Neutrophil Migration Across a Cultured Epithelial Monolayer Elicits a Biphasic Resistance Response Representing Sequential Effects on Transcellular and Paracellular Pathways

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Abstract. Migration of polymorphonuclear leukocytes across epithelia is a hallmark of many inflammatory disease states. Neutrophils traverse epithelia by migrating through the paracellular space and crossing intercellular tight junctions. We have previously shown (Nash, S., J. Stafford, and J. L. Madara. 1987. *J. Clin. Invest.* 80:1104–1113), that leukocyte migration across T84 monolayers, a model human intestinal epithelium, results in enhanced tight junction permeability—an effect quantitated by the use of a simple, standard electrical assay of transepithelial resistance. Here we show that detailed time course studies of the transmigration-elicited decline in resistance has two components, one of which is unrelated to junctional permeability. The initial decrease in resistance, maximal 5–13 min after initiation of transmigration, occurs despite inhibition of transmigration by an antibody to the common beta subunit of neutrophil β_2 integrins, and is paralleled by an increase in transepithelial short-circuit current. Chloride ion substitution and inhibitor studies indicate that the early-phase resistance decline is not attributable to an increase in tight junction permeability but is due to decreased resistance across epithelial cells resulting from chloride secretion. Since T84 cells are accepted models for studies of the regulation of Cl^- and water secretion, our results suggest that neutrophil transmigration across mucosal surfaces (for example, respiratory and intestinal tracts) may initially activate flushing of the surface by salt and water. Equally important, these studies, by providing a concrete example of sequential transcellular and paracellular effects on transepithelial resistance, highlight the fact that this widely used assay cannot simply be viewed as a direct functional probe of tight junction permeability.

A variety of human diseases, in their active phases, are characterized by transepithelial migration of leukocytes across epithelial surfaces. The transepithelial movement of neutrophils in response to chemotactic gradients of compounds such as the bacterially derived *N*-formylated peptides (Chadwick et al., 1988) is a crucial component of host defense. To model this event, we (Nash et al., 1987, 1988) and others (Milks et al., 1986; Cramer et al., 1986; Evans et al., 1983; Casale and Abbas, 1990) have used chemotactic gradients to drive transmigration of neutrophils across cultured epithelial monolayers. Using such model systems, it has been shown that epithelial permeability is increased (Nash et al., 1987; Milks et al., 1986; Evans et al., 1983) because of migration of neutrophils across intercellular tight junctions (Nash et al., 1987, 1988). We have shown that such transmigration requires a neutrophil–epithelial adhesive interaction for which the neutrophil integrin CD11b/18 is essential (Parkos et al., 1991). We have previously demonstrated, by measuring the fluxes of paracellular markers (Nash et al., 1987, 1988) and by performing dual Na^+-mannitol flux analysis, that the enhanced epithelial permeability seen after 60 min or more of transmigration represents enhanced paracellular (i.e., tight junction) permeability. Many studies, and all published time course data, including our own, used the convenient electrical assay of transepithelial resistance to passive ion flow (Nash et al., 1987, 1988; Milks et al., 1986; Evans et al., 1983). Use of the transepithelial resistance assay has become widespread by cell biologists as a means of assessing the permeability of the paracellular pathway and of the tight junction. However, it is often overlooked that this electrical parameter represents resistance to ion flow through both paracellular and transcellular pathways (Powell, 1981). Here we show that the transepithelial migration of neutrophils across a model intestinal epithelial monolayer (T84) initially decreases transepithelial resistance by transiently altering transcellular rather than paracellular resistance. Thus, the observed drop in resistance with transmigration represents sequential effects on the transcellular and paracellular pathways. Moreover, the transient initial decrease in transcellular resistance is shown to be due to electrogenic Cl^- secretion—the transcellular transport event which underlies hydration of epithelial surfaces such as those lining the respiratory and intestinal tracts (Frizzell et al., 1979; Welsh et al., 1983). These results (*a*) emphasize the

complex nature of transepithelial resistance measurements, (*b*) demonstrate that neutrophil transmigration across epithelia can influence transcellular as well as paracellular events, and (*c*) suggest that neutrophil transmigration may induce the active transcellular transport event which underlies mucosal hydration events such as diarrhea.

Materials and Methods

Cell Culture and Assay Systems

T84 cells (ATCC, passages 70–100) were grown, passaged, and plated on collagen-coated 0.33-cm^2 commercially available polycarbonate filters (Costar inserts; Costar Corp., Cambridge, MA) as previously described (Nash et al., 1987; Dharmsathaphorn and Madara, 1990). Electrical assays were performed as previously described (Parkos et al., 1991; Shapiro et al., 1991) with transepithelial resistance, transepithelial potential difference, and short-circuit current obtained using a dual voltage clamp (University of Iowa) with imposed current pulses of 25 μA.

