

SHORT REPORT

Severe inflammatory bowel disease associated with congenital alteration of transforming growth factor beta signaling



Samuele Naviglio^{a,*}, Serena Arrigo^b, Stefano Martellosi^c,
Vincenzo Villanacci^d, Alberto Tommasini^c, Claudia Loganes^c,
Antonella Fabretto^c, Silvia Vignola^b, Silvia Lonardi^d, Alessandro Ventura^{a,c}

^a University of Trieste, Trieste, Italy

^b G. Gaslini Institute for Children, Genoa, Italy

^c Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy

^d Spedali Civili, Brescia, Italy

Received 12 September 2013; received in revised form 14 January 2014; accepted 15 January 2014

KEYWORDS

Loeys–Dietz syndrome;
Connective tissue diseases;
Inflammatory bowel disease;
Colitis;
Transforming growth factor
beta

Abstract

Transforming growth factor beta is a pleiotropic cytokine which plays a central role in the homeostasis of the immune system. A complex dysregulation of its signaling occurs in Loeys–Dietz syndrome, a monogenic disorder caused by mutations of transforming growth factor beta receptors type 1 or type 2, characterized by skeletal involvement, craniofacial abnormalities, and arterial tortuosity with a strong predisposition for aneurysm and dissection. In addition, several immunologic abnormalities have been described in these patients, including an increased risk of allergic disorders as well as eosinophilic gastrointestinal disorders. The occurrence of inflammatory bowel disorders has been also reported, but it is poorly documented. We describe two unrelated children with Loeys–Dietz syndrome affected by severe chronic inflammatory colitis appearing at an early age. The intestinal disease presented similar features in both patients, including a histopathological picture of non-eosinophilic chronic ulcerative colitis, striking elevation of inflammatory markers, and a distinctly severe clinical course leading to failure to thrive, with resistance to multiple immunosuppressive treatments. One of the patients also presented autoimmune thyroiditis. Our report confirms

Abbreviations: TGF- β , transforming growth factor beta; LDS, Loeys–Dietz syndrome; TGFBR-1, transforming growth factor beta receptor type 1; TGFBR-2, transforming growth factor beta receptor type 2; Treg, regulatory T cells; IL, interleukin; Th17, IL-17-producing T helper cells; Th2, Type 2 helper T cell; UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease.

* Corresponding author at: Department of Pediatrics, University of Trieste, via dell'Istria 65/1, 34100 Trieste, Italy. Tel.: +39 0403785312; fax: +39 0403785452.

E-mail addresses: samuele.naviglio@gmail.com (S. Naviglio), serena.arrigo@ospedale-gaslini.ge.it (S. Arrigo), stefano.martellosi@burlo.trieste.it (S. Martellosi), villanacci@spedalicivili.brescia.it (V. Villanacci), alberto.tommasini@burlo.trieste.it (A. Tommasini), claudia.loganes@gmail.com (C. Loganes), antonella.fabretto@burlo.trieste.it (A. Fabretto), silvia.vignola@ospedale-gaslini.ge.it (S. Vignola), lonardi@med.unibs.it (S. Lonardi), alessandro.ventura@burlo.trieste.it (A. Ventura).

that chronic ulcerative colitis may be associated with Loeys–Dietz syndrome. This finding suggests that an alteration of transforming growth factor beta signaling may by itself predispose to inflammatory colitis in humans, and represent an invaluable model to understand inflammatory bowel diseases.

© 2014 European Crohn's and Colitis Organisation. Published by Elsevier B.V. All rights reserved.

