directed (basolateral-to-apical) transepithelial migration. Lastly, it is not known whether ICAM-1 on epithelia indeed serves as a major ligand for CD11b/CD18–mediated adherence.

In this study, we show that ICAM-1 is inducible in T84 intestinal epithelial cells and that ICAM-1 similarly appears focally on inflamed human intestinal epithelium in vivo. In both settings, ICAM-1 surface expression is restricted to the apical membrane domain. fMLP-driven transepithelial migration of PMN is attenuated by antibodies to ICAM-1 only when the direction of migration is apical-to-basolateral (nonphysiologic) thus affording PMN access to apically restricted ICAM-1. These antibodies do not block PMN transmigration in the physiologic direction, adhesion of suspended T84 cells to immobilized, purified CD11b/CD18, or adhesion of freshly isolated epithelial cells from inflamed intestine to purified CD11b/CD18. Thus, ICAM-1 interactions with the PMN integrin CD11b/CD18 play no role in physiologically directed transmigration of PMN, indicating that novel epithelial counterreceptors for CD11b/CD18 must exist on the basolateral membrane. The apical polarization of ICAM-1 together with data indicating that ICAM-1 can mediate interactions with PMN once they appear in the lumen, suggest that ICAM-1 may mediate post-transmigration interactions between PMN and epithelial apical membranes.

MATERIALS AND METHODS

Cell Culture

Monolayers of the human intestinal epithelial cell line T84 (30) were used to study ICAM-1

localization on intestinal epithelia and the functional role of ICAM-1 in epithelial-neutrophil interactions in vitro. T84 cells were grown in a 1:1 mixture of Dulbecco's modified Eagle's medium and Hams F-12 medium supplemented with 15 mM HEPES buffer (pH 7.5), 14 mM $NaHCO_3$, 40 μg/ml penicillin, 8 μg/ml ampicillin, 90 μg/ml streptomycin, and 5% newborn calf serum. Subculturing (or harvesting) was performed every 6–8 days by treatment with 0.1% trypsin and 1.0 mM EDTA in Ca^{++}- and Mg^{++}-free phosphate-buffered saline (30). For apical-to-basolateral transmigration experiments, T84 cells were grown on collagen-coated, polycarbonate permeable supports (inserts) with a surface area of 0.33 cm^2 (Costar Inc., Cambridge, MA, U.S.A.) as previously described (16,31). For physiologically directed (basolateral-to-apical) transmigration assays, T84 cells were plated on the underside of collagen-coated permeable filters resulting in inverted monolayers (16). Such inverted monolayers allow gravitational settling of PMN onto the filter and, subsequently, contact with the basolateral aspect of the monolayer. For surface-labeling experiments by ELISA, T84 cells were plated at 3/4 confluent density in 96-well microtiter plates and used 2–3 days later. Suspended T84 cells for use in adhesion or surface-labeling experiments were prepared by standard trypsin/EDTA (31) treatment of monolayers grown on plastic.

Transfected L cells expressing surface ICAM-1 were maintained in DME supplemented with 100 μM hypoxanthine, 400 nM aminopterin, and 16 μM thymidine as previously described (14). CHO cell transfectants expressing a glycosyl-phosphatidylinositol anchored form of human ICAM-1 (GPI–ICAM-1) or a domain-deleted form of ICAM-1 (F185–ICAM-1) were grown and maintained as described previously (32,33). As shown in the past, F185–ICAM-1 lacks domain 3 of ICAM-1, whereas GPI–ICAM-1 contains all five domains.

Isolation of Human Intestinal Epithelial Cells

Primary isolates of human colonic epithelial cells were obtained immediately following surgical resection of colonic segments containing normal mucosa or mucosa involved by medically refractory inflammatory bowel disease. Normal colonic epithelial cells were obtained from uninvolved mucosa of colon specimens resected for therapy of colonic adenocarcinoma. Using a procedure extensively modified from (34), approximately five 10-cm^2 sections of mucosa were washed in RPMI media followed by incubation in RPMI containing 1.5 mM dithiothreitol (DTT) (BioRad Inc., Richmond, CA, U.S.A.) and 2 mM EDTA for 20 min. After a second incubation for 20 min. in the same buffer without DTT, epithelial cells were dislodged by gentle scraping of the mucosa with fine-mesh stainless steel screen and used as epithelial cell suspensions.

Neutrophil Isolation

Neutrophils (PMN) were isolated from whole blood (anticoagulated with citrate/dextrose) obtained from normal human volunteers, using a gelatin sedimentation technique as previously described in detail (35). PMN were resuspended in modified HBSS without Ca^{++} and Mg^{++} (HBSS[−]) at a concentration of 4×10^7 cells/ml (4°C) and used for subsequent experiments.

Antibodies

Antibodies to ICAM-1 (Table 1) included the previously characterized R6.5 (IgG2a) (12,14,21) and RR1/1 (IgG 1) (36,37). Binding, noninhibitory antibody controls included mAb to major histocompatibility complex (MHC 1), W6/32 (38), anti–CD11a/CD18 (mAb TS1/22 [39]) and anti–CD11b/CD18 (mAb OKM1 [40]). Inhibition of CD11b-dependent binding was achieved using either mAb 44a (16,41) or mAb CBRM1/29 (33).

Additional mAbs to human ICAM-1 that were used were generated by previously described methods (33,42). In brief, female BALB/c mice were immunized with purified ICAM-1 (2 μg/immunization) (43) 28 days (intraperitoneally) and 3 days (intraperitoneally and intravenously) before fusion of splenocytes with the nonsecreting murine myeloma P3X63Ag8.653 (CRL 1580; American Type Culture Collection, Rockville, MD, U.S.A.). To select for mAb to ICAM-1, indirect immunofluorescence flow cytometry was used. ICAM-1 mAbs that blocked binding to Mac-1 but not to LFA-1 were identified by selecting hybridomas that preferentially recognized domain 3 of ICAM-1. Hybridomas (n = 672) were screened against CHO cell transfectants expressing a glycosyl-phosphatidylinositol–anchored form of human ICAM-1 (GPI–ICAM-1) (32) or a domain-deleted form of ICAM-1 (F185–ICAM-1) (14). All five domains of ICAM-1 are present on GPI–ICAM-1, whereas domain 3 is deleted on F185–ICAM-1. To permit

TABLE 1. Functional specificities of anti-ICAM-1 mAbs

mAb	Isotype	Inhibition of Binding to Purified CD11b/CD18[a]	Inhibition of Binding to Purified CD11a/CD18[b]
RR1/1	IgG1	−	++++
R6.5	IgG2a	++++	++++
CBRIC1/7	IgG1	++++	++
CBRIC1/11	IgG1	++++	+
CBRIC1/12	IgG1	−	−

[a]Adhesion of ICAM-1–transfected L cells to purified CD11b/CD18 was assessed in the presence of the antibodies listed as shown in Fig. 1. ++++, ≥80% inhibition; −, no inhibition.