Transmigration Experiments

Experiments were performed at 37°C on 0.33-cm^2 T84 monolayers (Shapiro et al., 1991) using human neutrophils, isolated as previously described (Parkos et al., 1991), in the presence or absence of a transepithelial chemotactic gradient of 1 μM *N*-formyl-Met-Leu-Phe (fMLP)[1] in a fashion analogous to previous studies (Nash et al., 1987, 1988). In summary, medium was gently aspirated from electrically tight (Dharmsathaphorn and Madara, 1990; Madara and Dharmsathaphorn, 1985) T84 monolayers and replaced with a modified HBSS (Sigma Chem. Co., St. Louis, MO; containing, in g/liter $CaCl_2$ 0.185, $MgSO_4$ 0.098, KCl 0.4, NaCl 8, Na_2HPO_4 0.048, glucose 1) to which was added 10 mM Hepes, pH 7.42. Inserts were then placed in 24-well tissue culture plates which contained, as a serosal bath, 1.0 ml of either HBSS or ion-substituted buffers described below. The chloride "free" buffer consisted of 135 mM Na gluconate (Sigma Chem. Co.), 5 mM K gluconate, 1 mM each of $CaCl_2$ and $MgCl_2$, 10 mM glucose, and 10 mM Hepes, pH 7.4. The control buffer for the ion substitution experiments consisted of 135 mM NaCl, 5 mM KCl, 1 mM each of $CaCl_2$ and $MgCl_2$, 10 mM glucose, and 10 mM Hepes, pH 7.4. For ion substitution experiments, T84 monolayers were rinsed once in the modified buffer before placement in the ion-substituted serosal bath. When used, Bumetinide (20 μM) (Hoffman LaRoche, Nutley, NJ) was added to the serosal bath 20 min before the start of an experiment.

After placement of inserts in 24-well tissue culture plates with serosal buffers, the apical reservoirs were gently aspirated dry and replaced with 0.16 ml of either HBSS or control buffer for ion substitution experiments. Experiments were initiated by the addition of 40 μl of a stock neutrophil suspension (5×10^7 cells/ml in either HBSS or control buffer modified only by omitting Ca^{++} and Mg^{++}) or control buffer without cells to the apical reservoir. Transepithelial resistance and short-circuit current were then serially monitored.

To further probe the effect of neutrophils on transepithelial resistance and short-circuit current under nontransmigrating conditions, we used an anti-β_2 integrin antibody which blocks neutrophil transmigration (Parkos et al., 1991). Mouse ascitic fluid containing previously characterized functionally inhibitory antibody against CD18 (TSl/18; Sanchez-Madrid et al., 1982; Mentzer et al., 1987; Smith et al., 1988) was used at dilutions known to inhibit polymorphonuclear leukocyte (PMN) adherence (1:200). Mouse ascites containing J5, an anti-CD10 monoclonal antibody known to bind to PMN but not inhibit adherence, was used as a control in some experiments (Ritz et al., 1980; Arnaout et al., 1983). For antibody experiments, saturating concentrations of antibodies were added to the apical reservoirs before the addition of neutrophils.

Chemotaxis Assay

Transmigration of neutrophils was also assessed by quantitation of the azurophil granule marker myeloperoxidase as described previously (Parkos et al., 1991). Briefly, at the end of an experiment, typically 110 min, the T84 monolayers were cooled to 4°C, washed with HBSS, and solubilized in Triton X-100–containing HBSS. The pH was adjusted to 4.2 with a 1:10 dilution of 1.0 M Na citrate, pH 4.2, and peroxidase activity (indicative of transjunctional migration; Parkos et al., 1991) was assayed by the addition of an equal volume of 1 mM 2,2'-azino-di-(3-ethyl) dithiazoline sulfonic acid and 10 mM H_2O_2 in 100 mM citrate, pH 4.2. To quantitate neutrophils in the serosal baths, Triton X-100 was added directly to the reservoir and assayed as above. T84 monolayers were found to have no significant myeloperoxidase activity in the absence of neutrophils. Furthermore, myeloperoxidase activity in the lower reservoirs was cell associated since 97% of the total activity appeared in a 1,000 *g* pellet with <3% remaining in the cell-free supernatant (data not shown).

Data Presentation

Because of variability in monolayer resistance among groups of monolayers and in neutrophils obtained from different donors, some resistance data were normalized with respect to baseline values of each individual monolayer. Data are expressed as mean ± SEM. *P* values were determined by an unpaired *t* test. Figures are representative of experiments repeated as described in the legends.

Results

Resistance Fall Associated with Transmigration

As we have previously shown (Nash et al., 1987, 1988; Parkos et al., 1991), transepithelial resistance falls when neutrophils transmigrate across T84 monolayers in response to a chemotactic gradient. This resistance response is depicted in Fig. 1 where, in the absence of a chemotactic gradient ([−] fMLP), there is no significant decline in transepithelial resistance (112.2% of baseline resistance at t = 110 min; NS). However, when neutrophils transmigrate across T84 monolayers in response to a chemotactic gradient, even in the presence of a control, noninhibitory antibody to an epitope unrelated to integrins (CD10, positive control, Parkos et al., 1991), there is a marked fall in transepithelial resistance (15.2% of baseline resistance in 110 min; $p < 0.001$). Neutrophil transmigration and the associated resistance fall can be prevented if the neutrophils are exposed to saturating concentrations of a blocking antibody to the common beta subunit of neutrophil β_2 integrins, CD18, (Parkos et al., 1991) (103.2% of baseline resistance at t = 110 minutes; NS). Fig. 1 also demonstrates that the resistance fall is correlated with actual numbers of transmigrating neutrophils. The positive control had transmigration of $8.9 \pm 2.2 \times 10^4$ neutrophils/ monolayer. There was virtually no measurable transmigration for the negative control (0.3×10^4 neutrophils/monolayer; near background value) (positive control vs. negative control, $p < 0.001$). Additionally, when compared to the negative control, there was complete inhibition of transmigration with anti-CD18 ($0.4 \pm 0.1 \times 10^4$ vs. 0.3×10^4 neutrophils/monolayer, respectively; NS). Thus, anti-CD18 serves as a control condition in which neutrophils can be exposed to a transepithelial chemotactic gradient but prevented from migrating because of a diminished ability to adhere to the epithelium.

