

Epithelial Exposure to Hypoxia Modulates Neutrophil Transepithelial Migration

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Summary

Polymorphonuclear leukocytes (PMN) are central to the pathogenesis of a number of intestinal diseases. PMN-induced damage to the protective epithelium occurs in hemorrhagic shock, necrotizing enterocolitis and conditions resulting in intestinal reperfusion injury. In such diseases, tissue hypoxia has been implicated as a pathophysiologic mediator. Thus, we hypothesized that exposure of intestinal epithelia to hypoxia may modulate PMN-epithelial interactions. In this study, T84 cell monolayers, a human intestinal crypt cell line, and isolated human PMN were used to examine the influence of hypoxia/reoxygenation (H/R) on PMN transepithelial migration. Confluent T84 cell monolayers were exposed to hypoxia (range 2–21% O₂ for 0–72 h) and reoxygenated with buffer containing PMN. Transmigration of PMN (basolateral to apical orientation) was driven by a transepithelial gradient of the chemotactic peptide fMLP. In response to hypoxia/reoxygenation (H/R), transmigration into, and across epithelial monolayer was increased in a dose- (EC₅₀ ~7% O₂) and time-dependent fashion (3.5 ± 0.3-fold increase at 2% O₂ for 48 h, *P* < 0.001). Such conditions of H/R were not toxic to epithelia and did not influence epithelial barrier function. The influence of H/R on PMN transmigration was protein synthesis-dependent (>80% decreased in the presence of cycloheximide) and could be inhibited by addition of functionally inhibitory antibodies to the PMN β2 integrin CD11b/18 (>80% attenuated) and to CD47 (>90% decreased compared to control). Hypoxia induced epithelial production and basolateral release of the PMN activating chemokine interleukin-8 (IL-8, nearly sixfold increase over normoxic control) which remained avidly associated with the epithelial matrix. Treatment of epithelial cells with IL-8 antisense oligonucleotides resulted in decreased monolayer-associated PMN but did not influence PMN transmigration, suggesting that epithelial IL-8 production may serve as a recruitment signal for PMN to the basal surface of polarized epithelia. The present observations indicate that H/R provides a relevant stimulus for novel biochemical crosstalk between epithelia and PMN.

The pathological hallmark of many acute and chronic intestinal diseases is the formation of crypt abscesses; accumulations of polymorphonuclear leukocytes (PMN) adjacent to crypt epithelial cells of the intestine (1). Significant tissue damage brought about by PMN migration across intestinal epithelium has been demonstrated in a variety of diseases including Crohn's disease, ulcerative colitis, hemorrhagic shock and ischemia/reperfusion injury (2, 3). The magnitude of PMN transepithelial migration, assessed quantitatively, has been shown to correlate with patients symptoms as well as with the degree of intestinal epithelial barrier dysfunction (4). Studies of human mucosa in such diseases suggest that PMN transepithelial migration predates focal breakdown of the epithelial surface (2), and that defective epithelial barrier function also predates struc-

tural discontinuities in the mucosa (5). At present, the pathophysiologic factors that lead to the formation of such inflammatory responses are poorly defined.

A common denominator of ischemia/reperfusion injury is tissue hypoxia. An extensive literature exists regarding hypoxic induction of endothelial cell function (recently reviewed in reference 6). Such studies have revealed that endothelial cell hypoxia leads to shifts in cellular metabolism, induction of specific cell surface proteins, regulation of cellular gene products and secretion of cytokines. Information regarding the impact of hypoxia on epithelial cell function, however, is clearly lacking.

We have previously modeled the event of PMN transmigration using human peripheral blood PMNs and cultured intestinal epithelial monolayers derived from the hu-

man cell line T84 (for recent review see reference 7). Such monolayers are composed of columnar epithelial cells with features similar to those of natural crypt epithelia (8, 9). These cells adjoin adjacent cells through apical circumferential tight junctions and maintain structure-function characteristics found in natural epithelia (8). Our prior studies using this epithelium as a model for examination of PMN-intestinal epithelial interactions have indicated that PMN transmigration elicits a reversible decrease in transepithelial resistance due to impalement of intercellular tight junctions, and is dependent on the integrin CD11b/CD18 and CD47. Moreover, PMN transmigration has been shown to depend on epithelial polarity in response to epithelial exposure to IFN- γ (10) or PMN exposure to lipoxin A₄ (11). Recent work further demonstrates that apical colonization of epithelia with pathogenic bacteria induces epithelial secretion of interleukin 8 and liberates additional soluble factor(s) which induce PMN transmigration across epithelial monolayers (12, 13, 14).

Here we establish an *in vitro* model of intestinal ischemia/reperfusion using hypoxia/reoxygenation (H/R)¹ of cultured intestinal epithelial cells in the presence of purified human PMN. Cultured epithelia exposed to H/R resulted in enhanced PMN transmigration in the physiologically-relevant basolateral-to-apical direction. Dissection of the transmigration pathway revealed that such enhancement did not result from epithelial toxicity, was dependent on new epithelial protein synthesis, and was blocked by anti-CD11b/18 and anti-CD47 mAbs. While hypoxia induced epithelia production and basolateral secretion of the chemokine IL-8, a direct role for IL-8 in driving H/R-elicited PMN transmigration could not be demonstrated. We speculate that epithelia may play a central role in directing leukocyte trafficking during intestinal ischemia/reperfusion.

Materials and Methods

Cell Culture. T84 intestinal epithelial cells (passages 67-85) were grown and maintained as confluent monolayers on collagen coated permeable supports as previously described in detail (15). Monolayers were grown on 0.33 cm² ring-supported polycarbonate filters (Costar Corp., Cambridge, MA) and used 6-12 d after plating as described previously (16). Inverted monolayers, used to study transmigration of PMN in the basolateral-to-apical direction were constructed as described before (17). To construct such monolayers, T84 cells were plated on the underside of permeable supports, effectively reversing the polarity of PMN-epithelial interactions by allowing gravitational settling of PMN onto the basolateral aspect of the monolayer (17).

Epithelial cultures were exposed to hypoxia as follows: growth media was replaced with fresh media equilibrated with hypoxic gas mixture and cells were placed in the hypoxic chamber (Coy Laboratory Products, Ann Arbor, MI). This hypoxia chamber consisted of an airtight glove box with the atmosphere continuously monitored by an oxygen analyzer interfaced with oxygen

and nitrogen flow adapters. Oxygen concentrations were as indicated in mmHg (normoxia equal to 21% O₂) with the balance made up of nitrogen, carbon dioxide (constant 5% CO₂) and water vapor from the humidified chamber. Samples of equilibrated media were collected and media hypoxia was monitored using a blood gas analyzer (Ciba-Corning, Essex, England). Transepithelial resistances were monitored in hypoxia by interfacing the voltage clamp from the outside through an airtight seal in the chamber.

Barrier function was assessed electrically by transepithelial resistance and by flux of fluorescein-conjugated dextran. To measure transepithelial resistance, the upper and lower reservoirs were interfaced with pairs of calomel and Ag-AgCl electrodes via 5% agar bridges. Resistance measurements were obtained using a dual voltage clamp (University of Iowa) as described previously (16). Flux of fluorescein isothiocyanate-labeled dextran (FITC-dextran, $M_r = 3$ kD; Molecular Probes, Eugene, OR) across epithelial monolayers was assayed exactly as described before (18). Samples were read on a fluorescent plate reader (Cytofluor™ 2300; Millipore Inc., Bedford, MA) and flux rates were calculated from a daily standard curve.

