Abstract

Pure red cell aplasia (PRCA) is an unusual cause of anemia in patients with chronic lymphoproliferative disorders. Here, we present two cases of PRCA, one associated with chronic lymphocytic leukemia (CLL) and the other with splenic marginal zone lymphoma, in which the PRCA responded dramatically to treatment with rituximab. We then review the literature on PRCA in lymphoma and response to rituximab. PRCA associated with CLL or lymphoma may be another indication for rituximab therapy.

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1. Introduction

Pure red cell aplasia (PRCA) is characterized by normochromic normocytic anemia, reticulocytopenia (reticulocyte count <1%), and an almost complete absence of erythroblasts from the bone marrow (erythroblasts <0.5%). Most cases of PRCA are idiopathic, but PRCA is reported in various hematological malignancies. Though acquired, PRCA is a relatively rare cause of anemia in chronic lymphocytic leukemia (CLL), its incidence may be as high as 6% [1]. It is also rare to find PRCA associated with non-Hodgkin lymphoma (NHL). For example, in a study of 47 PRCA cases that were evaluated over a 14-year-long period in Mayo Clinic, only 2 patients had NHL [2]. Most cases of PRCA are presumed to be autoimmune mediated by antibodies against either erythroblasts or erythropoietin, by T cells secreting factors selectively inhibiting erythroid colonies in the bone marrow or by natural killer (NK) cells directly lysing erythroblasts [3]. Viral infections, notably by parvovirus B19, may cause PRCA, especially in immunocompromised patients [4]. Immunocompromised patients (AIDS, post-transplantation, chemotherapy, etc.) may be unable to mount a neutralizing antibody response due to persistent bone marrow insufficiency.

Recently, there have been reports of PRCA caused by parvovirus B19 in patients with non-Hodgkin lymphomas that developed after instituting treatment with the monoclonal antibodies alemtuzumab (anti-CD52) [5–7] and rituximab (anti-CD20) [8].

Therapeutic approach to PRCA depends on the cause. Parvovirus may respond to intravenous immunoglobulin (IVIg). While autoimmune PRCA can be treated using corticosteroids, antithymocyte globulin, cyclosporine or splenectomy, the overall response to these treatments is only 68% [9]. For patients refractory to the above treatments or for those...
unable to tolerate these treatments, experimental approaches may be needed.

Rituximab has been found to be useful in treating autoimmune hemolytic anemia and thrombocytopenia associated with CLL [10,11]. More recently, there have been anecdotal reports of PRCA successfully treated with monoclonal antibodies, rituximab and alemtuzumab, in patients with chronic lymphocytic leukemia [12–15]. We report here two cases of B-cell lymphoproliferative disorders that responded to rituximab therapy. One is a case of CLL with PRCA, initially thought to be due to parvovirus B19 infection, whose PRCA did not respond to IVIg therapy, but dramatically responded to rituximab. We also report the first case of PRCA associated with splenic marginal zone lymphoma treated successfully with rituximab. We then review the reported cases of PRCA associated with B-cell lymphoproliferative disorders that responded to rituximab. We conclude that rituximab is an effective treatment for PRCA associated with B-cell lymphoproliferative disorders.

2. Patient 1

WM is a 54-year-old female initially diagnosed with stage I (Rai) CLL in April 1998 when she presented with extreme fatigue, cervical lymph node enlargement and a white blood cell count (WBC) of 30.8 × 10^9/L with 16.5 × 10^9/L lymphocytes. Her hemoglobin (Hgb) was 14.8 g/dL, platelet count was within normal limits and flow cytometry showed cells that were CD5+, CD23+ with dim CD20 and monoclonal kappa surface markers. Half of the B-cells were positive for CD11c. She also had recently been diagnosed with lymphocytic thyroiditis consistent with Hashimoto’s. She was treated with six cycles of fludarabine at 25 mg/m^2 daily for 5 days on a 4-week schedule from October 1998 to April 1999. CLL was in remission for 8 months, when she developed a thyroid nodule. Excision of the thyroid nodule showed CLL in a background of Hashimoto’s thyroiditis. Later, she received involved field radiation to the thyroid as the sole site of recurrence.