[b]Adhesion of ICAM-1–transfected L cells to purified CD11a/CD18 was assessed as described in Materials and Methods. R6.5 (21,36) inhibited adhesion to purified CD11a/CD18 by 91.5 ± 1.5% (++++), CBRIC1/7 inhibited 56 ± 10% (++), CBRIC1/11 inhibited 34 ± 7% (+), and CBRIC1/12 showed 0 ± 5% inhibition (−). RR1/1 (++++) inhibits ≥80% as reported elsewhere (21,36). Percentages represent mean ± SD; one of three experiments.

simultaneous screening, the two CHO transfectants were differentially labeled. GPI–ICAM-1–expressing CHO cells were labeled red with 10 ml of a filtered solution that contained the DNA-intercalating dye hydroethidine (40 μg/ml; Polysciences Inc., Warrington, PA, U.S.A.) and DME, 5% FCS for 30 min at 37°C (14). Excess dye was removed by washing four times with DME, 5% FCS. In contrast, F185–ICAM-1–expressing CHO cells were not labeled. Subsequently, CHO transfectants (2.5×10^4 of each population) were mixed, incubated in microtiter plates with hybridoma supernatants, labeled with FITC-conjugated secondary antibodies, and subjected to flow cytometry. Of 275 hybridomas that recognized the GPI–ICAM-1 expressing CHO cells, 15 did not recognize the F185–ICAM-1 CHO cells. Three of these mAbs, CBRIC1/7, CBRIC1/11, and CBRIC1/12 (IgG1 isotype) (44), are characterized here. Hybridomas were cloned twice by limiting dilution, isotyped (Immunopure mAb Isotyping Kit, Pierce, Rockford, IL, U.S.A.), and antibody purity was confirmed by reducing (5% β-mercaptoethanol) and nonreducing (50 mM iodoacetamide) SDS-8% PAGE.

Protein Purification

Mac-1 and LFA-1 were purified by immunoaffinity chromatography using peripheral blood leukocyte lysates as previously described (14,45). ICAM-1 was purified by immunoaffinity chromatography from detergent lysates of the erythroleukemic cell line K562 as described (43).

Antibody Labeling/Immunoprecipitation Experiments

To determine surface expression of ICAM-1, a combination of cell surface ELISA, immunofluorescence and biotinylation/immunoprecipitation techniques were used. Crude surface expression of ICAM-1 was assayed by ELISA. Briefly, T84 cell suspensions were treated with human recombinant IFNγ (generously provided by Genentech, 1000 U/ml) as previously reported (18), plated at 75% confluent density and cultured for 48 hr. To assay surface expression of ICAM-1, monolayers were washed with HBSS (HBSS (in g/l): $CaCl_2$ 0.185, $MgSO_4$ 0.098, KCl 0.4, KH_2PO_4 0.06, NaCl 8, Na_2HPO_4 0.048, glucose 1, and HEPES added to 10 mM, pH 7.4), cooled to 4°C and incubated with anti–ICAM-1 for 2 hr. After washing and incubation with peroxidase-conjugated secondary antibody (1 hr, 4°C), color development with ABTS (2,2'Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St Louis, MO, U.S.A.) was performed. Antibody binding was quantitated at OD 405 using a microtiter plate reader as previously described (46). The above ELISA was modified and used to assess sensitivity of ICAM-1 epitopes to cell trypsinization procedures. In such experiments, T84 cells were plated in the absence (media alone) or presence of IFNγ (1000 U/ml) on 25-cm^2 tissue culture flasks. After 48 hr, the cells were harvested using standard trypsinization techniques as described above. The washed cell pellets were divided into equal aliquots of 15 cm^2

(~1.5 × 10^7 cells) in microcentrifuge tubes, cooled to 4°C, and incubated for 2 hr with 10 μg/ml anti–ICAM-1. The cells were pelleted, washed, and incubated at 4°C with peroxidase-conjugated secondary antibody for 1 hr. After washing, color was developed with ABTS and the cell-free supernatant assayed at OD405 as described above.

Immunoprecipitation experiments were performed on T84 monolayers selectively biotinylated on the apical or basolateral surface (47,48). T84 monolayers cultured on 5-cm^2 permeable supports were washed free of media and selectively labeled on the apical or basolateral surface with a solution of 1 mM sulfo-NHS biotin (Pierce) in HBSS for 20 min (4°C). The biotinylation reaction was quenched with 150 mM NH_4Cl, and each 5-cm^2 monolayer was solubilized in 1 ml of buffer containing 100 mM KCl, 30 mM NaCl, 2 mM EDTA, 10 mM HEPES, pH 7.4, 2% Triton X-100, and protease inhibitors including 1.25 mM PMSF, 5 μg/ml chymostatin, 1 μg/ml each of leupeptin, pepstatin, and bestatin (4°C). The T84 cell lysate was precleared for 2 hr with 50 μl of IgG-Sepharose (normal mouse IgG coupled to CNBR activated Sepharose 6MB at a protein/sepharose ratio of 3 mg/ml according to the manufacturer's instructions (Pharmacia Inc., Upsala, Sweden) followed by incubation for 2 hr with 20 μl anti–ICAM-1–Sepharose (R6.5), prepared as above (4°C). The washed immunoprecipitates were denatured by heating to 100°C in the presence of 50 μl of nonreduced sample buffer followed by reduced SDS-PAGE and Western blotting using standard protocols. Labeled proteins were visualized after incubation with peroxidase-conjugated streptavidin using enhanced chemiluminescence, according to the manufacturer's instructions (Amersham).

For immunofluorescence, T84 monolayers were fixed in 3.7% paraformaldehyde in PBS (20 min, 20°C) and washed with gelatin-PBS. Fixed monolayers were then permeabilized with PBS containing 0.5% Triton X-100 (15 min, 20°C) followed by incubation with 10 μg/ml 1° antibody diluted in gelatin-PBS (2 hr, 20°C). After washing, monolayers were incubated with FITC-conjugated 2° antibody (1 hour, 20°C; Cappel Inc., Durham, NC, U.S.A.) and mounted in PBS-glycerol-*p*-phenylenediamine. Labeled monolayers were then viewed with a Zeiss/BioRad MRC-600 confocal fluorescence microscope. As a control for background labeling, control monolayers were incubated with comparable concentrations of normal mouse IgG. As a positive control for basolateral labeling, anti-β1 integrin was used (Telios Inc., San Diego, CA, U.S.A.).

Immunostaining of Human Tissue

ICAM-1 staining was also performed on 3μ frozen tissue sections of human colonic mucosa obtained from fresh surgical specimens. Tissue sections, mounted on glass coverslips, were air dried followed by fixation in 3.7% paraformaldehyde as above. Fluorescent labeling was performed as above.

Transmigration Experiments

FMLP-driven PMN transmigration experiments were performed using both standard (apical-to-basolateral migration) and inverted (basolateral-to-apical migration) T84 monolayers as previously described (16,31). Briefly, confluent T84 monolayers were washed free of media and preincubated with saturating concentrations of antibody (20 min, 20°C). For such antibody preincubation, 50 μl of antibody in HBSS was added to the upper chamber of the monolayer setup. After a 20-min preincubation, HBSS was added (100 μl) followed by 1 × 10^6 PMN in 25 μl HBSS(−) (HBSS as above but without $CaCl_2$ or $MgSO_4$). Transmigration was initiated by transfer of antibody/PMN containing monolayers to 24-well tissue culture plates containing 1 ml of 1 μM fMLP in HBSS. After incubation for 110 min at 37°C, neutrophil migration across monolayers to the fMLP-containing lower chambers was quantitated by myeloperoxidase assay (16). Migration across monolayers pretreated with IFNγ (1000 U/ml, 48 hr) was compared with that across control, untreated monolayers.

