Immunomodulators derived from host defence peptides

Albert van Dijk

Department of Infectious Diseases and Immunology
Faculty of Veterinary Medicine
Utrecht University
The Netherlands
Innate Host Defence

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

The first line of defence against infections
Host defence peptides

- <100 amino acids
- Cationic
- Amphipathic
- Classes/structure
- Antimicrobial
- Immunomodulatory
Differentiation

DNA binding/uptake

LPS inhibition

Chemotaxis

Immune activation

Phagocytosis

Wound-healing

Direct killing

Cationic peptides

Macrophage

B-cell

Monocyte

T-cell

Neutrophil

Gram-negative

Neutrophil

Dendritic cell

Macrophage

NO

Cytokines

Chemokines

PBMC

Chemokines

Chemokines

Gram-negative

Gram-positive

Fungi
Host defence peptides and susceptibility to infections

Strategies

• Feed additives that stimulate endogenous production of HDPs
• Use HDPs as markers for breed selection
• Alternatives to antibiotics based on host defence peptides (HDPs)
Chicken cathelicidin 2 (CATH-2)

- Cationic (+9)
- 26 amino acids
- Amphipathic

Van Dijk et al., 2005
Xiao et al., 2006
CATH-2 is produced by chicken heterophils

Heterophils

Mononuclear cells

Giemsa

anti-CATH-2

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

A

Control 8h p.i.

B

Control 48h p.i.

C

S. enteritidis 8h p.i.

D

S. enteritidis 48h p.i.

Van Dijk et al., 2009
Development of CATH-2 as an immunomodulator

ALTAN - ASIA

IMMUNO VALLEY
Connecting Human and Animal Health

Ministry of Economic Affairs

Universiteit Utrecht
Immunomodulation to enhance host defence

Natural HDPs

Strength Immune response

infection

enhanced trained response
time
In vivo models

CATH-2

- Chicken: Salmonella enteritidis, E. coli
- Zebrafish: Salmonella enteritidis

IDRs

- Mice: Staphylococcus aureus, Mycobacterium tuberculosis, Plasmodium berghei
**E. coli 506 intratracheal challenge model**

- **ED18**
- **ED21**
- **D7**
- **D14**

1 mg/kg D-CATH-2 or vehicle

Colibacillosis
- Mortality
- Mean Lesion Score (MLS)
- Air sac colonization

IT
1x10^6 CFU

N=41
7 d p.i.
E. coli 506 (IT) model: mean lesion scores at 7 d p.i.

Mean Lesion Score d7 p.i.

E. coli 506 (IT) model: mean lesion scores at 7 d p.i.

Cuperus / van Dijk et al. 2016
*E. coli* 506 (IT) model: bacteria in air sacs at 7 d p.i.
Conclusions
Colibacillosis model

D-CATH-2 in ovo

- 30% decreased mortality
- 52% reduction in number of birds with clinical symptoms
- 64% decrease in average lesion scores
- 93% reduction of *E. coli* colonisation in air sacs
Zebrafish model

- zebrafish embryos
- Yolk-injection
  2.6 ng/kg D-CATH-2
- Infection model:
  S. enteritidis pGMDs3
  10-100 CFU/embryo

→ 22 h p.i fluorescence
CATH-2 enhances number of phagocytic cells in zebrafish model

- In absence of infection:
  - 2.6 ng/kg D-CATH-2 → imaging (48 hpf)
D-CATH2 via in ovo route of administration

- Protective against different pathogens
- Protective in different species
- Prophylactic use
New immunomodulatory peptides show broad protection in mouse model infections

Bac2a
RLARIVVIRVAR-NH2

Immune Defence Regulators (IDRs)

IDR-1018:
VRLIVAVRIWRR-NH₂

Bob Hancock
University of British Columbia, Vancouver, Canada
Immune defence regulators (IDRs) protect against invasive *Staphylococcus aureus* infections

- C57BL/6 mice
- i.p. 4 mg/kg IDRs
- 4 h → i.p. challenge $10^8-10^9$ CFU *S. aureus*
- Peritoneal lavage (24h p.i.) → bacterial counts

![Graph showing bacterial counts in saline and IDR-1002 treatments.](image)

Nijnik et al. J Immunol 2010
Rivas et al. PLoS One 2013
IDR1018 protects against MDR-TB infections

- Balb/c mice
- $10^6$ CFU live multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB)
- 2 months infection
- treatment via IT route: 1 mg/kg IDR1018 every 2 days
- After 4 weeks treatment: bacterial load in lungs ➔ lung inflammation

*Rivas et al. PLoS One 2013*
Anti-malaria action of IDR1018

- C57BL/6 mice
- P. berghei ANKA model
- 90-100% mortality 6-8d p.i.
- 10-15% parasitic burden
- Adjunctive therapy: 24mg/kg IDR-1018 i.v. at d4, 5, 6 + anti-malarials d4-d11
- 75% survival at 13d p.i.

Achtman et al. Sci Trans Med 2012
Possible applications of cathelicidin-derived peptides

Prophylactic use:
- Immunomodulation *in ovo*
- Post-natal immunomodulation
- As adjunct to vaccines

Therapeutic use:
- Direct antimicrobial activity (not systemic)
- Indirect as adjunct to antibiotics