Manipulating the host response to treat infections

Guest speakers: R.E.W. (Bob) Hancock, Henk P. Haagsman
Moderator: Neeloffer Mookherjee
Host: Astrid Pentz-Murr (GARDP)

14 January 2021
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Today’s speakers

Manipulating the host response to treat infections

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UBC Killam Professor and Canada Research Chair
University of British Columbia (Canada)

Henk P. Haagsman
Professor of Molecular Host Defence
Utrecht University (Netherlands)

Moderator:
Neeloffer Mookherjee
Associate Professor
Departments of Internal Medicine and Immunology, University of Manitoba (Canada)
Introduction

Manipulating the Host Response to Treat Infections

Moderator:
Dr. Neeloffer Mookherjee

Associate Professor, Departments of Internal Medicine and Immunology
University of Manitoba, Canada

Chair, Canadian Institutes of Health Research Sex and Gender Science in Respiratory Health

Chair, WISDOM (Women in Science: Development, Outreach and Mentoring) Manitoba, Canada

https://www.mookherjeelab.com  @NMfromUManitoba
The Antibiotic Development Paradigm is Fractured

- Antibiotics are the single most successful medical intervention
- Dramatic increase in antibiotic resistance
- Dry pipeline; Traditional and genomics-based approaches to antibiotic development has not worked well.

Current Topics in Medicinal Chemistry (2017), 17, Issue 2
Defeating Antibiotic Resistance:
There is an urgent need for Alternate Strategies

More than 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people die as a result.

GLOBAL
A failure to address the problem of antibiotic resistance could result in:
10m deaths by 2050
Costing £66 trillion

Public Health England (Gov.UK)

https://www.cdc.gov
When do antibiotics fail?

1. Microbial Resistance (mutations/plasmids etc.)

2. Chronic Infections especially biofilms: 65% of all infections, highly resistant & associated with harmful inflammation

3. Sepsis: a dysregulated host response to infection (~20-35% death rate; 1 million deaths annually)

4. Individuals with dysregulated immune responses and immunosuppressed: chemotherapy, immuno-suppressive disease, genetic diseases, injuries or burns.

Hancock, R.E.W. 2020. The critical need for alternative approaches to address antibiotic treatment failure. https://revive.gardp.org/the-critical-need-for-alternative-approaches-to-address-antibiotic-treatment-failure/
Harnessing Host Defense: Immune Modulation

- Successful therapy with help from the host’s immune response
- Immune Modulation & Host Directed Therapies - used in antiviral and anticancer therapies

Cationic Host Defence Peptides

REGULATION AND REPROGRAMMING OF THE INNATE IMMUNE SYSTEM AS ALTERNATIVE TO ANTIBIOTICS

Henk P. Haagsman, Maaike R. Scheenstra, Edwin J. A. Veldhuizen & Albert van Dijk

Division of Infectious Diseases & Immunology
Organisation of the presentation

• Introduction: Host Defence Peptides (HDPs)
• Cathelicidin-derived peptides: paradigms for anti-inflammatory antimicrobials
• Cathelicidin-derived peptides: immunomodulatory activities
• Antimicrobial strategies involving HDPs
• Host-directed therapy by innate immune training via epigenetic reprogramming?
• Conclusions
Innate Host Defence

- Present in all organisms
- Limited repertoire of molecules
- Rapid
- Broad specificity
- Ancient

The first line of defence against infections

**Cellular defences**
- Neutrophils (heterophils)
- Macrophages, Innate Lymphoid Cells, Epithelial cells

**Effector molecules**
- Enzymes, Host Defence Peptides, Collectins, etc.
Examples of vertebrate Host Defence Peptides

CATH-2

HBD-2

HD-5

LL-37

Magainin-2

Amino acid side chains: red, hydrophobic; blue, basic; green, acidic

Mookherjee et al. Nat. Rev. Drug Discov., 2020
CATH-2 is produced by chicken heterophils

Van Dijk et al. Mol. Immunol., 2009
*Salmonella enteritidis* challenge of chickens results in recruitment of CATH-2 containing heterophils

