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## **Infliximab induces clinical resolution of sacroiliitis that coincides with increased circulating FOXP3<sup>+</sup> T cells in a patient with IPEX syndrome**

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## **ABSTRACT**

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare monogenic primary immunodeficiency due to mutations of FOXP3, a master transcription factor of regulatory T cells (Treg). IPEX syndrome leads to fatal course in most cases during early childhood or severe multi-organ immune-mediated disorders in patients who survive. Currently hematopoietic stem cell transplantation represents the only known effective cure for IPEX syndrome. However, older patients with a mild disease not severe enough to justify transplantation, raise concerns regarding the appropriate therapeutic management, which is therefore based on supportive and replacement therapies combined with pharmacological immunosuppression.

Herein, we report the case of a 22-year-old man with an incomplete IPEX syndrome without endocrine disorders having suffered from severe enteropathy since his birth treated with a combination of various immunosuppressant agents. He developed severe exacerbation of inflammatory low back pain in relation to sacroiliitis. Eventually, infliximab was initiated to control his back pain with rapid resolution as well as digestive improvement and also reduced biological inflammatory markers. In parallel, flow cytometry analysis revealed an increase in the frequency of circulating FOXP3+ CD4+ Treg cells. Altogether these data highlight that anti-TNF may represent a promising therapeutic option in patients with IPEX syndrome.

**Keywords:** IPEX syndrome, regulatory T cells, FOXP3, sacroiliitis, anti-TNF.

## 1. Introduction

Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome is a rare, monogenic, life-threatening, primary immunodeficiency due to loss-of-function mutations in the gene encoding the forkhead box P3 (FOXP3) transcription factor, which is critical for the differentiation and function of natural regulatory T cells (Tregs).[1] Tregs are essential for establishing and maintaining of self-tolerance and their impairment in IPEX syndrome leads to severe primary immunodeficiency associated with multi-organ autoimmunity. IPEX syndrome usually occurs in early childhood, most of the time leading to a fatal outcome. Allogeneic hematopoietic stem cell transplantation is the only curative therapy for IPEX syndrome to date. However, its use is limited by suitable donor availability and high risk-benefit ratio, in particular when organs have already suffered from irreversible damages from long-standing dysimmunity.[2] In addition, older patients with a mild disease not severe enough to justify transplantation, raise concerns regarding the appropriate therapeutic management, which relies mainly on various immunosuppressive treatments alone or in combination.

We report here a well-genotyped young adult patient with an incomplete IPEX syndrome who developed, in addition to a long-standing severe enterocolitis, a non-infectious sacroiliitis treated by adding anti-TNF (infliximab) to his usual low doses of oral steroids, cyclosporine, and mycophenolate mofetil.[3-7] Induction treatment with infliximab allowed a rheumatologic and digestive improvement as well as an increase in the frequency of circulating FOXP3+ CD4+ Tregs.

## 2. Case report

The patient was a 22 year-old male with an IPEX syndrome due to a deletion in exon 2 at codon 76 that started with severe and protracted neonatal diarrhea associated with early liver auto-immune dysfunction.[3,5-7] He often experienced abdominal pain, passing from one to three liquid and sometimes bloody stools a day, but his body weight remained stable with a good general condition. Upper and lower gastrointestinal endoscopic examinations revealed mucosal erythema, erosions and superficial ulcerations localized predominantly in the rectosigmoid and left colon without complication, such as stenosis or fistula. Histological analysis of the biopsies showed a mild non-specific chronic colitis with lymphocytic and eosinophilic infiltration of the *lamina propria* without granuloma. IPEX syndrome was incomplete, as the patient never experienced endocrine disorders. His usual treatment combined prednisone (5mg/day), cyclosporine (serum trough levels ranging between 60 to 100 µg/L) and mycophenolate mofetil (500 mg twice a day).[3,5,7]

