Long-Term Remission of T-Cell Large Granular Lymphocyte Leukemia Associated with Rheumatoid Arthritis after Rituximab Therapy

Running title: Rituximab in LGL Leukemia with RA

Divi Cornec (MD),1,2,3 Valérie Devauchelle-Pensec (MD-PhD),1,2,3 Sandrine Jousse-Joulin (MD),1,2,3 Thierry Marhadour (MD),1 Valérie Ugo (MD-PhD),2,3,4 Christian Berthou (MD-PhD),2,3,5 Nathalie Douet-Guilbert (MD-PhD),2,3,6 Alain Saraux (MD-PhD)1,2,3

1 CHRU Brest, Service de Rhumatologie, Brest, F- 29200 France
2 Université de Brest, Faculté de Médecine et des Sciences de la Santé, Brest, F-29200 France
3 Université Européenne de Bretagne, France
4 CHRU Brest, Laboratoire d’Hématologie, Brest, F- 29200 France
5 CHRU Brest, Service d’Hématologie Clinique, Brest, F- 29200 France
6 CHRU Brest, Laboratoire de Cytogénétique, Brest, F- 29200 France

Corresponding author:
Dr. Divi Cornec, Service de Rhumatologie, Hôpital de la Cavale Blanche, BP 824, F 29609 Brest cedex, France
Phone: +33 298 347 267. Fax: +33 298 493 627
E-mail: divi.cornec@chu-brest.fr

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Keypoints
1. Rituximab may be a safe and effective therapy in patients with both large granular lymphocyte leukemia and rheumatoid arthritis.
2. LGLL in some patients with rheumatoid arthritis may be a reactive manifestation of chronic autoantigen stimulation rather than a true concomitant malignancy.

Abstract

T-cell large granular lymphocyte leukemia (LGLL) is a rare clonal disease often associated with rheumatoid arthritis (RA) and manifesting chiefly as neutropenia and recurrent infections. Immunosuppressive agents are the mainstay of treatment, but long-term remissions are rare. We report two cases of LGLL in patients with RA successfully treated with rituximab, a monoclonal antibody specific of B cells and approved for treating RA. The first patient experienced a complete LGLL remission that was sustained during the 8-year follow-up after the first rituximab infusion. In the second patient, rituximab therapy was followed by immediate neutropenia recovery then by marked shrinkage of the LGLL clone 1 year later. The paradoxical efficacy of this specific anti-B-cell drug on a monoclonal T-cell disease suggests that some cases of LGLL may be reactive manifestations of chronic autoantigen stimulation rather than true malignancies.
INTRODUCTION

T-cell large granular lymphocyte leukemia (LGLL) is a rare clonal disorder in which a monoclonal T-cell population accumulates in the blood, bone marrow, spleen, and liver.\textsuperscript{1} The main clinical manifestations are cytopenia (mostly neutropenia), splenomegaly, and recurrent infections.\textsuperscript{2} T-cell LGLL is associated with rheumatoid arthritis (RA) in up to one-third of cases.\textsuperscript{3}

No large prospective trials have been conducted in LGLL.\textsuperscript{4} Low-dose methotrexate and short-term prednisone are the main first-line drugs.\textsuperscript{5,6} The overall 3-month response rate is only 55%, and most responders relapse after 1 year of treatment.\textsuperscript{7} Refractory disease may respond to cyclosporine, cyclophosphamide,\textsuperscript{4} or alemtuzumab.\textsuperscript{8,9} Complete remissions are rare.

Rituximab is a monoclonal anti-CD20 antibody approved for B-cell non-Hodgkin’s lymphoma\textsuperscript{10} and RA.\textsuperscript{11} Rituximab induces complete depletion of circulating B cells with no direct effects on T cells. No data have been published on rituximab therapy in patients with both RA and LGLL. Here, we describe two cases. This study is a retrospective review of clinical cases. No ethics committee consultation was required, but informed consent was obtained from both patients according to the Declaration of Helsinki.

