Pure Red Cell Aplasia: Association With Large Granular Lymphocyte Leukemia and the Prognostic Value of Cytogenetic Abnormalities

By Martha Q. Lacy, Paul J. Kurtin, and Ayalew Tefferi

From 1980 through 1994, we identified 47 adult patients with acquired pure red cell aplasia (median age, 64 years; range, 22 to 84 years). Associated clinical disorders included T-cell large granular lymphocytic (LGL) leukemia, thymoma, chronic lymphocytic leukemia, and non-Hodgkin’s lymphoma. Review of bone marrow findings in 40 patients showed absence of erythroid precursors in 14 patients and rare pronormoblasts in 26. None had morphologic evidence of myelodysplasia. T-cell receptor gene rearrangement studies with Southern blot technique in 14 patients showed clonal rearrangements in nine. Karyotypic analyses performed in 28 patients showed clonal abnormalities in four. Overall, 28 of 47 patients (60%) responded to immunosuppressive therapy, but none were the patients with cytogenetic abnormalities. There was a trend toward superior response to immunosuppressive agents in the patients with T-cell LGL leukemia. Cyclophosphamide, with or without corticosteroids, was the most useful treatment agent. Cyclosporine A was effective for refractory disease. Neither the presence of an associated clinical disorder nor the existence of detectable erythroid precursors affected overall survival. We conclude that (1) T-cell LGL leukemia is the disorder most commonly associated with pure red cell aplasia, (2) the presence of clonal cytogenetic abnormality predicts poor response to immunosuppressive therapy, and (3) oral cyclophosphamide and cyclosporine A are effective treatment regimens.

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Pure RED CELL aplasia (PRCA) is a rare hematologic syndrome characterized by anemia, reticulocytopenia, and severe erythroid hypoplasia of the bone marrow associated with quantitatively and qualitatively normal megakaryocytic and myeloid cell lines. In children, the syndrome is often congenital and referred to as Diamond-Blackfan anemia. Acquired PRCA in children is usually self-limited and referred to as “transient erythroblastopenia of childhood.” In adults, most of the cases are idiopathic, and the rest have been associated with lymphoproliferative disorders, parvovirus infections, and thymomas.

When PRCA marrow cells are assayed in semisolid media, 60% have a normal number of assayable erythroid progenitors. Increased erythropoietic proliferative capacity in vitro is correlated with therapeutic response to immunosuppressive therapy. The ability to proliferate in vitro, but not in vivo, is a notable feature of PRCA and suggests an inhibitor in vivo that blocks erythropoiesis. Humoral mechanisms have been demonstrated in vitro, including suppression of heme synthesis, inhibition of colony-forming units-erythroid (CFU-E) and burst-forming units-erythroid (BFU-E), and direct cytotoxicity to pronormoblasts.

For the patients with PRCA in whom an IgG inhibitor cannot be demonstrated, lymphocyte-mediated inhibition of erythropoiesis is thought to be the major mechanism of pathogenesis. Early investigations of a patient with T-cell chronic lymphocytic leukemia showed that blood lymphocytes inhibited normal marrow CFU-E growth, an effect that could be abolished by marrow treatment with antithymocyte globulin and complement. Subsequently, T cells with receptors for the Fc portion of IgG molecule (Ty) were shown to have an inhibitory effect on CFU-E. A similar inhibition of erythropoiesis in vitro by large granular lymphocytes has also been demonstrated. In patients with B-cell chronic lymphocytic leukemia and PRCA, an increased percentage of Ty cells was found in marrow aspirates and shown to inhibit the growth of erythroid colonies in vitro. CFU-E growth markedly increased after removal of the Ty cells by E-rosetting.

PRCA secondary to parvovirus B19 is thought to be due to a direct toxic effect of the virus on pronormoblasts. Serum that contains this DNA virus inhibits CFU-E growth in vitro, an effect that can be inhibited by antibody to the virus. Also, virus has been identified in proliferating CFU-E.

The aforementioned observations suggest a heterogeneous pathogenetic mechanism in adult PRCA. Clinically, the natural history of the disease, the associated conditions, and its response to treatment are incompletely understood, mainly because most published series are small. Also, few reports include long-term follow-up information. The report of the largest series to date has suggested that in most patients the disease responds to immunosuppressive therapy, and that PRCA is a chronic, relapsing disease with a median survival time greater than 10 years. Nevertheless, in a substantial number of patients, the disease fails to respond to therapy, with long-term survival less than the median. Identification of prognostic factors would be useful in counseling patients and in planning treatment.

