VERY EARLY ONSET IBD IN CHILDREN
- CAUSES, CURES, & CONUNDRUMS -

Scott B. Snapper, M.D., Ph.D.

Disclosures
I have the following financial relationships to disclose:

Abbvie – IBD Advisory Board; Cubist – IBD Advisory Board;
Eisai - IBD Advisory Board; Enterome – IBD Advisory Board;
Hoffman La-Roche – IBD Advisory Board, Consultant; Ironwoods –
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* No products or services produced by these companies are relevant to my presentation.

Learning Objectives

• Review immunodeficiencies that may present with intestinal inflammation

• Understand the phenotype, genetics and prognosis for IBD presenting in very young children

• Learn an appropriate immunological evaluation of a child with early IBD.
**Incidence of IBD is Increasing Dramatically Worldwide**


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**Genetics and IBD in the Adult and Pediatric Population**

- Increased risk of IBD in 1st degree relatives (26 fold increase for CD; 9 fold increase for UC)
- 30% of children have one or more family members with IBD
- Concordance rate much greater in monozygotic vs dizygotic twins
  - 10-15% in UC; 25-30% in Crohn's

Loftus et al., Gastroenterol. 2004
Bengston et al. J. Crohn’s Colitis 2009
Brant., IBD J. 2011
Ruemmele Curr Opin Gastroenterol 2010

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**GWAS Studies Have Identified over 180 Inflammatory Bowel Disease Susceptibility**

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Unique Aspects of Pediatric IBD

- ~20% of IBD presents in children
- Children with UC – more extensive disease
- Children with CD – upper intestinal tract involvement common
- Young children often present with Crohn’s colitis with perianal involvement

Unique Clinical Features of VEO-IBD*

**VEO - IBD**

- Colonic involvement - 80% at < 10 years of age
- Ileal involvement - less common at age < 10 yrs
- Family history – 40-50%
- Extension of disease – up to 40%

**Adolescent and Adult-Onset IBD**

- Colonic involvement - <20%
- Ileal involvement – up to 80%
- Family history – 14-20%
- Extension of disease – up to 16%

* Defined as Age < 10 by the Paris Classification

Unique Aspects of Infantile IBD (< 2yo)

- Often isolated Colonic Disease
- Severe Course – refractory to multiple immunosuppressant medications, often requiring surgery, occasionally fatal
- > 40% with one or more family members with IBD
- 25% first manifestation of underlying immunodeficiency

Ruemmele 2006 JPGN
Cannito 2009 EJP
Heyman 2005 I Ped

Greatest Increase in IBD Incidence
Very Early Onset IBD

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>IBD</th>
<th>CD</th>
<th>UC</th>
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<tbody>
<tr>
<td>6mo-4yr</td>
<td>+56.8% (P=0.11)</td>
<td>+51.0% (P=N/A)</td>
<td>+58.1% (P=0.95)</td>
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<tr>
<td>5-9 yr</td>
<td>+65.7% (P&lt;0.001)</td>
<td>+58.9% (P=0.003)</td>
<td>+57.9% (P&lt;0.0001)</td>
</tr>
<tr>
<td>10-14 yr</td>
<td>+74.1% (P&lt;0.0001)</td>
<td>+58.3% (P=0.002)</td>
<td>+58.3% (P&lt;0.009)</td>
</tr>
<tr>
<td>15-17 yr</td>
<td>+25.1% (P=0.009)</td>
<td>+12.1% (P=0.006)</td>
<td>+27.4% (P=0.03)</td>
</tr>
</tbody>
</table>

* By Poisson regression analysis, controlling for sex

Primary Immunodeficiencies Often Present with Intestinal Inflammation

- IPEX syndrome – frequent enteropathy
- Wiskott-Aldrich syndrome – 10% with colitis
- Chronic granulomatous disease
- Gaucher’s disease
- Crypts-like colitis
- NEMO (NF-kB Essential Modulator) Deficiency
- enterocolitis
- superficial cryptitis
- XIAP (X-linked inhibitor of apoptosis)
- severe fistulating perianal disease in about 20% of patients
- Common variable immunodeficiency – frequent enteropathy

Estimated Prevalence of Monogenetic Disorder that Can Present with an IBD like Immunopathology
Have Large Scale Genetic Efforts Missed Key Dominant Pathways Causing Infantile and VEO-IBD?

