Signal Transduction in Inflammatory Bowel Disease

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Ph.D
Overview

Inflammatory Bowel Diseases

1. Epidemiology, Symptoms and Pathogenesis
2. Phenotypes and Classification
3. Pathophysiology
4. Susceptibility loci and Pathways
5. Therapeutic intervention
Global map of IBD

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

1.5 million Americans

2.2 million people in Europe

IBD: Epidemiology

- UC: females ≥ males
- CD: males ~ females
- 2-3th decade of life
- 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 from mode of childbirth and early-life exposures like breastfeeding and antibiotic exposure in infancy, to exposures later on in adulthood like smoking, diet and lifestyle.
IBD: Symptoms

- Weight loss
- Diarrhea (bloody UC>CD)
  - Colonic inflammation
  - Food malabsorption
  - Bile acid malabsorption
  - Increased bowel motility
- Abdominal pain (bowel cramps)
- Fever, anemia, thrombocytosis
- Chronic bowel inflammation
- Extraintestinal manifestations (joints – skin – eyes)
- Associate diseases (psoriasis, spondylitis)
IBD: Pathogenesis

- Genetic variations
- Certain Environment
- Gut Microbiota
- Multi-factorial
- Infections

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

Location

E1: Ulcerative Proctitis
E2: Left-sided UC
E3: Extensive UC
Crohn's Phenotypes

Age at Diagnosis

Location

Behavior

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, Inflamm Bowel Dis 2000
IBD: Pathophysiology

- Genetic predisposition
- Dysbiosis
- Inflammation
- Deregulated immune response
- Oxidative stress
- Epithelial damage (↓ Tissue repair↑ Increased permeability)
Pathophysiology of IBD and risk for CRC

Experimental Biology and Medicine 2012;1;237(5).

Transl Gastrointest Cancer. 2013 January 1;2
Pathophysiology of IBD and risk for CRC

Sporadic colon cancer
Normal mucosa → Early adenoma → Intermediate adenoma → Late adenoma → Carcinoma
- Loss of APC function
- Microsatellite instability, KRAS mutations, COX-2 activity
- DCC and DPC4 mutations
- P53 mutations

Colitis-associated colon cancer
Normal mucosa → Indefinite dysplasia → Low-grade dysplasia → High-grade dysplasia → Carcinoma
- P53 mutations → P53 LOH
- DCC mutations
- SRC mutations
- KRAS mutations
- Loss of APC function

Sporadic CRC  Colitis-associated CRC

IBD: Susceptibility loci and Pathways
Timeline of Genetic Discoveries in Inflammatory Bowel Disease.

GWAS: Genome-wide association studies
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*, SLC2A4RG
- **ER stress**
  - CPEB4, ORMDL3, SERINC3, XBPI*
- **Intracellular logistics**
  - VAMP3, KIF21A, TTL8, FGFR1OP, CEPT2, TAPP
- **Oxidative stress**
  - PRDX5, BACH2, ADO, GPX4, GPX1*, SLC22A4, LRRK2, NOD2*, CARD9*, HSPA6, DLD, PARK7, UTSS2*, PEX13
- **Cell migration**
  - ARPC2, LSP1, AAMP

**IBD-related processes**

- **Epithelial barrier**
  - GNAI2*, HNF4A, CDH1, FRRF11, MUC19, ITLN1*
- **Restitution**
  - REL, FGFR4, NKX2-3, STAT3, ERRF11, HNF4A, PLA2G2A/E
- **Salute transport**
  - SLC9A4, SLC22A5, SLC22A4*, AQP12A/B, SLC9A3, SLC26A3
- **Paneth cells**
  - ITLN1*, NOD2*, ATG16L1*, XBPI*
- **Innate mucosal defence**
  - NOD2*, ITLN1*, CARD9*, REL, SLC11A1, FCGR3A/B
- **Immune cell recruitment**
  - CCL1/CCL2/CCL7/CCL8, CCR6, IL6RA/IL6RB, MST1*
- **Antigen presentation**
  - ERAP2*, LNPEP, DENND1B
- **IL-23/T_{H17}**
  - IL23R*, JAK2, TYK2*, STAT3, ICOSLG, IL-21, TNFSF15*
- **T-cell regulation**
  - NOD1, TNFSF8, TAGAP, IL2, IL2RA, TNFRSF9, PIM3, IL7R*, IL12R, IL23R*, PRDM1, ICOSLG, TNFSF8, IFNG, IL21
- **B-cell regulation**
  - IL5, IL22F1, BACH2, IL7R*, IRF5
- **Immune tolerance**
  - IL10, IL27*, SBNO2, CREM, IL1R1/IL1R5, NOD2*

