J Hematopathol (2009) 2:121–126
DOI 10.1007/s12308-009-0029-9

CASE REPORT

T cell lymphoproliferative disorders associated with anti-tumor necrosis factor alpha antibody therapy for ulcerative colitis: literature summary

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Received: 27 August 2008 / Accepted: 9 February 2009 / Published online: 18 March 2009

Abstract The enhanced risk of development of lymphoproliferative disorders in patients with inflammatory bowel disease has been attributed to immunosuppressive/immunomodulatory therapies. Infliximab is a chimeric monoclonal immunoglobulin G1 antibody directed against tumor necrosis factor alpha (TNF-α) that was approved by the Food and Drug Administration (FDA) in 1998 as an effective therapeutic agent against inflammatory bowel disease. Malignant lymphomas of both B and T cell lineage have been described in patients undergoing therapy involving TNF-α blockade. To date, eight cases of Epstein–Barr virus (EBV)-negative hepatosplenic T cell lymphoma associated with infliximab have been reported to the FDA's Adverse Event Reporting System, as well as several other T cell lymphoproliferative disorders with aggressive clinical outcomes. We present the histologic, immunophenotypic, and molecular features of a T cell lymphoproliferative disorder involving the axillary lymph node of a 33-year-old male following infliximab treatment for ulcerative colitis. These EBV-negative lymphomas suggest that lymphoproliferative disorders following infliximab treatment for inflammatory bowel disease may involve EBV-independent immune dysregulation. The spectrum of lymphoproliferative disorders associated with infliximab and the potential mechanisms by which they occur are discussed.

Keywords Inflammatory bowel disease · T cell lymphoma · Epstein–Barr virus · Infliximab · Anti-tumor necrosis factor alpha

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Introduction

There is an increased risk of development of lymphoproliferative disorders in patients with inflammatory bowel disease, represented by both ulcerative colitis and Crohn's disease. This increased risk may be due to altered lymphoid proliferation, immunologic defects, and ongoing chronic inflammation seen in patients suffering from inflammatory bowel disease. Most of the cases of lymphomas which have been described in inflammatory bowel disease patients have been non-Hodgkin lymphomas, although several cases of Hodgkin lymphoma have also been described. The emergence of a neoplastic clone within long-standing chronic inflammation may contribute to this predisposition to lymphoma [1].

Many of the approved therapeutics for inflammatory bowel disease involve immune modulation through immunosuppressive agents. Immunosuppressive agents such as azathioprine and mercaptopurines are associated with an increase in the relative risk of lymphoma development [2–4]. Many of these lymphomas are positive for Epstein–Barr virus RNA.

More recently, biologic therapeutic agents such as infliximab, which is a tumor necrosis factor alpha (TNF-α) receptor blocker, have become increasingly utilized in the treatment of inflammatory bowel disease. These agents work by decreasing T-cell-mediated effects of the immune system [5–7]. Recent literature has begun to suggest a correlation with the use of these agents with the development of malignant lymphomas. Currently, the US Food and Drug Administration's (FDA) Adverse Event Reporting system has received reports of eight hepatosplenic T cell lymphomas (HSTCL) in patients treated with infliximab for inflammatory bowel disease [8], suggesting a mechanism causing these lymphomas which is distinct from the

Epstein–Barr virus (EBV)-positive lymphomas seen in inflammatory bowel disease patients receiving immunosuppressive therapy such as steroids, azathioprine, and mercaptopurines. In this report, we describe the morphologic, immunophenotypic, and molecular features of an EBV-negative peripheral T cell lymphoproliferative disorder seen in a patient treated with infliximab for ulcerative colitis.