Case-reports

Case #1. A woman born in 1932 was diagnosed in 1998 with erosive rheumatoid factor (RF)-positive RA. Subsequent tests were positive for anti-citrullinated protein antibody (ACPA). Within a few months, severe neutropenia developed (Figure 1) and investigations showed a monotypic TCR-αβ+CD3+CD8+CD16+CD56-CD57+ T-cell LGL population in the blood and bone marrow. The absolute blood LGL count was 2.5 Giga/L (G/L), representing
50% of total lymphocytes. A TCR-gene rearrangement study detected a monoclonal T-cell population. No splenomegaly or focal splenic abnormalities were seen by ultrasonography. A low-level serum IgMκ component was detected. No evidence of B-cell lymphoma was found by bone marrow smear, biopsy, and flow cytometry; or by computed tomography (CT). Methotrexate and low-dose prednisone were started then changed in 2001 to cyclosporine 2 mg/Kg/day combined with weekly granulocyte colony-stimulating factor (G-CSF) injections as the patient had profound agranulocytosis revealed by severe stomatitis. In 2004, chronic fever and weight loss developed. Extensive investigations found no evidence of infection, and the RA was quiescent. The cyclosporine was stopped, after which the neutropenia worsened, requiring daily G-CSF injections. The monoclonal IgM component level increased to 21 g/L, the κ/λ free light-chain ratio increased to 5, and massive κ Bence-Jones proteinuria appeared. Again, histological, cytological, and flow cytometry studies of bone marrow showed no signs of B-cell lymphoma. No lymphadenopathy was found by physical examination or CT. Rituximab therapy was started in May 2005 (four infusions of 375 mg/m² each). The constitutional symptoms resolved rapidly and the neutrophil blood count increased progressively until normalization after 4 months without further G-CSF injections. The monoclonal IgM component level decreased gradually. Two additional rituximab courses (each consisting in two 1000-mg infusions) were given in 2008 and 2010 for RA flares. At last evaluation in May 2013, the neutrophil count was normal at 3.2 G/L, the monoclonal IgMκ titer was 2.1 g/L, and the blood lymphocyte immunophenotype was normal. The hemoglobin level and platelet count were also normal. No monoclonal T-cell population was detectable by TCR-gene rearrangement study. Thus, the LGCLL was in complete remission 8 years after the first rituximab infusion.
Case #2. This man born in 1948 started experiencing inflammatory pain in the hands and feet in March 2011. Neutropenia was first detected in June 2011 (Figure 2). Hematological investigations disclosed a monotypic TCR-αβ⁺CD3⁺CD8⁺CD16⁺CD56⁻CD57⁺ LGL population representing 50% of blood and bone marrow lymphocytes (absolute blood LGL count, 2.1 G/L). TCR-Vβ flow cytometry showed a defective repertoire, and clonality was demonstrated by TCR-gene rearrangement analysis. He had no splenomegaly or lymphadenopathy. T-cell LGLL was diagnosed, but the only treatment was a G-CSF injection in September 2011. In November 2011, RF- and ACPA-positive RA was diagnosed. Methotrexate and prednisone were started, but 3 months later the joint symptoms had worsened despite a prednisone dosage increase to 20 mg/d. The neutrophil blood count was 0.49 G/L and the lymphocyte count 6.6 G/L. A TNFα antagonist was not deemed appropriate given the clonal disease. Two 1000-mg rituximab infusions were given in February 2012 and rapidly induced a remission of the RA. The neutrophil count returned to normal after the first rituximab infusion and remained within the normal range subsequently. In July 2012, an increase in RA activity prompted a second rituximab course. The total lymphocyte count was normal (2.7 G/L) but the phenotype showed 40% of monotypic CD3⁺CD8⁺CD57⁺ cells (absolute LGL count, 1.1 G/L). In January 2013, despite prednisone discontinuation since September 2012, the RA remained in remission. The neutrophil count was 3.6 Giga/L and the CD3⁺CD8⁺CD57⁺ cells contributed only 13% of the total blood lymphocytes (LGL, 0.4 G/l). The monoclonal clone was still detectable by TCR-gene rearrangement molecular analysis.
DISCUSSION

To our knowledge, our two patients are the first reported cases of successful rituximab therapy in RA-associated T-cell LGPLL. The long-term follow-up in the first patient indicates that rituximab can produce a sustained improvement and even a complete remission of LGPLL. Rituximab was well tolerated in both patients.

Two pathogenic theories have been proposed for T-cell LGPLL. The initiating event leading to the monoclonal T-cell proliferation may be an acquired intrinsic molecular defect, such as a mutation. A recent study identified a somatic mutation in the signal transducer and activator of the transcription 3 (STAT3) gene in 31 (40%) of 77 LGPLL patients, with a higher proportion (73%) in patients with RA.12 Such a mutation may cause the development of a monoclonal T-cell population.

