Pseudomonas aeruginosa Isolates from Multiple Clinical Settings Induce Equal Levels of IL-8 Production by Airway Epithelial Cells

Kathleen K. Beasley[1], Wei Guang[1], K. Chul Kim[2], and Erik P. Lillehoj[1]

[1]University of Maryland School of Medicine, Baltimore, MD; [2]Villa Julie College, Stevenson, MD; [2]Temple University School of Medicine, Philadelphia, PA

Abstract

Rationale: *Pseudomonas aeruginosa* (PA) lung infection is a leading cause of morbidity and mortality among patients with cystic fibrosis, ventilator-associated pneumonia, community-acquired pneumonia, and in immunocompromised hosts. PA flagellin, the main structural protein of the flagellar filament, is a virulence factor with proinflammatory activity on respiratory epithelial cells. PA expresses one of two isoforms of flagellin (a-type or b-type) that differ in molecular weight due to the deletion of a ~ 50 amino acid sequence in the a-type proteins.

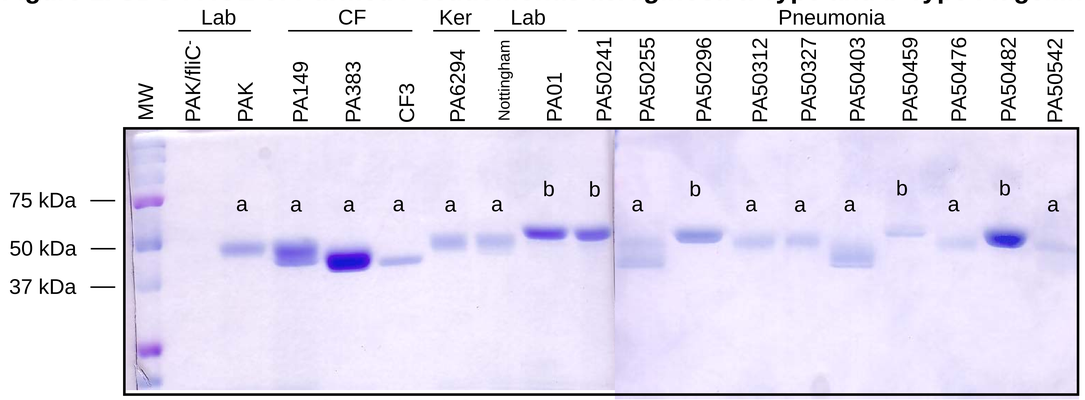
Methods: The distribution of a-type (45.0 - 52.0 kDa) and b-type (54.0 kDa) flagellins among a panel of 15 PA isolates from CF and pneumonia patients was determined by SDS-PAGE of the purified proteins and their abilities to stimulate IL-8 production by BEAS-2B human airway epithelial cells was compared.

Results: Both a- and b-type flagellins were purified with yields ranging from 21.8 to 96.8 µg/L of bacterial culture. All flagellins were free of detectable pilin and LPS contamination as assessed by Western blotting and the Limulus amebocyte test (< 0.1 E.U/µg). Compared with the PAK (a-type) and PA01 (b-type) prototype laboratory strains, 6/10 PA pneumonia isolates expressed a-type and 4/10 expressed b-type flagellin. All purified flagellins stimulated IL-8 production by BEAS-2B cells (range: 1,000-4,000 pg/ml). Moreover, no discernable differences in IL-8 production were evident using airway cells treated with the a-type or b-type flagellins.

Conclusions: We conclude that clinical isolates of PA are not distinguishable based on their expression of a- or b-type flagellins, and bacteria expressing both types of flagellins induce equivalent levels of IL-8 production by pulmonary epithelial cells.

Results

Figure 1. SDS-PAGE of Purified *Pseudomonas aeruginosa* a-Type and b-Type Flagellins



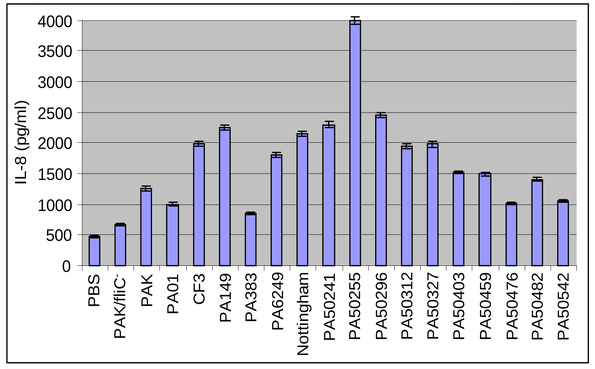
Flagellins were purified by $(NH_4)_2SO_4$ precipitation as described by Zhang *et al*. (4), resolved by SDS-PAGE, and stained with Coomassie Blue.

