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Loss of Interleukin-10 Signaling and Infantile Inflammatory Bowel Disease: Implications for Diagnosis and Therapy

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BACKGROUND & AIMS: Homozygous loss of function mutations in interleukin-10 (*IL10*) and interleukin-10 receptors (*IL10R*) cause severe infantile (very early onset) inflammatory bowel disease (IBD). Allogeneic hematopoietic stem cell transplantation (HSCT) was reported to induce sustained remission in 1 patient with IL-10R deficiency. We investigated heterogeneity among patients with very early onset IBD, its mechanisms, and the use of allogeneic HSCT to treat this disorder. **METHODS:** We analyzed 66 patients with early onset IBD (younger than 5 years of age) for mutations in the genes encoding IL-10, IL-10R1, and IL-10R2. IL-10R deficiency was confirmed by functional assays on patients' peripheral blood mononuclear cells (immunoblot and enzyme-linked immunosorbent assay analyses). We assessed the therapeutic effects of standardized allogeneic HSCT. **RESULTS:** Using a candidate gene sequencing approach, we identified 16 patients with IL-10 or IL-10R deficiency: 3 patients had mutations in IL-10, 5 had mutations in IL-10R1, and 8 had mutations in IL-10R2. Refractory colitis became manifest in all patients within the first 3 months of life and was associated with perianal disease (16 of 16 patients). Extraintestinal symptoms included folliculitis (11 of 16) and arthritis (4 of 16). Allogeneic HSCT was performed in 5 patients and induced sustained clinical remission with a median follow-up time of 2 years. In vitro experiments confirmed reconstitution of IL-10R–mediated signaling in all patients who received the transplant. **CONCLUSIONS: We identified loss of function mutations in IL-10 and IL-10R in patients with very early onset IBD. These findings indicate that infantile IBD patients with perianal disease should be screened for IL-10 and IL-10R deficiency and that allogeneic HSCT can induce remission in those with IL-10R deficiency.**

Keywords: Children; Genetic Defect; Immunodeficiency; Intestinal Inflammation.

Inflammatory bowel diseases (IBD) comprise a heterogeneous group of disorders, phenotypically classified as Crohn's disease, ulcerative colitis, and IBD unclassified.[1,2] The etiology of these disorders remains largely obscure. Experimental studies and genetic evidence suggest that chronic intestinal inflammation is triggered by various environmental factors in genetically susceptible individuals. During the last decade, several genome-wide linkage and association studies have revealed a number of genetic polymorphisms associated with an increased susceptibility to Crohn's disease and ulcerative colitis.[3–8] Genes that are implicated in the pathogenesis of IBD regulate various biological functions, such as immunomodulation, mucosal barrier integrity, and microbial homeostasis.[9] However, the functional significance of these genetic loci is controversial. Molecular analysis of rare Mendelian variants of IBD can also identify candidate genes implicated in IBD.[10,11] We and others identified children with mutations in the interleukin-10 receptor (*IL10R*)[12] and inter-

Abbreviations used in this paper: GVHD, graft-vs-host disease; HSCT, hematopoietic stem cell transplantation; IBD, inflammatory bowel disease; IL-10R, interleukin-10 receptor; LPS, lipopolysaccharide; PBMCs, peripheral blood mononuclear cells; TNF-α, tumor necrosis factor-α.

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leukin-10 (*IL10*)[13] genes. IL-10 represents a potent anti-inflammatory and immunosuppressive cytokine that mediates its pleiotropic effects on different immune cells through the transmembrane heterotetrameric complex composed of 2 IL-10R1 and IL-10R2 chains.[14] Although IL-10R1 is specific to IL-10, IL-10R2 is a shared cytokine receptor subunit mediating signals via IL-10, IL-22, IL-26, and interferon-λ.[15] Because IL-10 acts predominantly on hematopoietic and immune cells,[14] transplantation of allogeneic hematopoietic stem cells might be considered as curative therapeutic approach. In fact, proof-of-concept could be demonstrated in one IL-10R−deficient patient.[12]

Here, we analyzed a cohort of 66 patients with infantile (very early onset) IBD for mutations in the IL-10−related genes and describe our experience of standardized allogeneic hematopoietic stem transplantation (HSCT) in 5 patients with IL-10R deficiency.

Materials and Methods

Patients and DNA Sanger Sequencing

We collected blood samples from 66 early-onset IBD patients with clinical onset at younger than 5 years of age (subgroup of the A1a entity according to the Paris classification[16]), a severe course of disease, and different ethnic background. DNA Sanger sequencing of genes encoding for IL-10R1, IL-10R2, and IL-10 was performed upon parental written informed consent. The respective primer sequences are specified in Supplementary Table 1. Clinical information on IL-10− and IL-10R−deficient patients was collected by their attending physicians. The study was approved by the Institutional Review Board at Hannover Medical School, Germany.

