Characterization of an ExoS Type III Translocation-Resistant Cell Line

Elizabeth A. Rucks and Joan C. Olson
***Infect. Immun.* 2005, 73(1):638. DOI: 10.1128/IAI.73.1.638-643.2005.**

INFECTION AND IMMUNITY, Jan. 2005, p. 638–643 Vol. 73, No. 1
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.1.638–643.2005

Characterization of an ExoS Type III Translocation-Resistant Cell Line

Elizabeth A. Rucks and Joan C. Olson*

Department of Microbiology, Immunology, and Cell Biology, Health Sciences Center, West Virginia University, Morgantown, West Virginia

Received 11 July 2004/Returned for modification 4 August 2004/Accepted 9 September 2004

Pseudomonas aeruginosa **ExoS is a type III-secreted type III-secreted, bifunctional protein that causes diverse effects on eukaryotic cell function. The coculture of *P. aeruginosa* strains expressing ExoS with HL-60 myeloid cells revealed the cell line to be resistant to the toxic effects of ExoS. Differentiation of HL-60 cells with phorbol 12-myristate 13-acetate (TPA) rendered the cell line sensitive to ExoS. To understand the cellular basis for the alteration in sensitivity, undifferentiated and TPA-differentiated HL-60 cells were compared for differences in bacterial adherence, type III secretion induction, and ExoS translocation. These comparisons found that ExoS was translocated more efficiently in TPA-differentiated HL-60 cells than in undifferentiated cells. The studies support the ability of eukaryotic cells to influence *P. aeruginosa* TTS at the level of membrane translocation.**

The type III secretion (TTS) process allows the direct translocation of bacterial effector proteins into eukaryotic cells and is associated with the virulence of many gram-negative bacteria (15, 20). Four virulence factors, ExoS, ExoT, ExoU, and ExoY, are known to be translocated into eukaryotic cells by *Pseudomonas aeruginosa* TTS (47, 48). Unlike many gram-negative pathogens, both clinical and environmental *P. aeruginosa* isolates maintain the TTS system, implying that TTS has an integral role in *P. aeruginosa* survival in general (7, 8, 43). Also, consistent with the opportunistic lifestyle of *P. aeruginosa*, normal, healthy epithelial barriers and monolayers remain resistant to *P. aeruginosa* infection and TTS (9, 23, 31). However, when tissue barriers are compromised, *P. aeruginosa* can become a lethal pathogen. The purpose of these studies was to further understand the cellular basis of host-cell resistance to *P. aeruginosa* TTS by using the type III effector, ExoS, as a tool to monitor TTS.

In analysis of human epithelial cells, TTS-translocated ExoS has been found to cause a general inactivation of host cell function, as recognized by effects on cell growth, adherence, morphology, and phagocytic processes (13, 32, 33, 40). ExoS affects eukaryotic cell function through a bifunctional mode of action. The N-terminal region of ExoS includes a GTPase-activating (GAP) activity (17), which can affect eukaryotic cell morphology and phagocytosis (36, 40). The C-terminal region of ExoS includes an ADP-ribosyltransferase (ADPRT) activity that targets multiple, specific cellular proteins, including low-molecular-weight G (LMWG) proteins in the Ras superfamily (3, 10, 12, 19, 30). The ADPRT activity of ExoS is required for the irreversible effects of ExoS on cellular proliferation, readherence, and morphology (11, 32).

This study focuses on the effects of TTS-ExoS on HL-60 myeloid cell function. The HL-60 cell line originated from peripheral blood leukocytes of a patient with promyelocytic leukemia (4). When treated with specific compounds, HL-60 cells can be induced to terminally differentiate into granulocytes or monocytes (5, 29). We found HL-60 cells to be resistant to the toxic effects of ExoS in their undifferentiated form. However, upon differentiation with phorbol 12-myristate 13-acetate (TPA), HL-60 cells became sensitive to ExoS. The identification of a TTS-resistant cell line that can be induced to become TTS sensitive provides a model system for studying eukaryotic cell factors that influence the establishment of a functional *P. aeruginosa* TTS process.

Effects of ExoS on HL-60 cell function. As part of a series of experiments examining the effects of TTS-ExoS on different cell types, HL-60 cells (ATCC CCL 240; American Type Culture Collection, Manassas, Va.) were seeded at 5×10^5 cells/ml 48 h prior to coculture with *P. aeruginosa* strain 388 (21) or the isogenic non-ExoS-producing strain, 388ΔS (25), as previously described (33). Using [^{3}H]thymidine incorporation to measure effects on cellular proliferation (33), exposure to *P. aeruginosa* bacteria for 4 h (multiplicity of infection [MOI], 30:1) was found to cause approximately 45% inhibition of HL-60 cell DNA synthesis. However, no ExoS-specific effect on DNA synthesis was detected (strain 388 caused 45.0% ± 2.0% inhibition, and strain 388ΔS caused 44.7% ± 2.0% inhibition of DNA synthesis). Unlike epithelial cells, HL-60 cells have a round morphology, which precludes the use of cell rounding as a measure of the effects of ExoS on cell morphology. When scanning electron microscopy (SEM) was used to examine whether TTS-ExoS altered HL-60 cell surface structures, as has been previously observed for HT-29 epithelial cells and J774A.1 macrophages (32, 40), no alteration was evident (data not shown). The lack of ExoS-specific effects on DNA synthesis and cell morphology suggested that HL-60 cells were resistant to the effects of TTS-ExoS.

