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Components of intestinal epithelial hypoxia activate the virulence circuitry of *Pseudomonas*

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Kohler, Jonathan E., Olga Zaborina, Licheng Wu, Yingmin Wang, Cindy Bethel, Yimei Chen, James Shapiro, Jerrold R. Turner, and John C. Alverdy. Components of intestinal epithelial hypoxia activate the virulence circuitry of *Pseudomonas. Am J Physiol Gastrointest Liver Physiol* 288: G1048–G1054, 2005. First published December 9, 2004; doi:10.1152/ajpgi.00241.2004.—We have previously shown that a lethal virulence trait in *Pseudomonas aeruginosa*, the PA-I lectin, is expressed by bacteria within the intestinal lumen of surgically stressed mice. The aim of this study was to determine whether intestinal epithelial hypoxia, a common response to surgical stress, could activate PA-I expression. A fusion construct was generated to express green fluorescent protein downstream of the *PA-I* gene, serving as a stable reporter strain for PA-I expression in *P. aeruginosa*. Polarized Caco-2 monolayers were exposed to ambient hypoxia (0.1–0.3% O_2) for 1 h, with or without a recovery period of normoxia (21% O_2) for 2 h, and then inoculated with *P. aeruginosa* containing the PA-I reporter construct. Hypoxic Caco-2 monolayers caused a significant increase in PA-I promoter activity relative to normoxic monolayers (165% at 1 h; $P < 0.001$). Similar activation of PA-I was also induced by cell-free apical, but not basal, media from hypoxic Caco-2 monolayers. PA-I promoter activation was preferentially enhanced in bacterial cells that physically interacted with hypoxic epithelia. We conclude that the virulence circuitry of *P. aeruginosa* is activated by both soluble and contact-mediated elements of the intestinal epithelium during hypoxia and normoxic recovery.

epithelial cells; stress

PSEUDOMONAS AERUGINOSA IS an opportunistic pathogen that carries the highest case fatality rate (40%) of all nosocomial infections. Among critically ill patients, the primary site of colonization of *P. aeruginosa* is within the intestinal tract, where its mere presence is associated with a fourfold increase in mortality even in the absence of extraintestinal dissemination (10). Although the effects of bacteria on epithelial cell structure and function have been the subject of intensive study (3, 4, 11, 18), little is known about the effect of epithelial factors on bacterial gene expression.

We have identified a virulence-related attachment factor in *P. aeruginosa*, the PA-I lectin/adhesin, which plays a key role in adherence to and disruption of the intestinal epithelial barrier. The PA-I lectin is capable of causing a significant permeability defect that allows paracellular flux of known cytotoxins of *P. aeruginosa*, including exotoxin A and elastase, in both cultured intestinal epithelial cells and mouse intestine (9). The PA-I lectin is a 13,000-Da protein that is located within the cell cytoplasm when bacteria are grown in nutrient-rich media under ideal laboratory conditions. Yet, under conditions of physiological stress, such as occurs within the intestinal tract following surgical injury, expression of the PA-I lectin is increased and the protein relocates to the bacterial cell surface (21, 22).

Among the many characteristics of highly successful pathogens that infect and harm their hosts is the ability to sense and respond to changes in their local microenvironment. The environmental cues that typically signal a pathogen to respond with enhanced virulence are simple physicochemical properties such as changes in pH, redox state, and osmolality. More recently, host cell contact itself has been shown to activate specific virulence regulatory pathways in several pathogens of clinical importance including *P. aeruginosa* (13) (1, 2). Instillation of *P. aeruginosa* into the cecum of mice subjected to a 30% surgical hepatectomy results in severe sepsis and 100% mortality within 48 h, whereas sham-operated mice without hepatectomy, similarly exposed to *P. aeruginosa*, appear completely healthy with 100% survival (9). Previous studies (21, 22) in our laboratory have demonstrated that the PA-I lectin is expressed by bacteria within the cecum of mice following a 30% surgical hepatectomy. The putative role of PA-I in this response was established by the observation that mutant strains that do not express PA-I are completely apathogenic in this model. Because splanchnic vasosconstriction and intestinal epithelial hypoxia are a common consequence of surgical injury, the aim of this study was to determine the specific role of the intestinal epithelium in signaling to *P. aeruginosa* by examining the effect of epithelial cell hypoxia and reoxygenation on PA-I expression.

MATERIALS AND METHODS

Human epithelial cells. Caco-2_{BBe} cells expressing SGLT1 were maintained in DMEM with 25 mM glucose (high-glucose DMEM) with 10% fetal calf serum, 15 mM HEPES, pH 7.4, and 0.25 mg/ml geneticin, as previously described (17). Caco-2 cells were plated on 0.33-cm^2 collagen-coated, 0.4-μm pore size polycarbonate membrane Transwell supports (Corning-Costar, Acton, MA) for 20 days, and media were replaced with identical media without geneticin at least 24 h before bacterial inoculation.

