


Pathogen-associated molecular patterns: important in mastitis?  
 Otto Holst, Leibniz-Center for Medicine and Biosciences

**EADGENE** European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

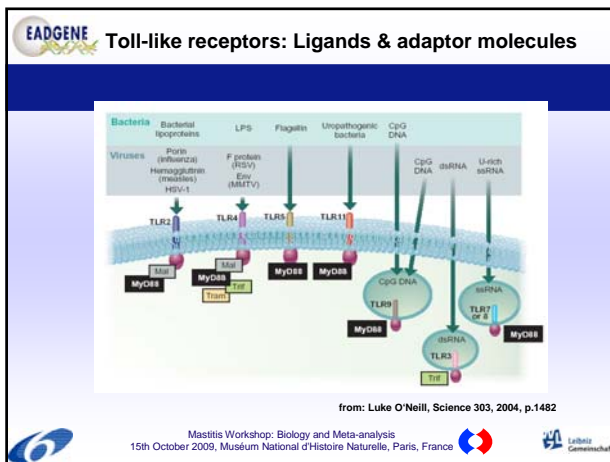
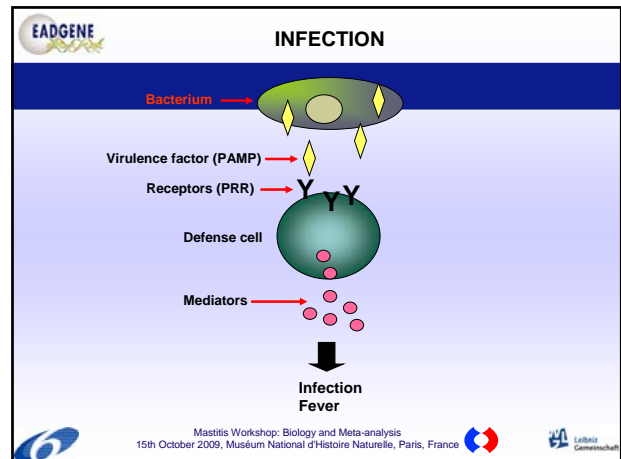
**Mastitis Workshop: Biology and Meta-analysis**  
 15th October 2009, Muséum National d'Histoire Naturelle, Paris, France



**Pathogen-associated molecular patterns: important in mastitis?**  
 Otto Holst

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| TLR | Ligand                   | Origin of the ligand  |
|-----|--------------------------|---|
| 1/2 | Triacyl lipopeptides     | Bacteria, Mycobacteria  |
|     | Porin PorB               | <i>Neisseria meningitidis</i>                                   |
|     | Soluble factors          | <i>Neisseria meningitidis</i>                                   |
| 2   | Lipoprotein/lipopeptides | Various   |
|     | Peptidoglycan            | Gram-positive bacteria  |
|     | Lipoteichoic acid        | Gram-positive bacteria  |
|     | Lipoarabinomannan        | Mycobacteria  |
|     | Glycolipids              | <i>Treponema maltophilum</i>                                    |
|     | Porins                   | <i>Neisseria meningitidis</i>                                   |
|     | .Atypical LPS*           | <i>Leptospira interrogans</i> , <i>Porphyromonas gingivalis</i> |
| 2/6 | Diacyl lipopeptides      | <i>Mycoplasma</i>   |

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| TLR | Ligand                              | Origin of the ligand                  |
|-----|-------------------------------------|---------------------------------------|
| 4   | LPS                                 | Gram-negative Bacteria                |
|     | Flavolipin                          | <i>Flavobacterium meningosepticum</i> |
|     | Taxol                               | Plants                                |
|     | Fusionsprotein                      | RSV                                   |
|     | HSP60                               | <i>Chlamydia pneumoniae</i> host      |
|     | Oligosaccharides of hyaluronic acid | host                                  |
|     | Oligosaccharides of heparan sulfate | host                                  |
|     | Fibrinogen                          | host                                  |

