Signal Transduction in Inflammatory Bowel Diseases
Overview

Inflammatory Bowel Diseases (IBD)

1. Epidemiology, Symptoms and Pathogenesis

2. Phenotypes and Classification

3. Pathophysiology

4. Susceptibility loci and Pathways

5. Therapeutic intervention
Global map of IBD

Increasing incidence and prevalence of the inflammatory bowel diseases with time

Established high-prevalence populations of IBD in North America and Europe

Emerging populations: Asia populations previously considered 'low risk' (such as Japan, Korea, India)

1.5 million Americans

2.2 million people in Europe

IBD: Symptoms

- Weight loss
- Food malabsorption
- Bile acid malabsorption
- Increased bowel motility
- Anemia, thrombocytosis
- Extraintestinal manifestations
- Associated diseases (psoriasis, spondylitis)
Inflammatory bowel diseases

Ulcerative colitis

• Diarrhea, bloody stool, fever
• Colonic (backwash ileitis)
• Continuous from the rectum to the proximal colon
• Diffuse inflammation of the mucosa and submucosa

Crohn’s disease

• Abdominal pain, cramping, malabsorption, fever
• Whole GI tract (right-sided colon, ileum)
• Discontinuous
• Transmural disease

Images of histological and endoscopic findings related to inflammatory bowel diseases.
IBD: Epidemiology

- UC: females ≥ males
- CD: males ~ females
- Age of onset: 15-30 years, 60-80 years
- Risk factors
  - CD: smoking, appendectomy
  - UC: non-smoking, appendectomy is protective
  - First degree relatives with IBD (CD>UC)
  - Suspected: measles infection, mycobacterium tuberculosis, oral contraceptives

Environmental influences: span the spectrum of life

- mode of childbirth
- early-life exposures like breastfeeding and antibiotic exposure in infancy,
- exposures later on in adulthood like smoking, diet and lifestyle
IBD: Crohn’s disease vs Ulcerative colitis

mucosa
submucosa
muscularis
IBD: Classification

- By disease activity – severity
- By disease biology – phenotype
- By disease mutations – genotype
- By response to therapy
- By causative factor
Ulcerative Colitis - Phenotypes

Types of Ulcerative Colitis

- Proctitis
- Proctosigmoiditis
- Distal colitis
- Extensive colitis
- Pancolitis
Crohn’s Phenotypes - Vienna classification

Age at Diagnosis

Location

Behavior

Vienna Classification of Crohn’s Disease


Age at Diagnosis

1 < 40 years
2 ≥ 40 years

Location

1 Terminal Ileum
2 Colon
3 Ileocolon
4 Upper GI

Behavior

1 Non-stricturing, non-penetrating
2 Stricturing
3 Penetrating

Further data to be collected

Sex: female / male
Ethnicity: caucasian / black / asian / other
Jewish: yes / no / partly
Family history of IBD: 1st degree relatives / other / none
Extraintestinal manifestation: yes / no

Gasche C, Inflammm Bowel Dis 2000
Montreal classification

A1 <16 years, A2 17–40 years, A3 >40 years

Major extraintestinal manifestations
Ulcerative colitis and risk for CRC

Risk factors

• Extent
• Severity
• Duration
• Age of onset
• Primary sclerosing cholangitis
• Family history of CRC
IBD and risk for CRC

Cumulative risk for colitis-associated cancer
UC
- 2% 10 years
- 8% 20 years
- 18% 30 years

CD: SIR 1.9

10-15% of all deaths in IBD

- Familial 10-30%  
- Sporadic 65-85%  
- Lynch syndrome 3-5%  
- Familial adenomatosis polyposis 1%  
- MutYH associated polyposis  
- Rare CRC syndromes 0.1-1%  
- Colitis-associated 2%
Pathophysiology of IBD and risk for CRC