Western Blot Analysis and Enzyme-Linked Immunosorbent Assay

Whenever potentially disease-causing mutations were identified, we performed additional functional studies to validate IL-10R deficiency. Experimental details have previously been published.[12] In brief, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation and were stimulated with recombinant human IL-10 (25 ng/mL; PeproTech, Hamburg, Germany) for various time periods, followed by Western blot analysis of signal transducer and activator of transcription 3 phosphorylation at the residue tyrosine 705 (Tyr705, Cell Signaling, Danvers, MA), a crucial downstream event of IL-10 signal transduction. In addition, to exemplify defective IL-10−mediated anti-inflammatory cytokine response, we harvested supernatants of lipopolysaccharide (LPS)-stimulated PBMCs (100 ng/mL; Sigma-Aldrich, Munich, Germany) and quantified tumor necrosis factor−α (TNF-α) secretion upon costimulation with IL-10 (25 ng/mL) using a commercially available OptEIA ELISA kit according to manufacturer's instructions (BD Bioscience, San Jose, CA).

To assess the functional impact of the identified IL-10 mutation, we followed a previously published protocol.[13] In brief, we synthesized wild-type and mutant IL-10 by lentiviral-mediated overexpression of respective complementary DNAs in 293T cells, quantified the concentration of secreted IL-10 in the supernatants by enzyme-linked immunosorbent assay, and analyzed the suppression of TNF-α secretion in LPS/IL-10−costimulated healthy donor PBMCs.

Allogeneic HSCT

Allogeneic HSCT was considered in patients with confirmed diagnosis of IL-10R deficiency (mutational analysis, functional studies). To date, 5 patients with a severe and refractory course unresponsive to treatment with steroids, immunosuppressive agents, and surgery were treated by allogeneic HSCT after a standardized protocol. Patients had a conditioning regimen including alemtuzumab (1 mg/kg body weight), fludarabine (180 mg/m^2 body surface area), treosulfan (42 g/m^2 body surface area), and thiotepa (10 mg/kg body weight). Strict gut decolonization was performed with the use of colistin, amphotericin B (oral), ciprofloxacin, metronidazole, vancomycin (oral), fluconazole, and total parenteral nutrition during the peritransplantation period. Graft-vs-host disease (GVHD) prophylaxis included ciclosporin and mycophenolate mofetil. Infection prophylaxis was intensified with additional aciclovir and itraconazole substituting fluconazole on day 3 after HSCT. Tapering of the immunosuppressive medication was started at day +100 in the absence of GVHD and included withdrawal of mycophenolate mofetil and dose reduction of ciclosporin by 10% every week. Hematopoietic chimerism was determined using restriction fragment length polymorphism. The reconstitution of IL-10R−mediated signal transduction in PBMCs from transplanted IL-10R−deficient patients was validated by Western blot analysis and enzyme-linked immunosorbent assay as described.

Results

Genetic Analysis

We sequenced the genes encoding for IL-10R1, IL-10R2, and IL-10 in 66 patients with early onset IBD (younger than 5 years of age). We identified 16 patients with mutations in IL-10−related genes: 5 patients had mutations in the *IL10RA* gene, 8 patients had mutations in the *IL10RB* gene, and 3 patients had mutations in the *IL10* gene. Of those, 9 mutations had not been previously documented in IBD (1 stop codon mutation, 4 amino acid substitutions, 2 compound heterozygous mutations, 1 3′UTR mutation, and 1 deletion). Results of this mutational screening are summarized in Table 1. Molecular details are displayed in Supplementary Figures 1A-C. In silico analysis using PolyPhen[17] predicted that all identified mutations with amino acid substitution are either probably or, in the case of patient 12, possibly damaging with respect to protein structure and function. In contrast, SIFT[18] analysis predicted tolerated protein function in IL-10RA$^{Arg117Arg/Cys}$ (patient 7) and IL-10RA^{Ile169Thr} (patient 12) mutations. Bioinformatic studies on the 3′UTR mutation suggested that this sequence variation targets a putative SR-protein binding site.

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Characterization of IL-10- and IL-10R-Deficient Patients

All patients with mutations in *IL10* and *IL10R* genes presented with severe and progressive colitis in the first 3 months of life, fulfilling the criteria of infantile (very early onset) IBD.[16] Colitis was associated with failure to thrive (15 of 16), recurrent fever and infections (15 of 16), bloody diarrhea (16 of 16), abscesses (15 of 16), perianal fistula (13 of 16), oral aphthous lesions (4 of 16),

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Table 1. Summary of Demographic, Molecular, and Clinical Information on IL-10- and IL-10R-deficient patients

