Novel Insights into IBD Pathogenesis

Maria T. Abreu, MD
Professor of Medicine
Chief, Division of Gastroenterology
University of Miami Miller School of Medicine
Etiologic Theories in Inflammatory Bowel Disease

- Genetic Predisposition
- Mucosal or Innate Immune System
- Environmental Triggers (luminal bacteria, infection)

IBD
Etiologic Theories in Inflammatory Bowel Disease

Genetic Predisposition

Mucosal or Innate Immune System

Environmental Triggers (luminal bacteria, infection)

IBD
Innate and Adaptive Immunity

- **Pattern recognition receptors**
  - TLRs, NODs

0–6 hours

- Immediate responses
- **Innate immunity**
  - Broad action

1–5 days

- Longer-term mobilization
- **Adaptive immunity**
  - Antigen-specific

- **PRIME**
- **ACTIVATE**
- **MODULATE**

- Release of antimicrobials
- Recruitment of cells
- Localized inflammation

KILL PATHOGENS
CLEAR INFECTION

TLRs Recognize Pathogen-Associated Molecular Patterns

Antigen Presenting Cells:
- Macrophages
- Dendritic Cells

T Cells

- TLR-2
- TLR-4
- TLR-5
- TLR-7,8
- TLR-9
- TLR-11

Bacterial lipopeptide
- Flagellin
- Lipopeptides

LPS
- MyD88

MD-2
- TRIF

CpG DNA
- UPEC/Profilin

ssRNA

dsRNA
- TLR-3

IRAK
- TRAF6

IRF3

NF-κB
- IκB

IFN-β
Leukocyte Diapedesis: A Multi-Step Process

Tethering and Rolling

Signaling (integrin activation)

Firm Adhesion

Diapedesis

Endothelial Cells
Key Adhesion Molecule Interactions

α4-Integrins: required for firm adhesion to and migration across endothelium
Genetic Susceptibility

• Familial incidence – 10%-15%
• Complex genetic disorders
• Conserved familial patterns of disease
• Twins: monozygotic > dizygotic concordance monozygotic twins:
  CD 44%-58%, UC 6%-18%
• Candidate gene approach – HLA region, TNF-α
• Genome-wide search: chromosome 1 IL-23R, 16 (NOD2 gene), 5 (OCTN)

The Brave New World of Genome Wide Scans!

- Genome wide association (GWA) studies
  - There are new “chips” that permit screening of 300K to 1 million single nucleotide polymorphisms (SNPs) at a single time
  - Every gene is represented but not every SNP in every gene is there (because one gene can contain several SNPs)
- Advantage: Unbiased approach
- Disadvantage: Statistical challenge (Bonferroni correction)
- Still need to figure out function and which gene in a region is really involved
- Next wave will be genetics stratified by phenotypes, rather than just UC versus Crohn’s disease
IBD Linkage Analysis and Crohn’s Disease
Genome-wide Association

Confirmed and replicated from independent genome searches
Suggestive from independent genome searches
Significant from meta-analysis
Suggestive from meta-analysis

- **GWA Crohn’s Disease**
- **IL23R**
- **ATG16L1**
- **PHOX2B**
- **S100Z**
- **SLC22A4**
- **TNFSF15**
- **CARD15**
- **PKD1L2**
- **FAM92B**
- **TXNDC11**
### Allelic Variants of NOD2 are Associated with Crohn’s Disease

<table>
<thead>
<tr>
<th>CARD1</th>
<th>CARD2</th>
<th>NBD</th>
<th>LRR</th>
<th>1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **P268S**
- **R702W**
- **G908R**
- **1007fs**
- **SNP5**
- **SNP8**
- **SNP12**
- **SNP13**

1. Carriage of NOD2 allelic variants¹-⁶:
   - Crohn’s disease 27-39%
   - Control population 14-16%
   - UC population 12-14%⁵

