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Case Report

Clonal T-cell Large Granular Lymphocytic Disorders Manifesting in Patients with HIV-1 Infection: Case Series and Review of the Literature

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Abstract. We report five patients with human immunodeficiency virus-1/acquired immunodeficiency syndrome (HIV-1/AIDS) who developed T-cell large granular lymphocytic proliferation (T-LGLP) or leukemia (T-LGLL). None of the patients fulfilled criteria for diagnosis of diffuse infiltrative lymphocyte syndrome (DILS) or HIV-associated CD8+ lymphocytosis syndrome at the time of diagnosis of LGL. The immunophenotype of malignant T-cells was identical in three patients with co-expression of CD3, CD8, CD57, and T-cell receptor (TCR) alpha/beta. Three out of five patients were also diagnosed with clonal disorders of B-cell origin including diffuse large B-cell lymphoma, Burkitt's lymphoma, and monoclonal gammopathy of undetermined significance (MGUS). Two patients developed cytopenias due to T-LGLL prompting initiation of therapy. Our study suggests that chronic viral infection with HIV can contribute to the evolution of T-LGLP. Clinical and laboratory characteristics of T-LGLP associated with HIV-1/AIDS resemble those of immunocompetent patients.

Keywords: T-cell LGLL, HIV-1, AIDS, Clonal, Large granular lymphocyte, Large granular lymphocytic leukemia, Large granular lymphocytic proliferation.

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Introduction. T-cell large granular lymphocytic leukemia (T-LGLL) is a rare, clonal lymphoproliferative disorder of mature T-cells manifesting with peripheral blood cytopenias, splenomegaly, and increased incidence of autoimmune disorders.[1,2] The diagnosis is based on clinicopathological characteristics. Early reports suggested a mandatory clonal LGL count of > $2x10^9$/L in peripheral blood and duration of lymphocytosis >6 months.[3] However, subsequent work postulated that diagnosis is possible in patients with lower LGL count manifesting with characteristic clinical features.[4,5] The spectrum of disease ranges from asymptomatic large granular lymphocytic proliferation to symptomatic leukemia which requires treatment. Long-term follow-up is recommended to distinguish between the two.

The association of T-LGLL with retroviral infections has been described previously. Two reports of patients with HIV-1 associated with T-cell or natural killer (NK) LGL have been previously documented.[6,7]

We report a cohort of patients with HIV-1/AIDS who subsequently or concurrently developed T-LGLP or T-LGLL. Clinical and

laboratory characteristics are analyzed and discussed.

Case 1. A 43-year-old Caucasian male presented with fatigue, sweats, weight loss, musculoskeletal pain, and recurrent pneumonia in November 2006. His complete blood count (CBC) revealed a white blood cell count (WBC) 19.6 $x10^9$/L, hemoglobin (Hb) 8.38 g/dL, platelet count (plt) 242 $x10^9$/L, absolute neutrophil count (ANC) 0.760 $x10^9$/L, and absolute lymphocyte count (ALC) 17.1 $x10^9$/L. Bone marrow biopsy (BMB) showed a hypercellular marrow (70%) with trilineage hematopoiesis and diffuse lymphocytic infiltration comprising 40% of the total cellularity. Lymphocytes were predominantly small to medium size with irregular nuclei and abundant granular cytoplasm. Flow cytometry of bone marrow aspirate revealed an atypical T-cell population co-expressing CD3, CD5, CD8, CD16, CD57 and TCR alpha/beta, consistent with T-LGL. Cytogenetics were normal. Serum protein electrophoresis showed elevated gamma globulin level of 57 g/L and M spike of 31 g/L. Immunofixation confirmed IgG kappa monoclonal gammopathy of undetermined significance (MGUS). CT scan, PET/CT scan, and bone survey were unremarkable.

HIV-1 antibody screen and western blot were positive. CD4 count was 0.30 $x10^9$/L and HIV-1 RNA viral load was 43,064 copies/mL. The patient was started on highly active antiretroviral therapy (HAART), and after five months of therapy, his viral load became undetectable. Repeat BMB and flow cytometry confirmed persistent infiltration of marrow with T-LGL. Due to persistent moderate neutropenia and three episodes of pneumonia, treatment with cyclosporine A (CSA) was initiated for symptomatic T-LGLL. The patient's most recent laboratory studies and response to therapy are not available.

