Immune (Dys)regulation in the intestine (and inflammatory bowel disease)

Crohn’s disease
Ulcerative colitis

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What is it with gastroenterologists?
Eczema

Psoriasis

Only an idiot would lump these together as “inflammatory skin disease”
Burrell Crohn, Mt Sinai NY, described regional ileitis (again) in 1932- why do we think that Crohn’s disease is even 1 disease because his pathologist noticed some similarities?
Crohn’s colitis

Distal ulcerative colitis

They are both in the colon and they are red

So they must be the same!
**Ulcerative colitis**
- Neutrophils
- Crypt abscesses
- Usually superficial
- Colon specific
- No granulomas
- Continuous lesions
- Proctitis initially, moves proximally

**Crohn's disease**
- Mononuclear cells
- Deep fissuring ulcers
- Granuloma
- Anywhere in bowel but mostly ileocecal
- Patchy lesions, cobblestoning
- Fat wrapping, fibrosis, fistulae etc
Gene wide association studies (GWAS)

GWAS typically focus on associations between single nucleotide polymorphisms (SNPs) and traits like major diseases.

Scan the whole genome and find SNPs which are more common or less common in 1000's of controls versus 1000's of patients with disease X.

These are not mutations, they are common variants which we all have.

Effect size is very, very small.
Clustering of the loci associated with IBD by disease type and function

**UC**
- Adaptive Immunity
  - IFNγ / IL26
  - IL2 / IL21
  - TNFRSF9
  - IL7R
  - TNFRSF14
  - IRF5
  - LSP1
  - FCGR2A
  - IL8RA / IL8RB
- Gut epithelium
  - HNF4A
  - ECM1
  - LAMB1
  - GNA12
  - CDH1
- Other
  - OTUD3/PLA2GE
  - CAPN10
  - PIM3
  - DAP

**Crohn’s**
- Adaptive Immunity
  - CCR6
  - PTPN22
  - DENNDIB
  - IL27
  - IL2RA
  - IL18RAP
  - VAMP3
  - ITLN1
  - ERAP2
  - TNFSF11
  - BACH2
  - CCL2/CCL7
  - MUC1/SCAMP3
  - TAGAP
- Other
  - SP140
  - THADA
  - ZP36L1
  - GCKR
  - ZMIZ1
  - PRD5X
  - CPEB4
  - FADS1
  - 5q31(IBD5)

**Adaptive Immunity**
- MST1*
- PRDM1*
- REL*
- SMAD3
- IL1R2*
- ICOSLG*
- PTPN2
- YDJC
- TNFSF15*
- IL10*
- Th17
- IL12B*
- TYK2
- CARD9*
- STAT3*J
- IL23R*
- AK2*
- Others
- C11orf30*
- CREM*
- RTE1*
- NPK2-3*
- ORMEL3*
- KIF21B*
- ZNF365
- CDKAL1
- PTGER4*
- HLA
- DRB*03

**Very small effect size**

**GWAS**

**Innate immunity**
- LRRK2
- IRGM
- NOD2
- ATG16
Candidate gene approach identified Nod2 in Crohn’s, not UC

NOD2

Long (q) arm of chromosome 16 at position 21

Mutations and frame shift

NOD2 mutations predispose to early disease and ileal disease. Loss of function mutations, 15% of patients, but not causal. Lots of healthy folk have these mutations.
ENVIRONMENTAL FACTORS

Smoking

- Crohn’s Disease
- Ulcerative Colitis

Appendicitis - Appendectomy

- Crohn’s Disease
- Ulcerative Colitis (< 20 years of age)
Colectomy “cures” ulcerative colitis
Crohn’s disease and ulcerative colitis are different diseases

“Understanding” of IBD is all about Crohn’s not UC

UC is still a complete mystery (organ-specific autoimmune disease?)
Why does this become this?
Children of immigrants get IBD, particularly Crohn’s

**WHY?**

Jamaica

No Crohn’s

India

Crohn’s
The clinical observation that changed the way we thought about Crohn’s disease—the Steve James paper plus others

HIV infection depletes folk of CD4 T cells
Crohn’s disease is caused by an excessive Th1 immune response to the normal microbes in the gut with damage being caused in half of patients by excess TNF-alpha.

Lots of Th17 cells but anti-IL-17A makes patients worse.

So it is not a Th1/Th17 disease.
Clinical Remission With Infliximab at 4 Weeks

Clinical remission defined as a CDAI score < 150.