Patient no.	Sex	Ethnicity, country of origin	Consanguinity	Age of diagnosis[b]	Mutation (gene; genotype)	Age of onset,[c,d]	Disease location/ behavior[d]	Extraintestinal manifestations	Treatment (outcomes)		Surgery
									Enteral/parenteral nutrition	Anti-inflammatory/ immunosuppressive drugs	
1[a]	M	Kurdish, Turkey	Yes	9 y 5 mo	*IL10RB,* Ex 4: c.G477A, p.Trp159X	30	L2, B2B3p	Folliculitis, gonarthritis	PN (p)	CS (p), ETN (n), IFX (n), MTX (n)	Ileocolostomy, colectomy (3 mo, p)
2	F	Kurdish, Turkey	Yes	6 y 9 mo	*IL10RB,* Ex 4: c.G477A, p.Trp159X	49	L2, B2B3p	Folliculitis, gonarthritis, renal abscesses	None	CS (g), IFX (g)	Ileostomy, colectomy (2 mo, p)
3[a]	F	Arab, Lebanon	Yes	13 y 3 mo	*IL10RA,* Ex 4: c.G421A, p.Gly141Arg	7	L2, B2B3p	Folliculitis, STH deficiency	None	CS (g), IFX (nt)	Colostomy, bowel resection (3 mo, p), colectomy (33 mo, p)
4[a]	M	Caucasian, Germany	Yes	7 mo	*IL10RA,* Ex 3: c.C251T, p.Thr84Ile	13	L2, B1p	Folliculitis	PN (p)	AZA (p), CS (p), CsA (p), IFX (n), MTX (n)	None
5	M	Turkish, Turkey	Yes	7 y 9 mo	*IL10RA,* Ex 3: c.C301T, p.Arg101Trp	7	L2, B2B3p	Folliculitis	None	AZA (n), CS (n)	Colectomy (44 mo, p)
6	M	Kurdish-Turkish, East Turkey	Yes	4 y 11 mo	*IL10RB,* Ex 4: c.G477A, p.Trp159X	60	L3L4a, B2B3p	Autoimmune hepatitis, folliculitis	EN (p)	ADA (p), AZA (nt), CS (p), IFX (nt), TAC (p)	Colostomy, bowel resection (28 mo, p)
7	M	Latin American, Brazil	No	2 y 2 mo	*IL10RA,* Ex 2: c.A170A/G, p.Tyr57Tyr/Cys *IL10RA,* Ex 3: c.C349C/T,p.Arg117Arg/Cys	41	L2, B1p	Arthralgia/arthritis (lower limbs), Kawasaki disease	None	CS (g), IFX (nt), MP (g), MTX (n)	None
8	F	South Asian, Bangladesh	Yes	9 mo	*IL10RB,* Ex 3: c.G197A, p.Cys66Tyr	45	L2, B1p	Folliculitis	EN (p), PN (p)	ADA (n), mesalamine (n), AZA (n), CS (p), IFX (n), TAC (n)	Ileostomy (6 mo, p)
9	M	Arab, Israel	Yes	6 mo	*IL10RB,* 3'UTR: c.*C52T	90	L2, B1p	Arthritis (large joints), folliculitis	PN (g)	Mesalamine (n), AZA (n), CS (p), ETN (g), IFX (nt), MP (n), MTX (n)	Ileostomy (6 mo, g)
10	F	Caucasian, Germany	No	1 y 5 mo	*IL10RB,* Ex 5: c.G611G/A, p.Trp204Trp/X *IL10RB,* Ex 6; c.C689C/A, p.Ser230Ser/X	21	L2L4a, B1p	Dermatitis, folliculitis	PN (g)	Mesalamine (n), (n), AZA (n), CS (p), IFX (p)	None
11	F	Caucasian, Poland	Unknown	5 y 4 mo	*IL10RB,* Ex 5: c.G611A, p.Trp204X	29	L2L4a, B3p	Folliculitis	PN (p)	Mesalamine (n), (n), AZA (p), CS (n), IFX (g)	Ileostomy (16 mo, p)
12	M	Black, Pacific Island	Yes	11 y 3 mo	*IL10RA,* Ex 4: c.T506C, p.Ile169Thr	7	L3L4a, B2B3p	Atopic dermatitis, folliculitis	EN (p), PN (p)	ADA (n), AZA (n), CS (n), IFX (n), MP (p), MTX (n)	PEG/ileostomy/colostomy (1 y, p), stricture-plasty (11 y), rectal stricture dilatations (12 y)
13[e]	M	Arab, Kuwait	Yes		*IL10,* Ex 5: c.G458A; p.Gly153Asp	26	L2, B1p	None	None	Mesalamine (n), (g), CS (g)	None
14	M	Arab, Kuwait	Yes	11 y 10 mo	*IL10,* Ex 5: c.G458A; p.Gly153Asp	31	L2, B1p	None	None	Mesalamine (n), (p), CS (g)	None
15	M	Arab, Kuwait	Yes	4 y 10 mo	*IL10,* Ex 5: c.G458A; p.Gly153Asp	34	L2, B3p	None	None	Mesalamine (n), (p), CS (g), IFX (g), MTX (p)	Fistula/abscesses
16[f]	M	Turkish, Turkey	Yes	2 y 1 mo	*IL10RB, c.331+907_574del*	65	L2L4a, B2B3p	Folliculitis	PN (p)	AZA (p), CS (g), IFX (p), CS (p)	Colostomy (g)

ADA, adalimumab; AZA, azathioprine; CS, corticosteroids; CsA, ciclosporin; EN, enteral nutrition; ETN, etanercept; F, female; g, good; IFX, infliximab; M, male; MP, mercaptopurine; MTX, methotrexate; n, none; nt, not tolerated; p, partial; PN, parenteral nutrition; TAC, tacrolimus.

