Structure and Function of the Skin: Cancer, Immunity and Inflammation

The Cutaneous Microbiome
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DISCLOSURE OF RELATIONSHIPS WITH INDUSTRY
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C010: Structure and Function of the Skin: Cancer, Immunity and Inflammation
The Cutaneous Microbiome

DISCLOSURES
No relevant conflicts to disclose

The Skin Microbiome
- Complex host-microbial interactions
- Host
  - Host defense
    - e.g. AMPs
- Microbes
  - Commensal/Pathogen
  - Culturing/Sequencing

The Skin Microbiome
- Physiology
  - Cornified keratinocytes
  - Temperature, pH, lipids
- Immunology
  - Adaptive
  - Innate
    - Toll-like receptors,
      - Antimicrobial peptides, etc.

Antimicrobial peptides
- 1200+ identified
- Widespread distribution (plants & animals)
- Ancient lineage
- Broad spectrum
- Grouped based on amino acid composition (10-50 a.a.), size, conformational structure

Proposed Mechanism of action:
- Cationic antimicrobial peptides associate with negatively charged bacterial membrane
- Leads to pores in the microbial membrane with subsequent osmotic lysis and microbial cell death

Many antimicrobial peptides have chemotactic properties

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Cell Source</th>
<th>Other Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-defensins</td>
<td>Infiltrating neutrophils, keratinocytes, dendritic cells</td>
<td></td>
</tr>
<tr>
<td>β-defensins</td>
<td>Keratinocytes</td>
<td>Chemotaxis of T cells and dendritic cells</td>
</tr>
<tr>
<td>Cathelicidin (LL-37)</td>
<td>Keratinocytes</td>
<td>Chemotaxis for T cells, monocytes, neutrophils, mast cells</td>
</tr>
<tr>
<td>Granulysin</td>
<td>Infiltrating T cells, keratinocytes, follicular epithelium, eccrine glands</td>
<td></td>
</tr>
<tr>
<td>Peptidin</td>
<td>Keratinocytes, follicular epithelium, sebocytes</td>
<td></td>
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<tr>
<td>Dermcidin</td>
<td>Eosinophils</td>
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<tr>
<td>RNAse 7</td>
<td>Keratinocytes</td>
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<tr>
<td>Lactoferrin</td>
<td>Keratinocytes</td>
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LL-37 (cathelicidin) and granulysin expression in skin

- Chemotaxis
- Angiogenesis
- Wound healing
- Granulysin:
- Chemotaxis
- Cytotoxic to tumor cells
- Anti-inflammatory
- Graft rejection

- LL-37 (in blue) is found in keratinocytes, follicular epithelium, eccrine glands and neutrophils.
- Granulysin (in yellow) is produced predominantly by T cells

HNPs (Human neutrophil peptides [α-defensins]) and HBD (human β-defensin) expression in skin

- HNPs (in tan) are found in azurophilic granules of neutrophils
- HBDs (in magenta) are produced by keratinocytes and macrophages

RNAse 7, Dermcidin, and Psoriasin expression in skin

- RNAse 7 (in orange) is present in keratinocytes in the epidermis
- Dermcidin (in red) is found predominantly in sweat glands
- Psoriasin (in green) is found in keratinocytes, sebocytes and in follicular epithelium

Antimicrobial and immunomodulatory properties

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<th>Regulation</th>
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<td>α-defensins</td>
<td>β-Defensins, HBD-2</td>
<td>Neutrophil elastase-dependent mechanism</td>
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AMPs are differentially expressed in psoriasis & atopic dermatitis

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Cathelicidin variant peptides induced by stratum corneum tryptic enzyme (SCTE), which is increased in rosacea, promotes enhanced pro-inflammatory responses

