## Is there a Crohn's pathogen?

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### Crohn's disease: Thanks

### Doug Reim

- Invitation & OrganizationDr. Phil Gold
- Introduction & Moderation
   Canadiens and Rangers
- No game tonight

### Crohn's disease: Goals

#### Dr. Behr is an MD

- i.e. provides medical advice
   Professor Behr is a scientist
- i.e. investigates new ideas
   These missions are on a continuum, minimal overlap
- Today's research aims to inform tomorrow's treatments

#### I will not give medical advice

This presentation is not for making clinical recommendations

### Crohn's disease: Biases

My funding: CIHR, FRSQ, CCFC

How bacteria cause disease

No pharmaceutical funding

### Crohn's disease: Overview

Crohn's & autoimmunity
Crohn's & immunedeficiency
Crohn's & mycobacteria
Concluding thoughts

### Crohn's as autoimmune disease

Have you been told that Crohn's is an auto-immune disease?

What is the evidence that Crohn's is in fact an auto-immune disease?

# Witebsky's postulates (1957) Causality for autoimmune diseases

You can detect Antibodies in the blood directed at 'self'

You can identify 'self' molecule recognized by these Antibodies

Called the Antigen

If you put together the Antibodies and the Antigen in an animal, together they will make the disease.

### Witebsky's postulates: Crohn's Disease

Antibodies in the blood directed at 'self'

- Not met (minority)
  - 'Self' molecule recognized by antibodies
- Depends on first part
   Antibodies and Antigen put together result in the disease.
- Not met

### Crohn's as auto-immune disease

Auto-immune model based largely on use of anti-inflammatory treatments

- Steroids, anti-TNF
   HOWEVER, these drugs are also used when we treat infectious diseases
- Meningitis, Tuberculosis: steroids
- Leprosy: steroids, anti-TNF
   No <u>direct</u> evidence of auto-immunity

## Crohn's as immunedeficiency

Have you been told that Crohn's is an immunedeficiency disease?

What is immune-deficiency?

What is the evidence that Crohn's meets the definition?

## What is an immune deficiency?

#### Immune defect

- Classically pediatric
  - » Many infections, many pathogens
- Then AIDS
  - » Many infections, many pathogens
- Increasingly recognized as selective
  - » Defective immunity to one pathogen
  - » In absence of that pathogen, no disease

### Crohn's as an immunedeficiency

Analogy Immunology Genetics

# CD as an immunedeficiency: Analogy

# Chronic granulomatous disease (C.G.D.)

- GI disease in 46/140 patients
  - » Similar to Crohn's pathology

Marciano, Pediatrics, 2004

# Common variable immune deficiency

Colonic lesions like Crohn's in 43%

Daniels, Am J Surg Pathol. 2007

# CD as an immunedeficiency: Immunology

- 1. Endoscopic biopsy
  - Biopsy the biopsy site later
    - » CD patients have defective repair

Marks, Lancet, 2006

- 2. Radio-labeled bacteria into the skin
  - Labelled leukocytes into the vein
    - » Crohn's patients manifest:

Less recruitment of leukocytes to site of infection Delayed clearance of the bacterial infection

Smith, J. Exp. Med, 2009

### CD as an immunedeficiency: Genetics

Crohn's genes: many are involved in immune responses to bacteria
Crohn's-associated mutations: loss-of-activity

**NOD2/CARD15** (Ogura, Nature, 2001)

 "...results suggest a link between...immune response to bacterial components and development of disease."

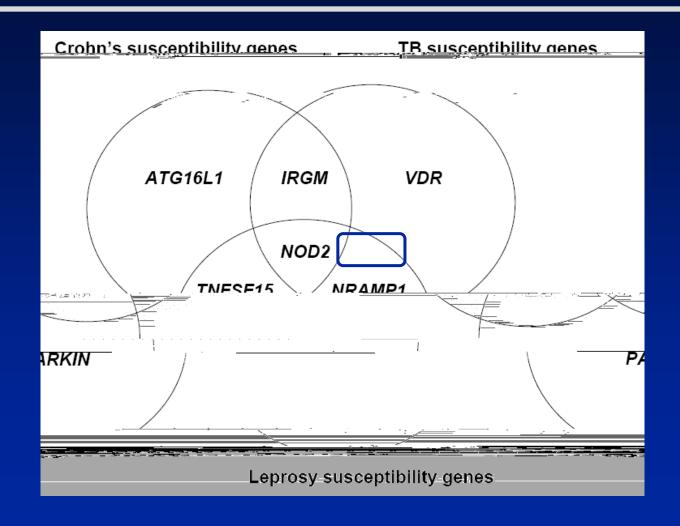
ATG16L1 (Hampe, Nature Genetics, 2007)

"The ATG16L1 gene encodes a protein in the...pathway that processes intracellular bacteria."

