Antimicrobial Peptides in Health and Disease

Michael Zasloff, MD., Ph.D.
Large, long lived animals survive without lymphocytes or antibodies
Discovery of Magainin Peptides

Usual healing

1 week old

1 month old

The rare case
Secretion of Magainins onto Frog’s Skin

The skin, x-section
Environmental microbes needed for regeneration of antimicrobial peptide skin arsenal

Frogs returned to “normal” aquarium

2 weeks later

Frogs returned to sterile water + antibiotics

[Graphs showing changes over time]

[Images of tissue samples]
Antimicrobial Peptides Bind Selectively To Microbial Cells

$S.\ aureus$

Endothelial cells

Magainin with fluorescent tag
Fundamental Design Principle: Amphipathic Distribution of **Cationic** and **Hydrophobic** Amino Acids

Human Neutrophil Defensin 3

Indolicidin

Magainin 2

Protegrin
New Locilex gets to the point.

Broad spectrum power of an oral antibiotic in an easy-to-apply topical

- Uniquely selective mechanism of action destroys a broad-spectrum of pathogens
- First-line topical with the seriously cidal power of an oral
- Patient-friendly application promotes proper wound care

LOCILEX cream
pexiganan acetate cream 1%

Gets to the point
Antimicrobial peptide mimetics
Polyarylamides

Adopts amphipathic secondary structures

*Adopted in vivo*
Hydrophobic interactions

Weak

Strong

Electrostatic and hydrophobic interactions

Prototypic plasma membrane of a multicellular organism (erythrocyte)

Bacterial cytoplasmic membrane

Cholesterol

Zwitterionic phospholipid

Anionic phospholipid
Frog skin antimicrobial peptides are “tuned” for specific niches.
Over 1000 different antimicrobial peptides have been discovered in animals and plants.
The secreted anti-infective protein/peptide arsenal of Innate Immunity

- Proteins that sequester nutrient metal ions
  - Lactoferrin
  - Lipocalin
  - Psoriasin
  - Calprotectin

- Proteins that attach and “mark”, but do not kill
  - Lectins
  - Pulmonary surfactants

- Proteins/peptides that rapidly and directly kill
  - Antimicrobial peptides
  - Antimicrobial proteins and their fragments:
    - Cytokines
      - PGYRP
    - Plasma Proteins
      - complement (C3a)
      - Apolipoprotein L-1
    - Very Basic Proteins
      - SLPI
      - RNAse
      - Phospholipases
      - Proteases
      - Histone 2A and buforin

- Enzymes that generate antimicrobial substances
  - Polyphenoloxidase, NO synthetase
Antimicrobial peptides are produced in the body wherever we find microbes.

Different types and numbers of microbes in different parts of the body

Sites of Antimicrobial Peptide Expression

- **WBC** (ex. Defensins, Cathelicidins)
- **Tongue** (ex. LAP, PBD-1)
- **Airways** (ex. TAP, HBD-1, HBD-2, LL-37, anionic peptides)
- **Kidney** (ex. HBD-1, Rk-1, MED-1)
- **Colon** (ex. EBD, SBD-1, SBD-2)
- **Small Intestine** (ex. Cryptdins, HD5, HD6, SBD-2)
- **Skin** (ex. LL-37, HBD-1, HBD-2)
- **Reproductive Tract** (ex. Cervix: HBD-1, HD6; Testes: Cryptdins, LL-37)
Tongue
Why is the tongue so rarely infected despite the hazards it faces?

A human tongue

A cow’s tongue
Antimicrobial peptides are induced by injury (dorsum of cow tongue)

Beta-Defensin mRNA

Intact epithelium

Acute abrasions, 1-2 d

Tongue epithelial Defensin appears after birth

birth

weaning

adult

Oral Infant Thrush
AMPs are not simply anti-infective: they communicate with specific receptors with “local” systems involved in defense and tissue repair.

LL-37

FMLP Receptor

Stimulates angiogenesis, neutrophil migration

HBD-2

P2X7 Receptor

Activates IL-1β secretion from macrophages

CXCR6, (MIP3α receptor)

Macrophages

Immature Dendritic cells
Skin
Why isn’t infection of the hair follicle the “normal” condition?
Concentrations of specific AMPs on human skin match the degree of protection needed: Psoriasin, as an example.

Psoriasin surface concentrations

Top of foot (low)  Bottom of foot (high)

Modified from (Glaser et al, Nat Immunology, 2005)
It is easy to demonstrate the presence of antimicrobial peptides on our skin. 

*E. coli* is very rapidly killed by psoriasin.

5 seconds on the skin

10 minutes on the skin
Most soaps wash off the powerful AMPs on our skin.

After using soap, remaining E. coli can actually grow more rapidly than before!!

Unwashed

Soap-washed

10 minutes
Cathelicidin ("mCRAMP") KO Mice Develop Invasive Group A Streptococcal Skin Infections

Nizet et al, Nature 2001
In epidermis, antimicrobial peptides and lipids comprise the permeability barrier of skin.

