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REVIEW

The pathogenesis and treatment of large granular lymphocyte leukemia

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ABSTRACT

Large granular lymphocyte (LGL) leukemia is a spectrum of rare lymphoproliferative diseases of T lymphocytes and natural killer cells. These diseases frequently present with splenomegaly, neutropenia, and autoimmune diseases like rheumatoid arthritis. LGL leukemia is more commonly of a chronic, indolent nature; however, rarely, they have an aggressive course. LGL leukemia is thought to arise from chronic antigen stimulation, which drives long-term cell survival through the activation of survival signaling pathways and suppression of pro-apoptotic signals. These include Jak-Stat, Mapk, Pi3k-Akt, sphingolipid, and IL-15/Pdgf signaling. Treatment traditionally includes immunosuppression with low dose methotrexate, cyclophosphamide, and other immunosuppressive agents; however, prospective and retrospective studies reveal very limited success. New studies surrounding Jak-Stat signaling suggest this may reveal new avenues for LGL leukemia therapeutics.

1. Introduction

Large granular lymphocyte leukemia embodies a spectrum of rare clonal lymphoproliferative disorders, all which involve inappropriate expansion of large granular lymphocytes (LGLs), either cytotoxic T-lymphocytes (CTLs) or natural killer (NK) cells [1,2]. In normal adults, LGLs represent 10–15% of peripheral blood mononuclear cells (PBMCs) and can be classified into two distinct lineages as either $CD3^+$ CTLs or $CD3^-$ NK cells. Both cell types play important roles in the immune system. LGLs become activated through antigen recognition and undergo significant expansion with subsequent death by apoptosis upon antigen clearance. In LGL leukemia, these LGLs persist [3]. In 1985, the term *LGL leukemia* was first introduced as a disorder involving clonal invasion of the blood, marrow, and spleen [4]. In 1993, the distinction between $CD3^+$ T-cell and $CD3^-$ NK-cell lineage subtypes of LGL leukemia was proposed [5]. In 1999, the WHO classification included T- and NK-cell LGL leukemia in the mature peripheral T-cell neoplasms subgroup [6]. In 2008, a provisional entity of chronic NK-cell lymphoproliferative disorder (CLPD-NK) was created by the WHO to separate it from the more aggressive NK-cell leukemia [7] (Table 1). This review paper will cover topics regarding clinical presentation, diagnosis, and treatment possibilities along with providing a future perspective on the LGL spectrum of disorders.

2. Epidemiology

The frequency of LGL leukemia has not been accurately determined but is estimated to account for 2–5% of chronic lymphoproliferative disorders in North America and up to 5–6% in Asia [1]. Indolent T-cell LGL leukemia represents the most frequent LGL disorder in Western countries, accounting for 85% of all cases. The median age at diagnosis is 60 years and both sexes are affected equally. Aggressive T-cell LGL leukemia is a rare disorder that has been suggested to be a separate clinicopathologic entity within the spectrum of LGL disorders [8,9]. It is possible that it arises from clonal evolution of indolent T-cell LGL leukemia but more likely it develops *de novo*. It typically affects a younger population with median age of 41 years. Most cases are poorly understood and have been reported in the literature using different terms [10,11]. CLPD-NK is an indolent disease comprising 5% of all LGL disorders. The median age at diagnosis is 58 years [12]. Finally, aggressive NK-cell leukemia is an extremely malignant disease with poor prognosis, early presentation (median age of 39 years) and predominantly in patients of Asian descent [12]. Approximately 10% of LGL disorders can be classified as aggressive NK-cell leukemia. An association with Epstein-Barr virus (EBV) has been demonstrated in these patients, and an initiating role for EBV in aggressive NK-cell leukemia has been suggested [13,14].

3. Clinical features

3.1. T-LGL leukemia

Most cases of T-LGL leukemia have an overall indolent behavior, but up to two-thirds will become symptomatic. Nearly 80% of symptomatic

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Table 1
Summary of LGL leukemia disorders.

Type	Median age	Clinical features	Markers
T-LGL, indolent	60	Asymptomatic OR Symptomatic with - Neutropenia - Anemia - Thrombocytopenia - Recurrent bacterial infections Autoimmune conditions - Eg. RA, PRCA, AIHA, ITP	$CD3^+$ $CD8^+$ $CD16^+$ $CD56^-$ $CD57^+$ $TCR\alpha\beta^+$ (10% are $TCR\gamma\delta^+$)
T-LGL, aggressive (rare)	41	Cytopenias Acute B-symptoms, hepatosplenomegaly	$CD3^+$ $CD8^+$ $CD57^+$ TCR-αβ
CLPD-NK	39	Similar to T-LGL, indolent Less prevalent autoimmune disease	$CD3^-$ $CD16^+$/ $CD56^+$ $CD57^+$
Aggressive NK-cell Leukemia	58	Fulminant B symptoms Cytopenias Hepatosplenomegaly	$CD3^-$ $CD16^+$ $CD56^+$ $CD57^+EBV^+$

patients will develop neutropenia and 45% will develop a severe neutropenia (absolute neutrophil count below 0.5 x 10^9/L) [5]. A range of hematologic manifestations are seen in patients with LGL leukemia. Neutropenia is the most common cytopenia that is seen in LGL leukemia, resulting in an increased frequency of bacterial infection. The mechanism of neutropenia is most likely multifactorial but includes secretion of pro-inflammatory cytokine, Fas ligand [15,16]. Pure red cell aplasia (PRCA) has been seen in 8–19% of patients and aplastic anemia has also been reported [17,18]. Thrombocytopenia occurs in about 20% of patients, and immune thrombocytopenic purpura (ITP) is seen at increased frequency in LGL patients. Myelodysplastic syndrome (MDS) has been reported in patients with T-cell LGL disease [19]. Some experts suggest that autoimmune disorders comorbid with T-LGL leukemia can occur in up to 40% of patients [1,20]. Rheumatoid arthritis appears to be the most frequent autoimmune disease in patients with LGL; it has been reported in up to 36% of cases [1]. Serologic abnormalities (rheumatoid factor, anti-nuclear antibody, and polyclonal hypergammaglobulinemia) are frequent [20].