1. Introduction

Transforming growth factor beta (TGF- β) is a pleiotropic cytokine which plays a central role in the regulation of the immune response to mucosal antigens.¹ Dysregulated TGF- β signaling occurs in Loeys–Dietz syndrome (LDS), an autosomal dominant disorder caused by heterozygous mutations of the genes encoding transforming growth factor beta receptor type 1 or 2 (TGFB β -1 and TGFB β -2, encoded by *TGFB β 1* and *TGFB β 2*, respectively). The disease is characterized by skeletal involvement (joint hyperlaxity, arachnodactyly, pectus deformity, scoliosis, vertebral instability) and arterial tortuosity with a strong predisposition for aneurysm and dissection. Craniofacial abnormalities (hyperthelormism, cleft palate or bifid uvula, craniosynostosis) may or may not be present.² Recently, an increased risk of immunologic abnormalities, including allergic disorders and eosinophilic gastrointestinal disorders, has been described in these patients.^{3,4} The occurrence of inflammatory bowel diseases (IBD) has been also reported,⁵ yet it is poorly documented. We report two unrelated children with LDS affected by severe inflammatory colitis appearing at an early age.

2. Case report

2.1. Case 1

Patient 1 is a female child who from the age of 13 months developed chronic bloody diarrhea and intermittent fever, eventually leading to failure to thrive. Laboratory tests showed persistently elevated erythrocyte sedimentation rate (ESR, 80–100 mm/h), hypergammaglobulinemia, and chronic anemia. There was no blood eosinophilia nor a story of allergic disorders. She also presented peculiar phenotypic features (hyperthelormism, proptosis, blue sclerae, joint hyperlaxity, arachnodactyly, mild dilation of the ascending aorta, and cervical spine instability) which had led to a diagnosis of LDS that had been confirmed by demonstration of a mutation of *TGFB β 2* already described in LDS (p.R528C).² Infectious causes of diarrhea (including bacterial and viral pathogens, tuberculosis, and parasites) were excluded. The evaluation of the immune system was normal except for a moderate deficiency of recent thymic emigrant T cells (defined as CD4+ CD45RA+ CD31+ cells, 23% of all CD4+ cells at 4 years of age; normal values 37–100%),⁶ while regulatory T cells (Treg, defined as CD4+ cells highly expressing CD25 and FOXP3) were normal. Testing for chronic granulomatous disease resulted negative. Genetic testing for interleukin (IL)-10 receptor mutations resulted negative. A computed tomography angiography scan did not show alterations of the mesenteric vessels. At colonoscopy, all the colonic mucosa

appeared fragile, edematous and diffusely ulcerated (Fig. 1). At esophagogastroduodenoscopy the gastric mucosa appeared hyperemic with some petechiae, while the duodenal mucosa appeared normal. Histopathological examination of the colonic biopsies appeared suggestive of chronic active ulcerative colitis (UC), with a moderate-to-severe inflammatory infiltrate made of lymphocytes, plasma cells, neutrophils and some eosinophils in the lamina propria; architectural distortion of the crypts (Fig. 2A); crypt abscesses (Fig. 2B); basal plasmocytosis (Fig. 2C); goblet cells depletion; and Paneth cell metaplasia. There was no evidence of granulomas. Immunohistochemical study of the colonic mucosa showed CD8+ cells being 25% of all CD3+ cells, GATA3+ cells 85% of all CD3+ (Fig. 3), and FOXP3+ cells being 5% of all CD3+ cells. A moderate lymphoplasmacellular infiltrate was also evident in the lamina propria of stomach and duodenum. Oral mesalazine and prednisone were started with good clinical response, yet the disease relapsed on prednisone tapering. Adding intravenous cyclosporine and oral azathioprine resulted only in limited improvement. Infliximab at 10 mg/kg was therefore added to azathioprine and prednisone, while cyclosporine was discontinued, yet with only modest clinical improvement, while tapering corticosteroids often resulted in clinical relapses that required an increase of prednisone and short courses of antibiotics (ciprofloxacin and metronidazole). After one year (13 infusions) infliximab was discontinued. At 4 years



Figure 1 Colonoscopy of patient 1. The colonic mucosa appears diffusely hyperemic and ulcerated.

