Clinical outcome in IL-10– and IL-10 receptor–deficient patients with or without hematopoietic stem cell transplantation

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Background: Inherited deficiencies of IL-10 or IL-10 receptor (IL-10R) lead to immune dysregulation with life-threatening early-onset enterocolitis.

Objectives: We sought to gather clinical data of IL-10/IL-10R–deficient patients and devise guidelines for diagnosis and management, including hematopoietic stem cell transplantation (HSCT).

Methods: We enrolled 40 patients with early-onset enterocolitis and screened for mutations in *IL10/IL10R* using genetic studies, functional studies, or both of the IL-10 signaling pathway. Medical records of IL-10/IL-10R–deficient patients were reviewed and compiled.

Results: Of 40 patients, we identified 7 with novel mutations, predominantly in consanguineous families with more than 1 affected member. IL-10/IL-10R–deficient patients had intractable enterocolitis, perianal disease, and fistula formation. HSCT was carried out in 2 patients with IL-10 deficiency and 1 patient with IL-10R α chain deficiency and proved to be an effective therapy, leading to rapid improvement of clinical symptoms and quality of life.

Conclusion: Because the defect in patients with IL-10/IL-10R deficiency resides in hematopoietic lineage cells and their colitis is resistant to standard immunosuppressive therapy, HSCT should be considered early as a potentially curative therapeutic option. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: *IL-10, IL-10 receptor, enterocolitis, mutations, colectomy, hematopoietic stem cell transplantation*

IL-10 and IL-10 receptor (IL-10R) deficiencies, which are caused by loss-of-function mutations in the genes encoding IL-10 or IL-10R, are primary immunodeficiencies that result in severe dysregulation of the immune system.[1,2] Affected patients primarily present early in life with inflammatory bowel disease (IBD). Perianal disease is particularly severe, but additional clinical features, including chronic folliculitis, recurrent respiratory diseases, and arthritis, have been described.[1-5] The severity of the inflammation and the formation of multiple abscesses, anal fissures, and fistulae, such as enterocutaneous and rectovaginal fistulae, frequently require surgical interventions and sometimes partial or total colectomy. On histopathology, ulcers of the intestinal mucosa with inflammatory infiltrates of the epithelium and abscesses are often found. Remarkably, IL-10– and IL-10R–deficient patients are typically unresponsive to immunosuppressive therapies with corticosteroids, methotrexate, thalidomide, and even anti–TNF-α mAbs.[1-5]

In view of the life-threatening nature of the IBD, its poor long-term prospects, and its resistance to conventional immunosuppressive therapies, allogeneic hematopoietic stem cell transplantation (HSCT) has been performed in IL-10/IL-10R–deficient patients, leading to sustained remission.[1,2,4]

IL-10 and IL-10R deficiencies are rare, with only a few cases published.[1-6] Here we summarize the medical histories and gene mutations of 7 new patients with IL-10R defects and 2 previously described patients with IL-10 deficiency[2] and discuss

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Abbreviations used
CMV: Cytomegalovirus
FOXP3: Forkhead box protein 3
HSCT: Hematopoietic stem cell transplantation
IBD: Inflammatory bowel disease
IL-10R: IL-10 receptor
IL-10R1: IL-10 receptor α chain
IL-10R2: IL-10 receptor β chain
NEMO: Nuclear factor κB essential modulator
NK: Natural killer
XIAP: X-linked inhibitor of apoptosis protein

the natural history and clinical outcome in patients with and without HSCT.

METHODS

Patients

We enrolled a total of 40 patients in this study with informed consent and approval by the responsible local ethics committees. The study patients represented a subgroup of pediatric patients with IBD in whom another defined primary immunodeficiency had been ruled out and who presented with onset of severe endoscopically and histopathologically confirmed colitis within the first 4 years of life. All had resistance to immunosuppressive therapy. All but one of the patients' families had possible or confirmed consanguinity or ethnic origin from a population with a high rate of consanguinity. By applying these criteria, we identified mutations in 1 patient (of 9) at Great Ormond Street Hospital London, London, United Kingdom; 1 patient (of 1) at the Department of Immunology, Royal Free Hospital London, London, United Kingdom; 2 patients (of 4) at the Department of Pediatrics, University of California San Francisco, San Francisco, Calif; 1 patient (of 2) at Akdeniz University School, Antalya, Turkey; 1 patient (of 21) at The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; and 1 patient (of 3) at the Department of Pediatric Immunology, Royal Manchester Children's Hospital, Manchester, United Kingdom. Patients were tested for the integrity of the IL-10 signaling pathways by carrying out functional assays, cell stimulation experiments, and Sanger genomic DNA sequencing, as described below. Details on medical history and clinical outcome with or without HSCT were gathered in 7 patients who harbored novel mutations in the IL-10R–encoding genes. In addition to these newly identified patients, we include follow-up of 2 previously published patients with loss-of-function mutations in *IL10* who underwent HSCT.[2]

In patients with regular IL-10 signaling, chronic granulomatous disease was ruled out based on a negative nitroblue tetrazolium dye reduction result or a flow-based neutrophil oxidative index test result. Other monogenic diseases associated with colitis, such as X-linked inhibitor of apoptosis protein (XIAP) deficiency, nuclear factor κB essential modulator (NEMO) deficiency, and immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (mutations in *XIAP2*, *NEMO*, and forkhead box protein 3 *[FOXP3]*, respectively), were ruled out by clinical examination, immune cell staining, or gene sequencing. Patients were not genotyped for polymorphisms/mutations in nucleotide-binding oligomerization domain protein 2 *(NOD2)*.

Immune cell phenotyping

T- and B-cell staining was performed in accordance with protocols published previously.[7,8] In brief, EDTA blood was collected, and PBMCs were isolated by using Lymphoprep (Axis-Shield, Oslo, Norway). PBMCs were stained with fluorochrome-labeled antibodies directed against CD3, CD4, CD8, CD16, CD56, and CD19 and subsequently analyzed by using fluorescence-activated cell sorting.

TNF-α ELISA

Commercially available TNF-α ELISA development kits (PeproTech, Hamburg, Germany) were used to detect TNF-α in cellular supernatants. In brief, 5×10^6 Ficoll-separated and purified PBMCs were stimulated overnight with 50 ng/mL *Escherichia coli* LPS (Sigma-Aldrich, St Louis, Mo) alone or in combination with recombinant human IL-10 (R&D Systems, Abingdon, United Kingdom) at a concentration of 20 ng/mL. Supernatants were analyzed in triplicate with a Tecan Sunrise ELISA microplate reader (Tecan, Mainz-Kastel, Germany).