GFP fusion constructs of wild-type P. aeruginosa. P. aeruginosa (ATCC-27853, American Type Culture Collection, Manassas, VA) was transformed with the plasmid pUCP24/PLL-EGFP. This con-

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struct harbors a PA27853 chromosomal DNA fragment containing an upstream regulatory region of PA-I followed by the entire *PA-I* gene fused at the COOH terminal with an enhanced green fluorescent protein (*EGFP*) gene excised from the pBI-EGFP plasmid (Clontech, Palo Alto, CA). Expression of the PA-I lectin was detected by fluorescence microscopy and fluorimetry of this reporter strain as previously described (21).

Dynamic fluorimetry. Caco-2 cells were grown to confluence on collagen-coated 96-well fluorimetry plates (Becton Dickinson Labware, Bedford, MA) and maintained in a 37°C incubator with 5% CO_2 and 21% O_2. The day before experiments, media were removed and replaced with 150 μl of antibiotic-free media. Three experimental conditions were created using a modification of the methods previously described by Xu et al. (23). In control conditions, Caco-2 cells were maintained in a 5% CO_2-21% O_2 incubator for 2 h. Hypoxic conditions were achieved by placing Caco-2 cells in a humidified hypoxia chamber at 37°C with 5% CO-95% N_2 for 2 h. Measured O_2 in the chambers varied between 0.1 and 0.3%. To simulate a reperfusion or reoxygenation state (normoxic recovery), after 2 h of Caco-2 cell hypoxia, hypoxic media were completely replaced with fresh, normoxic HDMEM media, and the cells were allowed to recover under normoxia (37°C, 5% CO_2-21% O_2) for 2 h before bacterial inoculation. The fluorescent reporter strain PA27853/PLL-EGFP was next added to the three groups of Caco-2 cells. Bacteria were cultured overnight in Luria-Bertani broth containing 20 μg/ml gentamicin at 37°C under shaking conditions. After ~12 h of growth, 50 μl of the bacterial suspension were added to the 96-well plates of Caco-2 cells. Care was taken to ensure that all bacterial samples were cultured for identical periods of time and that wells contained equal cell densities. Fluorescence was tracked immediately following bacterial inoculation and then hourly thereafter up to 3 h using a 96-well microplate fluorimeter (Synergy HT, Biotek, Winooski, VT). Plates were maintained in standard incubators at 37°C with 5% CO_2-21% O_2 between all measurements. Fluorescence values were calculated as follows: $\%\text{control} = 100 \times (RFUx_{t=n} - RFUx_{t=0})/(RFUc_{t=n} - RFUc_{t=0})$, where RFUx refers to the hypoxic or normoxic recovery groups and RFUc refers to the control at *time n*.

Exposure of bacteria to filtered media from Caco-2 cells and potential PA-I-inducing candidate molecules. In this set of experiments, reiterative conditions of control, hypoxia, and normoxic recovery (i.e., reperfusion/reoxygenation) were created in 96-well plates containing confluent Caco-2 cells. Media from all wells were then collected and passed through a 0.22-μm filter and stored on ice. Ninety-six-well fluorimetry plates without Caco-2 cells (Costar 3631, Corning, Corning, NY) were then prepared by adding a 20-μl bacterial suspension containing overnight growing cultures of PA27853/PLL-EGFP. Media from the three experimental groups were then added to the wells, and fluorescence was assessed over 5 h, with plates maintained at 37°C with continuous orbital shaking (100 rpm) between measurements. To screen for potential PA-I-inducing compounds that might be present in the media of hypoxic Caco-2 cell media, purified adenosine, D-lactate, and L-lactate (Sigma-Aldrich, St. Louis, MO) were added to wells containing HDMEM across a range of physiologically relevant dosages, which were then tested as described above.

Fluorescent microscopy. To visually correlate results from the above experiments to the spatiotermporal effects of PA27853/PLL-EGFP on hypoxic Caco-2 cells, cells were grown to confluence on Bioptechs dishes (Bioptechs, Butler, PA) and exposed to 2 h of hypoxia followed by inoculation with PA27853/PLL-EGFP. Experiments were performed on a 37°C microscopy stage and visualized using an inverted fluorescence microscope (Axiovert 100, Carl Zeiss, Thornwood, NY). Z-stacks were collected every 30 min for 3 h. Images were analyzed for bacterial distribution using ImageJ graphics analysis software (Version 1.31, National Institutes of Health, Bethesda, MD).

