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Indolent T-cell lymphoproliferative disease of the gastrointestinal tract after treatment with adalimumab in resistant Crohn's colitis

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Summary

We report a case of intestinal indolent T-cell lymphoproliferative disease (TCLPD) occurring after the initiation of tumor necrosis factor−α (TNF-α) inhibitor therapy for resistant Crohn's disease. A prominent T-cell infiltrate positive for CD8, TIA-1, and T-cell receptor–βF1 was associated with the foci of active inflammation. T-cell receptor gene clonality studies (BIOMED-2) demonstrated monoclonality. After the TNF-α inhibitor treatment was withdrawn, the T-cell infiltrates regressed, but 2 years later, the same monoclonal T-cell infiltrate reappeared at the only site of active inflammation. To the best of our knowledge, this report is the first to show a link between active inflammation and the TCLPD. In addition, it suggests a possible influence of the TNF-α inhibitor treatment on the evolution of the TCLPD. A high degree of suspicion is required in the presence of any unusual lymphoid infiltrate in inflammatory bowel disease to avoid over-looking an indolent TCLPD or misdiagnose an aggressive lymphoma.

Keywords

Indolent T-cell lymphoproliferative disease; Immunosuppression; Inflammatory bowel disease; Tumor necrosis factor–α inhibitor

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1. Introduction

Primary T-cell lymphomas of the gastrointestinal tract (GIT), the most common of which is enteropathy-associated T-cell lymphoma, account for almost 15% of all intestinal lymphomas and are aggressive neoplasms that do not respond to current therapies.

We describe an indolent CD8-positive cytotoxic $\alpha\beta$ T-cell lymphoproliferative disease (TCLPD) in a patient treated with tumor necrosis factor–α (TNF-α) inhibitor for resistant Crohn's disease. TNF-α inhibitors are increasingly used in the setting of autoimmune and chronic inflammatory disease in patients unresponsive to conventional treatments, including idiopathic inflammatory bowel disease (IBD). Data from the FDA adverse events reporting system and a review of published cases demonstrate an increased risk for the development of malignant lymphoma, especially hepatosplenic T-cell lymphoma (HSTCL), in Crohn's disease treated by immunosuppressive therapies including TNF-α inhibitors [1]. In contrast, most studies do not find any significant increase in the incidence of lymphoma in the absence of immunosuppressive therapy, neither in the total IBD population nor in patients with Crohn's colitis [2,3].

Indolent TCLPD of the GIT is a recently described rare entity with only a few cases reported since the 1990s [4–9]. Several cases occurred in patients with idiopathic IBD including Crohn's disease, and some of them were previously treated by immunosuppressive therapy [7,8]. These cases have variable phenotypes including CD4-positive T-cells, CD4/CD8 double negative, whereas most are CD8-positive, but nearly all are $\alpha\beta$ T-cells. The distinction between indolent TCLPD and an HSTCL of the GIT is still mostly based on the clinicopathological features. The histologic features of this entity, in conjunction with the clonality of the T-cell infiltrate in the context of an immunosuppressive treatment, can lead to confusion with HSTCL and to an unnecessarily aggressive therapy.

2. Materials and methods

2.1. Clinical story

A 27-year-old woman presented with a 15-year history of IBD diagnosed as Crohn's disease after several relapses. She did not undergo any surgical intervention during the course of her disease. She was initially treated with 5-aminosalycilic acid (Rafassal), and steroid enemas were added later. After 9 years, she became unresponsive; azathioprine was added, and after 21 months, discontinued due to leukopenia and patient intolerance. Endoscopic biopsies during this period were consistent with IBD. As a consequence of a poor response to the treatment, TNF-α inhibitor (adalimumab, Humira) was initiated without improvement after 7 months of therapy. The colonoscopy performed then showed a small inflammatory pseudopolyp with severe inflammation in the sigmoid colon, polypoid prominences in the ascending colon, edema and inflammation of the cecum without noticeable mass. Multiple biopsies were taken from the cecum to the rectum. At that time, her blood count and LDH levels were within normal limits. No hepatosplenomegaly was demonstrated by physical examination or imaging studies. Serologic studies were negative for Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus.

