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Behçet's Disease and T-Cell Large Granular Lymphocytic Leukemia: Two Case Reports and a Hypothesis on a Common Pathogenesis

Behçet Hastalığı ve T Hücreli Büyük Granüler Lenfositik Lösemi: İki Olgu Sunumu ve Ortak Bir Patogenez Üzerine Hipotez

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Abstract

T-cell large granular lymphocytic leukemia (T-LGL) is a rare disorder, characterized by a chronic course, autoimmune manifestations and autoantibodies, cytopenias and circulating cytotoxic T-lymphocytes. T-LGL leukemia usually manifests with hematological involvement and co-existing autoimmune and/or autoinflammatory conditions. Behçet's disease (BD) is a chronic inflammatory disorder with recurrent oral and genital ulcers, uveitis, other systemic findings such as neurologic involvement, vasculitis and arthritis. Pathogenesis of BD is still poorly understood. However, a polarization of the Th1/Th2 immune response toward the Th1 pathway, and Th17 involvement have been shown. Herein, we present two cases of T-LGL co-existing with BD, second and third cases in the literature. We review, discuss and hypothesize a possible pathogenetic association between BD and T-LGL.

Keywords: Behçet's disease, autoimmunity, STAT3, T-Cell large granular lymphocytic leukemia, TNFAIP3

Öz

T hücreli büyük granüler lenfositik lösemi (T-LGL), kronik bir seyri olan, otoimmün belirtiler, otoantikorlar, sitopeniler ve dolaşımdaki sitotoksik T lenfositleri ile karakterize nadir bir hastalıktır. T-LGL lösemi genellikle hematolojik tutulum ve birlikte mevcut otoimmün ve/veya otoenflamatuvar durumlarla kendini gösterir. Behçet hastalığı (BH) tekrarlayan oral ve genital ülser, üveit, nörolojik tutulum, vaskülit ve artrit gibi diğer sistemik bulguları olan kronik bir enflamatuvar hastalıktır. BH'nin patogenezi hâlâ tam olarak anlaşılamamıştır. Ancak, Th1/Th2 dengesinin Th1 yolağına kayması ve Th17 polarizasyonu gösterilmiştir. Bu vaka sunumunda, BH ve T-LGL birlikteliği olan, literatürün ikinci ve üçüncü vakaları sunulmaktadır. Ek olarak, BH ve T-LGL arasında olası patogenetik ilişkinin hipotezi tartışılmıştır.

Anahtar Sözcükler: Behçet hastalığı, otoimmünite, STAT3, T-hücreli büyük granüler lenfositik lösemi, TNFAIP3

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Introduction

T-cell large granular lymphocytic leukemia (T-LGL), a clonal proliferation of cytotoxic T-cells, is a rare disorder characterized by a chronic course, cytopenias and circulating cytotoxic T-lymphocytes.[1] T-LGL leukemia usually manifests with hematological involvement and autoimmune diseases.[1] Patients usually present with features related to neutropenia, such as bacterial infections involving skin, oral cavity and perirectal area. Severe sepsis is rarely reported, even though there is usually marked neutropenia present.[2]

Autoimmune diseases are commonly associated with T-LGL.[3] Most common manifestation is rheumatoid arthritis (RA); however, other conditions are also previously reported. Autoantibodies, most frequently rheumatoid factor (RF) and antinuclear antibodies (ANA) are reported in T-LGL, which underlines an autoimmune context.[3] B-cell dyscrasias, monoclonal gammopathy of undetermined significance or hyper/hypo-gammaglobulinemia without monoclonality are also observed.[4]

Behçet's disease (BD) is a chronic inflammatory disorder with recurrent oral and genital ulcers, uveitis, other systemic findings such as neurologic involvement, vasculitis and arthritis.[5] Pathogenesis of BD is still poorly understood. However, a polarization of the T-helper (Th) 1/Th2 immune response toward the Th1 pathway, and Th17 involvement have been shown in active BD.[5]

Herein, we present two cases of T-LGL co-existing with BD, second and third cases in the literature. With a review to the literature regarding the pathophysiology of BD and T-LGL, we discuss possible hypotheses for a unified pathology.