We reviewed the 14-year-long experience at our institution with adult PRCA. Given the known associations of PRCA with thymoma and chronic lymphocytic leukemia, we were particularly interested in looking for an association with lymphoproliferative disorders. We also sought to identify potential prognostic factors and to review response rates to various treatment regimens.

MATERIALS AND METHODS

We identified 47 patients who were evaluated at our institution between 1980 and 1994 for acquired PRCA. The cases of five of these patients have been included in previous reports. Patients with Diamond-Blackfan anemia or transient erythroblastopenia of childhood were excluded. The medical histories of the patients were reviewed retrospectively, and pertinent clinical and laboratory data...
were abstracted. Cytogenetic analysis and T-cell receptor (TCR) gene rearrangement studies with Southern blot technique are offered as routine clinical tests at our institution, and whenever available, the results of these investigations were recorded. Follow-up data were obtained by contacting the patient or his or her personal physician. All the patients had bone marrow aspirates and biopsies performed, and the findings were reviewed by one of our hematopathologists at the time of evaluation. Bone marrow specimens from 40 of the patients were available for review by a single hematopathologist (P.J.K.). The diagnosis of PRCA was made if the patient presented with a severe anemia and reticulocytopenia, the bone marrow aspirate and biopsy showed absence of erythrocyte precursors on maturation arrest at the pronormoblastic stage, and the leukocyte and platelet counts were normal. A diagnosis of large granular lymphocytic (LGL) leukemia was made if the patient had evidence of a clonal TCR gene rearrangement and lymphocyte phenotyping, either by immunochemical techniques or flow cytometry, confirmed the LGL phenotype. In three cases, patients with clonal TCR gene rearrangements did not have lymphocyte phenotyping available. In these patients, the diagnosis was made by finding large granular lymphocytes in the peripheral blood smear and by excluding other T-cell malignancies on clinical grounds. Patients with morphologic evidence of dysmyelopoiesis were excluded. A "complete response" was defined as a hemoglobin concentration greater than or equal to 11 g/dL sustained without transfusions. A "partial response" was defined as a hemoglobin concentration less than 11 g/dL in a patient who became transfusion independent. Two-sided x² tests were used to determine significance levels among different groups of patients with regard to treatment outcome. Survival data were estimated with the Kaplan-Meier method.

RESULTS

Patient Characteristics

Forty-seven patients who met the pathologic criteria for the diagnosis of PRCA were identified. All these patients were profoundly anemic for at least 1 month, and all required transfusions of packed erythrocytes at some time. There were 28 males and 19 females, the median age was 63 (range, 22 to 88 years). The median hemoglobin concentration at presentation was 6.3 g/dL (range, 3.0 to 8.4 g/dL), with a median reticulocyte count of 0.1% (range, 0.1% to 0.6%). All patients had radiographic examination or computed tomographic (CT) scans of the chest to look for thymoma. The median follow-up period was 4.5 years (range, 0.5 to 13 years). Nine patients had T-cell LGL leukemia, four had chronic lymphocytic leukemia, two had non-Hodgkin’s lymphoma, and four had thymoma (Table 1). One of the patients with thymoma also had LGL leukemia and, for the purposes of this review, was grouped with the LGL leukemia patients. Four patients had abnormal karyotypes. The karyotypic abnormalities are listed in Table 2. The 25 other patients were classified as having idiopathic PRCA. Acute leukemia did not develop in any of the patients. In one patient, the onset of PRCA coincided with the resection of the thymoma. In two other patients, PRCA coincided with recurrences of unresectable invasive thymomas. Thymoma was discovered in a fourth patient when he presented with PRCA. Although his thymoma was resected, his PRCA did not improve. One year later, he underwent peripheral blood lymphocyte phenotyping and was found to have a preponderance (86%) of CD3+/CD8+ lymphocytes. Subsequent TCR gene rearrangement studies confirmed clonality.