2. Carriage of 2 mutations is estimated to carry absolute risk of CD of 3%

---

NOD2 Role in Pathogenesis

NOD2 Phenotypes in Animal Models

**NOD2**<sup>-/-</sup>

**Commensal Bacteria**

**NOD2**<sup>-/-</sup>

**Control of Pathogenic Bacteria**

**NOD2<sup>2939IC</sup>**

**DSS-treatment**
Patients Carrying NOD2 Mutations Have a Decreased Response to its Ligand MDP

Van Heel et al. Lancet 365:1794, 2005
Crohn’s Disease May Represent Decreased Ability to Destroy Intracellular Bacteria—“Autophagy”

- Constitutive process for regulating intracellular homeostasis and organelle turnover
- Breakdown of bacteria: E coli, Salmonella, Legionella, M tuberculosis
- ATG16L1 SNP: Ileal CD associated with a nsSNP (A197T) in exon 8 of ATG16L1, chr 2q37.1
  - OR’s 1.45 (95% CI: 1.21–1.74) for carrying allele G
- Immune-regulated guanosine triphosphatase; impt M Tb (Chr 5q33.1)
  - OR= 1.38 (1.15–1.66)

“Newer” Th1/Th2/Th17 Paradigm

Therefore IL-12 versus IL-23 determine fate of Th1 or Th17 cells.
IL-12 Family of Cytokines

Plasma membrane

- IL-6
  - gp130
  - IL-6Rα
  - Cytokine-receptor homology domain
  - Fibronectin-like domain
  - Immunoglobulin-like domain

- IL-12
  - IL-12Rβ1
  - IL-12Rβ2
  - IL-12Rβ1
  - Cytokine-receptor homology domain
  - Fibronectin-like domain
  - Immunoglobulin-like domain

- IL-23
  - p19
  - IL-23R
  - gp130
  - Cytokine-receptor homology domain
  - Fibronectin-like domain
  - Immunoglobulin-like domain

- IL-27
  - p28
  - EB13
  - gp130
  - Cytokine-receptor homology domain
  - Fibronectin-like domain
  - Immunoglobulin-like domain
IL-12 and IL-23 Blockade in IBD

- IL-12 and IL-23 share common p40 subunit and common β1 receptor
- IL-12 and IL-23 activate different subpopulations of T cells
- IL-12 p40 neutralizing antibodies are therapeutic in mouse colitis models and in human CD

Absence of IL-23 Protects Against Colitis in IL-10⁻/⁻ Mice

IL-10⁻/⁻ KO  IL-10⁻/⁻ x p19⁻/⁻ (IL-23)  IL-10⁻/⁻ x p35⁻/⁻ (IL-12)

A Polymorphism in the IL-23R Gene Protects Against Crohn’s Disease

- Genome-wide association study: 3 SNPs identified (2 were Nod2)
- Uncommon variant of IL-23R: Arginine 381 → Glutamine
- Carriage of Arg381Gln is protective of Crohn’s disease: OR 0.26 (0.15-0.43)
- Functional signaling consequences unknown

Genes in the IL-23/IL-17 Pathway Interact to Increase Crohn’s Disease Susceptibility

Papadakis KA, et al. DDW. 2007. #454.

Odds Ratio for CD

\[ p_{\text{MH}} < 0.0001 \]

Number of “risk” haplotypes present

IL23R, IL17A, IL17RA, and IL12RB1
Dendritic cell
Peyer’s patch
NOD2
PAMPs
TLRs
Dendritic cell
Peyer's patch
NOD2
Nucleus
NF-κB
IL-23
Defect in autophagy
CARD CARD NOD2 LRRS
PAMPs TLRs
NATURE
IL-12p40
NF-κB
Adaptive Immune Responses to Microbial Products Increases With Decreased NOD2 Function

Level of Antimicrobial Abs

NOD2/CARD15 Variant Status

N = 499

N = 194

N = 39

P trend = .002

Normal Mucosal Adaptive Immune Response

sIgA
Abnormal Mucosal Adaptive Immune Response in IBD

- Crohn’s-like: T_{H17}, IFNγ, TNFα, IFN-κB, IL-23, IL-12, TGFβ, IL-10
- Ulcerative colitis-like: EBI3, NK T cells, IL-13, IL-4, IL-5, T_{H2}, IgG