Case Presentations

Case 1

A twenty-one-year-old female patient was referred for bicytopenia (anemia and neutropenia), lymphocytosis and elevated liver enzymes. She was previously diagnosed with T-LGL by splenectomy 3 years ago; and she was being followed up without medication. Six months ago, she was admitted to the hospital with an episode of uveitis. At that time, she also reported recurrent oral and genital aphtous ulcers, arthralgia and pleurisy. Complete blood count and routine biochemical tests were otherwise normal, apart from an elevated C-reactive protein (CRP, 4.2 mg/dL (<0.5)). Pathergy test was positive, and she was diagnosed as BD according to The International Clinical Criteria for Behçet's Disease. She had been treated with methylprednisolone (4 mg/day) plus colchicine (1 mg/day) for BD, however, colchicine had to be stopped due to aminotransferase elevation. During follow-up, liver enzyme elevation persisted, and cytopenias gradually developed. On admission, she had oral and genital aphtous ulcers; she had no other complaints that could be compatible with a recent infection. Physical examination was unremarkable apart from hepatomegaly (4 cm below costal margin). Laboratory results were: Hemoglobin: 9.6 g/dL (Reference range; 12–16), Mean Corpuscular Volume (MCV): 87 fL (Reference range; 80–102), leukocytes: $10.65x10^9$/L (Reference range; 3.91–8.77), neutrophils: $0.75x10^9$/L (Reference range; 2.06–7.02), lymphocytes: $4.75x10^9$/L (Reference range; 1.3–3.5), platelets: $279x10^9$/L (Reference range; 150–450) alanine aminotransferase (ALT): 496 U/L (Reference range; 10–49), aspartate aminotransferase (AST): 372 U/L (Reference; <34), gamma-glutamyl transpeptidase (GGT): 153 U/L (Reference; <73), alkaline phosphatase (ALP): 211 U/L (Reference range; 46–116), lactate dehydrogenase (LDH): 278 U/L (Reference range; 120–246), total bilirubin: 0.96 mg/dL (Reference range; 0.3–1.2), conjugated bilirubin: 0.3 mg/dL (Reference; <0.3), albumin: 3.47 g/dL (Reference range; 3.2–4.8), prothrombin time (PT): 11.2 seconds Reference range; (12–16.5), activated partial thromboplastin time (aPTT): 23.1 seconds (Reference range; 24-35). A work-up for viral, metabolic and autoimmune liver diseases were unremarkable. Liver biopsy showed LGL infiltration, immunophenotyping was positive for CD3, CD7, CD8, TIA-1 and granzyme B; negative for CD4, CD56 and CD20. She was then referred to hematology outpatient clinic for evaluation and treatment. Blood smear revealed large granular lymphocytes with abundant cytoplasms and azurophilic granules. Peripheral blood flow cytometry revealed CD3, CD8, TCRαβ positive and CD4, CD56, CD57, TCRγδ negative cells, being 32% lymphocyte population. Results were consistent with T-LGL. She has been treated with oral cyclophosphamide (100 mg daily) and colchicine (1 mg/day) for T-LGL and BD; symptoms that are related to BD and liver enzymes graudally improved. Patient continued to receive oral cyclophosphamide, she had no signs or symptoms, and she was still in remission after two years of follow up (Table 1).

Case 2

A fifty-five-year-old female patient was referred with oral and genital ulcers, fever, leukopenia, neutropenia and arthralgia. Five years ago, she had reported recurrent oral and genital aphtous ulcers, arthralgia. Pathergy test was positive, and she was diagnosed as BD according to The International Clinical Criteria for Behçet's Disease. She was treated with prednisolone (5 mg/day) and colchicine (1 mg/day). During follow-up, she had no remarkable complaints apart from oral ulcers that occurred several times per year. She had no symptoms regarding ocular or neurological involvement. She was admitted with a preliminary diagnosis of active BD and febrile neutropenia. Her physical examination revealed fever (38.7^0C), aphthous oral and genital ulcers and hepatomegaly (2 cm below the costal margin). There was no splenomegaly and palpable lymphadenopathy. Laboratory results showed a hemoglobin of 10.9 g/dL, a white blood cell count of $2.2x10^9$/L with a severe neutropenia (Reference range; $0.2x10^9$/L) and 72% lymphocytes ($1.58x10^9$/L). Erythrocyte sedimentation rate was 36 mm/h (Reference; <20), CRP was markedly elevated (14.4 mg/dL). Both

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Table 1. Demographic, clinical and laboratory comparison of three cases in the literature