PMN Transmigration Assay. The PMN transepithelial migration assay has been previously detailed (10, 17, 19). Briefly, human PMN were isolated from normal human volunteers by a gelatin sedimentation technique (20) and suspended in modified HBSS (without Ca²⁺ and Mg²⁺, with 10 mM Hepes, pH 7.4; Sigma) at a concentration of 5×10^7 /ml. Before addition of PMN, T84 monolayers were washed free of media with HBSS (containing Ca²⁺ and Mg²⁺). Transmigration assays were performed by the addition of PMN to the upper chambers after chemoattractant (1 μ M fMLP unless otherwise noted) was added to the opposing (lower) chambers. At time zero, 1×10^6 PMN were added and transmigration was allowed to proceed for 2 h at 37°C. All experiments were performed at 37°C. Transmigration was quantitated by assaying for the PMN azurophilic granule marker myeloperoxidase (MPO) as described previously (10, 17). In subsets of experiments, acellular permeable supports (T84 cells removed by EGTA, see below) were assayed for PMN transmigration using similar conditions as described above, with the exception that 10^{-8} M fMLP was used to establish the chemotactic gradient (21).

Cycloheximide (Sigma), where indicated, was prepared fresh as a 2 mg/ml stock in 95% ethanol and diluted to 1 μ g/ml in media at a concentration which we have previously found to inhibit >85% of radiolabeled leucine incorporation into precipitable protein (10).

Antibodies. Mouse ascitic fluids containing previously characterized, functionally inhibitory mAb to CD18 (22) (TS1/18, subclass IgG₁; American Type Tissue Collection [ATTC], Rockville, MD), CD11a (22) (TS1/22, subclass IgG₁; ATTC), or CD11b (23) (44a, subclass IgG_{2a}; ATTC) were used as described previously (10, 17). mAb C5/D5 (IgG₁), a functionally inhibitory mAb to CD47 was used as purified IgG as described before (21). A control mAb directed against MHC class I (24) (W6/32, subclass IgG_{2a}; ATTC) was used as a non-functional PMN and T84 cell binding control.

Under Agarose Chemotaxis Assay. Chemotaxis under agarose was used to assess the chemotactic activity of epithelial cell culture supernatants, exactly as detailed previously (25) using purified human PMN. PMN migration was measured microscopically by the leading front method (26) using an ocular micrometer as described previously (27). From each plate, directed migration was measured. In subsets of experiments, rabbit anti-IL 8 polyclonal Ab (50 μ g/ml; Endogen Inc., Boston, MA) was added to cell cul-

¹Abbreviations used in this paper: H/R, hypoxia/reoxygenation; MPO, marker myeloperoxidase.

ture supernatants collected from normoxic or hypoxic epithelia (see below) and incubated end-over-end for 30 min before addition to plates.

Quantitation of IL-8. Supernatants from normoxic and hypoxic epithelial monolayers were quantitated for IL-8 secretion using a capture sandwich ELISA and a daily IL-8 standard curve as described before (12). In subsets of experiments to determine polarity of secretion, supernatants were harvested from the apical or basolateral surface separately, volume adjusted to 1 ml with fresh T84 cell media and assayed for IL-8.

Detection of matrix-associated IL-8 was determined using monolayers of normoxic or hypoxic T84 cells which were stripped of cells and assayed for binding of anti-IL-8 antibodies using modifications of techniques described elsewhere (28). Briefly, confluent T84 cells were grown as inverted monolayers on permeable supports, incubated in normoxia or hypoxia as indicated, washed three times with warm normoxic HBSS and transferred to a solution of modified HBSS (without Ca^{2+} or Mg^{2+}) containing 5 mM ethylene glycol-bis(b amino ethyl ether) N,N,N',N' -tetraacetate (EGTA; Sigma) at 37°C. Monolayers were agitated for 2 h at 37°C and vigorously washed to remove cells, as has shown elsewhere (13). Monolayers were viewed under an inverted microscope to verify that the filters were acellular. Such acellular filters were cooled to 4°C, blocked with 100 μl media containing 10% BCS for 1 h at 4°C followed by addition of rabbit anti-human IL-8 polyclonal antibody (20 $\mu\text{g}/\text{ml}$ in media; Endogen) or control rabbit serum (Sigma; 1:500 dilution in media) for 2 h at 4°C. Monolayers were extensively rinsed five times with cold HBSS followed by addition of peroxidase conjugated goat anti-rabbit secondary Ab (1:1,000 in media; Cappel, West Chester, PA). Filters were washed five times in cold HBSS and exposed to peroxidase substrate (containing 1 mM 2,2'-azino-di-(3-ethyl) dithiazoline sulfonic acid with 10 mM H_2O_2 in 100 mM citrate buffer, pH 4.2) for 20 min at 37°C. Color development of supernatants was assayed at 405 nM on a microtiter plate reader (Molecular Devices, Sunnyvale, CA). Control monolayers for background subtraction consisted of collagen coated permeable supports without addition of epithelial cells.

CD47 ELISA. Expression of CD47 was determined exactly as described before (21) using mAb C5/D5 (purified IgG, 20 $\mu\text{g}/\text{ml}$ diluted in media containing 10% bovine calf serum) as a primary Ab on T84 epithelial monolayers in 96-well flat bottomed microtiter plates (Costar, Cambridge, MA). Negative controls included omitting the primary and/or secondary Ab. T84 cells exposed to IFN- γ (1,000 U/ml, 48 h) served as a positive control, which we have shown induces CD47 on the epithelial surface (21).

Antisense Oligonucleotide Treatment of Cells. Electrically confluent T84 cells grown as inverted monolayers on permeable supports were washed three times in warm T84 cell media. IL-8 phosphorothioate antisense oligonucleotide (40 base oligonucleotide) derived from the 5' untranslated sequence of exon 1 (29) were purchased from Oncogen Sciences (Cambridge, MA) were diluted at indicated concentrations in serum-free media containing N -[1-(2,3-dioleoyloxy)propyl]- N,N,N -trimethylammonium chloride (DOTMA, Lipofectin solution at 10 $\mu\text{g}/\text{ml}$; GIBCO BRL) and applied to cells (150 μl) to the basolateral surface only. Serum-containing media was used in the opposing well (i.e., apical surface). Cells were incubated in normoxia or hypoxia, as indicated, for 24 h followed by addition of 100 μl serum containing media to the basolateral surface. Cells were allowed to incubate for additional 24 h, then used as described above to assess IL-8 secretion or PMN transmigration. Controls consisted of media only or media containing DOTMA without antisense oligonucleotides.

Data Presentation. Since variations exist in both transepithelial resistance between groups of monolayers (baseline resistance range 650–1500 $\text{ohm}\cdot\text{cm}^2$) and in PMN obtained from different donors, individual experiments were performed using large numbers of uniform groups of monolayers and PMN from single blood donors on individual days. PMN transmigration results are represented as PMN cell equivalents derived from a daily standard PMN dilution curve. Values are expressed as the mean and SEM of n experiments.

Results

Influence of Hypoxia on Epithelial Function. Based on a number of cell viability measurements, T84 intestinal epithelial cell cultures exposed to hypoxia (as low as 1% O_2 for up to 72 h) alone or hypoxia followed by reoxygenation resulted in no significant damage. No changes in epithelial barrier function, a sensitive measure of epithelial toxicity (15), were detected using two separate techniques (see Table 1). First, measurement of baseline transepithelial resistance revealed no significant differences between control monolayers (normoxia, 48 h) and monolayers exposed to hypoxia (2% O_2 , 48 h) or hypoxia/reoxygenation (48 h hypoxia/3 h reoxygenation). To demonstrate that barrier function can be perturbed by a relevant biological stimulus, epithelial monolayers exposed to IFN- γ (1,000 U/ml, 48 h) were significantly attenuated in epithelial barrier ($P < 0.01$), as shown previously (30). As a second measure of epithelial barrier, we performed quantitative FITC-dextran fluxes ($M_r = 3$ kD) across epithelial monolayers (18). Similar to our findings with TER measurements, no differences in FITC-dextran flux was observed (Table 1). Pre-exposure of intestinal epithelia to IFN- γ (as above) resulted in a sig-

Table 1. Hypoxia/Reoxygenation Does Not Influence Epithelial Barrier Function

Condition	Transepithelial Resistance	Paracellular Flux
	$\text{ohm} \cdot \text{cm}^2$	$\text{pM}/\text{cm}^2 \cdot \text{h}$
Normoxia, 48 h	1145 \pm 96.8	14 \pm 3.3
Hypoxia, 48 h	1041 \pm 184.4*	12 \pm 4.1*
Hypoxia, 48 h Reoxygenation, 3 h	1165 \pm 140.5*	13 \pm 2.2*
IFN- γ , 48 h	551 \pm 64.7‡	93 \pm 8.2‡

T84 intestinal epithelial cells were grown to confluence on inverted monolayers and exposed to normoxia (21% O_2), hypoxia (2% O_2), hypoxia followed by reoxygenation or our positive control IFN- γ (1,000 U/ml) for indicated periods of time, followed by measurement of transepithelial resistance and paracellular flux of fluoresceinated dextran (3 kD M_r). Values represent transepithelial resistance measurements of eight monolayers in each condition.

