Neutrophil Migration across a Cultured Intestinal Epithelium

Dependence on a CD11b/CD18-Mediated Event and Enhanced Efficiency in Physiological Direction

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Abstract

Neutrophils (PMN) migrate across intestinal epithelia in many disease states. Although such migration serves as a histological index of disease activity, little is known concerning the molecular events underlying PMN-intestinal epithelial interactions. We have studied chemotactic peptide-driven movement of PMN across cultured monolayers of the human intestinal epithelial cell line T84. Using a transmigration microassay, we show that both the decreased transepithelial resistance (76±3%) and transmigration ($4\pm0.6 \times 10^5$ PMN · cm^{-2}, when PMN applied at $6 \times 10^6 \cdot cm^{-2}$) are largely prevented by MAbs which recognize either subunit of the PMN surface heterodimeric adhesion glycoprotein, CD11b/CD18. In contrast, such PMN-epithelial interactions are unaffected by MAbs recognizing either of the remaining two α subunits CD11a or CD11c. PMN from a leukocyte adherence deficiency patient also failed to migrate across epithelial monolayers thus confirming a requirement for CD11/18 integrins. By modifying our microassay, we were able to assess PMN transmigration across T84 monolayers in the physiological direction (which, for technical reasons, has not been studied in epithelia): transmigration was again largely attenuated by MAb to CD18 or CD11b (86±2% and 73±3% inhibition, respectively) but was unaffected by MAb to CD11a, CD11c. For standard conditions of PMN density, PMN transmigration in the physiological direction was 5–20 times more efficient than in the routinely studied opposite direction. (*J. Clin. Invest.* 1991. 88:1605–1612.) Key words: paracellular • integrin • inflammation

Introduction

PMN migration across intestinal crypt epithelium is a structural hallmark of a variety of active intestinal diseases such as ulcerative colitis, Crohn's disease, and infectious enterocolitis (1, 2). The degree of PMN transmigration into intestinal crypts and formation of crypt abscesses is indicative of disease severity (3), yet little is known concerning the mechanisms by which PMN migrate across epithelia. We previously modeled this transmigratory event in vitro using isolated peripheral blood human PMN and epithelial monolayers derived from T84 cells (4), a human intestinal epithelial cell line with crypt-like features (5–7). Using this model we have shown that PMN induced to migrate across T84 monolayers do so by impaling intercellular tight junctions (TJ)[1] and, putatively due to the resulting defects in TJ, transepithelial resistance falls and TJ permeabilities to inert paracellular solutes reversibly increase (4). Structural studies of this transmigratory event suggest that epithelial-PMN adhesive event(s) might be required (8). Although PMN-epithelial adhesive interactions have, in general, not been well characterized, studies of PMN-endothelial adhesive interactions indicate that several PMN surface ligands may participate in PMN adherence to blood vessel walls. Among the most prominent of these are three heterodimeric PMN surface proteins which share a common subunit (β_2, CD18) and belong to a superfamily of cell adhesive proteins termed integrins (9). Here we find that the CD11b/CD18 (Mo1/Mac-1) member of this glycoprotein family is required for PMN-T84 cell adhesive interactions which permit transepithelial migration to proceed. These studies were greatly aided by development (reported here) of an extremely simple, highly efficient assay of transmigration which permits large numbers of epithelial monolayers to be studied in parallel. Lastly, we have modified this assay to permit assessment of PMN transmigration in the "serosal-to-mucosal" direction; the direction which occurs naturally in the intestine but has been difficult to evaluate for technical reasons. These latter studies reveal that PMN transmigration is markedly more efficient in this "physiological" direction but that transmigration in this direction also requires a CD11b/CD18 mediated event.

Methods

Cell culture and assay systems. T84 cells (passages 70–100) were grown and passaged as previously described (5–7). In initial experiments, we used monolayers grown on 2-cm^2 polycarbonate filters that had been coated with ammonia-precipitated, glutaraldehyde–cross-linked collagen (10). In later experiments, to diminish reservoir volume and permit large numbers of parallel experiments to be performed, we used a commercially available ring-supported polycarbonate filter with a surface area of 0.33 cm^2 (Costar inserts; Costar Corp., Cambridge, MA). While T84 cells do not attach well to such filters unless collagen coated, we could accomplish acceptable collagen coating by placing 50 μl of viscous rat-tail collagen solution (10) diluted 1:100 with 60% ethanol in each well and allowing them to dry overnight (Fig. 1). Such treatment also substantially diminishes matrix depth and eliminates the matrix precipitation and crosslinking steps which impair the ability of PMN to transmigrate across the matrix (unpublished observations). As a result,

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1. *Abbreviations used in this paper:* LAD, leukocyte adhesion deficiency; MPO, myeloperoxidase; TJ, tight junctions.