Laboratory and Pathologic Review

Bone marrow specimens from 40 of the patients were available for review. Fourteen patients had an absence of erythroid precursors (Fig 1). Twenty-six patients had maturation arrest at the level of pronormoblasts (Fig 2). Three patients had giant pronormoblasts and vacuolations suggestive of parvovirus infection, and eight had bone marrow eosinophilia. None of the patients had morphologic evidence of myelodysplasia, as defined by the French-American-British Cooperative Group. Furthermore, on the basis of bone marrow morphology alone, it was difficult to identify prospectively those patients with LGL leukemia. The clonal LGLs were always a minor bone marrow cell population, accounting for 5% or less of the bone marrow cellularity.

The median hemoglobin concentration at presentation was 6.9 g/dL (range, 3.0 to 8.4 g/dL). The median reticulocyte count was 0.1% (range, 0.0% to 0.6%). The median platelet and leukocyte counts were normal. The patients who had abnormalities involving platelets or leukocytes had chronic lymphocytic leukemia or LGL leukemia. Blood or bone marrow specimens (or both) from 14 patients were tested for TCR gene rearrangements. The specimens from nine patients showed clonal rearrangement of TCR-β (Fig 3 and Table 3). Peripheral blood lymphocyte phenotyping, either with immunohistochemistry or flow cytometry, was available in six of the patients with rearranged TCR gene and confirmed the LGL phenotype. Five patients had lymphocytes positive for CD2 or CD3. Four of these patients were also positive for CD8, and one was positive for CD57. The lymphocytes from one patient expressed CD57, but were not typed for CD2 or CD3. Twenty-eight patients had cytogenetic analyses, and four had clonal karyotypic abnormali-

<table>
<thead>
<tr>
<th>Table 1. Conditions Associated With PRCA in 47 Patients</th>
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<tbody>
<tr>
<td>Condition</td>
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<tr>
<td>Idiopathic</td>
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<tr>
<td>T-cell large granular lymphocytic leukemia</td>
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<tr>
<td>Thymoma</td>
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<tr>
<td>Chronic lymphocytic leukemia</td>
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<tr>
<td>Non-Hodgkin's lymphoma</td>
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<td>Abnormal cytogenetics</td>
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Abbreviations: CR, complete response; PR, partial response.

* One of the patients also had large granular lymphocytic leukemia.

<table>
<thead>
<tr>
<th>Table 2. Cytogenetic Abnormalities Detected in Four of 28 Patients Tested who had PRCA</th>
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<tr>
<td>Cytogenetic Abnormality*</td>
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<tr>
<td>del(20)(q11.2q13.1)</td>
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<tr>
<td>delX(p11.2), t(5;7)(q13;7), +mar(18)</td>
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<tr>
<td>t(1;5)(p36;q35), +21</td>
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<tr>
<td>del(6)(p15.1), +21</td>
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* In one patient each.
ties. Eight patients had serologic studies for parvovirus IgM, and none were positive.

Results of Treatment

Because this was a retrospective review, the patients did not receive uniform treatment. Most patients initially received treatment with corticosteroids followed by various forms of immunosuppressive therapy until a response was achieved. Doses varied, but a typical starting dose was 0.5 to 1 mg/kg prednisone or its equivalent. Among the patients receiving cytotoxic therapy, most received cyclophosphamide with or without prednisone. The doses of cyclophosphamide varied from 25 to 100 mg daily. One patient received combination chemotherapy consisting of cyclophosphamide, vincristine, and prednisone. One other patient received chlorambucil with prednisone. Five patients in whom corticosteroids or cytotoxic therapy failed, received cyclosporine A. Of these five patients, four had starting doses of 12 mg/kg in divided doses. The other patient had an initial dose of 150 mg twice daily. The dose of cyclosporine A was tapered as tolerated.