		Reference case[6]	Case 1	Case 2
Age		56	21	55
Gender		Male	Female	Female
Presence of LGL		After BD	Before BD	After BD
BD duration		5 years (?)	-	5 years
LGL duration		NS	3 years	-
Means of LGL diagnosis		Flow cytometry analysis + TCR gene rearrangement	Splenectomy	Flow cytometry analysis + blood smear
Clinical and laboratory findings on BD diagnosis		Low grade fever Fatigue Oral and genital ulcers GI ulcers Anemia	Oral and genital ulcers Arthralgia Pleurisy Uveitis	Oral and genital ulcers Arthralgia
Clinical and laboratory findings on LGL diagnosis		NS?	Oral and genital ulcers Anemia Neutropenia Lymphocytosis Elevated transaminases	Neutropenic fever Oral and genital ulcers Leukopenia Neutropenia Arthralgia
Pathergy		Positive	Positive	Positive
Blood count				
	Hemoglobin (g/dL)	8.9	9.6	10.9
	WBC (10^9/L)	5.7	10.6	2.2
	Lymphocytes count (10^9/L)	4.4 (?)	4.75	1.5
	Platelets count (10^9/L)	186	279	NS
Flow cytometry				
	Positive	$CD3^+$ $CD8^+$ $CD16^+$ $TCR\alpha\beta^+$	$CD3^+$ $CD7^+$ $CD8^+$ TIA-1 Granzyme B^+ $TCR\alpha\beta^+$	$CD3^+$ $CD5^+$ $CD8^+$ $CD38^+$
	Negative	NS	$CD4^-$ $CD20^-$ $CD56^-$ $CD57^-$ $TCR\gamma\delta^-$	$CD4^-$ $CD56^-$
Treatment		Daily oral prednisolone (5 mg/day) + colchicine	cyclophosphamide (100 mg daily) + colchicine (1 mg/day)	methylprednisolone (24 mg/day) + methotrexate (15 mg/week orally)
Follow-up		NS	No signs or symptoms after two years	9 years, LGL in remission, mild symptoms of BD

LGL, large granular lymphocytic leukemia; BD, Behçet's disease; NS, not specified; TCR, T-cell receptor; GI, gastrointestinal; WBC, white blood cell; TIA, T-cell intracellular antigen-1.
(?) denotes assumed data from the reference.

urine and blood cultures were negative, but piperacillin/tazobactam was administered empirically for the treatment of febrile neutropenia. Patient exhibited rapid resolution of systemic symptoms and normalization of sedimentation and CRP levels after 7 days of antimicrobial therapy, but neutropenia and lymphocytosis continued. Therefore, a peripheral blood smear was performed, which revealed large granular lymphocytes constituting majority of the white blood cell population. Peripheral blood flow cytometry was consistent with T-LGL, expressing CD3, CD5, CD8 and CD38. The cells were negative for CD4 and CD56 expression. Patient was treated with methylprednisolone (24 mg/day) and methotrexate (15 mg/week orally); her symptoms and cytopenia gradually returned to normal. Patient was in remission from T-LGL after nine years of follow up, she still rarely reported oral

aphthous ulcers under weekly methotrexate and otherwise healthy (Table 1).

Discussion

Herein we report two cases with co-existing BD and T-LGL. To the best of our knowledge, at the time of writing this article these case reports are the second and third cases in the literature.[6] Reference case was a male, whereas our cases were female. While the first case was diagnosed with T-LGL first, and then BD, the opposite was observed in the second case, similar to reference case. Although reference case and case 2 were diagnosed by flow cytometry, bone marrow aspirate and/or biopsy might be required to confirm the diagnosis in some cases, especially those with low absolute numbers of circulating LGLs. Pathologic evaluation of the spleen is rarely required; as demonstrated in the first case.[7] Clinical and laboratory findings were generally similar, all patients were pathergy positive and had anemia. T-LGL should be considered in cases of unexplained cytopenias and relative lymphocytosis, especially in patients with autoimmune diseases and other inflammatory diseases such as BD. Treatment protocols were different from each other, we have decided our treatment regimens in multidisciplinary council meetings in concordance with patient data, current patient status, previous treatments and international recommendations for both diseases at the time of diagnosis. Therefore, we do not believe the effects were specific on BD or LGL.

