Pure red cell aplasia in autoimmune polyglandular syndrome with T lymphocytosis

ALBERTO GROSSI, CHIARA NOZZOLI, *RICCARDO GHERI, VALERIA SANTINI, CLAUDIA MARRANI, ANTONELLA ZOCCOLANTE, PIERLUIGI ROSSI FERRINI

*Division of Hematology, Florence, *Division of Endocrinology, Florence, Italy*

We report the onset of pure red cell aplasia (PRCA) in a patient with a history of polyglandular syndrome including Addison's disease, malabsorption syndrome, diabetes type I and gastric hyperplastic polyposis. An autoimmune origin for this complex disorder was not supported by the presence of organ specific antibodies, but T cells were found to be of polyclonal origin, as demonstrated by molecular analysis of T cell receptor (TCR) gene rearrangement. The pathophysiology of this case, based on laboratory findings and response to therapy, is discussed.

Pure red cell aplasia (PRCA) is a clinical syndrome characterized by severe normochromic and normocytic anemia, a reticulocyte count less than 1%, and normal platelet and granulocyte counts. Bone marrow shows a selective erythroid hypoplasia. The chronic form may occur as a primary disease or be associated with pre-existing autoimmune, viral or neoplastic diseases such as thymoma, rheumatoid arthritis, systemic lupus erythematosus, hepatitis, lymphoma, B-cell chronic lymphocytic leukemia (CLL), T-cell CLL and large granular lymphocytic (LGL) leukemia.[1-7] Immunologic mechanisms have been advocated to play a role in the pathophysiology of PRCA. Antibodies with selective cytotoxicity against marrow erythroid progenitors (BFU-E, CFU-E) or precursors (marrow normoblasts) or against erythropoietin have been demonstrated in the serum of some patients with PRCA.[8] In other reports, T-cells with suppressor/cytotoxic phenotype from patients with PRCA and thymoma, lymphoma, CLL, LGL leukemia or polyglandular type I syndrome, have been shown to suppress erythropoiesis *in vitro*.[8] T-lymphocytes have been suggested to have a clonal origin in some cases.[10]

We report the case of a patient who developed PRCA associated with T ($CD3^+$, $CD8^+$, $HLA\text{-}DR^+$) lymphocytosis, during the course of a polyglandular disorder including Addison's disease, malabsorption syndrome, diabetes type I and gastric hyperplastic polyposis. The pathophysiology of this case, on the basis of laboratory findings and response to therapy, is discussed.

A 42-year-old man was referred to our division because of severe anemia. He had suffered from atopic dermatitis since early childhood. At the age of 23 he developed primary adrenal failure and since then he had been receiving hydrocortisone replacement therapy. In 1990 he underwent gastric endoscopy because of a period of dyspepsia and gastric discomfort. A diagnosis of hyperplastic polyposis was made. Shortly after he also developed a malabsorption syndrome with pancreatic exocrine insufficiency. In June 1994 the patient was referred to us because of macrocytic anemia resistant to treatment with vitamin B12 and folic acid. Complete blood count was as follows: hemoglobin 8.2 g/dL with a mean corpuscolar volume of 113 fL, platelets 361×10^9/L and white blood cells 6.3×10^9/L (neutrophils 23%, lymphocytes 69%, eosinophils 4%, monocytes 4%). The blood smear morphology of lymphocytes did not show large granular cells. The percentage of reticulocytes was 0.3%. Direct and indirect Coombs' tests were negative. Serum ferritin and erythropoietin were 80 ng/mL and 360 mU/mL, respectively. Bone marrow aspiration and biopsy showed lymphocytosis with normal granulocytic and megakaryocytic lineages, while erythroid precursors were severely reduced and megaloblastic. The presence of thymoma was excluded by chest X-rays and CAT scan. Rheumatoid factor, antinuclear, anti-DNA, anti-thyroglobulin, anti-TSH, antiadrenal gland, anti-gastric mucosae antibodies and a microsome test were all negative. Serum immunoglobulin level was reduced (IgA 20 mg/dL, IgG 580 mg/dL, IgM 100 mg/dL). Serologic tests did not reveal evidence of recent or active CMV, Parvovirus B19, Epstein-Barr, HBV or HCV infections. Flow cytometric analysis of peripheral lymphocytes showed a T cell expansion (Figure 1) (CD3 90%, CD5 90%, CD7 90%, CD8 69%, CD4 21%, CD16/ CD56 3%, and HLA-DR 56%). Only 20% of CD8 were positive for γδ T cell receptor (TCR). TCR repertoire expressed by peripheral blood lymphocytes was analyzed by RT-PCR and no monoclonal or unappropriate rearrangement was detected. To discriminate the polyclonal from the monoclonal T-lymphocyte population, after RNA extraction from peripheral blood leukocytes of the patient, RT-PCR was performed employing a panel of TCR Vα and Vβ primers commercially available from Clontech (Palo Alto, CA, USA).

