Inflammatory Bowel Disease and IL-10/IL-10R defects

Prof. Bodo Grimbacher
CCI, Freiburg
bodo.grimbacher@uniklinik-freiburg.de
Inflammatory bowel disease

• Heterogeneous group of disorders characterised by chronic and relapsing inflammation

• Main entities: Crohn’s disease ulcerative colitis

• Complex etiology: genetics & environment

→ Dysregulation of the gastrointestinal immune system
Early-onset enterocolitis

- Onset **before 1 year** of age
- IBD with bloody diarrhoea
- **Severe perianal disease** with multiple abscesses and anal/recto-vaginal fissures and fistulae
- **Ulcers** of intestinal mucosa

Additional features:
- Chronic folliculitis
- Recurrent respiratory diseases
- Arthritis
Early-onset enterocolitis

Early-onset colitis can be an **autosomal recessive Mendelian trait**.

Patients have been diagnosed with mutations in:
- **IL-10**
- **IL-10RA**
- **IL-10RB**
Interleukin-10

• **Anti-inflammatory cytokine:**
  – Suppression of secretion of inflammatory cytokines such as TNF-α, IFN-γ, IL-1, IL-6
  – Downregulation of surface molecules such as MHCII or B7

• secreted by a **wide range of immune cells**
Interleukin-10

- IL-10 receptor is a heteromeric complex of two distinct cytokine receptor subunits consisting of the ligand binding subunit IL-10 R alpha (IL-10 R1) and the signal-transducing accessory subunit IL-10 R beta (IL-10 R2).

- IL-10 R1 alpha is specific for IL-10 and expressed by most hemopoietic cells.

- IL-10 R2 beta is a widely expressed shared subunit for IL-10, IL-22, IL-26 and the IFNλ proteins IL-28A, IL-28B and IL-29.
IL-10 Signalling

IL-10 inhibits transcription of cytokine genes such as IL-1, IL-6, TNF-α.
Consanguineous family with severe early-onset colitis
early onset colitis / inflammatory bowel disease involving the colon and the small intestine
early onset colitis
colonoscopy findings:
• strictures,
• ulceration,
• scarring,
• florid inflammation
Histological picture of an indeterminate colitis.
Identification of a point mutation in \textit{IL10RB}

- Healthy Father
- Healthy mother
- Healthy sibling 1
- Healthy sibling 2
- Patient 1
- Patient 2
Anti-inflammatory IL-10 Signalling

IL-10 → IL10R → STAT3 → SOCS3 → Nucleus

LPS → TLR4 → TNF-α → SOCS3
Functional Test

PBMCs

LPS

TNF-α

IL-10

LPS

PBMCs

TNF-α
Lack of wild type IL10R2 expression

as stained with polyclonal IL10Rbeta antibody from R&Dsystems
Patients’ cells do not phosphorylate STAT3 following IL10 stimulation
Patients’ cells are not responsive to IL-10

Stimulation of PBMCs with IL-10
SOCS3 expression
Patients’ cells are not responsive to IL-10

- Pre-incubation with IL-10
- Stimulation with LPS

Healthy control versus Patient (TNF release in pg/mL)

- Medium
- LPS
- LPS + IL-10
In patients, IL10 cannot suppress LPS-induced TNF secretion
In the absence of IL10 receptor signalling, multiple pro-inflammatory cytokines are overexpressed.
Reconstitution of IL10R2 results in restored IL-10 dependent STAT3-P
IL-10R2 Trp159X

FOXp3+ regulatory T cells

Healthy control

IL10RB-deficient patient
The IL10 receptor alpha mutations

obtained from Cristoph Klein, Hannover
• 4-year old girl of Pakistani origin
• intractable ulcerative colitis:
  – diagnosed aged 3 months
  – perianal and rectovaginal fistulae
  – esophageal lesions
  – failure to thrive
Patient M.A.

- DOB: 30/06/2009
- Pakistani origin
- **Same phenotype:**
  - Severe colitis
  - Esophageal lesions
  - Treatment failure
IL-10 Gly113Arg

Family D

Healthy father

Healthy mother

Patient M.A.

G113R
IL-10 Gly113Arg

IL-10: Exon 3

healthy mother

patient A.I.

Family C

Il-10: Exon 3
Amino acid exchange in pos 113

glycine → arginine
Amino acid exchange in pos 113

→ formation of hydrogen bonds between helix C and D
→ change of hydrophobicity
Functional Test

With *in vitro* synthesized wild type and mutant IL-10
IL-10/IL-10R mutations in early-onset colitis

New England Journal of Medicine 2009

**Inflammatory Bowel Disease and Mutations Affecting the Interleukin-10 Receptor**

Erik-Oliver Glöckler, 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 Alloth, M.D.,
Dharani Murugan, M.Sc., Nadine Hätscher, B.Sc., Dietmar Pfeifer, M.D.,
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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.