Since these are Rare Diseases an International Effort is Required to Advance our Understanding

Adapted from Kaser A, Zeissig S & Blumberg RS, Dig Dis 2010

Very Early Onset IBD Consortium

PIs: Aleixo Muise, Christoph Klein, Scott Snapper

Mission:
To diagnosis, treat, and develop new therapies for young children and infants with IBD.

>80 Centers; 250 Scientists; >1000 VEOIBD Patients

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Case

- Presented in 1st year of life with severe colitis
- Persian ancestry
- Multiple enterocutaneous fistulae, recurrent folliculitis, recurrent infections, impaired wound healing

**Genetic Evaluation Identified Mutation in IL-10 Receptor**

**IL10R Pathway**

- IL10 restricts excessive immune responses
- Inhibits secretion of pro-inflammatory cytokines
- IL10 Receptor has two subunits:
  - Alpha – IL10
  - Beta – IL10, -22, -26
- Acts through JAK1, TYK2, and STAT3
- IL10, TYK2, and STAT3 have been identified in IBD GWAS
IL10R Deficiency Results in Infantile-Onset IBD

- IL10RB and IL10RA mutations have now been found in numerous locations within each gene—up to date each having similar presentations and similar signaling defects
- Hematopoietic stem cell therapy can be curative


Case 2 – IS THIS ONLY RELEVANT TO VEOIBD?

- Patient presented with severe diarrhea and perianal fistulas in first weeks of life → diagnosed with IBD
- Didn’t respond to various immunosuppressive medications
- Colectomy at age 5 years
- Severe perianal fistulizing disease persisted

Shouval DS, *JPGN*, 2014

CG – Clinical Presentation

- At age 12 years presented with 2 months of abdominal pain and enlarged liver and spleen
- CT – multiple focal liver lesions; hypermetabolic on PET
- Biopsy – Large B cell lymphoma
- Responded well to chemo but relapsed after 3 years
- Awaiting autologous stem cell transplant
Functional IL-10R Testing

No stimulation
IL-6 20 ng/mL
IL-10 20 ng/mL

Targeted Sequencing of IL10RB

Diagnosis of loss of function mutations in IL10RB

Treatment changed to allogeneic SCT

>12 months s/p SCT – no signs of colitis or lymphoma recurrence

Functional Studies in Mice and Man Can Lead to Novel Therapeutic Approaches

- IL-10R patients develop severe infantile IBD
- IL-10R knock-out mice (Il10rb-/-) develop colitis
- Recent studies have shown that IL10-induced signals is important for T cells

What is the role of IL-10R signaling in innate immune cells in the intestine?

This is mouse!
Macrophages (Mϕ) Can Differentiate into Pro and Anti-inflammatory Subsets

Intestinal Macrophages

Bone Marrow Derived Macrophages (BMDM)

Hypothesis: IL-10R-dependent signals are required for the generation and function of pro- and anti-inflammatory Mϕ

Reduction of Anti-inflammatory Mϕ in the Intestine of Il10rb⁻⁻ Mice

Reduction of Anti-inflammatory Mϕ in the Bone Marrow of Il10rb⁻⁻ Mice

Shouval DS, Immunity 2014
Transfer of WT Macrophages (M2r) Prevents Colitis in II10r mice

Shouval DS, Immunity 2014

Animal Models Informing Human Studies

Is MΦ generation and function abnormal in these patients?

IL-10R Signaling is Required for the Generation and Function of Human Anti-inflammatory MΦ

10
IL-10R-Signals in Macrophages Regulate Intestinal Inflammation

Developing Gene Therapy Approaches for VEOIBD

Preclinical Model of IL10RB Gene Therapy
Transduction of Il10rb−/− Bone Marrow Precursors With IL10RB Expressing Lentiviral Vectors Protect Mice from Colitis

Case 2

- Patient ET
  - Presented at 2 months of age:
    - Blood in stool
    - Diagnosed with cow’s milk protein allergy
- Diagnosed < 1 yo with Crohn’s colitis.
- Developed perianal and small bowel disease < 2 years of age.
- No evidence of chronic infections or immunodeficiency.
- No family history of IBD, parents not consanguineous.
- Has abnormal low normal reactive oxygen species (ROS) production (3x).

Hypothesis:

Defects in the NADPH oxidase genes that do not cause overt Chronic Granulomatous Disease (CGD) are associated with susceptibility to IBD.