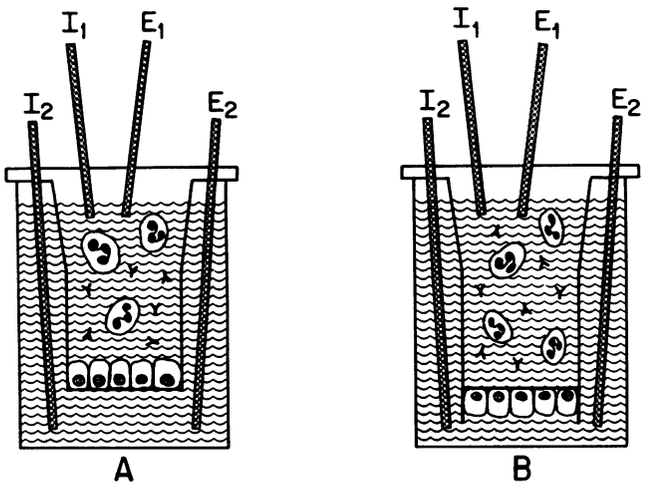

Figure 1. A simple efficient system for electrical assays of PMN transmigration across T84 monolayers. (*A*) T84 monolayers are shown growing in the conventional configuration in which PMN are layered, by gravity on "mucosal" surface of the monolayer. (*B*) Inverted monolayers for use in studies of PMN transmigration in the physiologically relevant (serosal-to-mucosal) direction. See Methods for details. Antibodies are depicted as Y in the upper chambers of *A* and *B*. The letters I and E correspond to agar bridges interfacing with current passing or voltage sensitive electrodes in inner (1) or outer (2) reservoirs.

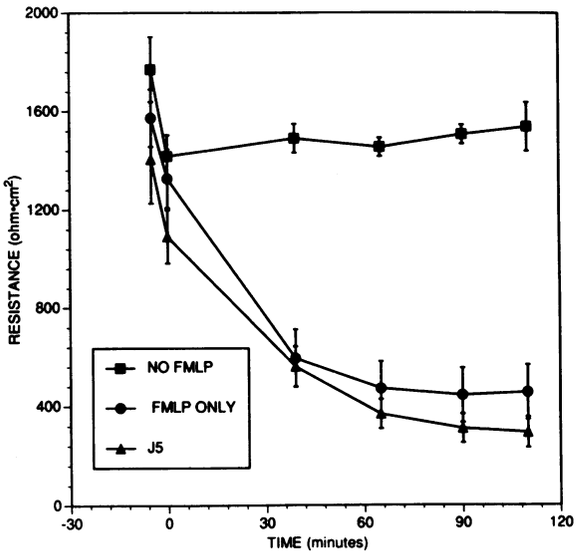

Figure 2. Representative experiment showing effect on transepithelial resistance of FMLP-elicited PMN transmigration (mucosal-to-serosal) across T84 monolayers. PMN were added to monolayers at time 0. In the absence of FMLP, resistance was stable from 0–120 min. In contrast, in the presence of FMLP with or without J5, an antibody that binds to non-CD11/CD18 related PMN surface epitopes at high density (19, 21), marked decreases in resistance were observed. Resistances were calculated as described in Methods and in references 5–7. Each condition is the mean of five monolayers±SE. One representative experiment of five.

serosal-to-mucosal PMN transmigration can also be studied by constructing inverted monolayers (Fig. 1). To make inverted monolayers (thus permitting gravitational settling of PMN on the serosal aspect of the monolayer), polycarbonate rings of the same diameter as the insert base and 1 mm in height were glued to the bottom of inserts (Fig. 1). Inverted filters were treated with rat-tail collagen as above, and T84 cells were plated onto the inverted inserts and allowed to attach overnight after which the inserts were righted into 24-well culture plates, maintained for 6–14 d to achieve electrical stability (11) and then used. Polycarbonate rings were glued to the inserts with RTV silicone rubber (General Electric Co., Wilmington, MA) which has the dual advantage of not releasing trace cytotoxic solvents after setting and of being nonconductive, thus preventing current leaks across the assembly. The volumes used in the upper and lower reservoirs of the inserts were 0.2 and 1.0 ml, respectively.

Electrical assays were performed on 2-cm^2 rings as previously described (5–7) but were modified for monolayers grown on inserts. Rather than being mounted in modified Ussing chambers (5–7) or sterile chambers (12) as we have previously described, inserts were studied in the 24-well plates in which they were grown. As shown in Fig. 1, the serosal and mucosal reservoirs were each interfaced with calomel and Ag-AgCl electrodes via 5% agar bridges made with HBSS. Measurements of resistance were obtained using a dual voltage clamp (University of Iowa) as described before (5–7) except that current pulses of 25 μA were used since 100-μA currents lead to time-dependent polarization effects that would have necessitated current pulses in the millisecond range for accurate reading. Electrical readings obtained from such inserts were highly reproducible (as will be reflected by the data shown below) and correlated well with data obtained from standard Ussing chambers (as can be seen by comparing the data from Fig. 2 with that from reference 4). The only positional effects noted in this system occur when the tips of the agar bridges in the outer well abut the bottom of the plate, a problem which can be avoided by cutting the ends of these bridges to a 45–60° taper. We have previously shown that such inserts can also be reliably used to obtain electrical measurements of Cl^- secretion in T84 monolayers so prepared (13). For studies reported here, ~ 700 monolayers were used, the vast majority of which were on 0.33-cm^2 inserts.

Transmigration experiments. To ensure constant temperature, all experiments were carried out in a constant 37°C environment. Insert-monolayer assemblies were lifted from wells, the medium was discarded, and the entire insert was gently washed in HBSS (Sigma Chemical Co., St. Louis, MO, containing, in grams per liter, $CaCl_2$ 0.185, $MgSO_4$ 0.098, KCl 0.4, KH_2PO_4 0.06, NaCl 8, Na_2HPO_4 0.048, glucose 1, type H1387) to which was added 10 mM Hepes pH 7.42.