*Van Dijk et al. Mol. Immunol., 2009*
Antimicrobial activities of HDPs

Visualisation of interactions of CATH-2 with bacteria
Fast membrane binding and permeabilisation of *E. coli* by CATH-2

Fast membrane binding and permeabilisation of *S. aureus* by CATH-2

Schneider et al. *mSphere*, 2017
Antibacterial mechanisms of Cationic Host Defence Peptides

Mookherjee et al. Nat. Rev. Drug Discov., 2020
Functions of host defence peptides

HDPs reduce the inflammatory response
Example:
CATH-2 “silently” kills *P. aeruginosa* *in vitro* and *in vivo*

Coorens et al. *Infect. Immun.*, 2017
HDPs increase the inflammatory response
Example: CATH-2 enhances DNA uptake by macrophages

Enhancement of TLR9 (mammals) or TLR21 (birds) activation by CpG in vaccinations

Coorens et al. J. Immunol., 2015
HDP-derived products as alternatives to antibiotics

**Strategies:**

- Direct administration:
  - Antimicrobial activity
  - Immunomodulatory activities

- Stimulation of endogenous production of HDPs
Host-directed therapy by increasing production of HDPs

Example:
Application of histone deacetylase (HDAC) inhibitors
Induction of innate effectors in phagocytes and epithelium

Bergman et al. Front. Immunol., 2020
Immunomodulation by Cationic Host Defence Peptides

Mookherjee et al. Nat. Rev. Drug Discov., 2020
Prophylactic potency of CATH-2 and derivatives in veterinary medicine

*In ovo* administration (chicken embryos)
*In ovo* D-CATH-2 administration protects from colibacillosis

*CATH*
1 mg/kg

**E. coli**
Intratracheal (i.t.)
1*10^6 CFU

**Peptide**
Day -3

**Hatch**
Day 0

**Infection**
Day 7

**Sacrifice**
Day 14

**Mortality**

- Uninfected
- Infected
- D-CATH-2

**Mean lesion score**

**Morbidity**

- Uninfected
- Infected
- D-CATH-2

*Cuperus et al. Sci. Rep., 2016*
Efficacy of D-CATH-2 in a zebrafish infection model

Zebrafish embryos

Yolk injection
2.6 ng/kg D-CATH-2

Salmonella enteritidis
10-100 CFU/embryo

# D-CATH-2 via embryonic route of administration

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<th>Challenge</th>
<th>Result</th>
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<td>Chicken</td>
<td><em>E. coli</em> (i.t.)</td>
<td>Mortality 30%</td>
</tr>
<tr>
<td>3 days pre-hatch</td>
<td>7 days post hatch</td>
<td>Morbidity 52%</td>
</tr>
<tr>
<td></td>
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<td>Bacterial load 93%</td>
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<tr>
<td>Chicken</td>
<td><em>S. enteriditis</em> (s.c.)</td>
<td>Mortality 50%</td>
</tr>
<tr>
<td>3 days pre-hatch</td>
<td>3 days post hatch</td>
<td>Morbidity 67%</td>
</tr>
<tr>
<td>Zebrafish</td>
<td><em>S. enteriditis</em></td>
<td>Mortality delayed 24 h</td>
</tr>
<tr>
<td>28 hpf</td>
<td>18-20 hpi</td>
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</tbody>
</table>

- Peptide doses in models are too low to be directly antimicrobial
- Efficacy despite 6 to 10 day ‘gap’ between treatment and challenge!
Amtimicrobial potency of HDPs in mammalian infection models

Presented by dr. Hancock in following presentation of this webinar
Peptide-induced innate immune memory?

CATH-2

trained immunity

infection

enhanced threshold

time

Strength Immune response

Strength Immune response
Epidemiological observations of non-specific effects of vaccines

• Live vaccines induce cross-resistance
  • BCG
  • Measles-containing vaccines
  • Oral polio vaccines
  • Vaccinia against smallpox

• Inactivated vaccines induce cross-resistance
  • Diptheria-tetanus-pertussis containing vaccines
  • Hepatitis B vaccine
  • Inactivated polio vaccine

Adapted from Jensen et al. Semin. Immunol., 2016
Pathogen-associated molecules and cross-protection

