The Pathophysiology of Pure Red Cell Aplasia: Implications for Therapy

By Robert J. Charles, Kathleen M. Sabo, Pamela G. Kidd, and Janis L. Abkowitz

To determine the utility of marrow culture in defining the less than 0.5% of nucleated marrow cells). For this analysis, patients were evaluated at the University of Washington before specific therapies (n = 21) or at the time of treatment failure (n = 16). Evaluation included a medical and drug exposure history, a physical examination, a chest x-ray or computed tomography to rule out thymoma, lymphocyte immunophenotype studies, anti-nuclear antibody and rheumatoid factor determinations, marrow cytogenetics, and marrow progenitor cell cultures. Retrospective Southern analyses to detect human parvovirus B19 was performed in the 27 patients for whom sera was stored. Clinical follow-up was obtained to document therapeutic responses. Normal burst forming unit-erythroid (BFU-E) growth (>30 bursts/10^5 marrow mononuclear cells (MMNC)) in culture proved an outstanding predictor of clinical response, as 27 of 29 individuals with normal frequencies of erythroid bursts in culture responded to immunomodulating therapies (sensitivity 96%, specificity 78%, predictive value 93%, P = .0001 with two-tailed chi square analysis). Overall, 28 patients responded to either immunomodulating therapies or drug withdrawal. Twenty-four patients obtained a normal hematocrit (complete response (CR)) and 4 additional patients became transfusion independent (partial response). Although responding patients often required several therapies, 20 of 24 (83%) patients who obtained a CR have sustained a normal hematocrit without maintenance therapy at the time of last follow-up (median 5 years). In contrast, of 8 patients with poor in vitro BFU-E growth (<6 bursts/10^5 MMNC), 7 failed to respond to any therapy and all died (median survival time 17 months). Our data suggest that in individuals, from whom BFU-E mature appropriately in culture, immunosuppressive drugs should be used sequentially until a CR is obtained and a durable remission is the expected outcome.

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PURE RED CELL aplasia (PRCA) is a clinical syndrome defined by the absence of mature erythroid precursors in an otherwise normocellular bone marrow (BM). Patients have severe anemia, a low reticulocyte count and normal platelet and granulocyte counts.1

The physiology of PRCA is likely heterogenous. It has been associated with autoimmune, viral, and neoplastic diseases including thymoma,2-7 rheumatoid arthritis,8-9 systemic lupus erythematosus,10-11 hepatitis,12 mononucleosis,13-14 lymphoma,15-18 B-cell chronic lymphocytic leukemia (CLL),19 T-cell CLL,20 and large granular lymphocytic (LGL) leukemia.21-22 In these settings, serum antibodies with selective cytotoxicity for marrow erythroid cells or erythropoietin have been reported.24-26 In other studies T-cells from patients with PRCA associated with thymoma,24,27 chronic Epstein-Barr virus (EBV),14,28 lymphoma,29 CLL,20-30,33 and LGL leukemia34,35 have been shown to suppress erythropoiesis in vitro. PRCA may also occur in association with myelodysplasia or as a consequence to chronic human parvovirus B19 (HPV B19) infection.36-38

The optimal treatment of PRCA remains uncertain. Because clinical and morphologic presentations of PRCA are overlapping, management decisions are difficult. In this report, we have prospectively studied 37 PRCA patients to evaluate the roles of clinical presentations and marrow culture studies as prognostic indicators of long-term response. To gain further insight into the pathophysiology and natural history of PRCA, we have separately analyzed those patients (n = 21) evaluated before any pharmacologic intervention.

MATERIALS AND METHODS

Criteria for admission. This series includes patients with PRCA evaluated at the University of Washington between 1982-1992. All patients fulfilled standard criteria for PRCA and had anemia with reticulocytopenia (reticulocyte count <1%), normal white cell counts, normal differential counts, and normal platelet counts.124 BM biopsies revealed no decrease in overall cellularity. Hemoglobinized cells comprised less than 3% of nucleated marrow cells on marrow aspirate. All patients but two (patients 9 and 19) met the stricter criteria described by Clark et al,39 (hemoglobinized cells comprising less than 0.5% of nucleated marrow cells). For this analysis, patients (n = 3) with trilineage dysmorphic abnormalities allowing the definitive diagnosis of meyelodysplasia were specifically excluded. Studies were approved by the Human Subjects Committee, University of Washington, Seattle.

Initial clinical evaluation. The initial clinical evaluation included a medical and drug exposure history, a physical examination, and a computer-assisted tomographic scan or x-ray to rule out thymoma. Blood was obtained to immunophenotype lymphocytes to detect excess natural killer (NK) cells or diagnose CLL; antinuclear antibody (ANA), rheumatoid factor and other rheumatologic studies; and a complete blood count and morphometry. A retrospective analysis for HPV B19 was performed on the 27 patients for whom serum was stored and has been reported separately.16 The presence or absence of proerythroblasts on the marrow aspirate was specifically noted. Marrow cytogenetics were obtained from all patients.