Case 2. A 39-year-old Caucasian male who was diagnosed with HIV-1 in 1994 developed oral mucositis in January 2007. WBC revealed leukopenia with an ANC close to 0 $x10^9$/L, and normal Hb and plt. He was non-compliant with HAART until February 2007 when he resumed emtricitabine, tenofovir, atazanavir, and ritonavir, along with G-CSF 480 mcg SC biweekly due to severe neutropenia.

In April 2007, his CBC revealed a WBC 2.96 $x10^9$/L, Hb 8.81 g/dL, plt 264 $x10^9$/L, ANC 0.26 $x10^9$/L, and ALC 1.35 $x10^9$/L. Peripheral flow cytometry revealed an atypical T-cell population with abundant granulated cytoplasm co-expressing CD3, CD5 weakly, CD7, CD8, CD57 and TCR alpha/beta. Molecular studies revealed clonally rearranged TCR beta gene, consistent with T-LGL. BMB and cytogenetics were normal.

Repeat BMB in August 2007 demonstrated a hypercellular marrow (70%) with low level infiltration of T-LGL. Flow cytometry of bone marrow aspirate was positive for a clonal T-LGL population with the identical immunophenotype as seen in peripheral blood.

HIV-1 RNA viral load was undetectable, and the absolute CD4 count was 0.045 $x10^9$/L. CT scan showed no adenopathy or hepatosplenomegaly. ANC stabilized above 1.0 x 10^9/L with G-CSF treatment, then remained normal for more than two years after discontinuation of G-CSF.

Case 3. A 47-year-old Caucasian male was diagnosed with HIV in 2000. He was treated with emtricitabine, tenofovir, lopinavir, and ritonavir, but became non-compliant with therapy from 2006 until 2008.

In August 2008, his CD4 count was $0.021x10^9$/L, and viral load was 53,000 copies/mL. He was restarted on his HAART regimen with improvement in CD4 counts, ranging from $0.090x10^9$/L to 0.100 $x10^9$/L. He was diagnosed with stage 3AE AIDS-associated diffuse large B-cell lymphoma (DLBCL) of the oral cavity in May 2008. He underwent therapy with five cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with intrathecal prophylactic methotrexate. He achieved complete remission.

In September 2009, he developed lymphocytosis of 7.19 $x10^9$/L. Peripheral flow cytometry revealed an abnormal T-cell population co-expressing CD3, CD8, CD57, TCR alpha/beta, CD5 weakly, +CD7 weakly, and HLA-DR. TCR gamma gene was clonally rearranged. His CBC showed otherwise normal blood counts. He was clinically asymptomatic and has been managed with observation.

Case 4. A 51-year-old Caucasian man presented with unintentional weight loss, diarrhea, and acute

renal failure in January 2009. His CBC revealed absolute lymphocytosis >10 x 10^9/L. Flow cytometry on peripheral blood was consistent with a CD8+ lymphoproliferative disorder. Esophagogastroduodenoscopy and colonoscopy with biopsies revealed non-specific inflammatory changes from the distal esophagus through the colon. Immunohistochemistry showed a colonic infiltration with an atypical lymphoid population co-expressing CD2, CD3, CD5, CD8 and TCR alpha/beta.

His CBC in February 2009 showed WBC 19 x10^9/L, Hb 9.8 g/dL, plt 156 x10^9/L, ANC 5.6 x10^9/L, and ALC 12.3 x10^9/L. BMB was normocellular with focal interstitial infiltration of CD8+ cytotoxic T-lymphocytes without aberrant antigen expression. Cytogenetics were normal. TCR beta gene was clonally rearranged. HIV-1 ELISA and confirmatory western blot were positive. HIV-1 viral load was 251,189 copies/mL and CD4 count was 0.53 x10^9/L CMV PCR revealed 400 copies/mL suggesting CMV reactivation. PET/CT revealed diffuse hypermetabolic lymphadenopathy and splenomegaly. Cervical lymph node excisional biopsy was consistent with follicular hyperplasia. Flow cytometry revealed no evidence of an atypical clonal T- or B-cell population. TCR beta gene rearrangement studies on lymphonodal tissue were positive. Repeat flow cytometry on peripheral blood showed the presence of CD8+ lymphocytosis without aberrant immunophenotype, despite rearrangement of both TCR beta and gamma genes.