IBD: Pathogenesis

Multi-factorial

Infections

Genetic variations

Nature Reviews | Gastroenterology & Hepatology

Ananthakrishnan, A. N. Nat. Rev. Gastroenterol. Hepatol. 12, 205-217 (2015);
IBD: Pathophysiology

- Genetic predisposition
- Dysbiosis
- Inflammation
- Deregulated immune response
- Oxidative stress
- Epithelial damage (↓Tissue repair ↑permeability)
IBD: Susceptibility loci and Pathways

Timeline of Genetic Discoveries in Inflammatory Bowel Diseases

- **1988**: Epidemiological twin studies
- **1996**: Genome-wide linkage studies
- **2001**: NOD2
- **2006–2007**: CD GWAS: *IL23R, ATG16L1, IRGM, NKX2.3*
- **2008–2009**: Th17 axis, autophagy
- **2010 and beyond**: CD meta-analysis
  - Pediatric GWAS
  - UC GWAS
  - ~50 confirmed IBD susceptibility loci
  - Detailed IBD genetic map, novel therapies, natural hx

Further GWAS: UC, early-onset IBD, geographical/ethnic subtypes, subphenotypes (e.g., severe disease)
Fine-mapping of loci and function of genes/causative variants: copy number variation, epigenetics, reclassification, diagnostics, prognostics, IBD CHIP

*Source: Expert Rev Gastroenterol Hepatol © 2009 Expert Reviews Ltd*
Inflammatory bowel disease susceptibility loci

163 IBD loci (GWAS)
A model for IBD pathways based on GWAS

**Cellular responses**
- **Autophagy**
  - ATG16L1*, IRGM, NOD2*, LRRK2, CUL2, PARK7, DAP
- **Apoptosis/necroptosis**
  - FASLG, THADA*, DAP, PUS10, MST1*
- **Carbohydrate metabolism**
  - GCKR*, SLC2A4R
- **ER stress**
  - CPEB4, QPMDL3, SERINC3, XBP1*
- **Intracellular logistics**
  - VAMP3, KIF21B, TLL1, FGFR1OP, CEP72, TPPP
- **Oxidative stress**
  - PRDX5, BACH2, ADO, GPX4, GPX1*, SLC22A4, LRRK2, NOD2*, CARD9*, HSPA6, DLD, PARK7, UTS2*, PEX13
- **Cell migration**
  - ARPC2, LSP1, AAMP

**IBD-related processes**
- **Epithelial barrier**
  - GNA12*, HNF4A, CDH1, ERRFI1, MUC19, ITLN1*
- **Restitution**
  - REL, PIK3CA, NCK2*-3, STAT3, ERRFI1, HNF4A, PLA2G2A/E
- **Salute transport**
  - SLC9A4, SLC22A5, SLC22A4*, AQP12AV/B, SLC9A3, SLC26A3
- **Paneth cells**
  - ITLN1*, NOD2*, ATG16L1*, XBP1 *
- **Innate mucosal defence**
  - NOD2*, ITLN1*, CARD9*, REL, SLC11A1, FGG2AV/B
- **Immune cell recruitment**
  - CCL11/CCL2/CCL7/CCL8, CCR5, IL1R1/IL1R2, MST1*
- **Antigen presentation**
  - ERAP2*, LNP1R, DEND1B
- **IL-23/TH17**
  - IL23R*, JAK2, TYK2*, STAT3, ICOSLG, IL21, TNFSF15*
- **T-cell regulation**
  - NFIP1, TNFSF8, TAGAP, IL2, IL2RA, TNFRSF9, DIM3, IL2R*, IL12b, IL23R*, PRDM1, ICOSLG, TNFSF8, IFNG, IL21
- **B-cell regulation**
  - IL5, IL21, ICOSLG, TGF*, IL7R*, IRF5
- **Immune tolerance**
  - IL10, IL27*, SNB20, CREM, ITI1/ITI19, NOD2*