DNA extraction, PCR, and sequencing

Isolation of DNA, PCR, and sequencing analysis was performed as previously described.[1] Primers (Life Technologies, Paisley, United Kingdom) used are listed in Table E1 in this article's Online Repository at www.jacionline.org. Mutations are reported by using published conventions.[9]

RESULTS

Patients with mutations affecting IL-10 and IL-10R

In our cohort of 40 new patients selected as previously described, 7 (18%) had molecular defects in IL-10 signaling, all with mutations in *IL10RA* or *IL10RB*, the genes encoding the IL-10R components IL-10 receptor α chain (IL-10R1) and IL-10 receptor β chain (IL-10R2), respectively (Table I).[2]

Patient 1 showed a homozygous point mutation in exon 3 of *IL10RA* (c.350G>A) leading to an amino acid exchange from arginine to histidine at position 117 (Arg117His) in the IL-10R1 protein. Patient 2 revealed a homozygous splice site mutation in *IL10RB* at nucleotide IVS3+1G>C, which resulted in skipping of exon 3, a frame shift, and a predicted truncated IL-10R2 protein with a stop codon at amino acid position 72 (Leu59fsX72). Patient 3 harbored a homozygous 1-nucleotide deletion in exon 2 of *IL10RB* (c.53delT), which led to a frame shift and premature stop codon at amino acid position 29 (Trp18fsX29) of the IL-10R2 protein. Patient 4 had a homozygous mutation in exon 5 of *IL10RB* (c.577 G>C), resulting in an amino acid exchange from glycine to arginine at position 193 (Gly193Arg) in IL-10R2. In patient 5 we detected a large homozygous deletion of unknown size spanning exons 1 to 3 in *IL10RA* (EX1_3del, data not shown). Patient 6 was compound heterozygous with a point mutation in exon 2 of *IL10RA* (c.170A>G) in 1 allele, leading to a replacement of tyrosine by cysteine at position 57 (Tyr57Cys), and a 5.5-kb deletion, including exons 2, 3, and 4 of *IL10RA*, in the other allele (EX2_4del), leading to a frame shift and a predicted truncated IL-10R1 protein (Val23fsX31). Patient 7 carried a homozygous point mutation (c.374T>G) in exon 4 of *IL10RA*, resulting in an amino acid exchange from leucine to arginine at position 125 (Leu125Arg) in IL-10R1 (Table I and see Figs E1 and E2 in this article's Online Repository at www.jacionline.org).

The possible effect of the detected amino acid alterations was assessed by using the online tool Polymorphism Phenotyping (Polyphen; http://genetics.bwh.harvard.edu/pph/)[1,2,4,10]: all mutations were predicted to be probably damaging. In contrast to healthy control subjects, PBMCs from 5 of our 7 patients with *IL10RA*/*IL10RB* mutations did not show any IL-10–mediated inhibition of LPS-mediated TNF-α release (see Fig E3 in this article's Online Repository at www.jacionline.org). These results confirmed that the detected mutations led to loss of function. Functional tests were not carried out in 2 patients: patient 4 had already died, and the parents of patient 5 did not consent to have their child's PBMCs tested.

TABLE I. Ethnicity, genetic information, parentage, family history, and summary of immunologic abnormalities

Patient no.	Ethnicity	Mutation	Parentage and family history	Immunologic abnormalities
1	Indian	IL-10R2: Arg117His (homozygous)	Consanguinity; brother with fatal severe enteropathy	Increased immunoglobulin levels; decreased CD4/CD8 T-cell ratio; increased T-cell percentages; low B-cell and NK cell numbers
2	Arabic	IL-10R2: splice site mutation at exon/intron 3 boundary (homozygous); results in skipped exon 3 and truncated protein: Leu59fsX72	Consanguinity; 2 siblings and uncle with fatal colitis	High IgA and IgG levels; normal T-cell, B-cell, and NK cell subsets
3	Pakistani	IL-10R2: 1 nucleotide deletion in exon 2 (homozygous); results in truncated protein: Trp18fsX29	Suspected consanguinity; no family history	Increased IgA levels; normal T-cell, B-cell, and NK cell subsets
4	Turkish	IL-10R2: Gly193Arg (homozygous)	Consanguinity; 2 siblings with fatal colitis	Not determined
5	Arabic	IL-10R1: exons 1, 2, and 3 deleted (Ex1_3del; homozygous)	Consanguinity; cousin with neonatal diarrhea, died age 8 mo	Low IgG levels (requiring IVIG); increased $CD3^+$ T-cell percentages; low B-cell percentage; reduced response to mitogens
6	White	IL-10R1: Tyr57Cys (in allele 1)/IL-10R1: Val23fsX31 (in allele 2; compound heterozygous)	No consanguinity; no family history	Increased IgA levels; normal T-cell, B-cell, and NK cell subsets
7	Pakistani	IL-10R1: Leu125Arg (homozygous)	Consanguinity; no family history	Before BMT: low IgG levels (requiring IVIG); high T-cell numbers After BMT: reduced response to mitogens; normal T-cell, B-cell, and NK cell subsets
8	Pakistani	IL-10: Gly113Arg (homozygous)	Consanguinity; aunt died in infancy with diarrhea and severe perianal rash	Before BMT: decreased CD4/CD8 ratio; high $CD8^+$ T-cell percentage After BMT: decreased CD4/CD8 ratio; high IgG levels; low $CD3^+/CD4^+$ T- and B-cell numbers; impaired response to mitogens
9	Pakistani	IL-10: Gly113Arg (homozygous)	Consanguinity; 1 sister with fatal colitis	Before BMT: increased IgM and IgA levels After BMT: decreased CD4/CD8 ratio; high T-cell numbers

For details, see Table E2. Patients 8 and 9 have been described in detail by Glocker et al.[2]
BMT, Bone marrow transplantation; *IVIG*, intravenous immunoglobulin.

Previously described patients 8 and 9 harbored an identical homozygous point mutation in exon 3 of the IL-10 gene (c.337 G>A), resulting in an amino acid replacement from glycine to arginine at position 113 (Gly113Arg).[2] Mutant IL-10 did not inhibit LPS-mediated TNF-α release in PBMCs when compared with wild-type IL-10 (see Figs E1 and E3).[2]

Patients with early-onset enterocolitis and wild-type *IL10/IL10R*

Within the subgroup of pediatric patients with IBD with onset of disease at less than 4 years and severe disease course, we compared the clinical course of patients without IL-10/IL-10R deficiency with that of patients with IL-10/IL-10R deficiency. Several patients had a similarly severe course of disease and also were from consanguineous families. However, the majority of these 33 patients had a later onset beyond 12 months and were more responsive to anti-inflammatory drugs.

Immunologic features of patients with mutations in *IL10* or *IL10R*

The immunologic work-up of our IL-10– and IL-10R–deficient patients revealed various minor abnormalities, including decreased $CD4^+/CD8^+$ T-cell ratios, increased or decreased serum immunoglobulin levels (in some cases low enough to require immunoglobulin substitution), and low or increased numbers of natural killer (NK), B, or T cells (summarized in Table I and Table E2[11] in this article's Online Repository at www.jacionline.org). Some patients presented with recurrent infections and complications, such as oral ulcers and EBV-positive lymphoma.