A Transient Early-Phase Resistance Decrease Can Be Dissociated from Neutrophil Transmigration

As shown in Fig. 2, under conditions where a chemotactic gradient is present but neutrophil transmigration is inhibited with anti-CD18, transepithelial resistance reversibly decreased. In contrast to the stable resistance of negative control monolayers without applied neutrophils, under che-

1. *Abbreviations used in this paper*: fMLP, *N*-formyl-Met-Leu-Phe; PMN, polymorphonuclear leukocyte.

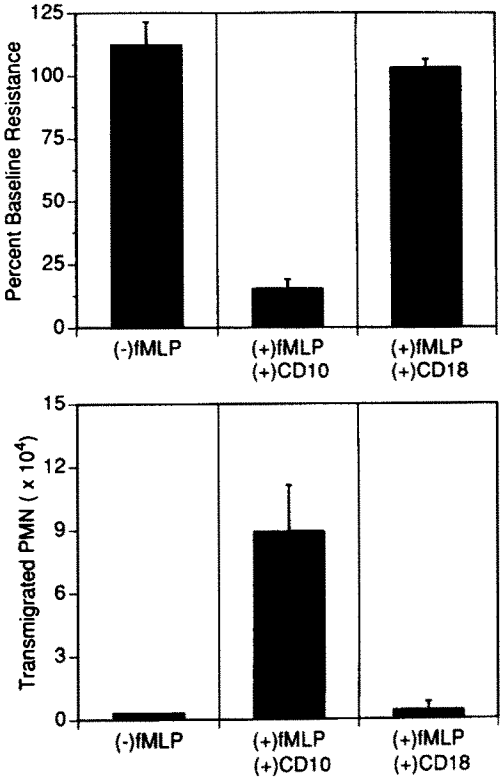

Figure 1. Transmigration of neutrophils across T84 monolayers is associated with a fall in transepithelial resistance which can be inhibited by anti-CD18. In both panels, neutrophils were layered on T84 monolayers in the presence or absence of a transepithelial gradient of fMLP (1 μM). The effects of the β_2 integrin antibody anti-CD18 on transmigration under chemotactic conditions were also evaluated (CD18). As a control for antibody binding to irrelevant neutrophil surface epitopes, anti-CD10 antibody was added as the positive control. After 110 min, percent of baseline transepithelial resistance (*top*) and numbers of transmigrated neutrophils per monolayer (*bottom*) were measured as described in Materials and Methods and are plotted for each condition. Data represent the mean ± SEM of three monolayers for each condition. One of more than five experiments.

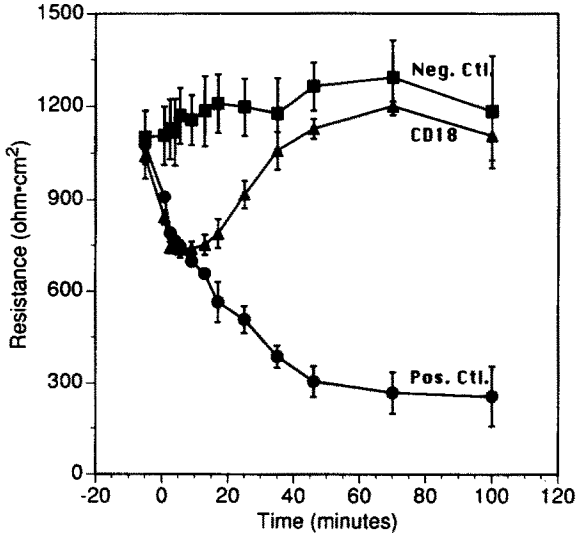

Figure 2. A transient, early-phase resistance decline can be dissociated from neutrophil transmigration. As described in both Fig. 1 and in Materials and Methods, PMN were layered on T84 monolayers in the presence (positive control) of a transepithelial gradient of fMLP. The effects of anti-CD18, which inhibits transmigration in the presence of chemotactic conditions, were assessed. The negative control represents a transepithelial gradient of fMLP without applied neutrophils. Transepithelial resistance was measured at the various time intervals shown. As can be seen, inhibition of transmigration with anti-CD18 is associated with a transient fall in resistance in the first 13 min which returns to baseline by 30–40 min. The response after treatment of PMN with anti-CD10 antibody was indistinguishable from that of the above positive control (data not shown). Treatment with anti-CD18 alone (no PMN) had no effect on transepithelial resistance (data not shown). Data represent mean ± SEM of three monolayers for each condition. One of three experiments.

motactic but anti-CD18–inhibited conditions, a transient resistance fall of 300 ohm × cm² (a 30% fall from baseline) between 4 and 13 min ($p < 0.01$) occurred. After 13 min, the resistance returned to baseline values at 25–35 min (916–1,058 ohm × cm²). Such transient and reversible decreases in resistance were also seen in the absence of anti-CD18 when neutrophils were stimulated by other means even if chemotactic conditions were absent (not shown). There was no significant difference between the degree to which resistance fell in the first 13 min whether transmigration proceeded normally or was inhibited by anti-CD18. The maximum rates of resistance fall for the positive transmigration control and anti-CD18 conditions were essentially equal (mean = 67 vs. 57 ohm × cm² per min; NS). Such data suggest that the early phase of the resistance fall during transmigration is related to an effect of neutrophil–epithelial interaction other than disruption of tight junctions due to transjunctional neutrophil migration.