She did well for over 2 years, when her disease progressed. She was again treated with fludarabine for one cycle. The following month, she was hospitalized elsewhere for fevers and dermatomal zoster infection. She was found to be anemic and was given transfusions. Her Hgb at the time of transfer to our institution was 9.8 g/dL. WBC was 19.4 × 10^9/L and platelets were 273 × 10^9/L. Three days later, her Hgb had decreased to 7.1 g/dL with reticulocyte of 0.26%. Coomb’s test was negative, LDH was only slightly elevated and haptoglobin was normal indicating a cause other than hemolysis. Lymph node biopsy did not support transformation. Bone marrow biopsy revealed 80% marrow cellularity with infiltration by small atypical lymphoid cells consistent with CLL. Cytogenetics showed karyotype of 46,XX, del(3)(q21q26.1),ins(3)(q26.2q21q26.2). The erythropoietic series represented only 2% of cells, mostly large pro-erythroblasts with intranuclear inclusion-like structures (Fig. 1). Based on this result, parvovirus B19 infection was suspected. Parvovirus B19 IgG antibodies were slightly positive (titer 1:64), but IgM titers were not elevated (0.05). Her fevers persisted without an identifiable cause. Empiric treatment for parvovirus infection was initiated with intravenous immunoglobulin (IVIg) at 1 mg/kg for 2 days. Her fevers then subsided, but she continued to require RBC transfusions. Subsequently, PCR for parvovirus B19, performed after treatment with IVIg and steroids, was negative.

The patient continued to require periodic transfusions for 6 months, despite weekly erythropoietin injections. While cyclosporine was considered, a trial of rituximab, given its utility in autoimmune processes and against CLL itself as well as its favorable side effect profile, was initiated. She received rituximab intravenously at 375 mg/m^2 weekly for

Fig. 1. Bone marrow biopsy and aspirate (insert) with interstitial infiltrates of small lymphocytes and scattered large pronormoblasts (arrows) with possible intranuclear inclusions.
4 weeks. By the day of her fourth treatment, the Hgb had improved to 10.6 g/dL with reticulocyte count of 5.91%. Her CLL remained in partial remission and she has remained transfusion independent for 21 months since rituximab treatment with her latest Hgb 12.8 g/dL, WBC $9.3 \times 10^9/L$ and platelets $198 \times 10^9/L$.

3. Patient 2

KR is a 57-year-old Iraqi immigrant female living in Sweden with past medical history significant for tuberculosis involving lymph nodes of the neck in 1997. She was successfully treated and remained in good health until late 2003 when she started developing low-grade fevers, fatigue and night sweats. In April 2004, she was admitted to the hospital for worsening symptoms. On examination, she was pale and her spleen was palpable 1 cm below the costal margin. There was no lymphadenopathy. Her Hgb was 6.9 g/dL with mean corpuscular volume (MCV) of 78 fl, WBC count was $3.6 \times 10^9/L$ with normal differential and platelets were $203 \times 10^9/L$. There was no evidence of iron deficiency (ferritin $239 \mu g/L$), Hgb electrophoresis was normal, Coomb’s test was negative and LDH was only slightly elevated, but reticulocyte count was low ($3 \times 10^9/L$). Chest X ray was clear. Computerized tomography scan of the abdomen revealed an enlarged spleen measuring $19 \text{ cm} \times 11 \text{ cm} \times 6 \text{ cm}$. The patient was initially suspected of having myelodysplasia and was treated with prednisone, erythropoietin and periodic red cell transfusions for 6 months. A second review of her bone marrow biopsy was requested.