Patients who did not undergo HSCT

Six of our 9 patients aged between 2 weeks and 25 years had not received HSCT as of this writing (summarized in Table II). The patients were of Pakistani, Arab, Indian, Turkish, and white origin; were primarily born to consanguineous parents; and often had a positive family history (Table I and see Fig E4 in this article's Online Repository at www.jacionline.org). In all patients an IBD phenotype predominated with perianal disease that was refractory to any immunosuppressive therapy, including azathioprine, steroids, and anti–TNF-α antibodies. Apart from patient 4, who died at age 40 days, all patients underwent surgical interventions, including partial or subtotal colectomy or stoma formation, in an attempt

TABLE II. Clinical information: sex, age of onset, age of transplantation and current age, major clinical findings, complications, treatment, and follow-up in our IL-10/IL-10R–deficient patients

Patient no.	Sex	Age of onset	Age of transplantation; current age	Major clinical findings	Complications	Immunosuppression/ surgical therapy	Follow-up
1	Female	3 y, 6 mo	No transplantation; 16 y	Severe colitis, therapy resistant Recurrent ear infections, chesty Oral ulcers	Toxic megacolon Bowel perforation and peritonitis	Steroids, azathioprine, infliximab, cyclosporine A Colectomy at the age of 3 y	Ongoing colitis, no remission
2	Female	1 mo	No transplanation; 25 y	Severe colitis, therapy resistant Frequent bronchitis in childhood Oral ulcers Gingivitis	Lymphadenopathy Hepatosplenomegaly Bilateral hydronephrosis EBV-lymphoma	Steroids, cyclosporine A Colectomy at the age of 5 mo	Ongoing colitis, no remission
3	Female	1 mo	No transplantation; 12 y	Severe colitis, therapy resistant Recurrent infections of the ear	EBV-lymphoma Hearing loss Anal stenosis	Steroids, azathioprine; colectomy at the age of 10 mo	Ongoing colitis, no remission
4	Male	10 d	No transplantation; died age 40 d	Severe colitis Life-threatening bacterial infections	Intestinal adhesions Died of septicemia	No immunosuppression/ no surgery	Patient died
5	Male	2 mo	No transplantation; 1 y	Severe colitis Severe otitis media/ urinary tract infections (*Pseudomonas aeruginosa*, *Enterobacter cloacae*) Enteritis (rotavirus/ adenovirus)	*Candida* species–induced septicemia	Prednisone, azathioprine	Ongoing colitis, no remission
6	Male	3 mo	No transplantation; 2 y	Severe colitis, therapy resistant Sinusitis	Colon perforation, ileostomy Ileostomy revision *Clostridium difficile* after antibiotics	Steroids, azathioprine, methotrexate, infliximab Ileostomy	Ongoing colitis, no remission; awaiting transplantation
7	Female	1 mo	Transplantation at age 3 y, 2 mo; now 4 y, 6 mo	Severe colitis, therapy resistant Recurrent infections: otitis media, urinary tract infections	Allergic reaction on infliximab Pancreatitis Several line sepsis with gram-negative rods and *Candida* species after BMT: *Pseudomonas* bacteremia CMV reactivation	Prednisone, infliximab	Colitis resolved
8	Female	1 mo	Transplantation at age 3 y, 11 mo; now 5 y, 7 mo	Severe colitis, therapy resistant	Moderate hearing loss High-output stoma after BMT: RSV infection, CMV and EBV infection/reactivation	Steroids, infliximab, adalimumab Colectomy at age 16 mo	Colitis resolved
9	Male	1 mo	Transplantation at age 1 y, 2 mo; now 2 y, 11 mo	Severe colitis, therapy resistant	*Klebsiella* species line sepsis	Steroids, azathioprine	Colitis resolved

Patients 8 and 9 have been described in detail by Glocker et al.[2]
BMT, Bone marrow transplantation; *RSV*, respiratory syncytial virus.

to control the progressive disease. However, complete remission was not achieved in any of the patients. The disease manifested very early within the first 2 months of life, except for patient 1, now a 16-year-old girl, who presented with frequent episodes of diarrhea with blood and mucus when aged 3 years, 6 months.

Patients who received HSCT

Three of 9 patients underwent HSCT (summarized in Table II). They were all Pakistani and born to consanguineous parents; both IL-10–deficient patients had positive family histories (see Fig E4).

Patient 7, a now 4½-year-old girl, was first hospitalized at 4 months of age with poor weight gain, low-grade fever, thrush, diarrhea, and buttock abscesses. At 10 months, endoscopy showed severe colitis with ulceration and multiple enteric and soft tissue fistulae (including rectovaginal fistula), leading to a diagnosis of Crohn disease. Despite treatments with high-dose prednisone, azathioprine (which caused pancreatitis), methotrexate, and infliximab (to which she was allergic with hives), she never achieved remission and required total parenteral nutrition on a continuous basis with several episodes of gram-negative rod and *Candida* species line sepsis. The birth of an unaffected, HLA-matched younger sibling made treatment by HSCT possible. She received a conditioning regimen, including busulfan, fludarabine, and rabbit anti-thymocyte globulin, when she was 3 years and 2 months old. Within 2 months of HSCT, despite an episode of cytomegalovirus (CMV) reactivation and *Pseudomonas aeruginosa*–induced sepsis, she had formed stools for the first time and was able to stop total parenteral nutrition. By 1 year after HSCT, she had greater than 90% stable donor engraftment and was in excellent health on oral nutrition with weaning of overnight nasogastric supplementation. Her fistulae healed, and her weight was at 25% for age; she had normal T- and B-cell numbers, greater than 50% of normal PHA response, and positive isohemagglutinin values (Table I and see Table E2). She had protective antibodies against tetanus (0.23 IU/mL) and polio types 1, 2, and 3 (1:128 each) after revaccination.

IL-10–deficient patients 8 and 9 have been described previously.[2] In brief, patient 8 presented at the age of 3 months with severe, chronic, active ulcerating enteritis; perianal and rectovaginal fistulae; and oral ulcers. Immunosuppression, including steroids, anti–TNF-α mAbs (infliximab and adalimumab), and methotrexate, did not induce remission, and colectomy was carried out at the age of 16 months. At age 3 years and 6 months, an undefined hearing loss was diagnosed. After her paternal grandfather was identified as a suitable donor and she had received a conditioning regimen, including alemtuzumab, fludarabine, and melphalan, HSCT was carried out at the age of 3 years and 11 months. She had prolonged admissions because of complications of high stomal output and had respiratory syncytial virus, CMV, and EBV infections/reactivation in the post-HSCT period (Table II); the CMV infection was controlled by administering ganciclovir, and the EBV and respiratory syncytial virus infection/reactivation resolved spontaneously.