2.2. Immunohistochemistry

Three-micrometer formalin-fixed paraffin-embedded (FFPE) sections were processed using an automated immunostainer (Benchmark; Ventana Medical Systems, Phoenix, AZ, USA) and stained with antibodies against CD3, CD2, CD4, CD8, CD5, CD7, CD10, Granzyme B, TIA-1, CD56, CD57, TdT, Bcl2, CD20, CD23, and T-cell receptor (TCR)–βF1.

2.3. Clonality studies

Molecular analysis for *TCRG*, *TCRB*, and *TCRD* genes rearrangement was performed after DNA isolation from the paraffin blocks of the intestinal biopsies, using the polymerase chain reaction (PCR) and GeneScan analysis, according to the recommendations and the established BIOMED-2 protocols of the EuroClonality consortium [10].

2.4. Genetic study

Mutational analysis was performed on DNA extracted from selected FFPE biopsies with prominent infiltration by CD8-positive and clonal T-cell populations, using next-generation sequencing approach targeting 40 genes involved in T-cell lymphomagenesis. Among the genes included in the panel are *JAK1, JAK2, JAK3, STAT3 STAT5B, TET2, DMNT3A, RHOA, IDH1, IDH2, NOTCH1, NRAS, KRAS, HRAS, BRAF,* and *PIK3CA*.

2.5. Histological, immunophenotypic, and molecular findings

The biopsies demonstrated foci of active chronic inflammation in the cecum, ascending colon, and sigmoid. The foci of active IBD were associated with a dense infiltrate of small lymphocytes widening the lamina propria without crypts destruction, necrosis, or ulcerations. The normal mucosa was devoid of lymphoid infiltrates. Most of the small lymphocytes were positive for CD3, CD2, CD5, CD7, CD8, TCR-βF1, and TIA-1, and negative for Granzyme-B, CD56, and CD57, with few CD4-positive T-cells (CD4/CD8 ratio approximately 1:4) and tiny aggregates of CD20+ B-cells (Fig. 1). Those findings initially raised a consideration for HSTCL, a recognized complication of immunosuppressive treatment [1,11]. Immunostain for cytomegalovirus was negative. In situ hybridization revealed no evidence of Epstein-Barr virus EBER-1 DNA. GeneScan analyses of the BIOMED-2 PCR studies for T-cell clonality on *TCRG*, *TCRB*, and *TCRD* genes, performed on lymphoid infiltrates associated with the foci of inflammation, disclosed a monoclonal population positive for *TCRG* and *TCRB* (Fig. 1 H–I) and negative for *TCRD* (data not shown), consistent for $\alpha\beta$ T-cells. The normal mucosa was free of lymphoid infiltrates and then no clonality studies were performed on these biopsies. The bone marrow biopsy showed a small interstitial infiltrate of CD8- and TIA-1–positive T-cells, only a few CD4-positive T-cells, and no intrasinusoidal T-cell infiltrate. The molecular studies for *TCRG* gene rearrangement performed on the FFPE tissue from the bone marrow biopsy demonstrated the presence of the same $\alpha\beta$ T-cell clone (data not shown). An upper GIT endoscopy was subsequently performed and did not reveal involvement by the IBD or by the TCLPD. Review of previous biopsies up to 6 years before did not show *TCR* gene rearrangement (Fig. 2A), although the CD4/CD8 ratio was abnormal with a predominance of CD8-positive T-cells in the infiltrates associated with the active inflammation (Fig. 2A). Targeted sequencing of 40 genes associated with several subgroups of T-cell lymphoma was

performed on selected FFPE biopsies showing a high percentage of clonal CD8-positive T-cells but failed to detect any mutations. In particular, mutations associated with HSTCL and aggressive enteropathy-associated T-cell lymphoma of both γ/δ and α/β T-cell origin lymphomas (including *STAT5B, STAT3, JAK1,* and *JAK3*) were not detected ([12] and unpublished data–MR).

