Recognition of intestinal epithelial HIF-1α activation by

Pseudomonas aeruginosa

Nachiket J. Patel,1 Olga Zaborina,1 Licheng Wu,1 Yingmin Wang,2 Donald J. Wolfgeher,3 Vesta Valuckaitė,4 Mae J. Ciancio,4 Jonathan E. Kohler,1 Olga Shevchenko,1 Sean P. Colgan,5 Eugene B. Chang,4 Jerrold R. Turner,2* and John C. Alverdy1*

1Departments of Surgery, 2Pathology, 3Proteomics, and 4Medicine, the University of Chicago, Chicago, Illinois; and 5Department of Anesthesia, Critical Care Medicine, Brigham Women’s Hospital, Harvard Medical School, Cambridge, Massachusetts

Submitted 20 June 2006; accepted in final form 8 August 2006

P. aeruginosa. Am J Physiol Gastrointest Liver Physiol 292: G134–G142, 2007. First published August 10, 2006; doi:10.1152/ajpgi.00276.2006.—Human intestinal epithelial cell monolayers (Caco-2) subjected to hypoxia and reoxygenation release soluble factors into the apical medium that activate the virulence of the opportunistic pathogen Pseudomonas aeruginosa to express the potent barrier-dysregulating protein PA-I lectin/adhesin. In this study, we defined the role of hypoxia-inducible factor (HIF)-1α in this response. We tested the ability of medium from Caco-2 cells with forced expression of HIF-1α to increase PA-I expression in P. aeruginosa and found that medium from Caco-2 cells overexpressing HIF-1α increased PA-I expression compared with medium from control cells (P < 0.001, ANOVA). To identify the components responsible for this response, medium was fractionated by molecular weight and subjected to mass spectroscopy, which identified adenosine as the possible mediator. Both adenosine and its immediate downstream metabolite inosine induced PA-I expression in P. aeruginosa in a dose-dependent fashion. Because inosine was not detectable in the medium of Caco-2 cells exposed to hypoxia or reoxygenating HIF-1α, we hypothesized that P. aeruginosa itself might metabolize adenosine to inosine. Using mutant and parental strains of P. aeruginosa, we demonstrated that P. aeruginosa metabolized adenosine to inosine via adenosine deaminase and that the conditioned medium enhanced the extracellular accumulation of inosine. Together, these results provide evidence that P. aeruginosa can recognize and respond to extracellular products of intestinal hypoxia that are released after activation of HIF-1α. The ability of P. aeruginosa to metabolize adenosine to inosine may represent a subversive microbial virulence strategy that deprives the epithelium of the cytoprotective actions of adenosine.

hypoxygen-inducible factor 1α; adenosine; epithelial cells

* J. R. Turner and J. C. Alverdy contributed equally to this work.

Address for reprint requests and other correspondence: J. C. Alverdy, Center for Surgical Infection Research and Therapeutics, Univ. of Chicago, Pritzker School of Medicine, 5841 S. Maryland MC 6090, Chicago, IL 60637 (e-mail: jalverdy@surgery.bsd.uchicago.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
this response because adenosine is released by hypoxic epithelial cells (16, 17), and our group (7) previously identified adenosine as a compound with PA-I-inducing activity.

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with

MATERIALS AND METHODS

Reagents. Adenosine, inosine, hypoxanthine, AMP, ADP, and ATP were purchased from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase obtained from bovine spleen was also purchased from Sigma-Aldrich.

Bacterial and human epithelial cells. Four bacterial strains of Pseudomonas aeruginosa were used in these studies. Strain PA27853 is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with is a prototype clinical strain used in our previous studies and was obtained from the American Type Culture Collection (Manassas, VA) (23). Strain PAO1 is a completely genomically sequenced strain with
medium from Caco-2BBe cells overexpressing HIF-1α to stock solutions of adenosine increased PA-I expression. We therefore tested the ability of inosine, the immediate downstream metabolite of adenosine, to induce PA-I expression. We therefore tested the ability of inosine, the immediate downstream metabolite of adenosine, to induce PA-I expression. A 100 mM stock solution of hypoxanthine was prepared in HDMEM and NaOH to dissolve the hypoxanthine. An equal amount of NaOH was added to the HDMEM alone to control for the possible pH effect. The 100 mM hypoxanthine solution was serially diluted in HDMEM to make 10, 5, 1, and 0.5 mM concentrations and tested in the GFP fluorescence assay.