Lancet 2010

**Infant colitis—it’s in the genes**

Erik-Oliver Glöckler*, Natalie Frede*, Mario Perro, Neil Sebire, Mamoun Elawad, Neil Shah, Bodo Grimbacher

Mutations in:
- IL-10RA (2 patients)
- IL-10RB (2 patients)
Defective IL10 Signaling Defining a Subgroup of Patients With Inflammatory Bowel Disease

Bernadette Begue1, Julien Verdier1, Frédéric Rieux-Laucat, PhD2, Olivier Goulet, MD, PhD1,3,4, Alain Morali, MD, PhD5, Danielle Canioni, MD, PhD3,4, Jean-Pierre Hugot, MD, PhD6, Cécile Daussy7, Virginie Verkarre, MD, PhD3,4, Bénédicte Pigneur, MD3,4, Alain Fischer, MD, PhD3,4, Christoph Klein, MD, PhD7, Nadine Cerf-Bensussan, MD, PhD1 and Frank M. Ruemmele, MD, PhD1,3,4

IL-10/IL-10R mutations in early-onset colitis

IL-10/IL-10R polymorphisms are associated with very-early-onset ulcerative colitis

Christopher J. Moran MD1,2,3, Thomas D. Walters MD4, Cong-Hui Guo MD5, Subra Kugathasan MD6, Christoph Klein MD, PhD7, Dan Turner MD, PhD6, Victorien M. Wolters MD, PhD4,5, Robert H. Bandsma MD, PhD4, Marialena Mouzaki MD4, Mary Zachos MD4, NEOPICS†, Jacob C. Langer MD9, Ernest Cutz MD10, Susanne M. Benseler MD11, Chaim M. Roifman MD12, Mark S. Silverberg MD13, Anne M. Griffiths MD4, Scott B. Snapper MD, PhD2,3,14,15,‡,*, Aleixo M. Muise MD, PhD4,5,‡,*

Clinical outcome in IL-10 and IL-10R-deficient patients with/without HSCT

Karin R. Engelhardt, PhDab, Neil Shah, MDc, Intan Faizura-Yeop, MDc, Dilara F. Kocacik Uygun, MDd, Natalie Fredeb, Aleixo M. Muise, MD, PhDe, Eyal Shteyer, MDI, Serkan Filiz, MDd, Ronnie Chee, MDA, Mamoun Elawad, MDC, Britta Hartmanng, NEOPICSb, Peter D. Arkwright, MD, PhDi, Christopher Dvorak, MDi, Christoph Klein, MD, PhDk, Jennifer M. Puck, MDj, Bodo Grimbacher, MDab, and Erik-Oliver Glocker, MDI
Mutations in:
- IL-10RA (3 patients)
- IL-10RB (6 patients)
- IL-10 (3 patients)
### Mutations occurring more than once:

- **IL-10 Gly113Arg** (2 patients from 2 families)
- **IL-10 Gly153Asp** (3 patients)
- **IL-10R2 Trp159X** (3 patients from 2 families)

### Table: Loss of Function Mutations in IL-10 and IL-10RA/RB

<table>
<thead>
<tr>
<th></th>
<th>Homozygous</th>
<th>Compound heterozygous</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>IL-10RA</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>IL-10RB</td>
<td>11</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

24 different loss of function mutations in IL-10 and IL-10RA/RB
## Treatment

### Failure of immunosuppressive drugs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Immunosuppression/Surgical therapy</th>
<th>Follow-up</th>
</tr>
</thead>
</table>
| 1       | - Steroids, azathioprine, Cyclosporine A, infliximab  
- Colectomy at the age of 3y | - Ongoing colitis, no remission |
| 2       | - Steroids, cyclosporine A  
- Colectomy at the age of 5m | - Ongoing colitis, no remission |
| 3       | - Steroids, azathioprine  
- Colectomy at the age of 10m | - Ongoing colitis, no remission |
| 5       | - Prednisone, azathioprine | - Ongoing colitis, no remission |
| 6       | - Steroids, azathoprine, methotrexate, infliximab  
- Ileostomy | - Ongoing colitis, no remission |
## Treatment: HSCT

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Complications</th>
<th>Follow-up</th>
</tr>
</thead>
</table>
| 7       | Transplanted aged 3y2m Now 4 ½ years old | - Allergic reaction on infliximab  
- Pancreatitis  
- Oral aversion  
- Candida + bacterial line sepsis - Colitis resolved  
*post BMT:*  
- Pseudomonas bacteremia  
- CMV reactivation | |
| 8       | Transplanted aged 3y11m; Now 5 years 7 months | - Moderate hearing loss  
- High output stoma  
*post BMT:*  
- RSV infection  
- CMV infection/reactivation  
- EBV infection/reactivation | - Colitis resolved |
| 9       | Transplanted aged 1y2m; Now 2 years 11 months | - Klebsiella line sepsis | - Colitis resolved |
Hematopoietic stem cell transplantation in patient II.3

only 5 weeks later...