To assess whether sensitivity to ExoS could be altered by treatments that induce HL-60 cell differentiation, cells were terminally differentiated into adherent monocytes by treatment

* Corresponding author. Mailing address: Department of Microbiology, Immunology, and Cell Biology, Robert C. Byrd Health Sciences Center, P.O. Box 9177, West Virginia University, Morgantown, WV 26506-9177. Phone: (304) 293-5843. Fax: (304) 293-7823. E-mail: jolson@hsc.wvu.edu.

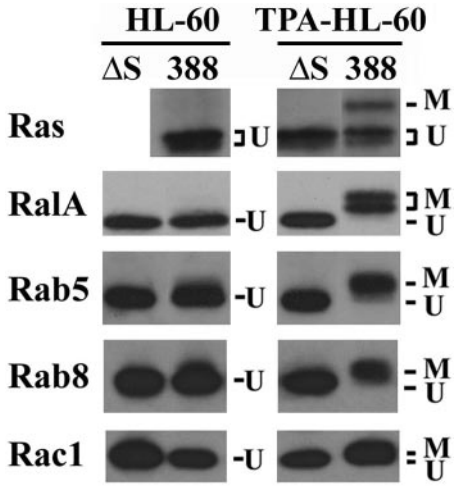

FIG. 1. ExoS ADPRT substrate modification in HL-60 and TPA–HL-60 cells. HL-60 cells were seeded and cultured for 48 h in the presence or absence of 20 ng of TPA/ml, prior to coculture for 4 h with strain 388ΔS (ΔS) or 388 (MOI, 30:1). Bacteria were removed and cells were processed and analyzed for ADP ribosylation of the indicated LMWG proteins based on altered mobility in SDS-PAGE and immunoblot analyses, as previously described (10, 12, 30, 42). RalA, Rab8, and Rac1 antibodies were obtained from BD Transduction Laboratories (San Diego, Calif.). Rab5 antiserum was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, Calif.). Proteins were detected using horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG) (Jackson Immuno Research, West Grove, Pa.) or anti-rabbit IgG (BD Transduction Laboratories) and were visualized by enhanced chemiluminescence (Amersham, Arlington Heights, Ill.). Results are representative of at least three independent experiments. Modified (M) and unmodified (U) proteins are labeled.

with 20 ng of TPA/ml for 48 h (22) and then were cocultured with strain 388 or 388ΔS or no bacteria, as described above. Terminal differentiation of eukaryotic cells halts cellular proliferation, thereby precluding the examination of effects of ExoS on TPA–HL-60 cell proliferation. In SEM analyses, exposure of TPA–HL-60 cells to ExoS-producing strain 388 resulted in a loss of cell surface microvilli compared to that with TPA–HL-60 cells exposed to 388ΔS or no bacteria (data not shown). The effect of strain 388 on cell surface microvilli provided an initial indication that TPA differentiation altered HL-60 cell sensitivity to TTS-ExoS.

TPA-induced alterations in HL-60 cell sensitivity to TTS-ExoS were also recognized at the level of ExoS ADPRT substrate modification. The ADP ribosylation of LMWG proteins by ExoS can be detected based on alterations in protein mobility, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analyses (3, 10, 12, 19, 30). When TPA–HL-60 cells were cocultured with strain 388, 388ΔS, or no bacteria for 4 h and analyzed for ADP ribosylation of LMWG proteins, shifts in the mobility of Ras, RalA, Rab proteins (Rab5 and Rab8 are shown), and Rac1 were apparent (Fig. 1). In comparison, only a slight modification of Rab5 was detected in undifferentiated HL-60 cells cocultured with strain 388. The studies support the notion that TPA differentiation induces HL-60 cell sensitivity to TTS-ExoS, as evident in ExoS ADP ribosylation of cellular substrates and alterations in microvilli structure.

Mechanisms of induction of ExoS sensitivity. Although ExoS is secreted by *P. aeruginosa* into culture supernatants, it is not toxic to eukaryotic cells in the absence of a TTS translocation apparatus (33, 47). Possible mechanisms by which TPA could induce alterations in sensitivity to ExoS include (i) indirect effects on TTS that facilitate bacterial adherence or non-TTS-mediated diffusion of ExoS into cells and (ii) direct effects that alter the induction or efficiency of the TTS translocation process.