Adhesion Experiments

The effects of anti–ICAM-1 antibodies on intestinal epithelial cell binding to CD11b/CD18 was assayed using a slightly modified form of a previously described method (14). Microtiter plates were coated with functionally active purified CD11b/CD18. For optimal coating with CD11b/CD18, a solution of purified integrin at ≥0.1 mg/ml was diluted 15-fold with 150 mM NaCl, 2 mM $MgCl_2$, and 25 mM Tris, pH 7.3, and allowed to bind to microtiter wells for 2 hr (20°C). Nonspecific binding was blocked by incubation with a solution of blocking buffer containing 0.5% bovine serum albumin (phosphate-buffered saline, pH 7.4, containing 2 mM $MgCl_2$, 10 mM

dextrose, and 0.5% BSA). T84 cells and human colonocytes were resuspended in blocking buffer and fluorescently labeled for 10 min at 37°C by incubation with 5 μg/ml BCECF-AM (2′,7′-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester; Molecular Probes Inc., Eugene, OR, U.S.A.), washed and immediately used in adhesion assays. Antibodies in blocking buffer were added to the CD11b/CD18–coated microtiter plates followed by a 20-min incubation (20°C). Subsequently, labeled epithelial cells (50 μl, ~2.5 × 10^5 cells per well) were added, followed by gentle, constant swirling for 15 min to allow antibody binding to cells while preventing adhesion (20°C). Plates were then held flat and stationary at 37°C for 1 hr to permit adhesion. To quantitate adhesion, each well was gently washed twice, and total fluorescence of each well was assayed at an excitation/emission wavelength of 485/535 nm using a fluorescence microtiter plate reader (Millipore Inc., Milford, MA, U.S.A.). The percentage of applied cells adherent to purified CD11b/CD18 in the absence of inhibition typically ranged from 25 to 55%. Adhesion was calculated as the fluorescence ratio (postwash fluorescence/prewash fluorescence) × 100 and expressed as the percentage of adherent cells.

The binding of ICAM-1–transfected (ICAM-1^+) L cells was examined as previously detailed (14). Briefly, purified CD11b/CD18 and CD11a/CD18 were diluted and absorbed (25 μl) to 6-cm petri dishes for 50 min at 37°C, and nonspecific sites were blocked by subsequent addition of PBS, 2 mM $MgCl_2$, 0.5% heat-treated BSA (blocking buffer). After removal from tissue culture plates with trypsin-EDTA (Gibco Laboratories, Grand Island, NY, U.S.A.), ICAM-1^+ L cells were washed twice and resuspended in PBS, 2 mM $MgCl_2$, 0.5% heat-treated BSA (5.0 × 10^6 cells/ml). For 30 min prior to the binding assay, cells (1.0 × 10^6 cells in 200 μl) were preincubated at 4°C with mAb (200 μl of culture supernatant or 20 μg/ml purified mAb). Subsequently, cells were washed once and resuspended (1.0 × 10^6 cells/ml) in blocking buffer, added in 1 ml to petri dishes, and incubated for 50 min at 37°C. The procedure for washing and quantitation of adhesion has been described (14).

Statistical Analysis

Data are presented as the mean ± SD and compared by Student's *t* test or by one-way analysis of variance (ANOVA).

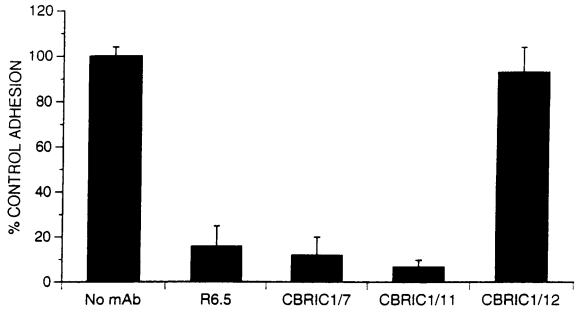

FIG. 1. Effect of mAbs on adhesion of ICAM-1 transfected L cells to purified CD11b/CD18

ICAM-1–expressing L cells were detached, washed, and resuspended (5 × 10^6/ml) in PBS containing 2 mM $MgCl_2$ and 2% heat-treated BSA as described in Materials and Methods. Cells were preincubated for 30 min with no antibody (No mAb) or with one of the anti–ICAM-1 mAb above (20 μg/ml, 4°C). Subsequently, cells were washed, resuspended (10^6/ml), and allowed to bind to 6-cm petri dishes coated with purified CD11b/CD18 for 50 min (37°C). Unbound cells were washed free and adherent cells were quantitated as described previously (22). Bars = mean ± SD of adherence normalized with respect to binding in the absence of antibody (100%); one of three experiments.

RESULTS

Antibody Inhibition of CD11b/CD18 Binding to ICAM-1

To characterize the ability of antibodies to inhibit adhesion, the binding of ICAM-1–transfected L cells to purified CD11b/CD18 was assessed (Fig. 1). R6.5 binds to the immunoglobulin superfamily (IgSF) domain 2 of ICAM-1 (21,22), has been previously characterized to inhibit both CD11a and CD11b/CD18–ICAM-1 binding (12), and blocked adhesion in this assay by >75%. Two newly generated antibodies, CBRIC1/7 and CBRIC1/11, appear to bind to IgSF domain 3 of ICAM-1. Both CBRIC1/7 and CBRIC1/11 recognized the ICAM-1 protein containing all five domains (GPI–ICAM-1) but not the ICAM-1 mutant lacking the CD11b/CD18–binding site (domain 3 of ICAM-1). These two mAbs inhibited adhesion to purified CD11b/CD18 by >75%. In contrast, a third newly generated antibody, CBRIC1/12, which also appears to bind to domain 3, did not inhibit adhesion. Since CBRIC1/12 binding to ICAM-1–expressing cells was comparable to that of CBRIC1/7 and CBRIC1/11 (not shown), it served as a noninhibitory binding control for the domain 3 of ICAM-1. An additional control, RR1/1 mAb, has

been previously shown to inhibit CD11a/CD18– but not CD11b/CD18–mediated binding to ICAM-1 and maps to domain 1 (21,36). Table 1 summarizes the inhibitory effects of the various anti-ICAM-1 mAbs on ICAM-1-mediated adhesive interactions with CD11b/CD18 and CD11a/CD18.