Caco-2 cell barrier function during hypoxia and normoxic recovery in the presence of P. aeruginosa or purified PA-I. Caco-2 monolayer transepithelial electrical resistance (TER), a measure of barrier function, was assessed using agar bridges and Ag-AgCl-calomel electrodes and a voltage clamp (University of Iowa Bioengineering, Iowa City, IA) as previously described (17). TER was calculated using Ohm's law. Fluid resistance was subtracted from all values (17). Two microliters of overnight cultures of PA27853 normalized to cell density or 50 μg of purified PA-I (Sigma-Aldrich) were added to the apical chamber of the Caco-2 cell transwells following exposure to hypoxia and normoxic recovery as detailed above. Caco-2 cell TER was assessed every hour, and cells were maintained at 37°C with 5% CO_2-21% O_2 throughout the experiment. To determine the effect of PA27853 on the barrier function of Caco-2 cells under conditions of sustained hypoxia, reiterative experiments were performed under continuous hypoxia (37°C, 5% CO_2-95% N_2), in which TER measurements were made every hour for 7 h within the hypoxic chamber using an EVOM resistance measurement apparatus (World Precision Instruments, Sarasota, FL).

Northern blot analysis. In selected experiments, PA-I expression was confirmed using Northern blot analyses as previously described (21).

Statistical analysis. Data were analyzed, and statistical significance was determined using Prism 4.0 (GraphPad Software, San Diego, CA). Statistical significance was defined as $P < 0.05$ by Student's *t*-test or two-way ANOVA, as appropriate.

RESULTS

PA27853/PLL-EGFP P. aeruginosa respond to the environment of Caco-2 cell hypoxia and normoxic recovery with enhanced fluorescence. To determine whether the green fluorescent protein (GFP) reporter strain PA27853/PLL-EGFP would display increased PA-I promoter activity when added to Caco-2 cells exposed to hypoxia (2 h at <0.3% O_2) and normoxic recovery (hypoxia followed by 2 h of recovery in normoxic conditions), reporter strains were added to the media of Caco-2 cells exposed to the two conditions. GFP reporter strains demonstrated significantly more PA-I promoter activity,

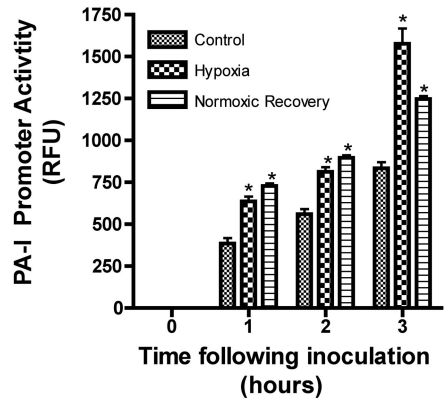

Fig. 1. Enhancement of PA-I expression, as measured by bacterial fluorescence, in the presence of Caco-2 cells exposed to hypoxia or normoxic recovery. Green fluorescent protein (GFP) reporter strain PA27853/PLL-EGFP was coincubated with Caco-2 monolayers in 96-well fluorimetry plates, following exposure of the epithelia to hypoxia (2 h at <0.3% O_2) and normoxic recovery (hypoxia followed by 2 h of recovery in normoxic conditions). Although normoxic Caco-2 cells induced a mild increase in fluorescence, GFP reporter strains demonstrated significantly higher PA-I promoter activity within 1 h of incubation with Caco-2 cells exposed to either hypoxia or normoxic recovery. Data normalized to baseline measurements at *time 0* (*$P <$ 0.001). RFU, relative fluorescence units.

as measured by fluorescence, within 1 h of incubation with Caco-2 cells exposed to either hypoxia or normoxic recovery (Fig. 1). The media pH in all experimental conditions was measured at all time points and demonstrated no significant difference among control, hypoxia, and normoxic recovery groups because all media were buffered (data not shown). However, to show that the pH of media did not influence fluorescence in PA27853/PLL-EGFP, strains were incubated in media at pH 6.5, 7.4, and 7.7. The percent change in fluorescence was not different among groups (6.5 = 106 ± 10, 7.4 = 100 ± 12, 7.7 = 112 ± 12; P = not significant). Similarly, to rule out an effect of hypercarbia or hypoxia alone on PA-I promoter activity in our reporter strains, strains were subjected to hypoxia (0.1% for 2 h) and hypercarbia (80% CO_2 for 2 h). No difference in fluorescence was observed between groups (data not shown). Taken together, these findings demonstrate that media from Caco-2 cells exposed to hypoxia with or without normoxic recovery activate PA-I promoter activity.

