Neutropenia in a Patient With Rheumatoid Arthritis Associated With αβ/γδ T-Cell Large Granular Lymphocyte Lymphoproliferative Disorder Manifesting as Felty’s Syndrome: From Diagnosis to Therapy

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Abstract

T-cell large granular lymphocytic (LGL) leukaemias are clonal proliferations of cytotoxic T-cells that are frequently associated with rheumatoid arthritis (RA) and neutropenia and are usually controlled with immunosuppressive drugs and growth factors. We report a case of a 51-years-old patient with RA, mild splenomegaly and severe autoimmune neutropenia, who have failed to respond to methotrexate, leflunamide and corticosteroids and had a suspicion of granulocyte-colony stimulating factor (G-CSF) induced thrombocytopenia. The diagnosis of Felty’s syndrome was done and phenotypically abnormal TCR-αβ and TCRγδ T-LGL were detected in blood, although PCR-based studies provided evidence only for clonal TCRG gene rearrangements. A second attempt to control neutropenia with G-CSF induced thrombocytopenia again. Cyclosporine A was effective in controlling neutropenia, the dose being adjusted in order to maintain acceptable neutrophil counts and to minimise side effects. These included hypertension, renal insufficiency and gingival hypertrophy, the later being worsened by the concomitant use of amiodipine.

Keywords: Large granular lymphocytes; Rheumatoid arthritis; Neutropenia; Cyclosporine A; Granulocyte-Colony Stimulating Factor; Amlodipine; Thrombocytopenia; Gingival hypertrophy.

Introduction

Large granular lymphocytes (LGL) represent up to 15% of the peripheral blood lymphocytes in normal adult individuals, where most LGL are natural killer cells and only about 15% are cytotoxic T cells (CTL). However, 85% of the LGL leukaemia (LGLL) cases are derived from CTL, the majority of cases being T cell receptor (TCR)- αβ CD8+ and a minority arising from TCR-αβ CD4+ or TCR-γδ T cells [1].

T-cell derived LGL lymphoproliferative disorders (LPD) do represent a continuum spectrum of transient or persistent polyclonal, oligoclonal and monoclonal CTL proliferations and are more frequent in patients with chronic inflammatory conditions, infectious or autoimmune diseases or that underwent splenectomy or transplantation; in order to establish the diagnosis of a T-cell LGL leukaemia (T-LGLL), the expanded T-cells should be proven to be monoclonal [1-3]. T-LGLL frequently associates with cytopenias and immunological abnormalities, including dysgammaglobulinemia, autoantibodies and circulating immune complexes [1-5]. They sometimes coexist with autoimmune disorders, rheumatoid arthritis (RA) occurring in up to 30% of patients [1]. Felty’s syndrome is a clinical entity defined by the triad of RA, neutropenia, and splenomegaly [6].

Although the aetiology of T-LGL-LPD is not known, there is evidence for an antigen-driven CTL proliferation that ultimately leads to the development of a clonal LGL expansion [2-5]. Chronic T-cell activation with auto-antigens has been proposed as the initial stimulus and evidence for a viral infection was provided for specific T-LGLL subtypes [7]. Deregulation of Fas/Fas ligand (FasL) induced CTL apoptosis have also been documented [8].

The diagnosis of T-LGL-LPD (including T-LGLL) should be suspected in patients with unexplained cytopenias and increased numbers of LGL in blood, documented either by morphology and/or by flow cytometry [1]. Flow cytometry is useful not only to demonstrate increased numbers of CTL, but also to evidence an aberrant CTL immunophenotype and an expansion of specific families of the variable domain of the TCR -α, -β, -γ and -δ chains; clonal rearrangements of the TCR genes are documented by Polymerase Chain Reaction (PCR) based molecular studies [1-3].