IFNγ Induction of ICAM-1 Expression on T84 Cells

ICAM-1 expression could not be detected by surface ELISA on unstimulated T84 cells (Fig. 2). In contrast, time- ($t_{1/2}$ ~30 hr) (Fig. 2A) and concentration-dependent (ED_{50} ~30 U/ml) (Fig. 2B) expression of ICAM-1 was induced by IFNγ preexposure. Binding of the various ICAM-1 antibodies to the surface of T84 monolayers was analyzed and compared by antibody dilution. While there was no detectable surface labeling in unstimulated monolayers, all ICAM-1 mAbs bound to IFNγ-pretreated (1000 U/ml, 48 hr) monolayers in a concentration-dependent manner with saturation at a concentration of 10 μg/ml or less (Data not shown).

Since T84 cells assume a polarized phenotype on permeable supports, and polarized epithelia exhibit biochemically distinct apical and basolateral membrane domains, we examined whether ICAM-1 expression was domain restricted. Immunoprecipitation studies were performed on T84 monolayers that were selectively biotinylated on the apical or basolateral surface (Fig. 3). We and others have used selective cell surface labeling to successfully label apical and basolateral epithelial membrane proteins on cells cultured on permeable supports (47–51). As shown in Fig. 3, ICAM-1 was not expressed on either apical or basolateral surfaces in the basal state. In contrast, treatment with IFNγ resulted in expression of ICAM-1 restricted to the apical domain (Fig. 3, (+) IFN, Lane A). In data not shown, strong labeling of a basolaterally restricted adhesive protein, β1 integrin, was observed in immunoprecipitations with basolaterally biotinylated T84 cells.

Apical domain restricted expression of ICAM-1 in fully polarized cells was confirmed by confocal microscopy localization (Fig. 4). En face images in the plane of the apical membrane revealed multifocal staining for ICAM-1 in IFNγ-exposed but not in untreated cells (Fig. 4 A and B). In Panel C of Fig. 4, reconstructed images confirmed that IFNγ induced expression was limited to the apical membrane. No labeling was observed in unstimulated control T84 cells (Fig. 4D). Of interest was the lack of detectable ICAM-1 expression on the basolateral surface of T84 monolayers (Fig. 4C). Such staining patterns were identical for T84 cell monolayers cultured in a standard or an inverted fashion. As controls for basolateral staining, Panels E and F of Fig. 4

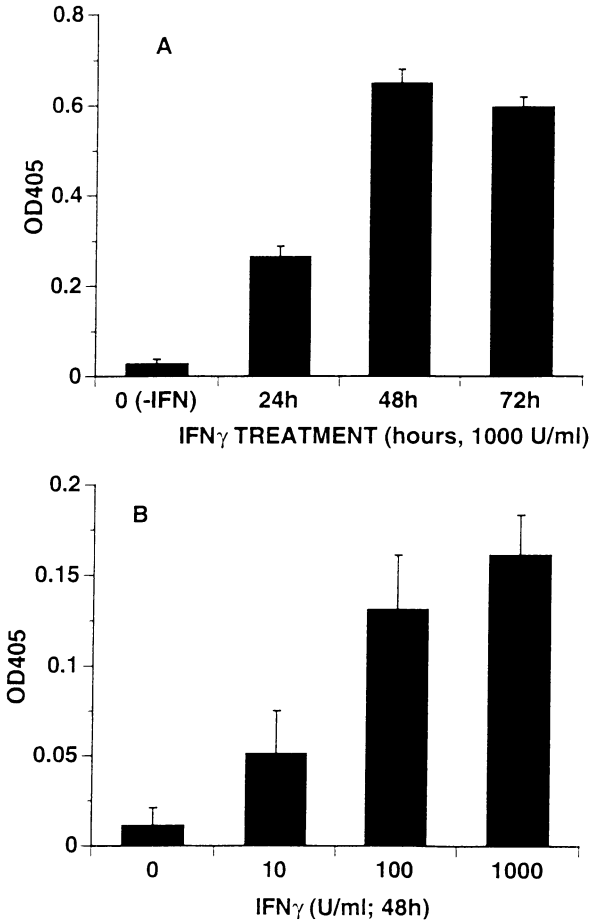

FIG. 2. Time course and concentration dependence of IFNγ-induced cell surface ICAM-1 expression on T84 cells

(A) As described in Materials and Methods, T84 cells cultured at 3/4 confluent density on 96-well tissue culture plates were exposed to IFNγ (1000 U/ml) for the times indicated and subsequently assayed for surface ICAM-1 expression using mAb R6.5 by enzyme-linked immunosorbent assay (ELISA). (B) T84 cells similarly prepared were exposed to IFNγ for 48 hr at the concentrations indicated and subsequently assayed for surface ICAM-1 by ELISA as in Panel A. Color was developed and quantitated at OD 405 using a microtiter plate reader as described in Materials and Methods. Optical density at 405 nm represents antibody-treated well values after subtraction of the values from wells treated with irrelevant nonbinding antibody control. Each value represents the mean ± SD of four individual monolayers.

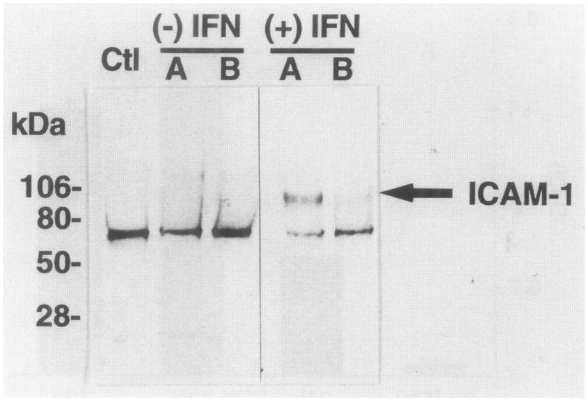

FIG. 3. Immunoprecipitation of ICAM-1 from confluent T84 monolayers following selective cell surface labeling (apical or basolateral biotinylation)

T84 monolayers cultured to confluency on 5-cm^2 permeable supports were cooled to 4°C and selectively labeled on the apical (Lanes A) or basolateral surface (Lanes B) with biotin followed by immunoprecipitation of ICAM-1 using mAb R6.5 as described in Materials and Methods. Immunoprecipitates were subjected to SDS-PAGE on 5–16% polyacrylamide gradient gels followed by Western blotting, incubation with streptavidin-peroxidase, and development by enhanced chemiluminescence. The immunoprecipitates of control, unstimulated monolayers ([−] IFN) were compared with those of monolayers maximally stimulated with IFNγ ([+] IFN). As can be seen, there is no ICAM-1 in the apically (A) or basolaterally labeled (B) unstimulated monolayers ([−] IFN). However, following IFNγ stimulation (1000 U/ml, 48 hr) there is the appearance of a ~100 kD ICAM-1 band in the apically labeled (A) but not the basolaterally labeled (B) lanes ([+] IFN). Ctl, control immunoprecipitation with normal mouse IgG; this lane shows a nonspecific, prominent 70-kD band which is also observed in the ICAM-1 lanes.

show the confocal images of a basolaterally restricted protein, β1 integrin (46,52), which is observed as lateral cell membrane labeling. These data suggest that ICAM-1 is not expressed on the basolateral domain with which PMN interact during transepithelial migration.

