Host – Pathogen Interactions: Molecular Basis and Host Defense Mechanisms

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Welcome to 4 part webinar series

"The Microscopic War: Microbes and Immunity”

Part 1: Host – Pathogen Interactions: Molecular Basis and Host Defense Mechanisms

Part 2: Microbiome: From Identification to Characterization


Part 4: Toll-like Receptors in Inflammation – The bridge between innate and adaptive immunity
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Agenda

- Introduction to the microbiome
  - Human microbiome - microbiota
  - The complex host-microbe relationship
  - The impact of microbiome on human health and diseases

- The host-pathogen interaction
  - The protection mechanisms of microbiota
  - The control of immune system over microbiota

- The host defense mechanisms
  - Innate immune response
  - Adaptive immune response

- Technologies for exploring host-pathogen interactions
  - Characterize the composition of the gut microbiome
  - Profile innate and adaptive immune response through gene expression
Introduction to the microbiome

Cellular composition of the organism

Estimations of the number of microbial cells that live in and on the human body, human cells are outnumbered by a factor of 10.

35 trillion human cells = 350 trillion microbial cells!

**What does “microbiome” mean?**

**Microbiota** are the microbes that live in a specific location, e.g. the human body, the gut, soil, etc.

**Metagenomics** is the study of the collection of genomes derived from a specific sample or community.

**Microbes** are microscopic organisms that can be either single or multicellular.

**Human microbiome** is defined as the collective genomes of the complete microbiota present in a human body.
The NIH Common Fund Human Microbiome Project (HMP)

**The first phase of HMP (FY2007-2012)**

- Characterize the composition and diversity of microbial communities which inhabit major mucosal surfaces of the human body, and evaluated the genetic metabolic potential of these communities.
  - ~10,000 organisms live with us (10 times more)
  - ~ $8 \times 10^6$ genes in this genome (20-60% are not cultivable)
  - Different body sites have unique communities (Each area of the body has its own microbiome)

**The current phase of HMP (FY2013-2015)**

- Develop tools and datasets for the research community for studying the role of these microbes in human health and disease.
Host – Microbiota Interaction

**Dual necessity:** peacefully coexist to achieve a mutually beneficial relationship

- The host provides the microbiota a niche with a stable nutrient supply
- The microbiota performs essential functions for host physiology, including metabolic, digestive, and immune mechanisms
  - Regulate the host’s metabolic function and energy balance
  - Provides the host with the capacity to hydrolyze complex plant sugars, synthesize vitamins, and detoxify xenobiotics in a mutualistic context

**Commensal gut microbiota protects the host from infection via both direct and indirect mechanisms**

The symbiosis between microbes and humans provides a stable and common metabolic pattern and well-balanced physiological homeostasis.

Direct and indirect protection mechanisms of microbiota

Direct and indirect protection mechanisms of microbiota

Host – Microbiota Interaction

Direct and indirect protection mechanisms of microbiota

The big question? How, what, who in the microbiota does what, when and how to the immune system?

How does microbiota shape immunity - the cellular and molecular mediators

- Impact lymphoid structure development and epithelial function
- Enhance innate immunity to pathogens
- Shape T cell subsets
- Provide protective roles against systemic infection
- Influence invariant T cells and innate lymphoid cells
- Trigger inflammation in immunocompromised hosts
- Protect against autoimmune disease

The crosstalk between the microbiome and the immune system

The control of immune system over microbiota

“The mammalian immune system plays an essential role in maintaining homeostasis with resident microbial communities, thus ensuring that the mutualistic nature of the host-microbial relationship is maintained.”

The critical controls of the immune system

1. Exerts control over stratification and compartmentalization of the microbiota
2. Exerts control over microbiota composition

The immune system exerts critical controls over microbiota composition, diversity, and location

The importance of microbiota in human health and diseases

When the mutualistic relationship between the host and microbiota is disrupted, the gut microbiota can cause or contribute to diseases.

**Diseases influenced by gut microbial metabolism**

The host responses

The challenge that the host faces

It is important to note that commensal bacteria do not always protect against pathogenic infection and in certain contexts they can facilitate it.

And under certain conditions, particular bacterial populations can acquire pathogenic properties.