AMPs and skin lipids are stored within "lamellar bodies" and secreted into the "mortar" space.

Lipids provide a barrier to reduce water loss.

AMPs (and lipids) provide antimicrobial function.

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Keratinocytes Store the Antimicrobial Peptide Cathelicidin in Lamellar Bodies

Marissa H. Braff,* Anna Di Nardo,*† and Richard L. Gallo*†

*University of California, San Diego, La Jolla, California, USA; and Department of Dermatology, IMMC, La Jolla, California, USA; (San Diego Dermatological J Invest Dermatol 124:394–400, 2005)
Restoration of the skin’s permeability/AMP barrier after “Superficial damage” is rapid and requires “drying”

Tape stripped skin

An occlusive dressing inhibits the repair

14 days after tape stripping the base of a diabetic’s foot

Remarkable absence of inflammation in the setting of an infected ulcer
Vitamin D
(and LL-37)
Tuberculosis infection of skin

(Lupus vulgaris)

Neils Rydberg Finsen

- Sunlight cured TB infections of the skin
- Nobel Prize, 1903

Faroe Islands, Denmark
Sunlight stimulates the antimicrobial defenses of skin and blood cells

Why is infection so unusual after sunburn?

Topical application of VitD to Skin

Weber et al, J Invest Derm 2005
Mallbris et al, J Invest Derm 2005
Wang et al, J Immunol 2004

Gene lacks VIT D response element

Liu, PT et al, Science 2006

Gene lacks VIT D response element
Winter and respiratory infections

Influenza

Figure 1. The seasonal and latitudinal distribution of outbreaks of type A influenza in the world, 1964-1975, extracted from the Weekly Epidemiological Record of the World Health Organization into major zones. The diagrams show for each calendar month the percentage of each zone's total outbreaks. In both north and south temperate zones the outbreaks are distributed around the local midwinter, whereas the tropical zones show a trendline, each approximating towards the distribution of its own temperate zone. The curve indicates the "midwinter" path taken initially by vertical solar radiation. The "epidemic path" seems to parallel it, but to lag six months behind it. (Reproduced with permission, Cambridge University Press, Hope-Simpson, 1981)

Seasonal Plasma [VitD]

Figure 2. Weekly rates of invasive pneumococcal disease in the United States, January 1996–December 1998. Weekly numbers of cases from active surveillance areas in California, Connecticut, Georgia, Maryland, Minnesota, Oregon, and Tennessee were divided by the population under surveillance that year and multiplied by 52 to give annualized weekly rates.

Vit D supplementation

Figure 2
Skin conditions associated with abnormal expression of antimicrobial peptides
Two common inflammatory skin conditions with very different tendencies to infection (bacterial and viral)

Atopic dermatitis (common)

Psoriasis (rare)

Henseler and Christophers, J Am Acad Dermatol, 1995
Antimicrobial peptide expression is turned on "high" in psoriasis, but is "relatively" depressed in eczema, considering the extent of epithelial damage.

AMP induction within keratinocytes are suppressed by Th2 cytokines IL4 and IL13

Ong et al. NEJM 347:1151(2002)
Psoriasis: Marked induction of diverse antimicrobial peptides and proteins

Harder et al, Nature 1997
Transgenic plants that express Frog skin antimicrobial peptides are protected from disease.

Control poinsettia

Poinsettia expressing Magainin I

Poinsettia expressing PGLα
Cystic Fibrosis: antimicrobial peptides fail to function in abnormal airway surface fluid


Smith et al., Cell 85, 229-236 (1996)
CFTR-targeted pigs (-/- or DF508/DF508) manifest impaired bacterial eradication in their airways at birth and spontaneously develop lung disease


CF airway surface fluid has a defect in killing *S. aureus* in vivo

Anesthetized pigs

Tracheal window

*CFTR+/* vs. *CFTR−/−* pigs

a-f indicate littermates
Airway Surface Liquid (ASL) pH from CF pigs is more acidic than wild type.

Methacholine-stim ASL ex vivo

ASL on primary epithelial cultures
Increasing pH of CF airway surface fluid restores antimicrobial activity.
Effect of pH on Antimicrobial Activity of two abundant antimicrobial proteins in ASL

Luminescence assay with *S. aureus*

**Lysozyme**

**Lactoferrin**
An unstudied miracle relevant to this Conference
A shark bite on a human
A shark bite on a dolphin

Healing without infection or inflammation; minimal blood loss, regeneration; no evidence of pain

The Biota of the Oceans are part of the Dolphin's Metagenome

Antibiotics are produced to protect these organisms against the microbes of the sea

The dolphin retains (in its blubber) the protective antimicrobial agents that have been selected as effective against the microbes of the sea (and that are compatible with its physiology)
The Biota of the Oceans are part of the Dolphin’s “Antimicrobial Metagenome”

Antibiotics are produced to protect these organism against the microbes of the sea

The dolphin retains (in its blubber) the protective antimicrobial agents that have been selected as effective against the microbes of the sea (that are compatible with its physiology)
“Nicky”
Healing… in harmony with her microbes
“Helpless”, in a pond?