[a]Patients have been reported previously by Glocker et al.[12]

[b]Diagnosis is defined as IL-10 or IL-10R deficiency.

[c]Defined as first symptoms of IBD.

[d]According to the Paris classification.[16]

[e]Patient died due to traffic accident.

[f]Patient died due to sepsis.

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folliculitis (11 of 16), and arthritis (4 of 16). Of note, all 16 patients had perianal disease. Immunological workup revealed normal numbers of B and T cells and normal proliferative responses of T cells. Analysis of serum immunoglobulins revealed in some patients elevated IgG, IgA, and IgM levels, but IgE levels were within normal range. All IL-10R−deficient patients developed specific antibodies in response to vaccination and displayed normal neutrophil reduced nicotinamide adenine dinucleotide phosphate-oxidase activity (data not shown). Colonoscopy demonstrated moderate to severe discontinuous colitis with deep ulcerations and pseudopolyps (Figure 1*A*). Histological analysis of colonic biopsies revealed unspecific acute colitis with multiple ulcerations and dense polymorphic infiltrations (Figure 1*B, C*), reminiscent of Crohn's disease. All patients had multimodal therapy, including exclusive enteral nutrition, anti-inflammatory and/or immunomodulatory agents. Most patients underwent ileostomy, colostomy, and/or surgical intestinal resections. Despite complex multimodal therapy approaches, none of the patients had a sustained response. Detailed information on demographics, clinical phenotype, and treatment of individual IL-10/IL-10R−deficient patients is provided in Table 1.

We assessed the functional consequences of the mutations and assayed IL-10R−dependent signaling in primary PBMCs from 10 patients. As shown in Figure 1*D* and Supplementary Figure 2*A*, deleterious mutations in *IL10R* genes abrogate IL-10−mediated signaling resulting in deficient phosphorylation of signal transducer and activator of transcription 3 at the Tyr705 residue, an essential downstream target for the physiological anti-inflammatory response of IL-10. As a consequence, IL-10R−deficient primary cells are unresponsive to IL-10−dependent negative feedback regulation and secrete high amounts of TNF-α upon costimulation with LPS and IL-10 (Figure 1*E, F* and Supplementary Figure 2*B*). To analyze the functional impact of the IL-10^{Gly153Asp} mutation, we quantified the inhibition of TNF-α release in LPS-stimulated PBMCs from healthy donors upon stimulation with lentivirally overexpressed and secreted wild-type or mutant IL-10 (Figure 2). Wild-type IL-10 induced suppression of TNF-α secretion comparable with recombinant human IL-10. In contrast, mutant IL-10 failed to exert anti-inflammatory effects.

Allogeneic HSCT

Based on our previous observation,[12] we performed allogeneic HSCT in 5 IL-10R−deficient patients unresponsive to immunosuppressive medication and surgical intervention. We used an immunosuppressive and myeloablative conditioning regimen consisting of alemtuzumab, fludarabine, treosulfan, and thiotepa (Figure 3). Ciclosporin and mycophenolate mofetil were administered for GVHD prophylaxis. Age at transplantation

