SHORT REPORT

Novel de novo mutations of the interleukin-10 receptor gene lead to infantile onset inflammatory bowel disease

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Abstract

Background and aims: Defects in the interleukin 10 (IL-10) signalling pathway have been shown to cause very early onset inflammatory bowel disease (IBD). We report a patient with severe infantile-onset IBD with a compound heterozygous IL-10 receptor alpha subunit (IL-10RA) mutation, one of which was paternally-inherited and the other occurring de novo. Methods:

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1. Case report

The patient was born at 35 weeks gestation with a birth weight of 2.5 kg (50th percentile) to non-consanguineous white Caucasian parents. He was breast fed and remained healthy during the first month of life. Following the introduction of a cow’s milk formula feeds he developed diarrhoea and eczema. These symptoms were attributed to cow’s milk protein allergy and the formula was changed to a hypoallergenic amino acid based feed. There was no improvement and at four months the patient was referred to a tertiary centre for investigation of failure to thrive and bloody diarrhoea. Clinical examination revealed anal fissures and ulcers. Endoscopy showed gastritis, duodenitis and florid colitis of the rectum and sigmoid colon; complete colonoscopy was abandoned due to friable mucosa. Immunological investigations including nitroblue tetrazolium (NBT), immunoglobulins, and lymphocyte subsets were normal, but there was evidence of chronic systemic inflammation with raised erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), thrombocytosis and hypoalbuminaemia.

The patient was initially treated with corticosteroid, aza-thioprine, gut rest and total parental nutrition (TPN) with minimal improvement. He also had multiple courses of antibiotics (ciprofloxacin and metronidazole) for perianal fissures and fistulae, with some symptomatic improvement. An induction course of infliximab (5 mg/kg at week 0, 2, 6) at 8 months of age resulted in a partial response enabling reintroduction of enteral feeding. However, his disease subsequently flared and was refractory to further doses of infliximab.

At 2 years of age, he had a further flare of his colitis with extensive fissuring and destructive fistulising perianal disease. This was further complicated by multiple episodes of severe Gram-negative sepsis. He was treated with systemic antibiotics, gut rest, TPN and finally a colectomy with formation of an end ileostomy. His perianal disease markedly improved thereafter, but he continued to have frequent abdominal pain.

Currently at 10 years of age, he has significant growth failure (height Z score −3.05; weight Z score −3.91). He continues to be symptomatic and has elevated systemic inflammatory markers. A recent Paediatric Crohn’s Disease Activity Index (PCDAI) was 45, indicating ongoing severe disease. A repeat gastroscopy shows microscopic oesophagitis, gastritis and duodenitis, without granulomata. He suffers from severe food aversion and receives his entire nutritional requirements in the form of an elemental formula via a gastrostomy. Besides mild eczema, the patient had pyoderma gangrenosum but not folliculitis. He does not have any other extra-intestinal manifestations of IBD or autoimmune disease.

2. Identification of novel IL-10RA mutation

To investigate a possible IL-10 signalling pathway defect causing early onset IBD, molecular genetic testing was performed as previously published. In brief, deep sequencing of IL-10, IL-10 receptor alpha subunit (IL-10RA) and IL-10 receptor beta subunit (IL-10RB) were performed. We identified a missense mutation in exon 4 of IL-10RA (c.583T>C) in one allele and a nonsense mutation in exon 7 of IL-10RA (c.1368G>T) in the other allele. Neither mutation has been reported previously. The patient has functional IL-10RA deficiency despite normal IL-10RA expression. Conclusion: This represents the first case report of a de novo mutation of IL-10RA that is associated with very early onset severe IBD. Therefore, IL-10 pathway defect should be considered in patients with infantile-onset IBD even if the parents are non-consanguineous.

3. IL-10 signalling pathway defect

Given the patient’s mutations are novel, it was important to demonstrate a functional defect in IL-10 signalling. Peripheral blood mononuclear cells (PBMC) were isolated via Ficoll-Paque centrifugation. Surface expression of IL-10RA was analysed by flow cytometry, which demonstrated comparable IL-10RA expression between the patient and the control (Fig. 2A). After IL-10 binds to its receptor, the principal signalling
pathway is mediated via phosphorylation and activation of signal transducer and activator of transcription 3 (STAT3).6 Independent of IL-10 receptors, interleukin-6 (IL-6) also phosphorylates STAT3.7 We therefore examined the IL-10 signalling pathway by measuring phosphorylated STAT3 (pSTAT3) in PBMC cultured in the presence of IL-6 or IL-10. Here the patient demonstrated normal STAT3 phosphorylation in response to IL-6, but near absent STAT3 phosphorylation in response to IL-10 (Fig. 2B), suggesting a specific defect of the IL-10 signalling pathway upstream of STAT3. Furthermore, in contrast with the controls, the patient’s monocytes stimulated with lipopolysaccharide (LPS) failed to respond to IL-10 mediated suppression of tumour necrosis factor alpha (TNFα) secretion (Fig. 2C). Collectively, these results indicated that the patient has functional IL-10RA deficiency despite normal IL-10RA expression. This is consistent with previously reported cases of IL-10RA defects in the literature.1–3,8,9 These assays however do not distinguish whether the lack of signalling is due to impaired IL-10 binding to its receptor, or a defective cytoplasmic domain of IL-10RA leading to impaired signalling.