The patient presented with a severe exacerbation in the last 18 months of unremitting inflammatory low back and buttock pain not improved with rest, and associated with awakening during the second half of the night and morning stiffness. He had been actually complaining of low back pain for about 10 years. He also displayed inflammatory cervicalgia and fingers tenderness without swelling. Physical exam found a bilateral positive FABER test. X-rays showed sclerosis of the right sacroiliac joint margins and Magnetic Resonance Imaging (MRI) an important bilateral sacroiliitis (figure 1). Retrospectively, an abdominal MRI performed 6 years ago already showed a right sacroiliitis. Human Leukocyte Antigen B27 (HLA-B27) test was negative. Laboratory tests showed both systemic and mucosal inflammation with high C-reactive protein (105 mg/L) and increased fecal calprotectin (7444 µg/g of stools), while white blood cell count and lymphocyte subset proportions (CD3, CD4/CD8 ratio, CD19) were normal.[7] Antinuclear, anti-extractable nuclear antigen, anti-dsDNA, anti-C1q, antineutrophil cytoplasmic, anti-chromatin, anti-cyclic citrullinated peptide antibodies and rheumatoid factor were absent. Finally, the diagnosis of axial spondyloarthritis was proposed and infliximab (5mg/kg at week 0, 2, 6 and every 8 weeks) was introduced to control both digestive and rheumatologic symptoms while the dose of mycophenolate mofetil was gradually reduced and finally tapered. Steroids and cyclosporine were kept at stable dose. As soon as few days following the first infusion of infliximab, inflammatory low back pain and the lumbar stiffness fully disappeared. Digestive symptoms were significantly improved three months after the onset of infliximab with abdominal pain resolution, as well as CRP which dramatically decreased to 10 mg/L and fecal calprotectin to 1926 µg/g of stools. The tolerance of infliximab was good and no adverse event was recorded after a 6-month follow-up period.

Given the previously published data regarding the impact of anti-TNF treatment on Treg compartment, we studied in our patient the effect of infliximab on this subpopulation. For this purpose, we measured before (day 0) and after induction treatment (day 45) Treg frequency and FOXP3 expression level within circulating CD4<sup>+</sup> T cells by flow cytometry. We observed an almost 3-fold increase of the frequency of circulating FOXP3<sup>+</sup> cells among CD4<sup>+</sup> T cells (0.22 % vs 0.61 % at day 0 and 45, respectively). This rise in peripheral Tregs after infliximab induction treatment was also noticed in absolute number of cells (1.2 vs 3.3 cells /mm<sup>3</sup>) and FOXP3 mean fluorescence intensity (1067 vs 1736) (figure 2A and B).

### **3. Discussion**

We report a rare case of IPEX syndrome which developed sacroiliitis. Infliximab initiated to control symptoms resulted in a significant clinical improvement coinciding with a substantial increase in circulating FOXP3<sup>+</sup> Tregs.

The most severe cases of IPEX syndrome are characterized by the early onset of a triad of clinical manifestations including severe diarrhea, type 1 diabetes and dermatitis.[2,8] Autoimmune enteropathy remains the hallmark of IPEX syndrome and the patients sometimes experience mucoid or bloody diarrhea, but more often a severe neonatal watery diarrhea due to severe extended enterocolitis, as it happened in our patient and also in his older brother who died at 6 weeks of age.[7] Nevertheless, a wide variety of autoimmune symptoms, with number increasing with age, can complicate the typical triad.[2,8] Peripheral arthritis involving one or more joints is a rare manifestation often associated with mild forms of IPEX syndrome. Arthritis onset is usually not acute and clinical manifestations are not typical, explaining why this related diagnosis could be delayed.[8] To our best knowledge, sacroiliac inflammation has never been reported so far in the literature in IPEX syndrome. Little is known about the clinical outcomes and therapeutic management in patients with IPEX syndrome over 20 years of age and it could not be deciphered whether this rheumatologic manifestation is related or not to IPEX syndrome. However, one can speculate that the spondyloarthritis occurrence in our patient has been promoted by an exaggerated intestinal permeability leading to a “leaky gut” and to substantial changes in the gut microbiota in the context of this severe enteropathy and colitis mimicking Crohn’s disease.[9] In addition, the negativity of HLA-B27 and the absence of family history of psoriasis, uveitis, spondyloarthritis or inflammatory bowel disease suggest in our patient a link with the IPEX syndrome.