Vα primers were 22 5'-primers for the same number of known major Vα families, PCR amplification was performed using one of the 5'-primers together with a 3'-primer annealing to the Cα region. Vβ primers were 24 5'-primers for 24 known major Vβ families, to be used in amplification with a 3'-primer annealing to the Cβ region. Primers were designed in order to avoid 5'-3' sequence complementarity between the C- and V- region primers. PCR amplification was carried on for 35 cycles, as indicated by the manufacturer's instructions, with step 1 at 95°C for 1 min, step 2 55°C for 1 min and step 3 72°C for 1 minute. After amplification, PCR products were analyzed by agarose gel 2% electrophoresis and bands visualized by ethidium bromide staining. Bands were visible with all primers' pairs, their sizes ranging from 240 to 430 bp for Vα primers and from 180 to 370 bp for Vb primers. Cα-Cα control was

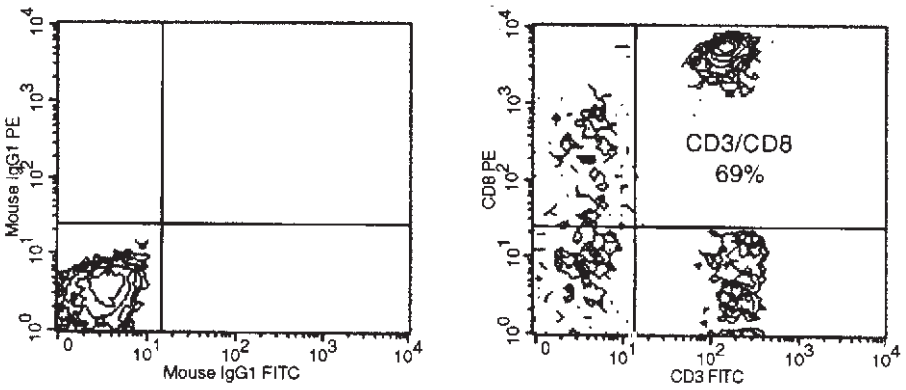

Figure 1. Flow cytometry analysis of peripheral lymphocytes (left) showing the co-expression of CD3 FITC with CD8 PE (right).

604 bp, whereas Cβ-Cβ was 146 bp.

No karyotypic abnormalities were found in bone marrow cells. A non T-depleted mononuclear cell population was used for cell growth studies, and an increased proliferation of patient's peripheral blood erythroid progenitors (BFU-E) was observed, even in the presence of autologous serum substituting for FCS in the culture medium. Moreover, the patient's serum did not inhibit the growth of normal erythroid progenitors.

The patient's refusal to undergo a new bone marrow aspiration made it impossible to evaluate CFU-E proliferation. At the time of admission, the patient was on substitution therapy with steroids for hypoadrenalism. After diagnosis, he was given cyclosporin treatment (5 mg/kg/day) for four months. Anemia was not corrected, but an improvement of his malabsorption syndrome was observed. Finally a course of antithymocyte globulin (ATG) induced reticulocytosis (4.7% at day 21) and corrected the anemia (12.3 g/dL), but the patient relapsed ten months later and refused an additional cycle of ATG. He is currently on periodic treatment with high dose immunoglobulin (0.4 g/kg/day for five days each month). Red cell transfusion supplementation was reduced from 1-2 U per week to 1 U every three weeks.

