Paneth cells, antimicrobial peptides and the regulation of the intestinal microbiota

Dr. Nita Salzman – Medical College of Wisconsin, USA

Paneth Cells, Antimicrobial Peptides, and the Regulation of the Intestinal Microbiota

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The human microbiome

- “The totality of microorganisms and their collective genetic material present in or on the human body”
- Comprised of bacteria, viruses, others (archaea, eukaryotes)
- Distinctive microorganisms at each body site (gut, lung, skin, mucosa etc.)
- The majority of bacteria comprising the microbiota are not culturable by currently available techniques

Gastrointestinal microbiome

- Intestinal microbes outnumber eukaryotic cells by 1.3 fold
- Composition of the microbiota
  - 500-1000 different species, predominated by Clostridium-Flavobacterium-Bacteroides (CFB) and Firmicutes divisions, falling into groups of Clostridium and Bacteroides
  - Anaerobic bacteria outnumber aerobic by 10-100 fold
- Complex, dynamic ecosystem which includes autochthonous (indigenous) and allochthonous (transitory) organisms

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Gastrointestinal microbiome

16S ribosomal RNA

16S ribosomal RNA

Prokaryotic ribosome  Eukaryotic ribosome

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16S ribosomal RNA

Prokaryotic ribosome  Eukaryotic ribosome

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<td>235</td>
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</tbody>
</table>

1540 nucleotides long

Analysis of bacterial composition using 16S ribosomal RNA

Intestinal tissue

PCR amplification from genomic DNA

Isolate genomic DNA

High throughput gene sequencing

Metagenomic analysis

- Composition analysis reveals consistency of individual over time, but inter-subject variability
- Metagenomic analysis reveals core functional microbiome common among individual subjects

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Quantification of bacterial groups by qPCR using 16S rRNA

What about metabolic activity?

Physiological roles of the intestinal microbiota

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Mammalian mucosal surfaces

Continual contact with microbes
Low incidence of infection
Infrequent inflammatory response

Effective mucosal defense mechanisms

How do we exist in the context of an enormous bacterial burden?

Microbiota

Epithelium

How does the host exist in the context of an enormous bacterial burden without becoming infected?

How is this achieved in the absence of excessive inflammation?

How does the host distinguish and effectively respond to pathogens without triggering excessive inflammation?

Microbial challenge at wet mucosal epithelia

Proteins
Lysozyme
PLA2

Antimicrobial peptides
Inorganics
NO, H2O2

Local antimicrobial activities

Epithelium

Hulten and Bienen, 1999. Pediatric Research

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The anatomy of the mucosal barrier

The anatomy of the mucosal barrier

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The anatomy of the mucosal barrier
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The human gastrointestinal tract

Paneth cell granules contents

N. nucleus
ER endoplasmic reticulum
SG secretory granule

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Paneth cell granules contents

- α-defensins
- β-defensins
- CRS peptides
- Lysozyme
- sTFα2
- RegIII
- Angiogenin-4
- α-1-antitrypsin
- TNF
- MMP7
- IL-17A
- α1A
- Leptin
- Adiponectin
- Serum amyloid A1

Activity of antimicrobial effectors secreted by Paneth cells

<table>
<thead>
<tr>
<th>Paneth cell antimicrobial</th>
<th>Biochemical classification</th>
<th>Antimicrobial activity</th>
<th>Transcriptionally induced</th>
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<tbody>
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<td>α-defensins</td>
<td>Antibacterial peptides</td>
<td>Against Gram-positive and Gram-negative bacteria</td>
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<td>CRP</td>
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<td>Angiogenin</td>
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<td>Yes</td>
</tr>
<tr>
<td>TNF</td>
<td>Cysteine-rich peptides</td>
<td>Against Gram-positive and Gram-negative bacteria</td>
<td>No</td>
</tr>
<tr>
<td>MMP7</td>
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<td>Serum amyloid A1</td>
<td>Cysteine-rich peptides</td>
<td>Against Gram-positive and Gram-negative bacteria</td>
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</table>

Paneth cells secrete antimicrobials in response to bacteria and bacterial products

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Activated crypt defensins in germ-free mice

- Analyzed on an acid/urea-polyacrylamide gel and stained with Coomassie Blue:
  - Proteins extracted from the small intestines of adult germ-free mice
  - Mice conventionalized for 1 day
  - Mice conventionalized for 7 days
  - A conventionally reared mouse
- Processed crypt defensins in the germ-free mice were equivalent to those seen in the conventionalized mice