<table>
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<tr>
<th>Component</th>
<th>Source</th>
<th>Cross-protection</th>
<th>Reference</th>
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<tr>
<td>LPS (endotoxin)</td>
<td>G(-) bacteria</td>
<td>Staphylococcus aureus</td>
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<tr>
<td>Peptidoglycan (muramyl dipeptide)</td>
<td>Bacteria</td>
<td>Toxoplasma</td>
<td>Krahenbuhl (1981)</td>
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<tr>
<td>Flagellin</td>
<td>G(-) bacteria</td>
<td>Streptococcus pneumoniae Rotavirus</td>
<td>Munoz (2010)</td>
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<td>FimH (adhesin)</td>
<td>Escherichia coli</td>
<td>Influenza virus</td>
<td>Abdul-Careem (2011)</td>
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<td>β-glucan</td>
<td>Fungi</td>
<td>S. aureus S. pneumoniae</td>
<td>Marakalala (2013)</td>
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<td>Chitin</td>
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<td>S. aureus E. coli</td>
<td>Rizzetto (2016)</td>
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</table>

Adapted from Sánchez-Ramón et al. Front. Immunol., 2018
Trained immunity regulatory pathways

Application of trained immunity inducers

Sánchez-Ramón et al. Front. Immunol., 2018
Applications of Host Defence Peptides

Therapeutic use:
• As immunomodulatory antimicrobials
• As adjunct to antibiotics

Prophylactic use:
• Regulation/reprogramming innate immune system in veterinary relevant species (generic protection)
• In vaccines as adjuvant
Section Molecular Host Defence

Andrea Bosso
Maarten Coorens
Tryntsje Cuperus
Soledad Ordonez
Viktoria Schneider
Weidong Zhang

Albert van Dijk
Martin van Eijk
Marina Kraaij
Maaike Scheenstra

Hanne Tjeerdsma
Edwin Veldhuizen
Antibiotic Resistance.
Immunomodulatory Strategies

R.E.W. (Bob) Hancock, PhD, OC
UBC, Vancouver, Canada

Conflict of Interest statement: Formed 2 virtual R&D companies to enable commercial development (through partnering) of peptides and sepsis diagnostics
Antibiotic Adjuvants/Adjuncts

Alternatives to antibiotics—a pipeline portfolio review

Antibiotics have saved countless lives and enabled the development of modern medicine over the past 70 years. However, it is clear that the success of antibiotics and vaccines has come at a cost. We now expect a long-term and perhaps never-ending challenge with antibiotic-resistant bacteria. A broader approach to address back this epidemic, we discuss alternatives to antibiotics, which we defined as non-compounds (other than classic antibacterial agents) that target bacteria or any

Host Directed therapies

Antibodies; Vaccines; Probiotics; Lysins; Wild Type and Engineered Bacteriophages; Immune Stimulation; Antimicrobial peptides; Innate Defence Regulators; Anti-biofilm Peptides
When do antibiotics fail?

1. Resistance (mutations/plasmids etc.)

2. Sepsis (~20-35% death rate; 11 million deaths annually). Dysfunctional immune response.

3. Chronic Infections especially biofilms (65% of all infections and very resistant). Associated with chronic inflammation.

4. Individuals with disturbed immune systems (chemotherapy, immuno-suppressive disease, genetic diseases, massive injuries or burns).

Indications 2 to 4 are rarely taken into account when developing new anti-infectives.
Immune Modulation

- Successful therapy against infections requires help from the host: immune response

- Immune modulation is highly used in antiviral and anticancer therapy

- Less commonly used for bacterial infections

Immune Modulation
Bacteria-based Products for Respiratory Diseases

- 35 placebo-controlled trials (4,060 participants) provided data in a form suitable for inclusion in the meta-analyses.
- Trial quality was generally poor and a high level of statistical heterogeneity was evident.
- By combining results, immunostimulants reduced incidence of Acute Respiratory Tract Infections by an average of 39% reduction cf. placebo.
- No difference in adverse events was evident between placebo and treated groups.
- Utilized in Latin America and Eastern Europe
- Many other immune modulators
- Need to understand mechanisms