PRCA is mostly commonly considered to be an autoimmune disorder due to antibody-mediated or T cell-mediated inhibition of erythropoiesis. Involvement of immunologic mechanisms in the pathogenesis of PRCA is further supported by its frequent association with other autoimmune diseases and response to immunomodulatory therapy.[8] Sequentially our patient developed Addison disease, gastric hyperplastic polyposis, malabsorption syndrome and diabetes type I. The features presented by our patient do not fit with the most commonly described syndromes of multiple endocrine gland insufficiency (type I and type II), that are frequently associated with HLA antigens B-8, DR 3.[9] In our case the autoimmune basis to the syndrome was not supported by the presence of organ specific autoantibodies, and hypogammaglobulinemia was constantly present. However, the absence of anti-adrenal antibodies cannot rule out the presence of organ specific autoantibodies, which may have disappeared after the target organs had been completely destroyed.[10] Autologous serum was not able to inhibit the proliferation of BFU-E, thus lending further support to the contention that the pathogenesis of our patients' PRCA was not antibody mediated. Peripheral T cells expressing $CD3^+$, $CD8^+$, $HLA\text{-}DR^+$, and $CD16/56^-$ phenotype were predominantly expanded, but the unaffected growth of peripheral blood non T-depleted cells suggested that BFU-E proliferation was not suppressed by T lymphocytes. On the other hand they may have been toxic for erythroid precursors, as shown by the severe reduction of bone marrow erythroid precursors.

Increased MCV and megaloblastic erythroid precursors are unusual in PRCA, while they are common signs of ineffective erythropoiesis. As our patient had neither folic acid nor vitamin B12 deficiency, this feature suggests that an underlying immune mechanism caused hypoplastic and ineffective erythropoiesis.

Correction of anemia, reduction of peripheral lymphocytosis by ATG and improvement of the malabsorbtion syndrome by cyclosporine further support the hypothesis of a cell-mediated autoimmune etiology for the PRCA and polyglandular syndrome in our patient. T lymphocytes showed neither cellular atypia nor monoclonal rearragements of αβ TCR, which were suggestive of non-neoplastic lymphocytosis. Therefore, the expansion of suppressor T cells could be regarded as a reactive phenomenon in the context of an underlying autoimmune disorder.

It must, however, be considered that T-cell clonality is not always demonstrable in patients with typical $CD3^+$ lymphocytosis even using RT-PCR for TCR gene rearrangements. In fact there is a risk of cross-reactivity of primers among different TCR families, as well

as of contamination by peripheral blood leukocytes known to express all major TCRs. Response to immunosuppressive protocols is not always achievable or long-lasting. If clonal T cell proliferation is refractory to this treatment, the use of chemotherapy may be justified.

Key words

Pure red cell aplasia, lymphocytosis, polyglandular syndrome

Correspondence

Alberto Grossi, M.D., Division of Hematology, Policlinico di Careggi, viale Morgagni 85, Florence, Italy. Phone: international +055-4277358 or 4277587 • Fax: international +055-4277794.

References

1. Schmid JR, Kiely JM, Harrison EG Jr, Bayrd ED, Pease GL. Thymoma associated with pure red-cell agenesis. Cancer 1965; 18:216-30.
2. Dessypris EN, Baer MR, Sergent JS, Krantz SB. Rheumatoid arthritis and pure red cell aplasia. Ann Intern Med 1984; 100:202-6.
3. Kiely PD, McGuckin CP, Collins DA, Bevan DH, Marsh JC. Erythrocyte aplasia and systemic lupus erythematosus. Lupus 1995; 4:407-11.
4. Wilson HA, McLaren GD, Dworken HJ, Tebbi K. Transient pure red-cell aplasia: cell-mediated suppression of erythropoiesis associated with hepatitis. Ann Intern Med 1980; 92:196-8.
5. Mangan KF, Chikkappa G, Farley PC. T gamma (Tγ) cells suppress growth of erythroid colony-forming units *in vitro* in the pure red cell aplasia of B-cell chronic lymphocytic leukemia. J Clin Invest 1982; 70:1148-56.
6. Loughran TP Jr. Clonal diseases of large granular lymphocytes. Blood 1993; 82:1-14.
7. Reid TJ III, Mullaney M, Burell LM, Redmond J III, Mangan KF. Pure red cell aplasia after chemotherapy for Hodgkin's lymphoma: *in vitro* evidence for T cell mediated suppression of erythropoiesis and response to sequential cyclosporin and erythropoietin. Am J Hematol 1994; 46:48-53.
8. Charles RJ, Sabo KM, Kidd JP, Abkowitz JL. The pathophysiology of pure red cell aplasia: implications for therapy. Blood 1996; 87:4831-8.
9. Trence DL, Morley JE, Handwerger BS. Polyglandular autoimmune syndromes. Am J Med 1984; 77:107-16.
10. Maung ZT, Norden J, Middleton PG, Jack FR, Chandler JE. Pure red cell aplasia: further evidence of T cell clonal disorder. Br J Haematol 1994; 87:189-92.