(i) Indirect mechanisms. To examine whether TPA treatment induces cell surface alterations that enhance *P. aeruginosa* adherence, rendering HL-60 cells more sensitive to the bacterial contact-mediated TTS process, bacterial association to undifferentiated and TPA–HL-60 cells was examined. In these analyses, cells were cocultured with strain 388 or 388ΔS at a high MOI (100:1) to optimize detection of bacterial association. Bacteria were removed after 4 h, and cells were washed three times with HEPES-buffered saline (10 mM HEPES [pH 7.4], 137 mM NaCl, 4 mM KCl, 11 mM glucose) and lysed in 0.25% TX-100. Lysates were plated to quantify associated bacteria as previously described (42). Bacterial association with undifferentiated HL-60 cells was determined to be 25.8 ± 2.6 bacteria per cell, compared to 8.5 ± 0.8 bacteria per TPA–HL-60 cell. Using the same method to compare the adherence of strain 388 or 388ΔS to eukaryotic cells, no significant difference in bacterial association was detected with the expression of ExoS (data not shown). The results support that TPA treatment did not affect HL-60 membrane properties in a manner that enhanced bacterial association.

Another means by which TPA treatment might indirectly affect ExoS internalization is through alterations in membrane properties that allow the direct diffusion of ExoS into cells. To examine this possibility, TPA–HL-60 cells were cultured with soluble ExoS for 4.5 h in the presence or absence of bacteria. To allow an endogenous form of ExoS to be evaluated in these studies, strain 388 culture supernatant (500 μl), containing 1.1 μg of ExoS in the presence of other *P. aeruginosa*-secreted factors, served as the source of ExoS. In addition to strains 388 and 388ΔS, TTS mutant strains 388-11 (47) and 388ΔPopD (46) (both provided by Dara Frank, Medical College of Wisconsin, Milwaukee, Wis.) were included in these studies to allow ExoS diffusion to be assessed independently of a functional TTS process. Strain 388-11 is a PscC mutant defective in TTS-mediated secretion and translocation, and strain 388ΔPopD is a PopD mutant defective in TTS-mediated translocation but not secretion. Previous studies have shown that in the absence of PscC and PopD, ExoS is unable to exert toxic effects on eukaryotic cell function (14, 32, 46). ExoS ADP ribosylation of cellular substrates was used to detect ExoS internalization. No substrate modification was detected in TPA–HL-60 cells cultured with soluble ExoS in the presence of strain 388ΔS, 388-11, or 388ΔPopD (data not shown). In addition, no enhancement of ExoS substrate modification was detected when soluble ExoS was added to TPA–HL-60 cells cocultured with strain 388. The results support that TPA treatment does not alter HL-60 membrane integrity in a manner that allows ExoS internalization independently of the TTS process.

(ii) Direct mechanisms. Differences in the sensitivity of undifferentiated and TPA–HL-60 cells to ExoS might also relate

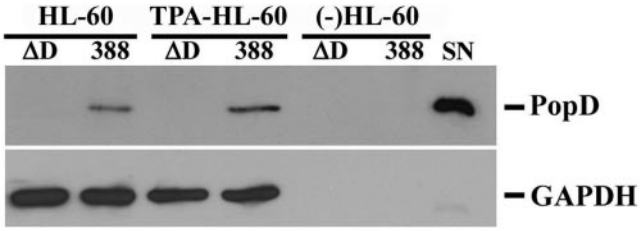

FIG. 2. Analysis of PopD induction by HL-60 and TPA–HL-60 cells. HL-60 cells or TPA–HL-60 cells were cocultured for 4 h with strains 388ΔPopD (ΔD) or 388 (MOI, 30:1). Total PopD production was determined by adding Laemmli sample buffer to tissue culture wells, and samples were resolved by SDS-PAGE and immunoblotted for PopD. Eukaryotic cell growth and protein loading were compared by immunoblotting for the eukaryotic housekeeping protein, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Chemicon International Inc., Temecula, Calif.). (−)HL-60 represents strain 388 incubated in coculture medium for 4 h in the absence of eukaryotic cells. PopD within a TTS-induced bacterial culture supernatant (SN) (21) was included as a positive control. Results are representative of at least three independent experiments.

to alterations in cellular properties that affect host cell induction of the TTS process. To examine whether altered sensitivity to ExoS was occurring at the level of TTS induction, prior to coculture, strains 388 and 388ΔPopD were cultured in a medium that does not allow TTS induction (TSBD [21] supplemented with 5 mM $CaCl_2$). Bacteria were then cocultured with undifferentiated or TPA-differentiated HL-60 cells for 4 h in Hanks balanced salt solution (Sigma, St. Louis, Mo.), which was found to minimize background induction of PopD while maintaining eukaryotic and bacterial cell growth. Total PopD production was examined by adding 4× Laemmli sample buffer (27) to tissue culture wells, which allowed the combined harvest of eukaryotic cells, bacteria, and coculture supernatants. Samples were resolved by SDS-PAGE and were immunoblotted using an anti-PopD antibody (provided by Dara Frank). Cells treated with strain 388ΔPopD, no bacteria, or bacteria cultured in the absence of eukaryotic cells served as negative controls in these experiments. As shown in Fig. 2, the induction of PopD by undifferentiated HL-60 cells closely resembled that of TPA-differentiated HL-60 cells. The results support that undifferentiated HL-60 cells are as effective as TPA–HL-60 cells in inducing TTS components that mediate membrane translocation.