TLR expression, IgG, IL-10, T_{Reg}
Treatment of Colitis by Inhibition of Th1/Th17

Colitis

Treatment

Anti-IL-12/23
Anti-IFNγ
Anti-TNFα
IL-10

Attenuated/
No Colitis

Th1/Th17

Th1/Th17
The TNF Superfamily: MHC Paralogs

Chr6
- LTα1β2
- TNF
- LTα3

Chr19
- LIGHT
- CD27L
- 4-1BBL

Chr1
- FasL
- OX40L
- AITRL

Chr9
- TL1A
- CD30L

LTβR
- TNFR1
- TNFR2
- HVEM
- CD27
- 4-1BB
- DcR3
- Fas
- OX40
- AITR
- DR3
- CD30

TRADD
- FADD
- Caspase8

TRAF2/3/5

Executioner
- Caspases

NFκB

APOPTOSIS

INFLAMMATION

CELL SURVIVAL
Key Actions Attributed to TNF-α

- **Macrophages**: Proinflammatory cytokines, Chemokines, Increased inflammation
- **Endothelium**: Adhesion molecules, Increased cell infiltration
- **Epithelium**: Ion transport, Permeability, Compromised barrier function
- **Fibroblasts**: Metalloproteinase synthesis, Acute phase response, Increased CRP in serum

**References**
Infliximab and Adalimumab Induce Apoptosis of Activated T-cells and Monocytes

Apoptosis

TNF

Anti-TNF

Fab fragment-PEG

Outside in-signaling

Lugering et al, Gastro v. 121, 2001
Ten Hove et al, Gut v. 50, 2002
Shen, C et al. IBD Journal v. 12, 2006
Shen, C et al. APT v. 21, 2005
Etiologic Theories in Inflammatory Bowel Disease

- Genetic Predisposition
- Mucosal or Innate Immune System
- Environmental Triggers (luminal bacteria, infection)
Luminal Bacteria Stimulate Immune-mediated Colitis

Mice
- IL-2^/-
- IL-10^/-
- TCR\(\alpha^-\)/
- CD\(_3\)E\(_26\) TG
- SAMP1/yit
- DSS
- CD\(_{45}\)RB\(^{hi}\) \(\rightarrow\) SCID

Rats
- B\(_{27}/\beta\)2M TG
- Indomethacin

Nonhuman Primate
- Cotton top tamarin

Bacteria
- IL-1\(\beta\)
- TNF\(\alpha\)
- TH\(_1\)

No Bacteria
- No Immune Activation
- No Colitis

No Colitis

No Bacteria
- No Immune Activation
- No Colitis

Bacteria
- No Immune Activation
- Colitis
“Bacterially” Generated Phenotypes

- **IL-10**/
  - *Germ-Free*
- **IL-10**/
  - *Commensal Bacteria*
- **IL-10**/
  - *E. faecalis*
- **IL-10**/
  - *E. coli*
- **IL-10**/
  - *E. faecalis and E. coli*

Bacteria Find Their Niche in the Mucosa

The Immunologic Spectrum of IBD

Indeterminate colitis

UC

CD

Th2

Th1
The Real Immunologic Spectrum of IBD

Th1
Th2

T_{IL-17}
Defective T_{regs}

IBD2
IBD3
IBD4
IBDx
CARD15

ASCA
CBir1
pANCA
OmpC
I2

IBD1
IBD2
IBD3
IBD4
IBDx
Pathogenesis: Definition of IBD Subtypes

Genetic Variants/Mutations
- NOD2?
- TLR5?
- TLR5/Chr 4
- Autophagy defect

Immunologic/Bacterial Phenotypes
- High antibody combo quantile
- OMPC+
- CBir1+
- Low antibody combo quantile

Clinical Phenotypes
- Aggressive SB dz
- Internal penetrating disease
- SB surgery
- Non-progressive disease