Determination of inosine concentration in adenosine solution treated with P. aeruginosa. Preliminary experiments indicated no detectable levels of inosine in HIF-1α-overexpressing and hypoxic cell medium as measured by LC-MS-MS (data not shown). We therefore speculated that P. aeruginosa may convert adenosine to inosine. From the annotation of the PAO1 genome sequence (15), PA0148 was found to have a 46% similarity to adenosine deaminase from Escherichia coli and 62% similarity to adenosine deaminase (putative) from Saccharomyces cerevisiae and therefore was characterized as probable adenosine deaminase (http://www.pseudomonas.com/). To test the ability of P. aeruginosa to metabolize adenosine to inosine, the PAO1 strain of P. aeruginosa was cultured overnight in tryptic soy broth (TSB) at 37°C under shaking conditions. The PAO1 strain was then added in a 1-to-10 ratio to TSB and 10 mM adenosine solution. After bacterial inoculation, samples were placed at 37°C and 300 rpm for 6 h. The four groups, 1) TSB, 2) TSB with PAO1, 3) 10 mM adenosine, and 4) 10 mM adenosine with PAO1, were analyzed by LC-MS-MS and thin-layer chromatography (TLC) for adenosine and inosine.

Determination that P. aeruginosa metabolizes adenosine to inosine via its potential adenosine deaminase. To further confirm that adenosine deaminase originating from P. aeruginosa was responsible for the conversion of adenosine to inosine, we used wild-type PAO1 and its derivative strain, ID35276, with an adenosine deaminase (PA0148) knockout mutation (5). The derivative strain was confirmed to be a knockout by PCR analysis (data not shown). PAO1 and its adenosine deaminase mutant strain of P. aeruginosa were cultured overnight in TBS at 37°C under shaking conditions. Mutant and PAO1 strains were added in a 1-to-10 ratio to 10 mM adenosine solution. After bacterial inoculation, samples were placed at 37°C and 300 rpm for 12 h. Samples were then centrifuged at 5,000 rpm for 3 min to separate the bacteria, and the supernatant was collected. The two groups, 1) 10 mM adenosine with PAO1 and 2) 10 mM adenosine with adenosine deaminase mutant strain of P. aeruginosa, were analyzed by TLC for adenosine and inosine.

Does P. aeruginosa change its metabolism of adenosine in the presence of medium from HIF-1α-overexpressing or hypoxic Caco-2BBe cells? Next, we sought to determine whether the dynamics by which P. aeruginosa metabolizes adenosine are altered by the medium from Caco-2BBe cells overexpressing HIF-1α or Caco-2BBe cells subjected to hypoxia, speculating that conditioned medium might itself change the kinetics of P. aeruginosa adenosine deaminase. The PAO1 strain of P. aeruginosa was grown overnight in TBS at 37°C under shaking conditions. The overnight culture was added at 1:10 to samples containing 1) HDMEM (control), 2) medium from parental Caco-2BBe cells, 3) medium from Caco-2BBe cells overexpressing HIF-1α, and 4) medium from parental Caco-2BBe cells subjected to...
2 h of hypoxia. All samples were placed at 37°C under shaking conditions for 8 h. After 8 h, all samples were inoculated with adenosine. Samples were kept at 37°C under shaking conditions and evaluated for adenosine and inosine via TLC at 0, 5, and 15 h.

Western blot analysis. In selected experiments, PA-I expression was confirmed by Western blot analyses as previously described (23).

Statistical analysis. Data analysis and statistical significance calculations were performed with Prism 4.0 (GraphPad Software, San Diego, CA). Statistical significance was defined as \( P < 0.05 \) by two-way ANOVA or Student’s t-test as appropriate.