Inflammation-Elicited Surface Expression of ICAM-1 in Natural Intestinal Mucosa

Since IFNγ is a dominant cytokine in intestinal mucosa in states of intestinal inflammation (19), we examined inflamed intestinal mucosa by ICAM-1 immunostaining in order to determine whether ICAM-1 was similarly focally expressed and, if so, whether it was apically restricted. As shown in Panels G and I of Fig. 4, human crypt intestinal epithelia from inflamed mucosa also expressed ICAM-1 focally in an apically restricted manner. The localized staining in the crypt is consistent with T84 phenotype which displays functional characteristics of crypt, but not surface, intestinal epithelial cells. Also consistent with our findings using T84 cells (see above) and with other reports (28,53,54), no detectable ICAM-1 expression was observed on control, noninflamed mucosa (data not shown).

The Role of ICAM-1 in Neutrophil Transepithelial Migration

APICAL-TO-BASOLATERAL MIGRATION OF PMN BECOMES ICAM-1 DEPENDENT FOLLOWING IFNγ ACTIVATION. While it has been previously shown that PMN transepithelial migration is dependent on the neutrophil β2 integrin CD11b/CD18, little is known about epithelial ligands for this integrin (16,18). Since an important vascular ligand for CD11b/CD18 is ICAM-1, we investigated the role of ICAM-1 in PMN-intestinal epithelial interactions using a panel of anti-ICAM-1 mAbs that block CD11b/CD18–ICAM-1 binding. As shown in Panel A of Figure 5, none of the anti-ICAM-1 antibodies tested affected fMLP-driven, apical-to-basolaterally directed PMN migration across resting T84 monolayers. In addition, transmigration in the absence of antibody was indistinguishable from the binding antibody control (data not shown).

In contrast, Fig. 5B shows that such non-physiologically directed, fMLP-driven PMN transepithelial migration was significantly inhibited by R6.5, CBRIC1/11, and CBRIC1/7 in IFNγ pre-exposed (i.e., ICAM-1–expressing) monolayers: (64.4 ± 4.3%, 73.8 ± 13%, and 50 ± 19% inhibition for R6.5, CBRIC1/7, and CBRIC1/11; $p < 0.01$, 0.02, and 0.05, respectively). Antibodies known to block either ICAM-1–LFA-1 interactions (RR1/1) or to recognize an ICAM-1 domain 3–dependent epitope but not to influence ICAM-1–CD11b/CD18 interactions (CBRIC1/12) did not influence PMN transmigration.

PMN MIGRATION IN THE PHYSIOLOGIC (BASOLATERAL-TO-APICAL DIRECTION) IS INDEPENDENT OF ICAM-1. PMN migration physiologically occurs in the basolateral-to-apical direction. In contrast to apical-to-basolaterally directed migration, none of the antibodies inhibited physiologically directed mi-

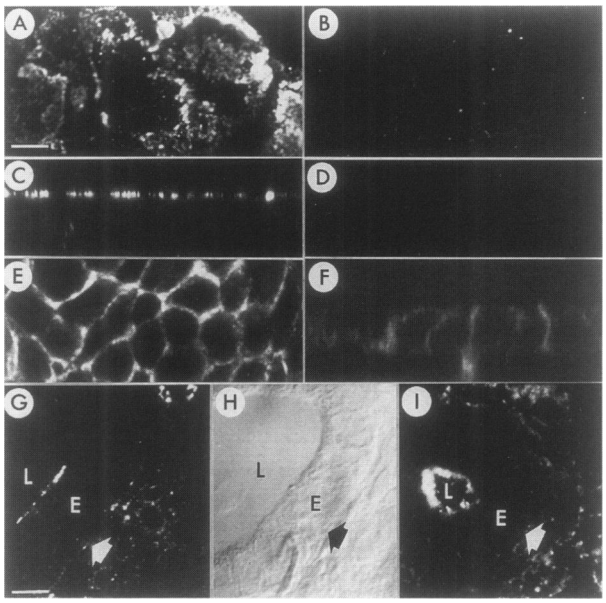

FIG. 4. Localization of ICAM-1 on T84 monolayers and natural human colonic epithelium by immunofluorescence

(A–F) T84 monolayers cultured on permeable supports were stimulated with IFNγ (1000 U/ml, 48 hr), fixed with paraformaldehyde, permeabilized with Triton X-100, followed by labeling with primary antibody as described in Materials and Methods. Primary antibodies included anti–ICAM-1 (R6.5), control normal mouse IgG, and anti–β1 integrin, an epithelial subjunctional adhesion molecule as a basolateral binding control (52). Shown are x-y (en face) and x-z computer reconstructed confocal fluorescence micrographs. (A and B) the x-y (en face) immunofluorescence on the apical surface after staining for ICAM-1 on IFNγ stimulated and control, unstimulated T84 monolayers, respectively. (C and D) the x-z reconstructed immunofluorescence staining pattern for ICAM-1 following IFNγ stimulation (C) or in the absence of IFNγ stimulation (D). (E and F) As controls for basolateral labeling, the immunofluorescence staining patterns for β1 integrin in the mid-zone (x-y) of the monolayer and as an x-z reconstructed image, respectively. As can be seen in Panel B, there is no detectable ICAM-1 immunofluorescence in the absence of IFNγ stimulation. However, there is bright apical ICAM-1 immunofluorescence after IFNγ stimulation (A and C). Treatment of monolayers with normal mouse IgG (not shown) resulted in no detectable immunofluorescence, identical to that shown in Panel D. (G–I) Three-micron–thick frozen sections of human colonic epithelium involved with active inflammatory bowel disease. Sections were air dried, fixed with paraformaldehyde, and fluorescently labeled for ICAM-1 (R6.5, 3 μg/ml). (G and I) The ICAM-1 staining in active Crohn's disease with focal staining of the apical membrane and sub-epithelial inflammatory cells (to the right of the closed arrows): L, lumen; E, epithelium; closed arrow, the basal pole of the epithelium which abuts the basement membrane. (H) For orientation, the Nomarski image corresponding to that shown in Panel G. In data not shown, there was no significant ICAM-1 staining of epithelium in noninflamed colon sections or with irrelevant IgG. Scale bars in Panels A and G = 20 μ.

gration, irrespective of IFNγ pretreatment (W6/32 versus RR1/1, R6.5, CBRIC1/7, CBRIC1/11, CBRIC1/12; NS; Fig. 6). These results suggest that the morphologically and biochemically defined restricted expression of ICAM-1 to the apical membrane in IFNγ exposed cells (and similarly imaged in inflamed colonic epithelia) precludes involvement of ICAM-1–CD11b/CD18–mediated adhesive interactions in physiologically directed migration.