Fluorescence imaging of GFP reporter strains in the Caco-2 cell environment. To determine whether epithelial cell contact contributes to the expression of GFP in our PA-I reporter strain, Caco-2 cells were imaged by fluorescent microscopy following exposure to hypoxia and apical inoculation with PA27853/PLL-EGFP. Fluorescence imaging demonstrated that PA27853/PLL-EGFP exposed to hypoxic Caco-2 monolayers appeared markedly more fluorescent than bacteria exposed to normoxic monolayers at the 120-min time point (Fig. 2). Multiple images of the bacterial/Caco-2 cell coculture demonstrated that more bacteria were located near or within the plane of the cell monolayers exposed to hypoxia than in nonhypoxic cells. Quantitative analysis of multiple microscopy images revealed an average of 658 ± 78 bacteria/high-powered field at the level of the surface of hypoxic epithelia, whereas no bacteria were seen in plane-matched controls ($P < 0.001$).

PA27853/PLL-EGFP reporter strains respond to a paracrine factor present in media from Caco-2 cells exposed to hypoxia and normoxic recovery. To determine whether soluble compounds released into the media in response to Caco-2 cell hypoxia are capable of activating PA-I expression independent of bacterial contact with the epithelium, we tested the ability of media from hypoxic Caco-2 cell cultures to enhance fluorescence in our reporter strain. PA27853/PLL-EGFP bacteria exposed to filtered media from Caco-2 cells exposed to hypoxia and normoxic recovery developed a significant enhancement of fluorescence that appeared greatest at the 5-h time point (Fig. 3; control: 3.7% ± SD 3.9; hypoxia: l2.6% ± SD 5.8; normoxic recovery: 13.1% ± SD 3.9; $P < 0.001$ by 2-way repeated measures ANOVA). Results were confirmed by Northern blot analysis (Fig. 3). To determine whether this paracrine factor was isolated to the apical or basolateral compartments, we performed reiterative experiments in which isolated media from the basolateral and apical compartments of hypoxic monolayers, as well as mixtures of apical and basolateral media, were added to wells containing the GFP-PA-I reporter strain PA27853/PLL-EFGP. Only those bacteria exposed to hypoxic media from the apical chamber or hypoxic mixed media showed a statistically significant increase over controls (Fig. 4; $P < 0.05$).

Adenosine alone induces PA-I expression in P. aeruginosa. To determine whether candidate compounds specifically released by hypoxic Caco-2 cells could induce the expression of PA-I, we tested the effect of D-lactate, L-lactate, and adenosine in our GFP-PA-I reporter strains. D- and L-lactate had no effect on PA-I promoter activity (data not shown); however, PLL/