Patient 9 presented at the age of 4 months with bloody diarrhea; endoscopic and histopathologic findings resembled those of patient 8. Enteral feeding and immunosuppressive therapies failed, and parenteral nutrition was instituted. The early genetic diagnosis of IL-10 deficiency and identification of a sibling as a suitable bone marrow donor avoided colectomy and allowed for timely HSCT on conditioning with alemtuzumab, fludarabine, and melphalan. HSCT was carried out at the age of 14 months without complications.

Both IL-10–deficient patients (the girl is now 5 years and 7 months, and the boy is now 2 years and 11 months) are now off immunosuppression, have been cured of colitis, and are doing well.

DISCUSSION

We analyzed a cohort of 40 patients with early-onset IBD and identified 7 (18%) with mutations in the genes encoding IL-10R: *IL10RA* (encoding IL-10R1) and *IL10RB* (encoding IL-10R2; previously published mutations are summarized in Table E3 in this article's Online Repository at www.jacionline.org). We did not see any genotype-phenotype correlations in this cohort of patients. In contrast to the other 33 pediatric patients without mutations, IL-10/IL-10R–deficient patients had a very early onset within the first months of life, particularly severe perianal disease, fistula formation, and resistance to immunosuppressive drugs. An exception was patient 1. Her relatively late onset was unusual and might suggest a hypomorphic mutation with partially retained IL-10R function; however, this was unlikely given the complete lack of IL-10R function.

Eight of 9 patients were born to consanguineous parents, and 6 had a positive family history, with siblings or other relatives who had died of a similar disease. The low numbers of patients identified thus far demonstrate that IL-10/IL-10R deficiencies are rare and that very early-onset IBD is a heterogeneous group of diseases.

Several monogenic primary immunodeficiencies, including Wiskott-Aldrich syndrome, NEMO deficiency, chronic granulomatous disease, and XIAP deficiency, can be associated with IBD and need to be considered in patients in whom *IL10/IL10R* mutations have been ruled out.[12-17] Particularly worth mentioning is the immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome caused by mutations in *FOXP3*: affected patients lack IL-10–producing $FOXP3^+$ regulatory T cells and have multiple autoimmune disorders, including autoimmune enteropathy, which is the predominant cause of morbidity and mortality.[18,19]

The unresponsiveness of IL-10R–deficient subjects' leukocytes to IL-10 and the lack of functional IL-10 in IL-10–deficient subjects substantiates the regulatory role of IL-10 to protect against extensive tissue damage by excessive inflammatory reactions and accounts for the severe course of the disease.[20,21] On binding of IL-10 to IL-10R, a complex of 2 α- (IL-10R1) and 2 β (IL-10R2) subunits, signal transducer and activator of transcription 3 is phosphorylated, dimerizes, and translocates to the nucleus to downregulate expression of proinflammatory genes.[22] In contrast to IL-10R1, which is unique to IL-10R, IL-10R2 is also a component of the receptors for IL-22, IL-26, and the λ-interferons IL-28A, IL-28B, and IL-29. Expression of IL-10R1 is restricted to hematopoietic cells and tissues, and IL-10R2 is found on a wide range of nonimmune cells, such as epithelial cells and keratinocytes.[22-26]

IL-10/IL-10R–deficient patients showed minor inconsistent immunologic abnormalities without any genotype-phenotype correlation. Increased serum immunoglobulin levels, most likely reflecting the ongoing active inflammation; normal or slightly decreased serum immunoglobulin levels; and variations in T-cell, B-cell, and NK cell numbers occurred. Several patients had recurrent infections, primarily affecting the respiratory tract, which might be the reason for the hearing loss observed in 2 of our patients. However, whether the immunologic alterations and occurrence of apparently frequent infections were caused by the underlying disease, nutritional deficiencies, or the strong immunosuppressive therapy our patients received remains unclear. Of interest are EBV-associated lymphomas that developed in 2 of our patients who harbored mutations in the gene encoding IL-10R2, which is also part of the receptor for λ-interferons. Thus a lack of λ-interferon signaling, which is a component of the antiviral defence,[27] might have combined with immunosuppressive therapy to predispose to lymphomas.

Because of the life-threatening clinical course and based on the identification of *IL10/IL10R* mutations, allogeneic HSCT was carried out in 3 of our 9 patients and induced sustained remission.[1,4] The positive outcomes support the hypothesis that IL-10 signaling in hematopoietic cells is critical to control hyperinflammation in the gut and suggest that HSCT should be considered early in patients with IL-10/IL-10R1 deficiency.[1,4] Despite the very much improved colitis, patients 8 and 9 had lower CD4/CD8 cell ratios, with patient 8 revealing strongly decreased CD4 T- and CD19 B-cell numbers and patient 9 having high CD8 T-cell numbers. In addition, patients 7 and 8 showed reduced T-cell responses to mitogens; however, these immunologic abnormalities might normalize over time. Of note, patient 7 had protective antibodies against polio and tetanus after post-HSCT revaccination.

In conclusion, our work shows that IL-10/IL-10R deficiencies are primary immunodeficiencies that need to be considered in patients with therapy-refractory early-onset IBD and severe perianal disease. Even though the colitis is at the center of attention, other subtle immunologic features might contribute to the phenotype of the disease, thereby making the disease a complex immunologic disorder. HSCT is the only current therapeutic approach that can cure the disease and provide the affected patients with a normal quality of life.

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Clinical implications: IL-10/IL-10R deficiency needs to be considered in patients with intractable early-onset enterocolitis. Although standard immunosuppressive treatments are not effective, HSCT is potentially curative.

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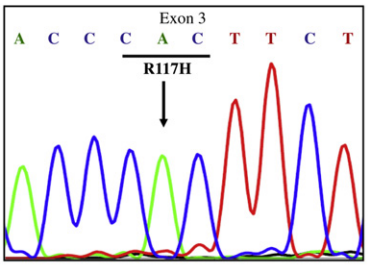
Pat. 1: Homozygous mutation in *IL10RA*, c.350G>A;

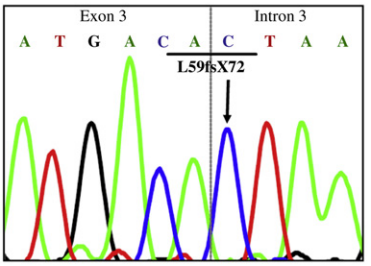
results in IL-10R1: Arg117His



Pat. 2: Homozygous mutation in *IL10RB*, IVS3+1G>C;

results in frame shift and pre-mature stop codon:

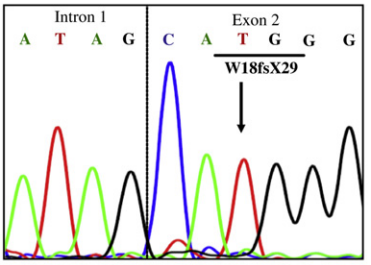
IL-10R2: Leu59fsX72



Pat. 3: Homozygous deletion in *IL10RB,* c.53delT;

results in frame shift and pre-mature stop codon:

IL-10R2: Trp18fsX29



Pat. 4: Homozygous mutation in *IL10RB*, c.577 G>C;

results in IL-10R2: Gly193Arg

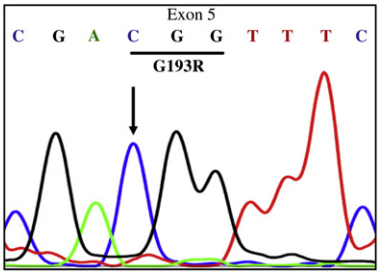


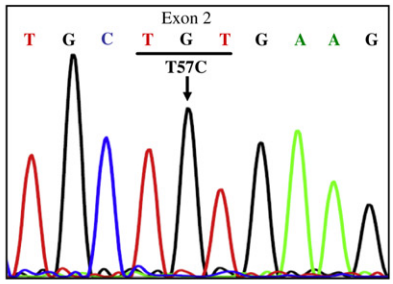
FIG E1. Sequence chromatograms of patients with mutations in *IL10RA* (encoding IL-10R1), *IL10RB* (encoding IL-10R2), and *IL10* (encoding IL-10). Affected bases are marked with an *arrow*, resulting mutant base triplets are underlined, and their effect on protein expression is indicated.

Pat. 6: Heterozygous mutations in *IL10RA*;

1) c.170A>G, results in IL-10R1: Tyr57Cys (allele 1);

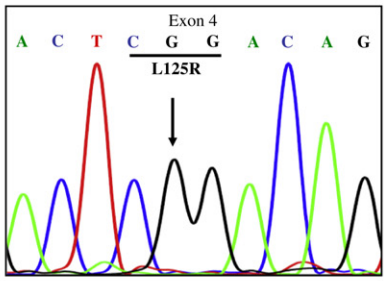
2) Ex2_4del, results in frame shift and

pre-mature stop codon: IL-10R1: Val23fsX31 (allele 2)



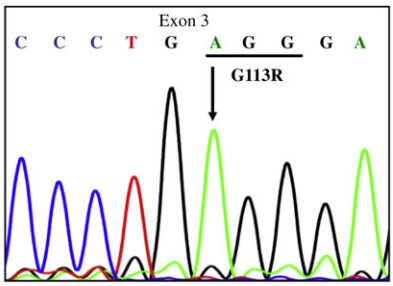
Pat. 7: Homozygous mutation in *IL10RA,* c.374T>G;

results in IL-10R1: Leu125Arg



Patient 8: Homozygous mutation in *IL10, c.*337G>A;

results in IL-10: Gly113Arg



Patient 9: Homozygous mutation in *IL10, c.*337G>A;

results in IL-10: Gly113Arg

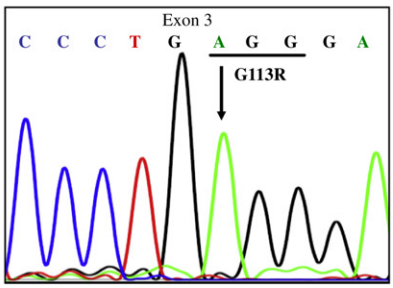


FIG E1. *(Continued)*

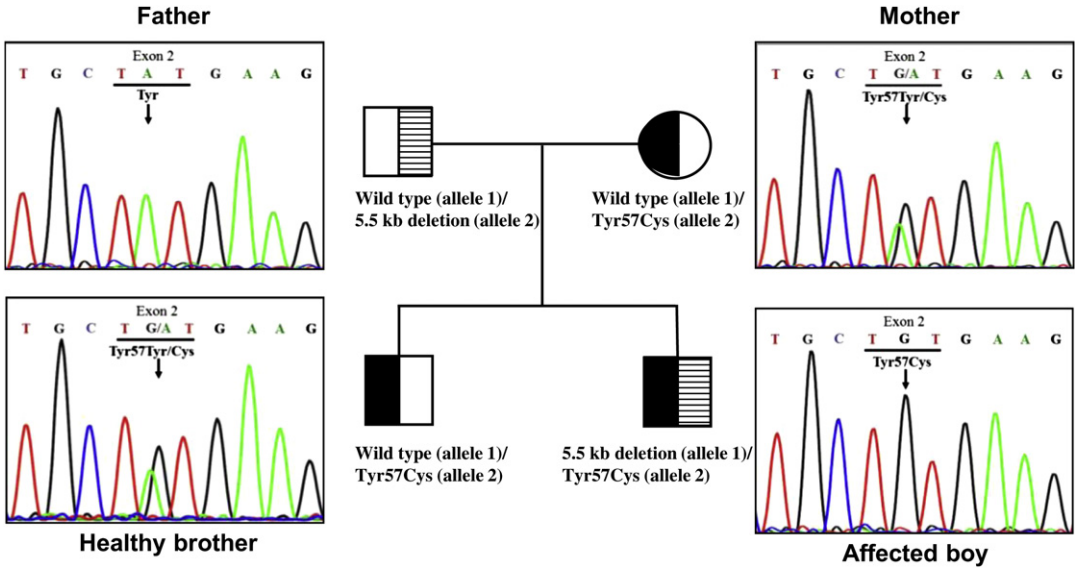


FIG E2. Genotypes at triplet 57 of *IL10RA* of patient 6's family. The mother and her healthy son are heterozygous, carrying a wild-type (tyrosine) and mutant allele (cysteine). The father is heterozygous, with 1 wild-type allele and 1 allele with a large deletion. The affected boy is compound heterozygous, with the mutant allele from his mother and the deletion from his father.