The Role of ICAM-1 in Intestinal Epithelial Cell Adhesion to Purified CD11b/CD18

T84 MONOLAYERS. The dependence of physiologically directed PMN transmigration on CD11b/CD18 (16), with the apparent independence of PMN transmigration on expression of ICAM-1, suggested that CD11b/CD18–mediated binding of PMN to T84 cells involves ligands other than ICAM-1. To test this hypothesis, we next examined whether suspended T84 cells bound to purified, surface immobilized, CD11b/CD18 and, if so, whether such interactions occurred independently of ICAM-1. We first confirmed that surface ICAM-1 epitopes remained intact in suspended epithelial cells preactivated with IFNγ. Aliquots of approximately 1×10^7 T84 cells harvested by standard methods were washed and incubated with 10 μg/ml anti–ICAM-1 or control IgG for 2 hr (4°C) followed by washing and incubation with peroxidase-conjugated secondary antibody. After washing the cell pellets, color was developed and the supernatants assayed using a microtiter plate reader as described in Materials and Methods. Control, unstimulated T84 cells were compared with T84 cells stimulated with IFNγ. Trypsin-insensitive surface ICAM-1 (R6.5) was readily detectable after IFNγ stimulation (0.4 versus 0.6 and 2.27 OD units for control IgG versus unstimulated and IFNγ-stimulated T84 cells, respectively).

The effects of anti–ICAM-1 mAbs on adhesion of suspended T84 cells (±IFNγ preactivation) to immobilized CD11b/CD18 were next as-

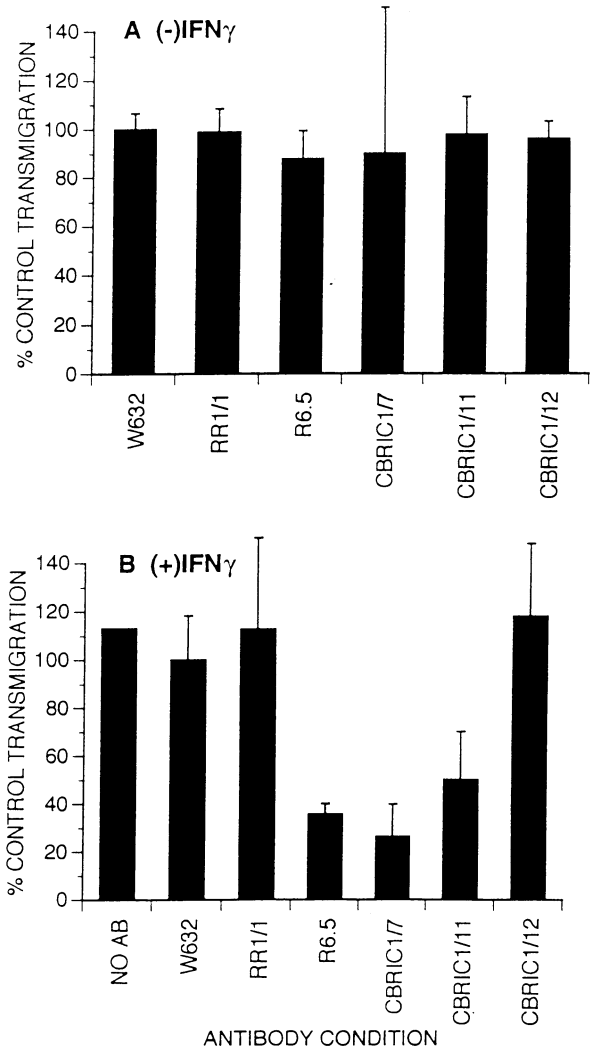

FIG. 5. Effect of anti–ICAM-1 mAbs on apical-to-basolaterally directed PMN transepithelial migration

Transmigration assays were performed in the apical-to-basolateral direction on both untreated (A) and IFNγ-pretreated (1000 U/ml, 48 hr; B) T84 monolayers cultured on 0.33-cm^2 permeable supports. As described in Materials and Methods, saturating concentrations of the antibodies (10 μg/ml) indicated were added to the apical side of confluent T84 monolayers and preincubated for 20 min (20°C) followed by addition of 1×10^6 PMN. The monolayers were transferred to 24-well tissue culture wells, each containing 1 ml of 1 μM fMLP in HBSS to initiate PMN transmigration which was allowed to proceed for 110 min (37°C). PMN which had transmigrated to the opposite reservoir or PMN within monolayers were quantitated by myeloperoxidase assay as described in Materials and Methods. Transmigration is normalized with respect to a control binding antibody, anti–MHC I W6/32 and expressed as a percentage of control. As can be seen in Panel A, none of the anti–ICAM-1 antibodies had any effect on transmigration in the control unstimulated monolayers. However, as shown in Panel B, following IFNγ stimulation mAbs R6.5, CBRIC1/7, and CBRIC1/11 significantly inhibited (>60%) transmigration. In data not shown, anti-CD11b (44a) but not anti-CD11a (TS1/22) markedly inhibited transmigration in such assays as previously reported (16,18). Data represents the mean ± SD of four monolayers for each condition; one of three experiments.

sessed (Fig. 7). Although anti–CD11b/CD18 mAb inhibited T84 cell binding by more than 90% (56.4 ± 6.9 versus 2.4 ± 0.4% adhesion for antibody control versus anti-CD11b/CD18; $p < 0.01$), none of the anti–ICAM-1 antibodies influenced T84 cell–CD11b/CD18 adhesive interactions, even when epithelial cells were pretreated with IFNγ (Fig. 7A).

Since transepithelial migration of PMN has been shown to be CD11b/CD18 dependent and CD11a/CD18 independent, it is likely that T84 cells selectively adhere to the CD11b/CD18 integrin. We thus tested this hypothesis using immobilized CD11b/CD18 or CD11a/CD18. T84 cells preferentially adhered to purified CD11b/CD18 but not CD11a/CD18 (61 ± 5% of applied cells adhered to Mac-1, while 5 ± 3.3% adhered to LFA-1; $p < 0.01$) (Fig. 7B). No significant adhesion to BSA alone was observed. Moreover, T84 cells adhered to purified CD11b/CD18 in a specific manner. Pretreatment of the CD11b/CD18–coated plastic with inhibitory mAb to CD11b (CBRM1/29 or 44a [33,41]) completely inhibited T84 cell binding to CD11b/CD18 but had no effect on the small amount of observed binding to CD11a/CD18 (51 ± 2 cells adherent to Mac-1 reduced to 0 ± 1 cell after anti-CD11b treatment versus 6.4 ± 1.1 cells adherent to CD11a/CD18 before anti-CD11b and 7.6 ± 2.1 adherent after incubation with anti-CD11b) (Fig. 7C).