The patient started efavirenz, emtricitabine, and tenofovir in April 2009. His CBC normalized and GI symptoms subsided. Repeat CT scans in February 2010 showed resolution of lymphadenopathy. However, repeat peripheral flow cytometry in February 2011 revealed a new clonal atypical T-cell population co-expressing CD3, CD8, CD57, TCR alpha/beta, and weakly CD5. TCR beta gene was clonally rearranged. Absolute LGL count was 0.773 x10^9/L. The patient has been clinically asymptomatic and has been managed with observation.

Case 5. A 58-year-old male presented with unintentional weight loss, night sweats, and fatigue in 2009. He was found to have extensive abdominal and retroperitoneal lymphadenopathy and was diagnosed with stage 3B Burkitt's lymphoma and HIV. At the time of diagnosis, his CD4 count was 0.050x10^9/L, and viral load was 69,000 copies/mL. He was started on cyclophosphamide, vincristine, doxorubicin, dexamethasone, cytarabine, and methotrexate (hyperCVAD) as well as emtricitabine, tenofovir, lopinavir, and ritonavir. He achieved complete remission, and his viral load became undetectable. Lopinavir was switched to efavirenz due to side effects. His viral load remained undetectable with CD4 counts > 1.0x10^9/L.

He was noted to have mild lymphocytosis on routine labs in August 2016 with CBC showing WBC 11 x10^9/L, Hb 9.31 g/dL, plt 222 x10^9/L, ALC 4.09 x10^9/L, and ANC 5.86 x10^9/L. Peripheral flow cytometry showed increased clonal CD8+/CD57+ large granular lymphocytic T-cells with an absolute LGL count of 0.92 x10^9/L. TCR beta and gamma genes were clonally rearranged. The patient has been clinically asymptomatic without cytopenias and has not required treatment.

Discussion. A hallmark of HIV infection is a depletion of infected helper CD4+ cells resulting in an increased incidence of opportunistic infections and AIDS-defining malignancies.[8] The introduction of HAART therapy results in a significantly decreased incidence of such malignancies, as well as an improved patient outcome.[9,10] HIV-associated mature T-cell malignancies comprised only 3% of all AIDS-related lymphomas in a single institutional study in the US.[11] However, significantly higher frequency (27%) was observed in a study from South America, suggesting geographical and ethnic differences.[12]

In contrast to the decrease in absolute CD4+ lymphocytes, transient expansion of CD8+ cells has been detected early in the course of HIV infection due to host immune response.[13] Sustained expansions of the CD8+ T-cell population have been reported in two conditions associated with HIV: diffuse infiltrative lymphocytosis syndrome (DILS) and HIV-associated CD8+ lymphocytosis syndrome.[14]

DILS was initially described in 1989 by Itescu *et al.* as a sicca syndrome in HIV infection. TCR gene rearrangement was detected in a significant proportion of patients with DILS, suggesting a clonal origin in these cells.[15] The immunophenotype of these virally expanded

CD8+ T-cells is similar to memory and effector T-cells with co-expression of CD8, CD11a, CD11c, and CD57.[15] A characteristic feature of DILS is a CD8+ lymphocytic infiltration of salivary glands, and less frequently, other visceral organs.[15] None of our patients presented with salivary gland infiltration. A recent report suggested that the incidence of DILS has decreased over time due to the introduction of HAART.[16] Four out of five patients in our series did not fulfill criteria for DILS at any time of our observation; they developed the expansion of clonal LGLs 2 to 13 years after diagnosis of HIV/AIDS. Case 4 manifested initially with lymphocytosis, infiltration of colon and bone marrow with clonal CD8+ T-cells, suggesting DILS before the diagnosis of HIV. The CD8+ clone associated with DILS disappeared from his circulation almost two years before a new immunophenotypically distinct clonal CD8+ population occurred in peripheral blood. His HIV infection was well controlled with HAART at the time he developed a new clonal T cell LGL population.