**Pathways**

- **UC**
- **CD**
- **IBD**

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*NATURE | VOL 474 | 16 JUNE 2011*
Intestinal homeostasis pathways

• Barrier function
• Epithelial restitution
• Microbial defense
• Innate immune regulation
• Regulation of adaptive immunity
• Reactive oxygen species (ROS) generation
• Autophagy
• Endoplasmic reticulum (ER) stress
• Metabolic pathways
Barrier defects allow bacterial products and dietary antigens to cross the epithelium and enter the lamina propria.
Intestinal Epithelial Barrier
Loss of barrier function visualised by confocal endomicroscopy.

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 exposed mucosa

Swidsinski et al
JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2009
Molecular mechanisms of IBD

IL-23 and IL-12 signaling axis

**shared signaling mediators**

**IL-12RB**

**JAK2**

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

nucleotide-binding-oligomerization-domain- (NOD)
Nod2 and Disease Location

Table 3  Genotype-phenotype correlations in Crohn’s disease

<table>
<thead>
<tr>
<th></th>
<th>L1 (ileal)</th>
<th>L2 (colonic)</th>
<th>L3 (ileocolonic)</th>
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<tbody>
<tr>
<td>Cuthbert 32</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lesage 16</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Murillo 52</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Ahmad 33</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Vermeire 58</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Hampe 48</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
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<tr>
<td>Abreu 59</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Mendoza 53</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Helio 47</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bairead 19</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Giachino 55</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+/-, trend but not statistically significant.

Gasche C, Gut 2005
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†
ER-stress/ Unfolded Protein Response pathway

Prolonged/unresolved ER stress leads to inflammation.
Molecular mechanisms of IBD
Pathways affecting Paneth cell

Impairment of ER stress resolution in Paneth cells can lead to intestinal inflammation
Autophagy gene Atg16L1 affects granule packaging
Loss of Nod2 results in diminished defensin production

Autophagic pathways regulated by ATG16L1; NOD2; the unfolded protein response (ER stress) via XBP1 affect Paneth cell function and thereby control the release of antimicrobial peptides
IL10 inhibits pro inflammatory role of grp78 within cytosol
Molecular mechanisms of IBD
Interleukin-10

- IL-10 is a cytokine with important immunoregulatory functions (e.g. TNF inhibition)

- IL-10<sup>-/-</sup> mice display enterocolitis similar to Crohn‘s disease (*Cell 1993*)

- Clinical trials with IL-10 for Crohn‘s disease show some but not broad efficacy.

- Homozygous loss of function mutations in IL10 and IL10R cause severe infantile (very early onset) IBD
Molecular mechanisms of IBD

IL-10 signaling

R. Sabat et al., Cytokine & Growth Factor Reviews 21 (2010) 331–344
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 erosions IL10⁻/⁻
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

Gasche C et al.
Two novel SNPs in the IL-10R1 cDNA

<table>
<thead>
<tr>
<th>SNP</th>
<th>position</th>
<th>Aa position</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>SNP1</td>
<td>G241A</td>
<td>A60A</td>
<td>1,3</td>
</tr>
<tr>
<td>SNP2</td>
<td>G520A</td>
<td>A153A</td>
<td>1,2,3</td>
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<tr>
<td>SNP3</td>
<td>A536G</td>
<td>S138G</td>
<td>2,3</td>
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<td>SNP5</td>
<td>G586A</td>
<td>P175P</td>
<td>2,3</td>
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<tr>
<td>SNP6</td>
<td>A731G</td>
<td>I203V</td>
<td>3</td>
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<tr>
<td>SNP7</td>
<td>C1033T</td>
<td>T324T</td>
<td>3</td>
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<tr>
<td>SNP4</td>
<td>G1112A</td>
<td>G330R</td>
<td>2,3</td>
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<tr>
<td>SNP8</td>
<td>C1320T</td>
<td>S420L</td>
<td>3</td>
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<tr>
<td>SNP9</td>
<td>C1929T</td>
<td>3’ UTR</td>
<td>3</td>
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<tr>
<td>SNP10</td>
<td>A3331G</td>
<td>3’ UTR</td>
<td>3</td>
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<tr>
<td>SNP11</td>
<td>A3524G</td>
<td>3’ UTR</td>
<td>3</td>
</tr>
</tbody>
</table>