HLA-compatible brother, conditioning with Campath-1H 1mg/kg, Fludarabine 180 mg/m2, Treosulfan 42 mg/m2, and Thiotepa 10 mg/kg
5 patients with IL-10R defects who were unresponsive to immunosuppressive therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at HSCT [years]</th>
<th>HSCT source</th>
<th>Chimerism</th>
<th>Complications</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pat 1</td>
<td>9 11/12</td>
<td>10/10 MSD</td>
<td>100 (d+721)</td>
<td>skin III*</td>
<td>clinical remission</td>
</tr>
<tr>
<td>Pat 3</td>
<td>13 9/12</td>
<td>10/10 MUD</td>
<td>100 (d+537)</td>
<td>none</td>
<td>clinical remission</td>
</tr>
<tr>
<td>Pat 4</td>
<td>10/12</td>
<td>9/10 MUD</td>
<td>none</td>
<td>graft rejection (d+86)</td>
<td>clinical remission</td>
</tr>
<tr>
<td></td>
<td>1 4/12</td>
<td>9/10 MUD</td>
<td>100 (d+362)</td>
<td>skin III*, gut II*, chronic skin (lim)</td>
<td>clinical remission</td>
</tr>
<tr>
<td>Pat 5</td>
<td>8 3/12</td>
<td>10/10 MSD</td>
<td>80 (d+375); DLI (d+235)</td>
<td>Rotavirus, SVC thrombosis</td>
<td>clinical remission</td>
</tr>
<tr>
<td>Pat 6</td>
<td>5 6/12</td>
<td>9/10 MUD</td>
<td>100 (d+250), SC-Boost (d+260)</td>
<td>skin I-II*</td>
<td>thriving, colitis improved, fistula not yet healed</td>
</tr>
</tbody>
</table>

Kotlarz et al., Gastroenterology 2012
Transplantation regimen

Conditioning regime

- Alemtuzumab (5 x 0.2 mg/kg)
- Fludarabine (6 x 30 mg/m²)
- Treosulfan (3 x 14 g/m²)
- Thiopeta (2 x 5 mg/kg)

Supportive Care

- Amphotericin B, Colistin
- Vancomycin, Ciprofloxacin, Metronidazole
- Fluconazole
- Itraconazole, Aciclovir

Kotlarz et al., Gastroenterology 2012
Colonoscopy

Acute colitis

Early cobble stone pattern

Intermittent serpiginous ulcers with fibrin layers

Histopathology of colon biopsies

Glandular distortion

Mild to moderate inflammation

Circumscribed and superficial mucosal defects

Kotlarz et al., Gastroenterology 2012
Colonoscopy
Normal intestinal mucosa
13 months after HSCT

Histopathology of colon biopsies
Almost complete reduction of glandular distortion
Inconspicuous, sparse leucocytic infiltration within lamina propria mucosa
13 months after HSCT

Kotlarz et al., Gastroenterology 2012
Reconstitution of IL-10 signalling

STAT3 tyrosine phosphorylation

Before HSCT

After HSCT

Kotlarz et al., Gastroenterology 2012
Reconstitution of IL-10 signalling

Inhibition of TNF-α secretion

Before HSCT

After HSCT

Kotlarz et al., Gastroenterology 2012
Summary

• Early-onset colitis can be caused by mutations in IL-10 or the IL10 receptor (alpha or beta chain)

• Early-onset colitis due to defective IL10 signalling can be substantially improved by stem cell transplantation

• The gut pathology in inflammatory bowel disease is characterized by hyperinflammation due to the lack of the dampening effect of IL10 signalling
Conclusion

• IL10 function is crucial for the gastrointestinal immune homeostasis

• The IL10 pathway may constitute a therapeutic target in inflammatory bowel disease

• The pathology is interestingly limited to the gut. Is IL10 dispensible or redundant in other organs?
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All patients
and their families

