Research Update:
Looking for the Answers

Ted Denson, M.D.

Schubert-Martin Inflammatory Bowel Disease Center

Research Support: NIH/NIDDK, CCFA, BMRP
Objectives

• Causes of IBD: genes & bugs
• Dietary approaches
• New medications in the pipeline
Multi-factorial Causes of IBD

Genetic Predisposition
n=200+

Gut Bacteria, Fungi & Viruses: Microbes

Environmental Factors: Diet & Antibiotics
Increasing trend of IBD in industrialized countries since the 19th century and in industrializing countries since the 20th century.
Global Prevalence of IBD: 6 Million

- North-South gradient in North America & Europe
- UC increased first in Asia
- CD now in Japan

Cosnes et al. Gastro 2011
Incidence has doubled in the pediatric age group over the past decade.

80,000 affected in the U.S.

60% males

Loftus, *Gastroenterology* 2003; 124:abstract 278

Factors Influencing IBD Development and Flares

- Exposome
- Infectome
- Microbiome

Inflammatory activity of IBD over time:
- Pre-clinical
- Clinical

Genetic susceptibility:
- Disease onset
- Diagnosis
- Flares
- Controlled disease
The genetic, environmental, and microbial determinants of IBD

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>Risk in Caucasians and Middle Eastern migrants</td>
<td>Protective in Caucasians and Asians</td>
</tr>
<tr>
<td>Antibiotic use in childhood</td>
<td>Risk in Caucasians, protective in Asians/Middle Eastern migrants</td>
<td>Risk in Caucasians, protective in Asians/Middle Eastern migrants</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>Protective in Asians and most studies in Caucasians</td>
<td>Protective in Asians and most studies in Caucasians</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>Risk in Caucasians</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>Risk in Caucasians</td>
<td>Protective in Caucasians</td>
</tr>
<tr>
<td>Low levels of vitamin D</td>
<td>Risk in Caucasians</td>
<td>Risk in Caucasians</td>
</tr>
<tr>
<td>Tea or coffee consumption</td>
<td>Protective in Asians</td>
<td>Protective in Asians</td>
</tr>
</tbody>
</table>

Hygiene hypothesis: Having pets in childhood, living on a farm, larger family size, and drinking unpasteurized milk were inversely associated with the risk of CD and UC.

Changing diet: Introduction of packaged food, fast food chains, increased use of antibiotics, increased fat (monounsaturated and polyunsaturated fatty acid) consumption and sugar intake, less dietary fibers is associated with risk of IBD.

Dietary chemicals: Food additives – saccharin, sucralose, carboxymethylcellulose and polysorbate-80, common emulsifiers (including polysorbates, sorbate esters, lecithin), might increase risk of IBD (data are derived from animal models).

Over 200 IBD risk loci (37 specific for Crohn’s disease and 32 for ulcerative colitis) have been discovered. However, modest fraction of predicted heritability can be explained by known genes or loci.
Genetic Causes of Crohn’s Disease

Intestinal barrier: Loss of bacterial sensing and killing

White cell over-activation
Genetic Causes of Ulcerative Colitis

Intestinal barrier: Poor healing

White cell over-activation
Up to 50 Genes with Strong Effects Cause IBD in Very Young Children: May be Cured with Bone Marrow Transplant (BMT)

Uhlig et al Gastro 2014

Anti-Inflammatory Pathways: BMT
Intestinal Lining Healing

White Cell Defects: BMT

Inflammatory Pathways: BMT
IBD Risk Genes and Chances of Developing IBD

 Uhlig et al IBDJ 2016
Environmental Triggers: The “Exposome”

- Air pollution
- Diet
- Drugs
- Stress
- Infections
- Food additives
- Water pollution

Direct effects on intestinal epithelial cells, mucosal immune cells, extraintestinal cells

Indirect effects via modulation of the intestinal microbiota

INFLAMMATORY BOWEL DISEASES
Environmental Factors

- Smoking: Crohns flares and medication effectiveness
- NSAIDs: Crohns and Colitis flares in 25%
- Vitamin D deficiency: IBD risk and flares
- Perinatal & childhood infections/antibiotic exposures?
- Stress?
- Food or food additives?
- Final measurable effect: epigenetic changes and microbial shifts
Approaches to Studying the “Exposome” Factors Which Trigger IBD Development and Flares