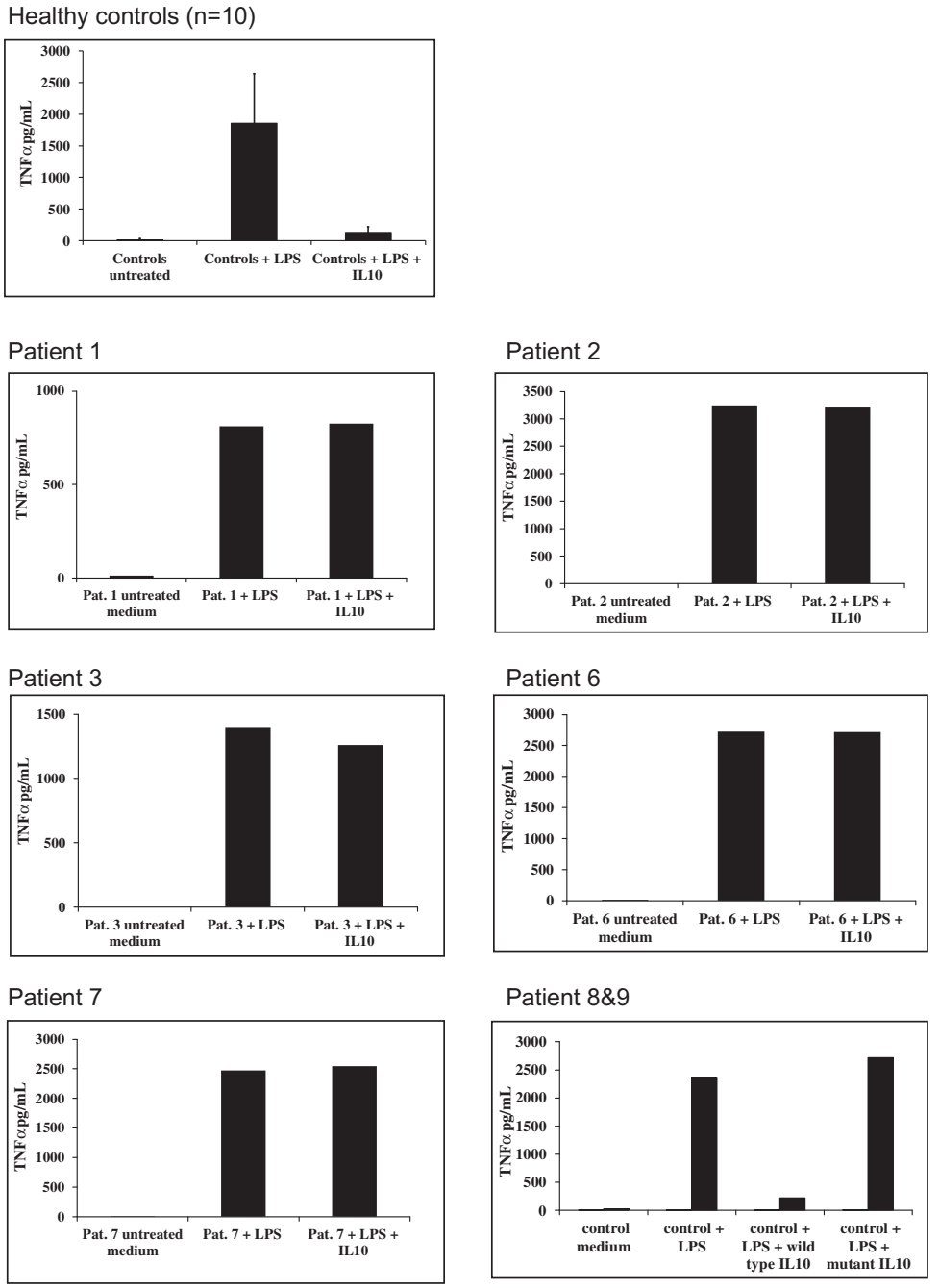


FIG E3. IL-10 did not inhibit TNF-α release in LPS-challenged PBMCs from IL-10R–deficient patients 1, 2, 3, 6, and 7 when compared with that seen in healthy control subjects (n = 10). Mutant IL-10, as expressed in patients 8 and 9, does not inhibit TNF-α release in LPS-challenged PBMCs from healthy donors. TNF-α concentrations in supernatants were measured by using ELISA.

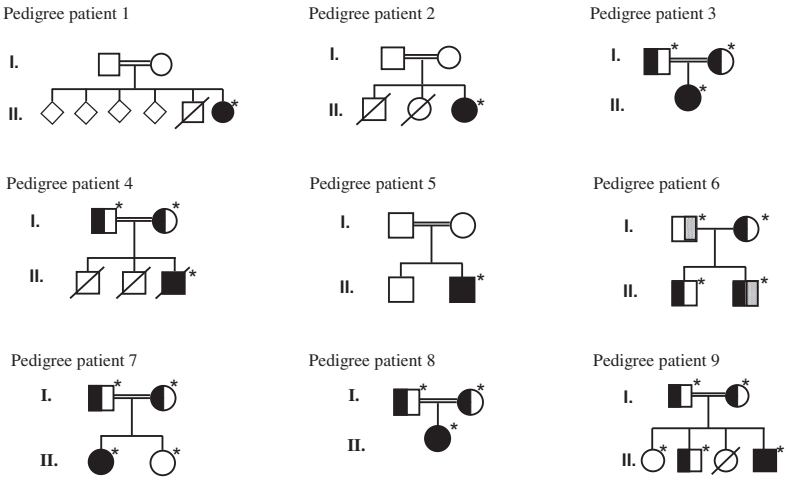


FIG E4. Patients' pedigrees. *Circles* represent female and *squares* represent male subjects. *Solid symbols* illustrate homozygous affected patients, *half-solid symbols* indicate heterozygous healthy carriers, and *open symbols* indicate wild-type healthy individuals. *Dashes* indicate deceased subjects and *asterisks* indicate sequenced subjects.

TABLE E1. Sequences of PCR primers used in this study and PCR product lengths

Exon	Forward primer	5′ → 3′ Sequence	Reverse primer	5′ → 3′ Sequence	Length
IL10 gene					
1	min176Fw	AAT CAT TTT TGC TTA CGA TGC	312Rv	GCA GGA GGA GGG TTC TTA TAG	488 bp
2	996Fw	CTC TAA ATG AAA GGG CAT CAA	1494Rv	ATC ACC TCC TCC AGG TAA AAC	499 bp
3	1243Fw	AAA GGA GAA GTG GGA AGA TGT	1710Rv	TTT ACA GCT AGC TCT GCC AGT	468 bp
4	2567Fw	CAG GAT CAC CAA CAC TTT CTC	2870Rv	CCC CAA AGA CAC TTA ACA GAA	304 bp
5	3523Fw	ATT TTG AAG ACA GTG CTT TGC	4018Rv	TAG AAA TGG GGG TTG AGG TAT	496 bp
IL10RA gene					
1	min223Fw	ACT TTG ATC CGA AGG CAC T	359Rv	CTT CTC TAT CCT TCC CAA TCC	582 bp
2	1906Fw	ACC TCC CTT TCT TCT TTG GTA	2244Rv	TCA ACT GCT GGC TAT GTG TTA	339 bp
3	2722Fw	CAA CAG GGG AAC ACT AAT GAC	3439Rv	AAA CAA CTC CAG TTC CCT TTT	718 bp
4	6805Fw	ATT CTG GAG GCA AAG TCT CG	7151Rv	AGT TCC CAA TGG CAC ACA AG	347 bp
5	7245Fw	GCC CTT GTA GAT GAA GCA ATA	7874Rv	TAT GTG AAG CAG CAC CTA ACA	630 bp
6	9051Fw	AGA GTT GTG GCC TGT AGT TTG	9555Rv	TGA TTC CAT GTG TCT CAT CTG	505 bp
7 I	12169Fw1	GGA TTC GAA AAC AAA ACA GAA	12877Rv1	TCT CTT CAG CAC ATC TGG TCT	709 bp
7 II	12706Fw2	GAT GAC AGT GGC ATT GAC TTA	13448Rv2	TGC ACT AGT TCA GTG AGT TGG	743 bp
IL10RB gene					
1	min356Fw	AGG GTA AAG AAG ACC CTC AAA	309Rv	CCT AGT TGC GTC TCA GCA G	666 bp
2	1933Fw	AGC CAT AGA GGA GAA CCA AGT	2523Rv	ACC TAG AGA TGA CAG CAG TGG	591 bp
3	9681Fw	ACC AAT AGA CTT GCT CAA TGC	10437Rv	ACA AGG CAA GAT GAT GAC ATT	757 bp
4	13231Fw	CTA CCC TTC TTA GCC ATG TCA	14007Rv	TCC GAT CAG ATC TTT TGA CTC	777 bp
5	16683Fw	CCT TCC ACT GCT TAG TCA TGT	17394Rv	TAT GGT GTG TGA AGG ACT GTG	712 bp
6	21656Fw	GGA TTG TGA TGG TTA AAA TGC	22400Rv	CCC TTT TAC AAA TAG CCT TCC	745 bp
7	29720Fw	ATA GAT TTT CCA GCC AGG AGT	30303Rv	GCC CTG TTT CTC ACA ATT AAA	584 bp
Additional sequencing primers for *IL10RB*					
3	Ex3 10147Fw	CCC CCT CCA AAT TAA GTA CCA			
5	Ex5 Seq Rv	TGT GGT CTC CTT CCT AG			