Collectively, these data point to the likelihood that the increased sensitivity of TPA–HL-60 cells to ExoS relates to an increased efficiency of ExoS TTS internalization. To assess the efficiency of ExoS translocation in HL-60 and TPA–HL-60 cells, time course studies were performed, examining ExoS ADPRT activity in cytosolic and TX-100 membrane-soluble fractions after a 3-, 4.5-, or 6-h coculture period. After removal of bacteria, cells were washed, treated with 0.5% trypsin–0.53 mM EDTA (Gibco-BRL, Gaithersburg, Md.) to harvest cells and remove extracellular ExoS, and lysed in cell permeabilization solution (0.2% saponin, 100 mM NaCl, 250 mM sucrose, 5 mM EDTA, and P8340 [Sigma] protease inhibitor cocktail) (42). Cellular lysates were centrifuged at 16,000 × *g* to obtain the cytosolic (supernatant) fraction. The TX-100 membrane-soluble fraction was obtained by treating cellular pellets with 1% Triton X-100, as previously described (42). ExoS ADPRT activity was quantified in an in vitro ADPRT reaction (8, 26) and was related to total cellular protein. In these analyses, ExoS ADPRT activity was found to be five- to sixfold higher in both the cytosolic and TX-100 membrane fractions of TPA–HL-60 cells than that of undifferentiated HL-60 cells (Fig. 3). Additionally, the efficiency of ExoS ADPRT translocation was found to directly correlate with the degree of ExoS ADPRT substrate modification (data not shown). For example, ADP ribosylation of RalA, Rab5, and Rac1 was most efficient in the TX-100 membrane fractions of TPA–HL-60 cells, in association with the highest levels of ExoS ADPRT activity. Conversely, minimal substrate modification (only a slight modification of Rab5) was detected in the TX-100 membrane fractions of undifferentiated HL-60 cells, correlating with the limited ExoS ADPRT activity detected in these samples.

To further assess ExoS internalization, undifferentiated HL-60 cells and TPA–HL-60 cells were cocultured with *P. aeruginosa* strain PA103ΔUT, expressing pUCP plasmid (44)-encoded ExoS tagged with a hemagglutinin epitope (ExoS-HA) (34), provided by Joseph Barbieri (Medical College of

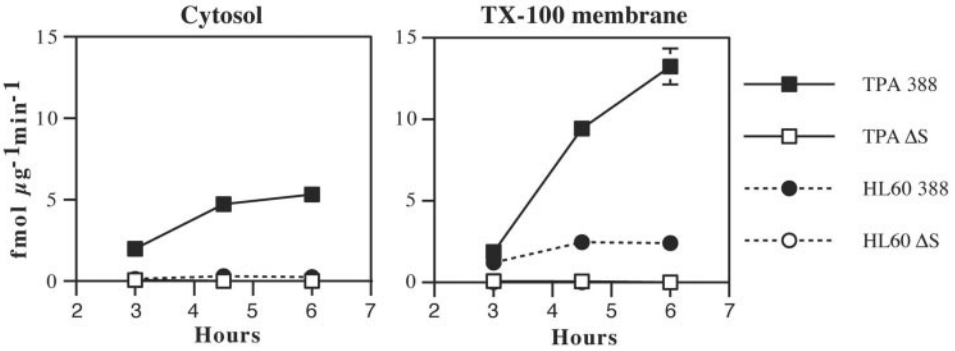

FIG. 3. Time-course analysis of TTS translocation of ExoS ADPRT activity. HL-60 and TPA–HL-60 (TPA) cells were cocultured with strain 388 or 388ΔS (ΔS) for 3, 4.5, or 6 h at a lower MOI (<10:1) to facilitate detection of differences in the efficiency of ExoS internalization. Bacteria were removed, cells were washed, and cytosolic and TX-100 (membrane-soluble) fractions were obtained. ADPRT activity was assayed using soybean trypsin inhibitor as an artificial substrate (26) and is reported as femtomoles per minute of ADP-ribose incorporated into substrate per microgram of cellular protein. Cellular protein was determined using the Sigma Diagnostics Lowry-based protein assay. The means and standard deviations of assays performed in duplicate from a single experiment are shown. The results are representative of three independent experiments.