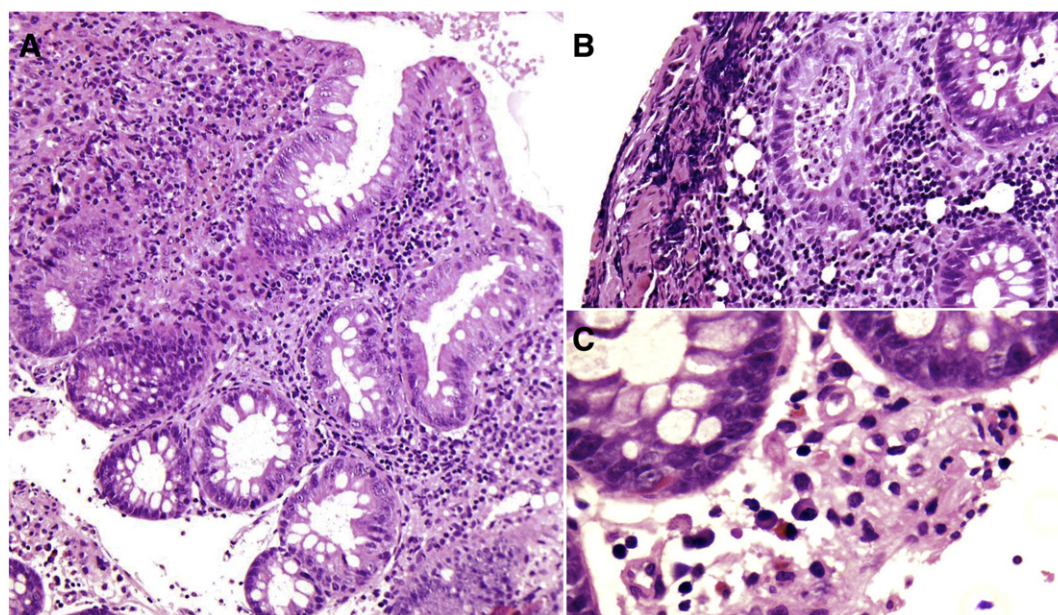


Figure 2 Colonic biopsy from patient 1. A: Ulcerative colitis, active phase, with architectural distortion of crypts and mixed inflammatory infiltrate in lamina propria (magnification $\times 20$). B: Crypt abscess ($\times 40$). C: Basal plasmocytosis ($\times 100$). Hematoxylin and eosin.

of age she developed two perianal extrasphincteric abscesses that were treated with antibiotic therapy. At 30 months of age she was also diagnosed with autoimmune thyroiditis because of subclinical hypothyroidism, anti-thyroperoxidase antibodies, and thyroid ultrasonography. Treatment with levothyroxine was started. Celiac disease had been excluded. Notably, treatment with losartan was also started for LDS at 20 months of age (at 0.7 mg/kg) with no improvements of the intestinal disease, which appears still active now at 4 years and 6 months of age.

2.2. Case 2

Patient 2 is a male child who had received a diagnosis of LDS because of typical phenotypic features (hyperthelormism, blue sclerae, bifid uvula, arachnodactyly, joint hyperlaxity, pectus carinatum, pes valgus, and dilation of the aortic root). The diagnosis had been confirmed by genetic analysis (de novo mutation of *TGFBR1*, p.G217R), and he had begun treatment with losartan and carvedilol. From 26 months of age he developed chronic bloody diarrhea, intermittent

fever and failure to thrive, with anemia, hypergammaglobulinemia and elevated ESR (60–70 mm/h). Infectious causes of colitis were excluded. Evaluation of the immune system was within normal limits, including the percentage of Treg in peripheral blood. Testing for chronic granulomatous disease resulted negative. Mutation analysis of the IL-10 receptor gene was normal. There was no story of allergic disorders. Colonoscopy showed involvement of the entire colon with normality of the terminal ileum. The colonic mucosa appeared diffusely congested, edematous, and ulcerated. Pathologic examination demonstrated severe involvement of the entire mucosal layer by a mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils and some eosinophils, with architectural distortion of the crypts, crypt abscesses, and no evidence of granulomas. The gastric and duodenal mucosa appeared grossly normal, yet a moderate lymphocytic and plasma cellular infiltrate was present in the lamina propria of the duodenum. Oral corticosteroids were started with good clinical response, but the disease relapsed on their discontinuation. A second short course of corticosteroids was started together with azathioprine (2.75 mg/kg), achieving remission for