HSTCL was ruled out because of the lack of B-signs and hepatosplenomegaly at physical examination confirmed by CT scan and abdominal ultrasonography, normal peripheral blood count, absence of intrasinusoidal T-cell infiltrate in the bone marrow biopsy, CD5/CD8/TCR-βF1–positive phenotype, and the *TCRB*-positive/*TCRD*-negative gene rearrangements. Assuming that the TCLPD was related to the TNF-α inhibitor treatment, adalimumab prescription was withdrawn after 7 months of treatment. The patient was left with Prednisone treatment only (40 mg) and a “wait and see” follow-up was adopted without any other immunomodulatory medication. The subsequent endoscopic biopsies up to 12 months after the TCLPD diagnosis demonstrate a decrease of the CD8 T-cell infiltrate (Fig. 2B). The clonality analysis shows the regression of the monoclonal peak of *TCRG* (Fig. 2B) and *TCRB* gene rearrangements to the background level after the withdrawal of the TNF-α inhibitor. Two years after the cessation of adalimumab (April 2015), 4 months after interruption of the steroid treatment, the endoscopic biopsies from colonoscopy showed a single focus of active inflammation associated with a CD8 T-cell infiltrate (Fig. 2C, left panel). Other biopsies taken from the terminal ileum and along the colon did not demonstrate active inflammation or significant T-cell infiltrate (Fig. 2C, right panel). A PCR study for *TCRG* gene rearrangement performed from the biopsy of the T-lymphocytes infiltrate in the sigmoid colon showed a monoclonal peak at the same location (250 bp) seen 2 years before, consistent with a relapse of the TCLPD (Fig. 2C, left panel). Lymphoid hyperplasia with no active inflammation was seen in terminal ileum without clonal *TCRG* gene rearrangement (Fig. 2C, right panel). At the last follow-up in April 2015 with no treatment, an extensive clinical investigation did not reveal any systemic disease or evidence for an aggressive T-cell lymphoma in the GIT, allowing the diagnosis of a still indolent TCLPD.

3. Discussion

We describe an indolent TCLPD with an inactivated CD8-positive cytotoxic $\alpha\beta$ T-cell phenotype in a patient with resistant Crohn’s disease treated with TNF-α inhibitor.

Indolent TCLPD of the GIT is a recently described entity with morphologic features mimicking HSTCL, an aggressive disease with a dire prognosis [7]. Only a few cases have been documented, mostly case reports with heterogeneous T-cells phenotypes (CD4+, CD4−/CD8−, CD8+) [4–9], suggesting the existence of different entities with a close clinical behavior. Some reported cases occurred in patients with Crohn’s disease [7,8] at least 2 after TNF-α inhibitor therapy (adalimumab and certolizumab) [7]. In addition to these previous reports, to the best of our knowledge, our case is the first to demonstrate a correlation between the active inflammation and the CD8-positive T-cells infiltrates (Figs. 1 and 2), and suggests a possible association between the adalimumab treatment and the development of the TCLPD.

The CD8 polyclonal T-cell aggregates observed in the biopsies before the initiation of the TNF-α inhibitor treatment were only associated with the IBD active inflammation, pointing to a link between the acute inflammatory process and the CD8 T-cells infiltrates. The development of a clonal subpopulation with an identical phenotype in the T-cell infiltrates, shortly after the introduction of the TNF-α inhibitor therapy, suggests that an inflammation-related TNF-pathway could be a key factor in the development of the TCLPD. Consistent with this hypothesis, a recent study of colitis in mice demonstrated the protective role of TNF-receptor 2 (TNFR2, TNFR1b) through the growth inhibition of the colonic mucosal CD8 T-cells [13,14]. TNFR2 has been associated with susceptibility to IBD, through the control of the expression of genes that regulate CD8 T-cells [15,16]. It has been demonstrated that the CD8 T-cells present in the lamina propria of the colonic mucosa play a critical role in the protection against inflammation [17] and in the pathogenesis of resistant Crohn's disease [18]. Disturbance of TNFR2 signaling might therefore be associated with the pathogenesis of IBD, via unrestricted proliferation of intramucosal CD8 T-cells in reaction to the inflammatory stimulus [13].