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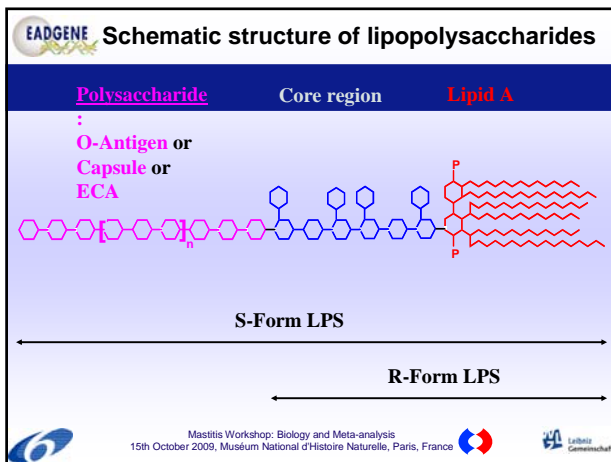
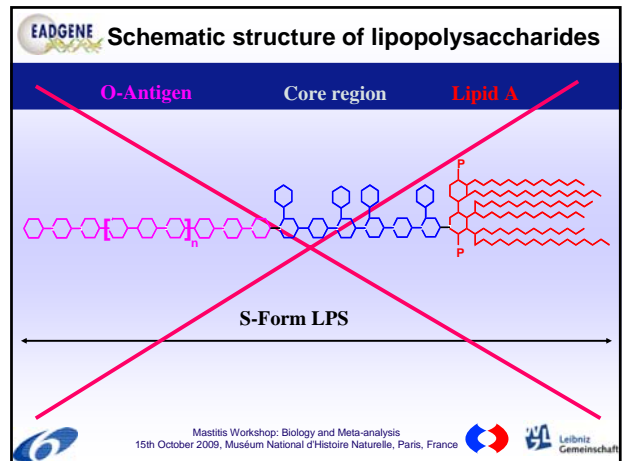
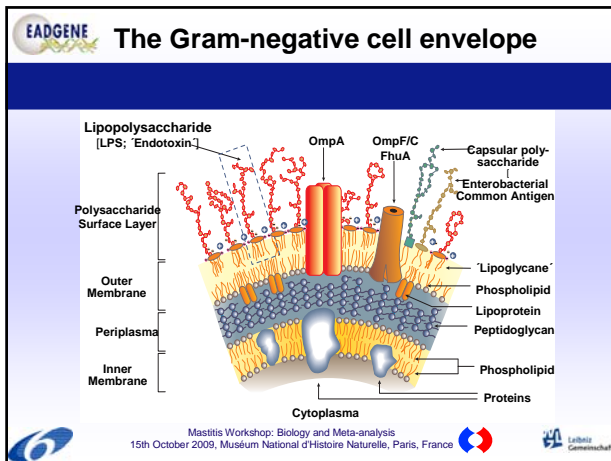
**Important bacteria in mastitis**

**Gram-negative: *Escherichia coli***

**Gram-positive: *Staphylococcus aureus*  
*Streptococcus uberis***

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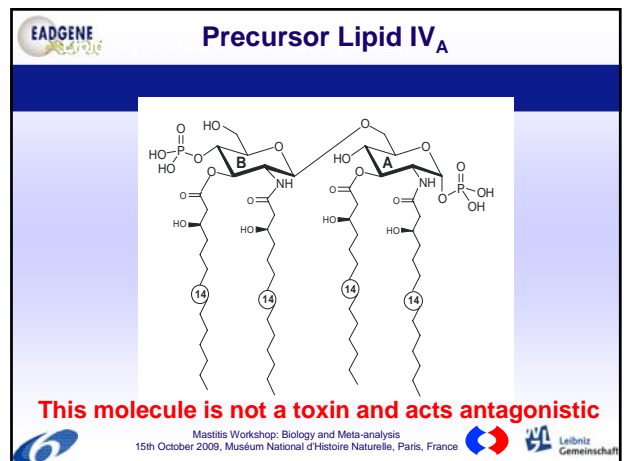
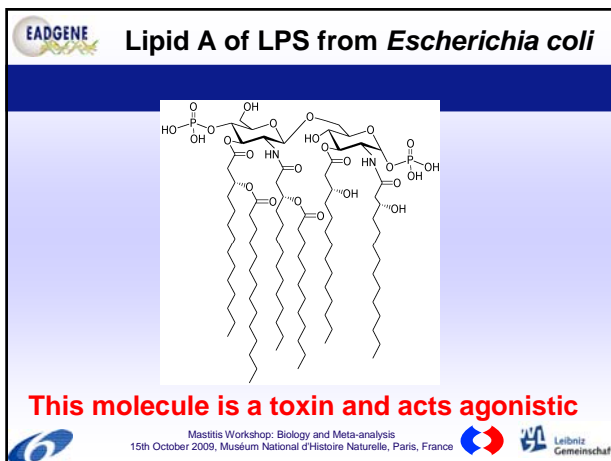
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### EADGENE

| Serotype  | Antigenic factors | Structure  |
|-----------|-------------------|--|
| Variant Y | -3,4              | $\rightarrow 3$ -GlcNAc <sup>6</sup> (1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 3)-Rha(1 $\rightarrow$ )                 |
| 1a        | I:4               | Glc<br>↓1,4<br>$\rightarrow 3$ -GlcNAc(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 3)-Rha(1 $\rightarrow$ )                |
| 1b        | I:6               | Glc<br>↓1,4<br>O:Ac<br>↓2<br>$\rightarrow 3$ -GlcNAc(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 3)-Rha(1 $\rightarrow$ )  |
| 2a        | II:3,4            | Glc<br>↓1,4<br>$\rightarrow 3$ -GlcNAc(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 3)-Rha(1 $\rightarrow$ )                |
| 2b        | II:7,8            | Glc<br>↓1,3<br>Glc<br>↓1,4<br>$\rightarrow 3$ -GlcNAc(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 2)-Rha(1 $\rightarrow$ 3)-Rha(1 $\rightarrow$ ) |