*How does the host discriminate between symbiotic and pathogenic bacteria to adjust its level of immune response?*
The innate immune system is the first line of defense against pathogens and is initiated by genome-encoded pattern recognition receptors (PRRs) that recognize PAMP:
- Non-specific and does not confer long-lasting immunity (memory)
- Immune cells: dendritic cells (DCs) and macrophages, intestinal epithelial cells and myofibroblasts

The adaptive immunity is highly specific, confers long lasting immunity, and adaptable:
- Cooperate with the molecules and cells of the innate immune system to mount an effective immune response
- Key players: T cells – Th1, Th2 or Th17 cell
Pattern recognition receptors (PRRs) include Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG1-like receptors (RLRs), etc.

- PRRs signaling cascades result in nuclear factor (NF)-κB activation of gene transcription and production of pro-inflammatory mediators.
- PRRs also play a crucial role in the crosstalk between innate and adaptive immune responses by promoting antigen presenting cell maturation and T cell activation.
- TLRs indues the expression of genes required for the inflammatory response, including inflammatory cytokines, chemokines, antimicrobial molecules, and major histocompatibility (MHC) and costimulatory molecules important for adaptive immune activation.
Adaptive immune responses

- Th1 and Th2 cells
- Th17 cells
- Regulatory T cells

During active inflammation, Th0 cells differentiate into T helper cells Th1, Th17 and Th2

- Th1 cells produce interferon (IFN)-γ and tumor necrosis factor (TNF)-α
- Th2 cells are a major source of IL-13
- Th17 cells release IL-17 and IL-21

Important adaptive immune cells

pDC detect viral antigen and release type I IFN
- Activation of mDC, mV, CD4, and CD8 T cells
- FRCs secrete chemokines and T cell survival factors
- FDCs coordinate B cell migration and B cell and CD4 T cell interactions
Host defense mechanisms - Summary

Host defense mechanism–Innate and adaptive immunity

Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Lance W. Peterson1 and David Artis
Characterize the composition and function of the gut microbiome

- Genomics – characterize DNA
- Transcriptomics – characterize RNA
- Metabolomics – characterize small molecules

Human microbiome sample

Extract DNA
- 16S rRNA gene sequencing
- 18S rRNA gene sequencing
- Total DNA sequencing (shotgun)
  - Bacteria and Archaea
  - Fungus / Yeast
  - Viruses

Extract RNA
- RNA expression profiling (transcriptomics)
  - Gene content
  - Gene expression

Extract small molecules
- Mass spectroscopy (metabolomics)
  - Metabolite characterization

Identify relative frequencies and pathways

What organisms are present and what is their relative abundance?

What are the functions of the community?
The Research Question

How can we monitor the microbiome and identify innate and adaptive immune responses?
Technologies for exploring host-pathogen interactions

- **Quick, High-Yield DNA or RNA Isolation**
  Regardless of what material you’re starting from, QIAGEN has the right kit for fast, easy, high-yield DNA or RNA isolation.
  - Find the right kit for your study

- **Pinpoint the Pathogens Provoking a Host Response**
  With more than 500 assays for profiling microbial species and genes, Microbial DNA qPCR Arrays and Assays will identify the responsible pathogen.
  - Detect microbial species, virulence genes, or antibiotic resistance genes

- **Innate Immunity — The First Line of Defense**
  The innate immune response attracts immune cells and activates the adaptive response to control novel foreign pathogens.
  - Identify an innate immune response

- **Which Toll-Like Receptors Are Signaling?**
  Toll-like receptors recognize broad classes of microbe, activating downstream signaling pathways that initiate a tailored immune response.
  - Profile TLR activity

- **RNA expression**

- **QIAGEN Kits**
  - QIAamp Fast DNA Stool Mini Kit
  - QIAamp UCP Pathogen Mini Kit
  - QIAamp UCP PurePathogen Blood Kit
  - QIAamp MinElute Media Kit
Detecting microbial metagenomes – a complete solution

QIAGEN provides next-generation sequencing technologies for metagenomics, as well as qPCR assays and arrays for verification of sequencing results and screening for specific bacterial species, virulence factor genes, and antibiotic resistance genes.