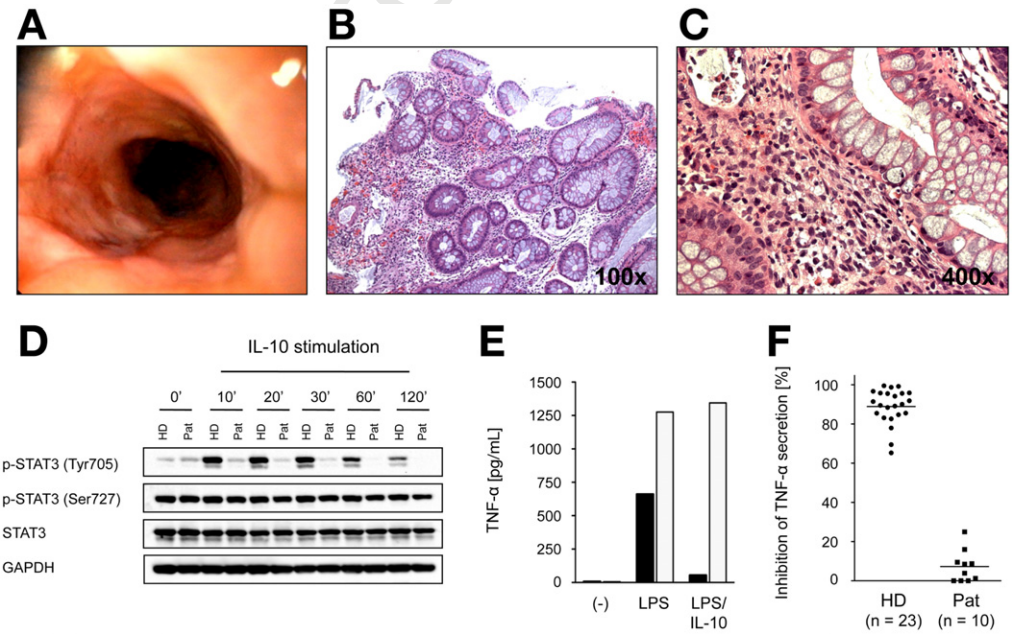


Figure 1. Clinical phenotype and defective IL-10-mediated signal transduction in IL-10R−deficient patients. (*A*) Representative colonoscopy image of patient 4 showing acute colitis accompanied by early cobblestone pattern and intermittent serpiginous ulcers with fibrin layers.(*B, C*) Histopathological analysis of colon biopsies of patient 4 revealed glandular distortion and a mild to moderate degree of inflammation with circumscribed and superficial mucosal defects. Infiltrate primarily consists of lymphocytes, macrophages, eosinophils, and polymorphonuclear granulocytes. (*D*) Representative Western blot analysis illustrating abrogated signal transducer and activator of transcription 3 phosphorylation at the Tyr705 residue in PBMCs of patient 4 upon stimulation with IL-10 (25 ng/mL) over time. Glyceraldehyde-3-phosphate dehydrogenase was used as loading control. (*E*) Enzyme-linked immunosorbent assay results demonstrating defective inhibition of TNF-α secretion in LPS-stimulated PBMCs upon costimulation with IL-10 in case of patient 4 (healthy donor [HD], *black bars*, Patient [Pat], *gray bars*). (*F*) Graphical representation of defective inhibition of TNF-α secretion in LPS-stimulated PBMCs from identified IL-10R−deficient patients upon costimulation with IL-10 (HD, n = 21; Pat, n = 9).

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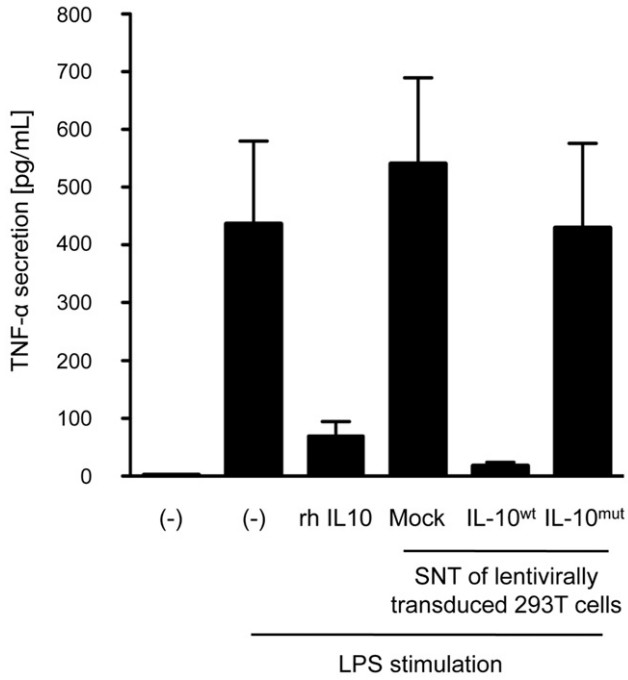


Figure 2. Defective anti-inflammatory function of mutated IL-10. To analyze the functional impact of the identified IL-10 mutation in patients 13, 14, and 15, we synthesized wild-type and mutant IL-10 (Gly153Asp) by lentiviral overexpression in 293T cells. Secreted IL-10 was quantified by enzyme-linked immunosorbent assay in supernatants of 293T cells (SNT) and the anti-inflammatory effect of in vitro synthesized IL-10 was assessed in LPS-stimulated healthy donor PBMCs.

ranged from 10 months to 13 years and 9 months with a median of 8 years and 3 months.

Further details of HSCT are summarized in Table 2. Patients 1 and 5 received hematopoietic stem cells from a matched sibling donor, and patients 3, 4, and 6 underwent HSCT from a matched unrelated donor. Donors were HLA-compatible in 10 of 10 (patients 1, 3, and 5) and 9 of 10 (patient 4 and 6) alleles, as determined by high-resolution molecular genotyping. Engraftment was achieved in all patients. However, patient 4 had graft rejection on day +80, patient 5 developed mixed chimerism (day +127, 93%), and patient 6 had a partial bone marrow failure. Donor-derived hematopoiesis post transplantation is displayed over time in Supplementary Figure 3. After rejection of first graft from a 9 of 10 matched unrelated donor, patient 4 was subjected to retransplantation from another 9 of 10 matched unrelated donor resulting in a prompt and durable engraftment. To prevent disease recurrence after the development of mixed hematopoietic chimerism patient 5 received one donor lymphocyte infusion (1×10^6 $CD3^{pos}$ cells/kg body weight, day +235) and the mixed chimerism remained stable (80%–82%, day +248 to 416) without any overt clinical symptoms. Furthermore, no hospitalization was required since day 60 after HSCT. Antiviral ganciclovir or cidofovir therapy of human herpes virus 6 infection in patient 6 was associated with partial bone marrow suppression resulting in red blood cell and platelet deficiency. He was treated with a stem cell boost on day +260 and recovered with his bone marrow leading to substantial clinical improvement of colitis.

