Biochemical and Biophysical Research Communications 428 (2012) 150–154

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mucoid *Pseudomonas aeruginosa* caused by *mucA* mutations result in activation of TLR2 in addition to TLR5 in airway epithelial cells

Trevor Beaudoin [a], Shantelle LaFayette [b], Dao Nguyen [a,b], Simon Rousseau [a,b,*]

[a] *Meakins-Christie Laboratories, McGill University, Montreal, Canada*
[b] *Department of Medicine, McGill University, Montreal, Canada*

ARTICLE INFO

Article history:
Received 4 October 2012
Available online 12 October 2012

Keywords:
Pseudomonas
Toll-like receptors
p38α Mitogen-activated protein kinase
Mucoid
Inflammation
Cystic fibrosis

ABSTRACT

The presence of the mucoid phenotype of *Pseudomonas aeruginosa* is a marker of poor survival in cystic fibrosis. As CF lung disease results from chronic infection leading to airway inflammation, we determined whether the switch to a mucoid phenotype by *P. aeruginosa* has an impact on the inflammatory response of airway epithelial cells. Exposure of airway epithelial cells to non-mucoid and mucoid *P. aeruginosa*-derived material leads to p38α MAPK activation, a key protein kinase involved in transmitting inflammatory signals. However, while the non-mucoid strain PAO1 activates p38α MAPK pathway solely via TLR5, the mucoid strain PACF508 activates p38α MAPK via both TLR5 and TLR2. Inactivation of *mucA* (the gene responsible for the mucoid phenotype) in PAO1 leads to p38α MAPK activation by both TLR2 and TLR5, as observed in the clinical mucoid isolate PACF508. Therefore, the switch to mucoid phenotype may contribute to more inflammation via TLR2 activation in addition to TLR5. Our findings highlight an important and under recognized role for TLR2 in the response of airway epithelial cells to infection.

1. Introduction

Cystic fibrosis (CF) is characterized by mucus hyper-secretion, chronic infection and inflammation associated with decreasing lung function [1,2]. Therefore signals that increase inflammation in chronically infected CF patients may worsen decline in lung function. Markers of inflammation are increased at the onset of pulmonary exacerbations [3] and following these episodes, a net decline in lung function has been documented from pre-exacerbation state [21]. Unfortunately, very little is known about pulmonary exacerbations. They are probably related to a complex relationship between host defense and airway microbiology. One model proposes that exacerbations are caused by the release and proliferation of planktonic bacteria from biofilm aggregates [4].

Pseudomonas aeruginosa is the most significant pathogen in CF with up to 80% of patients eventually chronically infected with *P. aeruginosa* [5]. Moreover, clinically the presence of the mucoid phenotype of *P. aeruginosa* is a marker of poor survival in CF [6,7]. The mucoid phenotype is typically attributed to mutations in the *mucA* gene, a negative regulator of the stress sigma factor AlgU [8].

Activation of innate immunity in response to pathogens is mediated via pattern-recognition receptors (PRRs) expressed by host cells. The p38α mitogen-activated protein kinase (MAPK) is an important mediator of inflammatory signaling that plays a role in host defenses against *P. aeruginosa* in vertebrates [9]. Activation of p38α MAPK in response to *P. aeruginosa* has been linked to the flagellin receptor Toll-like receptor 5 (TLR5) expressed at the surface of airway epithelial cells [10].

In this report, we investigated if the switch to a mucoid phenotype by *P. aeruginosa* has an impact on the activation of p38α MAPK in airway epithelial cells.

2. Materials and methods

2.1. Materials

All chemicals were bought from Fisher Scientific (Fair Lawn, NJ, USA). Zeocin, Hygromycin, Blasticidin, Normocin, FSL-1, LPS from *P. aeruginosa* and *S. typhimurium* flagellin were bought from InvivoGen (San Diego, CA, USA).

