REPROGRAMMING THE INNATE IMMUNE SYSTEM AS AN ALTERNATIVE

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Innate Host Defense

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

Cellular defenses
- Neutrophils (heterophils)
- Macrophages, NK-, IL-cells

Effector molecules
- Enzymes, Host defense peptides, Collectins

The first line of defense against infections
Examples of vertebrate host defense peptides

Amino acid side chains: red, hydrophobic; blue, basic; green, acidic
CATH-2 is produced by chicken heterophils

Heterophils

Mononuclear cells

Giemsa

Anti-CATH-2

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

Van Dijk et al., 2009a
Visualization of CATH-2 / *E. coli* interactions

![Diagram showing interactions between CATH-2 and E. coli, including RNA, LPS, LP, DNA, Flagellin, andMount in confocal microscope.]
Live imaging of CATH-2-mediated *E. coli* killing

Schneider et al., 2016
Fast membrane binding and permeabilization of *E. coli* by CATH-2
Fast membrane binding and permeabilization of *S. aureus* by CATH-2

Schneider et al., 2017
Antibacterial mechanisms of Cationic Host Defense Peptides

Mookherjee et al. Nat. Rev. Drug Discov. 2020
Functions host defense peptides

Cuperus et al. 2013
Antimicrobial activity vs. inhibition of macrophage activation

Viability

CATH-2 (µM)

E. coli (CFU/ml)

0
0.15
0.31
0.62
1.25
2.5
5
10
10^4 10^5 10^6 10^7

TNFα

CATH-2 (µM)

E. coli (CFU/ml)

0
0.15
0.31
0.62
1.25
2.5
5
10
10^4 10^5 10^6 10^7

Antimicrobial threshold

Inhibitory activity

CATH-2 (µM)

Coorens et al., 2017
Cathelicidin-mediated “silent killing”
Host Defense Peptides / Antimicrobial Peptides as anti-infectives

**Strategies:**

- Administered HDP-based peptides as alternatives to antibiotics
- Gut: feed additives that stimulate endogenous production of HDPs
  (presentation of dr. Guolong Zhang)
Immunomodulation by Cationic Host Defense Peptides

Mookherjee et al. Nat. Rev. Drug Discov. 2020
CATH-2 enhances DNA uptake by macrophages

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

Coorens et al. 2015
Zelnate® DNA Immunostimulant: a new chapter in BRD management

Bovine Respiratory Disease (BRD) is a complex disease with many contributing factors—including stress that compromises the immune system. Even though there are many antimicrobial options for BRD, veterinarians and producers continue to look for new technology to treat BRD. Zelnate DNA Immunostimulant is a novel, non-antibiotic technology that enhances the animal’s natural defenses against BRD. Learn more about BRD

Zelnate is the first licensed immunostimulant that aids in the treatment of BRD associated with Mannheimia haemolytica.
Prophylactic potency of HDPs and derivatives

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

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

Mortality

Mean lesion score

Morbidity

Cuperus, et al., 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

22h after infection

Schneider et al. (2016c)
D-CATH-2 via embryonic route of administration

<table>
<thead>
<tr>
<th>Target species</th>
<th>Challenge</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<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>
<td></td>
<td>Bacterial load 93%</td>
</tr>
<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>
<td></td>
</tr>
</tbody>
</table>

- Peptide doses in models are too low to be directly antimicrobial
- Efficacy despite 6 day to 10 day ‘gap’ between treatment and challenge!
New immunomodulatory peptides show broad protection in Mouse Model Infections

Observations by Hancock Laboratory (UBC, Vancouver)

Invasive Staph. aureus Mouse Model

MDR -TB Mouse Model

Cerebral Malaria Mouse Model

Science Transl. Med. 4:135ra64, 2012

Plos One 8:e59119, 2013

IDR 1018: VRLAVAVRIWRR-NH2

Also protects vs. E. coli, Salmonella, Klebsiella, Pseudomonas, MRSA, Tuberculosis, Pox & HSV viruses

IBD, CF, Sterile inflammation; LPS/hypoxia-ischemia

→ Wound Healing

Science Transl. Med. 4:135ra64, 2012

Grand Challenges in Global Health

Lars Steintraesser, Louis Schofield, Ariel Achtman, Bruno Rivas, Rogelio Hernandez Pando, Carina Mallard
Pharmacokinetics of IDR peptides
Hancock Laboratory (UBC, Vancouver)
Peptide-induced innate immune memory?