Materials and methods

Histologic and immunophenotypic studies

The morphologic features of the case were assessed on formalin fixed paraffin-embedded tissue sectioned into hematoxylin-and-eosin-stained slides. Immunohistochemical studies were performed using an avidin–biotin–peroxidase complex method and an automated immunostainer (Ventana-Biotech, Tucson, AZ, USA). The following immunostains were performed: CD2, CD3, CD4, CD5, CD7, CD8, CD15, CD20, CD21, CD30, CD56, fascin, Alk-1, TIA-1, and beta F1. Appropriate positive and negative controls were used for all immunohistochemical studies. The sources of antibodies used are as follows: anti-CD2 (Novocastra, Norwell, MA, USA), anti-CD3 (Ventana, Tucson, AZ, USA), anti-CD4 (Novocastra), anti-CD5 (Novocastra), anti-CD7 (Novocastra), anti-CD8 (Novocastra), anti-CD15 (Becton Dickinson, San Jose, CA, USA), anti-CD20 (Ventana), anti-CD21 (Dako, Carpenteria, CA), anti-CD30 (Dako), anti-CD56 (Accurate, Westbury, NY, USA), fascin (Dako), ALK-1 (Dako), TIA-1 (Biocare Medical, Concord, CA, USA), and beta-F1 (Endogen, Rockford, IL, USA).

Molecular studies

In situ hybridization

In situ hybridization for EBV was performed as previously described [9]. An automated in situ hybridization instrument was used to perform the hybridization. EBER-1, an EBV-encoded digoxigenin-labeled RNA riboprobe, was diluted in a 50% formamide-containing buffer and applied to the tissue sections and to appropriate positive and negative controls. An antisense riboprobe directed against a small nuclear ribonucleoprotein was used to confirm RNA integrity of the tissue sections.

PCR analysis

Polymerase chain reaction analysis for clonal rearrangements of the T cell receptor gamma chain gene was performed as previously described [9].

Results

Clinical history

The patient is a 33-year-old man who was diagnosed with ulcerative colitis based on colonic biopsies. He was subsequently treated with the tumor necrosis factor alpha blocker infliximab. He presented with an acute complaint of abdominal pain. Computed tomography scan of the abdomen and pelvis revealed retroperitoneal and mesenteric abdominal and pelvic lymph node enlargement as well as splenomegaly. Laboratory workup revealed the patient to be