Morphologically, the marrow was cellular with scattered paratrabecular lymphoid nodules (Fig. 2A and B). The lymphocytes were small, slightly irregular and CD20 positive by immunostaining (Fig. 2D). By flow cytometry, the B-lymphocytes were kappa monoclonal CD20+, CD5−, CD10− and CD23−. The myeloid series and megakaryocytes were within normal limits. The erythroid series was represented by scattered large pro-erythroblasts only (Fig. 2C). There was no evidence of myelodysplasia. Morphologic and immunophenotypic findings were consistent with PRCA and splenic marginal zone lymphoma. Parvovirus inclusions were not present. Cytogenetics of the marrow aspirate showed a normal female karyotype except for monosomy 18 in 3/25 metaphases, the significance of which was unclear. Following this revised diagnosis, she was treated briefly with cyclophosphamide with minimal improvement. She then received rituximab at a weekly dose of 375 mg/m². Response was seen by 1 week after the first dose and a total of five doses were given. Hgb increased to 13.7 mg/dL and other symptoms disappeared as well. Two months after the last dose

![Fig. 2. A and B (Hematoxylin and eosin stain): Low and higher magnifications of the core bone marrow biopsy showing a cellular marrow with paratrabecular lymphoid nodules (arrows). C (H and E): High magnification demonstrating scattered large pro-erythroblasts and otherwise absent lymphoid series. D: L-26 (CD20) immunostaining of a positive lymphoid nodule.](image)
4. Discussion

Rituximab, a genetically engineered chimeric anti-CD20 monoclonal antibody, can selectively deplete B-cells by mechanisms which include antibody-dependent cell-mediated cytotoxicity, complement-mediated cytotoxicity, and inhibition of cell proliferation with direct induction of B-cell apoptosis [16]. Antibody mediated autoimmune hematological disorders that have been successfully treated with rituximab include immune thrombocytopenia, cold agglutinin disease and autoimmune hemolytic anemia [17–20]. Its role in PRCA associated with mature B-cell neoplasms is not very well established.

We summarize (Table 1) the few published cases of PRCA associated with B-cell lymphoproliferative disorders that were treated with rituximab (including those presented above). Each of the four patients with a relapse of CLL developed PRCA within a few weeks after re-starting chemotherapy, in three cases with fludarabine. In one case, rituximab was started as the initial treatment for PRCA. The maximum time for onset of response was 4 weeks. In two cases, where PRCA and the primary disorder were simultaneously diagnosed, rituximab led to response in both PRCA and primary disorder (complete response in CLL and partial response in splenic lymphoma). One patient had response to rituximab lasting only for 4 weeks, but then responded to alemtuzumab. This patient also had the lowest hemoglobin, possibly indicating disease severity. Poor prognostic cytogenetics is one known reason for failure of PRCA to respond to immunosuppression [2]. Unfortunately, we do not have cytogenetics on any of these cases (excluding the ones presented in this report) to comment on whether they play a role in response to rituximab. No significant side effects were reported in any of these patients. There was no standard on the number of treatments given, although the standard dose and schedule appeared to be adequate.

There are two case reports in the literature of the efficacy of rituximab in the treatment of bone marrow aplasia associated with refractory CLL and refractory NHL. The patient with refractory CLL was treated with rituximab weekly for 4 weeks. Response was seen in all blood counts in less than a month of completing treatment. Though there was residual CLL in bone marrow, blood counts stayed within normal limits for at least 24 months [21]. Egerer et al. reported a case of follicular NHL with pancytopenia following fludarabine, and showed a complete response with respect to both NHL and marrow aplasia [22]. This patient was treated with maintenance rituximab every 3–4 months after initial weekly doses. It is possible that other cases of PRCA that did not respond to rituximab were not reported.