Partially «unbiased» approaches (some selection inevitably necessary)

- Patient cohort
  - Questionnaire-based EWAS, evaluation of socio-demographic factors (e.g. breastfeeding)
  - "unbiased" approach
  - Retrospective analysis possible
  - Factors before onset of disease "accessible"
  - Relatively cheap
  - Large sample possible

- Healthy control cohort

Selective approach

- "omics" EWAS, evaluation of blood/tissue/exhalation air levels of potential chemicals and factors in patients and control cohorts
  - "unbiased" approach
  - Quantitative measurement
  - Definite factors
  - Some causation possible, especially for pollution factors

- Single factor studies, animal models and selective experiments
  - Hypothesis driven
  - Causative relationship can be established
  - Animal models possible
  - Interventions and therapies easier
  - Selection bias
  - Expensive
  - Only possible after onset of disease
  - Limited time points
  - Low number of factors accessible
Dietary Patterns and Risk of IBD in Europe: Results from the EPIC Study

- Tested associations between dietary patterns and risk for Ulcerative Colitis or Crohn’s Disease
- Prospective case-control study among 366,351 participants, including 256 who developed UC and 117 who developed CD
- Tested for association with Mediterranean diet score or other dietary patterns
- Med. diet: high in vegetables, fruits & nuts, grains, fish, and olive oil and low in meats and dairy products
- No dietary pattern was associated with UC or CD risk; however, older than average age so results may not apply to children or young adults
- However, association between a diet high in sugar and soft drinks, and low in vegetables, and UC risk: Microbe imbalance?
- Drink more water and eat your vegetables!
Gut Bacteria

- Billions of bacteria in the intestine
- Bacteria change with genes, food, antibiotics
- Bacteria are different in IBD patients, and are the main activator of the immune system
Mechanisms of barrier function and immune regulation by the intestinal microbiota

A

Clostridium groups IV, XIVA
metabolic fuel
SCFAs
Histone deacetylase
restoration

F. prausnitzii
15kDa
B. fragilis
OMV

TLR2
NFκB
DC

Treg
TR1

Interleukin-10
IL10

CD4

TCR
MHC

CD4+ T cells

ILC3

IL-22

Apoptosis

B

Adherent invasive E. coli

Enterococcus faecalis
Mucolytic enzymes and proteases

E. coli

LPFA1

Propanediol
Fe metabolism

Bilophila wadsworthia

HS

IL17

IFNγ

Mucus

Autophagy

ATG16L1

IRGM

AIEC
Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability

[Graphs and statistical data from Xavier et al. Sci Transl Med 2016]
Intestinal Bacterial Shifts are Associated with Symptoms

We will now test a prebiotic dietary supplement to expand the non-inflammatory bacteria.

Gevers et al Cell Host Microbe 2014
Dietary Emulsifiers Alter Microbiota Localization and Promote Colitis in Mice Lacking the IL-10 Anti-Inflammatory Gene

CMC: carboxymethylcellulose
P80: polysorbate-80
Therapeutic Targets

1: Intestinal damage
   - Proteases
   - LTB$_4$

2: White cell activation by bacteria
   - NO
   - ROM

3: Inflammatory proteins
   - Resting Mφ
   - Activated Mφ
   - PMN
   - ICAM-1
   - MAdCAM-1
   - Lymphocyte
   - Selectins
   - PMN
   - Monocyte
   - TCR
   - CD4
   - CD28
   - CTLA4
   - MHC Class II
   - CD40L
   - IL-2
   - IL-12
   - IL-23
   - IL-17

4: White cell recruitment to the intestine
   - B7
   - B cell
   - T cell

Diet or fecal transplant to change microbes?