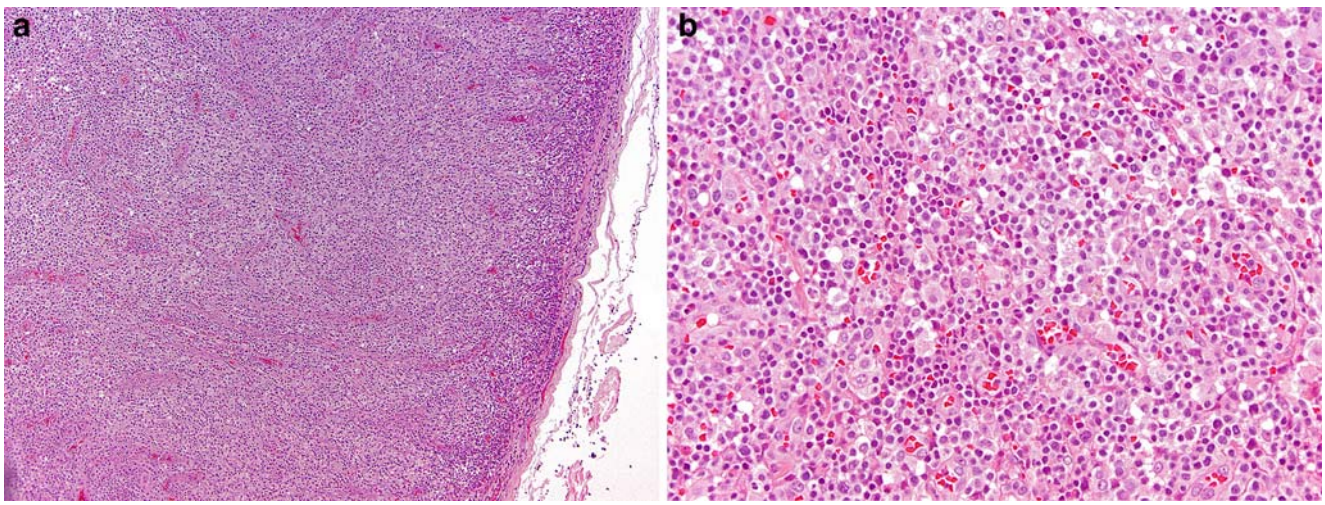

Fig. 1 Morphologic features of the T cell lymphoproliferative disorder in an axillary lymph node. **a** Effacement of lymph node architecture by a diffuse infiltrate of atypical lymphoid cells. **b** Atypical intermediate- to large-sized cells with irregular nuclear contours and variable prominent nucleoli

negative for infectious mononucleosis heterophile antibody, Epstein–Barr virus immunoglobulin G (IgG) and IgM, HIV antibody, and hepatitis A, B, and C serology. He subsequently underwent an axillary lymph node biopsy for histologic evaluation of generalized lymphadenopathy.

Light microscopy

Hematoxylin-and-eosin-stained sections from the axillary lymph node biopsy revealed an infiltrate of atypical lymphoid cells effacing normal nodal architecture (Fig. 1a). The infiltrate consisted of intermediate- to large-sized cells with marked cytologic atypia including irregular nuclear contours, prominent nucleoli, numerous mitosis, and areas of karyorrhectic debris (Fig. 1b).

Immunophenotyping

Immunohistochemical stains performed on the paraffin-embedded tissue showed the atypical cells to be CD3-positive T cells (Fig. 2a) with expression of CD5, CD4, and betaF1. Although most of the atypical cells were CD8 negative, a small subset was CD8 positive. The atypical T cells showed aberrant downregulation of both CD2 (Fig. 2b) and CD7 (Fig. 2c). CD20 and CD79a showed residual follicular B cells which were overrun by the neoplastic T cells; however, the large atypical cells were negative for both B cell markers. CD30 showed moderate expression, while CD15 and fascin were negative in the tumor cells. CD21 highlighted residual intact germinal centers. CD56, ALK-1, and TIA-1 were all negative.

Molecular studies

In situ hybridization for the EBER-1 probe was negative in the tumor cells (data not shown). T cell receptor gamma gene rearrangement analysis by polymerase chain reaction revealed a polyclonal T cell population (data not shown).

Discussion

Patients with inflammatory bowel disease are at an increased risk of developing malignant lymphoma independent of the use of immunosuppressive agents in their treatment, which is estimated to be not more than two times that of a normal individual [10]. Subtypes of malignant lymphoma that have been described in patients with inflammatory bowel disease who have not been treated with immunomodulatory therapy include high-grade B cell non-Hodgkin lymphomas, “granulomatous” T cell lymphoma, Hodgkin lymphoma, and several markedly polymorphic lymphomas that were unable to be further phenotyped [11, 12].

