13. ABSTRACT (Maximum 200 words)

Acquired pure red cell aplasia (PRCA) has been associated with various lymphoproliferative conditions but its occurrence with Hodgkin's disease is rare. We report a case of PRCA occurring immediately following the completion of induction chemotherapy in a patient with Stage III B nodular sclerosing Hodgkin's disease. In vitro erythroid colony studies documented evidence for T cell mediated suppression of erythropoiesis and lack of a serum inhibitor. Addition of cyclosporin to the in vitro cultures stimulated erythroid colony growth. Following in vivo treatment with cyclosporin peripheral blood CD4/CD8 ratios returned to normal. However, serum erythropoietin levels were inappropriately low. Subsequent treatment with erythropoietin induced a reticulocytosis and transfusion independence. Since discontinuing the erythropoietin, the patient has been able to maintain a hemoglobin of 100 g/L. This case illustrates that red cell aplasia occurring in the setting of Hodgkin's disease may be due to T cell mediated suppression of erythropoiesis. A response to cyclosporin may be masked by inappropriately low erythropoietin levels.
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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

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Acquired pure red cell aplasia (PRCA) has been associated with various lymphoproliferative conditions but its occurrence with Hodgkin’s disease is rare. We report a case of PRCA occurring immediately following the completion of induction chemotherapy in a patient with Stage IIIB nodular sclerosing Hodgkin’s disease. In vitro erythroid colony studies documented evidence for T cell mediated suppression of erythropoiesis and lack of a serum inhibitor. Addition of cyclosporin to the in vitro cultures stimulated erythroid colony growth. Following in vivo treatment with cyclosporin peripheral blood CD4/CD8 ratios returned to normal. However, serum erythropoietin levels were inappropriately low. Subsequent treatment with erythropoietin induced a reticulocytosis and transfusion independence. Since discontinuing the erythropoietin, the patient has been able to maintain a hemoglobin of 100 g/L. This case illustrates that red cell aplasia occurring in the setting of Hodgkin’s disease may be due to T cell mediated suppression of erythropoiesis. A response to cyclosporin may be masked by inappropriately low erythropoietin levels.

INTRODUCTION

Pure red cell aplasia is a syndrome characterized by hypoproliferative normochromic, normocytic anemia, reticulocytopenia, and selective erythroid hypoplasia with preservation of normal myelopoiesis and megakaryopoiesis [1]. A variety of diseases and clinical conditions have been associated with chronic acquired PRCA. The lymphoproliferative disorders are the neoplasms most commonly associated with pure red cell aplasia [2], especially chronic lymphocytic leukemia and the non-Hodgkin’s lymphomas [3]. The occurrence of PRCA with Hodgkin’s disease, however, is rare with only five previously reported cases [4–8]; in all cases, PRCA either preceded or was concomitant with the diagnosis of Hodgkin’s disease. Antibody or T cell mediated suppression of erythropoiesis is responsible for PRCA occurring in lymphoproliferative disorders [9,10]. However, the mechanism for the development of PRCA in Hodgkin’s disease is obscure. We report a case of PRCA occurring after completion of chemotherapy in a patient with stage IIIB nodular sclerosing Hodgkin’s disease. In vitro erythroid colony studies and in vivo response to sequential cyclosporin and erythropoietin was consistent with T cell mediated suppression of erythropoiesis. An initial response to cyclosporin was masked by suboptimal erythropoietin production.