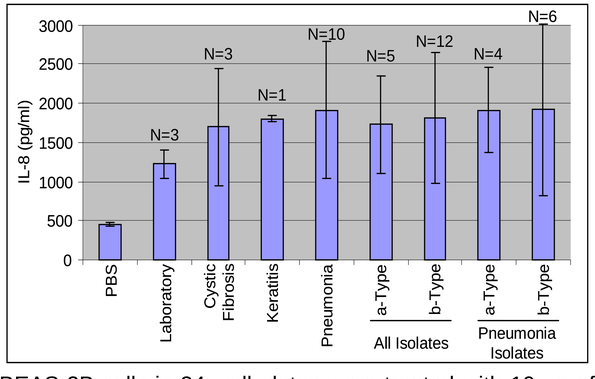
Table 1. *Pseudomonas aeruginosa* Flagellin Purification and Type Classification

PA Isolate	Source	Flagellin Yield[a]	LPS Content[b]	Flagellin MW[c]	Flagellin Type[d]
PAK/fliC-[e]	Laboratory	N/A[f]	< 0.1 E.U/µg	N/A	N/A
PAK	Laboratory	65.6 µg/L	< 0.1 E.U/µg	47.0 kDa	a
PA01	Laboratory	50.5 µg/L	< 0.1 E.U/µg	54.0 kDa	b
Nottingham	Laboratory	76.8 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA149	Cystic Fibrosis	42.7 µg/L	< 0.1 E.U/µg	46.0 kDa	a
PA383	Cystic Fibrosis	96.8 µg/L	< 0.1 E.U/µg	46.0 kDa	a
CF3	Cystic Fibrosis	40.6 µg/L	< 0.1 E.U/µg	50.0 kDa	a
PA6294	Keratitis	22.5 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA50241	Pneumonia	61.9 µg/L	< 0.1 E.U/µg	54.0 kDa	b
PA50255	Pneumonia	48.4 µg/L	< 0.1 E.U/µg	50.0 kDa	a
PA50296	Pneumonia	31.1 µg/L	< 0.1 E.U/µg	54.0 kDa	b
PA50312	Pneumonia	29.6 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA50327	Pneumonia	51.3 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA50403	Pneumonia	24.2 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA50459	Pneumonia	36.4 µg/L	< 0.1 E.U/µg	54.0 kDa	b
PA50476	Pneumonia	28.8 µg/L	< 0.1 E.U/µg	52.0 kDa	a
PA50482	Pneumonia	21.8 µg/L	< 0.1 E.U/µg	54.0 kDa	b
PA50542	Pneumonia	49.7 µg/L	< 0.1 E.U/µg	50.0 kDa	a

[a]µg of purified flagellin per liter of bacterial culture
[b]LPS determined by the Limulus amebocyte lysate test
[c]Flagellin molecular weight determined by SDS-PAGE with reference to prestained protein markers
[d]a-type flagellin MW = 54.0 kDa
b-type flagellin MW = 45.0-52.0 kDa
[e]PAK/fliC-, flagellin-deficient PAK
[f]Not applicable

Figure 2. IL-8 Production by Purified Flagellins





BEAS-2B cells in 24 well plates were treated with 10 µg of flagellin for 4 hr and IL-8 levels in supernatants were determined by ELISA (Pierce). Values are means ± SD.

Summary

- In general, PA isolates from acute pneumonia patients produced slightly more flagellin than cystic fibrosis or laboratory isolates.
- The non-flagellated PAK/fliC- strain stimulated significantly less IL-8 production compared with cells treated with any of the purified flagellins ($p < 0.05$).
- The distribution of a- and b-type flagellins had no specified pattern among the PA isolates from acute pneumonia patients.
- The difference of IL-8 production was only 7.1% between PA isolates from acute pneumonia patients compared with those from cystic fibrosis patients ($p > 0.05$)
- No difference in IL-8 production was noted between a-type and b-type flagellins ($p > 0.05$).

References

1. Chastre J, Fagon JY (2002) Ventilator-associated pneumonia. Am J Respir Crit Care Med 165:867-903.
2. Feldman M, Bryan R, Rajan S, Scheffler L, Brunnert S, Tang H, Prince A (1998) Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection. Infect Immun 66:43-51.
3. Brimer CD, Montie TC (1998) Cloning and comparison of fliC genes and identification of glycosylation in the flagellin of *Pseudomonas aeruginosa* a-type strains. J Bacteriol 180:3209-3217.
4. Zhang J, Xu K, Ambati B, Yu FS (2003) Toll-like receptor 5-mediated corneal epithelial inflammatory responses to *Pseudomonas aeruginosa* flagellin. Invest Ophthalmol Vis Sci 44:4247-4254.

Acknowledgments

Funded by U.S. Public Health Service grants ES013483 and AI072291.