The key role of Tregs in controlling autoimmunity is highlighted by the lethal autoimmune disease in Scurfy mice and its counterpart in human with IPEX syndrome. More subtle functional defects have been uncovered in Tregs isolated from patients with autoimmune diseases, including rheumatoid arthritis, psoriasis, type-1 diabetes mellitus, myasthenia gravis, and multiple sclerosis. Some of the dysfunctions observed in circulating Tregs from rheumatoid arthritis patients have been shown to be TNF-dependent, since infliximab induced a novel subset of effector Tregs with suppressive functions driven by interleukin (IL)-10 and TGF $\beta$ . [10] In addition, we and others have previously reported in patients with inflammatory bowel disease (IBD) and in rheumatoid arthritis that anti-TNF treatments drive Treg expansion.[11-13] Indeed, anti-TNF therapy has been shown to rapidly enhance functional circulating FOXP3 Treg frequency and number that correlated with improved clinical outcome.[14] It has been previously showed that adalimumab, but not etanercept, increases Treg number in rheumatoid arthritis patients and that these Tregs were capable of suppressing IL-17. Similarly, some findings report that anti-TNF may also potentiate the suppressive function of Tregs in IBD patients.[15-16] In contrast to IBD animal models, little is known about the contribution of Tregs in the control of induced-spondyloarthritis in rodent

models and conflicting results are available regarding Tregs in peripheral circulation in human spondyloarthritis.[15-18]

In the present report, infliximab treatment resulted in fast clinical improvement of both digestive and rheumatologic symptoms associated with a dramatic drop of objective biomarkers of systemic and intestinal inflammation (serum CRP and fecal calprotectin, respectively). This beneficial clinical outcome coincides with a weak but substantial increase in circulating Tregs. Although we acknowledge that we cannot formally claim that the impact on Tregs is directly associated with clinical improvement, the increase of the frequency but also of the absolute number of Treg suggests a critical role of these T cell subsets in the modulation of inflammation in various tissues or organs. Finally, the expression level of FOXP3, which is known to positively correlate with the suppressive function of Tregs, is also increased suggesting a potentiation of the immunomodulatory function of Tregs in the patient. Anti-TNF therapy is able to modulate Treg biology in both intended and unexpected ways, leading to Treg potentiation that may represent an unanticipated novel mechanism of action of biologics targeting TNF. Other drugs, including steroids have been shown to also impact FOXP3<sup>+</sup> Treg number. However, whether these drugs act directly on Tregs or indirectly by altering the inflammatory tissue microenvironment or target various pathogenic cells, remain still not clear.

Use of anti-TNF treatments in IPEX syndrome has been previously reported in only a few patients with variable results.[2] To our knowledge, it is the first report of a symptomatic sacroiliitis occurring in a well genotyped patient suffering from IPEX syndrome. The patient responded rapidly to infliximab therapy and interestingly his clinical and biological improvements coincide with enhanced circulating FOXP3 Tregs. This report suggests that anti-TNF may represent a promising therapeutic option in patients with IPEX syndrome.

### **Conflict of interest**

The authors declare no conflicts of interest.

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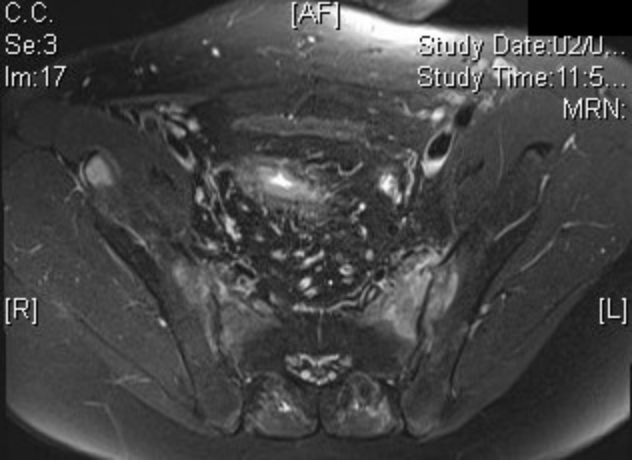
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**Figure 1. Representative picture MRI with the short tau inversion recovery (STIR) sequence showing abnormal increased signal intensity in sacral and iliac bones, bilaterally, typical for bone marrow edema due to inflammatory sacroiliitis. The lesions on STIR images are located close to the anterior part of the sacroiliac joints.**

**Figure 2. Comparison of quantitation of FOXP3 expression assessed by flow cytometry prior and after infliximab induction therapy (Day 45) on CD4+ cells extracted from peripheral blood samples in an IPEX patient.**

Immunolabeling of cell suspensions was performed in 1% bovine serum albumin and 3% human serum-phosphate-buffered saline, according to standard methods using specific antibodies directed against CD3, CD4 (both from BD Pharmingen, Pont de Claix, France) and FOXP3 (eBioscience, San Diego, CA). The cells were then quantified by flow cytometry on a FACS LSRII with FACSDiva software (Becton Dickinson, Pont de Claix, France) and analysis was performed with the FlowJo software (TreeStar, Ashland, Oregon). Percentages (A) and mean of fluorescence intensity (B) of FOXP3 cell subsets among CD4<sup>+</sup> cells were determined after gating on lymphocyte CD3<sup>+</sup> T cells.