Overall, 28 patients (60%) had a response to immunosup-
pressive therapy. After the patients with cytogenetic abnormalities were excluded, the response rate in the group was 65%. Among the 25 patients who received corticosteroids as first-line therapy, responses were seen in nine. The median time to response was 1.4 months (range, 1 to 3 months). Four other patients were given corticosteroids after having no response to other modalities; none of these patients had a response. Cytotoxic agents with or without corticosteroids were used as first-line therapy in 10 patients, five of whom had a response. Also, 15 other patients received cyclophosphamide after having no response to corticosteroids or other therapies, and eight of these patients had a response to cyclophosphamide. The median time to response in this group was 2.1 months (range, 1 to 3 months). All the patients who had treatment with cyclosporine A received it as second- or third-line therapy after failing to respond to corticosteroids or cytotoxic agents. Three of these patients had a response to cyclosporine A given initially at doses of 12 mg/kg, and one had a response to a dose of 150 mg twice daily. In all the patients who had a response, cyclosporine A was tapered as tolerated, and three patients have required ongoing maintenance therapy with low doses of the drug. All the patients who had a response to cyclosporine A had it in the first month of treatment. Two spontaneous remissions occurred, both in patients with idiopathic PRCA. The PRCA in these patients was not thought to be drug-induced, and the remissions were not associated with drug withdrawal. Six of the 28 patients who had a response, including the five who initially received treatment with corticosteroids alone, had a relapse when treatment was discontinued. Five of these six patients went on to achieve a second remission. The sixth patient refused further therapy. None of the patients with a response to cytotoxic agents had relapse. Fourteen patients achieved a response to their first therapy; most of the patients required more than one type of treatment. The responses by various clinical groups are listed in Table 1, and the results of all the regimens used are listed in Table 4. The median survival time for the group was 12 years. Among the 47 patients, there were 13 deaths. Eight of these resulted from failure of the PRCA, to respond to therapy, with resultant iron overload and organ dysfunction. Two patients died of progressive CLL. One patient died of progressive malignant thymoma. Two patients died of unrelated medical problems.

The patients with LGL leukemia had a better response to therapy than those with idiopathic PRCA (88% vs 56%). However, this result does not reach statistical significance (P = .07), and the difference in survival time between the two groups was not statistically significant. There was no difference when comparing the response rates and median survival times between the patients who had an absence of erythroid precursors and those with rare pronormoblasts (57% vs 50%; P = NS). None of the patients with karyotypic abnormalities achieved remission.

**DISCUSSION**

Our data and those in the literature support the concept that PRCA is pathogenetically heterogeneous. The blood and bone marrow findings are the final common pathway of at least four different disease processes: (1) humoral inhibition of erythropoiesis, (2) suppression of erythropoiesis by clonal T cells, as in LGL leukemia, or by nonclonal T cells, as in chronic lymphocytic leukemia and thymoma, (3) karyotypic abnormalities of bone marrow stem cells that probably represent a form of myelodysplastic syndrome, and (4) a direct toxic effect of parvovirus B19 on erythroid precursors.

The association of PRCA with lymphoproliferative disorders is of interest because of the growing evidence that in a subset of patients with PRCA the disease is mediated by clonal T-cell proliferations. The results of Mangan et al suggest that PRCA associated with chronic lymphocytic leukemia is mediated by T lymphocytes. Ablkowitz et al clearly demonstrated T-cell-mediated inhibition of erythropoiesis in marrow culture systems from patients with PRCA. Sivakumaran et al reported two patients with PRCA who

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**Table 3. TCR Gene Rearrangements**

<table>
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<tr>
<th>Patient</th>
<th>Specimen Studied</th>
<th>J(\alpha1) (EcoRI or BamHI)</th>
<th>J(\gamma1) (EcoRI)</th>
<th>T(\gamma) (EcoRI)</th>
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<tbody>
<tr>
<td>A</td>
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Abbreviations: BM, bone marrow aspirate; E, equivocal results; G, germline; ND, not done; PB, peripheral blood; +, rearrangement noted.
had TCR gene rearrangements that indicated T-cell clonality. Motoji et al reported a case of LGL leukemia associated with PRCA. The TCR gene rearrangement could not be detected after the PRCA was treated successfully with cyclophosphamide. Hara et al reported on a patient with PRCA and type I autoimmune polyglandular syndrome who had rearrangement of TCRγδ on Southern blot analysis and demonstrated that the TCRγδ+ lymphocytes inhibited BFU-E in culture. Oshimi et al reported on a series of 33 patients with LGL leukemia, four of whom met the criteria (proposed by Dessypris et al) for the diagnosis of PRCA. Eight other patients in that series had clinical presentations identical to PRCA, but did not meet the pathologic criteria for PRCA. Dhodapkar et al identified five patients with PRCA among 68 patients with T-cell LGL leukemia.