Biologic medications to block inflammatory proteins and now white cell recruitment
Cytokine Signaling Blockade with Biologics and Small Molecules

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>CYTOKINE</th>
<th>TARGET</th>
</tr>
</thead>
</table>

Biologics: monoclonal anti-cytokine antibodies (anti-TNF, anti-IL12/23, anti-α4β7)

Small molecules: specific oral inhibitors (JAK1/2/3 kinase activity; SMAD7 antisense)
Biologic Therapy: Antibody Blockade of Tumor Necrosis Factor Inflammatory Protein
Therapeutic Drug Monitoring to Optimize Long Term Benefit of Biologic Medications

Measurement of anti-TNF level and anti-drug antibodies

- Undetectable or low anti-TNF level\(^1\) and (-) ADA
  - Increase anti-TNF dose or decrease infusion interval

- High anti-TNF level and (+) or (-) ADA
  - Switch to another drug class.

- Undetectable or low anti-TNF level\(^1\) and (+) ADA
  - High ADA level\(^2\)
    - Switch anti-TNF or Drug Class
  - Low ADA level\(^2\)
    - Dose optimization and consider adding IMM

No response

Therapeutic Drug Monitoring of Anti-tumor Necrosis Factor Agents in Patients with Inflammatory Bowel Diseases

Yarur, Andres J.; Rubin, David T. Inflammatory Bowel Diseases. 21(7):1709-1718, July 2015.
doi: 10.1097/MIB.0000000000000380
Detection and treatment of IBD before symptoms

Figure 1

Colombel et al Gastro 2017
Early anti-TNF Prevents Penetrating but not Stricturing Complications

Figure 2: Development of Stricturing or Penetrating Complications during 5-year Follow-up in the matched cohort
Balance between Mitochondrial and Extra-Cellular Matrix Pathways

### A

<table>
<thead>
<tr>
<th>Pathway</th>
<th>B2 &gt; B3</th>
<th>B2 &lt; B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil chemotaxis (91)</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Neutrophil migration (101)</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>Response to TNF (73)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Cytokine activity (221)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Response to LPS (221)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Chemokine receptor binding (59)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Metalloproteinase activity (189)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Response to bacterium (567)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Response to wounding (1109)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Cytokine receptor binding (287)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Inflammatory response (669)</td>
<td>46</td>
<td>45</td>
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<tr>
<td>Extracellular matrix disassembly (136)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Biological adhesion (1518)</td>
<td>46</td>
<td>45</td>
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<tr>
<td>Growth factor binding (134)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Extracellular matrix organization (409)</td>
<td>46</td>
<td>45</td>
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<tr>
<td>Collagen binding (71)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Collagen catabolic process (78)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>TGF-β receptor binding (33)</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>Extracellular matrix structural constituent (70)</td>
<td>46</td>
<td>45</td>
</tr>
</tbody>
</table>

### B

![Graph showing fold change in gene expression](image)

- High in B2
- High in B1
- High in B2

### C

![Scatter plot showing log P-value](image)

- High in B1 Protected
- High in B2 Low Prob.
Targeting The IL-12 and IL-23 Inflammatory Proteins

1: Intestinal damage

2: White cell activation by bacteria

3: Inflammatory proteins

4: White cell recruitment to the intestine
Sources of and targets for IL17

- **SOURCE**
  - γδ T cells
  - Intraepithelial γδ T cells
  - Th17 cells
  - Innate lymphoid cells

- **CYTOKINE**
  - IL17

- **TARGET**
  - Epithelial cells
  - Fibroblasts
  - Neutrophils

IL23 and Th17 cell pathways

A

Cytokine

Cytokine receptor

Signaling molecules

Transcription factors

Potential levels for inhibition

Gene expression

B

MEDI2070
risankizumab

ustekinumab
briakinumab

p19
p40
p35

IL23
IL12

Th1

tofacitinib

JAK2
TYK2

IL-17
IL-21
IL-22

secukinumab
brodalumab

Th17

Ustekinumab Blocks the IL-12 and IL-23 Inflammatory Proteins & Provides Clinical Benefit for Two-Thirds of Patients With Crohn’s Disease Refractory to Anti–Tumor Necrosis Factor Agents

A

135 patients recruited

13 patients were excluded (follow-up less than 3 months)

122 patients included

B

122 patients included

90 mg SC weeks 0 & 4
90 mg SC q 8 wk

79 patients (65%) with clinical benefit at 3 months

43 patients (35%) with non response at 3 months

71/110 patients with luminal CD

8/12 patients with perianal CD

A clinical benefit was defined as a significant improvement in CD-related clinical symptoms and laboratory tests assessed by the patient's physician leading to continued ustekinumab treatment, associated with complete weaning from steroids if they were being taken at inclusion, without surgery, or immunosuppressant introduction.