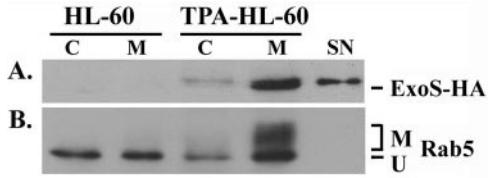

FIG. 4. TTS-mediated internalization of ExoS protein in HL-60 cells. HL-60 and TPA–HL-60 were cocultured for 4 h with strain PA103ΔUT expressing plasmid-encoded ExoS-HA (MOI, 30:1). Cytosolic (C) and membrane (M) fractions of HL-60 or TPA–HL-60 cells were obtained by digitonin extraction and centrifugation. Fractions were resolved by SDS-PAGE and were immunoblotted with (A) anti-HA antibody (Upstate, Waltham, Mass.) to detect ExoS-HA or (B) anti-Rab5 antibody to assess ExoS ADPRT substrate modification. Modified (M) and unmodified (U) Rab5 proteins are labeled. ExoS-HA, obtained from a TTS induced bacterial culture supernatant (SN), was included as a positive control.

Wisconsin, Milwaukee, Wis.). Strain PA103ΔUT is a derivative of strain PA103 that lacks functional *exoU* and *exoT* genes (46), and it was used to translocate ExoS into eukaryotic cells in the absence of other known TTS effectors. We have previously found it difficult to detect TTS-ExoS protein in eukaryotic cells by Western blot analysis (11). However, the addition of the HA tag to ExoS allowed detection of TTS-ExoS in eukaryotic cell extracts, without reported effects on ExoS function (34). Consistent with studies quantifying the internalization of ExoS ADPRT activity, ExoS-HA was found predominately in the membrane fraction of TPA–HL-60 cells (Fig. 4A), where two bands were evident (not obvious in Fig. 4A), presumably ExoS and processed ExoS (35). Only a higher mass, possibly an auto-ADP-ribosylated form of ExoS, was detected in the cytosolic fraction of TPA–HL-60 cells (39). ExoS-HA was not detected in either the membrane or cytosolic fractions of undifferentiated HL-60 cells. As shown in Fig. 4B, levels of internalized ExoS correlate with the efficiency of Rab5 ADP ribosylation in the respective fractions. These results are consistent with the resistance of HL-60 cells to ExoS relating to less efficient ExoS internalization and the notion that TPA differentiation induces cellular changes that allow the TTS apparatus to form and translocate ExoS.

It has become evident from studies of different cell lines that the host cell can influence the effectiveness of *P. aeruginosa* TTS. Polarized confluent epithelial monolayers remain resistant to *P. aeruginosa* infection and TTS but become sensitive to both when the epithelial layer is compromised (9, 31, 38). The host cell can also influence the toxicity and substrate targeting of TTS effectors, as evident in cellular differences in ExoS toxicity in association with alterations in LMWG protein substrate modification patterns (40, 42). In studying the effects of ExoS on promyelocytic HL-60 cell function, we identified another cell line that was resistant to *P. aeruginosa* TTS. A notable finding relative to HL-60 cells is that sensitivity to TTS-ExoS can be induced by treatment with the phorbol ester, TPA.

Several mechanisms were examined to explain the increased sensitivity to TTS-ExoS of TPA–HL-60 cells compared to that of undifferentiated HL-60 cells. These included (i) alterations in the efficiency of bacterial adherence to HL-60 cells, which is required for TTS-translocation, (ii) alterations in host cell induction of TTS proteins, and (iii) TPA-induced membrane alterations that allow the direct diffusion of ExoS independently of the TTS apparatus. None of these mechanisms accounted for the differences in the effects of ExoS on cell function and ADP ribosylation of substrates in HL-60 and TPA–HL-60 cells. Another possible mechanism that might explain the decreased sensitivity of HL-60 cells to TTS-ExoS is an increased rate of intracellular ExoS degradation or inactivation. In examining this possibility, no enhancement of TTS-ExoS ADPRT substrate modification was detected in either HL-60 cells or TPA–HL-60 cells following treatment with the irreversible proteosome inhibitor MG132 (Sigma) or protease inhibitors (data not shown). In addition, in in vitro ExoS ADPRT reactions, purified ExoS was found to efficiently ADP-ribosylate LMWG proteins in HL-60 cell lysates, as previously reported (3), and ExoS was not degraded or its activity inhibited by endogenous HL-60 proteins (data not shown). Alternatively, in support of HL-60 cell resistance to TTS-ExoS occurring at the level of membrane translocation, time course analyses revealed only a minimal increase in ExoS ADPRT activity in the membrane fraction of HL-60 cells with time, and no activity was detected within the cytosol. In comparison, a linear increase in ExoS ADPRT activity was detected in the membrane fraction of TPA–HL-60 cells with time, and detectable but smaller amounts translocated into the cytosol. Similar results were obtained from tracking bacterial translocation of ExoS-HA. Together these results support that differences in the sensitivity of HL-60 and TPA–HL-60 cells to ExoS relate to cellular influences on the *P. aeruginosa* TTS translocation process rather than cellular properties that affect membrane permeability, bacterial adherence, TTS induction, or ExoS degradation.