T-cell derived LGLL are usually clinically indolent, with a median survival time of over 10 years. Neutropenia is the most significant clinical problem in patients with TCR-
αβ<sup>+</sup>CD8<sup>+</sup> and TCR-γδ<sup>+</sup> T-LGLL (85%), severe neutropenia being present in about 45% of cases [1-3], although being rarely observed in TCR-αβ<sup>+</sup>CD4<sup>+</sup> T-LGLL [3]. Anaemia and thrombocytopenia are found in about 48% and 20% of patients, respectively [1]. Mild to moderate splenomegaly occurs in 20-50% of cases, whereas hepatomegaly is found in only 10-20% [1].

In spite of the indolent behaviour, the majority of patients with T-LGLL require treatment at some point during the disease course and the most common indications are symptomatic cytopenias, especially chronic neutropenia associated with recurrent infections [1, 9]. Improvement of cytopenias is usually achieved with immunosuppressive agents, including methotrexate (MTX), cyclophosphamide and cyclosporine A (CsA); corticoids alone normally do not produce sustained neutrophil increase, and secondary long-
term effects are not negligible; treatment with G-CSF may also improve neutropenia, being particularly beneficial for short periods of time during infectious complications [9-12].

Herein, we report a case of a patient with RA with 15 years of evolution and a proliferation of TCRαβ+ and TCR-γδ T-LGL who suffered from severe chronic neutropenia, refractory to MTX, leflunamide and corticoids. Attempts to correct the neutropenia with G-CSF during undercurrent infections resulted in thrombocytopenia for two times. Finally, CsA therapy made possible a sustained neutrophil recovery, dose adjusting being done in order to avoid secondary effects; one of them, gingival hyperplasia, was substantially worsened due to using amlodipine to control hypertension and renal insufficiency.

**Case Report**

A 51-year-old Portuguese Caucasian man with RA was referred to our haematology consultation in December 2009 for investigation of severe chronic neutropenia. On physical examination, he presented bilateral swelling, stiffness and deformity of the proximal interphalangeal and metacarpophalangeal joints, as well as a mild splenomegaly.

In the past, he has received several therapies. Shortly after the diagnosis of RA in 1995, he was treated with gold salts and two years later he started with oral prednisone (5 mg/day) and MTX (5 mg/week) which were maintained for about five years. In 2007, after consulting his doctor due to worsening of the osteoarticular complaints, he restarted on prednisone (10 mg/day) and MTX (10 mg/week), the later being suspended in January 2008, because of sustained neutropenia. Two months afterward, he started on oral leflunamide, which was discontinued one month later because of worsening of the neutropenia (neutrophils 0.1 × 109/L) and a serious skin infection on his hand, refractive to oral fluclucocaxilin. For that reason, he was hospitalized and received oral prednisolone (100 mg/day, with a rapid decrease in dosage) and subcutaneous G-CSF (300 μg/day). Neutrophil recovery was observed 10 days later, reaching 7.9 × 109/L (Fig. 1A). However, because of a progressive decrease in platelet counts from 181 to 87 × 109/L, it was decided to stop G-CSF, and neutrophils declined again to 0.5 × 109/L (Fig. 1A). From 2008 to 2009, he remained on oral prednisone 5 - 10 mg/day, with persistence of asymptomatic neutropenia.

**Investigations**

Blood counts revealed severe leukopenia and neutropenia (leucocytes 1.5 × 109/L; neutrophils 0.2 × 109/L; lymphocytes 1.4 × 109/L), as well as mild anemia (haemoglobin 118 g/L) and thrombocytopenia (platelet count 140 × 109/L). The bone marrow aspirate and biopsy showed a normal myeloid/erythroid ratio and a normal granulocytic maturation, without morphological evidence for lymphocytic infiltration.

X-rays of the hands and feet revealed subarticlar bone erosions, radial aspect of the proximal interphalangeal, metacarpal and metatarsophalangeal joints and soft tissue swelling. The abdominal echography confirmed the splenomegaly (longitudinal and antero-posterior splenic axes of 18 and 13 cm, respectively).

The rheumatoid factor was of 111 UI/mL (normal < 20 UI/mL), the anti-cyclic citrullinated protein antibodies were of > 2,500 UI/mL (normal < 20 UI/mL), and the erythrocyte sedimentation rate was of 35 mm at the end of first hour. Serum IgG was slightly increased (1,690 mg/dL; normal range: 793 - 1,590 mg/dL) and a small monoclonal band (IgG, k) was detected. Biochemical parameters, including creatinine serum levels were normal. Anti-neutrophil IgG antibodies were documented on the neutrophil membrane by flow cytometry.

