Molecular microbiology and microbe-host interactions: A road to novel anti-infectives?

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Approaches for novel anti-infective therapies

- Vaccination and/or immunotherapy targeting antigens expressed by globally spreading sensitive and resistant bacterial strains.
- Making use of better screening procedures in live organisms.
- Use directed evolution to evolve new antimicrobial products.
- New types of natural product libraries.
- There are still a number of candidate targets for novel antimicrobials.
- Targeting the micro-organisms own death signaling system.
- Targeting central mechanisms for bacterial pathogenicity disarming disease causing ability of the pathogen,
- Enhancing the host’s own antibacterial clearing system.
- Immunomodulation

Beta-lactams and vancomycin inhibit cell wall biosynthesis by blocking the penicillin binding proteins (PBP)

PBP inhibition by beta-lactams is not sufficient for bacterial killing!

- Beta-lactams allow cell wall hydrolases to lyse bacteria.
- Beta-lactams and other bacteriocidal antibiotics induce production of hydroxyl radicals that can kill bacteria.

Cell wall degrading enzymes, like pneumococcal LytA, lyse bacteria in the stationary phase, autolysis

Bacterial lysis caused by beta-lactams, are due to suicidal autolytic enzymes like pneumococcal LytA
The peptidoglycan substrate for LytA is located in a circular area on each side of the bacterial equatorial plane.

Novel small molecules that activate suicidal lysis by the LytA enzyme

Bactericidal antibiotics kill by stimulating bacterial production of hydroxyl radicals

Innate immune proteins (PGRPs) and antibacterial peptides may also kill via oxidative stress

The anionic membrane microdomain for localized secretion (Exportal) is also target for antibacterial peptides

Bacterial Pathogens Disrespect our Phagocytes. Can We Strike Back!

An enterococcal mprF mutant has larger hBD2 foci and is more hBD2 sensitive
**S. aureus Subversion of Host Phagocyte Defense**

Mechanisms of staphylococcal resistance:
- Resistance to oxidative burst killing
- Resistance to antimicrobial peptides
- Nonopsonic phagocytosis or degradation of immunoglobulins
- Intracellular persistence

**Aureolysin**

**First Steps of Staphyloxanthin Biosynthesis Resemble Those of Human Cholesterol Biosynthesis**

**One Cholesterol-Lowering Agent Blocked S. aureus Virulence In Vitro & In Vivo**

**Targeting the golden pigment on Staphylococcus aureus**

**Human squalene synthase inhibitors prevent staphylococcal pigment production**

**Small molecules blocking Type III secretion**
- Expressed by many important Gram-negative pathogens; Pseudomonas, Salmonella, Shigella, Chlamydia etc. These effector proteins perturb phagocytosis and other cellular functions
- TTS inhibitors block the transfer of effector proteins into host cells that perturb macrophage function
Type III secretion inhibitors block the cytotoxic effects of *Yersinia pseudotuberculosis*

![Type III secretion inhibitors block the cytotoxic effects of *Yersinia pseudotuberculosis*](image)

Type III secretion inhibitors block the intracellular growth cycle of *Chlamydia*

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Neutrophil extracellular traps (NETs)

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Statins boost production of neutrophil and macrophage extracellular traps

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Pneumococci lacks catalase and produce large quantities of hydrogen peroxide by the pyruvate oxidase gene *spxB*

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Immunomodulation after pneumococcal infections

- Study human monocyte derived dendritic cells (hMDDC) from buffy coats.
- Single infections as well as co-infections with influenza A virus
Human MDDC responses to pneumococcal infection

Pneumococcal peptidoglycan induces hMDDC cytokine production which in turn triggers a chemokine response in airway epithelial cells.

Supernatants from MDDCs added to D562 cells

Vitamin D3 modulates the human MDDC immune response to pneumococcal peptidoglycan

Peptidoglycan-stimulated human MDDCs promote Th1 (IFN-γ) and Th17 (IL-17) responses in CD4+ memory T cells

Buffy coats from 10 human donors

% T cells producing IFN-γ, IL-17, or CCR7 after co-culture with MDDCs

Supernatants from MDDCs added to T-cells

Model for human MDDC responses to pneumococcal peptidoglycan and the immunomodulatory effects of vitamin D3.
Mechanistic studies on how Influenza A virus sensitizes for *S. pneumoniae* infections using human dendritic cells

- human monocyte-derived dendritic cells
- X31: H3N2, reassortant of PR8 x A/Aichi/68(H3N2)
- TIGR4R: unencapsulated isogenic mutant of TIGR4 serotype

TLR ligands can substitute virus infection to gain synergism in co-infection studies

The duration of IAV infection influences IL-12p70 secretion

The uptake of pneumococci is influenced by the duration of IAV infection

No difference in killing of engulfed bacteria depending on the presence of IAV

Cytokine secretion of co-infected hMDDC

Expression of IL-12p70 and IL-6 is enhanced
Specific induction of the IL-12p35 subunit

mRNA level of IL12-p35 / p40

- Very few co-infected cells detectable

IL-12p70 positive cells are not IAV infected

Supernatants of IAV infected hMDDC:s can mediate synergistic IL-12p70 induction

What mediates synergistic induction of IL-12?

IFNα pretreatment partially mimicks a preceeding IAV infection
Infection with Influenza A virus leads to secretion of cytokines e.g. type I IFNs which confer a priming of uninfected neighbouring cells. This results in increased IL-12p70 secretion after exposure to *Streptococcus pneumoniae*.

IL-12p70 and IL-6 been recognized as a hallmark for critical illness in severe H1N1 pandemic influenza. Bermejo-Martin Crit Care 2009

**Summary**

- Antibiotic resistance is on the rise
- There are novel opportunities for bacterial killing
- There are approaches to sensitize already resistant bacteria
- Novel anti-infective principles; disarming the pathogen or boosting the host response

**Peptidoglycan-stimulated human MDDCs promote Th1 and Th17 responses in CD4+ memory T cells**

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