**Medizinische Universität Wien**
Intestinal homeostasis pathways

• Barrier function
• Epithelial restitution
• Microbial defense
• Innate immune regulation
  • Paneth cell dysfunction, impaired recognition of microbes
• Regulation of adaptive immunity
  • Imbalance of effector and regulatory T cells and cytokines, migration and retention of leukocytes
• Reactive oxygen species (ROS) generation
• Autophagy
• Endoplasmic reticulum (ER) stress
Intestinal Epithelial Barrier
Epithelial Barrier Defect in IBD

Barrier defects allow bacterial products and dietary antigens to cross the epithelium and enter the lamina propria

Claudin-2: channel-forming
Claudin 3, 4, 5, 8: barrier forming claudins
Loss of barrier function visualised by confocal endomicroscopy

Efflux of fluorescein at a site of local barrier loss

microerosions, where more than one cell is lost from a single site and the lamina propria is exposed to the lumen
**Mucosa associated flora in IBD**

Human colonic wall of healthy controls

Ulceration of the epithelial surface in a patient with UC
Bacteria attach to the mucosa
Bacteria in direct contact with lamina propria
Molecular mechanisms of IBD - NOD2/ CARD15

2001 – mutation in CARD15 (=NOD2, IBD1) gene
Linkage to Crohn’s disease

No linkage to ulcerative colitis

NOD2 mutation
  2.5x increase risk for heterozygous
  30x increase risk for homozygous or compound heterozygous

Intracellular pattern recognition receptor (muramyl dipeptide)

nucleotide-binding oligomerization domain-containing protein 2 (NOD2)
caspase recruitment domain-containing protein 15 (CARD15)
inflammatory bowel disease protein 1 (IBD1)
## Nod2 and Disease Location

### Table 3
Genotype-phenotype correlations in Crohn’s disease

<table>
<thead>
<tr>
<th>Study</th>
<th>L1 (ileal)</th>
<th>L2 (colonic)</th>
<th>L3 (ileocolonic)</th>
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<tr>
<td>Cuthbert</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>Lesage</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Murillo</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
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<tr>
<td>Ahmad</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Vermeire</td>
<td>+</td>
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<td>Hampe</td>
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<td>+/-</td>
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<td>Bairead</td>
<td>+</td>
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<tr>
<td>Giachino</td>
<td>+</td>
<td>-</td>
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</tbody>
</table>

+/-, trend but not statistically significant.
Nod2 Mutation in Crohn’s Disease Potentiates NF-κB Activity and IL-1β Processing

Shin Maeda,1 Li-Chung Hsu,1* Hongjun Liu,1* Laurie A. Bankston,1,3 Mitsutoshi Iimura,2 Martin F. Kagnoff,2 Lars Eckmann,2 Michael Karin1†

A

100
95
90
85
80
75
70

BW(%) 3% DSS

0 1 2 3 4 5 6 7 8 9 10

WT (n=19) m/m (n=16)

B

WT m/m

F

WT m/m

+DSS -DSS

NF-κB

HDAC

KA

IKKα

Science. 2005
Molecular mechanisms of IBD - Interleukin-10

- IL-10 is a cytokine with important immunoregulatory functions (e.g. TNF inhibition)
- Homozygous loss of function mutations in IL10 and IL10R cause severe infantile (very early onset) IBD
- Clinical trials with IL-10 for Crohn’s disease show some but not broad efficacy.
- IL-10−/− mice display enterocolitis similar to Crohn’s disease (Cell 1993)
Molecular mechanisms of IBD