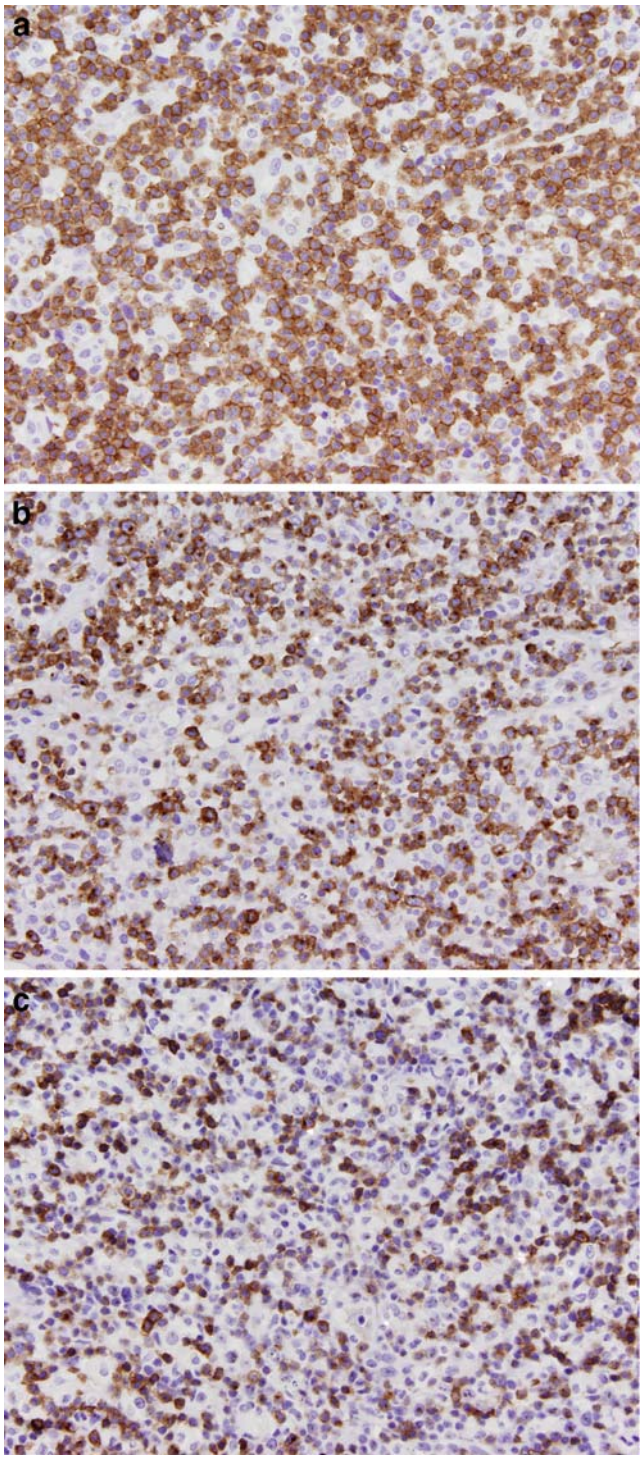

Fig. 2 Immunohistochemical features of the T cell lymphoproliferative disorder. **a** Atypical cells show diffuse reactivity to CD3. **b** Aberrant loss of CD2 expression. **c** Aberrant loss of CD7 expression

The use of immunosuppressive agents for the treatment of inflammatory bowel disease increases the risk of

developing malignant lymphoma. The use of azathioprine and 6-mercaptopurine for the treatment of inflammatory bowel disease began to increase in the 1990s [13]. Subtypes of lymphoma described in these immunosuppressed inflammatory bowel disease patients include diffuse large B cell lymphomas, marginal zone lymphomas, Hodgkin lymphoma, and plasmacytoma [13]. Seven of 18 azathioprine/6-mercaptopurine-associated lymphomas were positive for the Epstein–Barr virus (EBV) in one series [13].

The overall increase in EBV-positive lymphomas, which are the hallmark of immunosuppression-associated lymphomas, in patients treated with azathioprine and 6-mercaptopurine suggests a common pathogenesis. More than 80% of non-Hodgkin lymphomas occurring in transplant recipients are associated with EBV [14]. It is felt that these lymphoproliferative disorders develop due to a decreased cell-mediated immune surveillance, which in turn results in proliferation of EBV-infected B lymphocytes [15]. Studies evaluating the relative risk of developing lymphoma while undergoing treatment with azathioprine or 6-mercaptopurines show it to range between slightly greater than one to up to 60 [12].