Of note, the patient showed a consistent inverted CD4/CD8 ratio of the T-cells associated with the active inflammation before the development of the TCLPD (Fig. 2A), as shown also by the flow cytometry studies of her peripheral blood and bone marrow aspiration, suggesting a dysfunctional CD8 T-cell population. In 20 Crohn's disease control cases we examined, the CD4/CD8 ratio of the lymphoid aggregates associated with acute inflammation was within the normal limits (data not shown). Therefore, we postulate that in our reported case, the intramucosal CD8 T-cells are dysfunctional and not normally responding to the TNF-α regulatory pathway. Thus, the observed inverted CD4/CD8 ratio leads to the development of the colitis and the close association of the CD8 T-cell infiltrates with the foci of active inflammation. Hence, introduction of TNF-α inhibitor in this context could have led to the emergence of the CD8 T-cell clone. However, further studies should be performed to confirm or invalidate this hypothesis, particularly genomic studies for specific mutations aimed at the genes involved in the TNF-α/TNFR1/TNFR2 pathway for inflammatory response regulation of the CD8 T-cells in resistant IBD.

The prevalence of IBD is now roughly 0.5% of the total population, Crohn's disease representing nearly 50% of the IBD [19]. In a recent European publication about management of IBD, 20% of the patients are reported to be treated with TNF-α inhibitors [20], and this number is still growing. Our report suggests the need for a screening of the CD4/CD8 ratio and to exclude any dysfunction of the CD8 T-lymphocytes, before initiating a treatment with TNF-α inhibitors in IBD patients. Moreover, any unusually prominent lymphoid infiltrate needs to be analyzed with a high index of suspicion to exclude a monoclonal TCLPD.

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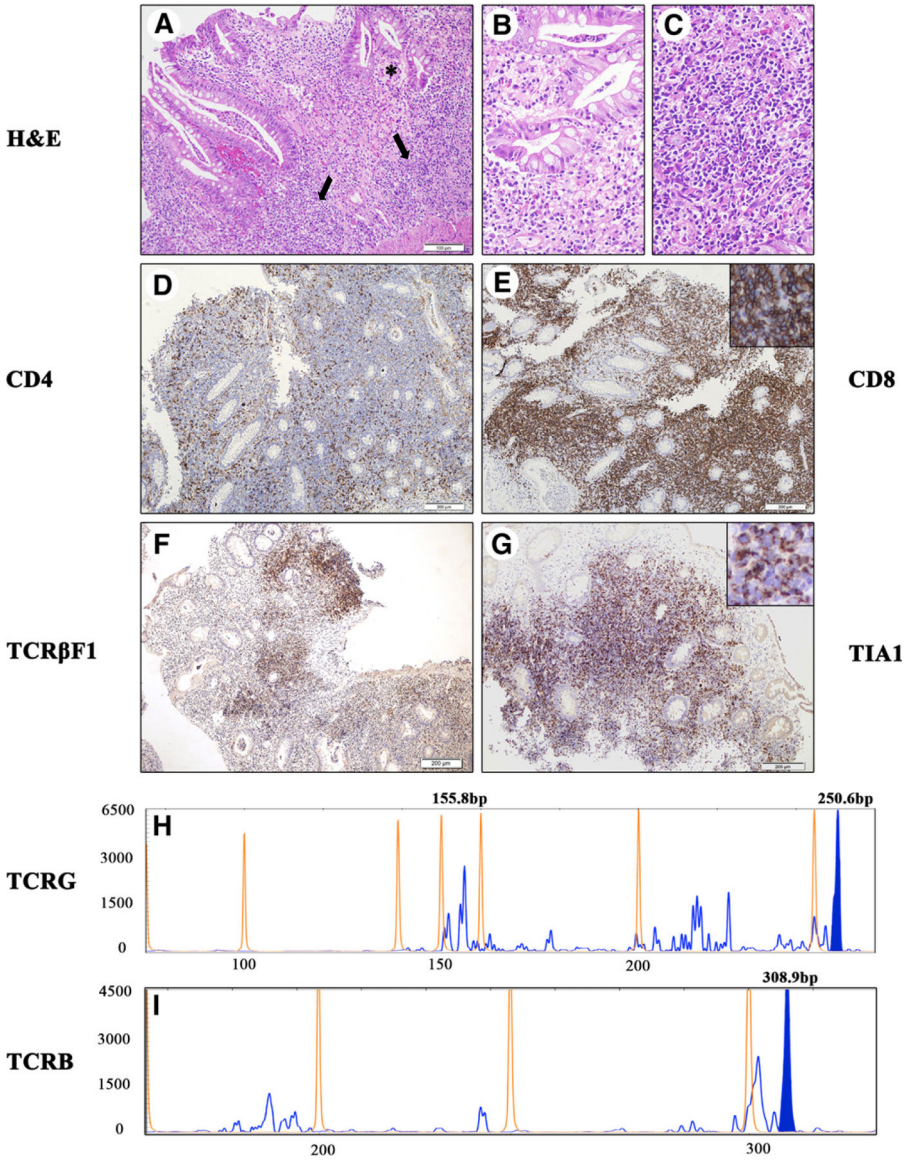

