A component of the innate immune response prevents bacterial biofilm development

A Little on Biofilms

- Biofilms are communities for bacteria
- Once established difficult to remove
- Safe haven for bacteria from biocidal compounds
- Link between environmental and clinical

Study Design

- Authors proposed that some factor of the innate immune response prevents biofilm formation.
- Lactoferrin - chelator of iron, present in bodily secretions.
- GFP expressing *P. aeruginosa* used.
- Flow cell chambers for biofilm development.
- +/- lactoferrin, conalbumin, or deferoxamine.
- Confocal microscopy with time lapse.
- Checked biofilm resistance to biocidal compounds (tobramycin and H₂O₂).
A Little on Flow Cell

- Monitor bacterial attachment in a system
- Control rate of flow
- Integrated with microscopy techniques
Iron...Who Cares?

- Bacteria, animals, and plants need iron
- Activity of some enzymes require iron
  - ex: NorR in *E. coli* (YgaA in *S. typhimurium*)
  - Activated by NO in anaerobic environment
  - NO produced by macrophage (iNOS)

Figure 1 - Growth Curve

Figure 1 Growth of *P. aeruginosa* in the presence of lactoferrin. Results are representative of three experiments.

Used 20 _μg/mL_
Figure 2 - Confocal Microscopy

- Lactoferrin prevented formation of biofilm
- What if other bacteria are present?

- 4 hrs
- 24 hrs
- 3 days
- 7 days
Figure 3 - Bacteria Behaving Badly

- Squatters - localized in 15 \_m circle until division
- Ramblers - remained attached, but moved outside circle
- Flyers - detached from surface
Time Lapse Microscopy

- Monitored bacterial behavior w/ or w/o lactoferrin
- Control video (no lactoferrin)
- Experimental video (with lactoferrin)
Figure 4 - Twitching Motility

- Specialized motility mediated by type 4 pili
  - Increase in type 4 genes or proteins?
  - 40 have been identified in *P. aeruginosa* (J. S. Mattick, *Annual Rev. Microbiol* 56: 289-314)

- Twitching motility stimulated by iron chelators and may prevent biofilm formation

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**a** No lactoferrin

**b** Fe sat. lactoferrin

**c** Fe unsat. lactoferrin

**d**

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<th>FeCl₃ (µM)</th>
<th>0</th>
<th>0.5</th>
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<td>Deferoxamine (mM)</td>
<td>-</td>
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**e** No lacto. (mutant)

**f** lacto. (mutant)
Figure 5 - Biofilm Protection

- Susceptibility to tobramycin and H$_2$O$_2$
- Presence or absence of iron chelator conalbumin
Questions/Comments/Concerns