While these studies provided evidence that TPA differentiation of HL-60 cells altered cell line responsiveness to TTS-translocated ExoS, the mechanism for this transition remains unknown. TPA induces HL-60 cells to differentiate into mature macrophages by activating protein kinase C (PKC) signaling pathways (45) and increasing protease activity (2). A hallmark of TPA differentiation is the ability of the cells to adhere to one another and to tissue culture substrate. This implicates the potential for TPA to induce alterations in HL-60 cell surface-interactive components that might affect TTS responsiveness. Several receptors for *P. aeruginosa* have been identified, including the cystic fibrosis transmembrane regulator (CFTR) (16, 37), asialo GM1 (1, 6, 18, 24), and β_1 integrins (38, 41). However, the identity of the eukaryotic receptor that is required for *P. aeruginosa* TTS-host cell interaction remains unknown. Interestingly, bacterial association with TPA–HL-60 cells was less efficient than with undifferentiated HL-60 cells, which might seem contradictory to the increased TTS sensitivity of TPA–HL-60 cells. However, this could also be an indication that *P. aeruginosa* adherence to TPA–HL-60 cells is more TTS specific. In this regard, candidate TTS-interactive proteins, such as $\alpha_5\beta_1$ integrins and intracellular adhesion molecule 1 (ICAM-1), show enhanced expression and avidity, respectively, in response to TPA differentiation (2, 28). These comparisons also draw attention to the potential use of HL-60 and TPA–HL-60 cells to identify TTS-specific eukaryotic cell-interactive components.

Presently, little is known about the host cell contribution to

P. aeruginosa TTS. Studies described here identify a link between HL-60 cell resistance to TTS-ExoS and host cell properties that affect the establishment of a functional TTS translocation apparatus. While the mechanism for this TTS resistance is not known, it appears to be reversed by treatments that affect cell polarity and adherence. Also of relevance to these studies is the identification of a TTS-resistant HL-60 cell line, which can be induced to become TTS sensitive, providing a model system to study eukaryotic cell properties associated with alterations in *P. aeruginosa* TTS sensitivity.

We thank Dara Frank (Medical College of Wisconsin, Milwaukee, Wis.) for providing the *P. aeruginosa* strains and the PopD antibody used in these studies and Joseph Barbieri (Medical College of Wisconsin) for providing the ExoS-HA construct. We thank Jennifer Fraylick, Claudia Rocha, and Timothy Vincent for their helpful comments during these studies, and we thank Carol Moskos for technical assistance with SEM. We also appreciate the editorial comments of Nyles Charon, Thomas Elliott, and Richard Bakker.

This work was supported by Public Health Services grant NIH-NIAID 45569, by the Medical University of South Carolina Institutional Research Funds, and by the Mary C. Babb Cancer Center Foundation Fund 2V882 from West Virginia University.