4. Discussion

IL-10 is an anti-inflammatory cytokine secreted by a range of immune cells including regulatory T-cells (Treg), macrophages and dendritic cells. IL-10 plays an important role in mucosal homeostasis and has been implicated in the pathogenesis of IBD. This is supported by the fact that IL-10 knockout mice developed spontaneous onset of colitis in the presence of gut bacteria.10 Large scale genome-wide association studies (GWAS) had identified IL-10 as an IBD risk allele.11 IL-10RA polymorphisms were shown to confer risk of developing very early onset ulcerative colitis6 and lately, Galatola et al. reported a case of very early onset ulcerative colitis due to synergistic effect of several variant alleles in the IL-10 receptor genes.12 Furthermore, recent studies showed that patients with loss-of-function mutation of either IL-10 or IL-10 receptor developed very aggressive, early onset IBD.1–3,8,9,13–15 To date 36 patients of various ethnicities with IL-10 signalling pathway defects have been described in the literature.1–4,8,9,12,14,15 Most of the patients were born to...
A

IL-10RA

B

IL-6

IL-10

Control

Patient

pSTAT3

C

Control

Patient

TNFα
consanguineous parents with only seven patients born to non-consanguineous marriages. Six cases of compound heterozygote mutations in IL-10RA/IL-10RB were described3,8,9,12,15; however a disease causing de novo mutation has not been reported.

Kotlarz et al. and Pigneur et al. provided a comprehensive description of the clinical phenotypic characterization of infantile-onset IBD due to defects of the IL-10 signalling pathway.1,3,15 Our patient had extremely early onset of symptoms, florid colitis and severe perianal disease consistent with the previously described IL-10R-deficiency phenotype. Therefore IL-10 and IL-10R were sequenced and based on the identification of inactivating mutations no further genetic testing was carried out. In addition, our patient had early inflammation of the upper gastrointestinal tract as evidenced by the presence of microscopic gastritis and duodenitis. Furthermore, technetium-tagged white blood cells scan (WBC-Tc99m) and barium small bowel study demonstrated small bowel enteritis. These findings differ to the of the disease extent described by Pigneur et al.15

In terms of treatment, our experience was consistent with the reported cases in the literature. Immunosuppressive therapy was not efficacious. Induction doses of infliximab resulted in a partial early response which was not sustained by further doses. Antibiotic therapy temporarily reduced perianal inflammation, but only colectomy and dysfunctioning ileostomy resulted in improvement of his perianal disease.

Other therapeutic strategies had been trialled in IBD. These include attempts to use parenteral IL-10 to treat IBD; however it was ineffective and had undesirable side effects. Milk fermented by genetically modified IL-10 producing Lactococcus lactis was shown to be an effective anti-inflammatory in IL-10 knockout murine model of IBD,16 but evidence is still lacking in humans.17 Notably, these therapies were trialled in unselected IBD patients rather than patients with IL-10 deficiency. These IL-10 replacement treatment strategies are unlikely to be successful in patients with IL-10 receptor mutations. However, it remains unknown if such strategies would be helpful in patients with IL-10 deficiency.

Haematopoietic stem cell transplantation (HSCT) was recently reported to be curative in IL-10 and IL-10 receptor deficient patients with IBD.1,3,8,15 This treatment option appeared to be the most promising. To date, 10 patients with an IL-10 signalling defect have had successful HSCT. However, long-term outcomes of HSCT are not yet available. In view of the life-threatening clinical course of IL-10 signalling pathway defect, we are currently considering the option of HSCT in this patient.

5. Summary

IL-10RA (c.583T>C) and IL-10RA (c.1368G>T) are novel mutations that have not been previously reported to cause severe infantile-onset IBD. Furthermore, this represents the first case report of a patient with de novo mutation in IL-10RA that leads to severe infantile-onset IBD. Therefore, IL-10 pathway defect should be considered in patients with infantile-onset IBD even if the parents are non-consanguineous.

Conflict of interest

There is no conflict of interest.

References


Figure 2 Functional study of IL-10 signalling pathway. A. Histogram showing IL-10RA expression of CD3+CD4+ T cells in the patient (dotted line), compared to a healthy control (solid line) and isotype (shaded). B. Histogram showing the expression of phosphorylated STAT3 (pSTAT3, pY705) in CD3+CD4+ T cells either without stimulation (shaded) or after stimulation by IL-6 (100 ng/ml) or IL-10 (100 ng/ml) in healthy control and patient (as indicated). C. Histogram showing intracellular TNFα expression of CD14+ monocytes after 4 hour stimulation with LPS (100 ng/ml) in the absence (solid line) or presence (dotted line) of IL-10, compared to isotype control (shaded) in patient and control as indicated.