Probiotics

- Use of bacteria to prevent infections
- Several mechanisms proposed, e.g. exclusion, immune modulation
- Already used successfully
  - As OTC medicines: *S. salivarius* clinically proven for Strep throat, Halitosis, Chronic sore throat
  - In fecal transplantation for *C. difficile*, and
  - Reduces sepsis (24% → 15%) & enterocolitis (4.6 → 2.5%) in 10,000 premature Very Low Birthweight Babies
- Consortia of defined bacteria can replace normal microbiota


*Denkel et al. 2015 Antimicrobial Resistance & Infection Control 2015, 4(S1):O39*
New immunomodulatory peptides show broad protection in Mouse Model Infections

Invasive Staph. aureus Mouse Model

MDR -TB Mouse Model

Cerebral Malaria Mouse Model

Also protects in animal models vs. E. coli, Pseudomonas, MRSA, Salmonella, Klebsiella, Tuberculosis; Candida; Pox & HSV viruses; Sterile inflammation; Malaria, Inflammatory Bowel Disease, Cystic Fibrosis (cells); LPS/hypoxia-ischemia;

\[ \text{Wound Healing} \]

IDR 1018: VRLIVAVRIWRR-NH₂

Lars Steinstraesser, Louis Schofield, Ariel Achtman, Bruno Rivas, Rogelio Pando, Carina Mallard, Octavio Franco
Balb/c mice infected intratracheally with high dose $10^6$ virulent live *MTb* strain; Multi-drug Resistant (MDR) or H37Rv.

After 2 months of infection, treatment via IT: 32 $\mu$g (~1 mg/kg) peptide every 2 days.

Groups of 5 animals sacrificed after 4 wks of treatment and lung bacilli counted.

RNA-Seq and bioinformatics studies on mouse lungs are deciphering mechanisms in vivo

Intranasal delivery (single dose prophylactic) *Pseudomonas aeruginosa* lung infections

**Protective Immunity↑**

**Harmful Inflammation↑**

**Effect of Microbes**

**Protective Immunity↑**

**Harmful Inflammation↓**

**Effect of Peptides**

**Interaction network from whole lungs of mice infected with PA103 vs. control.**

- 4,739 genes DE:
- Many immune (+ inflammatory) pathways↑
- Extracellular matrix↓

Network for PA103+IDR-1002 cf. PA103

- 2,111 genes DE
- Immune system pathways↓
- Metabolism, T	extsubscript{h} cell differentiation & VEGF regulation↑

IDR-1002 given intranasally 24 hr prior to infection

**NetworkAnalyst**
IDR peptides can treat sterile inflammation

- Mouse Ear sterile inflammation model
- Induced with phorbyl myristate (PMA)
- Treat with peptides topically @ -30 min

Assessing production of reactive oxygen and nitrogen species with probe LO-12

PMA + IDR-1002

Ear swelling @ 6 hours

Suppressive effects on IL-6, MCP-1, KC
Occurs at level of transcription (IRF8)
≡ Indomethacin

Modelling High Density Infections by *ESKAPE* pathogens in mice - Tracking infection and inflammation in a chronic model

*P. aeruginosa* LESB5

Infection: *lux*

Neutrophil tracker

Reactive Oxygen Species

Intravenous antibiotics at 6X the human dosage did not work

- Intra abscess worked poorly
- For some antibiotics 1,000X the human dose didn't work
- Challenging model for recalcitrant chronic infections (cf. biofilms)

Pletzer et al, 2017. mBio 8:e00140-17
Biofilms: an alternative growth state & highly resistant to most conventional antibiotics

- Biofilms are found everywhere; form on surfaces and are often an adaptation to stress
- Cause >65% of all bacterial infections (clinically relevant) e.g. devices, lung, bladder, wound, oral, ears, nose & throat, skin, etc.
- Chronic infections that lead to chronic and harmful inflammation
- Highly adaptively resistant
- No specific treatments!!