LGL cells exert their effective functions via two major pathways: i) perforin-granzyme mediated cytotoxicity, ii) a death-receptor mediated (such as Fas) pathway.[8] Leukemic LGL cells have been known to infiltrate bone marrow, spleen and liver; these cells are proficient at cytotoxicity, however, they are usually deficient in proliferation (mostly persist in G0/G1 cell cycle) and resistant to activation-induced cell death.[2,8-10] It was previously demonstrated that LGL clones upregulate cytotoxicity and adhesion genes, and downregulate protease inhibitors.[2,8] LGL leukemia is associated with an increase in proinflammatory cytokines, such as RANTES, MIP-1β and IL-18, as well as Fas ligand.[11] LGL cells express high levels of Fas and Fas ligand, however, they are resistant to Fas-mediated apoptosis.[12] Soluble Fas ligand was also detected in patients with LGL, possibly acting as a decoy receptor, it is therefore usually noted that soluble Fas ligand may contribute to cytopenic manifestations such as neutropenia and may be used as a marker in LGL.[1,2] In recent years, somatic gain-of-funtion mutations of STAT3 were observed in both T- and NK-LGL leukemia subsets, hinting a possible common pathogenesis.[13] This observation was also later adopted into WHO classification of lymphoid neoplasms, as a distinct subset.[14] Considering that STAT3 is important in balancing Th17-Treg populations and has a role in interferon-gamma signalling, it could be proposed that STAT3 governs multiple aspects of possible autoimmune and/or autoinflammatory processes.[15] Indeed, it was previously hypothesized that a mutation of STAT3 could be the major cause of resistance to apoptosis in LGL cells, as well as hematological involvement and and autoimmune and/or autoinflammatory manifestations, as a result of proinflammatory conditions.[1]

Behçet's disease is a multisystem, complex, chronic inflammatory disorder, involving oral and genital aphthous ulcers, papulopustular skin lesions, ocular lesions usually manifesting as uveitis.[16] Th1 and Th17 cell subsets play important roles in pathogenesis in BD.[5] Proinflammatory cytokines of Th1 origin; such as IL-2, IL-12, IL-18 and IFN-γ are known to be increased in BD.[17] IL-18 levels were found to be correlated with the disease activity in another study.[18] IL-18 promoter gene was also reported as a candidate susceptibility gene in BD.[19] Although IL-18 majorly activates NF-kB pathway, it was also previously demonstrated that IL-18/IL-18Rα/IL-18Rβ complex induces phosphorylation of STAT3 in NK cell line.[20] In addition, another study examining STAT3 pathway in BD demonstrated that both total and phosphorylated STAT3 expressions were higher in BD, possibly due to elevated Th1/Th17-type cytokines.[21] STAT3 is also a major factor in differentiation of Th cells to Th17; Th17 and associated cytokines were also previously associated with active BD as well.[22] Considering these findings, IL-18 and STAT3 form major pathogenetic pathways in BD.

Considering that STAT3 pathway possibly plays the most important pathogenic role in LGL, and upregulation of IL-18 and STAT3 pathways are reported pathologically active pathways is BD, we assume that there may be a common pathogenetic link between these two diseases. Moreover, possible links are not limited to these findings. Another pathogenetic link between LGL and BD have been found in recent years: *TNFAIP3* alterations, a gene encoding an NF-kB signaling inhibitor, A20, was found in 3 out of 39 patients with LGL, underlying a possible role of NF-kB pathway.[23] Additionally, STAT3 mutations were found

to be significantly associated with *TNFAIP3* mutations. Interestingly, a familial, autosomal dominant form of *TNFAIP3* mutation, resulting in a haplo-insufficiency of A20 protein, results in a BD-like phenotype, due to impaired inhibition of NF-kB pathway.[24,25]

Due to its indolent nature, not all patients with T-LGL require treatment.[7] Severe neutropenia, symptomatic anemia or thrombocytopenia, severe constitutional symptoms and co-existing autoimmune diseases are the most common indications for medical treatment in T-LGL.[1,3] In the present cases, immunosuppresive treatment improved not only T-LGL but also BD as well, therefore highlighting a possible common immunopathogenesis between the two diseases.

T-LGL should be considered in the differential diagnosis in patients with BD admitting with hematological findings such as cytopenias and lymphocytosis. We hypothesized that there may be a pathogenetic association between BD and T-LGL, due to the fact that upregulation of IL-18 and STAT3 pathways, along with a reduction in A20 protein resulting in reduced NF-kB inhibition that plays a major role in the pathogenesis of both diseases. Further studies are needed in patients with T-LGL accompanying inflammatory diseases including BD to confirm these immunopathological assumptions.

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