Inserts were then placed into new wells containing HBSS and allowed to equilibrate for 5–15 min. To ensure that contaminating serum factors did not influence the observed results, in a subset of experiments monolayers were washed in HBSS two days before the experiment and were subsequently maintained in insulin and transferrin-supplemented serum free media until use. PMN, isolated as described previously (14), were induced to transmigrate using a 1-μM gradient of FMLP (4). Where used, antibodies were added to the upper reservoir ("mucosal" for monolayers in unmodified inserts and "serosal" for inverted monolayers—see Fig. 1) 15 min before the addition of PMN. In apical-to-basolateral transmigration experiments, PMN were added to a final density of $6 \times 10^6 \cdot cm^{-2}$ from a 5× PMN stock suspended in HBSS without calcium or magnesium.

Chemotaxis assay. PMN contents of monolayers and lower reservoirs were quantitated by assaying the azurophil granule marker, myeloperoxidase (MPO) as described previously (15) with slight modifications. Briefly, inserts containing monolayers were washed five times with HBSS to remove nonmigrated PMN. Myeloperoxidase was then released by solubilization of monolayers in 1.0 ml of HBSS containing 0.5% Triton X-100. To the lower reservoirs, 50 μl of 10% Triton X-100 in H_2O was added for a final concentration of 0.5%. The pH was then adjusted to 4.2 with 100 μl of 1.0 M citrate buffer pH 4.2. For each sample, color development was assayed at 405 nm on a microtiter plate reader (Bio-Rad Laboratories, Richmond, CA) after mixing equal parts

of sample and a solution containing 1 mM 2,2'-azino-di-(3-ethyl) dithiazoline sulfonic acid and 10 mM H_2O_2 in 100 mM citrate buffer pH 4.2, 20°C. After appropriate color development the reaction was terminated by the addition of SDS to a final concentration of 0.5%. The assay was standardized with known concentrations of the same PMN used in each experiment and was linear in the range used ($0.3–50 \times 10^4$ cells/ml). MPO activity was negligible in lysates of T84 monolayers unexposed to PMN. The measured MPO contents of reservoirs (or filters) accurately reflected PMN-associated MPO since cell-free (microfuged) supernatants of both reservoirs contained < 3% of total MPO activity.

Morphologic studies. Monolayers were fixed in 2% glutaraldehyde in HBSS at the end of representative experiments and processed for 1-μm Epoxy sections as previously described (5, 11).

Antibodies. Mouse ascitic fluids containing previously characterized, functionally inhibiting monoclonal antibodies to CD18, CD11a (16, 17), CD11b (18, 19), or CD11c (20) were used at dilutions known to inhibit leukocyte adherence to endothelium and other surfaces. J5, an anti-CD10 monoclonal antibody known to bind to PMN but not inhibit adherence, was used as a control (19, 21). An irrelevant mouse ascitic fluid (NS1) served as an additional control.

Data presentation. Since variations may exist both in monolayer resistance between groups of monolayers and in PMN obtained from different donors, individual experiments were performed using large uniform groups of monolayers and PMN from single donors. The figures shown are of representative experiments each of which were repeated as indicated in the legend. Data are presented as means±standard error.

Results

Mucosal-to-serosal transmigration

Inserts: A simple reliable assay system for epithelial transmigration. We have previously shown that FMLP induced mucosal-to-serosal transmigration across 2-cm^2 monolayers subsequently mounted in Ussing-type chambers results in a fall in transepithelial resistance putatively due to reversible disruption of T84 tight junctions (4). The transmigration-associated resistance responses across the 0.33-cm^2 inserts used for these studies are shown in Fig. 2. In this group of 15 monolayers, the baseline resistance was 1,581±80 ohm · cm^2. After 110 min of FMLP-induced transmigration, in the absence of antibody, the resistance fell by 71% to 458±99 ohm · cm^2. This FMLP-induced response was not significantly different from that occurring in the presence of a control antibody, J5, where resistance fell by 79% to 295±55 ohm · cm^2. The time course of the resistance fall in Fig. 2 had a $t_{1/2}$ of 30 min with a near maximal response at 90 min. With different PMN preparations, the $t_{1/2}$ value varied by only 10 min from that shown in Fig. 2. The PMN dose response of transepithelial resistance fall was also similar to that we previously reported in 2-cm^2 monolayers grown on crosslinked collagen and studied in modified Ussing chambers (4); maximal responses were generally elicited with PMN densities of $3–6 \times 10^6$ cells · cm^{-2} with small responses occurring at cell densities $< 4 \times 10^5$ cells · cm^{-2} (maximal fall from baseline resistance at 90 min; 13%).

The transepithelial resistance response seen under conditions promoting PMN transmigration involves a CD11b/CD18-mediated event

To investigate the possibility that the transmigration-related resistance response (and/or transmigration itself) might be dependent on integrin-mediated adhesion between PMN and epithelial cells, antibody inhibition studies were performed as described above. As shown in Fig. 3, there was no difference between the anti-CD18 treated PMN resistance response and the negative control (mucosal PMN without FMLP gradient). Anti-CD18 (TS18, IgG1 subclass) completely inhibited the transmigration-associated resistance fall when compared to the positive controls J5 and FMLP without antibody ($P < 0.001$). Mouse ascites NS1 also had no effect on the PMN induced transepithelial resistance fall (i.e., resistance fall was comparable to that of J5 or FMLP without antibody controls; data not shown).