The introduction of new biologic agents specifically designed to target cytokines involved in immune dysregulation has led to new questions about the link between lymphoma and inflammatory bowel disease. This includes infliximab, a TNF-α blocker approved by the FDA in 1998. TNF-α is thought to be a key cytokine involved in the autoinflammatory mechanism of inflammatory bowel disease [16]. Infliximab is a chimeric immunoglobulin G1 monoclonal antibody directed against TNF-α, which binds TNF-α and blocks its interaction with cell surface receptors. In 1998, infliximab became the first "biological therapy" approved by the FDA for the treatment of adults with moderate to severe Crohn's disease unresponsive to conventional therapy. It was then approved for the pediatric population for the same indication in May 2006, followed by approval for the use in adults with moderate to severe ulcerative colitis in October 2006 [8]. Infliximab has also been used in treating other immune-mediated diseases, such as rheumatoid arthritis and psoriatic arthritis. Now, almost 10 years after the introduction of this novel drug into the treatment of inflammatory bowel disease, data have begun to show an association between infliximab and malignant lymphoma.

Large population-based studies report the incidence of non-Hodgkin lymphoma in patients receiving infliximab for Crohn's disease range from 0.2% [17] to 1.4% [18]. A review of the adverse event surveillance system run by the FDA published in 2002 found 26 cases of lymphoproliferative disorders following treatment with anti-TNF-α agents in the first two and a half years following the introduction

Table 1 Summary of EBV-negative lymphomas associated with anti-tumor necrosis factor alpha therapy

Patient no. [reference]	Age, year/sex (indication for use)	TNF-α blocker duration of use before onset	Tumor subtype	Involved sites	Outcome/time to death after diagnosis (if applicable)
1 [8]	31/M (CD)	3 years	HSTCL	Not reported	Death/12 months
2 [8]	15/M (CD)	16 months	HSTCL	Not reported	Death/5 days
3 [8]	12/M (CD)	58 months	HSTCL	Not reported	Alive
4 [8]	17/F (CD)	29 months	HSTCL	Not reported	Death/3 months
5 [8]	19/M (CD)	36 months	HSTCL	Not reported	Death/10.5 months
6 [8]	18/M (CD)	7 months	HSTCL	Not reported	Death/12 months
7 [8]	19/M (CD)	2 months (lymphoma developed 31 months later)	HSTCL	Not reported	Death/8 months
8 [8]	22/M (UC)	1 dose only (lymphoma developed 56 months later)	HSTCL	Not reported	Alive
9 [20]	69/M (psoriatic arthritis)	18 months	Sezary syndrome	Cutaneous	Death/3 months
10 [20]	81/F (CD)	4 months	Systemic ALCL	Cutaneous/ bone marrow	Death/2 weeks
11 [22]	75/M (ankylosing spondylitis)	17 months	Sezary syndrome	Cutaneous	Regression with discontinuation of infliximab
12 [20]	47/M (psoriasis)	4 doses (lymphoma developed 4 months later)	CD30+T cell lymphoma	Cutaneous	In remission
13 [this case]	30/M (UC)	Unknown	Peripheral T cell lymphoproliferative disorder	Axillary lymph node	Unknown

CD Crohn's disease, *ALCL* anaplastic large cell lymphoma, *HSTCL* hepatosplenic T cell lymphoma, *UC* ulcerative colitis

of these agents for clinical usage [19]. Patients ranged in age from 29 to 84 years; the time from first infliximab dose to lymphoma diagnosis ranged from 2 to 52 weeks. The subtypes of lymphoma described in this initial report include diffuse large B cell lymphoma, follicular lymphoma, large cell non-Hodgkin lymphoma, small T cell lymphoma, mantle cell lymphoma, Hodgkin lymphoma, Burkitt lymphoma, and small lymphocytic B cell lymphoma. Notably, two of the cases regressed following the discontinuation of the anti-TNF-α therapy without cytotoxic therapy directed at the lymphoma. One case was EBV positive, one EBV negative, and the EBV status of the remaining 24 cases was not reported.