RESULTS

Soluble factors from the lumen of mouse intestine subjected to segmental ischemia and reperfusion induce PA-I expression in *P. aeruginosa*. As seen in Fig. 1, filtered perfusates from the lumen of mouse intestine subjected to 10 min of vascular occlusion induced the expression of PA-I in *P. aeruginosa* strain PA27853/PLL-EGFP (*\( P < 0.001 \), ANOVA, \( n = 6 \)). This finding was also observed when PA27853/PLL-EGFP was

![Figure 2](image2.png)

**Fig. 2.** A: effect of medium from Caco-2 cells overexpressing hypoxia-inducible factor (HIF)-1α and their parental controls on PA-I expression in *P. aeruginosa*. Results demonstrate a time-dependent increase in PA-I expression in PA27853/PLL-EGFP reporter strains exposed to medium from Caco-2 cells overexpressing HIF-1α (*\( P < 0.01 \), ANOVA). B: results were confirmed by Western blot. C: effect of <3-kDa medium fraction on PA-I expression *P. aeruginosa* from 1) parental Caco-2 cells exposed to normoxia (21% for 2 h), 2) Caco-2 cells overexpressing HIF-1α, and 3) parental Caco-2 cells exposed to 2 h of hypoxia (<0.3% \( \text{O}_2 \)). Results demonstrate a significant increase in PA-I expression in Caco-2 cells exposed to hypoxia and Caco-2 cells overexpressing HIF-1α (*\( P < 0.001 \), ANOVA). D: extracellular concentration of adenosine in Caco-2 cells overexpressing HIF-1α and Caco-2 cells exposed to hypoxia for 2 h (<0.3% \( \text{O}_2 \)). A significant increase in extracellular adenosine concentration is seen in Caco-2 cells overexpressing HIF-1α and in Caco-2 cells exposed to hypoxia compared with control (*\( P < 0.001 \), Student’s t-test).

![Figure 3](image3.png)

**Fig. 3.** A: effect of adenosine on PA-I expression in *P. aeruginosa*. PA-I expression was significantly increased in the PA27853/PLL-EGFP reporter strain in response to 10 mM adenosine (*\( P < 0.001 \), ANOVA). B: increased expression was confirmed by Western blot analysis (**\( P < 0.05 \), Student’s t-test).
exposed to luminal perfusates and after 10 min of reperfusion (Fig. 1). In contrast, blood components did not induce PA-I expression compared with medium from HIF-1α-overexpressing cells alone (*P < 0.001, ANOVA), raising the possibility that the factors responsible for PA-I expression are released from the intestinal tissue itself. To rule out the possibility that the in vivo expression of the PA-I expression was not due to secondary effects of surgical stress such as physicochemical changes in the local microenvironment, stock strain PA27853 and reporter strain PA27853/PLL-EGFP were exposed to ambient hypoxia (0.3% O2), pH changes (6–8), and 80% CO2. None of these conditions induced PA-I expression (data not shown).

Medium from Caco-2BBe cells overexpressing HIF-1α induces PA-I expression in P. aeruginosa. The PA27853/PLL-EGFP reporter strain of P. aeruginosa exposed to medium from HIF-1α-overexpressing cells demonstrated significant time-dependent induction of PA-I as measured by fluorescence (**P < 0.05, Student’s t-test, n = 3) (Fig. 2A). Results were confirmed by Western blot analysis (P < 0.05, Student’s t-test, n = 3) (Fig. 2B).

Medium fractions <3 kDa induce PA-I expression in P. aeruginosa. To identify specific molecular mass fractions from the medium of Caco-2BBe cells that induce PA-I expression, media from parental Caco-2BBe cells, Caco-2BBe cells exposed to hypoxia (2 h <0.3% O2), and Caco-2BBe cells with forced expression of HIF-1α was fractionated into four molecular mass fractions and tested for their ability to induce PA-I expression by the GFP fluorescence assay. Results demonstrated that medium fractions with a molecular mass of <3 kDa in both HIF-1α-overexpressing and hypoxic cell media significantly induced PA-I expression (P < 0.001, ANOVA, n = 6) (Fig. 2C). The remaining fractions had no effect on PA-I expression (data not shown).