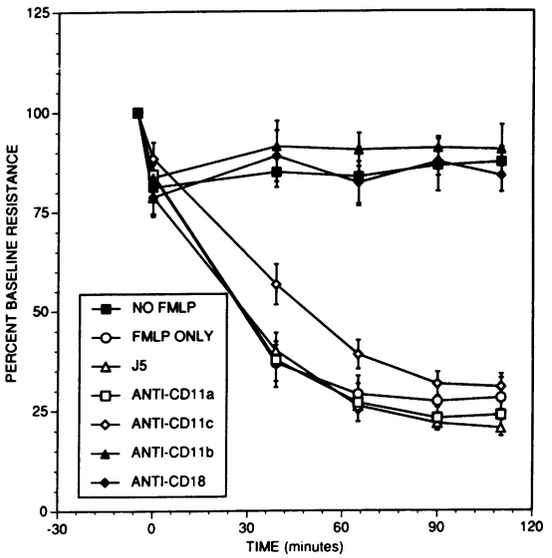

Figure 3. Effect of antibodies on the PMN transmigration-associated transepithelial resistance response. PMN were incubated with 1:200 dilutions of antibody-containing mouse ascitic fluid and induced to migrate across T84 monolayers in the mucosal-to-serosal direction with 1-μM gradients of FMLP. Transepithelial resistance is normalized with respect to each monolayer's resistance immediately before the addition of PMN (baseline resistance). Anti-CD11b and CD18, but not anti-CD11a, CD11c, or J5, completely inhibit the resistance response. One of five experiments.

We next tested which of the specific α-subunit(s) of β_2 integrins was required for PMN transmigration across T84 monolayers, using functionally defined inhibitory antibodies to CD11a, CD11b, and CD11c (16, 17, 19, 20). As shown in Fig. 3, the anti-CD11a (TS22, IgG_1 subclass) result was indistinguishable from the positive transmigration controls, J5 (IgG_{2a} subclass) or FMLP without antibody. The anti-CD11c (L29, IgG_1 subclass) condition was also similar to the positive controls. The suggestion of a slight blunting of the resistance fall seen with anti-CD11c in Fig. 3 was inconsistently demonstrated between groups of experiments. Lastly, a combination of anti-CD11c and anti-CD11a also did not substantially inhibit the transmigration elicited fall in resistance in two experiments where a saturating combination of anti-CD11a and anti-CD11c were used. There was a 50±4% fall in baseline resistance as compared to a 63±5% fall for J5 treated internal positive controls ($P = 0.07$; six monolayers for each condition). In contrast to the above results, anti CD-11b (44a, IgG_{2a} subclass) inhibited the fall in transepithelial resistance as com-

pletely as anti-CD18 (Fig. 3) suggesting that a CD11b/CD18 mediated T84-PMN adherence event is required to elicit the transmigration-associated decrease in resistance. The concentration dependence of inhibition of the transepithelial resistance fall by anti-CD11b is shown in Fig. 4. At antibody dilutions between 1:1,000 and 1:2,000, there was ~ 50% inhibition with complete inhibition achieved at dilutions as high as 1:500. Such concentrations are similar to those which have been described for CD11b/CD18-dependent endothelial–PMN adhesive interactions (22).

PMN transmigration is dependent on a CD11b/CD18-mediated event

We next sought to ensure that the absence of the transmigration-related resistance decline associated with anti-CD11b or anti-CD18 exposure represented inhibition of PMN transepithelial migration as opposed to inhibition of a functional effect associated with, but not required for, transmigration. First, PMN transmigration-associated resistance response values were compared with numbers of transmigrated PMN. As shown in Fig. 5, there is an inverse relationship between the number of transmigrated PMN and resistance. A maximal decrease in resistance was seen when > 1–2 × 10^5 PMN equivalents transmigrated (i.e., PMN between epithelium and filter plus PMN in lower reservoir).

Having demonstrated a correlation between the resistance response and the number of transmigrated PMN, we next determined whether anti-CD11b or anti-CD18 inhibition of the resistance response was also indicative of diminished PMN transmigration. The results of such studies, depicted in Fig. 6, show no PMN transmigration in the absence of FMLP, and transmigration of 1.54±0.47 × 10^5 and 1.17±0.18 × 10^5 PMN/monolayer under the positive control conditions (FMLP only and antibody J5, respectively). Indistinguishable from these positive controls were experiments performed in the presence of anti-CD11a or anti-CD11c (1.51±0.14 × 10^5 and 1.14±0.15 × 10^5 transmigrated PMN/monolayer, respectively). In contrast, anti-CD18 or anti-CD11b treatment, as in negative controls, resulted in no measurable PMN transmigration. Thus, the inhibition of the transmigration-associated resistance fall by antibodies to CD11b or CD18, is associated with inhibition of PMN transmigration. In the experiments depicted in Fig. 6, transmigrated PMN were found both between the monolayer and filter and in the serosal reservoir (44±1 and 56±1% of total transmigrated PMN in the serosal reservoir vs. between the monolayer and filter for J5 control; and no measurable PMN in either compartment for anti-CD11b). Examination of 1-μm sections (data not shown) correlated well with the above data and showed that PMN adhesion to the apical surface was a rare event while PMN were routinely found under the monolayer during transmigration. These morphology studies indicated that filter-associated MPO could be equated with transmigrated PMN.