*Not significantly different than normoxia control

‡Significantly different than normoxia, hypoxia and hypoxia/reoxy, $P < 0.01$

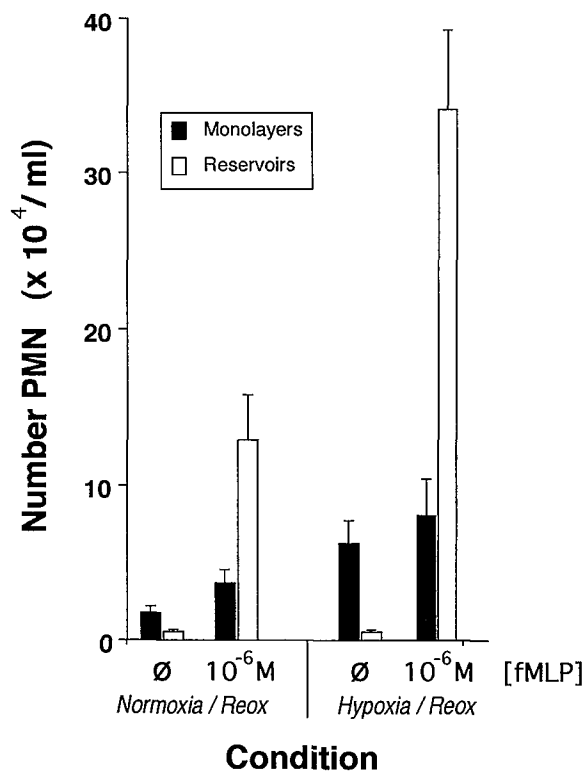


Figure 1. Epithelial exposure to hypoxia modulates neutrophil-epithelial interactions. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers (i.e., basolateral surface upward) on permeable supports. Confluent monolayers were exposed to hypoxia (2% O₂, 48 h) and reoxygenated with buffer containing PMN (20 μl at 5 × 10⁷/ml) in the presence or absence of a 1 μM transepithelial gradient of the chemotactic peptide fMLP. PMN were allowed to transmigrate for 2 h at 37°C. Transmigration was quantitated by assaying for monolayer-associated (the number of PMN which remain firmly associated with washed monolayers, closed bars) and reservoir-associated (the number of PMN which traverse the epithelial monolayer into the adjacent reservoir, open bars) PMN as the PMN-specific marker myeloperoxidase. Results represent the mean ± SEM for 12 individual monolayers in each condition.

nificantly increased flux ($P < 0.01$). Thus, these results indicate intestinal epithelial barrier function is not influenced by hypoxia and it is unlikely that the conditions used here influence viability of T84 cell monolayers. In addition, no distinct changes in morphology were observed and no evidence of cell death was apparent (based on soluble LDH measurements from supernatants and trypan blue exclusion, data not shown).

Hypoxia/Reoxygenation Modulates Epithelial-PMN Interactions. As shown in Fig. 1, exposure of T84 epithelial monolayers to hypoxia, followed by reoxygenation with buffer containing a suspension of PMN resulted in significantly increased fMLP-stimulated (1 μM) PMN transepithelial migration. This hypoxia-elicited modulation of PMN-epithelial interactions was reflected as an increased number of PMN which traversed the epithelial monolayer into the bottom reservoir (Fig. 1, 12.7 ± 2.6 × 10⁴ for normoxic controls vs 36.2 ± 5.61 × 10⁴ PMN for hypoxia/reoxygenated monolayers, 2% O₂ for 72 h, $P < 0.025$) and the

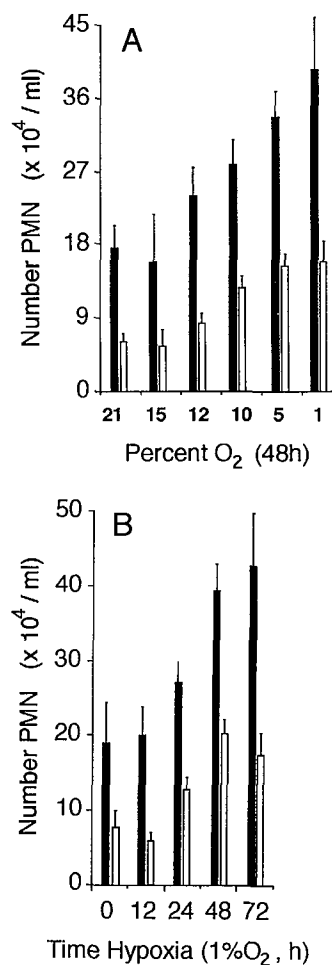


Figure 2. Epithelial exposure to hypoxia modulates neutrophil-epithelial interactions. Inverted monolayers of the human intestinal epithelial cell line T84 were grown on permeable supports. Confluent monolayers were exposed to hypoxia for 48 h at indicated O₂ concentrations (left) or for indicated periods of time (right) and reoxygenated with buffer containing PMN (20 μl at 5 × 10⁷/ml) in the presence of a 1 μM transepithelial gradient of the chemotactic peptide fMLP. PMN were allowed to transmigrate for 2 h at 37°C. Monolayer-associated PMN (open bars) and PMN transmigration (reservoir-associated PMN, closed bars) were quantitated by assaying for the PMN-specific marker myeloperoxidase. Results represent the mean ± SEM for 12 individual monolayers in each condition. ■, Reservoirs; □, monolayers.

number of PMN which migrated into monolayers and remained tightly associated with washed monolayers (Fig. 1, monolayer-associated, 3.7 ± 0.87 × 10⁴ for normoxic controls vs 8.2 ± 1.31 × 10⁴ PMN for hypoxia/reoxygenated monolayers, 2% O₂ for 72 h, $P < 0.001$). In the absence of a gradient of fMLP, hypoxia/reoxygenation increased the number of PMN which remained associated with epithelial monolayers (Fig. 1, 2.4 ± 0.3-fold increase over normoxia, $P < 0.01$), but did induce transepithelial PMN migration (Fig. 1, $P =$ not significant).