**Pseudomonas aeruginosa**

- **MIC** = 0.2 µg/ml
- **MIC**_{cipro} = 0.25 µg/ml

**S. aureus**

MRSA

$\leftarrow$ MIC = 0.2 µg/ml

Biofilms are found everywhere; form on surfaces and are often an adaptation to stress. Cause >65% of all bacterial infections (clinically relevant) e.g. devices, lung, bladder, wound, oral, ears, nose & throat, skin, etc. Chronic infections that lead to chronic and harmful inflammation. Highly adaptively resistant. No specific treatments!!
Broad Spectrum Anti-Biofilm Activity when added at 0 or 2 days of biofilm formation

Kills all ESKAPE pathogens

Peptides work rapidly (3 min) vs multispecies oral biofilms & synergize with EDTA & Chlorhexidine →

And work on dentin biofilms (left) ↓

Anti-biofilm peptides are effective against high density infections and synergize with conventional antibiotics in vivo

- Peptides in combination with antibiotics work better than either agent alone.
- Observed for all ESKAPE pathogens + E. coli (several antibiotics)


NB: Larger effect on abscess formation (pathology) than bacterial numbers. Can we also impact on biofilm-mediated inflammation?

DJK5 + Azithromycin

NB: Larger effect on abscess formation (pathology) than bacterial numbers. Can we also impact on biofilm-mediated inflammation?

Random Screening for New Peptides: Spot Synthesis of Peptides using a Pipetting Robot

Solid phase synthesis (peptide array) method synthesizes peptides from C-terminus on cellulose sheets.
Structure Activity Relationships of Immune Modulation & Anti-Biofilm activities overlap

Based on lead peptides. Substituting every position with 9 most common amino acids → Identification of many peptides with better or similar activities (dark red cf. grey).

Evan Haney et al. Peptides 71:276-85, 2015; Sci. Reports 8:1871, 2018

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Chemokine induction

Anti-inflammatory
Peptides work in a Human Skin Model reducing biofilms and suppressing inflammation

Catherine Wu. NPJ Biofilms and Microbes in press

↑ Staphylococcus aureus
↓ Pseudomonas aeruginosa

4 sec thermal injury at 100°C
Treating infection plus inflammation: In-vivo chronic lung/sinus infections using LESB65 in alginate

- Sinusitis very common; >29.2 million in USA; est. aggregated cost $8B.
- CRS is caused by a bacterial biofilm infection of the sinus cavity that causes chronic inflammation.
- Antibiotics are used a lot but are not effective for CRS. No approved drugs for management of CRS.

Grace Choi
Morgan Alford

Potent inflammation

P. aeruginosa (lux) LESB65 in alginate

DJK5 treatment (Respimat)
Crispr mutant library methods reveal 185 mutant genes that are required for *Salmonella* infection of macrophages

GeCKOv2 gRNA human library

Mutant cells of Cas9-THP-1 monocytes

PMA

30min infection

*S. Typhimurium* MOI = 400

FACS sort resistant macrophages

Hi-Seq to identify gRNAs → Resistance

Mutant macrophages - No GFP *Salmonella* (control)

Mutant macrophages + GFP *Salmonella*

Amy Yeung, Gordon Dougan, Allan Bradley, Chris Hale, Sanger Team. 2019. mBio 10(5):e02169-19,
New Model for finding Host-directed therapies: Human stem Cells

Chlamydia

Chlamydia is the world’s leading cause of STD & preventable blindness.

Human Chlamydia only grows in human cells; Macrophages involved in pathology

hIPSdM provide a tractable model

 Enables study of infection genetics for essential genes/targets (Cas9/CRISPR)

Chlamydia → 2,029 genes with altered expression in hIPSdM & human macrophages

Effect of IRF5-/- on Gene expression after Chlamydia infection; Amy Lee

Chlamydia infection at 24 hr

Yeung et al. 2017. Nature Communications 8:15013
Conclusions

- We need new anti-infectives. Resistance is very complex. And we are not properly addressing the reasons for antibiotic failure.
- Given our dismal recent success, what if we do not discover new antibiotics?
- Immune modulation as an adjunctive treatment provides significant opportunities. Not discussed - Vaccines, Therapeutic Monoclonal Antibodies

Acknowledgements: Cast of dozens of trainees, Much funding from taxpayers and companies, Many excellent collaborators
Thank you for joining us

Visit now revive.gardp.org/webinars to find more webinars about antimicrobial drug R&D