There are few documented reports of patients with HIV and T-cell or NK-cell LGLP. Smith *et al.* reported 18 patients with HIV-1 who had persistent expansions of T-cell LGLs for 6 to 30 months. However, only five patients revealed clonal TCR gene rearrangement, and no cytopenias or LGL infiltration of bone marrow were reported.[14] Ghrenassia *et al.* reported 14 patients, three of which had HIV/AIDS, with CD8+ T-cell expansion. Six patients had non-clonal, symptomatic organ infiltration and nine patients had at least one cytopenia. Some patients with cytopenias were found to have rearrangements consistent with T-LGL, while others did not.[17]

A large retrospective study of patients with LGLL found that 45.6% never required therapy. Of those, peripheral blood LGL population percentage ranged from <0.5 to >2.0 x10^9/L. At the time of diagnosis, 24.6% of patients with LGLL had levels <0.5 x10^9/L. Patients with intermediate LGL counts (0.5-2.0 x10^9/L) required the highest mean number of therapies compared to those with high (>2.0 x10^9/L) or low (<0.5 x10^9/L) LGL counts.[2]

Increased incidence of B-cell dysregulation resulting in the development of autoimmune disorders and B-cell malignancies were reported in patients with LGL leukemia as well as patients with DILS, which could suggest a causative role of chronic viral antigenic stimulation or the presence of a putative autoantigen. Two separate studies identified a high frequency of B-cell dyscrasias in patients with T-LGLL; MGUS, chronic lymphocytic leukemia, Hodgkin and non-Hodgkin lymphoma were reported in 20% to 43% of T-LGLP patients.[18,19] Another study described 20 patients with a dual diagnosis of T-LGLP and either a B-cell or plasma cell lymphoproliferative disorder.[20] Interestingly, three of five patients in our series developed B-cell malignancies. Since HIV-positive patients also have an increased incidence of B-cell lymphoproliferative disorders, both HIV/AIDS and T-LGLL could be implicated in the development of B-cell malignancies. In three patients, the diagnosis of HIV-1 infection was made prior to the development of T-LGLP. The median time from diagnosis of HIV/AIDS to the diagnosis of T-LGLP was two years (range 0-13 years).

Conclusions. We describe five unique patients with HIV/AIDS who developed persistent expansions of clonal T-cell LGLs while their HIV infection was controlled with HAART (**Table 1**). All patients fulfilled earlier or more recent criteria for the diagnosis of LGLL (**Table 2**).

All patients demonstrated expansion of an immunophenotypically abnormal clonal T-cell

Table 1. Characterization of HIV/AIDS Method of highly active antiretroviral therapy (HAART) utilized is recorded, where available. Response to HAART is characterized by CD4 count and HIV viral load. (N/A = not available).

Case	Initial		HIV Treatment	Response	
	CD4 count (x10^9/L)	HIV viral load (copies/mL)		CD4 count (x10^9/L)	HIV viral load (copies/mL)
1	0.300	43,064	N/A	N/A	N/A
2	0.045	undetectable	Emtricitabine/Tenofovir; Atazanavir; Ritonavir	0.077	undetectable
3	0.021	53,000	Emtricitabine/Tenofovir; Lopinavir/Ritonavir	0.100	N/A
4	0.530	251,189	Efavirenz/Emtricitabine/Tenovir	N/A	N/A
5	0.050	69,000	Emtricitabine/Tenofovir; Lopinavir/Ritonavir; Efavirenz/Emtricitabine/Tenovir	1.228	undetectable

Table 2. All five patients met criteria for T-cell LGL leukemia or large granular lymphocytic proliferation. (ANC-absolute neutrophil count, ALC-absolute lymphocyte count, LGL-large granular lymphocyte, TCR-T cell receptor gene, BMBx-bone marrow biopsy, CSA: cyclosporine A, G-CSF-granulocyte-colony stimulating factor, obs-observation.).

Case	ANC 10^9/L	ALC 10^9/L	LGL count 10^9/L	Duration of LGL lymphocytosis (months)	Clonality TCR gene rearrangement	BMBx (% of involvement)	Therapy
1	0.83	10.69	6.42	12	B+γ+	40	CSA
2	0.3	1.31	0.36	30	B+γ+	10	G-CSF
3	2.69	4.49	2.8	60	B+γ-	ND	obs
4	2.38	10.15	4.0	24	B+γ-	ND	obs
5	2.78	6.65	2.1	60	B+γ+	ND	obs

large granular lymphocytic population which persisted for more than six months. Furthermore, two out of five patients developed sustained neutropenia requiring therapy, which is the most common cytopenia diagnosed in patients with LGLL. The rest of patients demonstrated an indolent course of disease which was previously reported in 30-50% of immunocompetent patients with LGLL. Our observation expands a spectrum of T-cell large granular lymphocyte disorders associated with HIV/AIDS, and supports the hypothesis that a chronic antigenic stimulation with viral antigens could be implicated in the etiopathogenesis of T-LGLP and T-LGLL.

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