As shown in Fig. 2, pre-exposure of epithelial monolayers to hypoxia resulted in an O₂-dependent (ED₅₀ ~7% O₂, ANOVA $P < 0.025$) as well as a hypoxia time-dependent increase ($t_{1/2}$ ~30 h, ANOVA $P < 0.01$) in the number of PMN which traversed the epithelial monolayer. Similar increases in monolayer-associated PMN were observed (Fig. 2, A and B, ANOVA, both $P < 0.025$) Such results indicate that epithelial pre-exposure to hypoxia/reoxygenation elicits a specific, quantitative increase in fMLP-stimulated PMN transmigration. A similar proportionate

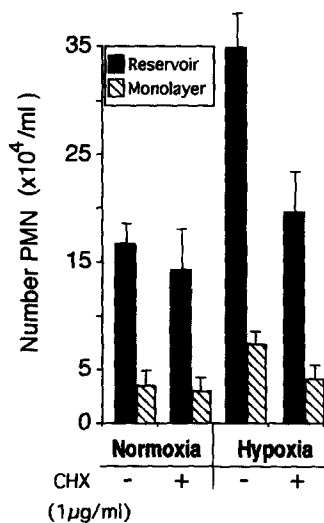


Figure 3. Hypoxia-elicited increase in PMN transmigration is protein synthesis dependent. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers on permeable supports. Confluent monolayers were exposed to hypoxia (2% O₂, 48 h) or normoxia in the presence (+) or absence (-) of non-toxic concentrations of the protein synthesis inhibitor cycloheximide (CHX, toxicity determined from dose response measuring epithelial barrier function). Monolayers were reoxygenated with buffer containing PMN (20 µl at 5 × 10⁷/ml) in the presence of a 1 µM transepithelial gradient of the chemotactic peptide fMLP. PMN were allowed to transmigrate for 2 h at

37°C. Transmigration was quantitated by assaying for monolayer-associated (hatched bars) and reservoir-associated (closed bars) PMN as the PMN-specific marker myeloperoxidase. Results represent the mean ± SEM for 10 individual monolayers in each condition.

increase was present in the number of monolayer-associated PMN.

Such hypoxia-elicited increases in PMN transmigration did not require reoxygenation. Experiments performed within the hypoxia chamber (i.e., all buffers pre-equilibrated to hypoxia, PMN resuspended in hypoxic buffer, cells washed in hypoxic buffer and transmigration in hypoxic atmosphere) resulted in similar results as with reoxygenation (PMN transmigration of 13.2 ± 1.89 × 10⁴ for normoxic controls vs 39.2 ± 4.31 × 10⁴ PMN for hypoxic monolayers, 2% O₂ for 72 h, *P* < 0.001), indicating that the elicited functional effect is hypoxia-dependent and not reoxygenation-dependent.

We next determined whether a gradient of fMLP was necessary to elicit increases in PMN transmigration. Normoxic and hypoxic (2% O₂ for 72 h) monolayers were assayed for transepithelial migration in the presence (fMLP, 1 µM) and absence (buffer) of a chemotactic gradient. Our results indicate that in the absence of a gradient of fMLP, the number of monolayer-associated PMN increased in response to hypoxia (3.7 ± 1.90 × 10⁴ for normoxic controls vs 11.6 ± 3.11 × 10⁴ PMN for hypoxic monolayers, 2% O₂ for 72 h, *P* < 0.05), but no significant transepithelial migration (i.e., PMN which completely traverse the epithelial monolayer) was observed across either normoxic or hypoxic monolayers.

Protein-Synthesis Dependence. To determine whether the effects of hypoxia on PMN transmigration required the synthesis of new protein by epithelial cells, transmigration was assessed after exposure of epithelial cells to hypoxia (2% O₂ for 48 h) in the presence and absence of 1 µg/ml cycloheximide. In performing these experiments, and as shown previously (10), we found that monolayers maintained baseline electrical characteristics such as high transepithelial

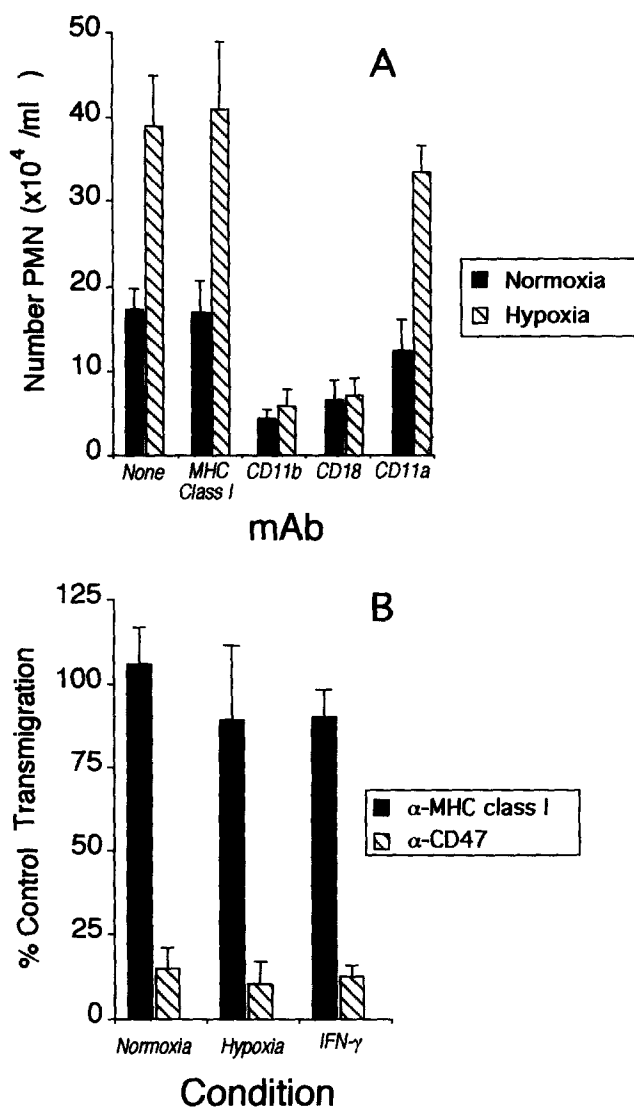


Figure 4. Hypoxia-elicited increases in neutrophil transmigration are dependent on the PMN β₂-integrin CD11b/18 and CD47. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers on permeable supports. Confluent monolayers were exposed to hypoxia (2% O₂, 48 h) or normoxia and reoxygenated with buffer containing PMN (20 µl at 5 × 10⁷/ml) preincubated with: (A) MHC class I mAb (clone W6/32, 1:200 dilution of ascitic fluid), CD11b mAb (clone 44a, 20 µg/ml), CD18 mAb (clone TS1/18, 20 µg/ml) or CD11a mAb (clone TS1/22, 20 µg/ml). (B) MHC class I mAb (clone W6/32, 1:200 dilution of ascitic fluid) or CD47 (clone C5/D5, 20 µg/ml). PMN were driven to migrate by the presence of a 1 µM transepithelial gradient of the chemotactic peptide fMLP. PMN were allowed to transmigrate for 2 h at 37°C. PMN transmigration was quantitated by assaying for the PMN-specific marker myeloperoxidase. Results represent the mean ± SEM for 10 individual monolayers in each condition.

resistance, even after 48 h exposure to cycloheximide (1251 ± 163 ohm-cm² vs 1084 ± 281 ohm-cm² for cycloheximide treated control monolayers, *P* = NS). As shown in Fig. 3, the addition of cycloheximide largely ablated the increase in PMN migration into and across hypoxic epithelial monolayers, but did not influence PMN migration

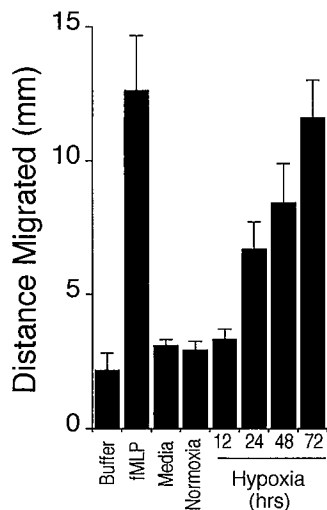


Figure 5. Hypoxia elicits a soluble epithelial factor which stimulates neutrophil chemotaxis. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers (i.e., basolateral surface upward) on permeable supports. Confluent monolayers were exposed to hypoxia (2% O₂, range 12–72 h) or normoxia (21% O₂, 72 h). Cell culture supernatants were collected from monolayers and used in an under agarose chemotaxis assay to determine chemotactic activity. Controls included buffer, chemotactic peptide fMLP (10⁻⁸ M) or unconditioned media. Results represent the mean ± SEM for 12 individual chemotaxis wells in each condition.

across normoxic monolayers, consistent with previous observations (10). These data suggest that new protein synthesis is, at least in part, necessary for the hypoxic modulation of PMN transepithelial migration.