TABLE E2. Immunologic data, including serum immunoglobulins, T-cell subsets, B cells, NK cells, response to mitogens, and phagocyte function tests, in our IL-10/IL-10R–deficient patients

	Patient no.			
	1	**2**	**3**	**4**
Age	16 y	25 y	12 y	Died age 40 d
Mutation	IL-10R1: Arg117His	IL-10R2: Leu59fsX72	IL-10R2: Trp18fsX29	IL-10R2: Gly193Arg
IgM (g/L)	3.1 (0.5-1.8), increased	2.1 (0.4-2.3), normal	1.25 (0.5-1.8), normal	Not determined
IgA (g/L)	3.93 (0.5-2.4), increased	14.1 (0.7-4.0), increased	3.65 (0.5-2.4), increased	Not determined
IgG (g/L)	19.6 (5.4-16.1), increased	26.6 (7.0-16.0), increased	6.33 (5.4-16.1), normal	Not determined
$CD3^+$ (absolute counts) $\times 10^9$/L	2.23 (0.7-4.2), normal	1.28 (0.7-2.1), normal	1.55 (0.7-4.2), normal	Not determined
$CD3^+/CD4^+$ (absolute counts) $\times 10^9$/L	0.93 (0.3-2.0), normal	0.66 (0.3-1.4), normal	0.86 (0.3-2.0), normal	Not determined
$CD3^+/CD8^+$ (absolute counts) $\times 10^9$/L	1.39 (0.3-1.8), normal	0.62 (0.2-0.9), normal	0.57 (0.3-1.8), normal	Not determined
$CD19^+$ (absolute counts) $\times 10^9$/L	0.12 (0.2-1.6), decreased	0.13 (0.1-0.5), normal	0.51 (0.2-1.6), normal	Not determined
$CD16^+CD56^+$ (absolute counts) $\times 10^9$/L	0.03 (0.09-0.9), decreased	0.09 (0.09-0.6), normal	0.11 (0.09-0.9), normal	Not determined
CD4/CD8 ratio	0.67 (1.0-3.6), decreased	1.07 (1.0-3.6), normal	1.07 (0.9-2.6), normal	Not determined
$CD3^+$ (% lymphocytes)	94% (55-78), increased	84% (55-83), increased	70% (55-78), normal	Not determined
$CD3^+/CD4^+$ (% lymphocytes)	39% (27-53), normal	40% (28-57), normal	39% (27-53), normal	Not determined
$CD3^+/CD8^+$ (% lymphocytes)	58% (19-34), increased	37% (10-39), normal	26% (19-34), normal	Not determined
$CD19^+$ (% lymphocytes)	5% (10-31), decreased	9% (6-19), normal	23% (10-31), normal	Not determined
$CD16^+/CD56^+$ (% lymphocytes)	1% (4-26), decreased	6% (7-31), decreased	5% (4-26), normal	Not determined
Response to PHA	Normal	Not determined	Not determined	Not determined
Nitroblue tetrazolium test	Negative	Negative	Negative	Not determined

	Patient no.			
	5	**6**	**7a (before HSCT)**	**7b (after HSCT)**
Age	1 y	2 y	3 y, 2 mo	4 y
Mutation	IL-10R1: Ex1_Ex3del	IL-10R1: Tyr57Cys (allele 1); deletion (allele 2)	IL-10R1: Leu125Arg	
IgM (g/L)	3.9 (0.4-1.6), increased	1.3 (0.5-2.2), normal	1.31 (0.5-2.2), normal	0.67 (0.5-2.2), normal
IgA (g/L)	<0.4 (0.15-0.7)	2.50 (0.3-1.2), increased	0.79 (0.3-1.2), normal	1.00 (0.3-1.2), normal
IgG (g/L)	4.2 (3.0-9.0), normal (on IVIG)	8.74 (3.1-13.8), normal	4.75 (3.1-13.8), normal	9.29 (3.1-13.8), normal
$CD3^+$ (absolute counts) $\times 10^9$/L	Not available	2.39 (0.9-4.5), normal	7.58 (0.9-4.5), increased	1.75 (0.9-4.5), normal
$CD3^+/CD4^+$ (absolute counts) $\times 10^9$/L	Not available	1.43 (0.5-2.4), normal	4.39 (0.5-2.4), increased	1.03 (0.5-2.4), normal
$CD3^+/CD8^+$ (absolute counts) $\times 10^9$/L	Not available	0.89 (0.3-1.8), normal	3.64 (0.3-1.8), increased	0.77 (0.3-1.8), normal
$CD19^+$ (absolute counts) $\times 10^9$/L	Not available	0.61 (0.2-2.1), normal	2.03 (0.2-2.1), normal	1.47 (0.2-2.1), normal
$CD16^+CD56^+$ (absolute counts) $\times 10^9$/L	Not available	0.34 (0.1-1.0), normal	0.86 (0.1-1.0), normal	0.24 (0.1-1.0), normal
CD4/CD8 ratio	2.3 (1.3-3.9), normal	1.6 (0.9-2.9), normal	1.2 (0.9-2.9), normal	1.3 (0.9-2.9), normal
$CD3^+$ (% lymphocytes)	85% (54-76), increased	70% (43-76), normal	71% (43-76), normal	50% (43-76), normal
$CD3^+/CD4^+$ (% lymphocytes)	60% (31-54), increased	42% (23-48), normal	41% (23-48), normal	28% (23-48), normal
$CD3^+/CD8^+$ (% lymphocytes)	26% (12-28), normal	26% (14-33), normal	34% (14-33), increased	21% (14-33), normal
$CD19^+$ (% lymphocytes)	10% (15-39), decreased	18% (14-44), normal	19% (14-44), normal	43% (14-44), normal
$CD16^+/CD56^+$ (% lymphocytes)	3% (2-13), normal	10% (4-23), normal	8% (4-23), normal	7% (4-23), normal
Response to PHA	Reduced	Normal	Reduced	Reduced (50% of control)
Nitroblue tetrazolium test	Negative	Normal neutrophil oxidative index	Normal neutrophil oxidative index	Not determined