Wils et al Clin Gastro Hep 2015
Targeting White Cell Recruitment to the Gut

1: Intestinal damage

2: White cell activation by bacteria

3: Inflammatory proteins

4: White cell recruitment to the intestine

- NO
- ROM
- Proteases
- LTB₄
- PMN
- Integrins
- ICAM-1
- MAdCAM-1
- Selectins
- Activated Mφ
- Resting Mφ
- TCR
- CD4
- CD28
- CTLA4
- B7
- MHC Class II
- Naive T cell
- CD40L
- B cell
- T cell
- CD40
- IL-2
- IL-4
- IL-5
- IL-8
- IL-12
- IL-17
- IL-23
- IL-28
- Th1
- Th2
- Th17
- IFNγ
- TNF
- IL-17
- IL-5
Vedolizumab – anti-adhesion molecule ($\alpha 4\beta 7$)

- $\alpha 4\beta 7$ adhesion molecules are specifically needed for white cell intestinal recruitment
- FDA approval for adults with Crohns and UC in 2014

Association Between Response to Etrolizumab which Blocks White Cell Retention in the Gut and Expression of Biomarkers in Colon Biopsies of Patients With Ulcerative Colitis

Etrolizumab, a humanized monoclonal antibody against the β7 integrin subunit, blocks both α4β7:MAAdCAM-1 and αEβ7:E-cadherin interactions to reduce homing of leukocytes to the gut mucosa and retention of lymphocytes in the intraepithelial compartment, respectively.
Using Mesenchymal Stem Cells to Heal Fistulas

A. MSCs escape immune surveillance

- No expression of MHC-II
- No expression of co-stimulatory molecules
- Low expression of MHC1

B. Immunomodulatory properties of MSCs

- Cell-cell contact
- Soluble factors
- Generation of Tregs

C. Proposed fistula tissue repair properties of MSCs

1. Immune suppression
2. Growth factor production
3. Tissue repair
Treatment of IBD by altering microbial composition or function

**Correct dysbiosis/microbial function**

- **Traditional**
  - Antibiotics
  - Probiotics
  - Prebiotics
  - Synbiotics (combination probiotics/prebiotics)

- **Developing**
  - FMT
    - Random donor
    - Matched donor/recipient
    - Prepared donor
  - Synthetic Mixtures
    - For all patients
    - Targeted for individual recipients
  - Dietary
    - Complex foods
    - Simplified (synthetic)
    - Improved prebiotics
  - Bacteriophage targeting aggressive bacteria
  - Block attachment/invasion AIEC
  - Anaerobic environment-sequester $O_2$

**Normalize mucosal barrier function**

- ↑ SCFAs
  - Clostridium IV, XIVA
  - F prausnitzii
  - Roseburia
- ↑ Mucolytic spp (E faecalis)
- Block attachment/invasion E coli
- Agonists EGFR
- Agonists FXR (i.e. bile acids)

**Reverse immune dysfunction**

- **Diet**
  - ↑ SCFA substrates
  - ↓ HS substrates
  - ↑ Omega 3 FA
  - ↓ milk fat
  - **Selected Commensal Bacteria**
    - Clostridium groups IV, XIVA
    - Faecalibacterium prausnitzii
    - Bacteroides fragilis
  - **Novel Synthetic Bacterial Metabolites**
    - F prausnitzii 15kDa
    - Bacteroides fragilis OMV
  - **Selected Bacteria**
    - SFB Bilophila wadsworthia
    - Adherent/invasive E coli
  - **Recombinant Bacteria**
    - IL10 producing

*Induce remission with corticosteroids or biologics*
Gut Bacteria and Fungi Change within One Week of Starting Exclusive Enteral Nutrition (EEN)