SNP3: Serine 138 to Glycine
SNP4: Glycine 330 to Arginine

Gasche C, J Immunol 2003
S138G and G330R are loss-of-function alleles

Reduction in IL-10-induced STAT1 and STAT3 activation in IL-10R1-S138G (SNP3) but not in IL-10R1-G330R (SNP4)

IL10R1-G330R alters duration of STAT phosphorylation

G330 is important in stabilizing the STAT signal.
ORIGINAL ARTICLE

The IL-10R1 S138G loss-of-function allele and ulcerative colitis

P Grundtner¹, S Gruber¹, SS Murray², S Vermeire³, P Rutgeerts³, T Decker³, PL Lakatos⁵ and C Gasche¹,⁶

¹Division of Gastroenterology and Hepatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria; ²Scripps Genomic Medicine, La Jolla, CA, USA; ³Department of Gastroenterology, University Hospital Gasthuisberg, Leuven, Belgium; ⁴Max F Perutz Laboratories, University of Vienna, Vienna, Austria; ⁵Ist Department of Medicine, Semmelweis University, Budapest, Hungary and ⁶Christian Doppler Laboratory on Molecular Cancer Chemoprevention, Vienna, Austria


An Intracytoplasmic IL-10 Receptor Variant Permits Rapid Reduction in STAT3 Activation

Michaela Finsterbusch, Vineeta Khare, Christoph Campregher, Rayko Evstatiev, and Christoph Gasche*

Medical University of Vienna, Department of Internal Medicine III, Division of Gastroenterology and Hepatology and Christian Doppler Laboratory for Molecular Cancer Chemoprevention, Vienna, Austria
IL-10 homolog cytokines and their receptors
Three distinct loss of function, homozygous mutations in genes IL10RA and IL10RB in 4 of 9 patients with early-onset colitis.
Oxidative stress and DNA damage response in IL-10 KO mice

unpublished data; Frick A, Khare V et al
Inflammation driven colon carcinogenesis in IL-10 KO mice

unpublished data; Frick A, Khare V et al
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
<table>
<thead>
<tr>
<th>Ref.</th>
<th>Human</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombel et al [25]</td>
<td>65 patients having recently undergone intestinal resection surgery</td>
<td>4 μg/kg daily or 8 μg/kg twice weekly for 12 wk</td>
<td>No clear evidence of effect</td>
</tr>
<tr>
<td>Fedorak et al [26]</td>
<td>95 mild to moderately active CD (CDAI 200-350)</td>
<td>1, 5, 10 or 20 μg/kg of daily for 4, 20 wk follow up</td>
<td>Improved clinical response (based on CDAI score) and improved endoscopic appearance</td>
</tr>
<tr>
<td>Schreiber et al [26]</td>
<td>329 therapy-refractory chronic active CD (CDAI 200-400)</td>
<td>1, 4, 8 or 20 μg/kg of Tenvil subcutaneously for 28 d</td>
<td>Non-significant clinical improvements</td>
</tr>
<tr>
<td>van Deventer et al [29]</td>
<td>46 patients with active steroid-resistant CD (CDAI 200-350)</td>
<td>0.5, 1, 5, 10 or 25 μg/kg daily for 1, 3 wk follow up</td>
<td>Reduction in the average score of CDAI</td>
</tr>
<tr>
<td>Braat et al [28]</td>
<td>10 patients with moderate to severe CD</td>
<td>10 enteric-coated capsules containing 10^8 chn of LL-Thy12 twice daily for 7 d</td>
<td>Clinical benefit observed in 8 of 10 patients, including 5 showing complete remission</td>
</tr>
<tr>
<td></td>
<td>Animal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barbara et al [28]</td>
<td>DNB induced colitis Spf Sprague-Dawley rats</td>
<td>Ad5IL-10 (5 × 10^7-1 × 10^8 pfu)</td>
<td>Improved colitis macroscopically and histologically and decreased MPO activity and LTB4 levels</td>
</tr>
<tr>
<td>Grool et al [25]</td>
<td>40 male NZ white rabbits formalin-immune complex induced colitis</td>
<td>100 or 500 μg/kg single IV infusion of rIL-10</td>
<td>Anti-inflammatory response as measured by decreased mucosal damage, leukocyte recruitment, MPO and LTB4</td>
</tr>
<tr>
<td>Ribbons et al [24]</td>
<td>TNBS induced colitis in 74 Sprague-Dawley rats</td>
<td>0.5, 5, 50, 500 μg/kg rIL-10 subcutaneous injection twice daily for 5 d</td>
<td>Mild anti-inflammatory effects</td>
</tr>
<tr>
<td>Sasaki et al [25]</td>
<td>3% DSS induced C57Bl6 mice</td>
<td>Intra-peritoneal administration of adIL-10</td>
<td>Significant reduction in MPO</td>
</tr>
<tr>
<td>Tomoyose et al [28]</td>
<td>4% DSS induced colitis BALB/c mice</td>
<td>Recombinant mouse rIL-10 (1, 100, 1000 unit/mL)</td>
<td>Marked improvement in intestinal inflammation Inhibition of tissue damage and production of pro-inflammatory cytokines</td>
</tr>
<tr>
<td>Steidler et al [27]</td>
<td>DSS induced and spontaneous IL10^-/- mouse models of colitis</td>
<td>Daily intragastric inocula of 2 × 10^7 or 10^8 LL-mIL10</td>
<td>Reduced histological score by 50% in DSS and prevented onset of colitis in IL10^-/- mice</td>
</tr>
</tbody>
</table>
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)
Endoscopic mucosal healing in IBD

