Antimicrobial Peptides in Defense and Inflammatory Skin Diseases

Richard L. Gallo, M.D., Ph.D.
Professor of Medicine and Pediatrics,
Chief, Division of Dermatology

University of California, San Diego

The Problem

- Anthrax
- Pseudomonas
- HHV 6
- Malasessia furfur
- Mycobacterium TB
- HIV
- Staph aureus
- HHV 6
- Pseudomonas
- Malasessia furfur
- Anthrax
- HIV
- Staph aureus
Microbes multiply quickly

62,914,560

Streptococcus

15 Streptococcus pyogenes

6.5 hrs

Innate Immunity

Key characteristics

• Evolutionarily ancient
• No need to learn
• Very fast
• Sensitive
• Broad activity
• Links to adaptive response
The Skin Innate Immune System

Multiple active layers

- Physical Barrier
- Keratinocyte
- Dendritic cell
- Neutrophil
- Mast Cell

Immediate
- Physical and chemical barrier
  (stratum corneum, pH, constitutively produced ROS, lipids, peptides)

Early
- Pattern recognition, inducible chemicals
  (TLRs etc., iNOS, antimicrobial peptides, chemokines)

Intermediate
- Cell recruitment
  (Neutrophils, Monocyte, Macrophage, NK, NKT cells)

Late
- Cell education and clonal proliferation
  (Dendritic cells, T-cells, B-cells)

AMPs

Timing is Key to the immune response
Antimicrobial peptides

- Most studied in human skin are cathelicidins and β-defensins
- Over 1200 known and many can be found on skin
- Broad spectrum of antimicrobial action (bacteria, viruses, fungi)

Peptides usually kill by disrupting the membrane

Barrel Stave Pore Model

Carpet Model

Toroidal Pore Model

Bechinger 1999
Cathelicidins are induced during injury.

Mouse skin immunostaining for cathelicidin

Removing Cathelicidin from mice increases infection

By making more AMPs we can increase resistance to disease

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<th>Plants</th>
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<tr>
<td>Amphibian antimicrobial peptide</td>
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<td>expressed in tobacco</td>
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<td>confers resistance to <em>Pseudomonas</em></td>
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<td><em>Biochem.J, 2003</em></td>
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<td>expressed in mouse Paneth cells</td>
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<td>confers resistance to <em>Salmonella</em></td>
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<td>Pig antimicrobial peptide</td>
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<td>expressed in mouse skin</td>
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<td>confers resistance to <em>Group A</em></td>
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<td><em>Streptococcus</em></td>
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Is the information from lab investigation relevant to humans?

What can this teach us about skin disease?
The human skin surface is antimicrobial

Atopics have less AMPs than expected

Atopic Dermatitis with history of eczema herpeticum are most suppressed

Conclusion: Lack of appropriate production of AMPs leads to increased infections in Atopics

Atopic Dermatitis
Underproduction ➔ Infection

Two pathways for AMPs

"Oh, if only it were so simple."
The result of inflammatory actions of AMPs

- Augments innate defense, cell recruitment
- Enhances tissue repair

Excessive AMP production leads to disease!

AMPS and Rosacea

What Rosacea Looks Like—If Untreated

Second-Stage Rosacea
Rosacea begins as a redness in the nose that comes and goes. If untreated, it becomes more permanent, and tiny blood vessels become visible. In addition, slight swelling, pimples, and pustules develop. If it spreads to the patients, it can reach the point where everything that comes across the face blurs, turns, or irritates.

Ocular Rosacea
Rosacea can lead to watery, teary eyes and irritation of the eyelids. Gently washing the eyelid margins with diluted baby shampoo can help relieve symptoms.

Bulbous Nose Rosacea
In advanced cases of rosacea, the nose may become bumpy, swollen and red from excess tissue (phlyctenulae).
Functions associated with Cathelicidin Antimicrobial peptides are similar to findings in Rosacea

- Cathelicidins are stimulated by UV, microbes, trauma
- Cathelicidins and other AMPs can stimulate inflammation
- Cathelicidins can stimulate angiogenesis

3 Major abnormalities discovered in Rosacea

1. Cathelicidin is increased

2. Altered processing of cathelicidin peptides

3. Increased kallikrein 5 (SCTE) expression

Peptide induces erythematotelangectatic response

Normal peptide (KR-20) vs. Rosacea peptides (LL-37)

320 µM, BID, for 2 days
cathelicidin concentration in rosacea skin; 40 - 1500 µM

Proposed model of AMPs in pathogenesis of Rosacea

Triggers

Rosacea (high susceptibility)
- Cathelicidin (mRNA / hCAP18)
- Kallikrein 5
- Altered peptides (LL-37 / FA-29 etc)

Normal (low susceptibility)
- Normal balance of cathelicidin and kallikrein
- No symptoms

AMPs and Psoriasis

AMP LL37 enables self DNA to activate TLR9

Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptides.


Antimicrobial peptides and self-DNA in autoimmune skin inflammation.

Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide.
Keratinocytes express TLR9 in psoriasis

Keratinocytes increase interferons in response to LL37
Keratinocytes increase IL-23 in response to LL37

**mRNA**

IL23A

**Protein**

IL-23

Evolving Model: A central role for AMPs in Psoriasis

Injury or infection

Psoriasis

Neutrophil

LL37

DNA/LL37 complexes

TLR9

IL-23

TLR9

IL-17, IL-22

IFN-α/β

Th1 or Th17 cells

Myeloid DC

Plasmacytoid DCs

IL-17, IL-22

IFN-α/β
Can we apply this information?

Maybe

Vitamin D-
A mechanism to enhance innate immunity?

• Vitamin D stimulates monocyte action against TB
  • Liu et al. *Science* 2006

• Wounding stimulates metabolism of Vitamin D in skin and induces TLR2 and AMPs
  • Schaubert et al. *JCI* 2006
Overview of Vitamin D

Keratinocytes express CYP27B1 and CYP27A1.

The innate immune pathway for Vitamin D in skin

Schauber et al. JCI 2007
More UV is not the answer

Governor of California: Arnold Schwarzenegger
My ultimate boss as a faculty of U of California

Oral supplementation of Vitamin D reverses Cathelicidin deficiency in Atopics

Hata et al. JACI 2008

Patient Group

4000 IU daily X 3 wks
Perhaps we are just reinventing the wheel?……

Take home messages:

• Innate immunity is essential to defense
• Skin deploys innate defense in several ways
• AMPs are part of innate immunity
  provide antibiotic action
  amplify host cell response
• Atopics have too little AMP response
• Psoriasis and Rosacea have too much
• Potential new therapeutic directions
THANK YOU!!!!

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