The microbial flora of the human gut—the antigen in Crohn’s

400 or maybe even 1000 species—$10^{13}$ bacteria
Microbiota is driving Th1 responses in Crohn’s

• Disease occurs where flora is abundant

• Anaerobic bacteria in the mucosa

• Lamina propria T cells react to the endogenous flora

• Lots of IL-12 in Crohn’s disease to drive Th1 response

• Beneficial effects of metronidazole in peri-anal disease

• Endoscopic remission maintained by diverting fecal stream

• Endoscopic lesions activated by fecal stream
Crohn's disease is due to a CD4 Th1 response to gut bacteria (delayed type hypersensitivity)

Normal gut bacteria

Healthy

Crohn's

Granuloma

IL-12

TNF

IFN

Macrophages

Granuloma
T cells drive inflammation but ulceration is caused by metalloproteinases

Activated T cells in CD

- APC
- T
  - IFN-γ
  - IL-17
  - TNF-α
  - IL-1β

Lamina propria fibroblasts

MMP-3 (an enzyme which degrades tissue)

Ulcer

Mucosa
Is there a colitogenic microflora in IBD?

Major changes in microbiota in active disease

Loss of F. prausnitzii

But active disease changes the gut environment (oxygen, plasma)

Dysbiosis not seen in pediatric Crohn’s but is seen in enteroendocrine tumors

So rush to say there is a dysbiosis in Crohn’s was premature
A very popular idea in IBD research is that there is a problem with regulatory T cells.

Essentially that defective T regs fail to control CD4 effector T cells with specificity for the gut microflora.

In normal gut lamina propria, CD4 T cells derived from Peyer’s Patches are activated- DR+, CD62L-, CD69+ and make interferon-gamma.
A Healthy Individual's Gut

Effector T Cells

Regulatory T Cells
In IBD

Effector T Cells

Regulatory T Cells
Increasing T regs will shift the balance and restore homeostasis

Cell therapy
CD4+CD25+ cells are naturally occurring regulatory T cells

Mouse without T cells or B cells

Normal mouse

CD4+CD25- cells ONLY
(Autoimmune disease)

CD4+CD25- cells (effector)
+ CD4+CD25+ cells (regulatory)

No Gastritis
Naturally-occurring regulatory T cells (CD4+CD25+ T-regs)

They have a TCRαβ that is thought to recognise self-antigens.

They express a gene called Foxp3 ("Master" gene for T-regs)

Foxp3 turns on many genes that turn T cells into regulatory T cells.

- e.g. CTLA-4, GITR, CD25 etc
- CTLA-4: a molecule that can bind to CD80/86 with higher affinity
IPEX syndrome
Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked

Human syndrome resulting in a mutation in Foxp3

Autoimmune disease strikes soon after birth

X-linked - so mostly affects boys

90% of patients develop diabetes

~70% develop thyroiditis

High mortality rate

This is not a typical IBD, it used to be called autoimmune enteropathy

The gut epithelium is full of enteroendocrine cells
TRANSFER MODEL OF COLITIS

COLITIS PREVENTED BY INJECTION OF Tregs
There is a problem with this though

IBD inflamed gut contains lots of T regs


Huge literature on T regs in IBD in man, and mouse models

But I am not sure......................

Why should there be one pathway to chronic inflammation of the gut?

Dozens of different mouse models where tinkering with the immune system results in an IBD

Anyway as patients only present when they have symptoms, the causative event is in the past
A final word about ulcerative colitis

Folk tried to shoehorn it as an atypical Th2 response with lots of IL-13 made by NK T cells (cf Crohn’s being a Th1 disease)

We did not find increased IL-13 in UC by a number of methodologies Biancheri et al 2014 Eur J Gastro 44:370-85.
Anti-IL-13 therapy failed in 2 trials in ulcerative colitis

UC is now being shoe-horned as a Th9 mediated disease
Blocking IL-9 won’t work- we can’t detect IL-9 in UC
But there has been some good news with newer therapies, ie more varied and better treatments
Patients with Corticosteroid-free Clinical Remission (Panel A) and Mucosal Healing (Panel B) at Week 26.

SONIC TRIAL

Combination of Infliximab and azathioprine
Combination therapy
Tofacitinib

A pill that contains an inhibitor of the JAK signalling pathway
Worked in UC, but not Crohn’s

Vedolizumab binds to alpha4 Beta 7 integrin and stops T cells getting into the gut.