| Table 1
| Summary of cases of PRCA associated with B-cell lymphoproliferative disorders treated with Rituximab (375 mg/(m² week)) + Reference, age (years)/gender | B-cell disorder, duration | Treatments for B-cell disorder | Hgb (g/dL) | Treatments for PRCA | Time from PRCA diagnosis to starting rituximab | Onset of response | Duration of response |
|---|---|---|---|---|---|---|---|---|
| Ghazal [12], 79/M CLL-B-cell (PRCA diagnosed simultaneously) | Same as that for PRCA | 7.0 | CyA, 4 months CS, 3 months IVIg, 2 days | Rituximab, 8 weeks | 6 months | 2 weeks | 10+ months (PRCA and CLL) |
| Ghazal [12], 47/F CLL-B-cell, 7 years relapse | Flud—5 cycles (just preceded PRCA) | 7.1 | Flud, 1 weeks | Rituximab, 8 weeks | – | – | 10 weeks | 4 weeks | 11+ months (PRCA and CLL) |
| Batlle [13], 58/M CLL- B-cell, 7 years relapse | CS + Chl + CsA 12 months, CHOP—6 cycles | 6.0 | CsA, 2 months | Rituximab, 4 weeks | – | – | 2 months | 4 weeks | 6+ months (PRCA and CLL) |
| Ru [14], 68/M CLL- B-cell, 6 months (normal WBC at PRCA diagnosis) | Chl—1 cycle, Flud—1 cycle (just preceded PRCA) | 1.5 | CS—3 week, IVIg—3 days | CsA+Cy, 3 weeks | Rituximab, 8 weeks | – | – | 6 months | 4 weeks | 21+ months (PRCA and CLL) |
| Pantelidou [15], 50/M CLL- B-cell, 4 years relapse | Chl—2 months (just preceded PRCA) | 7.8 | Rituximab, 8 weeks | – | – | – | Few days | 2 weeks | 3+ months (PRCA) |
| Present patient 1, 54/F CLL-B-cell, 2 years, relapse | Flud—4 cycles, Repeat Flud—1 cycle (just preceded PRCA) | 7.1 | IVIg + CS, 2 days | Rituximab, 4 weeks | – | – | 6 months | 4 weeks | 21+ months (PRCA and CLL) |
| Present patient 2, 57/F Splenic marginal lymphoma, (PRCA diagnosed simultaneously) | Same as that for PRCA | 6.9 | Cy + CS, 6 weeks | Rituximab, 5 weeks | – | – | 4 months | 1 week | 5+ months (PRCA and lymphoma) |

CsA: cyclosporine A, Cy: cyclophosphamide, Chl: chlorambucil, Flud: fludarabine, CS: corticosteroids, -NA-: not available. (*) Response to Alemtuzumab lasted 9+ months with onset of response in 1 week.

(a) Patient was excluded from this chart.

(b) Response to rituximab was included in all the others.
Parvovirus B19 is a potential cause of acquired PRCA in immunocompromised patients. IVIG may be effective for treatment of B19-associated PRCA, although its course in immunocompromised patients is not well defined. Patients with acquired PRCA should be evaluated for evidence of B19 infection [23]. The patient presented in Case 1 had CLL treated with fludarabine before she developed PRCA, rendering her immunocompromised. In this situation, serological tests are not reliable as antibody production is absent or minimal [24]. Usual pathological changes are giant pronormoblasts (Fig. 2), which are early erythroid cells with a diameter of 25–32 μm with large eosinophilic nuclear inclusion bodies and cytoplasmic vacuolization. Although the presence of giant pronormoblasts in either BM or blood is suggestive of B19 infection, their presence is not sufficient to make a diagnosis of B19 infection [4]. The sensitivity and specificity of giant pronormoblasts were only 63 and 92%, respectively, in one study [23]. Definitive diagnosis of parvovirus B19 virus induced chronic PRCA rests on detection of viral DNA by direct hybridization or the more sensitive PCR [4]. Whether Patient 1 really had parvovirus B19 infection remains questionable, since the negative PCR was performed after several therapeutic interventions, however the dramatic response to rituximab is beyond question.

The mechanism of action of rituximab in PRCA is not entirely clear. The most obvious explanation is the elimination of antibody producing tumor cells. However, the rapid and dramatic response, even with the persistence of residual neoplastic cells in some patients raises the possibility of other mechanisms, too. Investigating the action of rituximab and its action in PRCA may elucidate the role of rituximab in B-cell disorders in general. Regardless of the mechanisms, our own experience and those of the few published reports summarized here indicate that PRCA can be another indication of the use of rituximab in the treatment of active B-cell neoplasms.

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References