CD11b/CD18–MEDIATED, ICAM-1–INDEPENDENT ADHERENCE OF NATURAL HUMAN COLONOCYTES. The adhesion results using T84 cells above imply that intestinal epithelial cells adhere avidly to CD11b/CD18 and do so, even in the IFNγ stimulated state, by an ICAM-1–independent mechanism. To determine whether such observations were

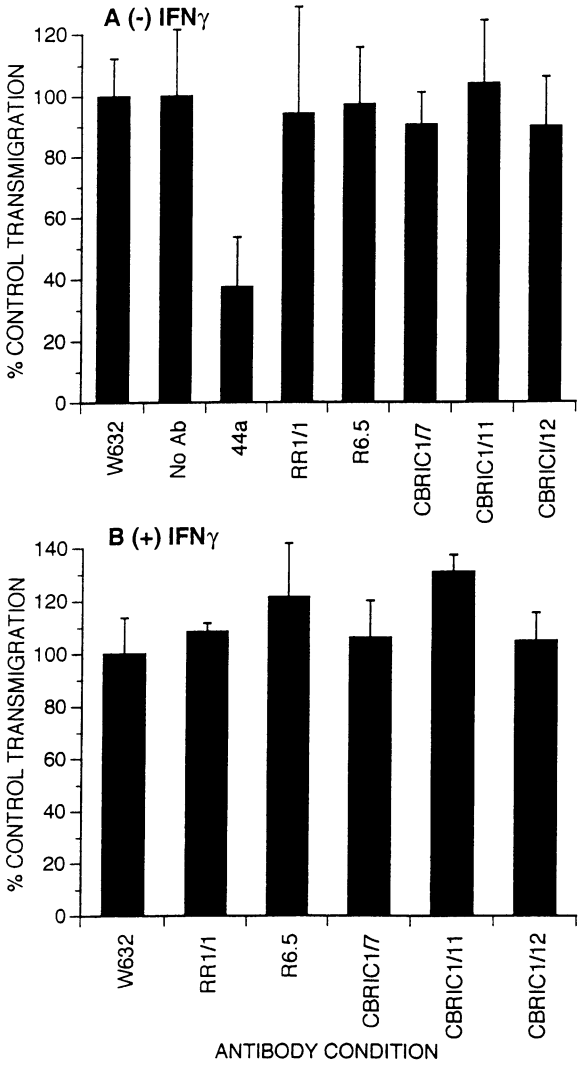

FIG. 6. Effect of anti-ICAM-1 mAbs on physiologically directed (basolateral-to-apical) PMN transepithelial migration

Transmigration assays were performed as in Fig. 6 except that inverted T84 monolayers were used and antibodies were applied to both apical and basolateral surfaces. As described in Materials and Methods, transmigration assays with such inverted monolayers allow gravitational settling of PMN onto the filter and subsequently, contact with the basolateral aspect of the monolayers prior to initiation of migration. Transmigration is normalized as a percentage of transmigration in the presence of the noninhibitory control antibody W6/32. As can be seen, there is no inhibitory effect of any of the ICAM-1 antibodies in unstimulated (A) or IFNγ-activated (B) monolayers. In addition, transmigration in the absence of antibody (no Ab; A) was not statistically different than that in the presence of W6/32. In data not shown, anti-CD11b (44a) but not anti-CD11a (TS1/22) markedly inhibits (>65%) transmigration in such assays, as previously reported (16,18). Data represents the mean ± SD of four monolayers for each condition; one of three experiments.

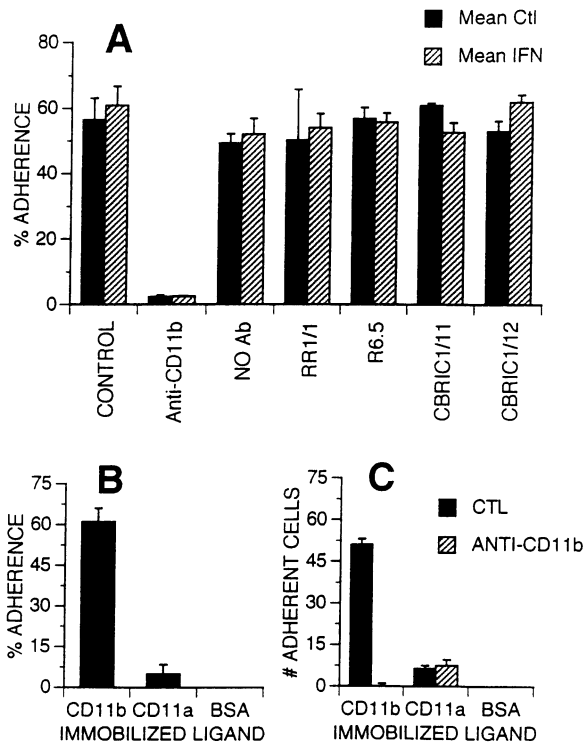

FIG. 7. ICAM-1–independent adherence of T84 cells to purified CD11b/CD18.

Suspensions of BCECF-AM–labeled T84 cells (~8 × 10^6/ml) were preincubated with saturating concentrations of the antibodies shown followed by adhesion to purified CD11b/CD18 in 96-well tissue culture plates as described in Materials and Methods. (A) Percentage of applied cells adherent to CD11b/CD18 after various antibody treatments is shown. Adhesion of control unstimulated T84 cells was compared with T84 monolayers stimulated with IFNγ. As an antibody control, adhesion in the presence of a noninhibitory anti-CD11b antibody, OKM1, was used. As a functionally inhibitory antibody to CD11b, mAb 44a was used by pretreating the CD11b/CD18 coated wells for 30 min at 10 μg/ml. (B) Adhesion of unstimulated T84 cells to purified CD11b/CD18 is compared with adhesion to CD11a/CD18 and bovine serum albumin (BSA). (C) The effect of saturating concentrations of functionally inhibitory anti-CD11b antibody (44a) on unstimulated T84 cell adhesion to CD11b/CD18, CD11a/CD18, and BSA is shown. Adherent T84 cells (nonlabeled) within a defined area were quantitated by microscopy as described previously (14). Values representative of one of two experiments showing the mean ± SD of quadruplicate determinations.

physiologically relevant, we studied adherence of natural intestinal epithelial cells to immobilized CD11b/CD18. For these studies, epithelial cells were obtained from inflamed mucosa to bias in favor of detecting potential ICAM-1–mediated interactions with CD11b/CD18. Adhesion of

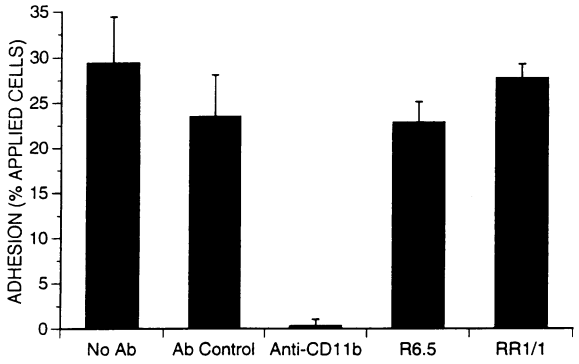

FIG. 8. ICAM-1–independent adherence of natural human colonocytes to purified CD11b/CD18

Natural human colonocytes obtained from a surgical resection specimen of a patient with severe inflammatory bowel disease were labeled and used in adhesion assays with purified CD11b/CD18 as described in Fig. 7 and in Materials and Methods. As described in the previous figure, the negative antibody control (Ab Control) represents adhesion after treatment of wells with OKM1. As a functionally inhibitory antibody to CD11b, mAb CBRM1/29 was used (Anti-CD11b). Pretreatment of T84 cells with saturating concentrations of functionally inhibitory anti-ICAM-1 antibody R6.5 had no effect on the adhesion to CD11b/CD18. Data represent the mean ± SD of quadruplicate determinations.

colonocytes was reduced from 23.5 ± 4.6% (for Ab control OKM1) to 0.3 ± 0.7% after incubation with a functionally blocking mAb to CD11b (Fig. 8). However, pretreatment of the colonocytes with anti–ICAM-1 mAbs failed to inhibit adhesion (27.7 ± 1.6% and 22.8 ± 2.3% versus 23.5 ± 4.6% adherence for RR1/1 and R6.5 versus OKM1, respectively; NS) (Fig. 8). Additional studies were performed on natural human colonocytes obtained from uninflamed colon with indistinguishable results (data not shown). Such results indicate that normal and inflamed natural human intestinal epithelial cells, like T84 cells, can adhere to CD11b/CD18 in an ICAM-1–independent fashion.