CD
Azathioprine
Adalimumab
Certolizumab
Infliximab

UC
5-ASA
Corticosteroids
Tacrolimus, CsA
Adalimumab
Infliximab
Azathioprine

MH

Neurath M F, and Travis S P L Gut 2012;61
Mechanism of 5-ASA activity

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

Khare V, Biochem Pharmacol. 2013 Jan 15;85
5-ASA restores epithelial Adherens Junctions

Khare V, Biochem Pharmacol. 2013 Jan 15;85
Overexpression of PAK1 in IBD

PAK1 overexpression increases cell proliferation and decreases apoptosis

Khare V, Dammann et al. 2015, Inflamm Bowel Dis. 2015
PAK1 is activated in intestinal inflammation

Dammann K, Khare V, unpublished data
# IBD: Therapeutic intervention

## Emerging Biological Treatments

<table>
<thead>
<tr>
<th>Biological target</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Innate immune cell signalling</strong></td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>Infliximab, adalimumab, certolizumab pegol</td>
</tr>
<tr>
<td>TLR</td>
<td>DIMS0150 (Kappaproct), BL-7040 RDP58</td>
</tr>
<tr>
<td>MyD88</td>
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<tr>
<td><strong>Pro-inflammatory cytokines</strong></td>
<td></td>
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<tr>
<td>JAK</td>
<td>Tafocitinib</td>
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<tr>
<td>IL-12/23</td>
<td>Ustekinumab, SCH900222, briakinumab</td>
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<tr>
<td>IL-17</td>
<td>Secukinumab, brodalumab, vidofludimus</td>
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<tr>
<td>IFNγ</td>
<td>Fontolizumab</td>
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<tr>
<td>IL-17</td>
<td>Secukinumab, brodalumab, vidofludimus</td>
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<tr>
<td>IL-13</td>
<td>QAX576, anrkinzumab, tralokinumab</td>
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<tr>
<td>IL-6 and IL-6R</td>
<td>Tocilizumab, PF04236921, C326</td>
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<tr>
<td><strong>T cell activity</strong></td>
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<td>T cell proliferation</td>
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<tr>
<td>CD3</td>
<td>Visilizumab</td>
</tr>
<tr>
<td>CD25</td>
<td>Basiliximab, dadilizumab, sotraastaurin</td>
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<tr>
<td>Protein kinase C inhibitor</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>Chemotaxis</strong></td>
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<td><strong>Regulatory T cells</strong></td>
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<tr>
<td>OvaSave</td>
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</tbody>
</table>
IBD: Therapeutic intervention

Anti - tumor necrosis factor α: Effective in CD

Anti-p40 monoclonal antibodies (Blocks IL23, IL12 pathway: CD, Psoriasis

Antibiotic therapy

Probiotics

Experimental diet

Specific treatment (anemia, osteoporosis, vitamin deficiencies…)

Surgery