Fig. 1.

Representative features of the endoscopic colonic biopsy with foci of indolent T-cell LPD at diagnosis (2013). A-I, Histology and immunohistochemistry. A, Infiltrates of indolent TCLPD (arrows) are associated with the foci of active IBD (*). The colonic mucosa shows the classic picture of active chronic IBD with numerous eosinophils and proliferation of small blood vessels with prominent endothelial cells in the lamina propria. The crypts are deformed and infiltrated by neutrophils with epithelial reactive changes and formation of crypt abscesses. B, Multiple infiltrates of small lymphocytes expand the lamina propria of the inflamed mucosa without crypt destruction. The indolent TCLPD infiltrate is composed by small lymphocytes with irregular nucleus and scant clear cytoplasm; few lymphocytes infiltrate the crypts without destruction of the epithelium. C, Hematoxylin and eosin stain, original magnification, ×200; scale bar, 100 μm. D-G, Most small lymphocytes in the infiltrate express (E) CD8, (F) TCRβ-F1, and (G) TIA-1, a phenotype of inactivated

cytotoxic $\alpha\beta$ T-cells. Only a few cells express (D) CD4 with an inverted CD4/CD8 ratio. (D-G, original magnification, ×100; scale bar, 200 μm). H-I, The BIOMED-2 PCR studies for (H) *TCRG* and (I) *TCRB* gene rearrangement after TNF-α inhibitor therapy revealed a reproducible monoclonal peak, which is observed at 309 bp for *TCRB* (I, expected range, 170–210 bp, 285–325 bp) and 250 bp for *TCRG* (H, expected range, 145–255 bp).