In our series, LGL leukemia was the disorder most commonly associated with PRCA. Nine of the 14 patients tested showed clonal TCR gene rearrangements. It is possible that the association with LGL leukemia is even stronger than our data suggest, because most of our patients were examined before the Southern blots for TCR gene rearrangements were routinely available at our institution.

In our experience, a clonal cytogenetic abnormality predicts a poor response to immunosuppressive therapy. It is rare to find a cytogenetic abnormality in association with PRCA, and no large series of such patients has been reported that might help to assess its clinical significance. There have been reports of two patients with PRCA associated with 5q- karyotypes, and neither of them had a response to treatment. Dessypris et al reported a series of 31 patients with PRCA in whom chromosome analyses were performed and found one patient with an abnormal karyotype (47, XG). The patient had no response to immunosuppressive therapy and subsequently developed acute leukemia. It is of interest to note that three of our four patients had abnormalities involving chromosome 5 and that the other patient had a 20q- abnormality, an abnormality associated with disorders of the erythroid series. None of our patients and none of those described in the literature had a response to immunosuppressive therapy. We postulate that the PRCA seen in these patients is actually a form of myelodysplasia rather than secondary to autoimmune or lymphoproliferative disease and that such patients are unlikely to have a response to immunosuppressive therapy.

Our data do little to elucidate the role of parvovirus B19 in the pathogenesis of PRCA. Only eight of our patients had IgM serologic studies for parvovirus, and none were positive. The data of Frickhofen et al suggest that as many as 15%
of cases of acquired PRCA are associated with parvovirus infection. This distinction is important because the treatment of choice for these patients would be immunoglobulin given intravenously rather than immunosuppressive therapy.

Our data are consistent with those of Clark et al. with regard to treatment regimens. In our experience, treatment with corticosteroids alone was of limited value. Only 30% of the patients had a response, and most of these were not durable responses. Three of nine patients who responded to corticosteroids were able to maintain remissions after corticosteroid therapy was stopped. The others either had a relapse and required cytotoxic agents or became dependent on corticosteroids. Thus, corticosteroids may still have a useful role in the treatment of PRCA, particularly in young people who may be spared the long-term consequences of cytotoxic therapy or treatment with cyclosporine A. However, most patients will require additional agents to achieve or to maintain remission. Treatment with cytotoxic agents, generally a combination of cyclophosphamide and prednisone, was effective in 50% of our patients. Of more importance, none of these patients had relapse. In our experience, androgens were of no benefit. On the basis of our data, plasmapheresis is seldom justified. The experience with antithymocyte globulin (ATG) presented here was very limited. However, Ackowitz et al. found six of nine patients with PRCA improved with use of ATG. Despite our small number of patients, we found the most effective agent was cyclosporine A. Four of the five patients who received this agent had a response. All of those who had treatment with cyclosporine A received it as second- or third-line therapy after not having a response to corticosteroids or cytotoxic agents. The only patient who did not have a response to cyclosporine A had a cytogenetic abnormality. Means et al. used a combination of cyclosporine A and corticosteroids and obtained responses in six of nine heavily pretreated patients. Raghavachari summarized the experience reported in the literature with treating PRCA with cyclosporine A and reported that the overall response rate was 65%. Therefore, consideration should be given to using cyclosporine A earlier to treat PRCA.

In conclusion, we found that LGL leukemia is the disease most commonly associated with PRCA. This association predicts superior response to immunosuppressive therapy, but is not correlated with improved survival. We found that a cytogenetic abnormality predicts poor response to immunosuppressive therapy and that such patients may be better served by treatment strategies suitable for clonal myeloid disorders. The presence or absence of erythroid precursors in the bone marrow does not correlate with clinical outcome. Various immunosuppressive agents are effective in the treatment of PRCA.

REFERENCES

5. Krantz SB, Kao V: Studies on red cell aplasia. I. Demonstration of a plasma inhibitor to heme synthesis and an antibody to erythroblast nuclei. Proc Natl Acad Sci USA 58:493, 1967


Pure red cell aplasia: association with large granular lymphocyte leukemia and the prognostic value of cytogenetic abnormalities [see comments]

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