CASE REPORT

A 25-year-old female presented in January 1989 with a 6 month history of diffuse adenopathy, fevers, and night sweats. ...
sweats. Physical examination revealed bilateral cervical, supraclavicular, and axillary adenopathy but no hepatosplenomegaly. A biopsy of a left cervical lymph node showed nodular sclerosing Hodgkin’s lymphoma. Staging evaluations including CT of the chest, abdomen, and pelvis revealed bilateral hilar and massive retropitoneal and pelvic adenopathy. An initial bone marrow aspirate and biopsy showed complete trilineage maturation in erythroid, myeloid, and megakaryocyte lines with no evidence of Hodgkin’s disease. Initial blood counts revealed a hemoglobin 97 gm/L, reticulocyte count 0.7%, white blood cell count 14.9 × 10^9/L with a normal differential, and platelets of 535,000/mm³. A diagnosis of clinical stage IIIB Hodgkin’s disease was made. No staging laparotomy or splenectomy was performed. The patient was treated with MOPP/ABV chemotherapy for six cycles (CALGB 8695) and a complete response was achieved after three cycles as judged by follow-up physical examination and CT scans. Three additional cycles of MOPP/ABV were administered and the patient completed her sixth course by July 1989. At that time, a complete blood count revealed hemoglobin 119 gm/L, white cell count 9.5 × 10^9/L, platelets of 205,000/mm³, and reticulocytes 0.8%. One month later, the patient became acutely ill with fever, acute sinusitis, *Pseudomonas aeruginosa* bacteremia, and *Pneumocystis carinii* pneumonia. She was treated with various antibiotics including erythromycin, vancomycin, piperacillin, mezlocillin, gentamicin, ampicillin, bacitracin, pentamidine, cefuroxime, and ceftazidime. Following a prolonged hospitalization, the patient eventually recovered. At discharge, the white cell count was 3.3 × 10^9/L (normal differential counts) and the platelets were 332,000/mm³. However, the patient was profoundly anemic with a hemoglobin of 53 gm/L and a reticulocyte count of 0.1%. There was no evidence of gastrointestinal bleeding. The anemia persisted and she became transfusion dependent; posttransfusion hemoglobin were ≤10.8 g/dL. All medications were discontinued. A bone marrow aspirate and biopsy revealed a cellular marrow with normal myelopoiesis and megakaryopoiesis. However, there was nearly complete absence of erythroid precursors. Rare pronormoblasts and basophilic erythroblasts were observed. There were no dysplastic or megaloblastic changes. Reticulin and iron stains were normal. Marrow cytogenetics showed a normal female karyotype. A diagnosis of pure red cell aplasia was made. Additional studies, including serum folate, B₁₂, direct and indirect Coomb's test, and sugar water tests were all normal or negative. A serum erythropoietin level by radioimmunoassay revealed a level that was appropriately elevated at 2.440 U/L, taken when the hemoglobin was 55 gm/L and after receiving a total of 24 units of packed red cells since initiation of chemotherapy. The chest radiograph and CT scans were normal. In vitro erythroid colony studies (see Results) were consistent with T cell mediated suppression of erythropoiesis.

The response to treatments are shown in Figure 1. No reticulocytosis was observed following cessation of all antibiotics. The patient was given a single course of i.v. immune globulin (400 mg/kg/dose; Sandoglobulin; Sando Pharmaceuticals, East Hanover, NJ) for 5 days (total dose 2 gm/kg). There was no evidence of a reticulocytosis after 4 weeks. On the basis of the in vitro colony studies, the patient was started on cyclosporin A (Sandimmune; Sandoz Pharmaceuticals, East Hanover, NJ) at a dose of 6 mg/kg p.o. b.i.d. The dose was adjusted to maintain the serum cyclosporin level of 200–440 ng/mL. Immunophenotyping of peripheral blood lymphocytes before initiation of cyclosporin showed a depressed CD4/CD8 ratio of 0.87. There was a relative decrease in total T cells marking with CD2 (34 × 10^6/L, normal range 66–246 × 10^6/L) and in the CD4 helper T cell population (20 × 10^6/L, normal range 41–148 × 10^6/L). The patient tolerated the cyclosporin well. After 3 months of treatment, however, the patient failed to develop a reticulocytosis. Sequential erythropoietin levels drawn when the hemoglobin was between 55 and 108 gm/L showed a decline in erythropoietin levels to a nadir of 15 IU/L (normal 2–14 IU/L). Cyclosporin was discontinued after 3 months. Repeat immunophenotyping studies following discontinuance of cyclosporin showed a correction of the CD4/CD8 ratio to 1.26 due to a relative increase in total T cells (110 × 10⁶/L) and CD4 positive helper T cells (65 × 10⁶/L). The patient was started on a trial of recombinant human erythropoietin (rEPO) on November 4, 1989. An brief 2 month course of rEPO was later given when the hemoglobin fell to 82 gm/L. The patient has been able to maintain a stable hemoglobin of 100 gm/L in the absence of recombinant erythropoietin (rEPO) for 5 days. Platelet counts remained in the normal range and the white cell counts were 4.4–9.4 × 10⁹/L with normal differential counts. The patient remains in a continuous complete remission from Hodgkin’s disease 3 years after completion of chemotherapy.