All transplanted patients had viral infections or reactivations after HSCT (cytomegalovirus, adenovirus, rotavirus, human herpes virus 6) and some required antiviral treatment (cidofovir, ganciclovir, foscarnet, and aciclovir). Viral infections could be controlled in all patients without any long-term side effects. Patients 1, 4, and 6 developed acute GVHD responding to a short-term course of prednisone therapy. Patient 4 developed limited chronic skin GVHD without sclerosis or ulcers requiring treatment with steroids and tacrolimus.

To date, 4 patients achieved fast and sustained complete clinical remission at a median follow-up of 2 years after HSCT. Improvement of the clinical status was furthermore evidenced by ameliorated wound healing, accelerated growth, and reduced need for hospitalization. Importantly, colonoscopy (Figure 4*A*) and histopathological examination (Figure 4*B, C*) of ileal and colonic biopsies revealed no evidence of active inflammation at 6 months after HSCT. Furthermore, functional studies on PBMCs isolated from transplanted IL-10R–deficient patients showed phosphorylation of signal transducer and activator of transcription 3 at Tyr705 upon stimulation with IL-10 comparable to healthy donors (Figure 4*D* and Supplementary Figure 4*A*). In addition, we could detect a

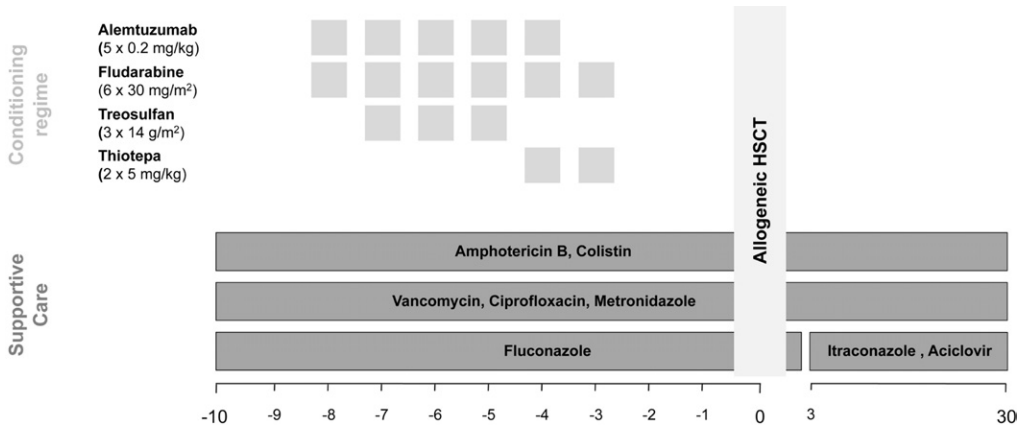


Figure 3. Hematopoietic stem cell transplantation (HSCT) in IL-10R–deficient patients.Scheme of the conditioning regimen and supportive care for HSCT. In addition, patients received standard care for HSCT following guidelines of *The European Group for Blood & Marrow Transplantation*.

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Table 2. Details of HSCT (Donor, Cell Source) and Clinical Course of Transplanted IL-10R−Deficient Patients

Patient no.	Age at HSCT	HSCT source		Engraftment			Complications			Clinical outcomes
		Donor	Cell source, *cells/kg*	WBC (>1000/μL)	ANC (> 500/μL)	PLT (>50,000/μL)	Chimerism	GVHD	Others	
1	9 y 11 mo	10/10 MSD	7×10^8 BMC	d+23	d+21	d+25	100 (d+721)	skin III°	Adenovirus	Clinical remission
3	13 y 9 mo	10/10 MUD	12.5×10^8 BMC	d+23	d+24	d+28	100 (d+537)	None	Bacillus sp, CMV	Clinical remission
4	10 mo	9/10 MUD	10×10^8 BMC	d+24	d+26	d+26		None	Graft rejection (d+86)	
	1 y 4 mo	9/10 MUD	8.5×10^8 BMC	d+13	d+13	d+32	100 (d+362)	Skin III°, gut II°, chronic skin (lim)	Rotavirus, SVC thrombosis	Clinical remission
5	8 y 3 mo	10/10 MSD	3.4×10^8 BMC	d+25	d+21	d+20	80 (d+375); DLI (d+235)	None	Rotavirus	Clinical remission
6	5 y 6 mo	9/10 MUD	6.7×10^5 PBSC	d+11	d+12	d+15	100 (d+250), SC-Boost (d+260)	skin I−II°	CMV, adenovirus, BK virus cystitis, systemic HHV-6 infection (encephalitis, pericarditis), partial BM failure (d+260)	Thriving, colitis improved, fistula not yet healed