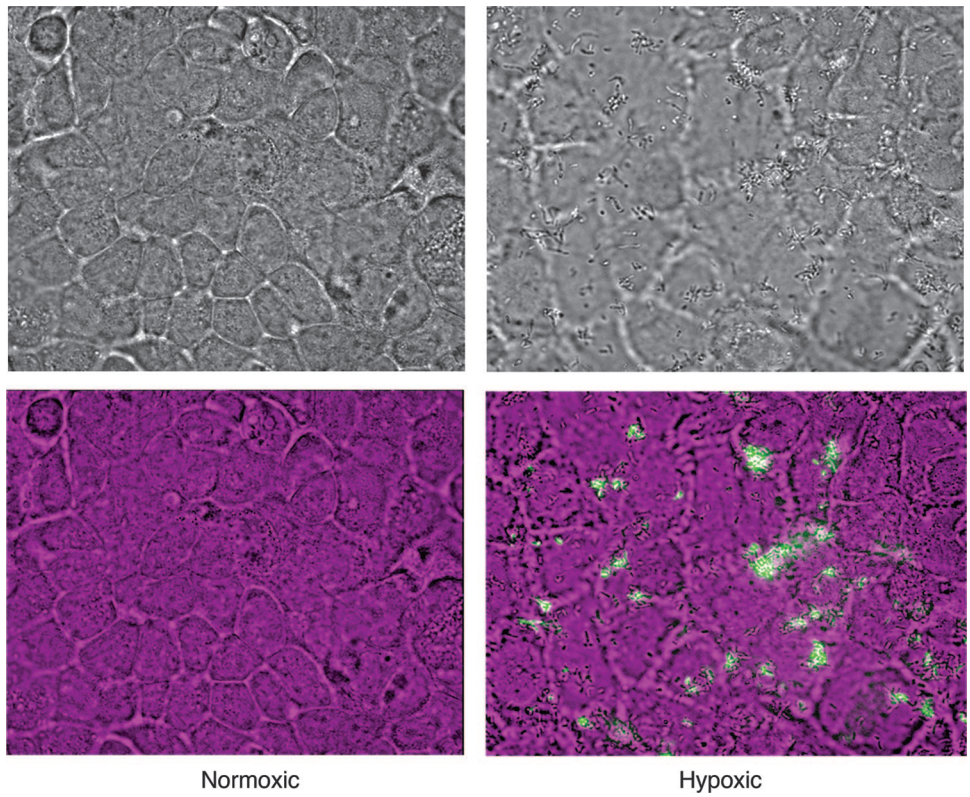

Fig. 2. PA-I promoter activity in the presence of hypoxic Caco-2 cells. *P. aeruginosa* PA-I reporter strains exposed to Caco-2 cells following 90 min of normoxic recovery from hypoxia show enhanced fluorescence vs. controls and increased numbers of bacteria in the plane of the epithelial cells, as demonstrated by fluorescence microscopy. *Top*: transmitted light images. *Bottom*: pseudocolor fluorescence images.

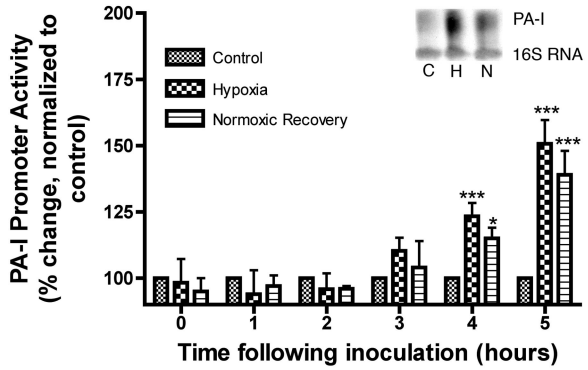

Fig. 3. Enhancement of PA-I promoter activity when *Pseudomonas* is exposed to filtered media from hypoxic and normoxic recovery variants. Filtered media from Caco-2 cells exposed to hypoxia and normoxic recovery were inoculated with the PA-I reporter strain and developed a significant enhancement of fluorescence over controls, although at later time points than those demonstrated when bacteria were able to directly contact epithelial monolayers (*$P <$ 0.05, ***$P < 0.001$, data normalized to control). Results were confirmed by Northern blot analysis for expression of PA-I mRNA in wild-type *P. aeruginosa*. C, control; H, hypoxia; N, normoxic recovery.

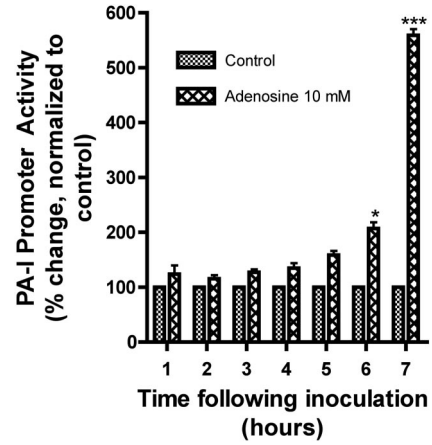

Fig. 5. Adenosine exerts a direct effect on PA-I promoter activity in GFP-PA-I reporter strain PLL/EGFP. Dilutions of adenosine in HDMEM were tested against our GFP-PA-I reporter strain. Although PA-I promoter activity was enhanced in response to 10 mM of adenosine (*$P < 0.05$, ***$P < 0.001$), it required at least 6 h of exposure to the compound, much longer than that observed with hypoxic media alone.

PA27853 responded with enhanced fluorescence to 10 mM adenosine (Fig. 5), raising the possibility that adenosine released by hypoxic Caco-2 cells could be the putative mediator of the increased PA-I response observed in the above studies. However, the time required for upregulation of PA-I expression was longer than that observed in response to hypoxic cell media, suggesting that other factors may be involved in the signaling pathway.

Caco-2 cells exposed to hypoxia and normoxic recovery resist the barrier-dysregulating effect of purified PA-I. To determine whether conditions of hypoxia and normoxic recovery enhance or attenuate the barrier-dysregulating properties of PA27853 against Caco-2 cells, TER was measured in Caco-2 cells apically inoculated with either PA27853 (Fig. 6) or purified PA-I (Fig. 7) following exposure to hypoxia and normoxic recovery. Despite the ability of media from hypoxic and reoxygenated Caco-2 cells to increase the expression of PA-I in *P. aeruginosa*, the TER of Caco-2 cells exposed to these conditions were unchanged in response to a *P. aeruginosa* challenge. However, hypoxic Caco-2 cells apically inoculated with purified PA-I exhibited an attenuated drop in TER compared with normoxic cells (Fig. 7; $P < 0.05$).