IRGM (Parkes, Nature Genetics, 2007)

 "Taken together, the genetic evidence regarding IRGM, ATG16L1, CARD15 and IL23R strongly implicates defects in innate immune pathways and handling of intracellular bacteria"

## CD as an immunedeficiency: Genetics



Lalande and Behr, Exp Rev Clin Immunology, 2010

## CD as an immunedeficiency: Genetics – 75,000 patients

# CD as an immunedeficiency: Which bacteria?

Analogy Immunology Genetics

Which bacteria can exploit these mutations to cause a chronic inflammatory disease?

### Simplified introduction to bacteria

Bacteria can be Gram-positive or Gramnegative

# Mycobacteria and the gene NOD2: Two questions

## Is NOD2 important for a mycobacterial infection?

- YES
- Mice disrupted for NOD2 have impaired resistance to experimental TB

Divangahi et al, J. Immunology, 2008

- Is NOD2 specific for a mycobacterial infection?
- YES
- Mycobacteria make a special form of the molecule recognized by NOD2 (*N*-glycolyl MDP)

Coulombe et al, J. Exp. Med, 2009 Hansen et al, J. Infectious Diseases, 2014

### NOD2 and different bacteria

Gram-positive

Intracellular

Produces **N**-glycolyl MDP

Gram-negative

Extracellular

Produces N-acetyl MDP

Favors a role for NOD2 in resistance to this microbial threat

e.g. mycobacteria

Favors ability of host to eliminate microbe in absence of NOD2

e.g. *E. coli* 

Vinh and Behr, Exp Rev Clin Immunology, 2013

# Immunedeficiency and mycobacteria

Data point to a problem with recognition & killing mycobacteria

At least in a subset of CD patients
 Why an intestinal disease?
 Which mycobacterium?

### M. avium paratuberculosis (MAP)



Analogy: Inflammatory bowel disease

Poor productivity Weight loss Diarrhea

Causal link proven by experimental infection studies (3-5 years in cattle, deer, etc.)

### MAP and Crohn's disease

MAP DNA has been detected in Crohn's biopsies by PCR

Feller et al, Lancet ID, 2007

Mycobacteria seen in Crohn's tissue by highpower microscopy

Jeyanathan et al, Microbes and Infection, 2007

Lymphocytes from Crohn's biopsies recognize *MAP* antigens

Olsen et al, PLoS ONE, 2009

N.B. Association Causation A B; B A; C A & B

### Can MAP Crohn's disease?

Classically, causation for gastroenteritis has been established with human volunteers

- E. coli
- Helicobacter pylori
- Campylobacter jejuni

We CANNOT give **MAP** to human volunteers

- MAP causes a chronic disease

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### Can *MAP* Crohn's disease? - 2

# We CAN infect mice disrupted for Crohn's genes We CAN control:

- Microbe (*MAP* vs. other)
- Duration of infection (weeks / months)

#### We CAN then monitor outcomes:

- Bacteria:
  - » What happens to *MAP* infection?
- Immunology
  - » Do we find cells that cause inflammation?
- Pathology
  - » Do we see Crohn's-like pathology?

### Mouse model: utilities

#### Conceptual advance:

- Ask about causation
  - » We control experiment
  - » We assign directionality
- Ask about mechanism

#### Translational advance

- Ask about benefit of treatment options
  - » Antibiotics
  - » Anti-TNF
  - » Antibiotics PLUS anti-TNF
- We do not set out to prove that something works
- We investigate whether something works

### Concluding thoughts

I do NOT consider Crohn's to be an autoimmune disease

Immunedeficiency model fits with analogy, immunology, genetics AND microbiology

Which bacteria are implicated remains a field of active investigation

Data are consistent with a mycobacterial infection, but science incomplete

### Concluding thought

"New facts, collected in old ways under the guidance of old theories, rarely lead to any substantial revision of thought. Facts do not "speak for themselves"; they are read in the light of theory"

## Acknowledgements:

#### The lab:

Mathy Jeyanathan
Maziar Divangahi
Francois Coulombe
Jesse Hansen
Jean-Daniel Lalande
Damien Montamat-Sicotte

Fiona McIntosh



#### **Collaborators:**

Philippe Gros
Michael Reed
James Gleason
Bhushan Nagar
Marilene Paquet