PMN β_2 -Integrin (CD11b/18)-Dependence. As shown previously (10, 17), PMN migration across T84 intestinal epithelial monolayers is dependent on the PMN β_2 -integrin CD11b/CD18. To investigate whether hypoxia-elicited PMN transmigration involves a pathway dependent on the β_2 -integrins, epithelial monolayers were exposed to hypoxia as described above (2% O₂ for 72 h) and subsequently assessed for PMN transmigration in the presence of functionally inhibitory concentrations (20 μ g/ml) of β_2 -integrin monoclonal antibodies. As shown in Fig. 4 A, the presence of functionally inhibitory concentrations of mAbs to CD11b and CD18 inhibited the number of transmigrating PMN by 85 ± 7% and 80 ± 10%, respectively, across control, normoxic monolayers. Epithelial monolayers pre-exposed to hypoxia (2% O₂, 72 h) and PMN in the presence of anti-CD18 and anti-CD11b mAbs inhibited this PMN transmigration event by 94 ± 8% and 93 ± 6%, respectively. Transmigration in the presence of a mAb to MHC class I (W6/32, binding control), or CD11a were not different from control in monolayers untreated or pre-exposed to hypoxia ($P = \text{NS}$ compared to no mAb control). Anti-CD18 and anti-CD11b mAbs also inhibited the number of monolayer-associated PMN under conditions of normoxia (decrease of 64 ± 10% and 58 ± 6% for anti-CD18 and anti-CD11b, respectively, compared to no antibody control) and hypoxia/reoxygenation (decrease of 81 ± 13% and 66 ± 14% for anti-CD18 and anti-CD11b, respectively, compared to no antibody control). Control mAbs (anti-CD11a and anti-MHC class I) did not influence the number of monolayer-associated PMN (data not shown). These data indicate that increased monolayer-associated PMN and increased PMN transmigration elicited by epithelial exposure to hypoxia largely involves a pathway

which is dependent on the PMN β_2 -integrin CD11b/CD18.

Dependence on CD47. The above data indicate that hypoxia-elicited increases in PMN transmigration is CD11b/18-dependent and results from new epithelial protein synthesis. Recently, a monoclonal antibody (mAb C5/D5) was described which inhibits PMN transepithelial migration but not CD11b/CD18-mediated adhesion (21). The antigen for mAb C5/D5 was determined to be C47 and is expressed on a broad variety of cell types, including epithelia and PMN. Thus, we examined the influence hypoxia on cell surface expression of CD47 and the impact of mAb C5/D5 on hypoxia-elicited PMN transmigration. Hypoxia (2% O₂ for 48h) did not influence cell surface expression of CD47 (mean OD 0.32 ± 0.08 vs 0.29 ± 0.091 for hypoxia and normoxia, respectively, $P = \text{NS}$, $n = 4$). As a positive control for CD47 induction, T84 epithelial cells were pre-exposed to IFN- γ (1,000 U/ml, 48 h) and resulted in significant induction CD47 expression (mean OD 0.71 ± 0.068, vs 0.29 ± 0.091 for IFN- γ and control, respectively, $P < 0.01$, $n = 6$), as previously reported (21). Immunoprecipitation and western blotting studies with surface labeled T84 cells (21) confirmed such findings (data not shown).

As shown in Fig. 4 b, pre-incubation of normoxic, hypoxic or IFN- γ exposed monolayers with saturating concentrations (20 μ g/ml) of mAb C5/D5 followed by PMN transmigration resulted in a significant decrease in transmigration (87 ± 8%, 89 ± 10% and 87 ± 5 decrease compared to no mAb control for normoxia, hypoxia and IFN- γ exposure, respectively, all $P < 0.001$). Addition of our control mAb W6/32 did not significantly influence PMN transmigration (Fig. 4 b).

The addition of anti-CD47 mAb to normoxic, hypoxic or IFN- γ exposed monolayers resulted in consistently increased PMN accumulation within epithelial monolayers (2.3 ± 0.8-, 3.2 ± 1.0-, and 3.7 ± 0.6-fold increase compared to no mAb control, all $P < 0.001$). Such results are consistent with our previous data that anti-CD47 mAb increases epithelial monolayer-associated PMN, likely because CD47 mediates post-adhesive interactions between epithelia and PMN and blocks a step distal to initial interactive events between epithelia and PMN(21). Overall, these results indicate that hypoxic modulation of PMN transmigration remains CD47-dependent.

Hypoxia Induces Epithelial Liberation of a Soluble PMN Chemoattractant. We next determined whether a soluble PMN chemotactic factor was liberated from hypoxic T84 intestinal epithelia. Cell culture supernatants from normoxic epithelial cells and cells exposed to hypoxia were harvested and assayed for stimulation of chemotaxis using an under agarose assay. As shown in Fig. 5, supernatants from epithelial monolayers exposed to hypoxia (2% O₂) stimulated PMN chemotaxis. A correlation existed between time in hypoxia (range 0–72 h) and the distance PMN migrated under agarose. Liberation of this chemoattractant was evident as early as 24 h ($P < 0.025$ compared to normoxia) and maximal at 72 h (4.1 ± 0.6-fold increase

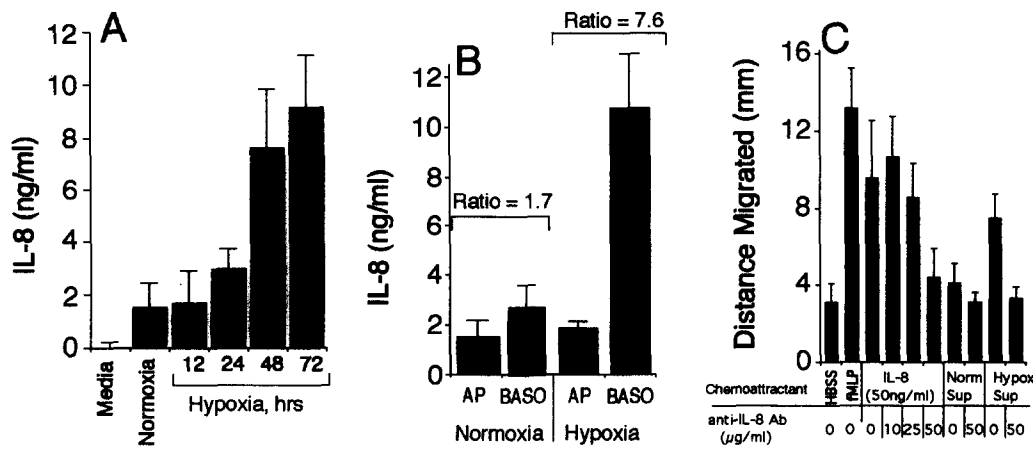


Figure 6. Hypoxia elicits polarized interleukin-8 release. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers on permeable supports. Electrically confluent monolayers were exposed to hypoxia (2% O₂ for indicated time) or normoxia (21% O₂, 72 h). Cell culture supernatants were collected and pooled (A), or collected separately (B, 72 h exposure to hypoxia or normoxia) from the apical (AP) or basolateral (BASO) surfaces of epithelial monolayers and assayed for presence of interleukin-8 by ELISA. Results represent the mean \pm SEM for 12 individual determinations in each condition. Shown in panel C are chemotaxis under agarose experiments using known IL-8 concentrations or supernatants harvested from hypoxic monolayers (72 h exposure). Conditions consisted of buffer only (HBSS), gradient of fMLP (fMLP, 10⁻⁸ M), gradient of a known concentration of IL-8 (50 ng/ml) with or without addition of indicated concentrations of anti-IL-8 polyclonal antibody, or supernatants harvested from normoxic or hypoxic monolayers and incubated with or without anti-IL-8 (50 μ g/ml). Results represent the mean \pm SEM for 12 individual chemotaxis wells in each condition.

compared to normoxia, $P < 0.001$). Values obtained from conditioned media of normoxic cells (72 h conditioned media) was not different than non-conditioned media. Buffer served as our negative control for non-stimulated random migration, and fMLP (10⁻⁸ M) served as our positive control.