<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>CYBB: gp91phox</td>
<td>X-Linked Recessive</td>
<td>~65%</td>
</tr>
<tr>
<td>CYBA: p22phox</td>
<td>Autosomal Recessive</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>NCF1: p47phox</td>
<td>Autosomal Recessive</td>
<td>~25%</td>
</tr>
<tr>
<td>NCF2: p67phox</td>
<td>Autosomal Recessive</td>
<td>~5%</td>
</tr>
<tr>
<td>NCF4: p40phox</td>
<td>Autosomal Recessive</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

Lam et al., 2010

Sequencing of NADPH Oxidase Genes in Infantile and VEO-IBD Patients Identifies Deleterious Mutations

- Identified a novel NCF2 variant - (c.113 G/A) resulting in a mutation in p67phox R38Q
- Variant results in aberrant Rac2 binding
- Patient responded to antibiotic treatment
- Examined this mutation in 2 independent VEO-IBD cohorts
  - 4% of VEO-IBD patients (11/268)
  - 0.3% of older IBD patients (1/330)
  - 0.2% of healthy controls (1/480)

Dhillon et al, Gastro 2014
How do we assess the heritability of the remaining fraction?

1. Deep sequencing of GWAS loci for identification of rare variants
2. Immunochip analysis of selected genes in similar cohort
3. Whole Exome Sequencing (WES)/Whole Genome Sequencing (WGS)

WES in VEO-IBD patient leads to identification of mutation in XIAP

Case 3 – WES
Multiple Intestinal Atresia (MIA), SCID and Apoptotic Enterocolitis

- A female patient born at term
  - Unrelated parents
  - Presented with high output secretory hematochezia at birth
  - Lymphopenia and hypogammaglobulinemia
- Colonoscopy demonstrated
  - Chronic inflammation with severe friability
  - Sloughed mucosa within the colonic lumen
  - Crypt apoptosis and exploding crypts
- WES

Mutations in Tetra-tronopeptide Repeat Domain 7A Result in a Severe Form of Very Early Onset Inflammatory Bowel Disease

Gastroenterology 2014

JACI 2013

TTC7A mutations disrupt intestinal epithelial aglomerobasal polarity

JMG 2013
TTC7A Mutations Cause Apoptotic Enterocolitis

- Little is known about the function of the Tetratricopeptide Repeat Domain 7 (TTC7A) gene
- Studies suggest it plays a critical role in PI4KIIIα regulation
- Defects in murine Ttc7 gene
  - result in the flaky skin (fsn) mutant mice
  - develop pleiotropic abnormalities, including runting syndrome, anemia, psoriasis, diarrhea, and intestinal apoptosis

Conclusions: TTC7A-Deficiency and VEOIBD

- Severe intestinal inflammatory process likely at least partially driven by a primary epithelial defect
- Associated with multiple intestinal atresia (and recurs post-resection)
- Associated with SCID (severe combined immunodeficiency)
- Intestinal disease seems to not respond to hematopoietic stem cell transplant

Case 4

- Pulm:
  - Bronchiectasis; Pulmonary nodules
  - rec. sinusitis, rec. URTI
- Heme:
  - Autoimmune thrombocytopenia & hemolytic anemia
  - Infiltration of BM by CD8γδ T cells
- CNS
  - Seizures
  - Infiltrative lymphocytic lesions
- GI
  - Enteropathy
  - Increased lamina propria and intra-epithelial lymphocytes

Dascha Weir/Alan Leichtner
GI Manifestations

- Presented at age 12
- Inflammation of upper & lower GI tract
- Biopsies – villous atrophy, crypt hyperplasia and absent Paneth and goblet cells, increased enterocyte apoptosis and lymphocytic inflammation
- Resistant to 5ASA, steroids, 6-MP, rituximab, prograf, cyclophosphamide, infliximab & sirolimus; Dependent on PN
- Recurrent C. difficile colitis and recurrent herpes zoster infections
- Passed away at age 22 - MRSA sepsis

Identification of CTLA4 Mutations

What is CTLA-4?