**Crohn’s Week 6**

- **Clinical Remission**: Placebo (N=148) vs Vedolizumab (N=220)
  - Placebo: 6.8%
  - Vedolizumab: 14.5%
  - P = 0.02

- **CDAI-100 Response**: Placebo (N=148) vs Vedolizumab (N=220)
  - Placebo: 25.7%
  - Vedolizumab: 31.4%
  - P = 0.23

**Ulcerative colitis**

- **Placebo (N=149)** vs **Vedolizumab (N=225)**
  - Mean Partial Mayo Clinic Score
  - Placebo vs. vedolizumab at 6 wk, P<0.001

**“Partial” Mayo Score**

Scores range from 0 to 3 pts for each variable:

1. **Bowel movement (BM) frequency**
   - Normal (0 pts); 1-2 BM > nil (1 pts); 3-4 BM > nil (2 pts); > 5 BM > nil (3 pts)

2. **Rectal bleeding**
   - None (0 pts); Streaks on stool < 50% BMs (1 pts); Obvious blood with most BMs (2 pts); Blood alone (3 pts)

3. **No Endoscopy**

4. **Physician Global Assessment (PGA)**
   - Normal (0 pts); Mild (1 pts); Moderate (2 pts); Severe (3 pts)

SMAD7 antisense

A pill that allows the immune system’s own endogenous pathways which dampen inflammation to become dominant in active Crohn’s disease
Why doesn't TGF β switch off inflammation in Crohn's?
In IBD (and Helicobacter infection) Pro-inflammatory T cells and macrophages become resistant to TGF-beta. Even though activated TGFβ is abundant, Smad 7 is the reason.

Switches-off cytokines

T cell in normal gut

T cell in IBD or infection gut

If you have an infection you do not want immunosuppressive cytokines to switch off the T cells which are getting rid of the pathogen.
Organ culture

Serum-free tissue culture medium with or without Smad7 sense or antisense oligonucleotide
SMAD7 antisense restores TGF-β1 signaling and inhibits cytokine production in Crohn’s disease (CD) tissue

CD tissue

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<th>sense</th>
<th>antisense</th>
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- SMAD7
- p-SMAD3
- SMAD3
- TNF-α
- IFN-γ

CD tissue culture supernatants

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<tr>
<th>IFN-γ (pg/mg tissue)</th>
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<tbody>
<tr>
<td>medium</td>
</tr>
<tr>
<td>sense</td>
</tr>
<tr>
<td>antisense</td>
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</tbody>
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![Graph showing IFN-γ levels in medium, sense, and antisense conditions]
Open label anti-sense clinical trial - Smad7 anti-sense

Patients with active right-sided Crohn’s disease

Molecular Therapy 2012

Knocking down Smad 7 allows endogenous TGF-beta to dampen inflammation
REMISSION

A Primary End Point

![Graph showing the percentage of patients in clinical remission with different doses of Mongersen.]

- Placebo: 10%
- 10 mg/day: 12%
- 40 mg/day: 55%
- 160 mg/day: 65%

P-values:
- Placebo vs. 10 mg/day: P < 0.001
- Placebo vs. 40 mg/day: P < 0.001
- Placebo vs. 160 mg/day: P < 0.001
- 10 mg/day vs. 40 mg/day: P < 0.001
- 10 mg/day vs. 160 mg/day: P < 0.001

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Can we get clues from patients about immune regulation in the gut and what is important?
Inflammatory Bowel Disease and Mutations Affecting the Interleukin-10 Receptor

Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis.

Interleukin 1β Mediates Intestinal Inflammation in Mice and Patients With Interleukin 10 Receptor Deficiency.

Treated 2 cases of early onset IBD with Anakirna- IL-1R antagonist
Summary

• The IBD's are unpleasant inflammatory diseases of the gut

• Crohn's disease and UC are obviously different diseases

• Crohn's at least is due to an exaggerated Th1 cell response to the microbiota

• Crohn's has a genetic component, but only in the developed world

• Why Crohn's is only penetrant in developed world is not known
  • (infections early in life teach the gut immune system to switch off?)

• Thinking that there is a single pathway to a heterogeneous disease is a bit 20th century, eg T regs

• Neutralising TNF with monoclonal antibodies has proven quite effective

• The future is with pills that target the intracellular pathways that drive disease and healing