	Patient			
	8 (before HSCT)	**8 (after HSCT)**	**9 (before HSCT)**	**9 (after HSCT)**
Age	3 y, 11 mo	5 y, 7 mo	1 y, 2 mo	2 y, 11 mo
Mutation	IL-10: Gly113Arg		IL-10: Gly113Arg	
IgM (g/L)	Not determined	1.01 (0.5-2), normal	1.66 (0.4-1.6), increased	1.33 (0.5-2.2), normal
IgA (g/L)	Not determined	1.39 (0.4-2.0), normal	1.48 (0.15-0.7), increased	0.55 (0.3-1.2), normal
IgG (g/L)	Not determined	28.9 (4.9-16.1), increased	8.84 (3.0-9.0), normal	4.38 (3.1-13.8), normal
$CD3^+$ (absolute counts) $\times 10^9$/L	1.92 (0.9-4.5), normal	1.92 (0.9-4.5), normal	Not determined	6.31 (0.9-4.5), increased
$CD3^+/CD4^+$ (absolute counts) $\times 10^9$/L	0.67 (0.5-2.4), normal	0.21 (0.5-2.4), decreased	Not determined	2.21 (0.5-2.4), normal
$CD3^+/CD8^+$ (absolute counts) $\times 10^9$/L	1.17 (0.3-1.8), normal	1.62 (0.3-1.8), normal	Not determined	3.03 (0.3-1.8), increased
$CD19^+$ (absolute counts) $\times 10^9$/L	0.37 (0.2-2.1), normal	0.11 (0.2-2.1), decreased	Not determined	1.07 (0.2-2.1), normal
$CD16^+CD56^+$ (absolute counts) $\times 10^9$/L	0.2 (0.1-1.0), normal	0.11 (0.1-1.0), normal	Not determined	0.66 (0.1-1.0), normal
CD4/CD8 ratio	0.57 (0.9-2.9), decreased	0.13 (0.9-2.6), decreased	2 (1.3-3.9), normal	0.7 (0.9-2.9), decreased
$CD3^+$ (% lymphocytes)	77% (43-76), increased	90% (43-76), increased	71% (50-77), normal	77% (43-76), increased
$CD3^+/CD4^+$ (% lymphocytes)	27% (23-48), normal	10% (23-48), decreased	47% (33-58), normal	27% (23-48), normal
$CD3^+/CD8^+$ (% lymphocytes)	47% (14-33), increased	76% (14-33), increased	23% (13-26), normal	37% (14-33), increased

(Continued)

TABLE E2. *(Continued)*

	Patient			
	8 (before HSCT)	**8 (after HSCT)**	**9 (before HSCT)**	**9 (after HSCT)**
$CD19^+$ (% lymphocytes)	15% (14-44), normal	5% (14-44), decreased	21% (13-35), normal	13% (14-44), decreased
$CD16^+/CD56^+$ (% lymphocytes)	8% (4-23), normal	5% (4-23), normal	7% (2-13), normal	8% (4-23), normal
Response to PHA	Not determined	Impaired	Normal	Normal
Nitroblue tetrazolium test	Negative	Not determined	Negative	Not determined

Values are compared with age-related reference ranges according to Comans-Bitter et al,[11] and patients 8 and 9 have been described in detail by Glocker et al.[2]
IVIG, Intravenous immunoglobulin.

TABLE E3. Sex, age of onset, mutation, and references of thus far published patients with mutations in IL-10, IL-10R1 (encoded by *IL10RA*), and IL-10R2 (encoded by *IL10RB*)

Patient no.	Sex	Age of onset (mo)	Mutation		Reference
1	Male	1	IL-10R2: Trp159X	Homozygous	Glocker et al, 2009[1]
2	Female	2	IL-10R2: Trp159X	Homozygous	Glocker et al, 2009[1]
3	Female	1	IL-10R1: Gly141Arg	Homozygous	Glocker et al, 2009[1]
4	Male	1	IL-10R1: Thr84Ile	Homozygous	Glocker et al, 2009[1]
5	Female	1	IL-10: Gly113Arg	Homozygous	Glocker et al, 2010[2]
6	Male	1	IL-10: Gly113Arg	Homozygous	Glocker et al, 2010[2]
7	Male	1	IL-10R1: Arg262Cys	Homozygous	Begue et al, 2011[3]
8	Male	3	IL-10R2: Glu141X	Homozygous	Begue et al, 2011[3]
9	Male	1	IL-10R1: Arg101Trp	Homozygous	Kotlarz et al, 2012[4]
10	Male	2	IL-10R2: Trp159X	Homozygous	Kotlarz et al, 2012[4]
11	Male	2	IL-10R1: Tyr57Tyr/Cys; *IL10R1*: Arg117Arg/Cys	Compound heterozygous	Kotlarz et al, 2012[4]
12	Female	2	IL-10R2: Cys66Tyr	Homozygous	Kotlarz et al, 2012[4]
13	Male	3	*IL10RB*: 3′ UTR: c.*C52T	Homozygous	Kotlarz et al, 2012[4]
14	Female	1	IL-10R2: Trp204Trp/X; *IL10R2*: Ser230Ser/X	Compound heterozygous	Kotlarz et al, 2012[4]
15	Female	1	IL-10R2: Trp204X	Homozygous	Kotlarz et al, 2012[4]
16	Male	1	IL-10R1: Ile169Thr	Homozygous	Kotlarz et al, 2012[4]
17	Male	1	IL-10: Gly153Asp	Homozygous	Kotlarz et al, 2012[4]
18	Male	1	IL-10: Gly153Asp	Homozygous	Kotlarz et al, 2012[4]
19	Male	1	IL-10: Gly153Asp	Homozygous	Kotlarz et al, 2012[4]
20	Male	2	*IL10RB*: c.331+907_574del	Homozygous	Kotlarz et al, 2012[4]
21	Female	3	IL-10R1: Pro206X	Homozygous	Moran et al, 2012[5]
22	Male	1	IL-10R1: Thr84Thr/Ile; *IL10R1*: Arg101Arg/Trp	Compound heterozygous	Mao et al, 2012[6]

UTR, Untranslated region.