ANC, absolute neutrophil count; BMC, bone marrow cells; CMV, cytomegalovirus; DLI, donor lymphocyte infusion; HHV-6, human herpes virus 6; MSD, matched sibling donor; MUD, matched unrelated donor; PLT, platelets; PBSC, peripheral blood stem cells; SC, stem cells; SVC, XXX; WBC, white blood cells.

substantial inhibition of TNF-α secretion in LPS/IL-10−costimulated PBMCs from transplanted patients (Figure 4*E, F* and Supplementary Figure 4*B*). Taken together, the clinical data and functional studies suggest reconstitution of the IL-10R−mediated signal transduction in IL-10R−deficient patients after allogeneic HSCT.

Discussion

We here provide a systematic study on the genetic etiology and a tailored therapy approach using allogeneic HSCT in children with IL-10R deficiency. Analyzing 66 early onset IBD patients, we identified 16 patients with loss-of-function mutations in *IL10* or *IL10R* genes. A variety of mutations were discovered. Most patients were born to consanguineous parents and had homozygous biallelic mutations (point mutations or deletions). However, some patients also presented with compound heterozygous mutations.

Clinically, all patients with documented IL-10 or IL-10R deficiency presented in their first 3 months of life. However, our results do not exclude that patients with first presentation of symptoms later during infancy or early childhood may have defects in the IL-10−mediated signaling. Our study suggests that patients with clinical history of severe refractory course of infantile IBD should be genotyped for mutations in *IL10* and *IL10R* genes. We could not observe clear genotype−phenotype correlations in our cohort of IL-10- and IL-10R−deficient patients. For example, patient 1 and patient 2 (siblings sharing the same homozygous *IL10RB* mutation) had a remarkably distinct level of disease severity, suggesting that the phenotypic manifestation is dependent on other intrinsic or extrinsic factors that remain presently unknown.

Very-early-onset IBD represents a rare variant within the pediatric IBD population. In a prospective European registry including 2054 unselected newly diagnosed pediatric IBD patients (EuroKids[19]), only 21 (1%) of 2022 informative patients manifested their bowel disease during infancy and, of those, only 5 (0.25%) within the first 3 months of life (de Bie CL & Escher JC, personal communication). Our cohort of 66 patients is prone to bias with respect to ethnic background and predominance of genetic factors in the pathogenesis of IBD. Nevertheless, mutations in IL-10−related genes were only discovered in approximately one third of the patients with onset in the first year of life, indicating that infantile IBD is heterogeneous. Other monogenetic conditions associated with IBD must be taken into consideration. For example, mutations in *XIAP,*[20,21] *NCF1 (p47-phox),*[22] and *PIK3R1*[23] can present with early onset IBD. We expect that additional monogenetic defects contributing to the pathogenesis of early-onset IBD will be discovered in the near future.

Current therapeutic strategies for pediatric IBD include the use of exclusive enteral nutrition, corticosteroids, mesalamine, sulfasalazine, immunomodulators (azathioprine, 6-mercaptopurine, methotrexate), and anti−TNF-α antibodies.[24] Knowledge of the underlying genetic etiol-

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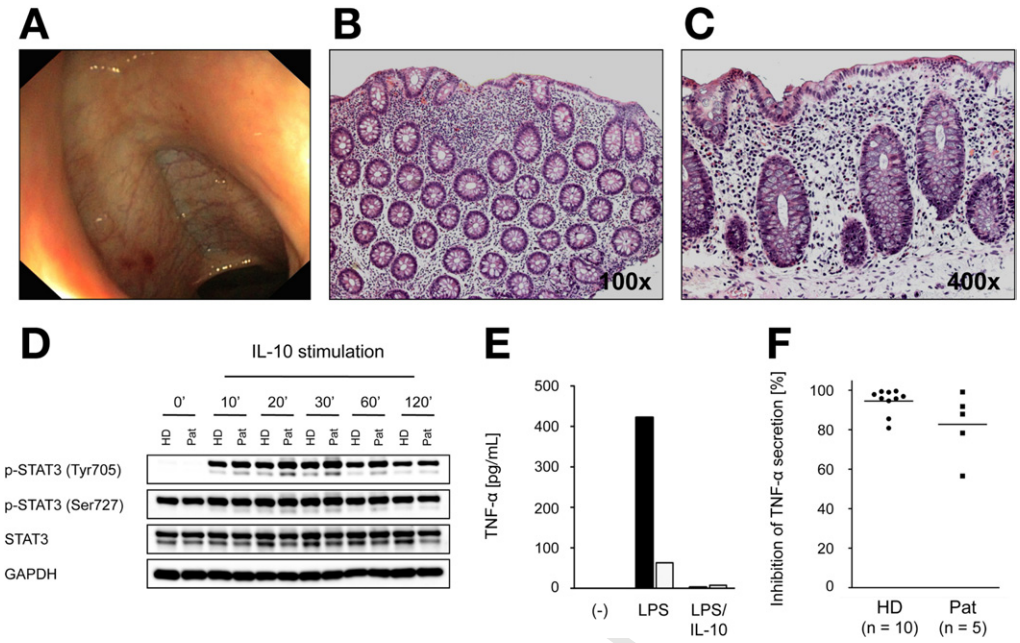