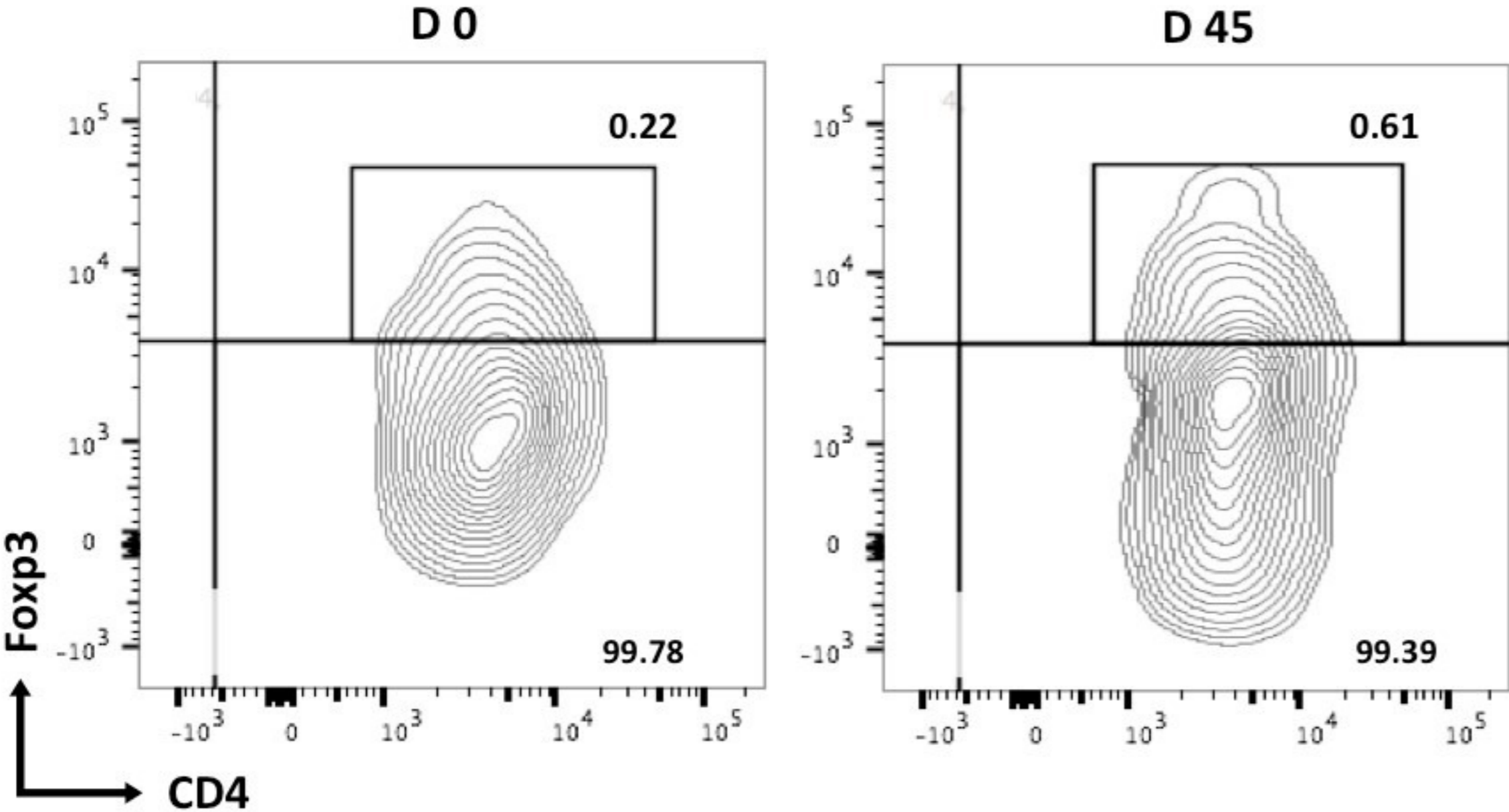
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Figure 2

A



B