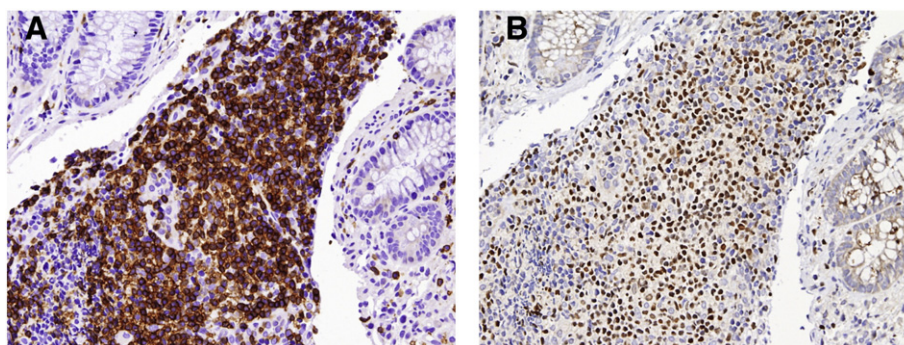


Figure 3 Immunohistochemical study of colonic mucosa from patient 1. A: CD3+ cells. B: GATA3+ cells.

15 months. The intestinal disease nevertheless relapsed, while still on azathioprine therapy, and subsequently became resistant also to systemic corticosteroids. Subcutaneous adalimumab at 3 mg/kg was started, leading only to partial improvement, and was therefore discontinued after one year. The intestinal disease is still active now at 6 years of age.

3. Discussion

The occurrence of gastrointestinal complaints is common in LDS. In the article by Frischmeyer-Guerrero et al.³ about half of the patients reported gastrointestinal complaints including repetitive vomiting, chronic abdominal pain, or dysphagia. Ten of these patients had gastrointestinal biopsies performed: six showed overt histological evidence of eosinophilic esophagitis, and of these six, five were found to have eosinophilic gastritis and four had eosinophilic colitis. The occurrence of non-eosinophilic chronic IBD has been also reported in LDS,⁵ but it is poorly documented. To our knowledge this is the first full clinical and histopathological description of IBD associated with LDS. Remarkable features in our patients were the early age at onset; the histopathological picture of chronic UC, not suggestive of eosinophilic colitis; moderate inflammatory involvement of the upper gastrointestinal tract – a common finding in pediatric UC;⁷ prominent systemic symptoms with fever, persistently elevated ESR and hypergammaglobulinemia; and a distinctively severe chronic clinical course leading to failure to thrive. Good clinical response to oral corticosteroids was observed, yet with frequent relapses on their tapering and marked resistance to several other therapeutic regimens. In patient 2 resistance to systemic corticosteroids eventually developed. Notably patient 1 also developed extrasphincteric abscesses, a feature more commonly associated with Crohn's disease (CD) than with UC. In this respect, while the histopathological features were considered more akin to active UC than CD, the whole clinical picture may be difficult to categorize in the standard IBD classification. In fact, classification of IBD appearing in the very first years of life may not always be straightforward. Of further interest, patient 1 presented autoimmune thyroiditis, which is also suggestive of an alteration of immunologic tolerance.