More recently, data have been published regarding the development of EBV-negative T cell lymphomas in patients treated with infliximab. HSTCL is a rare aggressive lymphoproliferative disorder comprising about 5% of peripheral T cell lymphomas. Of the more than 100 cases of HSTCL reported in the literature, eight of these cases were reported to the FDA's Adverse Event Reporting System as associated with infliximab use in patients with ages ranging from 12 to 31 years old being treated for inflammatory bowel disease [8]. In all cases, the lymphoma followed an aggressive course, with six patients dead of disease within 12 months. Other T cell lymphoproliferative disorders in patients receiving infliximab therapy include four cases of aggressive cutaneous T cell lymphomas [20–22], as well as a case of CD30+ T cell lymphoma which was EBV negative [23]. Table 1 summarizes the cases of TNF-α-blocker-related T cell lymphomas.

The index case represents an additional case of an EBV-negative T cell lymphoproliferative disorder in a young patient following treatment with infliximab for inflammatory bowel disease. Although T cell gene rearrangement studies did not identify a clonal rearrangement, the aberrant decreased expression of T cell antigens such as CD2 and CD7 in the neoplastic T cells strongly supports the presence of a T cell neoplastic population. Neither TNF-α nor infliximab itself has any direct effect on the apoptosis of B lymphocytes or EBV-positive cell lines, making these T cell lymphoproliferative disorders less likely to be related to a direct effect on B cells. Rather this suggests they are related to an impaired immune surveillance by T cells [5]. Data also show that infliximab significantly increases the percentage of CD19-positive cells while decreasing the percentage of CD3-, CD4-, CD8-, and HLA-DR-positive (activated) T cells in peripheral blood by inducing T cell apoptosis, resulting in a decrease in the number of activated T cells in the inflamed intestinal mucosa of inflammatory bowel disease patients [6, 7]. Perhaps in blocking the tumor-necrosis-factor-alpha-mediated apoptosis of circulating T lymphocytes, infliximab allows for the unrestricted growth of existing lymphoma cells [20].

CD4-positive T lymphocytes can be divided into two subtypes: Th1 cells and Th2 cells. Th1 cells secrete interferon and are involved in cell-mediated immunity while Th2 cells secrete other cytokines such as interleukin (IL)-4, IL-5, IL-10, and transforming growth factor beta and are involved in humoral immunity [24]. Autoimmune disease is often associated with the activation of Th1 cells. Th1 cytokines are involved in the inflammatory dysregulation seen in disorders such as inflammatory bowel disease, rheumatoid arthritis, and psoriasis, while Th2 cytokines predominate in some lymphomas [20]. Membrane-bound tumor necrosis factor alpha is present primarily on Th1 lymphocytes. Therefore, blockage and subsequent apoptosis of Th1 cells causing autoimmune disease may allow existing Th2 lymphoma cells to proliferate unchecked [7]. We can hypothesize that T cell lymphomas associated with infliximab are of the Th2 phenotype.

The apoptosis induced by tumor necrosis factor alpha blockers of Th1 T lymphocytes may prove to be the mechanism responsible for the development of T cell lymphoproliferative disorders in inflammatory bowel disease. This drug-mediated apoptosis may allow unchecked proliferation of Th2 T lymphocytes, leading to the development of malignant lymphoma.

Inflammatory bowel disease is an independent risk factor for the development of multiple types of malignant lymphoma. Therapeutic agents that suppress the immune system are associated with EBV-positive B cell lymphoma whereas immunomodulatory agents such as infliximab are associated with EBV-negative T cell lymphoma. Selective Th1 apoptosis induced by infliximab may be implicated in the pathogenesis of these lymphomas. Continued study is needed to further identify a more precise mechanism for the development of lymphoproliferative disorders in inflammatory bowel disease treated with TNF-α blockade. The FDA is currently beginning to probe the association of these drugs with the development of lymphomas [25]. With these medications being approved for use in more and more immune-related disorders, clinicians need to keep aware of this often aggressive consequence of treatment.

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