Transmigration of Neutrophils across T84 Monolayers Elicits a Transepithelial Current

Transepithelial ion currents, assayed as short-circuit currents (Horowicz et al., 1978), are indicative of net transepithelial transport of an anion or cation in the absence of a counterbalancing anion or cation. As shown in Fig. 3, a transient, reversible short-circuit current was observed with transmigration. However, comparable peak currents were observed under chemotactic conditions in which transmigration was blocked with anti-CD18 (61 vs. 54 μA × cm^{-2} in the presence and absence of anti-CD18, respectively; NS). Furthermore this short-circuit current response exactly mirrored the early-phase decrease in transepithelial resistance (Fig. 4). Thus, both early-phase electrical events (initial resistance fall and short-circuit current) occur in parallel, and, as assessed from the CD18 experiments, have no absolute requirement for transmigration. Such data suggest that these two electrical events occurring in the early phase of transmigration may stem from the same neutrophil-elicited functional event.

Early-Phase Electrical Responses Accompanying Neutrophil Transmigration Are Secondary to Cl⁻ Secretion

Since T84 cells are a model for the study of electrogenic Cl⁻ secretion (the basis of hydration of mucosal surfaces; Frizzell et al., 1979; Welsh et al., 1983) which produces a short-circuit current as an electrical signal (Frizzell et al., 1979; Welsh et al., 1983), and since such Cl⁻ secretion is largely regulated by opening of plasma membrane Cl⁻ chan-

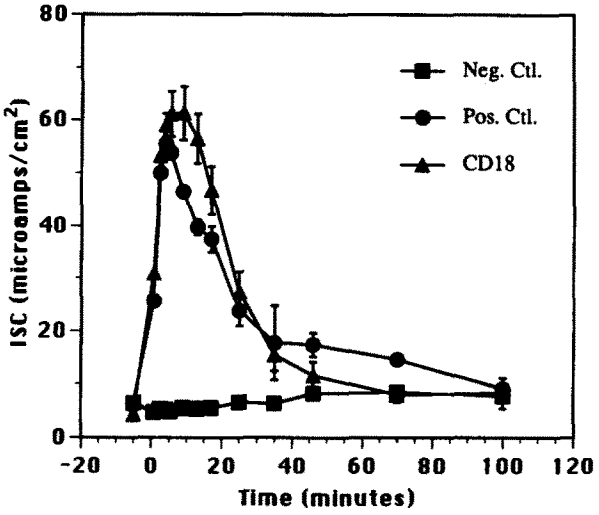

Figure 3. Neutrophils induce a transient short-circuit current in T84 monolayers. For each of the experimental conditions, short-circuit current (ISC), measured as described in Materials and Methods, is plotted against time. In all conditions, a transepithelial gradient of fMLP was present. In the negative control shown here, no neutrophils were added and no short-circuit current was detected. However, under chemotactic conditions, neutrophils, whether in the presence (CD18) or absence (positive control) of inhibitory concentrations of anti-CD18 antibody, induce a transient short-circuit current which peaks at 13 min and then declines to near baseline in 30 min. The response after treatment of PMN with anti-CD10 antibody was indistinguishable from that of the above positive control (data not shown). Treatment with anti-CD18 alone (no PMN) had no effect on short-circuit current (data not shown). Data represent mean ± SEM of three monolayers for each condition. One of three experiments.

nels (Cliff and Frizzell, 1990; Frizzell and Halm, 1990), we investigated whether the initial decrease in resistance, seen under chemotactic conditions, reflected opening of this transcellular pathway for anion flow. As shown in Fig. 5,

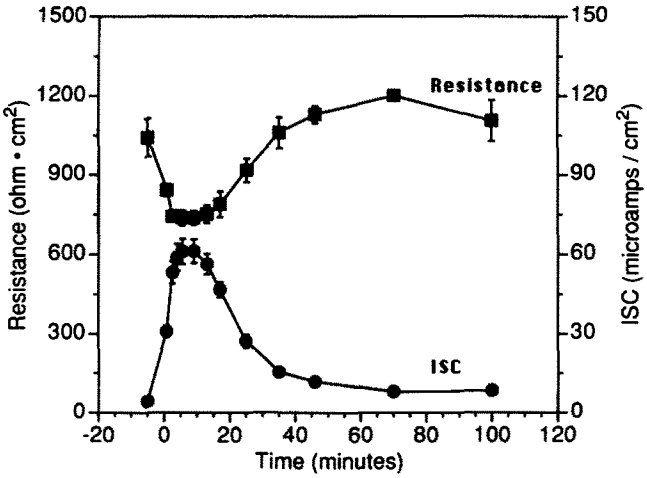

Figure 4. The neutrophil-induced early-phase resistance decline mirrors the short-circuit current time course and is not associated with transmigration. Short-circuit current (ISC) is plotted under the anti-CD18–inhibited resistance curve derived from Fig. 2. As can be seen, even when transmigration is inhibited, the rise and fall of the short-circuit current parallels, in an opposite fashion, the transient fall and rise in the resistance.