- Suppressive immunoregulatory surface protein
- Activation of T naïve cells induces expression of CTLA4  
  binds CD80 or CD86  
  inhibitory signal to activated T cells
- Shares homology to CD28

Zeissig S; Gut 2014

Kuehn HS; Science 2014

Zeissig S; Gut 2014

Kuehn HS; Science 2014

Schubert; Nat Med 2014
CTLA4 Deficiency

Immunological Analysis of Patients with CTLA4 Mutations

Immunologic And Genetic Evaluation

- All patients with low immunoglobulin levels
- Thyrombocytopenia, Hemolytic anemia
- Fewer regulatory T cells; markedly fewer naïve T cells; many activated T cells
- Autosomal dominant inheritance; Incomplete penetrance

Treatment: ? BMT

Not all patients young! Some diagnosed in 40's
Conclusions – When to suspect CTLA4 Mutations?

- Multiple autoimmune features
- Low immunoglobulins
- Pulmonary/CNS involvement
- Positive family history

LRBA Mutations Can Initially Present as VEO-IBD +/- CVID

Extended Spectrum of LRBA Deficiency

- Not all have low immunoglobulins
- Report of a family with IPEX-like phenotype
Treatment Options for LRBA Deficiency

Conclusions – When to suspect LRBA Mutations?
- Multiple autoimmune features (pulmonary, endocrine, GI)
- Low immunoglobulins
- Can present with IPEX-like phenotype
- Positive family history

Evolving Approach to VEO-IBD
- First – Screen for Genes known to cause VEO-IBD
- Next Examine Genes in Common Pathways
- Examine Novel Variants
- Functional Examination of Variants
I: Functional Screening: Genetic Confirmation

- Test
  - CBC
  - Neutrophil esterases activity
  - Pharmacoprotein
    - NLRP15
    - NLRP1
  - Lymphocyte subsets
  - Genomic testing
- Disease groups/pathology
  - Neutropenia
  - Thrombocytopenia
  - Lymphopenia
- Genetic confirmation
- Limited functional reactivity

II: Genetic Screening: Functional Confirmation

- Genetic screening
  - Whole exome sequencing
  - Whole genome sequencing
- Take home points
  - GWAS have shown that genetics in adult and adolescent pediatric IBD overlap considerably.
  - Immunodeficiencies account for a significant percentage of patients presenting with infantile IBD.
  - Unique genetic abnormalities may be more dominant in VEO-IBD (e.g., IL-10R; NCF2); however, data is limited.
  - Whole exome sequencing (and ultimately whole genome sequencing) will greatly expand our ability to detect rare variants in individual patients.

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Program Manager, Boston

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There is an Urgent Need to Develop In Vivo Models to Study Basic Biology of Human Cells and Disease

Hematopoietic stem cells (HSCs)

Jeremy Goettel, PhD

Unknown patient risk
Multifactorial

Tractable model
No risk to patient
Controlled environment

CD4 T cells interact with MHC class II

Mouse innate cell Mouse innate cell Mouse innate cell

Mouse MHC II Mouse MHC II Human MHC II

Good Immune Response Poor Immune Response Immune Response??

Goettel et al., Blood 2015
Generation of immune-humanized mice using CD34+ HSCs

Hematopoietic stem cells (HSCs)

Enhanced T cell and B cell responses!

HLA-DR1*01:01 donor

Goettel et al, Blood 2015

IPEX: (immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome)

• Due to loss-of-function mutation in FOXP3 (critical for regulatory T cells)
• Autoimmune enteropathy, eczema, type-1 diabetes, elevated IgE
• Often fatal in the first 2 years of life
• Foxp3−/− mice also develop systemic inflammation, autoimmunity, and premature death

Can human HSCs from an IPEX patient transfer clinical phenotype to humanized mice?

Hematopoietic stem cells (HSCs)

Patient CD34+ cells or IPEX CD34+

Goettel et al, Blood 2015
Increased mortality and multisystem organ failure in IPEX(huDR1) mice

Goettel et al, Blood 2015

Summary

- huDR1 mice exhibit greater T cell diversity, T cell recall responses, B cell maturation, and antibody class switching compared to NSG mice.
- CD34+ HSCs from a patient with IPEX syndrome cause multi-organ inflammation, development of autoantibodies, and increased mortality in huDR1 mice similar to that observed in Foxp3−/− mice.

Future directions

- Evaluate CD34+ HSCs from very-early onset IBD patients (e.g., IL10R deficiency, Wiskott-Aldrich syndrome, CGD) in huDR1 mice.
- We have re-derived huDR1 mice into germ free conditions to evaluate the role of specific microbes, byproducts, and metabolites in regulating mucosal immune function and promoting homeostasis.