DISCUSSION

These studies identify several aspects of human intestinal epithelial ICAM-1 expression potentially relevant to the biology of transepithelial migration of PMN. First, ICAM-1 is either not expressed or expressed at exceedingly low levels on the surface of resting T84 cells and intestinal epithelial cells from the uninflamed human colon. Second, when ICAM-1 surface expression is induced in vitro by IFNγ exposure or in vivo by intestinal inflammation, it is exclusively restricted to the apical domain. Lastly, it is probable that ICAM-1 does not serve as an epithelial counterreceptor for PMN during basolateral-to-apical (i.e., physiologically directed) transepithelial migration because of its apically restricted expression. These findings provide yet another contrast to transendothelial migration of neutrophils, in which ICAM-1 is positioned to function as a ligand for CD11b/CD18, since PMN first encounter ICAM-1 on the lumenal surface of blood vessels before migrating from the microvasculature.

ICAM-1 has been reported to be expressed in various epithelia in response to cytokines. Thus, epithelial ICAM-1 expression has implicitly been suggested to play a crucial role in leukocyte-epithelial adhesive interactions (23–26,29,55). For example, ICAM-1 is expressed on (i) renal tubular epithelia during allograft rejection (56,57); (ii) alveolar and bronchial epithelial cells, an effect increased in asthmatics and following infection, ozone injury, or inflammatory diseases (29,58–61); (iii) primary cultures of urothelia when exposed to LPS and urothelial cell lines (55,62); (iv) intestinal epithelia of patients with ulcerative colitis and graft-versus-host disease (27,28); and (v) gastric epithelia in association with colonization by *Helicobacter pylori* (23).

However, most studies have not addressed the polarized nature of epithelia when localizing the ICAM-1 protein expression on a cellular level (23,25,26,62). Additionally, where shown, surface polarity of ICAM-1 has been strictly defined by immunohistochemistry. Biochemical assays of polarity and analyses of functional consequences of polarized ICAM-1 expression have not been reported. In the mouse and rat lung, immunoelectron microscopic localization of ICAM-1 has revealed restricted expression to the alveolar type I cell apical membrane (63,64), just as we report here for intestinal epithelia. Similar studies also reveal induction of apical ICAM-1 on alveolar type II epithelial cells as a consequence of pneumonia (63,65). In two inflammatory intestinal disease states (ulcerative colitis and graft-versus-host disease), evaluation of published figures stained by immunoperoxidase techniques for ICAM-1 appear to reveal focal ICAM-1 expression on the lumenal epithelial surface (27,28), also in agreement with our results.

A study of an intestinal epithelial cell line with a high level of basal ICAM-1 expression concluded that ICAM-1 served as a ligand for PMN during transepithelial migration (25). However, PMN were added to the apical compartment, and thus apical-to-basolaterally directed migration was analyzed in this study. PMN migration across urothelial cells cultured on permeable supports has also been studied and transmigration found to be inhibited by mAb to ICAM-1 (55). However, the best available models to date for studies of cultured urothelial cells are, unfortunately, restricted to cell lines that do not form uniform monolayers with highly restricted permeability (i.e., are not likely to form the polarized monolayers that characterize normal human urothelia). In the skin, a multilayered and geometrically contrasting epithelium, ICAM-1 is also expressed after stimulation with IFNγ or TNFα (66) and may play a role in the inflammatory response at this site (66–68). Thus, while the results of the current study may apply to polarized epithelial monolayers (gastrointestinal tract, respiratory tract, kidneys), different paradigms may apply to stratified (multilayered) epithelia such as skin.

Although the natural epithelial ligand(s) for CD11b/CD18 have not been identified, their existence is strongly implied since T84 cells and natural colonic epithelial cells strongly adhere to purified CD11b/CD18 even under conditions that strongly inhibit ICAM-1–dependent adhesion. However, if other epithelial ligand(s) for CD11b/CD18 exist, it is possible that binding of such ligand(s) could substitute for ICAM-1–mediated binding. Candidate epithelial receptors for neutrophils and/or CD11b/CD18 include carbohydrate structures. PMN transepithelial migration is markedly inhibited by mannose 6-phosphate, glucose 6-phosphate, and complex carbohydrates such as fucoidin. However, such carbohydrate-induced effects do not appear to be secondary to inhibition of E, L, or P selectins (17). In addition, we have recently shown that heparan sulfate glycans can interact directly with CD11b/CD18 (69). Thus, carbohydrate-mediated adhesive interactions between neutrophils and epithelia are likely to be important.

Given the apparent apically restricted distribution of ICAM-1 on intestinal epithelium, it is worthwhile considering what physiological role such defined expression may subserve, especially given the distinct differences from endothelial ICAM-1 expression. In the lung, there may be a role for apically expressed ICAM-1 which could provide a tether for alveolar macrophages which normally reside in the air-filled alveolar space. While it is possible that lumenal ICAM-1 might be important in "docking" inflammatory cells in or near sites of inflammation, such cells do not normally reside in the gut lumen. However, receptor-mediated pathways for secreted PMN products are present on the apical membrane of intestinal epithelium. We have shown that activated PMN release 5'AMP which is converted to adenosine by an apical 5' ectonucleotidase (CD72) (5,6,70). Adenosine then binds to an apical A2b receptor resulting in electrogenic chloride secretion (8). Such PMN-induced chloride secretion is the basis of secretory diarrhea and might serve to rid the intestine of noxious agents. Thus, under conditions of active inflammation, induction of adhesive ligands such as ICAM-1 on the lumenal epithelial surface might serve to preserve PMN-apical membrane interactions which play key regulatory roles for epithelial transport events.

In addition, ICAM-1–based interactions between PMN and the apical intestinal epithelial membrane might serve a role in the clearance of pathogens from the surface of the intestinal mucosa. *Salmonella typhimurium*, for example, after binding to the apical membrane of intestinal epithelial cells, induces the basolateral release of IL-8 and apical release of other undefined chemotactic factors that promote PMN transepithelial migration (71,72). Under such conditions, apical ICAM-1 expression might serve as an adhesive ligand which retains PMN at the specific site of bacterial attachment to epithelia.

In summary, the data reported here indicate that ICAM-1 cannot serve as a counterreceptor for CD11b/CD18 during PMN transepithelial migration as it normally occurs during inflamed states. Such observations strongly suggest the presence of a novel counterreceptor(s) for CD11b/CD18 on epithelial basolateral membrane domains. These data also suggest that, in order to define a potential role for ICAM-1–mediated interactions between PMN and epithelia, it is necessary to focus on post-transmigration events in which PMN are positioned to interface with appropriate ICAM-1–expressing domains.

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