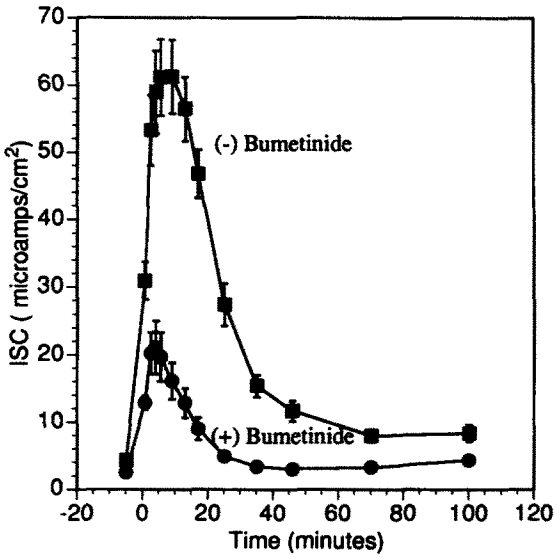

Figure 5. The transient, early-phase short-circuit current is bumetinide sensitive. Short-circuit current (ISC), measured as described in Materials and Methods, is plotted against time for T84 monolayers incubated with neutrophils in the presence of both a transepithelial gradient of fMLP and anti-CD18 and either in the presence (+) or absence (−) of 20 μM bumetinide (present in lower reservoir, or "serosal" bath, only). Bumetinide, an inhibitor of the basolateral Na:K:2Cl cotransporter, required for the generation of Cl^- secretion, effectively inhibits 70% of the short-circuit current. Data represent mean ± SEM of three monolayers for each condition. One of two experiments.

bumetinide, which inhibits transcellular electrogenic Cl^- secretion by inhibition of the basolateral mechanism for cellular uptake of Cl^- (Na/K/2Cl cotransporter; Gazitua and Robinson, 1982), substantially inhibits the observed short-circuit current response (61 vs. 21 $\mu A \times cm^{-2}$ peak currents for control and bumetinide treated, respectively; $p < 0.001$). As shown in Fig. 6, substitution of Cl^- with the plasma membrane–impermeant anion gluconate ablated both the transient, early-phase short-circuit current and the early-phase resistance decrease normally seen under chemotactic conditions (8.1 ± 1.3 vs. 0-fold increase in short-circuit current, $p < 0.001$ and 42 ± 13.5 vs. 11 ± 3.6% decline in resistance, $p < 0.02$, respectively, for time 0 vs. 10 min). Furthermore, under transmigration conditions in the absence of inhibitory antibodies, there is ablation of the short-circuit current and early-phase resistance decline by substitution of basolateral Cl^- with gluconate (9.6 ± 2.3 vs. 0.74 ± 0.1-fold increase in Isc and 38.9 ± 3.3% vs. 0 ± 7% decline in resistance from time 0 to 4 min for control [+ Cl^-] and gluconate substitution [$-Cl^-$], respectively). The above data indicate that Cl^- secretion is responsible both for the early-phase decrease in transepithelial resistance (opening of Cl^- channel) and the associated, transient short-circuit current (electrogenic anion secretion).

Isolation of the Paracellular Resistance Decline: Comparison with Onset of Neutrophil Transmigration

The above data indicate that one element of the electrical response resulting from neutrophil migration across T84 monolayers is Cl^- secretion—an event which is known to

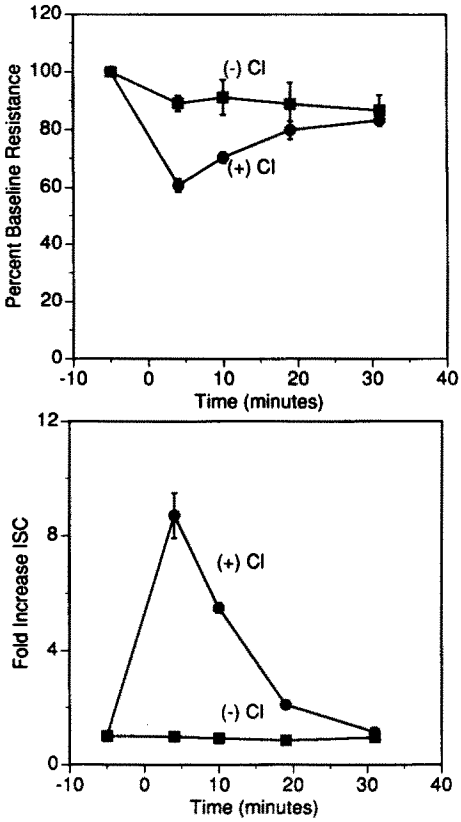

Figure 6. The transient, early-phase short-circuit current is due to chloride secretion. Transmigration experiments were performed in the presence (+) or absence (−) of basolateral reservoir chloride ion. Neutrophils were layered on T84 monolayers in the presence of both anti-CD18 and transepithelial gradients of fMLP as described in Materials and Methods. Plotted against time in the top panel is percent of baseline transepithelial resistance and in the bottom panel is "fold increase ISC" from baseline. As can be seen, omission of chloride ion from the basolateral bath completely inhibits both the transient early-phase resistance fall and short-circuit current. Data represent mean ± SEM of three monolayers for each condition. One of two experiments.

