Characterising the Lipopolysacchride of *Achromobacter xylosoxidans* - An emerging Cystic Fibrosis pathogen

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Introduction

Achromobacter xylosoxidans (AX) is an emerging opportunistic pathogen known to cause infections in the lungs of cystic fibrosis (CF) and bronchiectasis patients. It is understudied compared to other known respiratory opportunistic pathogens such as *Pseudomonas aeruginosa* (PA) and *Burkholderia cepacia* (BC). Previous research has indicated that one of the main virulence factors of opportunistic pathogens is the bacterial lipopolysaccharide (LPS)[1]. LPS plays an important role in prediction of serum sensitivity, antibiotic resistance and also triggering the host inflammatory response and driving disease progression. CF pathogens may adapt LPS in late stage CF.

Aim

To investigate and compare the LPS of clinically derived *A.xylosoxidans*, taken from CF and non-CF (bronchiectasis) patients.

- To determine the LPS chemotype using SDS-PAGE and silver staining.
- To determine the inflammatory potential of LPS rich lysates prepared from AX clinical samples by stimulating human THP-1 cells and measuring cytokine responses.

Methods

Bacterial Strains – *Achromobacter xylosoxidans* strains used in this study are listed in Table 1

LPS extraction and analysis– LPS was extracted using the Chembio LPS Extraction kit according to the manufacturers instructions. AX LPS samples were separated on a 12% acrylamide gel using SDS-PAGE. Gel was run overnight at a 5mA constant. LPS was visualised using a Pierce silver staining kit and dried.

LPS rich Whole cell lysate (WCL) preparation – Cultures harvested into solution and standardised at optical density of 0.2 at 600nm. Bacterial suspensions sonicated and incubated with deoxyribonuclease II (200µg/ml) at 37 °C for one hour. Lysates were then treated with Proteinase K (2mg/ml) at 60°C for 2 hours, boiled for 20 minutes (inactivating Proteinase K).

Cytokine stimulation - Differentiated human THP-1 cells (0.5 x 10^6 cells/ml in 300µl volume) were stimulated with AX WCL for 6 hours. Cytokine profiles (IL-8, IL-1β and TNF-α) of cell supernatants were measured by ELISA. A negative control of phosphate buffered saline (PBS) and a positive control of *E. coli* LPS were used.

Results

AX LPS chemotypes were determined by extracting LPS from AX strains and analysing by SDS-PAGE. Figure 1 shows a silver stained SDS-PAGE gel of AX LPS samples from strains AX1 to AX10. Results indicate which strains have smooth LPS and which have rough LPS. Characteristic banding of AX5 and AX7 samples indicate they have rough LPS. Laddering pattern of AX9 indicates it has smooth LPS.

Table 1. Bacterial strains used in this study

A. xylosoxidans strain	Strain number	Clinical source
AX1	269	Bronchiectasis
AX2	296	CF
AX3	353	Bronchiectasis
AX4	452	CF
AX5	551	Bronchiectasis
AX6	553	Bronchiectasis
AX7	735	CF
AX8	230	CF
AX9	2467	CF
AX10	301	Bronchiectasis

Figure 1. SDS-PAGE of all 10 strains of AX LPS

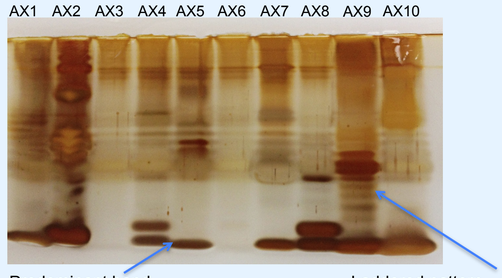


Figure 2. IL-8 response from differentiated THP-1 cells to WCL of each strain.

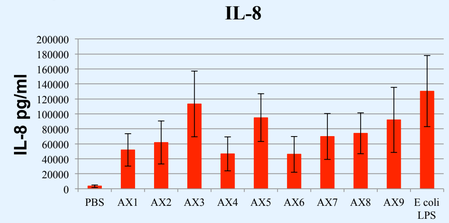


Figure 3. IL-1β response from differentiated THP-1 cells to WCL of each strain.

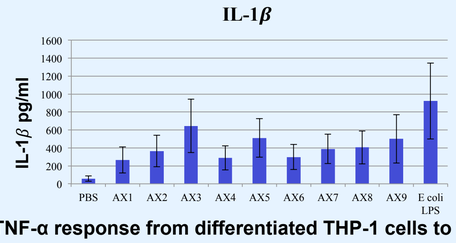
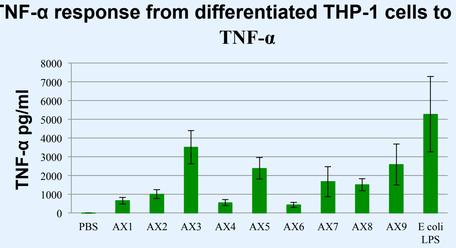


Figure 4. TNF-α response from differentiated THP-1 cells to WCL of each strain.



Results

The cytokine responses of differentiated human THP-1 cells exposed to whole cell lysates of AX strains was investigated. Figures 2, 3, and 4 show the IL-8, IL-1β and TNFα responses respectively.

The cytokine response for each strain followed a similar pattern across TNF-α, IL-1β and IL-8.

WCL from strains AX3, AX5 and AX9 elicited a strong cytokine response. Strains AX1, AX2 and AX6 elicited a weak cytokine response.

MMP-1 and MMP-3 from human macrophages exposed to AX WCL were not detectable using ELISA (not shown) .

Conclusion

AX LPS chemotype varies from strain to strain, most strains tested were found to be smooth, but AX5 and AX7 were rough. AX LPS chemotype did not correlate with clinical origin of bacterial samples.

AX whole cell lysates elicited varied cytokine responses from differentiated THP-1 cells. Responses show strain to strain variation which also did not correspond with clinical origin.

These variations need further study in infection models and with pathogen genome sequencing (underway with Prof Valvano, Belfast).

Discussion

•Better understanding of structure to function relationships in LPS and lipid A may allow targeting of inflammatory pathways and also allow potential vaccine candidates to be developed.

•Characterisation of the lipid A component of LPS from high and low cytokine inducers is underway in collaboration with Prof Molinaro University of Naples.

•References *1. De Soyza et al AJRCCM 2004 2. Cigana, Molinaro et al PLOSone 2012*