RegIIIγ expression is triggered by intestinal bacteria

[Cash et al., 2006. Science]

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RegIIIy expression is triggered by intestinal bacteria

INTESTINAL ANTIMICROBIAL PEPTIDES

Paneth cell  Enterocyte  Neutrophil  Bacteria

Structure of defensins

- α-defensing
  - PMNs, Paneth cells (constitutive production & secretion)
- β-defensing
  - Constitutive or inducible secretion
- θ-defensing
  - Monocyte PMNs, made of 2 α-defensins molecules

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Structure of defensins
- α-defensin
  - PMNs, Paneth cells (constitutive production & secretion)
- β-defensin
  - Constitutive or inducible secretion
- δ-defensin
  - Monoerythrocyte PMNs, made of 2 α-defensins molecules

Functions of defensins
- Antimicrobial activity
- Regulate epithelial cell growth
- Act as growth factors
- Promote tissue repair

Processing of defensins
- HBD5
  - Signal: 30 aa, Pro-piece: 94 aa, Active: 28 aa
- Cryptdin
  - Signal: 28 aa, Pro-piece: 100 aa, Active: 22 aa

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MMP7 KO mice: a model for defensin deficiency
- MMP7 is a matrix metalloproteinase, expressed in mouse PC's
- Processes prodefensins to active peptides
- MMP7 KO mice lack processed active defensins in both PC's and the small intestinal lumen, and are less able to clear enteric bacterial infections

A HD5 (DEFA5) transgenic mouse model

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A HD5 (DEFA5) transgenic mouse model

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HD6 self assembles into nanonets and blocks *Salmonella* invasion

Chu et al., 2022, Science

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Paneth cells express CD24 and support growth of Lgr5 stem cells

Regulation of Paneth cell RegIIIγ expression

Paneth cell NOD2 and bacterial homeostasis

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LRRK2 and Nod2 regulate lysozyme sorting in Paneth cells

Bachs et al., 2015, Nature Immunology.

LRRK2 and Nod2 regulate lysozyme sorting in Paneth cells

Bachs et al., 2015, Nature Immunology.

Quantification of total bacteria by intestinal segment in HD5 and MMP7 mice

Total bacterial numbers are not altered by the absence or change in defensin composition

Gollan et al., 2010, Nature Immunology.

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There are significant differences in microbial communities, when comparing HD5 mice to WT littermates and MMP7/-/- mice to WT littermates.

There are reciprocal shifts in the dominant bacterial classes found in the gut. Firmicutes and Bacteroidetes, comparing HD5 TG and MMP7 KO mice.

There is a complete absence of SFB in the HD5 mouse litters.

The expression of HD5 results in loss of SFB from the mouse small intestine.

SFB is abundant in MMP7/-/- (defensin deficient) mice.

What is the immunological significance of SFB presence/absence?

SFB (Candidatus arthromitis)

- Gram positive, spore forming, uncultivable
- Member of the gut biome of diverse species
- Directly contacts IEL
- Sensitive to IgA, antibiotics
- Induces complete complex maturation of mucosal immune responses
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LPL from HD5 mice lack IL17 expression

- LPL CD4+ T cells isolated, stimulated with PMA + ionomycin, analyzed by flow cytometry
- T cells from HD5 mice lack IL17A expression (no Th17 cells)
- IL17A expression in T cells (and Th17 cell abundance) is directly proportional to SFB abundance in the small intestine
- Defensins may regulate mucosal T cell responses through regulation of intestinal colonization

Paneth cell defensins regulate the microbiota

- ↑ Defensins
- ↑ Microbiota
- ↑ Mucosal Immune Responses

Paneth cell defensins regulate the microbiota

- Implications for understanding IBD pathogenesis
  - Abnormalities of bacterial colonization (dysbiosis) is a hallmark of Crohn’s disease
  - Several of the primary genetic defects are associated with Paneth cell dysfunction (NOD2, ATG14L1, XBPI; TCF-4)
  - Paneth cell defensins regulate the microbiome and consequently mucosal immune responses

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Patients with CD have reduced Paneth cell defensin expression

- All CD patients have reduced HD5 expression compared to controls
- CD patients with SNP13 NOD2 mutations have significantly less HD5 expression

Defensins, regulation of the microbiota and implications for intestinal homeostasis

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Paneth cell antimicrobials employ diverse approaches to maintain homeostasis at the mucosal surface

Several diseases are associated with Paneth cell abnormalities

Homeostasis and dysbiosis of Paneth cells

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