Not really, but so much remains unknown
Keratin filament turnover cycle is dynamic and regulated

Proposed Model:

Homeostasis: Assembly $\rightleftharpoons$ Disassembly
Basal level of KDAMPs

Stress: Disassembly $>$ Assembly

Soluble pool of keratin oligomers
Quantity and activity of KDAMPs

Schematic from Windoffer et al. J Cell Biol 2011;194:669-678
In vivo knockdown of keratin 6A in mouse corneas enhances bacterial adherence on the epithelium

K6A or scrambled siRNA

![Images showing experimental results](image_url)

Graph showing bacterial adherence and normalized gene expression.
How does the cornea remain healthy?

Bar = 20 µm.
As bacteria attach a “puff” of the antimicrobial peptide LL-37 is released killing them.

The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection.
Skin HBD3 is induced in a sterile wound. Dependent on EGFR stimulation. Injury leads to the cleavage of an EGF ligand by a protease.

Wound is protected as the wave of neutrophils disappears, and new epithelial growth is initiated.

_Sorensen et al, JCI 2006_
Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide

Roberto Landè, Josh Gregorio, Valeria Facchinetti, Bithi Chatterjee, Yi-Hong Wang, Bernhard Homey, Wei Cao, Yui-Hsi Wang, Bing Su, Frank O. Nestle, Tomasz Zal, Ira Mellman, Jens-Michael Schröder, Yong-Jun Liu & Michel Gilliet
Psoriasis is associated with increased β-defensin genomic copy number

Edward J Hollox, Ulrike Huffmeier, Patrick L J M Zeeuwen, Raquel Palla, Jesús Lascorz, Diana Rodijk-Olthuis, Peter C M van de Kerkhof, Heiko Traupe, Gys de Jongh, Martin den Heijer, André Reis, John A L Armour & Joost Schalkwijk

Most have 2-7 copies/diploid, some 9-12

Table 1: Influence of β-defensin gene copy number on the relative risk of psoriasis

<table>
<thead>
<tr>
<th>Copy number</th>
<th>Dutch cases</th>
<th>German cases</th>
<th>Combined cases</th>
<th>Dutch cases</th>
<th>German cases</th>
<th>Combined cases</th>
<th>Copy number</th>
<th>Population frequency (%)</th>
<th>Relative risk</th>
<th>90% CI relative risk</th>
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</table>

Counts are given in the table, together with the relative risk of psoriasis given the observed copy number, with a relative risk of 1.00 representing the population mean risk (25%). Population frequency shows an estimate of diploid copy number frequencies in native Northern Europeans derived from the combined controls columns. Relative risk values were calculated using Poisson regression with copy numbers of 6 or more into a single category. Bold type indicates a two-sided significant difference from a relative risk of 1.00 at the 5% level.
Epithelial damage/microbes

Pathophysiology of psoriasis, with a focus on antimicrobial peptides

**AMP** induction (excessive)

**Defensin**

Attraction of dendritic cells into the immediate neighborhood of the skin lesion

**LL-37 (and DNA)**

Induction of IFN α by plasmacytoid DC’s

Activation of the adaptive immune system (excessive)
ileum

cecum

Rectosigmoid

Normal vascular pattern of colon

The Intestine: How does the epithelium maintain its "health" considering…
Antimicrobial peptides protect the bowel wall, stem cells, and (possibly) regulate gut flora.

Alpha-defensins and other AMPs
Secreted into lumen; Limits microbial growth; Protect stem cells; Regulates composition of commensals

Beta-defensins; LL-37
Secreted into mucous biofilm; limit microbial access to epithelial layer (chemical shield)

Colon (enterocyte)

Small intestine (Paneth cell)

Figure 3. Paneth cells in the crypts of the small intestinal mucosa
Paneth cells, located at the base of the crypts of Lieberkühn (inset), discharge their defensin-rich granules into the crypt lumen. Illustrated by David Schumick, Department Medical Illustration, © 2000, The Cleveland Clinic Foundation. [Reproduced with permission from 140].
Bacterial analysis from microdissected compartments

Laser-capture microdissection

- Stratum corneum + Epidermis
- Non-tissue control (to check exogenous bacteria contamination)
- Laser
- Facial skin
- Hair follicle
- Adipose tissue
- Dermis
The subcutaneous microbiome is diverse.

Pyrosequencing for 16S rRNA gene
Bacteria products are detected below epidermidis by independent techniques.

Conclusions

→ Previously unexpected microbiome exists below the epidermis.

→ Skin-residing cells are influenced by direct interaction with commensal microbes below epidermis.
Crohn’s Disease: “harmony” disturbed

1. Microbial sensor (“NOD2”) is genetically defective in 25% of patients

2. The “front-line” antimicrobial defense is not effectively regulated

3. The bowel defends itself by calling in white blood cells from the blood stream that wreak havoc