Fig. 4. A: metabolites and precursors of adenosine and their rate-limiting enzymes. B: effect of medium from Caco-2 cells overexpressing HIF-1α treated with adenosine deaminase (Ado Deam) to deplete samples of adenosine. Treatment of samples with adenosine deaminase resulted in a significant increase in PA-I expression compared with medium from HIF-1α-overexpressing cells alone (*P < 0.001, ANOVA). C: effect of adenosine plus adenosine deaminase on PA-I expression in P. aeruginosa. Similar to results in B, addition of adenosine deaminase to samples with adenosine induced an increase in PA-I expression greater than that with adenosine alone (**P < 0.05, ANOVA), raising the possibility that metabolism of adenosine to inosine induces PA-I expression. D: effect of inosine on PA-I expression. Exposure of P. aeruginosa to inosine resulted in a dose-dependent increase PA-I expression at a concentration 10-fold less than adenosine (*P < 0.001, ANOVA).

Fig. 5. Effect of hypoxanthine, the next downstream metabolite of inosine, on PA-I expression in P. aeruginosa. PA-I expression was significantly increased in the PA27853/PLL-EGFP reporter strain in response to a 10 mM hypoxanthine (**P < 0.01, ANOVA).
Depletion of adenosine in the cell medium from Caco-2_BBe cells overexpressing HIF-1α with adenosine deaminase significantly increases PA-I expression. To determine whether adenosine was the putative component within the medium of HIF-1α-overexpressing cells that induces the expression of PA-I, the enzyme adenosine deaminase was added to deplete the medium of adenosine. Remarkably, the addition of adenosine deaminase to the medium resulted in a significant increase in PA-I expression (P < 0.001, ANOVA, n = 6) (Fig. 4B). Adenosine alone treated with adenosine deaminase also resulted in a significant increase in PA-I expression (P < 0.05, ANOVA, n = 6) (Fig. 4C), raising the possibility that inosine, the immediate downstream metabolite of adenosine, might play a role in PA-I expression. We next determined whether inosine alone could induce PA-I expression by testing varying concentrations of inosine in our GFP fluorescence assay and found that inosine significantly induced PA-I expression at 1 mM (P < 0.001, ANOVA, n = 12), a concentration of 10-fold less than that of adenosine (Fig. 4D). Next, we determined whether the next downstream metabolite of inosine, hypoxanthine, could also induce PA-I expression using the GFP fluorescence assay. Hypoxanthine significantly induced PA-I expression at 10 mM (P < 0.01, ANOVA, n = 8), the same concentration required for adenosine to induce PA-I (Fig. 5).
was measured in HIF-1α-overexpressing and hypoxic cell media via LC-MS-MS. No detectable levels of inosine were found (data not shown). Therefore we speculated that P. aeruginosa itself might convert adenosine to inosine. When adenosine solutions were inoculated with overnight cultures of wild-type PAO1, significant levels of inosine were detected by LC-MS-MS ($P < 0.001$, Student’s t-test) [lane 1: adenosine; lane 2: inosine; lane 3: P. aeruginosa cultured in the presence of high-glucose DMEM (HDMEM; control); lane 4: P. aeruginosa cultured in the presence of parental control Caco-2 cell media; lane 5: P. aeruginosa cultured in the presence of medium from Caco-2 cells overexpressing HIF-1α; lane 6: P. aeruginosa cultured in the presence of Caco-2 cells exposed to hypoxia].

### DISCUSSION

Data from the present study add to the small but growing body of evidence demonstrating that certain bacteria are fully capable of recognizing and responding to host-derived elements released during physiological stress. Under conditions of physiological stress or immune activation, soluble compounds released by the host, such as epinephrine and IFN-γ, have been shown to activate the virulence of important intestinal bacteria such as E. coli and P. aeruginosa (14, 22). That intestinal bacteria are signaled to upregulate their virulence by host-derived compounds released during physiological stress may have important implications in the pathogenesis by which intestinal bacteria cause sepsis in critically ill patients. This
may be particularly relevant for the human opportunistic pathogen \textit{P. aeruginosa} whose prevalence within the intestinal tract of critically ill patients approaches 50% and whose mere presence in this site is associated with a fourfold increase in mortality (10).