Peripheral blood immunophenotyping revealed that 93% of lymphocytes were T-cells, from which 74% were TCR-αβ+ (CD4+: 36%, CD8+: 33%) and 26% were TCR-γδ+ CD4+ CD8−/dim T-cells. T-LGL, defined as CD26-, CD27- and CD28- T-cells expressing CTL associated markers, accounted for 66% of CD8+ TCR-αβ+ and for 99% of the TCR-γδ+ T-cell compartments, respectively. TCR-αβ+ CD8+ and TCRγδ+ T-LGL had similar abnormal patterns of expression of CD2, CD5, CD7, CD11c, CD45RA and CD57 antigens (Fig. 2). TCR-Vδ repertoire studies confirmed a major Vδ1 LGL expansion (96% of the TCR-γδ+ T-cells) whereas those from TCR- Vδ showed a dilution pattern, indicating an expansion of one or more TCR-Vδ families that are not detected with the panel of monoclonal antibodies used [2]. Molecular studies using the five EuroClonality (BIOMED-2) standardized PCR-based TCR multiplex tubes [13], provided evidence for a monoclonal TCRG gene rearrangement (Fig. 3A) and revealed a polyclonal TCRB gene rearrangement pattern (Fig. 3B).

**Differential diagnosis**

Felty’s syndrome and T-LGL-LPD (including T-LGLL) are not mutually exclusive entities, as the distinction depends mainly on the clinical presentation and on the exhaustiveness of the laboratory investigation rather than being conceptual [6]. In fact, by definition, Felty’s syndrome consists of the triad of AR, splenomegaly, leukopenia with neutropenia and variable lymphocyte counts, whereas T-LGL-LPD, which may also occur in patients with RA, usually manifests as lymphocytosis, with or without splenomegaly and/or neutropenia [6]. Moreover, patients with Felty’s syndrome frequently show clonal T-LGL proliferations, suggesting that these are overlapping entities, and that the distinct clinical manifestations could probably be explained by differences in the homing and functional properties of the clonally expanded T-LGL and in clone size [6].
Figure 2. Flow cytometry dot plots showing the abnormal TCRαβ⁺CD8⁺ strong (yellow dots) and TCRγδ⁺CD8⁻/dim (green dots) T-LGL clones, detected in the peripheral blood. Red dots represent normal CD3⁺ TCRαβ⁺CD8⁺ strong T-cells. Other peripheral blood lymphocytes are represented by gray dots. Phenotypically abnormal CD3⁺ TCRαβ⁺CD8⁺ strong (yellow dots) and CD3⁺ TCRγδ⁺ (Vγ)CD8⁻/dim (green dots) T-LGLL cells had a similar immunophenotype for the majority of the markers: CD2⁺/dim, CD3⁺, CD5⁻/dim, CD7⁺/dim, CD11c⁺, CD16⁻, CD28⁻, CD45RA⁺, CD45RO⁻, CD57⁻/dim. Other markers (data not shown): CD11a⁺/dim, CD56⁻, CD94⁻, HLA-DR⁻/dim.
Figure 3. GeneScan fragment analysis of PCR products from TCR gene rearrangements of the DNA extracted from the peripheral blood of the patient, using the five BIOMED-2/EuroClonality multiplex tubes for TCRG (A) and TCRB (B) genes. TCRG and TCRB multiplex tubes are designed with different Vγ/Jγ and Vβ/Jβ primers, respectively, allowing for the amplification PCR products with different sizes: Vγ/Jγ (240 - 285 nt) and Vβ/Jβ (285 - 325 nt). Shown are the results obtained in TCRG-tubes A and B (A) and TCRB-tubes A, B and C (B). The analysis of the TCRG gene rearrangements reveals a clonal peak in a polyclonal background (TCRG-tube A, with the arrow indicating the clonal peak) whereas analysis of the TCRB gene rearrangements show a polyclonal pattern, with a typical Gaussian.
Treatment

Shortly after being admitted to our consultation, when treatment options were still being discussed, the patient had a skin infection on the face and started on ciprofloxacin and G-CSF (300 μg/day). Five days after, the platelet counts decreased from 123 to 41 × 10⁹/L, without evidence for neutrophil recovery (Fig. 1B). Thus, G-CSF was stopped and the patient was medicated with oral CsA (200 mg twice a day).