Supernatants from normoxic epithelial cells and cells exposed to hypoxia were also used to examine stimulation of PMN transmigration. Conditioned supernatants from epithelial monolayers exposed to hypoxia (2% O₂, 48 h) or normoxia (48 h) were placed on the apical surface of T84 monolayers and PMN transmigration was assessed in the basolateral-to-apical direction. Neither hypoxic or normoxic supernatants stimulated significant PMN transmigration ($0.8 \pm 0.09 \times 10^4$ for normoxic controls vs $0.6 \pm 0.01 \times 10^4$ PMN for hypoxic monolayers, 2% O₂ for 72 h, $P = \text{NS}$) compared to the positive control (1 μ M fMLP, $12.3 \pm 2.01 \times 10^4$ PMN, $P < 0.01$ versus both hypoxic and normoxic supernatants). These results indicate that while hypoxia induces epithelial secretion of a factor which stimulates PMN chemotaxis under agarose, the hypoxia-elicited transmigration effect can not be recapitulated using conditioned supernatants.

Hypoxia Induces Basolateral Release of Interleukin 8. Since the chemokine IL-8 has been shown to be produced by activated epithelial cells and specifically by T84 cells (12), we explored the possibility that hypoxia might induce epithelial secretion of IL-8, and if so, whether secretion of this chemokine was polarized. T84 cells were grown on permeable supports and exposed to ambient oxygen tension (normoxia, 72 h) or hypoxia (2% O₂, 12–72 h). Cell

culture supernatants were harvested, pooled and assayed for IL-8 by ELISA (12). As shown in Fig. 6 A, hypoxia induced a time dependent increase in soluble IL-8 production ($P < 0.001$ by ANOVA) with maximal production at 72 h. To determine whether IL-8 was produced in a polarized fashion, cell culture supernatants from the apical or basolateral surface were separately assayed for IL-8. As shown in Fig. 6 B, hypoxia induced polarized epithelial release of IL-8. Some baseline IL-8 release was evident from normoxic monolayers, although no distinct polarity was evident (basolateral/apical ratio: 1.7 ± 0.59 , $P = \text{NS}$). However, under hypoxic conditions, this ratio of basolateral/apical IL-8 secretion significantly increased to 7.6 ± 0.31 , ($P < 0.01$), indicating a distinct, hypoxia-elicited stimulation of IL-8 release preferentially to the physiologically-relevant basolateral surface.

As shown in Fig. 6 C, addition of anti-IL-8 Ab (at concentrations of 10, 25, or 50 μ g/ml) to a solution of known IL-8 concentration (50 ng/ml) and subsequent chemotaxis under agarose resulted in decrements of chemotaxis (ANOVA, $P < 0.025$) and was especially pronounced at 50 μ g/ml (compared to no Ab, $P < 0.01$). Addition of anti-IL-8 polyclonal antibody (50 μ g/ml final concentration) to supernatant harvested from hypoxic epithelia resulted in a $55 \pm 12\%$ decrease the distance PMN migrated ($P < 0.01$ compared to no Ab controls) toward a gradient of the undiluted supernatant. No significant decrease was observed when anti-IL-8 Ab was added to supernatants harvested from normoxic epithelial monolayers ($P = \text{not significant}$ compared to no Ab control), or to a solution of 10⁻⁸ M fMLP ($105 \pm 11\%$ of no Ab control, $P = \text{NS}$). Finally, in

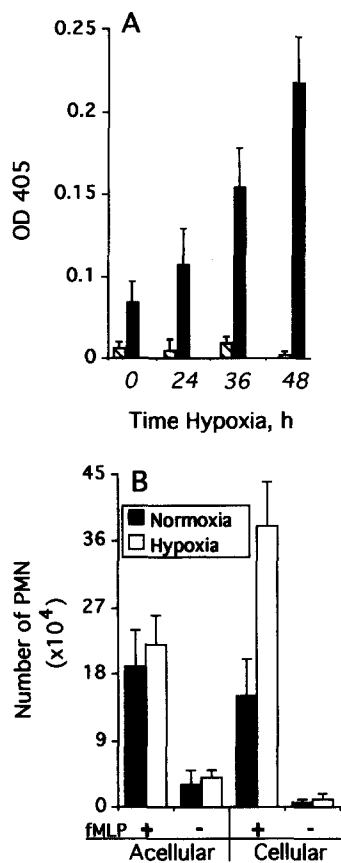


Figure 7. Basolaterally released interleukin-8 remains associated with acellular permeable supports but does not drive transmigration. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers on permeable supports. (A) Electrically confluent monolayers were exposed to hypoxia (2% O₂, range 24–48 h) or normoxia (21% O₂, 48 h). Epithelial cells were stripped from permeable supports by agitation in a solution of EGTA (see Material and Methods). Acellular monolayers were assayed for binding of anti-IL-8 antibody (solid bars) or equivalent dilutions of normal rabbit serum (stippled bars) by ELISA. Results represent the mean \pm SEM for six individual monolayers per condition. (B) Epithelia were exposed to hypoxia (2% O₂, 48 h) or normoxia (21% O₂, 48 h). Epithelial cells were stripped from permeable supports as described above (Acellular) or left intact as electrically confluent monolayers (Cellular). Transmigration was assessed across such monolayers in the presence and absence of a gradient of fMLP (1 μ M for cellular conditions and 10 nM for acellular conditions) for 2 h and results are presented as the number of PMN which traversed the monolayer. Results represent the mean \pm SEM for six individual monolayers per condition.

data not shown, addition of a control mAb (W6/32) at similar concentrations (50 μ g/ml) to hypoxic supernatants did not influence chemotaxis under agarose (110 \pm 25% of no Ab control, $P = \text{NS}$).

IL-8 Released from Epithelia Remains Tightly Associated with the Epithelial Matrix. The above results indicate that hypoxic epithelial cells secrete IL-8 in a polarized manner. T84 intestinal epithelia grown on permeable supports lay down an extensive extracellular matrix (9). Others have shown that T84 cells imprint the matrix with biologically active IL-8 (13), likely through affinity for specific matrix components (31, 32). Thus, we determined whether hypoxic monolay-

ers might have increased matrix-associated IL-8. To address this, T84 monolayers were exposed to hypoxia or normoxia (conditions as above) followed by stripping of epithelial cells from the polycarbonate supports using EGTA (see Material and Methods). No visible cells remained after such treatment (data not shown). SDS PAGE and Western blotting of solubilized filters prepared in this fashion failed to visualize protein bands consistent with IL-8 (data not shown). However, as shown in Fig. 7, increased periods of hypoxia resulted in increased binding of anti-IL-8 antibody to acellular membrane supports by ELISA (one way ANOVA, $P < 0.05$) and was maximal by 48 h exposure to hypoxia. These data indicate that hypoxic epithelial cells produce increased levels of IL-8 and detectable chemokine levels remain associated with acellular matrices.