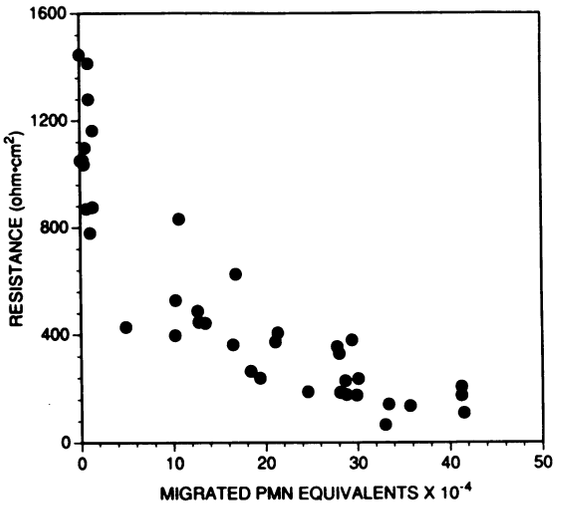

Figure 5. Comparison of numbers of transmigrated PMN as assessed by MPO with effects on transepithelial resistance (variable transmigration densities elicited by using FMLP gradients ranging from 10^{-6}–10^{-7} M and harvesting monolayers at 100 min). PMN on monolayers with no FMLP gradient were used as controls. When migrating in the mucosal-to-serosal direction, the fall in resistance correlates well with the number of transmigrated PMN and saturates after migration of > 1–2 × 10^5 PMN equivalents.

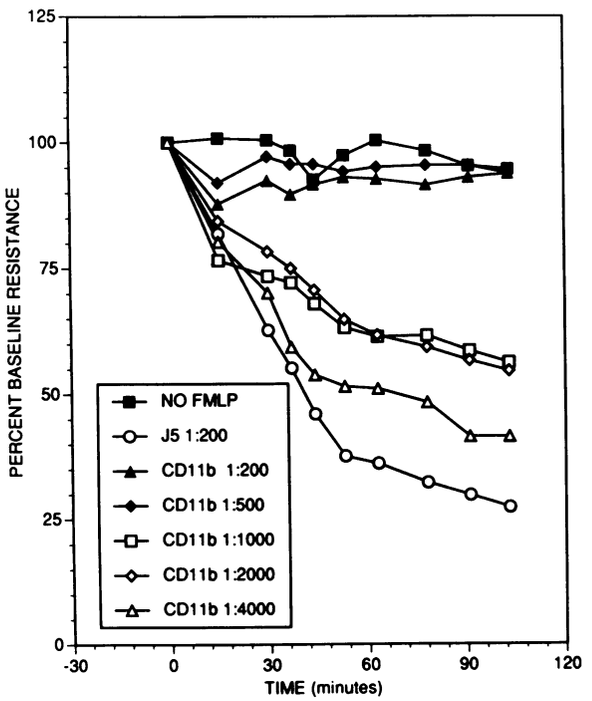

Figure 4. Dose response of anti-CD11b effect on PMN transmigration-associated resistance response. PMN were incubated with varying dilutions of mouse ascitic fluid containing anti-CD11b and induced to transmigrate across T84 monolayers in the mucosal-to-serosal direction with FMLP as described in Methods. Transepithelial resistance is normalized with respect to each monolayer's resistance immediately before the addition of PMN (baseline resistance). Complete inhibition is seen at a 1:500 dilution and partial inhibition can be seen at dilutions of at least 1:2,000. Each condition is the average of data from two monolayers.

PMN from a patient lacking surface CD11/CD18 fail to transmigrate

PMN were obtained from a patient with leukocyte adhesion deficiency (LAD) (kindly provided by Dr. Raif Geha, Division of Immunology, Children's Hospital, Boston, MA), a rare disorder in which PMN lack surface expression of CD11/CD18 molecules (22, 23). LAD or control PMN were placed on T84

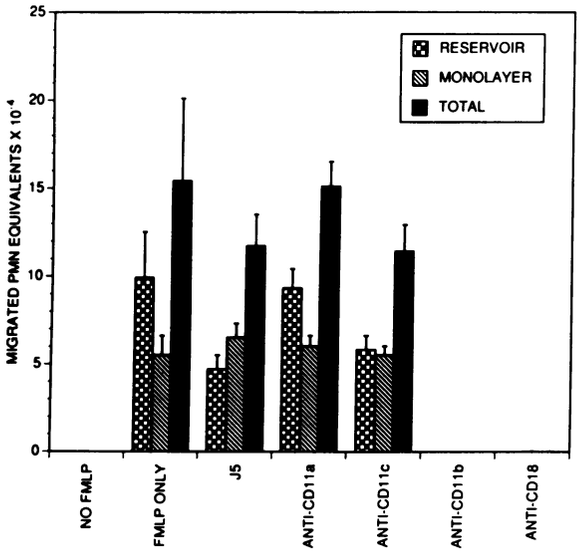

Figure 6. Effect of antibodies on numbers of PMN crossing T84 monolayers in the mucosal-to-serosal direction as assessed by measurement of MPO. PMN were incubated with saturating concentrations of monoclonal antibodies and induced to migrate across T84 monolayers as described in Methods. After 110 min, MPO of washed monolayers and lower reservoirs was measured. MPO activity was quantitated and standardized to known concentrations of starting PMN. Anti-CD18 and CD11b largely inhibit PMN transmigration but antibodies to CD11a, CD11c, and J5 do not. Numbers of PMN equivalents in monolayers, lower reservoirs, and monolayers plus lower reservoirs (total) is shown for each of the conditions. Each condition is the mean of five individual monolayers±SE. One of four experiments.

monolayers (each $n = 3$) at a density of 5×10^6 PMN · cm^{-2} in a 10^{-7}-M FMLP gradient for 45 min. As shown in Fig. 7, transmigrated PMN were easily identified in large numbers in monolayers coincubated with control PMN, but were virtually absent in monolayers coincubated with LAD PMN. Such data confirm that CD11/CD18 integrins are crucial for PMN migration across epithelia.