2.2. P. aeruginosa strains

Two strains of *P. aeruginosa* were investigated: the common laboratory strain PAO1, and PACF508, a stable mucoid clinical isolate from the sputum of a patient with CF (CFTRΔF508 homozygous;

Abbreviations: AEC, airway epithelial cells; CF, cystic fibrosis; CFTR, CF transmembrane regulator; MAPK, mitogen activated protein kinase; PRR, pattern recognition receptor; PsaDM, *P. aeruginosa* diffusible material; SCFM, synthetic CF media; TLR, Toll-like receptors.

* Corresponding author. Address: Meakins-Christie Laboratories, 3626 St-Urbain, Montréal, Canada H2X 2P2. Fax: +1 514 398 7483.
E-mail address: simon.rousseau@mcgill.ca (S. Rousseau).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.030

Hôpital Sainte-Justine, Montréal) [11]. The *flgK* and *mucA* mutants are transposon mutants obtained from the PAO1 transposon library [12].

2.3. P. aeruginosa diffusible material preparation

P. aeruginosa diffusible material from planktonic bacteria (PsaDM) was obtained from bacteria grown in peptone (Fisher Scientific) or synthetic CF medium SCFM [13] as previously described [14,15]. Prior to use, bacterial filtrates were heat inactivated at 95 °C for 10 min (to inactivate proteases) and allowed to cool to room temperature.

2.4. Antibodies

Neutralizing antibodies against TLR2, TLR4 and TLR5 were used at 5 μg/mL and purchased from InvivoGen (CA, USA). Anti-GAPDH (used at a concentration of 1/4000) and anti-phospho p38 MAPK (Thr180/Thr182; used at 1/1000 dilution) were purchased from Millipore (Temecula, CA). Anti-p38 MAPK (used at a dilution of 1/1000) was purchased from Cell signaling (Boston, Ma). Goat anti-rabbit IgG DyLight™800 (35,571; 1:15,000) and Goat anti-mouse IgG DyLight™680 (35,518; 1:15,000) were bought from Thermo Scientific (Rockford, IL, USA).

2.5. Cell culture

BEAS-2B AECs were cultured as previously described [11]. HEK-Blue TLR5 cells and HEK-Blue Null1 cells were purchased from InvivoGen (San Diego, CA). HEK-Blue TLR5 were grown and maintained in DMEM supplemented with 10% FBS with 100 U/mL penicillin G, 100 μg/mL of streptomycin, 100 μg/mL normacin, 100 μg/mL Zeocin and 30 μg/mL of blasticidin. 24 h prior to stimulation, cells were starved in DMEM without antibiotics. HEK-Blue Null1 were grown and maintained in DMEM supplemented with 10% FBS with 100 U/mL penicillin G, 100 μg/mL of streptomycin, 100 μg/mL normacin and 100 μg/mL zeocin. 24 h prior to stimulation, cells were starved in DMEM without antibiotics. Human airway epithelial cell line NuLi was derived from a normal lung of a 36-year-old male patient and CuFi airway epithelial cell line derived from lung of a 14-year-old female patient with cystic fibrosis homozygous for the CFTRΔF508 mutation were cultures as previously described [15].

2.6. Cell lysis and immunoblotting

Following stimulation, cells were lysed in ice-cold buffer (50 mM Tris–Cl pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% (v/v) Triton X-100, 1 mM sodium orthovanadate, 5 mM sodium pyrophosphate, 0.27 M sucrose, complete mini protease inhibitor cocktail and 2 mM DTT). Proteins were quantified using the Bradford