**METHODS**

Immunophenotyping studies on peripheral blood lymphocytes were performed using direct or indirect immunofluorescence using the following monoclonal antibod-
Case Report: Reid et al.

50

Fig. 1. Clinical course of a patient with pure red cell aplasia developing after treatment for Hodgkin's disease. Intravenous immunoglobulin (IVIg) was given at 40 g/kg/day for 5 days. Cyclosporin A was given at 6 mg/kg p.o. b.i.d. with the dose adjusted to maintain a serum cyclosporin level of 200–400 ng/mL. Erythropoietin (rEPO) was initially given at 50 U/kg i.v. TIW and increased to (*) 75 U/kg i.v. TIW. The patient received 3–4 units packed red cells during each transfusion.

ies: anti-Leu-4 (CD3), anti-Leu-3a (CD4), anti-Leu2a (CD8), anti-Leu-16 (CD20), anti-Leu-19 (CD56): Beckton-Dickinson, San Jose, CA; anti-T11 (CD2): Coulter Immunology, Hialeah, FL. In vitro erythroid colony studies for day 7 CFU_E and day 14 BFU_E were performed in a methocellulose culture system as previously described [9]. For day 7 CFU_E, 0.5 IU/dish recombinant erythropoietin (Amgen, Thousand Oaks, CA) was added. 1.0 IU recombinant erythropoietin and 1.0 IU Interleukin-3 (Amgen) was present in each dish for day 14 BFU_E. Target cells for these studies were the patient’s light density mononuclear marrow cells. T cells were isolated by sheep erythrocyte rosetting techniques as previously described [9]. For serum studies, patient’s serum at a final concentration of 10% was added directly to the plates in the presence or absence of complement (Pel Freeze Biologicals, Rogers, AK). For cyclosporin studies, all mononuclear bone marrow target cells were preincubated with varying doses of cyclosporin (Sandoz Pharmaceuticals, East Hanover, NJ) diluted in alpha minimum essential medium (Alpha MEM, GIBCO, Grand Island, NY). Student’s two tailed t-test was employed to compare results between control and test cohorts.

RESULTS

The effects on erythroid colony growth (day 7 CFU_E and day 14 BFU_E) of patient sera, autologous T cells, and cyclosporin are summarized in Table 1; 127 ± 14 (mean ± ISD) day 7 CFU_E and 23 ± 3 day 14 BFU_E were present in patient marrow and media controls and in absence of autologous serum. The addition of 10% autologous serum in the presence or absence of complement resulted in stimulation rather than inhibition of erythroid colony growth. Similar responses in CFU_E (data not shown) were seen with heat inactivated patient serum as well as with normal type AB serum control (untreated, heat inactivated, or with the addition of complement). Removal of T cells from marrow whole mononuclear cells resulted in significant augmentation of erythroid colony growth in T cell depleted (TD) cells cultured alone (255 ± 23 CFU_E (P < 0.01) and 43 ± 8 BFU_E (P = 0.06)) compared to media controls. The addition of either 10^5 or 2 × 10^5 autologous T cells to TD marrow reduced day 7 CFU_E to 63% and 62% of the TD controls, consistent with T cell mediated suppression of erythropoiesis (P < 0.05). BFU_E growth decreased to 74% and 81% of TD controls (P > 0.05). To determine whether cyclosporin would augment erythroid colony growth, patient’s marrow whole mononuclear cells were preincubated with graded doses of cyclosporin in the range of 10 to 1,000 ng/mL for 1 hour, washed, and then replated. Compared to media controls, there was significant augmentation of CFU_E in the 100–1,000 ng/mL cyclosporin range (P < 0.05) and for BFU_E at 10 and 500 ng/mL dose levels (P < 0.05).