Serosal-to-mucosal transmigration

Serosal-to-mucosal transmigration is CD11b/CD18 dependent. Since PMN migration across intestinal epithelia in disease states normally occurs in the serosal-to-mucosal direction, the direction opposite to that generally studied in epithelia due to technical considerations, we devised a method for investigating transmigration across "inverted" T84 cell monolayers using a modified version of our simple assay system (see Fig. 1 and Methods).

Under conditions identical to those described in Fig. 6, we investigated the effects of the anti-CD antibodies on PMN chemotaxis across the inverted monolayers. As shown in Fig. 8, $< 2 \times 10^4$ PMN adhere to or transmigrate across inverted monolayers in the absence of a transepithelial FMLP gradient. In contrast, the addition of a 1-μM FMLP transepithelial gradient results in transmigration of $8.82 \pm 7.2 \times 10^5$ PMN in 110 min. As shown in Fig. 8, the effects of the anti-CD antibodies on serosal-to-mucosal PMN transmigration are comparable to those seen in the opposite direction and depicted in Fig. 6. Treatment of PMN with saturating concentrations of anti-CD11a or anti-CD11c results in PMN transmigration of $7.0 \pm 1.24 \times 10^5$ and $7.78 \pm 0.56 \times 10^5$ PMN/monolayer, respectively (NS compared to positive controls). In contrast, treatment of PMN with saturating concentrations of anti-CD18 or anti-CD11b resulted in 86% and 73% inhibition of transmigration when compared to the J5 positive control (both $P < 0.001$); although when compared to the negative control, there was still modest residual transmigration of PMN in the presence of these antibodies ($1.13 \pm 0.23 \times 10^5$ and $2.26 \pm 0.23 \times 10^5$ PMN for anti-CD18 and anti-CD11b, respectively, compared to $< 2 \times 10^4$ PMN for negative controls; $P < 0.001$).

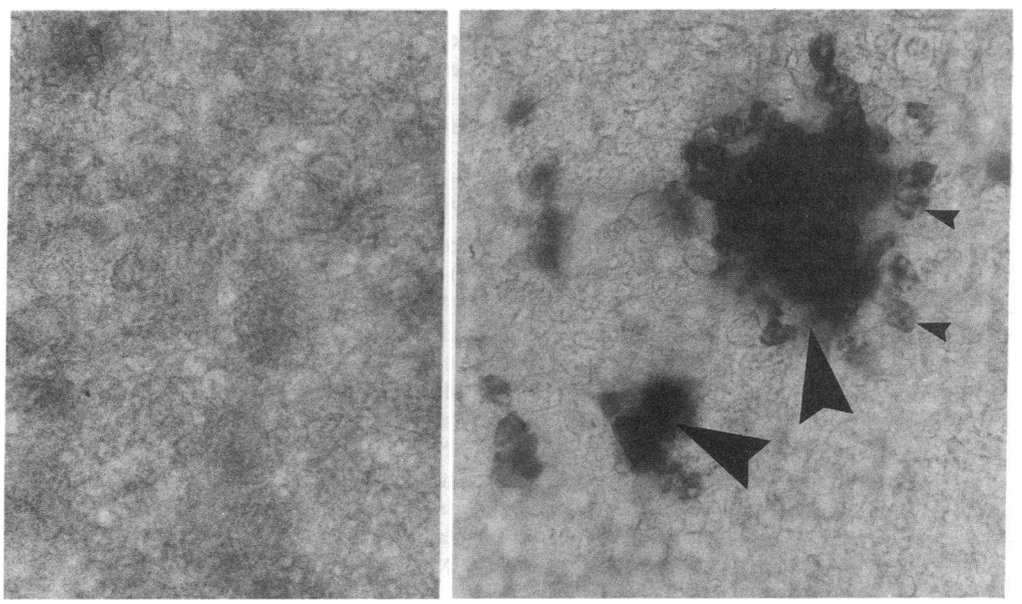

Figure 7. En face micrographs highlighting transepithelial migration of control (*right*) or LAD (*left*) PMN. (*Right*) As we have previously shown (4) transmigrated PMN can be identified by stains which highlight peroxidase localization. Clusters of transmigrated PMN trapped under the epithelium (*large arrowheads*) are prominent in control monolayers (5×10^6 PMN · cm^{-2}; 10^{-7} M FMLP gradient). Small arrowheads show individual transmigrated PMN. The density of PMN transmigration seen by this morphological assay has previously been shown by us to correlate well with the mucosal to serosal transmigration resistance response (4). (*Left*) LAD PMN fail to migrate across T84 monolayers (both × ~ 300).

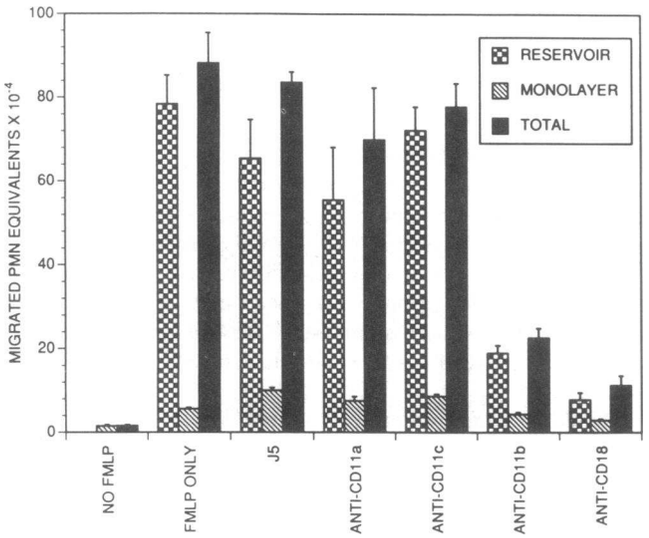

Figure 8. Effect of antibodies on numbers of PMN (MPO) crossing T84 monolayers in the serosal-to-mucosal direction. Inverted, electrically tight, T84 monolayers were grown on the underside of the polycarbonate filters, as described in Methods, and shown in Fig. 1. The transmigration experiment was then done exactly as described for Fig. 6. Anti-CD18 and CD11b largely inhibit transmigration in the serosal-to-mucosal direction as well. Each condition is the mean of five individual monolayers±SE. One of four experiments.