Alternatively, the T-cell expansion may be driven by a combination of persistent antigenic stimulation and immunogenetic factors. LGPLL cells exhibit the phenotype and genotype of cells subjected to chronic antigen activation, which may initiate the clonal transformation. Serial measurements in 71 patients with T-cell LGPLL showed that the dominant clonal population contracted or even disappeared over time in 26 (37%) patients, with or without a link to treatment, and was replaced by another clonal population expressing a different Vβ region.13 These findings suggest that, in some cases, development of the clonal population may be a reactive process unrelated to an intrinsic molecular defect. In RA, the effects of rituximab on the immune system may consist not only in transient B-cell depletion, but also in changes in the microenvironment and cytokine balance capable of impairing LGL clone survival without directly affecting the leukemic LGLs. This mechanism would explain the gradual shrinkage of the monoclonal population in our patients, until complete disappearance after several months in our first patient.
Late-onset neutropenia is a rare and usually transient complication of rituximab therapy in patients with malignant and rheumatic disorders.\textsuperscript{14,15} In some of these patients, an increase in CD3\textsuperscript{+}CD8\textsuperscript{+}CD57\textsuperscript{+} T-cell LGLs in the peripheral blood and bone marrow was described, with no monoclonality by TCR gene analysis.\textsuperscript{16,17}

As illustrated by our first patient, T-LGLL is frequently associated with concomitant low-grade B-cell dyscrasias, such as monoclonal gammopathy of unknown significance or chronic lymphocytic leukemia, suggesting that a common antigen may drive both T- and B-cell proliferation.\textsuperscript{18}

In conclusion, rituximab therapy may be safe and effective in LGLL-related neutropenia in RA patients and may produce a long-term complete remission of LGLL. The paradoxical efficacy of this specific anti-B-cell therapy on a T-cell monoclonal disease suggests that, in some cases, T-cell LGLL may be a reactive manifestation of chronic autoantigen stimulation rather than a true malignancy. Studies are warranted to assess our preliminary findings.
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Disclosure of Conflicts of Interest

No conflicts of interest to declare.

Authorship Contributions

All authors participated in the management of these two patients. Divi Cornec and Alain Saraux wrote the manuscript, and all authors revised it.
References

Figure legends

**Figure 1.** Patient #1 with rheumatoid arthritis. Neutropenia associated with large granular lymphocyte leukemia and monoclonal IgM gammopathy were diagnosed in 1998. Low-dose methotrexate was given from 1998 to 2001 then stopped because of agranulocytosis with severe stomatitis. Cyclosporine A and granulocyte colony-stimulating factor were started in 2001 then discontinued in 2004 because of chronic fever and weight loss attributed to monoclonal IgM progression. The first rituximab infusions were given in 2005 and ensured control of the joint symptoms, a decrease in monoclonal IgM titers, and prolonged complete remission of the leukemia.

MTX, methotrexate; CsA, cyclosporine A; G-CSF, granulocyte-colony stimulating factor

**Figure 2.** Patient #2, 63-year-old man diagnosed with T-cell large granular lymphocyte leukemia in July 2011. Seropositive erosive rheumatoid arthritis was diagnosed in November 2011 and low-dose methotrexate and prednisone were started. Three months later, neither the joint disease nor the leukemia was improved. Two 1000-mg rituximab infusions provided a rapid remission of the joint disease accompanied with immediate neutrophil count normalization despite persistence of the monoclonal T-cell population. Six months later, a second rituximab course was given for an exacerbation of the joint symptoms. In January 2013, the rheumatoid arthritis was still in remission despite prednisone discontinuation, neutrophil counts remained normal, and the leukemic cell count was substantially decreased.

MTX, methotrexate; DAS28, RA disease activity score evaluating 28 joints; G-CSF, granulocyte-colony stimulating factor
Figure 1

![Graph showing changes in various blood cell counts and monoclonal IgM levels over time, with indications of MTX, CsA + G-CSF, and Rituximab cycles.](image-url)

- **Neutrophil count (Giga/L, left axis)**
- **Lymphocyte count (Giga/L, left axis)**
- **Monoclonal IgM (g/L, right axis)**
- **Rituximab cycles**
Figure 2
Long-term remission of T-cell large granular lymphocyte leukemia associated with rheumatoid arthritis after rituximab therapy

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