Alternative to Antibiotics: Selective Modulators of Innate Immunity

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Approaching the Antibiotic Resistance Era

- Multidrug resistance
- Dry pipeline
- Emerging problems
- Animal Uses

There is an urgent need for new strategies to treat infections.
Cationic Peptides

*Birds do it, bees do it, even educated fleas do it....*  Cole Porter

- Important **host defense** mechanism in all complex species of life.

- >1000 peptides known. Diverse amino acid sequences and structures.

- 12 to 40 (or more) amino acids. Net charge +2 to +9 (Lys; Arg). Amphipathic.

- Role in Innate Immunity involves both *antimicrobial* and *immunomodulatory* activities; “Host Defence” peptides.
Peptide Arrays and Machine Learning (QSAR) creates improved 9-mer peptides

- Relates activity to structure (based on primary sequence and physical and inductive “descriptors”); using Neural Networks.
- Trained on tested peptides; predicts activity of virtual peptides
- Very effective and quite accurate for 100,000 peptide library

**Protects in mice (IP and IV)**

**MICs = 0.8 to 6 μg/ml**

- **vs. most Superbugs**
- **10X better than MX-226**;
- **Overall better than 4 leading antibiotics**

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<th>HHC-10</th>
<th>HHC-36</th>
<th>MX-226</th>
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New Therapeutic Possibilities-Anti-Biofilm Action

Peptides kill bacteria in biofilms

Peptides work vs. MDR Gram positive and Gram negative bacteria & AMP resistant bacteria: IC$_{50}$ < 0.25 μg/ml vs. *Pseudomonas*

*AAC 56:2696-704, 2012*
Many natural antimicrobial peptides have very weak direct antimicrobial activity e.g. Human LL-37 vs. *S. aureus*

Ability to stimulate innate immunity may be more important

Dawn Bowdish, Monisha Scott
Immune Modulation Approaches

- Successful antimicrobial therapy requires assistance from the host: immune response
- Immune modulation highly used in antiviral and anticancer therapy


IDSA “Bad Bugs No Drugs”
Modulating Immunity for Therapeutic Benefit

This is an Adjuvant Therapy - Designed to work with Antibiotics

Signal Integration (e.g. TFs, Hubs)

New immunomodulatory peptides show broad protection in Mouse Model Infections

Also protects vs. TB, *E. coli*, *Salmonella*, MRSA, VRE, *P. aeruginosa*, IBD, CF, cerebral malaria, sterile inflammation, etc.

Safety demonstrated in Phase I trials

These peptides developed independently for Grand Challenges Program.

In pig model no effect on infectious load

Lars Steinstraesser, Louis Schofield, Sandra Pillat, Lisa Thorsen, Sarah Mullaly, Bruce Vallance, Brett Finlay
Therapeutic IDR-1018 protects in conjunction with Anti-Malarial Therapy

- Only via IV
- No effect on Parasite load
- Anti-inflammatory

Systems Biology
Network analysis – IDR peptides

IDR1 treatment of Human Monocytes

Top Hubs
TRAF2; CRK; HSPA1B; RELA (MAPK & Chemokine Induction)
TNFAIP3; SMAD3; CUL-1 (Anti-Inflammatory & TGFβ)
CREBBP1; IRF7; AKT2; GRB2 (JAK-STAT & IFN)

Interactions between differentially expressed Genes

NB. RNA-Seq delivers 897 genes

Highly connected Nodes (Hubs) & Bottlenecks

688 nodes
2895 Edges
Monocytes/Macrophages are essential and recruitment is enhanced in infection model

Mechanism in animal models

- Peptide protects with pre and post treatment (A)
- Pro-inflammatory cytokines TNFα (C) and IL6 reduced. Anti-inflammatory cytokine IL-10 increased (D)
- Macrophages increase at the site of infection (B)
- Macrophages (liposomal chlodronate) but not neutrophils or lymphocytes are essential for protection

Balancing of Inflammation in vivo

Summary: Normal immunity protects but can lead to potentially harmful inflammation.

Bacteria can lead to potentially harmful inflammation.

LPS/LTA

Effector Mechanisms To Kill Microorganisms

Potentially Harmful Inflammatory Responses

Stimulation of Innate Immunity

Infections cause 33% of all deaths worldwide; 100,000 Americans die from antibiotic resistant infections.

Sepsis kills >200,000 people in USA annually & 5 million worldwide.
Summary: Peptides Boost Innate Immunity and Block Harmful Inflammation

Selective boosting of innate immunity represents a new adjunctive approach to treating infections: Increasing recruitment of immune cells while suppressing inflammation. Much needed as antibiotic resistance rises.
Triple adjuvant formulation (TAF) gives the potential for single dose protection vs. pertussis.

Overcoming the Roadblock of Multiple Dosing

**Graph: High Titre Mixed Th1, Th2 Protective Pigs, Cattle, Mice Works in Neonates**

- **PTd with TAC**
- **PTd & PP & CpG**
- **PTd & PP**
- **PTd & CpG**
- **PTd & HH2**
- **PTd (pertussis toxoid alone)**

**Vaccination Site: Ivory Coast**

**References:**

- *Vaccine* 27:2055-2064, 2009
- *Vaccine* 27:4662-4671, 2009

**Key Components:**

- CpG ODN
- Polyphosphazene; PP
- Host Defence Peptide (HH2)

*Volker Gerdts, Lorne Babiuk, Bob Hancock and many others*
Features of the Adjuvant Formulation

Duration of immunity > 2 years (mice)
>10 months in pigs

Comparison to Alum (2 different doses)

Protective – Neonatal pigs

Works mucosally at very low doses

Volker Gerdts, VIDO
Acknowledgements

Lab: Laure Janot, Matt Mayer, Chris Fjell, Ana Nijnik, Neeloffe Mookherjee, Jason Kindrachuck, Reza Falsafi,

Collaborators: Volker Gerdts; Bruno Rivas, Rogelio Hernandez; Edie Dullaghan and CDRD; Louis Schofield; Brett Finlay

Grand Challenges in Global Health

Pathogenomics of Innate Immunity