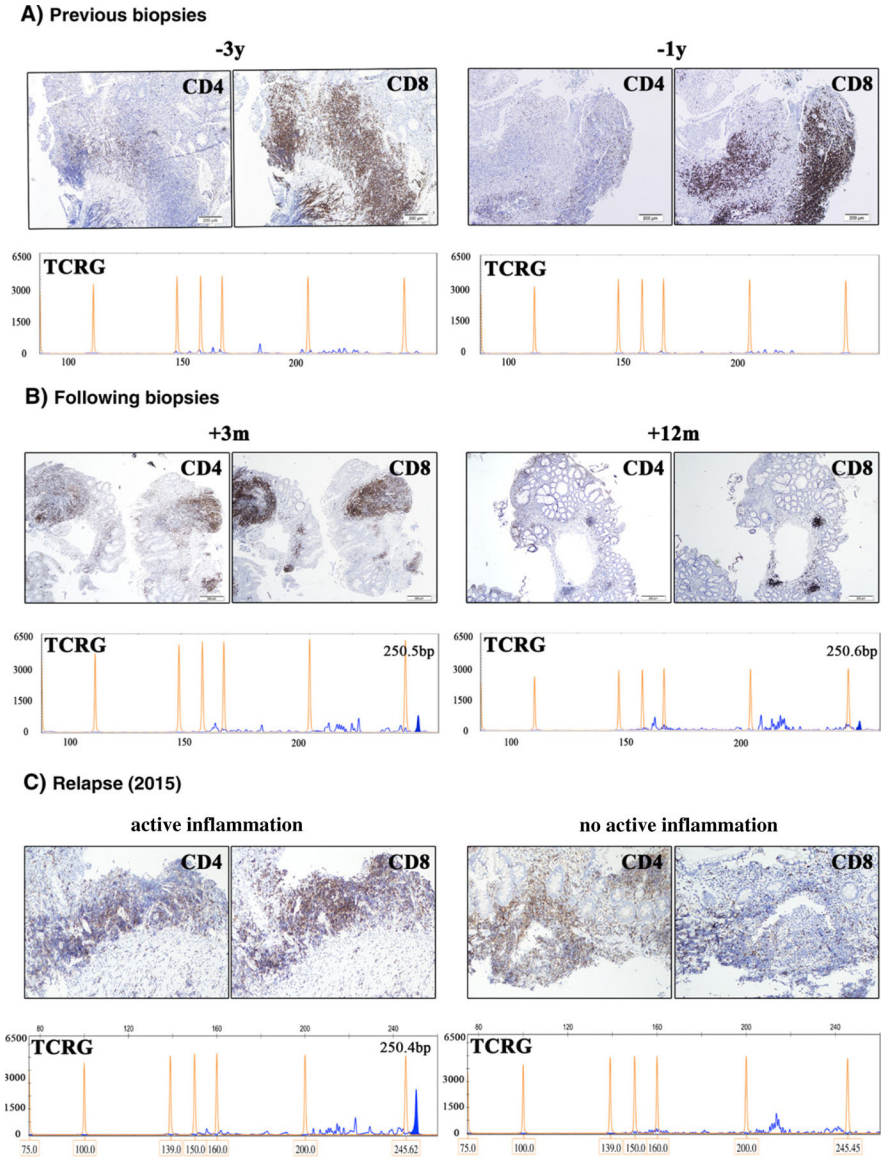

Fig. 2.

CD4 and CD8 immunostains of the lymphoid infiltrates and PCR studies for *TCRG* gene rearrangement before and after TNF-α inhibitor treatment. A, Before the occurrence of the indolent TCLPD. The colonic biopsies taken 3 years (2010) and 1 year (2012) prior the development of the indolent TCLPD show the same picture of a more or less prominent lymphocytic infiltrate closely associated with the foci of active inflammation and predominantly composed of CD8-positive T-cells with a marked inversed CD4/CD8 ratio. (Original magnification, ×100; scale bar, 200 μm). The BIOMED-2 PCR studies for *TCRG* gene rearrangement made from the infiltrates of CD8+ T-cells do not show evidence of monoclonality. B, After withdrawal of the TNF-α inhibitor treatment. Three months and 1 year after the cessation of the TNF-α inhibitor, the follow-up biopsies demonstrate a progressive attenuation of the T-cells infiltrates with a less prominent CD8-positive population and slight increase in the number of CD4-positive T-cells (original magnification,

×40; scale bar, 500 μm). The *TCRG* clone at 250 bp progressively regressed to the level of the polyclonal background. C, Representative colonic and terminal ileum biopsies at recurrence of the indolent TCLPD (2015). The active IBD inflammation in sigmoid colon is associated with a predominantly CD8-positive T-cells infiltrate. The *TCRG* gene rearrangement of this focus demonstrates the recurrence of the clonal peak at 250 bp, identical to the previously observed peak of the indolent TCLPD (2013). The terminal ileum biopsy shows a prominent lymphoid infiltrate without active inflammation. The CD4/CD8 ratio is within normal limits (original magnification, ×100; scale bar, 200 μm). The *TCRG* gene rearrangement of this infiltrate shows a reactive polyclonal T-cell population.