Figure 4. Clinical phenotype and reconstitution of the IL-10–mediated signal transduction in IL-10–deficient patients after allogeneic HSCT. (*A*) Representative colonoscopy of patient 4 demonstrates normal intestinal mucosa without evidence of inflammatory processes 13 months after HSCT. (*B, C*) Histopathological examination of colon biopsies after 13 months of HSCT revealed almost complete reduction of glandular distortion and an inconspicuous, sparse leukocytic infiltration within the lamina propria mucosa. (*D*) Representative Western blot analysis of signal transducer and activator of transcription 3 phosphorylation at Tyr705 residue demonstrating reconstitution of IL-10R–mediated signal transduction in patient 4 following 1 year of HSCT. (*E*) Representative enzyme-linked immunosorbent assay showing substantial inhibition of TNF-α secretion in LPS-stimulated PBMCs upon costimulation with IL-10 in patient 4 after 1 year of HSCT. Healthy donor, *black bars*; patient, *gray bars*. (*F*) Grouped column scatter graph showing reconstituted inhibition of TNF-α secretion in PBMCs from the cohort of transplanted IL-10R–deficient patients upon stimulation with LPS and IL-10.

ogy may open novel horizons for rational therapeutic strategies. Proof-of-principle for allogeneic HSCT has been provided in one patient with IL-10R deficiency.[12] In previous sporadic cases, allogeneic HSCT has been performed in adult IBD patients and favorable outcomes with respect to IBD were reported.[25–36] Thapar et al showed that bone marrow transplantation can be used for the treatment of intractable ulcerating enterocolitis of infants with undefined genetic defects.[37] Although our study suggests that allogeneic HSCT represents a causal therapeutic approach for IL-10R–deficient patients, a word of caution is warranted. Early-onset IBD might also be caused by dysfunction of nonhematopoietic cells, which would obviously not be corrected by allogeneic HSCT. Our experience and the data in the published literature should not be used as proof-of-concept for all early-onset IBD patients. A cautious attitude is supported by experimental studies in mice. Using a random mutagenesis screen, Brandl et al showed that MyD88/Trif-deficient mice are highly susceptible to dextran sulfate sodium-induced colitis.[38] Because MyD88 and Trif represent Toll-like receptor adaptor proteins, one could argue that MyD88-dependent signaling controls innate immune cells and that an allogeneic HSCT may correct this defect. However, mutant mice subjected to allogeneic HSCT had a marked aggravation of their clinical colitis phenotype and died much earlier than control animals.[38] This experimental mouse study exemplifies that the identification of a classical pathway controlling immune cells does not necessarily imply that an allogeneic HSCT approach is a promising therapeutic strategy. Disciplined and well-designed animal studies might be needed to determine whether defined monogenetic defects in humans with early onset IBD can be cured by allogeneic HSCT. HSCT should only be considered in the context of controlled clinical studies or on the basis of defined genetic mutations, such as in patients with IL-10R deficiency. Our study used a highly immunosuppressive conditioning regimen, including in vivo deletion of dendritic cells, in conjunction with intense gut decolonization. This strategy was chosen because, in contrast to patients with classical immunodeficiency disorders, IL-10R deficiency is characterized by hyperinflammation. Increased levels of inflammatory cytokines, such as TNF-α, have been shown to correlate with the severity of acute GVHD after HSCT.[39] Similar to many other rare diseases, it is very difficult to provide empiric evidence for or against certain constituents of the conditioning regimen. Future studies might help to refine transplantation procedures.

In summary, our study highlights that IL-10 and IL-10R deficiencies represent a defined subgroup of pediatric IBD patients, manifesting within the first 3 months of life. This condition is characterized by a severe and progressive course of colitis associated with perianal disease and variable extraintestinal manifestations, such as folliculitis and arthritis. We conclude that infantile IBD pa-

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tients with perianal disease should be genotyped for mutations in *IL10* and *IL10R* genes. Furthermore, our results suggest that allogeneic HSCT can induce sustained remission of IL-10R deficiency without undue toxicities or severe GVHD.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.04.045.

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Drs Kotlarz, Beier, and Murugan contributed equally to this work and should be considered *aequo loco*.

Conflicts of interest

The authors disclose no conflicts.

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