The occurrence of early onset chronic inflammatory colitis in our patients confirms the possibility of a true association between LDS and IBD, and the monogenic nature of LDS suggests a direct role for the alteration of TGF- β signaling in the pathogenesis of the immunologic disorders in LDS.³ In most cases of LDS, the disease-causing mutations affect either TGFBR-1 or TGFBR-2 intracellular kinase domain, resulting in a dysfunctional receptor. This alteration, however, seems to cause a paradoxical increase of downstream effectors,² possibly because of impaired autoregulation, altered receptor trafficking, or alternative signaling cascades.⁸ Recently Frischmeyer-Guerrero et al.³ demonstrated that this enhanced signaling also occurs in CD4⁺ lymphocytes from LDS patients, who also have an increased frequency of peripheral IL-13-producing Tregs. The authors concluded that the primary defect of TGF- β signaling in LDS seems to confer lymphocytes with an intrinsic propensity to acquire Th2 effector functions when stimulated with TGF- β . While this may explain the increased prevalence of allergic disorders in LDS patients, it

may be also associated with UC. In fact, UC has been described as an atypical Th2 immune response, characterized by an increased production of IL-13 in the lamina propria and mediated by non-classical IL-13-producing natural killer T-cells.⁹ Also in our case, the immunologic study of the colonic mucosa of patient 1 showed a strong presence of GATA3⁺ CD3⁺ cells, suggesting a prevalence of Th2 inflammatory cells.

Notably, mice bearing an inactivating mutation in the intracellular kinase domain of TGFBR-2 on T lymphocytes do develop severe inflammatory intestinal involvement, autoimmune cholangitis, hypergammaglobulinemia, and autoimmune antibodies.¹⁰ It should be remembered that TGF- β has a central role in directing the immune responses to mucosal antigens, with both anti-inflammatory and pro-inflammatory effects. In fact, both Treg, which control inflammation triggered by resident gut microflora, and IL-17-producing T helper cells (Th17), which are mainly pro-inflammatory, are thought to arise from a common precursor, and TGF- β helps to direct differentiation of both subsets. While TGF- β alone induces the differentiation of Treg cells, TGF- β together with other signals, including microbial signals and pro-inflammatory cytokines, induces the differentiation of Th17 cells.^{11,12}

Both patients were also receiving losartan, which is used in LDS because of its inhibiting effect on TGF- β signaling.¹³ Frischmeyer-Guerrero et al. demonstrated that losartan mitigates TGF- β signaling alterations in lymphocytes of LDS patients, and suggested that this treatment may hold promise to modify the clinical immunologic disorders. In our limited experience, treatment with losartan did not result in any clinical improvement of IBD. While another angiotensin receptor blocker, olmesartan, has been linked to a spruelike enteropathy,¹⁴ this has never been reported for losartan, therefore any adverse effect of the drug seems unlikely, also because in patient 1 intestinal symptoms predated any therapy.

In conclusion, our report supports the possibility of a true association of IBD with LDS. If confirmed, this suggests that dysregulated TGF- β signaling may be sufficient to predispose to the development of inflammatory colitis in humans, and represent an invaluable model for our understanding of IBD in general population.

4. Methods

Genomic DNA was extracted from 1 to 2 ml EDTA-anticoagulated blood using the EZ1 DNA Blood 350 μ l Kit (Qiagen, The Netherlands). Polymerase chain reaction amplification was performed for the entire coding, for the flanking regions and for the untranslated region at 5' and 3' of the genes IL10RA, IL10RB, using the KAPA 2G Fast Hot Start Readymix (Resnova, Italy). Purified polymerase chain reaction products were directly sequenced in both directions using the amplification primers by the Sanger method and used the ABI PRISM 3130XL automated DNA sequencer (Life technologies, Carlsbad, CA, USA). Sequences were analyzed with Seqman II Software (DNASTAR I Lasergene). Primer used for PCR and sequencing was designed using Primer Blast based on the relative sequence deposited in the GenBank.

Mutational analysis for *TGFBR1* and *TGFBR2* was performed at the Biomedical Sciences and Biotechnology Department Laboratory at University of Brescia, Italy.

Immunofluorescence staining for peripheral blood Tregs was performed by using the Human Treg Flow TM kit (Biolegend, San Diego, CA, USA) and according to the manufacturer's instruction. Samples were acquired with a CyAn™ ADP flow cytometer (Dako, Denmark) and collected data were analyzed by using the FlowJo software (Tree Star inc., Ashland, OR, USA).