REFERENCES

1. **Bentzmann, S. D., P. Roger, F. Dupuit, O. Bajolet-Laudinat, C. Fuchey, M. C. Plotkowski, and E. Puchelle.** 1996. Asialo GM1 is a receptor for *Pseudomonas aeruginosa* adherence to regenerating respiratory epithelial cells. Infect. Immun. **64:**1582–1588.
2. **Bestilny, L. J., and K. T. Riabowol.** 2000. A role for serine proteases in mediating phorbol ester-induced differentiation of HL-60 cells. Exp. Cell Res. **256:**264–271.
3. **Coburn, J., and D. M. Gill.** 1991. ADP-ribosylation of p21^{ras} and related proteins by *Pseudomonas aeruginosa* exoenzyme S. Infect. Immun. **59:**4259–4262.
4. **Collins, S. J., R. C. Gallo, and R. E. Gallagher.** 1977. Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature **270:**347–349.
5. **Collins, S. J., F. W. Ruscetti, R. E. Gallagher, and R. C. Gallo.** 1978. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. Proc. Natl. Acad. Sci. USA **75:**2458–2462.
6. **Comolli, J. C., L. L. Waite, K. E. Mostov, and J. E. Engel.** 1999. Pili binding to asialo GM1 on epithelial cells can mediate cytotoxicity or bacterial internalization by *Pseudomonas aeruginosa.* Infect. Immun. **67:**3207–3214.
7. **Feltman, H., G. Schulert, S. Khan, M. Jain, L. Peterson, and A. R. Hauser.** 2001. Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa.* Microbiology **147:**2659–2669.
8. **Ferguson, M. W., J. A. Maxwell, T. S. Vincent, J. d. Silva, and J. C. Olson.** 2001. Comparison of the *exoS* gene and protein expression in soil and clinical isolates of *Pseudomonas aeruginosa.* Infect. Immun. **69:**2198–2210.
9. **Fleiszig, S., D. Evans, N. Do, V. Vallas, S. Shin, and K. Mostov.** 1997. Epithelial cell polarity affects susceptibility to *Pseudomonas aeruginosa* invasion and cytotoxicity. Infect. Immun. **65:**2861–2867.
10. **Fraylick, J. E., J. T. Barbieri, M. J. Riese, T. S. Vincent, and J. C. Olson.** 2002. ADP-ribosylation and functional effects of *Pseudomonas* ExoS on cellular Ral. Biochemistry **41:**9680–9687.
11. **Fraylick, J. E., J. R. LaRocque, T. S. Vincent, and J. C. Olson.** 2001. The independent and coordinate effects of the ADP-ribosyltransferase and GTPase-activating activities of exoenzyme S on HT-29 epithelial cell function. Infect. Immun. **69:**5318–5328.
12. **Fraylick, J. E., E. A. Rucks, D. M. Greene, T. S. Vincent, and J. C. Olson.** 2002. Eukaryotic cell determination of ExoS ADP-ribosyltransferase substrate specificity. Biochem. Biophys. Res. Commun. **291:**91–100.
13. **Frithz-Lindsten, E., Y. Du, R. Rosqvist, and A. Forsberg.** 1997. Intracellular targeting of exoenzyme S of *Pseudomonas aeruginosa* via type III-dependent translocation induces phagocytosis resistance, cytotoxicity and disruption of actin microfilaments. Mol. Microbiol. **25:**1125–1139.
14. **Frithz-Lindsten, E., A. Holmstrom, L. Jacobsson, M. Soltani, J. Olsson, R. Rosqvist, and A. Forsberg.** 1998. Functional conservation of the effector protein translocators PopB/YopB and PopD/YopD of *Pseudomonas aeruginosa* and *Yersinia pseudotuberculosis.* Mol. Microbiol. **29:**1155–1165.
15. **Galan, J. E., and A. Collmer.** 1999. Type III secretion machines: bacterial devices for protein delivery into host cells. Science **284:**1323–1328.
16. **Gerceker, A. A., T. Zaidi, P. Marks, D. E. Golan, and G. B. Pier.** 2000. Impact of heterogeneity within cultured cells on bacterial invasion: analysis of *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi entry into MDCK cells by using a green fluorescent protein-labeled cystic fibrosis transmembrane conductance regulator receptor. Infect. Immun. **68:**861–870.
17. **Goehring, U.-M., G. Schmidt, K. J. Pederson, K. Aktories, and J. T. Barbieri.** 1999. The N-terminal domain of *Pseudomonas aeruginosa* exoenzyme S is a GTPase-activating protein for Rho GTPases. J. Biol. Chem. **274:**36369–36372.
18. **Hazlett, L. D., S. Masinick, R. Barrett, and K. Rosol.** 1993. Evidence of asialo GM1 as a corneal glycolipid receptor for *Pseudomonas aeruginosa* adhesion. Infect. Immun. **61:**5164–5173.
19. **Henriksson, M., C. Sundin, A. Jansson, A. Forsberg, R. Palmer, and B. Hallberg.** 2002. Exoenzyme S shows selective ADP-ribosylation and GTPase-activating protein (GAP) activities towards small GTPases in vivo. Biochem. J. **367:**617–628.
20. **Hueck, C. J.** 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol. Mol. Biol. Rev. **62:**379–433.
21. **Iglewski, B. H., J. Sadoff, M. J. Bjorn, and E. S. Maxwell.** 1978. *Pseudomonas aeruginosa* exoenzyme S: an adenosine diphosphate ribosyltransferase distinct from toxin A. Proc. Natl. Acad. Sci. USA **75:**3211–3225.
22. **Katagiri, K., S. Hattori, S. Nakamura, T. Yamamoto, T. Yoshida, and T. Katagiri.** 1994. Activation of Ras and formation of GAP complex during TPA-induced monocytic differentiation of HL-60 cells. Blood **84:**1780–1789.
23. **Kazmierczak, B. I., K. Mostov, and J. N. Engel.** 2001. Interaction of bacterial pathogens with polarized epithelium. Annu. Rev. Microbiol. **55:**407–435.
24. **Krivan, H. C., V. Ginsburg, and D. D. Roberts.** 1988. *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). Arch. Biochem. Biophys. **260:**493–496.
25. **Kulich, S. M., D. W. Frank, and J. T. Barbieri.** 1995. Expression of recombinant exoenzyme S of *Pseudomonas aeruginosa.* Infect. Immun. **63:**1–8.
26. **Kulich, S. M., D. W. Frank, and J. T. Barbieri.** 1993. Purification and characterization of exoenzyme S from *Pseudomonas aeruginosa* 388. Infect. Immun. **61:**307–313.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **Laouar, A., F. R. Collart, C. B. H. Chubb, B. Xie, and E. Huberman.** 1999. Interaction between $\alpha_5\beta_1$ integrin and secreted fibronectin is involved in macrophage differentiation of human HL-60 myeloid leukemia cells. J. Immunol. **162:**407–414.
29. **Leglise, M. C., G. A. Dent, L. H. Ayscue, and D. W. Ross.** 1988. Leukemic cell maturation: phenotypic variability and oncogene expression in HL-60 cells: a review. Blood Cells **13:**319–337.
30. **McGuffie, E. M., D. W. Frank, T. S. Vincent, and J. C. Olson.** 1998. Modification of Ras in eukaryotic cells by *Pseudomonas aeruginosa* exoenzyme S. Infect. Immun. **66:**2607–2613.
31. **McGuffie, E. M., J. E. Fraylick, D. J. Hazen-Martin, T. S. Vincent, and J. C. Olson.** 1999. Differential sensitivity of human epithelial cells to *Pseudomonas aeruginosa* exoenzyme S. Infect. Immun. **67:**3494–3503.
32. **Olson, J. C., J. E. Fraylick, E. M. McGuffie, K. M. Dolan, T. L. Yahr, D. W. Frank, and T. S. Vincent.** 1999. Interruption of multiple cellular processes in HT-29 epithelial cells by *Pseudomonas aeruginosa* exoenzyme S. Infect. Immun. **67:**2847–2854.
33. **Olson, J. C., E. M. McGuffie, and D. W. Frank.** 1997. Effects of differential expression of the 49-kilodalton exoenzyme S by *Pseudomonas aeruginosa* on cultured eukaryotic cells. Infect. Immun. **65:**248–256.
34. **Pederson, K. J., R. Krall, M. J. Riese, and J. T. Barbieri.** 2002. Intracellular localization modulates targeting of ExoS, a type III cytotoxin, to eukaryotic signaling proteins. Mol. Microbiol. **46:**1381–1390.
35. **Pederson, K. J., S. Pal, A. J. Vallis, D. W. Frank, and J. T. Barbieri.** 2000. Intracellular localization and processing of *Pseudomonas aeruginosa* ExoS in eukaryotic cells. Mol. Microbiol. **37:**287–299.
36. **Pederson, K. J., A. J. Vallis, K. Aktories, D. W. Frank, and J. T. Barbieri.** 1999. The amino-terminal domain of *Pseudomonas aeruginosa* ExoS disrupts actin filaments via small-molecular weight GTP-binding proteins. Mol. Microbiol. **32:**393–401.
37. **Pier, G. B., M. Gout, and T. S. Zaidi.** 1997. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. Proc. Natl. Acad. Sci. USA **94:**12088–12093.
38. **Plotkowski, M. C., S. D. Bentzmann, S. H. M. Pereira, J.-M. Zahm, O. Bajolet-Laudinat, P. Roger, and E. Puchelle.** 1999. *Pseudomonas aeruginosa* internalization by human epithelial respiratory cells depends on cell differentiation, polarity, and junctional complex integrity. Am. J. Respir. Cell Mol. Biol. **20:**880–890.
39. **Riese, M. J., U.-M. Goerhring, M. E. Ehrmantraut, J. Moss, J. T. Barbieri, K. Aktories, and G. Schmidt.** 2002. Auto-ADP-ribosylation of *Pseudomonas aeruginosa* ExoS. J. Biol. Chem. **277:**12082–12088.
40. **Rocha, C. L., J. Coburn, E. A. Rucks, and J. C. Olson.** 2003. Characterization of *Pseudomonas aeruginosa* exoenzyme S as a bifunctional enzyme in J774A.1 macrophages. Infect. Immun. **71:**5296–5305.
41. **Roger, P., E. Puchelle, O. Bajolet-Laudinat, J.-M. Tournier, C. Debordeaux,**