decrease transcellular resistance as a consequence of opening of Cl^- channels (Cliff and Frizzell, 1990; Frizzell and Halm, 1990). Using dual Na+/mannitol fluxes and other approaches, we have previously shown that paracellular resistance is also ultimately diminished during such transmigration. To examine the separate contributions of the paracellular and transcellular pathways to the transepithelial resistance response during transmigration, we first used the approach of Yonath and Civan (1971). This approach is shown in Fig. 7. Conductance, the reciprocal of transepithelial resistance, is plotted against short-circuit current for each of the three phases of the transmigration-associated electrical response (phase 1, period of increase in short-circuit current, 0–13 min; phase 2, period of decrease in short-circuit current due to attenuation of the Cl^- secretory response as clearly defined in experiments using anti-CD18, 13–30 min, and phase 3, period during which short-circuit current stabilizes and approaches baseline values, 30–100 min). By regression analysis, the value for conductance at the y-axis intercept (where short-circuit current would equal zero) is determined and the reciprocal of this value is used to ascertain the relative paracellular resistance during each of the three phases (resistance [ohm × cm^2] = 1/conductance [mmohs × cm^{-2}] × 1,000). The values derived for relative paracellular resistance, using this analysis, are shown in Fig. 8 and compared to the measurements of transepithelial resistance, under chemotactic conditions, in the presence or absence of anti-CD18. During transmigration, these relative paracellular resistance values for phases 1, 2, and 3 are 1,111, 370, and 192, respectively (Fig. 8 *B*). Such data indicate that the paracellular resistance is high during phase 1, but in the declining phase of the Cl^- secretory process (phase 2), decreases in both transcellular (Cl^- secretion through open channels) and paracellular resistance contribute to the observed net fall in transepithelial resistance. Also, the rate at which conductance increases (i.e., paracellular resistance decreases; Fig. 7) accelerates from phase 2 to phase 3 suggesting an increase in perturbation of the paracellular pathway which, as we have previously shown,

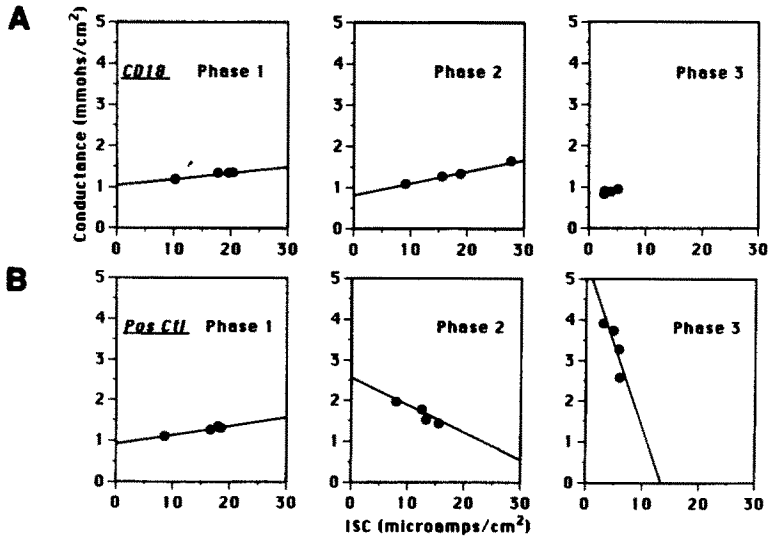

Figure 7. Analysis of conductance vs. current plots to reveal relative contribution of paracellular resistance. (*A*) Phases refer to three components of the current/resistance response—see text. In the presence of anti-CD18, (absence of transmigration across tight junctions) the conductance values for zero short-circuit current are ~1.0 mmohs × cm^{-2} in all phases, corresponding to a resistance of 1,000 ohm × cm^2. Thus, the resistance response observed under these conditions does not represent altered paracellular resistance. (*B*) In contrast, during active transmigration of neutrophils across tight junctions, the conductance values at zero current are 0.9, 2.7, and 5.2 for phases 1, 2, and 3, respectively. Such data indicate that, during transmigration, the phase 1 transepithelial resistance change represents a purely transcellular effect. However, during transmigration, the increasing conductance values after phase 1 indicate a mixed transcellular and paracellular effect in phase 2, and a purely paracellular effect in phase 3. All points represent an average of values from three monolayers.