**Disease**
Crohn’s disease vs. healthy controls in absence of antibiotics

**Decreased**
- Akkermansia
- Eubacterium
- Odoribacter
- Parabacteroides
- Prevotella
- Roseburia

**Increased**
- Eggerthella
- Escherichia
- Veillonella
- Candida
- Clavispora
- Cyberlindnera
- Kluvyveromyces

**Antibiotics**
Antibiotic use among patients with Crohn’s disease

**Decreased**
- Alistipes
- Bacteroides
- Blautia
- Desulfovibrio
- Eggerthella
- Ruminococcus

**Increased**
- Bifidobacterium
- Enterococcus
- Gemella
- Granulicatella
- Klebsiella
- Lactobacillus
- Pedococcus
- Rothia

**Diet**
EEN among patients with Crohn’s disease

**Decreased**
- Dialister
- Dorea
- Gordonibacter
- Haemophilus
- Streptococcus

**Increased**
- Candida
- Clavispora
- Cyberlindnera

**Inflammation**
Resolution of inflammation adjusted for antibiotics and treatments

**Decreased**
- Actinomyces

**Increased**
- Lactococcus
- Roseburia

**Baseline** Week 1 Week 4 Week 8

Lewis et al Cell Host Microbe 2015
Gut Bacteria Shift Towards Normal with Resolution of Inflammation with Either Nutritional or anti-TNF (Remicade) Therapy

Lewis et al Cell Host Microbe 2015
Clinical and Mucosal Improvement With Specific Carbohydrate Diet in Pediatric Crohn Disease

Cohen, Stanley A.; Gold, Benjamin D.; Oliva, Salvatore; Lewis, Jeffrey; Stallworth, Angela; Koch, Bailey; Eshee, Laura; Mason, David

doi: 10.1097/MPG.0000000000000449

- Improved symptoms and intestinal inflammation after 3 and 12 months
- Improved weight after 12 months

**The Specific Carbohydrate Diet**

**Foods that may be eaten**
- Fresh/frozen vegetables and legumes
- Fresh/raw/dried fruits, unsweetened juices (not from concentrate)
- Navy beans, lentils, peas, split peas, most nuts (unroasted preferably, nuts coming directly from shells so that nothing is added), natural peanut butter (with no sugar), lima beans, string beans
- Fresh/frozen meats, poultry, fish, eggs
- Some (natural/hard) cheeses (cheddar, Colby, Swiss, Havarti), homemade yogurt fermented >24 hours (no sugar added), dry curd cottage cheese
- Honey
- Tea, coffee, mustard, vinegar, most oils

**Foods to avoid**
- Canned vegetables
- Canned fruits, unless packed in own juices
- All grains, including flours
- Potatoes, yams, parsnips
- Chickpeas, bean sprouts, soybeans, mung beans, fava beans, and garbanzo beans
- Seaweed and byproducts, including agar and carageenan
- Processed, canned, breaded, smoked meats/fish
- All milk, buttermilk, commercially prepared yogurt and sour cream, heavy cream, soy/rice/potato/oat/hemp milk
- Instant tea or coffee, coffee substitutes, beer
- Canola oil, mayonnaise (due to additives), cornstarch, chocolate or carob, bouillon cubes or instant soup bases, all products made with refined sugar, sugar substitutes, Stevia, pectin, ketchup, ice cream, molasses, corn or maple syrup, baking powder, medication containing sugar, all seeds, balsamic vinegar, fructo-oligo saccharides
Fecal Microbiota Transplant

- Healthy donor screened for infectious agents
- Filtered diluted fecal preparation administered as enema, ND tube, scope
- CDiff infection and IBD (mainly UC)
- McMasters University: first randomized controlled trial in adult UC with negative results – 7/31 remission compared to 2/30
Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial

Remission rate: FMT 24% versus placebo 5% p=0.03
The human milk oligosaccharide 2′-fucosyllactose augments the adaptive response to extensive intestinal resection
Relative abundance of bacterial families discovered in the luminal contents at the time of (Preop) and following (Postop) ileocecal resection.
IBD: New Therapies

- New agents are being tested in over 100 clinical trials in the United States:
  - [www.ccfa.org](http://www.ccfa.org), [www.clinicaltrials.gov](http://www.clinicaltrials.gov)