M.-C. Plotowski, J. H. M. Cohen, D. Sheppard, and S. D. Bentzmann. 1999. Fibronectin and $\alpha_5\beta_1$ integrin mediate binding of *Pseudomonas aeruginosa* to repairing airway epithelium. Eur. Respir. J. **13:**1301–1309.
42. **Rucks, E. A., J. E. Fraylick, L. M. Brandt, T. S. Vincent, and J. C. Olson.** 2003. Cell line differences in bacterially translocated ExoS ADP-ribosyltransferase substrate specificity. Microbiology **149:**319–331.
43. **Rumbaugh, K. P., J. A. Griswold, and A. N. Hamood.** 1999. *Pseudomonas aeruginosa* strains obtained from patients with tracheal, urinary tract and wound infections: variations in virulence factors and virulence genes. J. Hosp. Infect. **43:**211–218.
44. **Schweizer, H. P.** 1991. *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19. Gene **97:**109–112.
45. **Tonetti, D. A., C. Henning-Chubb, D. T. Yamanishi, and E. Huberman.** 1994. Protein kinase C-β is required for macrophage differentiation of human HL-60 leukemia cells. J. Biol. Chem. **269:**23230–23235.
46. **Vallis, A. J., V. Finck-Barbancon, T. L. Yahr, and D. W. Frank.** 1999. Biological effects of *Pseudomonas aeruginosa* type III-secreted proteins on CHO cells. Infect. Immun. **67:**2040–2044.
47. **Yahr, T. L., J. Goranson, and D. W. Frank.** 1996. Exoenzyme S of *Pseudomonas aeruginosa* is secreted by a type III pathway. Mol. Microbiol. **22:**991–1003.
48. **Yahr, T. L., L. M. Mende-Mueller, M. B. Friese, and D. W. Frank.** 1997. Identification of type III secreted products of the *Pseudomonas aeruginosa* exoenzyme S regulon. J. Bacteriol. **179:**7165–7168.

Editor: D. L. Burns