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### EADGENE Conformations of lipid A

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### EADGENE Architecture of the Gram-positive cell envelope

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### EADGENE Lipopeptides

| Time (h) | SaLP SA 113 | ePG SA 113 | Pam,CSK | FSL-1 | Ctrl  |
|----------|-------------|------------|---------|-------|-------|
| 0        | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |
| 1        | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |
| 3        | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |
| 6        | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |
| 12       | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |
| 24       | ~1000       | ~1000      | ~1000   | ~1000 | ~1000 |

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### EADGENE Peptidoglycan

*Staphylococcus aureus*

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### EADGENE NOD-activating PG-fragments

|      |                         |                                      |
|------|-------------------------|--------------------------------------|
| NOD1 | GMTriP <sub>DAP</sub>   | GlcNAc-MurNAc-L-Ala-γ-D-Gln-meso-DAP |
| NOD2 | Muramyl dipeptide (MDP) | MurNAc-L-Ala-D-γ-Gln                 |
|      |                         | MurNAc-L-Ala-L-Lys-γ-D-Gln-meso-DAP  |
|      |                         | MurNAc-L-Ala-L-Orn-γ-D-Gln-meso-DAP  |

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### EADGENE Wall teichoic acids

poly(alditol-P) — linkage unit — peptidoglycan

Linkage unit

poly(alditol-P)-β-D-ManpNAc-(1→4)-β-D-GlcpNAc-(1→P-6)-MurpNAc

PG

PG

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$$\begin{array}{c} \text{-(P(O)}_2\text{-O-CH}_2\text{-CH-CH}_2\text{-O-P(O)}_2\text{-O-} \\ | \\ \text{OR} \end{array}$$
 -Glycerinbisphosphat

$$\begin{array}{c} \text{-(P(O)}_2\text{-O-CH}_2\text{-CH-O-P(O)}_2\text{-O-} \\ | \\ \text{CHOR} \end{array}$$
 2,3-Glycerinbisphosphat

**Poly(glycerolphosphate) type (e.g. in *Bacillus subtilis*)**

$$\begin{array}{c} \text{-(P(O)}_2\text{-O-CH}_2\text{-CH-CH-CH-CH}_2\text{-O-P(O)}_2\text{-O-} \\ | \quad | \quad | \\ \text{OH} \quad \text{OH} \quad \text{OH} \end{array}$$
 1,5-Ribitolbisphosphat

$$\begin{array}{c} \text{-(P(O)}_2\text{-O-CH}_2\text{-CH-CH-O-P(O)}_2\text{-O-} \\ | \\ \text{OH-CH}_2\text{CH}_2\text{OH} \\ | \\ \text{OH} \end{array}$$
 3,5-Ribitolbisphosphat

**Poly(ribitolphosphate) type (e.g. in *Staphylococcus aureus*)**

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### Lipoteichoic acids

a)  $\alpha\text{-Glc-Gal-(1}\rightarrow\text{2)-}\alpha\text{-Glc-(1}\rightarrow\text{3)-O-CH}_2\text{-CH-CH}_2$

b)  $\beta\text{-Glc-(1}\rightarrow\text{6)-}\beta\text{-Glc-(1}\rightarrow\text{3)-O-CH}_2\text{-CH-CH}_2$

c)  $\beta\text{-Glc-(1}\rightarrow\text{6)-}\alpha\text{-Gal-(1}\rightarrow\text{2)-}\alpha\text{-Glc-(1}\rightarrow\text{3)-O-CH}_2\text{-CH-CH}_2$

a) With Glc, e.g. in *Streptococcus*, *Lactococcus*, *Enterococcus*;  
 with Gal, e.g. in *Streptococcus*, *Lactobacillus*, *Listeria*.

b) In *Staphylococcus*, *Bacillus*.

c) In *Lactobacillus*.

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### Lipoteichoic acids

*Enterococcus faecalis*

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### Functions of the (lipo-)teichoic acids

- Adhesion to epithel- and endothel cells (*S. aureus*), most likely receptor-specific.
- Lysozyme resistant.
- Resistent against antimicrobial peptides.
- Bind cations, in particular  $\text{Mg}^{2+}$  („Ion reservoir“).
- Biofilm formation.

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### Functions of lipoteichoic acids

- Important in cell division.
- Is believed to be a PAMP.

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