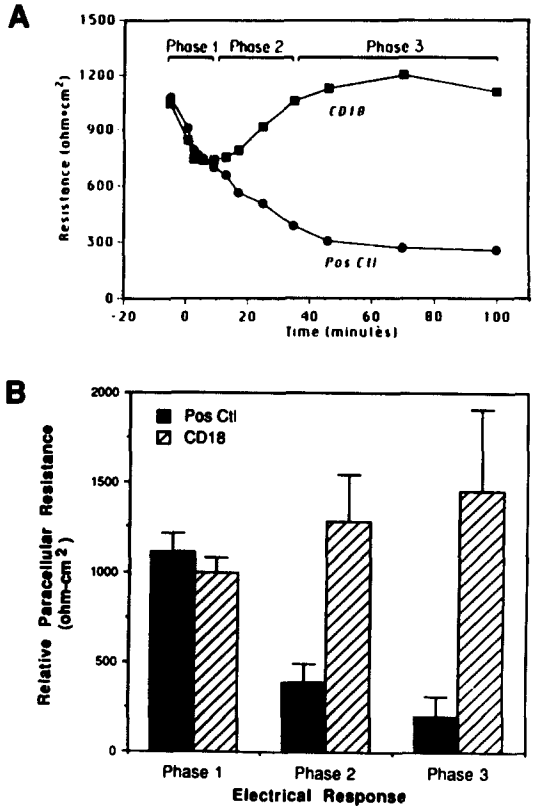

Figure 8. Comparison of paracellular and transcellular contributions to transepithelial resistance in the presence and absence of transmigration. (*A*) Time course of the change in net transepithelial resistance under chemotactic conditions in the presence (CD18) or absence (positive control) of anti-CD18. These data, in association with those shown in Figs. 2 and 3, indicate that the resistance response can be divided into three phases: phase 1, 2, and 3 (corresponding to the increase in short-circuit current and decrease in resistance due to Cl^- secretion; the return of current to baseline value; and the subsequent experimental period, respectively—see text). (*B*) Relative paracellular resistances obtained from analysis of Fig. 7 (see Results). Paracellular resistance remains high in phase 1 indicating that the observed fall in transepithelial resistance is via a transcellular mechanism. In phase 2, paracellular resistance remains high if transmigration in blocked (anti-CD18), but falls as transmigration commences. Thus, the observed transepithelial resistance fall in phase 2 is due to mixed transcellular and paracellular effects. In phase 3, in the absence of transmigration (anti-CD18), paracellular resistance remains at a high constant value, but paracellular resistance markedly declines in the presence of transmigration. Thus, transmigration elicits a progressive fall in transepithelial resistance due sequentially to transcellular effects (phase 1), transcellular and paracellular effects (phase 2), and paracellular effects (phase 3).

is due to impalement of tight junctions by transmigrating neutrophils. In contrast, in the presence of chemotactic conditions but in the absence of transmigration (anti-CD18; Fig. 8 *B*), phase 1 and phase 2 values are similar and indicative of a highly restricted paracellular pathway (1,000 and 1,176 ohm × cm^2, respectively). Such data indicate that the resistance fall seen under chemotactic conditions in which anti-CD18 is present is purely transcellular and due to Cl^- secretion, confirming the observations seen in the Cl^- substitution experiments (vida supra). By comparison of data in Fig. 7, *A* and *B*, and Fig. 8 *B* it is apparent that phase 1 values

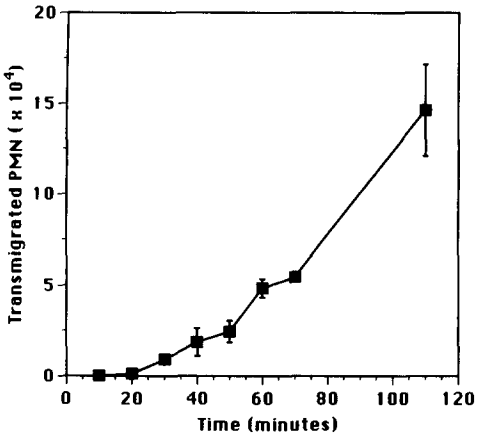

Figure 9. Time course of numbers of neutrophils migrating across T84 monolayers. Neutrophils were layered on T84 monolayers in the presence of a transepithelial gradient of fMLP as described in Materials and Methods. After the time points indicated on the curve, neutrophils which had transmigrated were quantitated using a myeloperoxidase assay. There is no measurable transmigration before 20 min. The first measurable transmigration, observed at 20 min, is modest (1–2,000 neutrophils/monolayer), but transmigration progressively rises subsequently. Data represent mean ± SEM of three monolayers for each condition. One of two experiments.

are similar. Thus paracellular resistances are unaffected in phase 1 even under conditions permitting transmigration. Cross comparison of phase 2 values (Fig. 8 *B*) confirms the observation of transmigration eliciting a mixed paracellular and transcellular resistance response during this phase (370 vs. 1,176 ohm × cm^2 for relative paracellular resistance in the presence and absence of transmigration, respectively). The interpretations of Figs. 7 and 8 are in good agreement with the time course of neutrophil movement through tight junctions as measured quantitatively by a myeloperoxidase assay (Fig. 9). At 10 min no measurable movement of neutrophils across tight junctions had occurred, corresponding to the lack of a paracellular resistance effect during phase 1 of Fig. 8 *B*. By 20 min, transmigration had just commenced (1,000–3,000 neutrophils per monolayer). After 20 min, the rate of transmigration of neutrophils across tight junctions dramatically increases, accounting for the increasing paracellular resistance change seen in phases 2 and 3 (Fig. 9).

The data above allows several approaches to obtain a resistance curve which isolates the paracellular resistance fall associated with neutrophil movement across tight junctions from the transcellular resistance component. As one example (Fig. 10), the resistance response observed under chemotactic conditions in the presence of anti-CD18 can be used to add the absolute value of the transcellular resistance decline due to opening of Cl^- channels to the net change in transepithelial resistance seen with transmigration. The resulting "corrected" resistance curve is representative of the isolated paracellular resistance response due to transmigration. The time course of the corrected "paracellular only" fall in resistance (Fig. 10) shows good correlation with the time course of neutrophil movement across tight junctions (Fig. 9).

Discussion

We show that the decrease in transepithelial resistance which

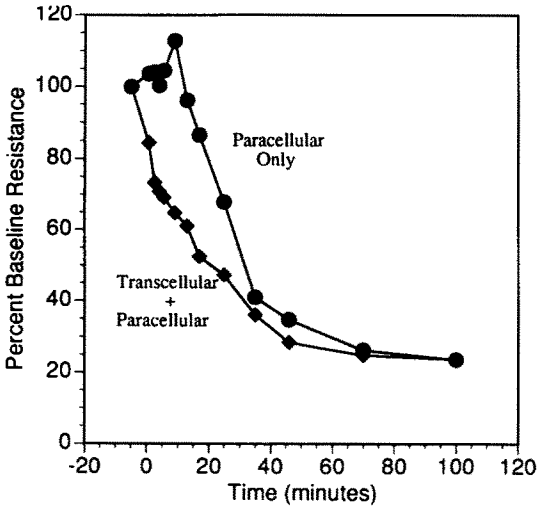

Figure 10. Isolation of the paracellular component of the resistance decline. To identify the "paracellular" component of the transepithelial resistance response elicited by neutrophil transmigration, effects on transcellular resistance must be corrected for. As shown in prior figures, anti-CD18 completely inhibits neutrophil transmigration but has no effect on neutrophil-elicited Cl^- secretion—the process which accounts for the early-phase transcellular resistance fall (i.e., paracellular effects are absent under these conditions). Thus, the PMN-induced changes in resistance observed under conditions which completely inhibit PMN transmigration ([+] anti-CD18) were used to "correct" the PMN-induced changes in resistance observed during uninhibited transmigration. As can be seen, the resultant "paracellular only" resistance curve is shifted to the right for the first 40 min and has a greater downslope after transmigration has commenced (20 min, see Fig. 8).