Outcome and follow-up

Treatment with CsA resulted in a progressive increase in neutrophil counts and after one month the patient had already 1.1 × 10⁹ neutrophils/L (Fig. 1C). The secondary effects included muscle cramps, hypertension, renal insufficiency and gingival hypertrophy. Muscle cramps were easily controlled with oral magnesium. Mild hypertension (systolic: 140 - 160 mmHg; diastolic: 90 - 100 mmHg) and increased creatinine serum levels (maximum of 2.1 mg/dL) were noted for the first time nearly one month after starting CsA; by adjusting the CsA dosage and adding a long-acting calcium-channel blocker (amlodipine, 5 mg/day), we could control hypertension and prevent further deterioration of the renal function. However, the patient developed mild peripheral oedema with normal cardiac function and progressive gingival hypertrophy, the later being difficult to manage. Besides of the cosmetic effect, it affected the oral hygiene, leading to gingival bleeding and tooth caries and requiring periodically partial gingivectomy. After suspending amlodipine, by July 2012, the peripheral oedema disappeared and the gingival hypertrophy reduced markedly. On the last evaluation, by October 2012, the patient persisted on CsA (125 mg twice a day) and prednisone (5 mg/day), and had normal blood counts, controlled joint complains and absence of infectious episodes.

Discussion

We reported on a case of a αβ⁺/γδ⁺ T-LGL-LPD manifesting as Felty’s syndrome in a patient with longstanding RA and severe neutropenia, which deserves discussion concerning the diagnosis, the mechanisms underlying the disease manifestations and the therapeutic decisions.

Biological bases for Felty’s syndrome and T-LGL lymphoproliferative disorders

This case fulfils the criteria both for the diagnosis of a Felty’s syndrome and a TCR-αβ⁺/TCR-γδ⁺ T-LGL-LPD with restricted TCR-V β and TCR- Vδ repertoires and similar aberrant immunophenotyping features. The fact that only monoclonal TCRG genes rearrangements were documented in the case reported herein, could be explained by limitations of the method in distinguishing polyclonal, oligoclonal and monoclonal T cell proliferations in specific conditions, such as low representation of the expanded T-cell clones, large polyclonal background and coexistence of clonally expanded TCR-αβ⁺ and TCR-γδ⁺ T cells [14]. The possibility of the TCR-αβ⁺ and the TCR-γδ⁺ T-LGL expansions being driven by a common antigen can be hypothesised. In fact, it is now accepted that T-LGL proliferations are triggered and sustained by antigen driven CTL expansions that develop non-randomly in the context of an immune response [5, 15]. This hypothesis could explain why in patients with T-LGL-LPD, similar genetic sequences are shared by multiple dominant and co-dominant T-cell clones, as defined by the VB-chain complementarity-determining region 3 (CDR3), the antigen-specific portion of the TCR [15].

Possible mechanism for the neutropenia

Neutropenia is usually the most significant problem both in Felty’s syndrome and T-LGLL patients and in generally it cannot be explained by tissue infiltration by the LGL [1, 6, 9]. Possible pathogenic mechanisms include autoantibody or immune complex mediated peripheral destruction of mature neutrophils, induction of Fas/FasL mediated neutrophil apoptosis, autoimmune destruction of the myeloid precursor cells and deregulation of the granulocytic cell maturation [10], probably related to the production of proinflammatory cytokines [16, 17]. In the case reported herein, positive antineutrophil autoantibodies and a normal bone marrow, argue for an autoimmune mechanism.