IL-10 family receptor complexes

Sharing of receptors by cytokines
Molecular mechanisms of IBD
Interleukin-10

Interleukin-10-Deficient Mice Develop Chronic Enterocolitis

Ralf Kühn,* Jürgen Löhler,† Donna Rennick,‡ Klaus Rajewsky,* and Werner Müller*†

Colon IL10−/− Pathogen-free

Duodenum IL10−/−

Duodenum IL10wt

Duodenum erosion IL10−/−
Molecular mechanisms of IBD
Interleukin-10

Inflammatory Bowel Disease and Mutations Affecting the Interleukin-10 Receptor

Erik-Oliver Glocker, M.D., Daniel Kotlarz, M.D., Kaan Boztug, M.D.,
E. Michael Gertz, Ph.D., Alejandro A. Schäffer, Ph.D., Fatih Noyan, Ph.D.,
Mario Perro, M.Sc., Jana Diestelhorst, B.Sc., Anna Allroth, M.D.,
Dhaarini Murugan, M.Sc., Nadine Hätscher, B.Sc., Dietmar Pfeifer, M.D.,
Karl-Walter Sykora, M.D., Martin Sauer, M.D., Hans Kreipe, M.D.,
Martin Lacher, M.D., Rainer Nustede, M.D., Cristina Woellner, M.Sc.,
Ulrich Baumann, M.D., Ulrich Salzer, M.D., Sibylle Koletzko, M.D.,
Neil Shah, M.D., Anthony W. Segal, M.D., Axel Sauerbrey, M.D.,
Stephan Buderus, M.D., Scott B. Snapper, M.D., Ph.D., Bodo Grimbacher, M.D.,
and Christoph Klein, M.D., Ph.D.

Three distinct loss of function, homozygous mutations in genes IL10RA and IL10RB in 4 of 9 patients with early-onset colitis.
Splice–site mutation in IL10R1 (G84150A): truncated protein

**Wildtype**

- Genomic: 15093 bp
- mRNA: 3672 bp
- Protein: 578 aa

**EX3del**

- Genomic: 15093 bp
- mRNA: 3493 bp
- Protein: 94 aa
IL-10 Therapy for Crohn‘s Disease: Individual Experience

Before therapy

After 28 day of s.c. IL-10 therapy

Patients respond differently to IL-10 treatment
Molecular mechanisms of IBD - Interleukin-10

Interleukin-10-Deficient Mice Develop Chronic Enterocolitis

Ralf Kühn, * Jürgen Löhler, † Donna Rennick, †
Klaus Rajewsky, * and Werner Müller †

Colon IL10−/−
Specific pathogen-free

Duodenum IL10−/−

Duodenum IL10wt

Duodenum erosions IL10−/−

Oxidative stress and DNA damage response in IL-10 KO mice

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Non-inflamed</th>
<th>Inflamed</th>
<th>Dysplastic</th>
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<tbody>
<tr>
<td>DSB</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>Oxidative stress</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>DDR</td>
<td>![Image]</td>
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<tr>
<td>HR</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>NHEJ</td>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
Oxidative stress - γH2AX as a surveillance marker in patients with IBD?

Frick A, Khare V, Gasche C et al

Mol Cancer Res. 2018
IBD: Therapeutic intervention

- to induce and maintain remission
- mucosal healing
- reduce surgical procedures and restore quality of life

Endoscopic mucosal healing in IBD

CD
Azathioprine

UC
Adalimumab
cCertolizumab
ingInfliximab

MH

5-ASA
Corticosteroids
Tacrolimus, CsA

Adalimumab
Infliximab
Azathioprine

Neurath M F and Travis S P L Gut 2012;
IBD: Therapeutic intervention

- to induce and maintain remission
- mucosal healing
- reduce surgical procedures and restore quality of life.