- Step 1: Sample collection
- Step 2: DNA purification
- Step 3: Sample enrichment
- Step 4: Library preparation
- Step 5: Verification by qPCR
QIAGEN’s Microbial DNA qPCR assay pipeline

Reveal the mysteries of the microbiome with over 500 assays that target species-specific or gene-specific microbial DNA

- >300 Bacteria identification assay
- 8 Fungi identification assay
- 1 Protist identification assay
- 87 Antibiotic resistance genes
- 87 Virulence factor genes
- 16 Arrays

- Antibiotic Resistance Genes
- Bacterial Vaginosis
- Biodefense
- Food testing: Dairy
- Food testing: Meat
- Food testing: Poultry
- Food testing: Seafood
- Food testing: Vegetable
- Intestinal infections
- Metabolic Disorders
- Oral Disease
- Respiratory Infections
- Sepsis
- Urinary Tract Infections
- Vaginal Flora
- Water Analysis

DNA Isolation ➔ Detection by qPCR ➔ Data Analysis

Part 2: Microbiome: From Identification to Characterization
June 9, 1 – 2 p.m. EDT
A complete workflow for host responses profiling

- Profiling gene expression from immune cells (especially cytokines, chemokines and other immune molecules) can be interpreted into immune system “status”
  - Inflammation?
    - Early
    - Chronic
    - Resolution
    - Type of response (bacterial, viral, other?)
Host Response Profiling: Gene expression

- Antifungal Response
- Antiviral Response
- Antibacterial Response
- Innate & Adaptive Immune Responses
- Inflammatory Cytokines & Receptors
- Dendritic & Antigen-Presenting Cells
- Inflammasomes
- Th1 & Th2 Responses
- Toll-Like Receptor Signaling Pathway
- IFN-a/b Response
- NFkB Signaling
- NFkB Signaling Targets
- MAPK Signaling
- PI3K/AKT Signaling
- 140+ pathways, including custom arrays
- Wet-bench validated assays for all genes of 13 species
PCR Arrays format and contents

- 84 Pathway-Specific Genes of Interest
- 5 Housekeeping Genes
- Genomic DNA Contamination Control
- Reverse Transcription Controls (RTC) n=3
- Positive PCR Controls (PPC) n=3

B2M, HPRT, RPL13A, GAPDH, HGDC
How RT² Profiler PCR arrays work?

### Isolate Total RNA
1. Convert Total RNA to cDNA.

- **Control**
  - Sample
  - cDNA 1
  - cDNA 2

2. Add cDNA to RT² qPCR Master Mix & Aliquot Mixture Across PCR Array.


4. Data Analysis.

### cDNA Synthesis
- Genomic DNA Removal Step (5 min.)
- Reverse Transcription Step (20 min.)

### Load Plates
- 1 Sample per PCR Array
- 2 minutes with multi-channel pipet

### Run 40 cycle qPCR Program
- Standard cycling conditions for all Real Time PCR Instruments
- 2 hours

### Upload and Analyze Data (FREE)
- 15 minutes from Raw Ct to Fold Change Data
RT2 Profiler PCR Array data analysis

- Free complete & easy analysis with web/excel-based software
- Multiple analysis formats to interpret gene expression results

Scatter Plot

Volcano Plot

Clustergram
How cytokines are regulated under PMA-Ionomycin treatment

Human PBMCs were treated with PMA and ionomycin, and then analyzed using the Common Cytokines RT² Profiler PCR Array. This volcano plot shows both fold-change and the statistical significance, and demonstrates that 23 genes, including IL-10, IFN-gamma, IL-2, and TNF were upregulated, while IL-1beta and 5 other genes were downregulated in response to treatment.

Next step- Validate results by ELISA for only the cytokines which are changing!
Host Response Profiling: Gene expression

Innate Immunity — The First Line of Defense
The innate immune response attracts immune cells and activates the adaptive response to control novel foreign pathogens.

- Identify an innate immune response

Which Toll-Like Receptors Are Signaling?
Toll-like receptors recognize broad classes of microbes, activating downstream signaling pathways that initiate a tailored immune response.

- Profile TLR activity

Attend the part 3 & 4 webinars

June 16, 1 – 2 p.m. EDT

Part 4: Toll-like Receptors in Inflammation – The bridge between innate and adaptive immunity
June 23, 1 – 2 p.m. EDT
Thank you for attending

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