Treatment options

Treatment options for neutropenia associated with T-LGL-LPD (including T-LGLL) should be discussed in the light of the best biological and clinical evidence, taking into account the mechanism of action, the efficacy and the side effects, as well as the costs. When this patient was referred to our hospital, the major difficulty was to select the optimal therapy, as he was chronically medicated with corticosteroids, and has been previously treated with immunosuppressive agents and G-CSF without success.

G-CSF effectiveness and drug induced thrombocytopenia

Treatment with G-CSF often improves neutropenia in patients with T-LGL-LPD (including T-LGLL) and Felty’s syndrome, being especially useful when used for short periods of time, during intercurrent infections [9, 10, 18]. Unfortunately, this patient did not show a satisfactory response to G-CSF and suffered from a rare side effect, which is thrombocytopenia. Drug induced thrombocytopenia can be a consequence of decreased platelet production or accelerated platelet destruction [19]. Platelet consumption may occur by different mechanisms, including antibodies that recognize platelet glycoproteins in the presence of the sensitizing drug.
or of its metabolites, immune complexes or true autoimmune phenomena [20]. Slight thrombocytopenia is commonly observed during G-CSF mobilization of haematopoietic stem cells and has been attributed to the leukapheresis procedure and/or to G-CSF therapy [19], a possible mechanism being G-CSF-induced splenomegaly and hypersplenism [21]. However, the low number of reports on G-CSF induced thrombocytopenia contrast with the high number of patients receiving G-CSF, indicating some kind of individual drug sensitivity. Indeed, only a few cases of clinically significant G-CSF induced thrombocytopenia have been published to date [22-25], one of which documented a severe thrombocytopenia in a patient treated with G-CSF, whose subsequent investigations suggested an underlying immune mechanism [22], and another occurred in a patient with Felty’s syndrome [24]. In our patient, the fact that second exposition to G-CSF resulted in a more rapidly and profound decrease in platelet counts would suggest an immune-mediated mechanism, with an anamnestic response.

Cyclosporine a effectiveness and drug induced gingival hypertrophy

The immunosuppressive agents are the mainstay of therapy in T-LGLL, being effective in about 50-60% of patients [9-12]. However, responders usually require indefinite therapy to prevent relapse, as reversion of neutropenia with CsA and MTX occurs despite the persistence of the abnormal clonally expanded T-LGL, suggesting that the main effect is immunosuppressive or immunomodulatory [11, 12]. CsA acts on the expression of the genes coding for the interleukin 2 (IL-2) and the IL-2 receptor, suppressing both the cellular and the antibody-mediated immune responses [25].

Toxicities of long-term CsA therapy should not be neglected. Thus, after achievement of the best response, the dose should be tapered down slowly in order to obtain the lowest effective dose and to control side effects [9-12]. This patient maintained acceptable and stable neutrophil numbers at the CsA dosage of 125 mg twice/day, with minimal lateral effects except for gingival hypertrophy. In concerning this side effect, it should be emphasised that gingival hyperplasia has also been reported in association with calcium channel blockers, especially with dihydropyridines or benzothiazepine derivatives [26], and adding amlodipine in an attempt to control hypertension and renal dysfunction has markedly contributed to worsening of this condition.

Learning points/take home messages

Consider the diagnosis of a T-LGL-LPD (including T-LGLL) in patients with chronic neutropenia, especially if associated with RA, and keep in mind that it may be manifest as Felty’s syndrome.

Remember that clonal T-LGL expansions may occur without lymphocytosis, and their identification requires laboratory expertise concerning morphology, immunophenotyping and molecular studies.

Ponder CsA for long-term treatment of severe and/or symptomatic neutropenia associated with clonal T-LGL proliferations, adapting CsA dosage to each patient in order to obtain the desired therapeutic benefit with minimal toxicity and avoiding other medications with overlapping side effects, such as calcium channel blockers because they may also cause gingival hypertrophy.

Consider G-CSF to control neutropenia for short periods of time, providing that the patient is responsive, and investigate the possibility of drug induced thrombocytopenia when it develops de novo (or worsens) in patients treated with G-CSF.

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