Biopsies were formalin-fixed and paraffin embedded, then 4 mm-thick serial-cut sections were used of immunohistochemical staining. CD3+ cells were stained using CD3 rabbit monoclonal SP7, dilution 1:100 (Thermo Scientific, Fremont, CA, USA) and CD8: Mouse IgG1, clone C8/144B dilution 1:30 (Dako, Denmark); Foxp3: Rat IgG2a, clone PCH101, dilution 1:200 (eBioscience, San Diego, CA, USA); GATA3: Mouse IgG1, clone L50-823, dilution 1:300 (BD Biosciences, San Jose, CA, USA): after the appropriate antigen retrieval and revealed using EnVision (Dako, Glostrup, Denmark) and Novolink Polymer (Novocastra Laboratories, Newcastle upon Tyne, UK), respectively. Diaminobenzidine was used as chromogen and Meyer's Ematoxylin as counterstaining.

Acknowledgments

Samuele Naviglio cared for the patients, wrote the first draft of the manuscript, and approved the final manuscript as submitted.

Serena Arrigo, Stefano Martelossi, Silvia Vignola, and Alessandro Ventura cared for the patients, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Vincenzo Villanacci and Silvia Lonardi performed and reviewed pathological studies, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Alberto Tommasini and Claudia Loganese performed and reviewed immunologic studies, reviewed and revised the manuscript, and approved the final manuscript as submitted.

All authors read and approved the final manuscript. None of the authors have any competing interests to declare. There is no funding source for this manuscript.

References

- [1]. Di Sabatino A, Pickard KM, Rampton D, Kruidenier L, Rovedatti L, Leakey NAB, et al. Blockade of transforming growth factor beta upregulates T-box transcription factor T-bet, and increases T helper cell type 1 cytokine and matrix metalloproteinase-3 production in the human gut mucosa. *Gut* 2008;**57**(5):605–12.
- [2]. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. *Nat Genet* 2005;**37**(3):275–81.
- [3]. Frischmeyer-Guerrero PA, Guerrero AL, Oswald G, Chichester K, Myers L, Halushka MK, et al. TGFβ receptor mutations impose a strong predisposition for human allergic disease. *Sci Transl Med* 2013;**5**(195):195ra94.
- [4]. Abonia JP, Wen T, Stucke EM, Grotjan T, Griffith MS, Kemme KA, et al. High prevalence of eosinophilic esophagitis in patients with inherited connective tissue disorders. *J Allergy Clin Immunol* 2013;**132**(2):378–86.
- [5]. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. *N Engl J Med* 2006;**355**(8):788–98.
- [6]. Schatorjé EJH, Gemen EFA, Driessen GJA, Leuvenink J, van Hout RWNM, de Vries E. Paediatric reference values for the peripheral T cell compartment. *Scand J Immunol* 2012;**75**(4):436–44.
- [7]. Abdullah BA, Gupta SK, Croffie JM, Pfefferkorn MD, Molleston JP, Corkins MR, et al. The role of esophagogastroduodenoscopy in the initial evaluation of childhood inflammatory bowel disease: a 7-year study. *J Pediatr Gastroenterol Nutr* 2002;**35**(5):636–40.
- [8]. Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. *Nature* 2011;**473**(7347):308–16.
- [9]. Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest* 2004;**113**(10):1490–7.
- [10]. Gorelik L, Flavell RA. Abrogation of TGFβ signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 2000;**12**(2):171–81.
- [11]. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;**441**(7090):235–8.
- [12]. Zhou L, Lopes JE, Chong MMW, Ivanov II, Min R, Victora GD, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORγt function. *Nature* 2008;**453**(7192):236–40.
- [13]. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 2006;**312**(5770):117–21.
- [14]. Rubio-Tapia A, Herman ML, Ludvigsson JF, Kelly DG, Mangan TF, Wu T-T, et al. Severe spruelike enteropathy associated with olmesartan. *Mayo Clin Proc* 2012;**87**(8):732–8.