3.2. Chronic lymphoproliferative disorder of NK Cells

Clinical features of CLPD-NK are similar to those of T-LGL leukemia. Generally, CLPD-NK is an indolent disorder with a good prognosis. In most cases, CLPD-NK is detected on routine blood studies with persistent elevated circulating LGLs. Like T-LGL leukemia, CLPD-NK patients can be neutropenic, anemic, or thrombocytopenic and can also present with a wide array of autoimmune conditions, though this occurs at a lower frequency [20].

3.3. Aggressive NK-cell leukemia

Aggressive NK-cell leukemia is a highly aggressive hematological malignancy with a poor prognosis, younger age of presentation (median age of 39 years), and is most often seen in patients of Asian descent [6]. Aggressive NK-cell leukemia patients generally experience fulminant B symptoms, hepatosplenomegaly, and a wide range of cytopenias. EBV appears linked to pathogenesis.

4. Pathogenesis of LGL Leukemia

Research into LGL leukemia is focused on understanding the pathogenesis and etiology of the disease, with the idea that if we can understand the pathogenesis, we can develop drugs to combat these dysregulations. The activation of survival pathways and the evasion of apoptosis are major dysregulations seen in LGL leukemia. These include dysregulated Fas and Fas ligand (FasL) signaling, growth factor signaling, Map kinases (Mapk), Pi3k-Akt, nuclear factor kappa-light-chain-enhancer of activated B cells (Nfκb), and Jak-Stat signaling. A summary of these dysregulations is included in Table 2.

4.1. Fas and FasL

Fas and FasL signaling normally induces apoptosis and plays a fundamental role in regulation of the immune system. Fas-binding FasL produces the death-inducing signaling complex (DISC), leading to activation of caspase-dependent apoptosis. This is a major mechanism through which cytotoxic T cells (including LGLs) induce cell death in

Table 2
Summary of known dysregulations in LGL leukemia.

Pathway	Details	Potential as treatment modality
Fas and FasL and inhibition of activation-induced cell death (AICD)	Leukemic LGLs have elevated Fas-FasL levels in sera and are resistant to Fas-FasL-mediated apoptosis AICD. Soluble Fas (FasS) is elevated in patient sera and can block AICD.	
Interleukin-15 and platelet-derived growth Factor (Pdgf) signaling	Computational network modeling suggested that constitutive activation of interleukin-15 (IL-15) and Pdgf are sufficient to reproduce all known dysregulations in LGL leukemia.	Phase I clinical trials targeting IL-15 with a humanized antibody (Mikβ1) does not appear to be an effective treatment.
Map kinase signaling	Constitutively, activated Map kinase signaling has been demonstrated as a critical survival mediator in CLPD-NK.	*In vitro* pharmacologic Erk inhibition using PD098059 led to apoptosis in the NKL cell line.
Pi3k-Akt signaling	Pi3k-Akt signaling is constitutively active in T-LGL leukemia, due to overactive Src family kinases, which leads to inhibition of pro-apoptotic signaling.	*In vitro* treatment with Pi3K inhibitor LY 294002 significantly inhibited the activity of NFκB and induced apoptosis in patient T-LGL leukemia PBMCs.
Sphingolipid rheostat	Imbalance of sphingolipids has been demonstrated in LGL leukemia. Pro-apoptotic ceramide is decreased and anti-apoptotic sphingosine-1-phosphate is elevated (S1P). S1P receptor 5 is over-expressed in leukemic LGLs.	*In vivo* inhibition of acid ceramidase and delivery of C6-ceramide into a rat model of NK-LGL leukemia led to apoptosis of leukemic LGLs. *In vitro* pharmacological inhibition of Sphingosine kinase 1 via FTY750 led to apoptosis in leukemic LGLs and remission of leukemia in a rat model.
Nuclear-factor κB signaling	Nfκb has been shown to be activated in leukemic LGLs downstream of Akt and promotes the expression of anti-apoptotic Bcl-2 proteins.	Pharmacologic inhibition of NF-κB with BAY 11–7082 in T-LGL cells led to apoptosis *in vitro*. Bortezomib proteasome inhibitor has shown promise in preclinical studies in T-LGL leukemia cells.
Jak-Stat signaling	Jak-Stat pathway activation has been demonstrated in LGL leukemia; 30–40% of NK and T-LGL patients have somatic activating mutations in the SH2 dimerization and activation domain in the *STAT3* gene.	*In vitro* treatment with Stat3 inhibitors in both wild-type and mutant Stat3 patients results in apoptosis in leukemic LGLs. Mutant Stat3 is predictive of an earlier time to treatment failure and may contribute to the auto-immune phenotype in LGL leukemia and related diseases. Y640F Stat3 mutation was predictive of favorable methotrexate response.

LGLs = large granular lymphocyte cells; PBMCs = peripheral blood mononuclear cells.