As shown in Fig. 7 B, hypoxia-elicited increases in PMN transmigration required the presence of epithelial cells. No differences in transmigration were evident across acellular hypoxic or normoxic monolayers in the presence ($19.2 \pm 4.89 \times 10^4$ for normoxic controls vs $22.2 \pm 4.81 \times 10^4$ PMN for hypoxic monolayers, $P = \text{NS}$) or absence of 10 nM fMLP ($3.2 \pm 1.71 \times 10^4$ for normoxic controls vs $4.2 \pm 0.81 \times 10^4$ PMN for hypoxic monolayers, $P = \text{NS}$). The presence of confluent epithelial cells, as described above (Figs. 1–4), resulted in hypoxic-induction of PMN transmigration in the presence of 10^{-6} M fMLP ($20.0 \pm 3.99 \times 10^4$ for normoxic controls vs $39.2 \pm 5.86 \times 10^4$ PMN for hypoxic monolayers, $P < 0.01$), but not in the absence of fMLP ($0.5 \pm 0.49 \times 10^4$ for normoxic controls vs $0.9 \pm 0.8 \times 10^4$ PMN for hypoxic monolayers, $P = \text{NS}$). Significantly increased numbers of PMN remained associated with hypoxic acellular monolayers in the presence (3.3 ± 0.8 -fold increase over normoxic control, $P < 0.01$) or absence (2.2 ± 0.4 -fold increase over normoxia control, $P < 0.01$) of a gradient of fMLP (as above). Finally, addition of mAbs (20 μ g/ml, as above) directed against both CD11b and CD18, but not CD11a, blocked PMN transmigration across acellular monolayers exposed to conditions of normoxia (81 ± 10 , 76 ± 8 and $25 \pm 3\%$ decrease compared to no mAb control for anti-CD11b, CD18 or CD11a, respectively) and hypoxia (70 ± 13 , 84 ± 18 and $4 \pm 1\%$ decrease compared to no mAb control for anti-CD11b, CD18, or CD11a, respectively).

Influence of IL-8 Antisense Oligonucleotides on Hypoxia-Elicited Increases in PMN Transmigration. To better assess the contribution of IL-8 in PMN migration across reoxygenated monolayers, antisense oligonucleotide probes were used to determine whether inhibition of IL-8 mRNA in hypoxic epithelial monolayers would normalize transmigration responses. Controls for these experiments consisted of media alone or media containing lipofectin reagent. These conditions did not influence cell viability (judged morphologically and measured as transepithelial resistance, data not shown) As shown in Fig. 8 A, hypoxic epithelia incubated with IL-8 antisense oligonucleotides for 48 h liberated decreased amounts of IL-8 with increasing concentrations of oligonucleotide (ANOVA, $P < 0.01$). Indeed, at the highest oligonucleotide concentration (0.2 μ M), a $70 \pm 15\%$

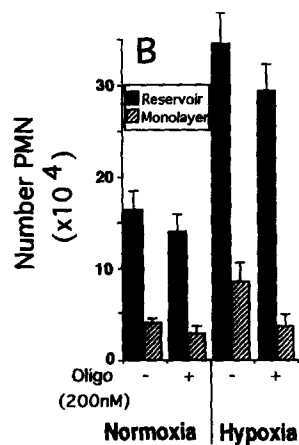
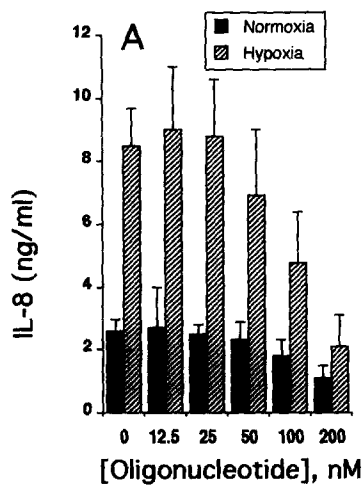


Figure 8. IL-8 antisense oligonucleotides diminish polarized IL-8 release and diminish PMN accumulation within epithelial monolayers. Monolayers of the human intestinal epithelial cell line T84 were grown as inverted monolayers on permeable supports. Electrically confluent monolayers were exposed to hypoxia (2% O₂) or normoxia (21% O₂) the presence of lipofectin reagent alone or with addition of indicated concentrations of IL-8 antisense oligonucleotide. (A) Cell culture supernatants were collected from the basolateral surface of epithelial monolayers and assayed for

presence of interleukin-8 by ELISA. Results represent the mean \pm SEM for six individual determinations in each condition. (B) Hypoxic (48 h) or normoxic monolayers treated or untreated with oligonucleotide were assayed for PMN were allowed to transmigrate for 2 h at 37°C and transmigration was quantitated by assaying for monolayer-associated (hatched bars) and reservoir-associated (closed bars) PMN as described above. Results represent the mean \pm SEM for six individual determinations in each condition.

diminution in IL-8 secretion was evident for hypoxic epithelia ($P < 0.01$ compared to no oligonucleotide). Normoxic monolayers exposed to antisense oligonucleotides liberated decreased IL-8 ($35 \pm 10\%$ at $0.2 \mu\text{M}$ oligonucleotide), although to a lesser extent than hypoxia.

Using such oligonucleotide treated epithelial cells ($0.2 \mu\text{M}$ oligonucleotide), PMN transmigration was assessed in response to a transepithelial gradient of fMLP (10^{-6} M , Fig. 8 B). Compared to normoxic monolayers, epithelia preloaded with IL-8 oligonucleotides did not result in a specific reduction in PMN transmigration (ratio of transfected to non-transfected transmigration 0.85 ± 0.09 and 0.87 ± 0.13 for normoxia and hypoxia, respectively, $P = \text{NS}$). IL-8 antisense oligonucleotides did, however, result in an hypoxia-specific decrease in monolayer-associated PMN (Fig. 8 B, $62 \pm 9\%$ decrease for hypoxia compared to $10 \pm 4\%$ decrease for normoxic monolayers, $P < 0.01$), indicating

that decreased hypoxia-elicited IL-8 secretion manifests as decreased migration into monolayers but not across monolayers.

Discussion

In a number of diseases, there exists a strong correlation between reperfusion injury and the acute inflammatory response (3). The direct role of PMN have been implicated in tissue damage resulting from reperfusion injury, although mechanisms remain only partially understood (3). In the intestine for instance, PMN accumulation at the level of the epithelium have been shown to play a central role in mucosal injury during intestinal reperfusion injury (33, 34, 35). In disorders such as Crohn's disease, there is evidence that hypoxia/ischemia may play a role in endpoint tissue damage (36). Thus, it seems likely that intestinal ischemia and PMN-epithelial interactions may be related events. The direct role of the epithelium is unclear. We report here that hypoxia provides a signaling mechanism for directing the physiologic movement of PMN across cultured intestinal epithelial monolayers. Such modulation is not secondary to epithelial toxicity, is dependent on new epithelial protein synthesis, and remains dependent on CD11b/18 and CD47. Additionally, while epithelial exposure to hypoxia was associated with increased basolateral secretion of IL-8, it is unlikely that this chemokine orchestrates increased PMN transmigration; rather, IL-8 likely serves to recruit PMN to the epithelial basolateral membrane.

Little is known about the direct influence of hypoxia on epithelial function. A relevant paradigm is the endothelium, and a number of elegant studies have examined the direct influence of hypoxia on endothelial cell function (recently reviewed in reference 6). Our studies demonstrate that clear differences exist between endothelial cells and epithelial cells with regard to functional sequelae of hypoxia. For example, while others have demonstrated that hypoxia significantly modulates endothelial barrier (37), we find no significant effect of hypoxia on epithelial barrier. Using both electrical resistance and paracellular flux assays, even relatively long periods of exposure to hypoxia (72 h) resulted in no observable change in barrier function. Such differences may lie at the level of barrier regulation in the respective cell types. Endothelial barrier is tightly coupled to intracellular cAMP levels (38), and hypoxia-dependent decrements in endothelial levels of cAMP seem to adequately describe such hypoxia-elicited barrier defects in confluent endothelial monolayers (39). Epithelial barrier function, however, is less dependent on intracellular levels of cAMP (40), and T84 cells, used in these studies, lack a cAMP component in tight junction regulation (41). Whether epithelial generation of second messengers such as cAMP are influenced by hypoxia, as they are with endothelia, is not known at the present time.