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<th>Innate immunity disorder</th>
<th>Abnormality of skin microflora</th>
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<td>Inflamator</td>
<td>Upregulation of MHC and TLR4</td>
<td>Overgrowth of Propionibacterium acnes</td>
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<td>Lower induction of cathelicidin and MHCs than those in parietal frontal skin despite the presence of inflammation</td>
<td>Impaired function of MHC and MHCs following acute wound repair, increased expression of TNFα and IL-1β</td>
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<td>Psoriasis</td>
<td>Abnormal expression of cathelicidin and MHCs</td>
<td>Inhibitory function of acanthocytes and MHCs</td>
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<td>Rosacea</td>
<td>Abnormal expression of cathelicidin and MHCs</td>
<td>No pathogenic bacteria found in rosacean skin</td>
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**Humans microbes are complex and multi-faceted**
- Microbes are more often considered pathogenic
  - **Mycobacterium tuberculosis**
  - Herpes simplex virus
- Microbes perform functions important for human health
  - Vitamin synthesis
  - Development and activity of immune system
  - Inhibition of skin colonization by pathogens

**Bacteria commonly found on skin**
- Duality of skin bacteria
  - Commensal vs pathogenic microbe
- **Staphylococcus epidermidis**
- **Staphylococcus aureus**
- **Corynebacterium spp.**
- **Propionibacterium acnes**

DNA sequencing is a powerful microscope for microbial identification

**Pipeline for skin microbiome studies**
1. Collect superficial skin sample
2. Isolate DNA
3. Amplify DNA with primers (bacteria = 16S rRNA gene)
4. DNA sequencing/analysis

**Skin diseases are associated with specific bacteria, antimicrobial peptides and TLRs**

**DNA sequencing**

**Sequencing instrument**

**Inflamator**
- Upregulation of MHC and TLR4
- Overgrowth of Propionibacterium acnes
- Impaired function of MHC and MHCs

**Atopic dermatitis**
- Lower induction of cathelicidin and MHCs than those in parietal frontal skin despite the presence of inflammation
- Impaired function of MHC and MHCs following acute wound repair, increased expression of TNFα and IL-1β

**Psoriasis**
- Abnormal expression of cathelicidin and MHCs
- Impaired function of acanthocytes and MHCs

**Rosacea**
- Abnormal expression of cathelicidin and MHCs
- No pathogenic bacteria found in rosacean skin
Duality of skin microbes: commensal vs pathogen

**Staphylococcus epidermidis**

- Major skin inhabitant
- Frequent cause of nosocomial infections
- Produce antibacterial products
  - Bacteriocins, Enzymes (Esp, a serine protease), Phenol soluble modulins, etc.

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Skin injury releases host dsRNA activating TLR3 in keratinocytes. *S. epidermidis* lipoteichoic acid (LTA) inhibits excess inflammation via TLR2-dependent mechanism.

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**S. epidermidis can inhibit excess *P. acnes*-induced inflammation**

- *S. epidermidis* lipoteichoic acid (LTA) inhibits excess *P. acnes*-induced inflammation via miR-143/TLR2 interaction.

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Skin commensals tune skin-resident T cells

- Mouse model of leishmania infection
- In germ-free mice:
  - Reduced local skin inflammation and effector T cell response
  - Adding *S. epidermidis* restores immunity

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In mice, neonatal skin exposure to commensal can lead to immune tolerance

- Adult or neonatal mice
- *E. coli* with or without skin abrasion
- Reduced 286-specific immune responses in 12D.11.4
- Adding *S. epidermidis* restores immunity
Well-known pathogen
• Self-limited to invasive infections
• Atopic dermatitis
• Job’s/AD Hyper IgE syndrome (STAT3 mutation)
• Asymptomatic nasal colonization
  • 20% permanently colonized
  • 30-50% transiently colonized
  • S. aureus preferentially hemolyzes human blood to utilize iron from human hemoglobin as a nutrient

Interactions with host & other bacteria
• Hemolysins
• Affinity for human hemoglobin
• Phenol-soluble modulins
• High levels produced by CA-MRSA
• Streptococci very sensitive to PSMs (may partially explain CA-MRSA dominance)
• Bacteriocins

Staphylococcus spp increase during AD flares

Commensal staphylococci can produce AMPs to selectively kill S. aureus

Duality of skin microbes: commensal vs pathogen

Staphylococcus aureus

Duality of skin microbes: commensal vs pathogen

Propionibacterium acnes

Duality of skin microbes: commensal vs pathogen

Corynebacterium spp.