Intestinal ischemia and hypoxia are physiological disturbances that invariably complicate the course of critically ill patients as blood flow is redistributed away from the intestinal tract to more vital organs. As a compensatory response, HIF-1\(\alpha\), a highly conserved global transcriptional regulator, is activated in direct response to both hypoxia and inflammation. As previously reported, hypoxia and HIF-1\(\alpha\) expression result in the extracellular accumulation of the cytoprotective compound adenosine that develops as a result of \textit{J}\' upregulation of 5'-ectonucleosidase (CD73), which accelerates the conversion of AMP to adenosine; 2) downregulation of adenosine deaminase, which prevents adenosine metabolism to inosine; and 3) downregulation of adenosine kinase, which prevents recycling of adenosine back to AMP (6, 17). Upregulation of CD73 with resultant accumulation of adenosine has been shown to be cytoprotective by enhancing tight junctional barrier function via mechanisms that involve adenosine activation of the 28-kDa adenosine receptor (2, 13, 18). We have previously reported that hypoxic intestinal epithelial cells remain resistant to the barrier-dysregulating effect of \textit{P. aeruginosa}, whereas, in the absence of hypoxia, \textit{P. aeruginosa} induces a profound and rapid effect on the barrier function of cultured intestinal epithelial cells (Caco-2BE) via expression of the PA-I lectin (7). However, over time, the medium of hypoxic intestinal epithelial cells directly upregulates the expression of the potent barrier-dysregulating PA-I protein in \textit{P. aeruginosa}, eventually leading to a decrease in transepithelial electrical resistance of cultured intestinal epithelial cell monolayers (7). Taken together, data from the present study, in conjunction with our previous data and data from others, suggest that, during intestinal epithelial hypoxia, eukaryotic cells activate a cytoprotective barrier-enhancing response to invading pathogens in association with HIF-1\(\alpha\) expression and extracellular adenosine release. However, at the same time, prokaryotic cells (\textit{P. aeruginosa}) can intercept and use these signals to develop a countermeasure to this response by expressing potent barrier-dysregulating proteins such as the PA-I lectin.

Data from the present study suggest that \textit{P. aeruginosa} may have developed a system to not only recognize and respond to end products of intestinal epithelial hypoxia but also to metabolize these products into molecules that can participate in bacterial cell-cell communication networks such as QS. The observation in the present study that medium from HIF-1\(\alpha\)-expressing Caco-2 cells or medium from hypoxic Caco-2 cells alters the metabolism of \textit{P. aeruginosa} such that inosine accumulates may provide an example whereby \textit{P. aeruginosa} itself uses eukaryotic signals as its own QS molecule. Whether inosine can act as a surrogate QS molecule in \textit{P. aeruginosa} similar to that described for epinephrine in \textit{E. coli} remains to be clarified (14). The precise mechanisms by which adenosine and inosine activate \textit{P. aeruginosa} to express the QS-dependent virulence factor PA-I will require further studies.

It has been recently shown that apical exposure of Caco-2 cells to \textit{P. aeruginosa} results in HIF-1\(\alpha\) expression (8). When Caco-2 cells were cocultured with \textit{P. aeruginosa}, even during the reoxyenation phase, HIF-1\(\alpha\) protein levels remained elevated, unlike cells exposed to hypoxia and reoxyenation in the absence of \textit{P. aeruginosa}, whereby HIF-1\(\alpha\) expression is rapidly degraded (8). The fact that the HIF-1 response is potentiated by \textit{P. aeruginosa} is interesting, given that results of the present study, and provides further evidence that the molecular dialogue between intestinal pathogens and the intestinal epithelium is bidirectional and highly dynamic. During severe critical illness where intestinal ischemia is often present and where intestinal colonization with \textit{P. aeruginosa} is highly prevalent, the final interplay of this dynamic interaction may be highly predictive of the development of severe sepsis and a systemic inflammatory response (12). The observation that intestinal ischemia is lethal when accompanied by intestinal colonization with \textit{P. aeruginosa} could be explained in part by the findings in the present study that show that the virulence circuitry of this pathogen has evolved to recognize and respond to end products of epithelial hypoxia (24). That the enzyme of \textit{P. aeruginosa} that metabolize adenosine (adenosine deaminase) is upregulated in response to medium from hypoxic epithelial cells also suggests that \textit{P. aeruginosa} may have evolved a very clever virulence tactic to deplete epithelial cells of a major cytoprotective compound, rendering them all the more vulnerable to the effects of this highly feared pathogen. In summary, data from the present study suggest that the coevolution of bacteria and epithelial cells, unique to the intestinal tract environment, may have resulted in a bacterial-epithelial molecular dialogue that is much more complex than previously appreciated.

REFERENCES