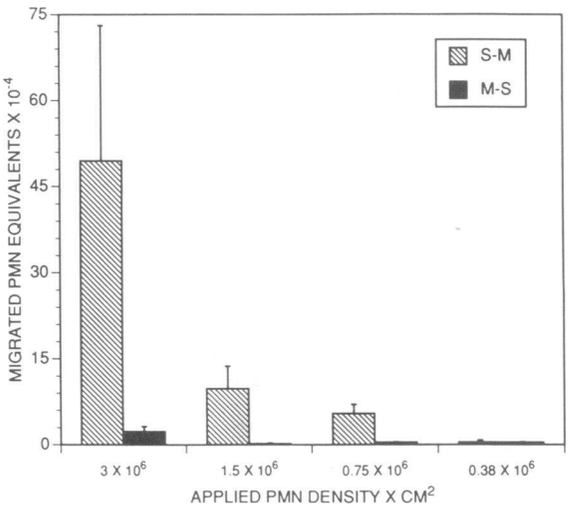

Figure 9. Effect of PMN density on numbers of migrated PMN in serosal-to-mucosal versus mucosal-to-serosal transmigration experiments. PMN were layered onto the mucosal or serosal aspect of T84 monolayers at the densities shown (per cm^2) and induced to transmigrate for 110 min described in Methods. PMN appearing in lower reservoirs were quantitated by the MPO assay as described in Methods. S-M, serosal-to-mucosal transmigration; M-S, mucosal-to-serosal transmigration. There is a 5–20-fold increase in transmigration in the physiologic, or serosal-to-mucosal direction. Each bar is the mean of three monolayers±SE.

Serosal-to-mucosal transmigration is highly efficient

To compare the efficiency of transmigration of PMN across the epithelium in the serosal-to-mucosal vs. the mucosal-to-serosal direction, experiments were performed using uniform conditions (passage of epithelia, neutrophil donor, experimental conditions). As shown in Fig. 9, PMN transmigration in the natural serosal-to-mucosal direction was markedly more efficient than that in the opposite direction. For the mucosal-to-serosal experiment, dilution of PMN to a density of 1.5×10^6 PMN · cm^{-2} (1/4 the standard density) yielded no measurable PMN equivalents in the lower reservoir after 110 min. In contrast, for the serosal-to-mucosal experiment, the dilution of PMN resulting in no measurable transmigration was 0.38×10^6 PMN · cm^{-2} (1/16 the standard density). Comparison of MPO data from all experiments done in both directions under standard conditions revealed that serosal-to-mucosal transmigration is consistently 5–20 times more efficient than in the opposite direction.

Electrical assays in evaluating serosal-to-mucosal transmigration

PMN transmigration in the serosal-to-mucosal direction elicits a large decrease in transepithelial resistance (22±3% of baseline value, at 110 min for positive control). However, given the markedly enhanced efficiency of PMN transmigration in this direction, it was not surprising that the transmigration-associated resistance decline under standard conditions (6×10^6 PMN · cm^{-2}) was not attenuated by antibodies to CD11b or CD18. For example, although under such conditions, anti-CD18 resulted in a 73% reduction in transmigration, 1.1×10^5 PMN still transmigrated (see Fig. 7), which is a sufficient number to induce a near maximal resistance response (see Fig. 5). In experiments in which PMN were applied to the serosal reservoir at 20% of the standard density, correspondingly fewer total PMN transmigrated and such transmigration was again largely inhibited by anti-CD18 or anti-CD11b (1.3±0.4 and 1.8±0.5 vs. $9.5 \pm 0.2 \times 10^4$ PMN/monolayer for anti-CD18 and CD11b, respectively, vs. positive control; both $P < 0.001$). Inhibition of transmigration under such reduced PMN densities also resulted in inhibition of the transmigration-associated resistance response although, even at such very low PMN densities, the final resistance value in the inhibited state was still substantially less than the baseline value. These data indicate that under conditions promoting serosal-to-mucosal transmigration, the effect on resistance is also much greater than that encountered in the mucosal-to-serosal direction. Indeed, the epithelium is so sensitive to transmigration in this direction that we find direct measures of transmigration, such as MPO, are preferable to resistance assays in these experiments.