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b.grimbacher@ucl.ac.uk
Immunophenotyping:
• Normal distribution of: T cells, CD 16+ NK cells, CD20+ B cells
• Normal proliferation of lymphocytes following stimulation
• Normal function of NK cells by ACDC
• Cytotoxicity in comparison to control was decreased

**IL-1 beta**  
NL<3.9 pg/ml  
• 14.12.05: 16.6 pg/ml  
• 19.12.05: 10.1 pg/ml  
• 23.12.05: 32.2 pg/ml  
• 27.12.05: 15.3 pg/ml

**IL-6:** 13 ng/l

**DD:** Undefined immunodeficiency syndrome  
Crohn’s disease

**Treatment:** Antibiotics and Infliximab
Patient 2 (sibling)
Birthday: 09.02.2000

Clinical presentation:
• Chronic inflammatory bowel disease with ileostoma and retrovaginal fistula
• Kidney abscesses both sides
• Muscular Dystrophy
• ? Mental retardation
• Hypochromic anemia
• EBV infection
• Pneumonia
• Skin folliculitis (acneiform)
• Chronic CRP elevation

01.08.2002
IgG: 15.3 g/l
IgA: 2.09 g/l
IgM: 2.24 g/l
IgE <2 IE/ml
IgG1: 14.4 g/l
IgG2: 1.5 g/l
IgG3: 0.24 g/l
IgG4< 0.02 g/l
IgG seropositive for: EBV, Rubella, and Parvovirus B19

DD: Crohn’s disease
Behcet’s disease

Treatment:
various antibiotics,
Infliximab from 11/03 to 01/05
Allergic reaction to Infliximab 01/05
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Immunological abnormalities</th>
</tr>
</thead>
</table>
| 1       | IL-10R1: Arg117His (homozygous) | - increased IgA, IgM and IgG  
- decreased CD4/CD8 T cell ratio  
- increased CD3+ cells (percentage)  
- increased CD3+/CD8+ cells (percentage)  
- **decreased CD19+ cells (abs. counts & percentage)**  
- decreased CD16+/CD56+ cells (abs. counts & percentage) |
| 5       | IL-10R1: Exon 1, 2 and 3 deleted (homozygous); Ex1_3del | - increased IgM, decreased IgA and IgG (requiring IVIG)  
- decreased CD3+ cells (percentage)  
- decreased CD3+/CD4+ cells (percentage)  
- **decreased CD19+ cells (percentage)**  
- reduced response to mitogens (50%) |
| 6       | IL-10R1: Tyr57Cys/Ex2_4del (compound heterozygous) | - increased IgA  
- reduced response to conjugated pneumococci vaccine |
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Immunological abnormalities</th>
</tr>
</thead>
</table>
| 7       | IL-10R1: Leu125Arg (homozygous) | **before BMT:**  
- decreased IgG (requiring IVIG)  
- increased CD3+ cells (abs. counts)  
- increased CD3+/CD4 cells (abs. counts)  
- increased CD3+/CD8+ cells (abs. counts & percentage)  
- reduced response to mitogens  
- reduced response to conjugated pneumococci vaccine  

**post BMT:**  
- reduced response to mitogens |
## Immunological abnormalities: IL-10R2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Immunological abnormalities</th>
</tr>
</thead>
</table>
| 2       | **IL-10R2:** splice site mutation at exon/intron 3 boundary (homozygous); results in skipped exon 4: Leu59fsX72 | - increased IgA and IgG  
- increased CD3+ cells (percentage)  
- decreased CD16+/CD56+ cells (percentage) |
<p>| 3       | <strong>IL-10R2:</strong> one nucleotide deletion in exon 2 (homozygous); results in frameshift and premature stop codon: $\rightarrow$Trp18fsX29 | - increased IgA                                  |</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Immunological abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td><strong>IL-10:</strong> Gly113Arg (homozygous)</td>
<td></td>
</tr>
</tbody>
</table>

*before BMT:*
- decreased CD4/CD8 ratio
- increased CD3+ cells (percentage)
- increased CD3+/CD8+ cells (percentage)

*post BMT:*
- increased IgG
- decreased CD4/CD8 ratio
- increased CD3+ cells (percentage)
- decreased CD3+/CD4+ cells (abs. counts & percentage)
- increased CD3+/CD8+ cells (percentage)
- decreased CD19+ cells (abs. counts & percentage)
- impaired response to mitogens

| 9       | **IL-10:** Gly113Arg (homozygous) | 

*before BMT:*
- increased IgM and IgA

*post BMT:*
- decreased CD4/CD8 ratio
- increased CD3+ cells (abs. counts & percentage)
- increased CD3+/CD8+ cells (percentage)
- decreased CD19 (percentage)