Caco-2 cells exposed to sustained hypoxia completely resist the barrier dysregulating effect of PA27853. To determine whether Caco-2 cells exposed to sustained hypoxia could resist the barrier-dysregulating effect of PA27853, the TER of Caco-2 cells apically inoculated with PA27853 in an environment of sustained hypoxia was measured. Caco-2 cells maintained TER equal to hypoxic Caco-2 cells without bacteria and completely resisted the predicted decrease in TER at the 7-h time point (Fig. 8). That Caco-2 cells partially resist the

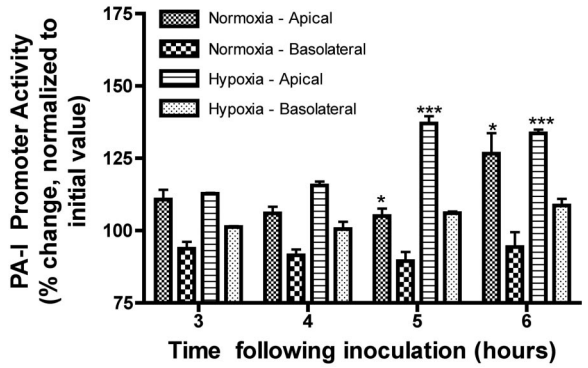

Fig. 4. Media from the apical chamber of Caco-2 cells is necessary and sufficient for induction of PA-I expression. Isolated media from the basolateral and apical compartments of hypoxic monolayers were added to wells containing the PA-I reporter strain. Only bacteria exposed to apical chamber media displayed an increase in PA-I expression. Hypoxic apical chamber media induced PA-I to a greater degree and at earlier time points than normoxic apical chamber media (*$P < 0.05$, ***$P < 0.001$).

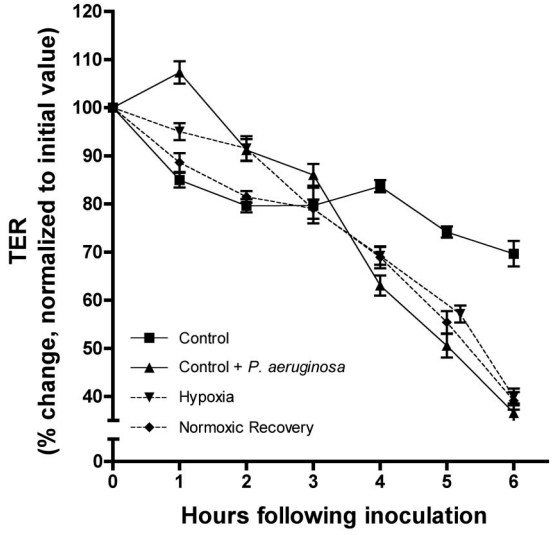

Fig. 6. Change in TER of Caco-2 cells apically inoculated with *P. aeruginosa* does not vary significantly between hypoxic or normoxic recovery cells and normoxic cells. Transepithelial electrical resistance (TER) was measured in Caco-2 cells apically inoculated with PA27853 following exposure to hypoxia and normoxic recovery. Despite the ability of media from hypoxic and reoxygenated Caco-2 cells to increase the expression of PA-I in *P. aeruginosa*, the TER of Caco-2 cells exposed to these conditions were unchanged in response to a *P. aeruginosa* challenge. Data normalized to control.

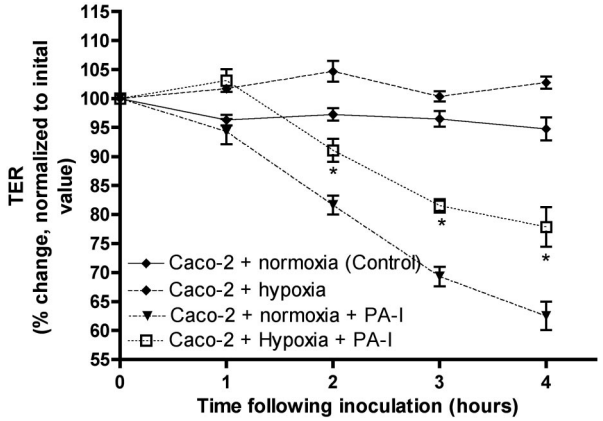

Fig. 7. Hypoxic Caco-2 cells resist the barrier-dysregulating effects of purified PA-I. TER was measured in Caco-2 cells apically inoculated with purified PA-I following exposure to hypoxia. Hypoxic Caco-2 cells apically inoculated with purified PA-I exhibited an attenuated drop in TER compared with normoxic controls. (*$P < 0.05$).

barrier-dysregulating effect of strains of PA27853 despite increased PA-I expression could be explained by previous observations suggesting that epithelial cells normally respond to hypoxia with an enhancement of local mucosal defense proteins and barrier function (5).