CATH-2

trained immunity

infection

enhanced threshold

time

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
  - Diphteria-tetanus-pertussis containing vaccines
  - Hepatitis B vaccine
  - Inactivated polio vaccine

Jensen et al. (2016)
### Pathogen-associated molecules and cross-protection

<table>
<thead>
<tr>
<th>Component</th>
<th>Source</th>
<th>Cross-protection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS (endotoxin)</td>
<td>G(-) bacteria</td>
<td><em>Staphylococcus aureus</em></td>
<td>Breyne (2017)</td>
</tr>
<tr>
<td>Peptidoglycan (muramyl dipeptide)</td>
<td>Bacteria</td>
<td>Toxoplasma</td>
<td>Krahenbuhl (1981)</td>
</tr>
<tr>
<td>Flagellin</td>
<td>G(-) bacteria</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>Munoz (2010)</td>
</tr>
<tr>
<td>FimH (adhesin)</td>
<td><em>Escherichia coli</em></td>
<td>Influenza virus</td>
<td>Abdul-Careem (2011)</td>
</tr>
<tr>
<td>β-glucan</td>
<td>Fungi</td>
<td><em>S. aureus</em> <em>S. pneumoniae</em></td>
<td>Marakalala (2013)</td>
</tr>
<tr>
<td>Chitin</td>
<td>Fungi</td>
<td><em>S. aureus</em> <em>E. coli</em></td>
<td>Rizzetto (2016)</td>
</tr>
<tr>
<td>CpG oligonucleotide</td>
<td>Bacteria (synthetic)</td>
<td><em>E. coli</em> Influenza virus</td>
<td>Ribes (2014); Jiang (2011); Norton (2010)</td>
</tr>
</tbody>
</table>

Adapted from Sánchez-Ramón et al.
Trained immunity regulatory pathways

Mulder et al. (2019)
Innate immune training of THP-1 cells by CATH-2

van Dijk et al. unpublished
Innate immune training of THP-1 cells by CATH-2

van Dijk et al. unpublished
Innate immune training of THP-1 cells by CATH-2

induces glycolysis

- primes cytokine response to multiple TLR ligands (TLR4 & 2)
- involves changes in cell metabolism (mTOR pathway)
- requires MAPK p38 signaling, but not NFkB signaling
- is probably epigenetic regulation
- involves P2/P2X7 activation
- requires peptide uptake

van Dijk et al. unpublished
Innate immune training *in vivo* (mice)

CATH D-CATH-2 (1-21)

1 mg/kg

Peptide

Day 0

Sacrifice

Day 1

Sacrifice

Day 7

Weight difference from start

Blood

Spleen

T cells

B cells

Scheenstra et al. unpublished
Innate immune training *in vivo* (mice)

**Spleen**

**Macrophages**

- Control
- DC(1-21)

**Dendritic cells**

- Control
- DC(1-21)

**Scheenstra et al. unpublished**
Total splenocyte activation

**CATH**

D-CATH-2 (1-21)

1 mg/kg

Single cell suspension

Activation (24h)

Peptide

Day 0

Sacrifice

Day 1

- **Control**
- **DC(1-21)**

### Extracellular TLRs

- **LPS**
  - TLR-4
  - IL-6 (pg/ml)
  - 50 ng/ml
  - 100 ng/ml

- **PAM2CSK4**
  - TLR-2/6
  - IL-6 (pg/ml)
  - 10 ng/ml

- **PAM3CSK4**
  - TLR-1/2
  - IL-6 (pg/ml)
  - 10 ng/ml

### Intracellular TLRs

- **R848**
  - TLR-7 & TLR-8
  - IL-6 (pg/ml)
  - 100 ng/ml

- **PGN**
  - NOD-1 & NOD-2
  - IL-6 (pg/ml)
  - 100 ng/ml

- **ODN-1826**
  - TLR-9
  - IL-6 (pg/ml)
  - 100 nM

- **PAM3CSK4**
  - TLR-1/2
  - IL-6 (pg/ml)
  - 10 ng/ml

**Scheenstra et al. unpublished**

• Increased response against different TLR agonists
Enhanced activation of bone marrow derived macrophages

**CD86 expression**

**Macrophages**

- Control
- DC(1-21)

**Dendritic cells**

- Control
- DC(1-21)

Peptide

- Day 0

Sacrifice

- Day 7

**Peptide**

- LPS-EB
- PAM2
- PAM3
- PGN-EB

**Scheenstra et al. unpublished**
Trained immunity at the epigenetic, cellular and systems level

- **Cellular level**
  - Naive myeloid cell
  - Activated myeloid cell
  - Quiescent myeloid cell
  - Activated myeloid cell

- **Systems level**
  - 'Trained' myeloid cell

- **Targeted immunotherapy**
  - GM-CSF
  - IL-1β
  - PAMPs
    - β-Glucan
    - Peptidoglycan
    - BCG

Mulder et al. (2019)
Application of trained immunity inducers

Sánchez-Ramón et al. (2018)
Applications of cathelicidin-derived peptides

Therapeutic use:
• Direct antimicrobial activity
• As adjunct to antibiotics

Prophylactic use:
• Immunomodulation (generic protection)
  • mammals, fish, birds (*in ovo*)
• In vaccines as adjuvant
Division Molecular Host Defence

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