Intracellular adhesion molecule 1 (ICAM-1) MAb does not inhibit PMN transmigration

Since an epitope on the ICAM-1 may serve as a receptor to CD11b/CD18 (24) and this epitope is functionally blocked by MAb Fab R6.5 (24), we performed serosal-to-mucosal PMN transmigration studies in the presence of R6.5 Fab (50 μg/ml) (monolayers preincubated for 30 min in Fab R6.5). At a density of 1.2×10^6 PMN · cm^{-2}, R6.5 Fab failed to inhibit transepithelial migration as compared to controls (each $n = 4$; total transmigrated PMN = $6.4 \pm 1.5 \times 10^4$ vs. $5.3 \pm 1.3 \times 10^4$ PMN equivalents for control and R6.5 Fab, respectively, NS). A repetition of this experiment yielded the same result (control $n = 3$, R6.5 Fab $n = 4$; total transmigrated PMN = $16.4 \pm 6.9 \times 10^4$ vs. $16.5 \pm 3.5 \times 10^4$ PMN equivalents for control and R6.5 Fab, respectively). Similar experiments done at two higher PMN densities (3×10^6 PMN · cm^{-2} and 6×10^6 PMN · cm^{-2}, all n

= 3–4 for experimental and control), also failed to show an inhibitory effect of R6.5 Fab on PMN transmigration.

In data not shown we have demonstrated that immunoglobulin-sized molecules applied to the basolateral surface of T84 monolayers on inserts (coated with dilute collagen as detailed in Methods) have ready access to the basolateral membranes of T84 cells.

Discussion

In a variety of organs (lung, kidney, urinary bladder, etc.) including the intestine, neutrophils traverse both the endothelial lining of blood vessels and subsequently the epithelium during acute inflammation. While the complex events related to PMN transendothelial migration are being widely addressed (22, 23, 25, 26), those related to transepithelial migration have received less attention (27–30). However, such transepithelial migration of PMN may be of substantial clinical importance. For example, this migratory event elicits diminished epithelial barrier function, a known functional deficit present in inflammatory bowel disease (31). In addition, we have recently described that PMN when exposed to conditions present in the intestinal lumen, produce a small molecular weight hydrophilic substance that effectively elicits electrogenic Cl^- secretion (13), the transport event which relates to the clinical setting of secretory diarrhea. However, this neutrophil-derived secretagogue activity only occurs upon exposure of the apical membrane of intestinal epithelial cells (which would require transepithelial migration).

One factor impeding characterization of PMN transepithelial migration is the lack of availability of a microassay system that circumvents the use of Ussing chambers and thus permits large numbers of parallel experiments to be performed. The assay system we describe fills this need and can be used for studies of transmigration in either direction by either simple electrical or MPO assays.

A major finding of this study is that transepithelial migration of PMN, as modeled here, largely requires the PMN surface adhesion molecule CD11b/CD18. Our observation that LAD PMN, which lack CD18 surface expression (22, 23), fail to migrate across T84 monolayers, is consistent with a CD11/CD18 requirement. These observations fit well with our previous description of morphologically defined PMN-epithelial cell "adhesion" plaques which occur during transmigration (8) and which are analogous to those occurring between PMN and endothelial cells at sites of PMN emigration (32). CD11b/CD18 is a known receptor for factor x (33), the complement degradation product iC3b (19, 34), and fibrinogen (35). Additionally, this $\beta 2$ integrin may play an important role in general leukocyte functions such as phagocytosis and chemotaxis (36, 37) and is perhaps regulated by subunit phosphorylation (38). In aggregate, the three CD18 associated heterodimers on the PMN surface (CD11a, CD11b, CD11c), under certain conditions, are also known to play a significant role in adherence to endothelial (22, 23, 25, 26) and cardiac muscle (39) cells. The ligand to which CD11b/CD18 bound in our experiments is unclear, as the ligands enumerated above should not have been present. Indeed, to further ensure that an absorbed serum factor was not present for the results we obtained, subsets of experiments (not shown) were performed, with identical results to those reported, in which monolayers were kept in serum-free conditions for two days before the experiment. CD18-dependent PMN adherence to and transmigration across endothelia appears, at least in part, to depend on interactions with ICAM-1, or CD54, a member of the immunoglobulin superfamily of adhesion receptors that can be widely distributed on epithelia particularly after induction by inflammatory stimuli (24, 40). However, in endothelia, CD11a/CD18 rather than CD11b/CD18 has been shown to be the receptor for CD54. Recently it has been suggested that CD54 can interact with CD11b/CD18 and that such interaction takes place at a CD54 epitope distinct from that at which CD11a/CD18 binding occurs (24), although the physiological significance of such an interaction is presently unclear. We show that a MAb to CD54 that inhibits this CD11b-mediated interaction (24) had no effect on serosal-to-mucosal transmigration.

Lastly, we report a distinct polarity to the efficacy of PMN migration across epithelia. An increase in available receptors due simply to surface area considerations is unlikely to explain the degree of enhanced efficiency of transmigration in the serosal-to-mucosal direction. However, factors other than differences in the biochemical composition/receptor density and/or type of apical and basolateral membranes may well play a role in determining the efficiency of migration. For example, when migrating in the serosal-to-mucosal direction, PMN first encounter matrix components (those applied to the filter [type I collagen] and those synthesized by the epithelial cells), and it is known that PMN interactions with matrix can play a "priming" role in PMN functions such as the respiratory burst (41, 42). Thus, it would not be surprising if the process of PMN epithelial transmigration during disease states were substantially influenced by matrix interactions before direct PMN-epithelial cell interactions. It is also important to note that, although CD11b/CD18 plays a major role, the transmigration event is likely to involve a complex cascade of epithelial-PMN interactions, including initial adhesion, subsequent adhesion events, spreading, TJ impalement, and perhaps other events. Interruption of this putative cascade at any one of many events could be sufficient to dramatically impede transmigration. Thus, identification of elements of this cascade, particularly organ-specific interactions, might have promise in future efforts geared toward development of organ-specific antiinflammatory strategies.

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