• Anti-inflammatory
  - 5-aminosalicylates (oral, rectal, enema)
  - Corticosteroids (oral, iv, topical)

• Immunosuppressive reagents
  - Azathioprine
  - 6-Mercaptopurine
  - Cyclosporine
  - Methotrexate

Medical Management
Surgical Intervention
Lifestyle Changes (Smoking, Diet)
Mechanism of 5-ASA activity

PAK1; p-21 activated kinase 1
Serine/threonine kinase and effector of RAC1/CDC42
Overexpression of PAK1 in IBD

Human

Normal  |  CD  |  UC  |  CAC  |  CRC

Mouse (C57BL/6)

Normal  |  DSS-colitis  |  AOM/DSS carcinoma  |  IL-10⁻/⁻ carcinoma  |  APC^Min adenoma
PAK1 signaling is activated in intestinal inflammation
IBD: Therapeutic intervention

- Lifestyle Changes (Smoking, Diet)
- Pre- and probiotics

- Antibiotics
- 5-ASA or sulfasalazine
- Prednisone or budesonide
- Immunomodulators (AZA or 6-MP or MTX)
- Cyclosporine
- FMT
- Biologic agents
- Surgery
- Nutritional support

## IBD: Therapeutic intervention

### Emerging Biological Treatments

<table>
<thead>
<tr>
<th>Biological target</th>
<th>Drugs</th>
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<tbody>
<tr>
<td><strong>Innate immune cell signalling</strong></td>
<td></td>
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<tr>
<td>TNFα</td>
<td>Infliximab, adalimumab, certolizumab pegol</td>
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<td>TLR MyD88</td>
<td>DIMS0150 (Kappaproct), BL-7040 RDP58</td>
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<td><strong>Pro-inflammatory cytokines</strong></td>
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<td>JAK</td>
<td>Tafocitinib</td>
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<td>IL-12/23</td>
<td>Ustekinumab, SCH900222, briakinumab</td>
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<td>IL-17</td>
<td>Secukinumab, brodalumab, vidofludimus</td>
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<tr>
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<td>IFNγ</td>
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<td>IL-13</td>
<td>QAX576, anrulinzumab, tralokinumab</td>
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<td>IL-6 and IL-6R</td>
<td>Tocilizumab, PF04236921, C326</td>
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<td><strong>T cell activity</strong></td>
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<td>T cell proliferation</td>
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<td>CD3</td>
<td>Visilizumab</td>
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<td>CD25 Protein kinase C inhibitor</td>
<td>Basiliximab, daclizumab</td>
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<td>Sotraustarin</td>
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<td><strong>Chemotaxis</strong></td>
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<td>CCR9</td>
<td>CCX-025, CCX282-B</td>
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<td>α4β7 integrin</td>
<td>Vedolizumab, natalizumab, ELND-004, AJM-300, etrolizumab</td>
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<td>MAdCAM-1</td>
<td>PF-547659</td>
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<td>IP-10</td>
<td>MDX-1100</td>
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<td><strong>Regulatory T cells</strong></td>
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<td>OvaSave</td>
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**Biological therapy**

**anti-TNF-α antibodies:** effective for the treatment of UC and CD

Side effects: non melanoma skin cancer
psoriasis like skin lesions
Molecular mechanisms of IBD
IL–23 and IL–12 signaling axis

Risankizumab

Ustekinumab

shared signaling mediators

PS=psoriasis; AS=ankylosing spondylitis, Behçet’s disease (BD) and primary biliary cirrhosis (PBC) all IBD associated diseases

IL-12RB
JAK2

Cho JH; Brant SR
anti-α4β7 integrin antibodies

- **Targeting lymphocyte homing**
- **Vedolizumab**
  - humanised monoclonal IgG1 antibody targeting integrin α4β7.
  - activity is focused on α4β7-MAdCAM-1 binding.
  - MAdCAM-1 is expressed almost exclusively in the gastrointestinal tract.
Therapy – Fecal microbiota transplantation

FMT for the treatment of ulcerative colitis

Figure 2: Case examples of primary outcome after faecal microbiota transplantation

A. and C Baseline endoscopic appearance; B and D. Endoscopic appearance at end of week 8 after FMT
IBD: Outlook

Genetic variations

Certain Environment

Gut Microbiota

Multi-factorial

Infections

IBD Phenotype

genetic risk factors act in synergy with the external environment as well as the internal ‘environment’ (gut microbiota)