DISCUSSION

The association of Hodgkin’s lymphoma and PRCA is rare [4–8]. In two of the cases, an IgG inhibitor to eryth-
T cell coculture studies
- TD alone: 255 ± 23
data not shown
- TD + 2 x 10^5 T cells: 160 ± 3
data not shown
- TD + 10^5 T cells: 158 ± 12

cyclosporin A studies
- WMNC + 10 ng/mL CyA: 164 ± 6
- WMNC + 100 ng/mL CyA: 193 ± 4
- WMNC + 500 ng/mL CyA: 212 ± 6
- WMNC + 1,000 ng/mL CyA: 197 ± 21

*Values represent mean ± ISD for triplicate culture dishes.
*Values in parentheses represent % control (27 CFU, or 23 BFU, for serum and cyclosporin studies and 22 ± 11 for TD alone. Controls for T cell coculture studies were expressed as % TD alone (255 CFU, or 43 BFU,).
*P = 0.01 by Student's two-tailed t-test vs. control.
*P = 0.05 by Student's two-tailed t-test vs. control.
*Similar responses in CFU, (data not shown) were seen with heat inactivated patient serum as well as with normal type AB serum control (untreated, heat inactivated, or with the addition of complement).
*Not significant.

The majority of cases of acquired PRCA that have been carefully studied are due to either T cell mediated suppression or antibody mediated inhibition of erythropoiesis [1]. Autoantibodies or auto-reactive T lymphocytes may attack erythroid precursors (pronormoblasts) or erythroid progenitor cells (CFU, or BFU,). Natural killer (NK) cells have also been implicated in the development of PRCA [17-19]. Patients with lymphoproliferative disorders may apparently develop erythroid suppression by either T cell mediated or antibody mediated mechanisms [9,20]. T lymphocytes may suppress in vitro erythropoiesis in patients with chronic lymphocytic leukemia associated with PRCA [21-25] and some of these patients have become transfusion-independent following treatment with cyclosporin or antithymocyte globulin [21-24]. In the present case, we provided evidence for T cell mediated suppression of erythroid progenitor cell growth in vitro. There was no evidence for a serum inhibitor. In addition, in vitro preincubation of patient's marrow with cyclosporin showed a dose effect of cyclosporin on erythroid colony growth. Cyclosporin A may interfere with the function of activated T cells [26]. Following in vivo
TABLE II. Literature Review of PRCA Associated With Hodgkin’s Disease

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<tr>
<th>Reference</th>
<th>Age/sex</th>
<th>Histology</th>
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*PRCA noted at diagnosis of Hodgkin’s disease.
†Not reported.
‡Complete response.
§Immunoglobulin mediated. Ig class not specified.
∥Radiation therapy and vincristine.
¶Transient response.
**Mixed cellularity.
†IgG mediated.
*Lympocyte depleted.
|Not immunoglobulin mediated (probably T-cell mediated).
|Cytoxan. doxorubicin, vincristine and prednisolone.
|PRCA developed after treatment for Hodgkin’s disease.
"Nodular sclerosing.

administration of cyclosporin, our patient had a dramatic increase in T helper cells with normalization of the CD4/CD8 ratio. Collectively, the in vitro studies and in vivo T cell subset response to the administration of cyclosporin implicates T cells in the pathogenesis of the PRCA in our patient.

Although the weight of the evidence was consistent with T cell mediated suppression in this case, the patient did not develop an immediate reticulocytosis during a 3 month trial of cyclosporin. Cyclosporin is well known to impair erythropoietin production in humans [27-29] and in mice [30]. As was documented, inappropriately low erythropoietin levels developed in our patient following cyclosporin administration. Upon institution of subcutaneous erythropoietin, a brisk reticulocytosis was seen and a sustained erythropoietic response was subsequently observed in the absence of both erythropoietin and cyclosporin.

CONCLUSION

It is likely that the MOPP/ABV therapy and/or the Hodgkin’s disease induced and immunoregulatory disturbance, allowing the emergence of autoreactive erythroid suppressor T cells which triggered the PRCA in our patient. The immediate response to cyclosporin was masked by the development of impaired erythropoietin production. Correction of this problem resulted in a dramatic reversal of the anemia. Erythropoietin levels should be monitored in patients with PRCA or aplastic anemia receiving cyclosporin if there is no reticulocytosis. The addition of subcutaneous erythropoietin concomitantly or following cyclosporin may unmask a defective response due to inappropriate erythropoietin levels.

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REFERENCES

Case Report: Hodgkin's Disease and Pure Red Cell Aplasia