Soluble factors present in the media of hypoxic Caco-2 cells induce increased barrier resistance in normoxic cells. To determine whether the normoxic Caco-2 cells could be induced to increase their resistance to barrier dysregulation by *P. aeruginosa* through signals present in hypoxic cell media, we exchanged the apical and basolateral media of normoxic Caco-2 cells with filtered media from the apical and basolateral compartments of hypoxic Caco-2 cells and tested the barrier function of these cells when apically inoculated with *P. aeruginosa*. Normoxic Caco-2 cells exposed to media from hypoxic

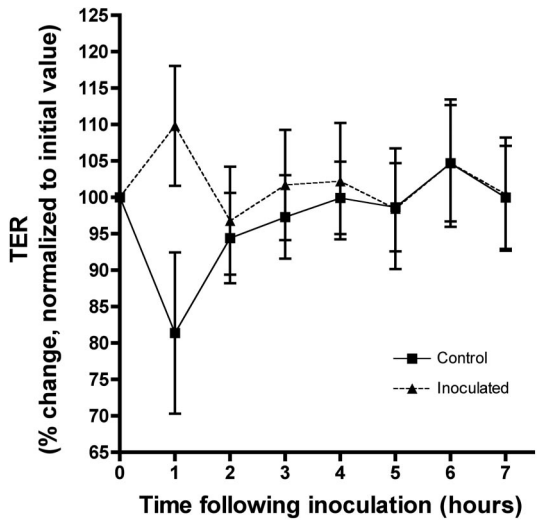

Fig. 8. Caco-2 cells maintained under hypoxic conditions completely resist the barrier dysregulating effects of apically inoculated *P. aeruginosa*. TER of Caco-2 cells apically inoculated with wild-type *P. aeruginosa* in an environment of sustained hypoxia was measured. Caco-2 cells maintained TER equal to hypoxic Caco-2 cells without bacteria and completely resisted the predicted decreased in TER (control = sustained hypoxia; inoculate = sustained hypoxia and inoculation with PA27853). Data normalized to initial value.

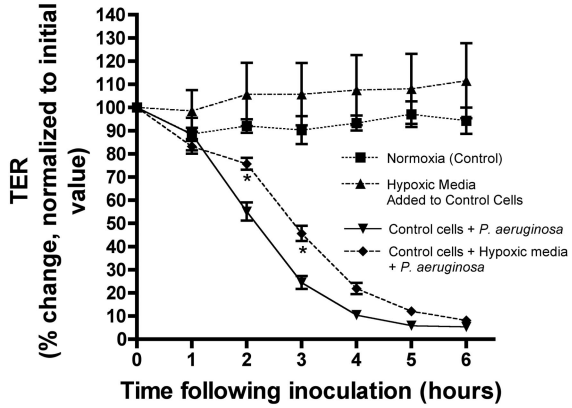

Fig. 9. Media from hypoxic Caco-2 cells transferred to untreated Caco-2 cells attenuates the barrier-dyregulating effect of *P. aeruginosa*. Media of normoxic Caco-2 cells were exchanged with media from hypoxic Caco-2 cells. Epithelial monolayers were then apically inoculated with *P. aeruginosa*, and barrier function was measured. Normoxic Caco-2 cells exposed to media from hypoxic epithelia displayed a prolonged resistance to barrier dysregulation induced by *P. aeruginosa*, suggesting that normoxic epithelia may be activated to enhance their barrier function in the presence of soluble mediators produced during hypoxia. (*$P < 0.05$).

epithelia displayed a prolonged resistance to barrier dysregulation induced by *P. aeruginosa* (Fig. 9), suggesting that normoxic epithelia may be activated to enhance their barrier function in the presence of soluble mediators produced during hypoxia.

DISCUSSION

Although *P. aeruginosa* is not considered to be an intestinal pathogen in the classic sense, it induces one of the most rapid and profound decreases in intestinal epithelial TER of any bacteria reported to date. We have previously reported, in both Caco-2 and T-84 cells, that *P. aeruginosa* (PA27853) can induce an 80% decrease in TER within 4 h following its apical inoculation (9). If defined by this criterion alone, *P. aeruginosa* is among the most pathogenic organisms to the intestinal epithelium yet described. The observation that as many as 5% of the normal population harbor this pathogen within their intestinal tracts (6), coupled with our animal studies demonstrating that control mice do not develop any symptoms of infection following the direct introduction of large quantities of *P. aerugionsa* into the cecum (9), suggest that this organism behaves like a classic opportunist, switching virulence genes on and off in response to selected environmental cues. Although it is well established that environmental cues such as pH, redox state, and nutrient composition can activate virulence gene expression in bacteria through a variety of membrane-bound biosensor kinases (24), there are no previous reports suggesting that bacterial signaling compounds are released by host cells following physiological or ischemic stress. From the standpoint of the evolutionary fitness of the microbe, however, it is logical that a pathogen might recognize the biochemistry of host cell stress, because possessing a system that recognizes host susceptibility would allow for a more accurate assessment of the costs versus benefits of host invasion. Yet, whereas it is well established that intestinal pathogens can communicate directly with the cells to which they adhere, that such a molecular dialogue might be bidirectional is poorly described (7, 15).