occurs as neutrophils migrate across T84 monolayers represents two sequential events. Specifically, effects on transcellular and paracellular pathways, which exist in epithelia (Powell, 1981), occur sequentially. The early phase of the resistance response represents a reversible decrease in transepithelial resistance. This phase, which occurs over the first 30 min of transmigration, is associated with the appearance of a short-circuit current which, in T84 cells, is indicative of electrogenic Cl^- secretion (Dharmsathaphorn and Madara, 1990). Such chloride transport is known to commence when secretory agonists, such as cAMP analogs, stimulate the regulated opening of Cl^- channels on plasma membranes (Dharmsathaphorn and Madara, 1990; Cliff and Frizzell, 1990; Frizzell and Halm, 1990). Opening of such channels diminishes the resistance to passive ion flow of the apical plasma membrane (Cliff and Frizzell, 1990; Frizzell and Halm, 1990) thereby decreasing transepithelial resistance (Welsh et al., 1983; Frizzell and Halm, 1990). We have shown that this early-phase resistance decline and the parallel increase in short-circuit current are indeed due to such a process. If the membrane-impermeant anion gluconate is substituted for Cl^-, no short-circuit current or fall in resistance are detected during the early phase of transmigration. This maneuver prevents the basolateral uptake of Cl^- by the Na:K:2Cl cotransporter and thus the cells cannot generate a transcellular Cl^- current. Similarly, since gluconate cannot move through the highly anion-selective Cl^- channel, transepithelial resistance measurements will not even detect open Cl^- channels under conditions in which the major anion is gluconate. Bumetinide, a widely used inhibitor of Cl^- secretion (Gazitua and Robinson, 1982), which acts by inhibiting the basolateral Na:K:2Cl cotransporter, is also shown to ablate the early-phase short-circuit current response.

We were able to isolate the early-phase electrical responses seen with transmigration after observations with antibodies that inhibit transmigration. We have previously shown that antibodies to components of the neutrophil integrin CD11b/18 inhibit neutrophil transmigration in response to transepithelial chemotactic gradients by interfering with required neutrophil–epithelial adhesive interactions (Parkos et al., 1991). Since neutrophils, activated by chemotactic gradients, but not able to transmigrate or adhere by CD18-dependent mechanisms, elicit the early-phase electrical responses indicative of Cl^- secretion, it is likely the agonist for Cl^- secretion during transmigration is the small hydrophilic, but as yet uncharacterized, neutrophil-derived secretagogue (NDS) activity which we have recently described in supernatants of activated neutrophils (Nash et al., 1991).

Electrogenic Cl^- secretion, the basis of the early-phase fall in resistance during transmigration, is not a peculiarity of the cell line used in these studies. The four transport proteins involved in electrogenic Cl^- secretion (Cl^- channels; K+ channels; Na:K:2Cl cotransporter, and the ubiquitous Na+K+ATPase; Dharmsathaphorn and Madara, 1990), are all represented in T84 cells as they are in other epithelia which secrete salt and water via this mechanism. This process is present in respiratory tract epithelia (Welsh et al., 1983), intestinal epithelia (Frizzell et al., 1979), and in a wide variety of other salt secreting epithelia (bird nasal gland, shark rectal gland, and fish gill [Frizzell and Halm, 1990]). Furthermore, since regulation of Cl^- secretion in T84 cells is highly comparable to that found in natural tracheal and intestinal epithelium (Dharmsathaphorn and Madara, 1990), this cell line has become a widely used model for mechanistic studies of Cl^- secretion. In disease states of the intestine, electrogenic Cl^- secretion is the basis of secretory diarrhea (Sullivan and Field, 1991). Since neutrophil transmigration across epithelia likely occurs in response to a mucosal threat (bacterial chemoattractants, etc.), it makes teleological sense that a mucosal flushing response be coupled to neutrophil movement, as we report here for the first time.

We have previously shown that the decrease in transepithelial resistance seen after transmigration of neutrophils across T84 monolayers for 60 min (second-phase resistance response) is entirely due to enhanced tight junction permeability (Nash et al., 1987, 1988). This confirmed the interpretations of others, based on electrical data (Milks et al., 1986; Evans et al., 1983), that the decrease in resistance seen 40–90 min after the onset of neutrophil transmigration across various epithelia is due to enhanced junctional permeability. However, we now show transmigration can also affect transcellular transport pathways in such a way as to produce decreased resistance unrelated to tight junction permeability. Several of the simple commercially available "chopstick" devices for electrical recordings provide only crude resistance measurements and are not able to assess transepithelial currents reflective of electrogenic ion transport. Our findings thus indicate the care required in interpreting simple transepithelial resistance readings obtained from epithelia as direct parameters of tight junction permeability.

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