Fatal myelofibrosis following fludarabine administration in a patient with indolent lymphoma

Luis Palomera, Gemma Azaceta, Maria José Varo, Joaquín Soria,* Martin Gutierrez

*Departments of Hematology and *Pathology, Hospital Clínico Universitario de Zaragoza, Spain*

We report a case of fulminant myelofibrosis after administration of fludarabine in a patient diagnosed as having refractory low-grade lymphoma, progressing fatally. Myelofibrosis in the setting of an indolent lymphoma is very rare; this fact, and the short period between drug administration and fibrosis suggest an etiopathogenic link, although this potential and severe adverse effect of fludarabine has not been previously reported in the literature.

Fludarabine is a purine analog which has remarkable activity in several lymphoproliferative disorders, including chronic lymphocytic leukemia and low-grade non-Hodgkin's lymphoma (NHL). Its administration carries some side effects, usually mild or moderate and reversible. Non-hematologic toxicity includes nausea, vomiting, diarrhea, fatigue and neurologic or pulmonary complications. However, the major toxicity reported has been infection related to both myelosuppression and immunosuppression.[1] Uncommon adverse effects are immune disease and bone marrow necrosis.[2,3]

We report the first case, to our knowledge, of fulminant myelofibrosis after administration of fludarabine in a patient with low-grade NHL.

A 62-year-old male with a diagnosis of follicular NHL (grade II, REAL classification) in 1988, was treated with chlorambucil achieving partial response. In October 1995 he was admitted because of tenderness over the lumbar region, night sweats and weight loss. Physical examination was unremarkable, except for an enlarged left cervical lymph node. Peripheral blood count showed Hb: 9.4 g/dL, WBC: 9×10^9/L (normal differential counting) and platelet count: 211×10^9/L. The erythrocyte sedimentation rate was 85 mm in the first hour. Renal and liver function were normal. Lactate dehydrogenase (LDH): 519 IU/L (normal< 480 IU/L), β_2-microglobulin: 2.8 mg/L (normal < 2.4 mg/L). Bone marrow biopsy showed no lymphomatous infiltration. Chest and abdominal tomography (CT) revealed splenomegaly, retroperitoneal adenopathy and enhanced density of two lumbar vertebrae (L_3, L_4). Combination chemotherapy was started (CHOP) and continued until May 1996, achieving resolution of B symptoms and shrinkage of lymph nodes and spleen. The bone marrow remained without neoplastic infiltration. In July 1996 the patient was admitted with severe weakness, night sweats and fever. Cervical adenopathy was present. The hemoglobin was 7.7 g/dL, WBC 7.9×10^9/L (normal differential counting) and platelet count 278×10^9/L. Fludarabine was started (25 mg/m^2 daily for 5 days). After the second course of fludarabine, peripheral blood count showed pancytopenia (Hb 4 g/dL, WBC 1.6×10^9/L, platelets 15×10^9/L), LDH: 590 IU/L, alkaline phosphatase: 335 IU/L (normal < 250 IU/L). A bone marrow biopsy was performed which showed diffusse fibrotic and sclerotic reactions, and foci of large polymorphic Sternberg-like cells. Despite receiving granulocyte colony-