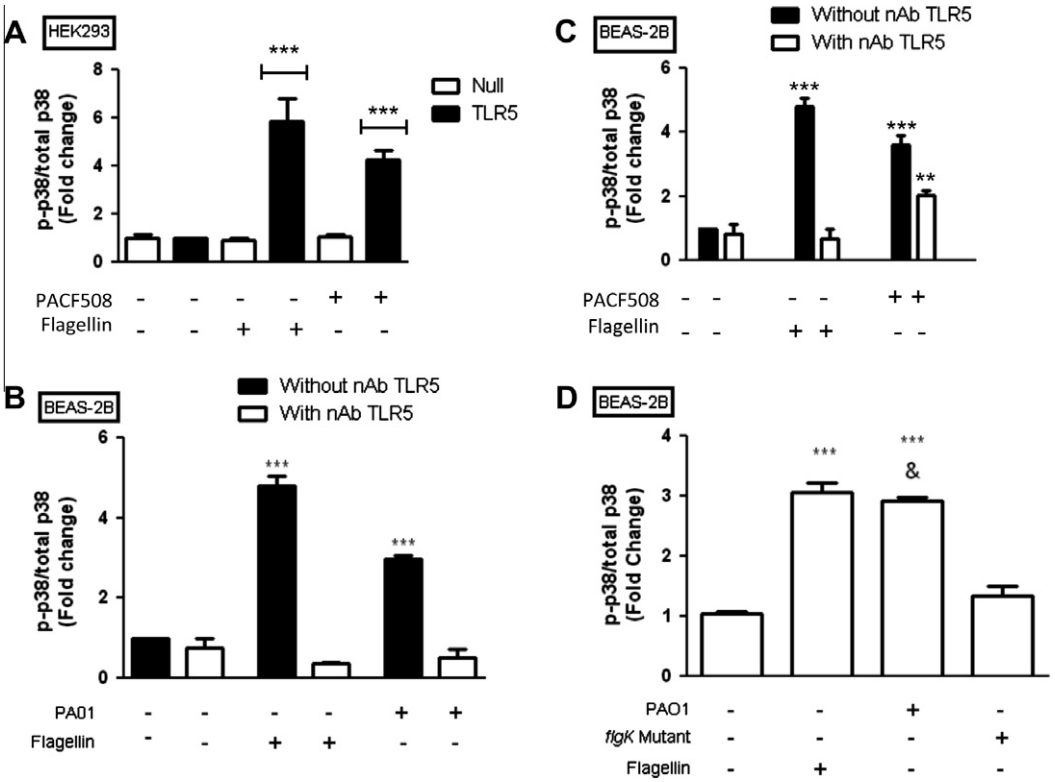

Fig. 1. TLR5 leads to p38 MAPK activation in AECs exposed to Planktonic PsaDM. (A) Human embryonic kidney (HEK) cells lacking (null, white bars) or expressing TLR5 (TLR5, black bars) were left untreated (−) or treated (+) with 0.4 μg/mL flagellin or 1 μg/mL of planktonic PACF508 PsaDM grown in SCFM for 30 min. Following stimulation, cells were lysed and 20 μg of Triton-soluble materials was subjected to SDS–PAGE. After transfer to nitrocellulose, the membranes were probed with antibodies recognizing only the phosphorylated forms of p38α MAPK or antibodies that recognize all forms of p38α MAPK. Quantitative analysis of the signals from each antibody was performed using Li-Cor infrared Odyssey imaging system. (B and C) BEAS-2B AECs were left untreated (−) or pretreated for 30 min without (black bars) or with 5 μg/mL neutralizing TLR5 antibody (white bars) followed by exposure (+) for 30 min to 0.4 μg/mL of flagellin or 1 μg/mL of planktonic PACF508 PsaDM. p38α MAPK activation was determined as in Fig. 1A. (D) BEAS-2B AECs were left untreated (−) or stimulated (+) for 30 min with 1 μg/mL planktonic PsaDM from wildtype PAO1 or a *flgK* mutant. p38α MAPK activation was determined as in Fig. 1A. TLR5 nAb: TLR5 neutralizing antibody. The mean of three experiments is shown +/− SEM. Statistics were performed using one-way ANOVA with Bonferroni post-test analysis. $^{***,**}p \leqslant 0.001$ or 0.01, respectively compared to unstimulated; $^{\#}p \leqslant 0.05$ in absence of TLR5 nab compared to its presence. $^{\&}p \leqslant 0.05$ wildtype PAO1 compared to PAO1*flgK*.