The present results indicate that exposure of epithelia to hypoxia promotes enhanced PMN movement into, and across intestinal epithelial monolayers during reoxygenation.

ation. Our studies have dissected the transmigration response in an attempt to define hypoxia-elicited factors which might drive this response. The signal(s) responsible for such enhanced transmigration are generated during the period of epithelial hypoxia, and do not depend on a period of reoxygenation, since experiments without reoxygenation also resulted in enhanced transmigration (see results). Moreover, hypoxia is not a stand alone signal for inducing transepithelial migration of PMN, rather, a second transepithelial signal (provided in our model by fMLP, at concentrations which approach luminal concentrations in vivo [42]) was necessary to drive this response. Of note, however, hypoxia alone did enhance movement of PMN into the basolateral space of epithelial monolayers (see results), suggesting that hypoxia may liberate an epithelial signal which recruits PMN to the basolateral space. We show in these studies that hypoxia induces epithelial basolateral release of IL-8, a potent PMN activator (43, 44). Such secretion is markedly enhanced and polarized in response to hypoxia. Significant evidence indicates that basolateral release of IL-8 may serve as a recruitment signal, although it is unlikely that such IL-8 promotes successful movement of PMN entirely across the epithelium. First, secretion of IL-8 is polarized to the basolateral surface of epithelia, as we show here (Fig. 6) and as demonstrated previously (12, 13). Such polarized release disallows the establishment of transepithelial gradients favorable for transmigration, and thus may well serve to promote PMN movement to the basolateral space. Secondly, T84 cells grown on permeable supports lay down an extensive extracellular matrix (9). IL-8 is a basic charged peptide with affinity for matrix heparin groups (31, 32), and we demonstrate that IL-8 remains avidly associated with acellular permeable supports (Fig. 7). These data confirm a recent study by McCormick et al. who demonstrated that intestinal epithelia exposed to a luminal pathogen (*Salmonella typhimurium*) imprint basolateral epithelial matrices with bioactive IL-8 gradients (13). Of note on this regard, proteoglycans (such as heparin) serve as ligands for PMN β_2 -integrin CD11b/18 (45) and CD47 appears regulate integrin interactions with matrix components (46). Our findings indicate that hypoxia-elicited responses are both CD11b/18- and CD47-dependent (Fig. 4). Thus, matrix bound IL-8 may provide a especially potent setting for recruitment of PMN to the basolateral surface. Finally, inhibition of IL-8 secretion using antisense oligonucleotides demonstrated no specific effects on PMN transepithelial migration in response to hypoxia (Fig. 8), but significantly dampened PMN movement into epithelial monolayers. Thus, while IL-8 may serve to recruit PMN to the basolateral space, additional unidentified factors likely play a role in driving enhanced PMN transmigration in response to epithelial hypoxia.

The mechanism(s) whereby hypoxia enhances IL-8 secretion is not clear. NF- κ B, a transcription factor important in IL-8 production (47), may be indirectly activated through intracellular changes in redox potential (48). We find, however, that hypoxia in the absence of reoxygenation en-

hances such IL-8 secretion (see Results), and thus minimal changes, if any, in redox potential are necessary for IL-8 induction. Others have shown that NF- κ B can be activate directly by hypoxia (49), although the details of such induction remain to be elucidated. It is unlikely that NF- κ B activation by hypoxia fully explains our IL-8 findings here. While we show that the hypoxia-elicited modulation of PMN transmigration is protein-synthesis dependent, other NF- κ B-dependent functions are not similarly induced (i.e., expression of cell surface ICAM-1 was not induced, data not shown). Finally, such enhanced secretion of IL-8 could result from generation of a soluble factor which in turn stimulates epithelial secretion of IL-8 through autocrine mechanisms. Transfer experiments using conditioned media from hypoxic epithelia and exposure to normoxic monolayers have proven negative on this accord (data not shown). Thus, mechanisms to explain induction of basolateral secretion of IL-8 remain to be elucidated at this time.

Several possibilities exist to explain increased transmigration of PMN in the physiological basolateral-to-apical direction. While the molecular aspects of PMN-epithelial interactions are clearly in their infancy, several recent studies have contributed to knowledge on this front and are important to highlight in context of the present results. First, hypoxia-elicited increases in PMN transmigration remain dependent on CD11b/18 (Fig. 4). The counter-receptor for this integrin on epithelia has not been defined, though a putative ligand is ICAM-1 (50). It is unlikely that ICAM-1 plays a significant role in hypoxia-elicited PMN transmigration, since T84 cells express little or no ICAM-1 in the baseline state (51) or in response to hypoxia (see above). Thus, future work will determine whether hypoxia and/or hypoxia/reoxygenation could induce or regulate CD11b/18 ligands on epithelia. Second, PMN transmigration across

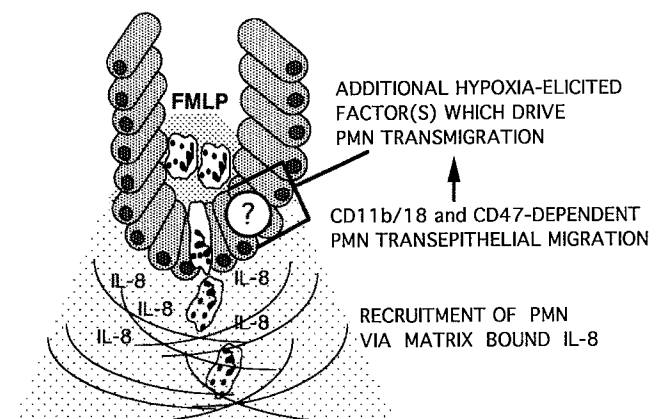


Figure 9. Model by which epithelial hypoxia modulates PMN-epithelial interactions. Hypoxia elicits basolateral release of IL-8. PMN are recruited to the subepithelial space by matrix-associated IL-8. At the basal surface of the epithelia, enhanced PMN transmigration occurs in response to a gradient of second signal (i.e., fMLP). Translocation across the epithelia occurs in a CD11b/18- and CD47-dependent manner.

T84 monolayers in response to hypoxia is CD47-dependent. CD47 was recently shown to mediate post-adhesive events in transepithelial (21) as well as transendothelial migration (52). Our results indicate that hypoxia does not appear to regulate epithelial surface expression of CD47 (see results), though we have not determined whether hypoxia may elicit structural changes in CD47 which might differentially influence PMN-matrix interactions or PMN-epithelial interactions. Thus, given that hypoxia-elicited increases in PMN transepithelial migration do not require reoxygenation (see Results), depends on new epithelial protein synthesis (Fig. 3), lacks a direct involvement of a soluble factor (see Results and Figs. 7 and 8), and requires the presence of intact epithelial cells (Fig. 7 B), a final possibility is the direct role of a novel or as yet undetermined molecule important in such PMN-epithelial interactions.

A model of hypoxia-elicited modulation of PMN transepithelial migration is shown in Fig. 9. In this model, secretion of IL-8 from hypoxic epithelia recruits leukocytes to the basal surface of epithelia. Subsequent PMN migration across epithelia is dependent on CD11b/18 and CD47 and is induced by transepithelial gradients of a second stimulus, such as fMLP. Additional components which may be hypoxia-elicited remain to be elucidated. The present observations suggest that the epithelium may direct trafficking of PMN in response to a number of stimuli. Such cell-cell crosstalk may occur through a number of mechanisms, including expression of cell surface proteins, secretion of soluble signals, and establishment of matrix bound cell-cell signals. A better understanding of the signal transduction pathways important during hypoxia will be fundamental to developing tissue specific therapies for such disorders.

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