To demonstrate that bacteria sense and respond directly to host cells, we used the PA-I lectin/adhesin of *P. aeruginosa* as a reporter gene. The PA-I lectin is under tight regulatory control of two key systems of virulence gene regulation in *P. aeruginosa*: the quorum-sensing signaling system and the alternative sigma factor RpoS. The quorum-sensing signaling system and RpoS are interconnected systems of virulence gene regulation in *P. aeruginosa* that control the expression of hundreds of virulence genes in this pathogen. Because PA-I expression is dependent on the function of both quorum sensing and Rpos, it serves as a relevant biological readout for generalized virulence gene activation in *P. aeruginosa* (19, 20). The finding that soluble elements of intestinal epithelial cells and, in particular, adenosine can activate PA-I expression, suggests that specific host cell-derived compounds may be released that signal colonizing pathogens such as *P. aeruginosa* to a weak and susceptible host. That adenosine alone can activate PA-I expression is an important finding given that adenosine is released and can accumulate in the extracellular milieu of hypoxic tissues at high concentrations. During active intestinal inflammation, 5′-AMP derived from migrating polymorphonuclear leukocytes is converted to adenosine by the apical surface epithelium of the intestine. Strohmeier et al. (14) have demonstrated that under normal conditions, the human intestinal epithelial cell line T-84 can convert substantial amounts of 5′-AMP that accumulate to as much as 5 mM adenosine in the apical media within 30 min. Although in the present study, activation of PA-I promoter activity in *P. aeruginosa* required what appeared to be an unphysiological dose of adenosine, the precise concentration of adenosine to which *P. aeruginosa* might be exposed within the intestinal tract during prolonged hypoxia and reoxygenation is unknown. In addition, adenosine exposure required 6 h before PA-I promoter activity was observed, whereas with hypoxic media PA-I promoter activity was observed at 4 h. As a matter of speculation, an opportunistic organism like *P. aeruginosa* may require an inordinately potent and prolonged host-derived signal for it to invest the resources and energy required to mount a toxic offensive against the intestinal epithelium. Under such circumstances, *P. aeruginosa* might "sense" that the host on which its survival depends is subjected to an extreme degree of inflammation and vulnerability and hence represents a liability to its survival.

Given that PA-I expression was increased in response to Caco-2 cell hypoxia and normoxic recovery, we expected to see a more profound decrease in TER when *P. aerugionsa* was apically inoculated onto Caco-2 cells exposed to these conditions. That enhanced PA-I expression in *P. aeruginosa* did not decrease Caco-2 cell TER during hypoxia could be explained by the enhancing effect of hypoxia itself on Caco-2 cell barrier function. This possibility is supported by the finding that hypoxic media transferred to normoxic Caco-2 cells enhanced their resistance to *P. aeruginosa* (Fig. 5). This notion is further supported by the finding that hypoxic Caco-2 cells resist the barrier-dysregulating property of purified PA-I, again suggesting that hypoxia enhanced epithelial barrier function to the barrier-dysregulating effects of the PA-I protein of *P. aeruginosa*. These findings are also in agreement with the known enhancing effect of hypoxia on intestinal epithelial barrier function (8). Furuta and colleagues (5) have demonstrated that exposure of Caco-2 cells to hypoxia increases the expression of both mucin and trefoil peptides, and they have also observed TER to be preserved or even increased in Caco-2 cells during hypoxia. This response makes physiological sense given that under such circumstances, the intestinal epithelial surface will be vulnerable to a potentially hostile flora. However, during reperfusion, which here we have termed normoxic recovery, Caco-2 cells eventually succumb to the potent barrier-dysregulating effect of *P. aerugionosa*. This is consistent with both clinical and animal studies where the greatest alteration in intestinal permeability and systemic proinflammatory activation occurs during the reperfusion phase following ischemic injury to the intestine (12, 16).

In summary, herein we demonstrate that *P. aeruginosa* is capable of sensing and responding to local elements of host cell stress. Host-derived bacterial signaling compounds appear to be released by intestinal epithelial cells in response to hypoxia and normoxic recovery, which are often present during critical illness and its treatment. Further elucidation of the precise host compounds or signals that are sensed by colonizing nosocomial pathogens, such as *P. aeruginosa*, could lead to a better understanding of how infection continues to complicate the course of the most critically ill patients.

GRANTS

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