The Microbiome and the Pathogenesis of Inflammatory Bowel Disease

Demystifying Medicine Lecture
Feb 9, 2016

Warren Strober, Laboratory of Host Defenses, NIAID
Crohn’s Disease: Clinical Features

- A chronic, relapsing, full-thickness inflammation of the gut

- Affects small bowel (30%), colon (30%), or both (40%)

- Fistulization, strictures

- Abdominal pain, bleeding, diarrhea, weight loss, fever, lethargy

- Associated uveitis/episcleritis, arthritis, skin disease, oral ulcers, kidney stones

- Up to 20% chronically active, 70% chronic relapsing, 10% in sustained remission; 70% will require surgery
Crohn’s Disease Results from a Dysregulated Response to Mucosal Ligands and Antigens

Colitis

Normal Bowel

Ligands
Antigens

DC

MLN

T cell

IL-12
IL-23
IL-6
TNF-α
IFN-γ
IL-17
TNF-α

Cytokines

Ligands
Antigens

Colitis
I. The Microbiome and Gut Inflammation
Characteristics of Microbiome Abnormalities in Crohn’s Disease

- Firmicutes Depleted and Proteobacteria Increased in a Subset of Patients
- Abnormalities limited to Small Bowel in CD Patients
- Bacteroidetes decreased or increased
- Faecalibacterium prausnitzii and Clostridium decreased
- No evidence of a pathogenic organism
- Decreased Bacterial Diversity
Organisms in the Gut Microbiome with Specific Regulatory/Host Defense Functions
Organisms in the Gut Microbiome May Prevent or Cause Intestinal Inflammation
Mechanism of Regulatory T Cell Induction by Clostridial Organisms
The Gut Microbiome May Contain Two Types of Colitogenic Organisms
Gut Microbiome of NOD2-Deficient Mice with DSS-Colitis Transmits Colitis to WT Co-housed Mice
NOD2-Deficient Mice Exhibit More Severe DSS-Colitis and Dysbiosis
Organisms in the Gut Microbiome May Prevent or Cause Intestinal Inflammation
Adherent/Invasive E. coli (AIEC) a Colitis-Promoting Organism
II. NOD2 Risk-Polymorphisms and the Pathogenesis of Crohn’s Disease
CARD15 encodes NOD2, a member of the NLR family of proteins.

NOD2 is an intra-cytoplasmic sensor of MDP, a component of peptidoglycan.

10-15% of patients with Crohn’s disease patients bear CARD15 mutations.
NOD2 and Disease

Disease-associated Mutations

Card

NBD/NOD

R334W
R334Q

?? Uncontrolled NF-κB activation

Blau Syndrome

LRRs

R702W
G908R
L1007fs

Deficient NF-κB activation

Crohn’s Disease
Paneth Cells and Defensin Production
IL-12p40 and IL-6 production by Hu Dendritic Cells Pre-treated with MDP

1st                          2nd

Medium       TLR ligands
Medium       TLR ligands + MDP
MDP         TLR ligands
MDP         TLR ligands + MDP

1st                          2nd

Medium       TLR ligands
Medium       TLR ligands + MDP
MDP         TLR ligands
MDP         TLR ligands + MDP

**IL-12p40 and IL-6 production by Hu Dendritic Cells Pre-treated with MDP**
NOD2 Stimulation Leads to Induction of IRF4 Expression

<table>
<thead>
<tr>
<th>Protein</th>
<th>Medium</th>
<th>MDP</th>
<th>Medium</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyD88</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>TAK1</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>TRAF6</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>IKKγ</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>IRF3</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>IRF4</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>IRF5</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>IRAK-M</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>SOCS-1</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Actin</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
Prevention of TNBS-colitis by administration of MDP
Inhibition of DSS-induced Colitis by MDP Administration

Day

% Body Weight

4% DSS

MDP i.p.
Failure of MDP Administration to Inhibit DSS-Colitis In IRF4-Deficient Mice
NOD2 Signaling Inhibits All TLR Responses

TLR2
PGN

TLR4

MDP

TLR9
CpG

RICK+IRF4
Nod2

c-Fos

IL-12

CD4

IFN-γ

Decreased Development of Th1 cells and Inflammation
IAP Proteins are E3 Ligases that PolyUbiquitinate RIP2
NOD2 Pre-Stimulation Induces IRF4 Binding to RICK, TRAF6 and MyD88 …and Inhibits NF-κB Activation
NOD2 Pre-Stimulation Induces IRF4 Binding to RICK, TRAF6 and MyD88 – Duolink Staining
NOD2 Pre-Stimulation of Hu Dendritic Cells Induces IRF4-Mediated Inhibition of K63-Linked Polyubiquitination - I
NOD2 Pre-Stimulation of Hu Dendritic Cells Induces IRF4-Mediated Inhibition of K63-Linked Polyubiquitination - II
MDP Administration Down-Regulates TNBS-Colitis
MDP Treatment Induces IRF4 Interactions and Inhibition of K63-Polyubiquitination in Colonic LPMCs
Prevention of TNBS-Colitis by Administration of an IRF4-Expressing Plasmid - I
Prevention of TNBS-Colitis by Administration of an IRF4-Expressing Plasmid - II
Treatment of TNBS-Colitis by Administration of an IRF4-Expressing Plasmid
NOD2 Regulation of TLR Signaling

- Muramyl Dipeptide
- NOD2
- RICK
- IRF4
- MyD88
- NF-kB
- TLR Ligand
- TLR