Marrow culture studies. To quantify the frequencies of erythroid progenitors, 10^5 marrow mononuclear cells (MMNC) were suspended in 1 ml α-medium containing final concentrations of 1.2% methylcellulose, 1% bovine serum albumin, 10^-4 mol/L beta mercaptoethanol, penicillin/streptomycin, 30% fetal calf serum (FCS), 1 U/ml recombinant human erythropoietin and 2.5% human lymphocyte conditioned medium.40 Cultures were incubated at 37°C in 5% CO2 and erythroid colonies (from colony forming unit-erythroid [CFU-E] and erythroid bursts (from burst forming unit-erythroid [BFU-E]) were enumerated on days 7 and 14, respectively, with inverted microscopy. Granulocyte macrophage (GM) colonies (from CFU-GM) were quantified on day 14 on the same plates.
to control for any technical issues. All studies were performed in triplicate.

To determine if aberrant T cells or a serum antibody suppressed erythroid differentiation, coculture studies were performed. Peripheral blood (PB) E-rosette (+) cells were added to 10^5 ER-MMNC in ratios of 1:1, 2:1, and 3:1. T-cell mediated inhibition of erythropoiesis was defined as a 40% decrease in the numbers of detectable erythroid bursts or erythroid colonies (compared to baseline ER-cultures) and with no change in the numbers of CFU-GM colonies. To investigate antibody mediated inhibition, marrow cells were incubated for 30 minutes at room temperature with autologous serum or normal AB serum, then for 30 minutes at room temperature with an equal volume of rabbit complement before culture. As an additional assay, 15% autologous or normal AB serum was added directly to marrow cultures (replacing 15% of the FCS). Antibody mediated inhibition was considered present if the numbers of erythroid bursts or erythroid colonies decreased by 35%, and if no change in GM colony numbers was detected, when MMNC were incubated with autologous serum then complement, or were cultured in the presence of autologous serum.

Follow-up studies. Follow-up has been acquired from contact with patients or their physicians, and ranged from 1 month to 12 years. Patients were classified to have a complete response (CR) if their hemoglobin or hematocrit became normal and a partial response (PR) if their need for red cell transfusions ended. These results were correlated with results from marrow culture and coculture studies to determine significant prognostic indicators of therapeutic success. Among patients with a CR or PR, median follow-up was 5 years.

Relapses occurred in several patients. Relapses were defined as a transfusion need which recurred after a patient obtained a therapeutic remission. Marrow aspirations and biopsies were not generally performed at this time, and therefore it cannot be stated that these patients fulfilled all criteria for PRCA at relapse.

Statistical analysis. Results were analyzed by two-tailed chi square analysis employing the Statview 512 plus program (Abacus Concepts, Inc, Berkeley, CA).

RESULTS

Patient characteristics. Clinical data are summarized in Table 1. Patients ranged in age from 4 to 83 years with a median of 57 years. Twenty-two patients were men and fifteen women. Twenty-one of the patients were evaluated before specific therapies for PRCA. The other 16 patients were referred following treatment failure. Fifty-one percent (19 of 37) of patients had a CR following drug withdrawal. Three patients had a CR following thymectomy and another had a CR following thymectomy and prednisone therapy. Other forms of therapy including vincristine, splenectomy, and androgens were attempted without benefit in a few patients failing prednisone, ATG, or cytotoxic agents.

To consider possible selection bias, response rates to various agents were independently analyzed in the group evaluated before specific therapies for PRCA. Of these 21 patients, 17 received prednisone alone as their initial treatment and 8 patients (47%) obtained a complete remission. In the patients failing prednisone, 78% responded to either ATG, cyclo-

ease process (P > .05). HPV B19 infection was found retrospectively in 4 (11%) patients using Southern blot analysis of sera. One of these patients had concurrent HIV infection, I had Sweet’s syndrome, I admitted to marijuana abuse that may have contributed to immune incompetence, and I had no immunologic abnormality.

As previously stated, LGL leukemia was diagnosed in three patients. These patients fulfilled the diagnostic criteria of Loughran, and had both an absolute increase in large granular lymphocytes (>700/μL) and either an absolute increase in CD8+ lymphocytes (>1,000/μL) or NK (CD 56+ or CD 57+) cells (1,000/μL). In addition, 1 patient (patient 25) developed LGL leukemia during the follow-up period. T-cell receptor gene rearrangement studies were performed in 2 of these patients and confirmed a clonal abnormality. Seven additional patients had elevated percentages of NK cells (ranging from 21% to 51%) and greater than 200 NK cells but failed to meet the diagnostic criteria for LGL leukemia. None of these 7 patients were evaluated with T-cell receptor gene rearrangement studies.

Although no patient had trilineage morphologic abnormalities to allow the clinical diagnosis of myelodysplasia, micro-megakaryocytes were present in the marrow aspirate and a single unilobed neutrophil was seen in the PB of patient 31. Marrow from patient 7 could not be aspirated and biopsy revealed myelofibrosis, but no additional abnormality. Cyto genetic abnormalities were present in three patients. These included 5q- deletions in patients 17 and 20 and trisomy 8 in patient 31.

Responses to therapy. Individual responses to therapy are summarized in Table 1. Twenty-eight patients (76%) obtained hematocrits adequate to end transfusion requirements (24 CR, 4 PR). Twenty-five of these patients had remissions secondary to immunomodulating therapies. Three patients had a CR following drug withdrawal. Although not specified in the study design, 33 of 37 patients received prednisone as their initial therapy. Nine of 33 (27%) patients obtained a complete remission. No additional therapy was required in 6 patients.

Patients often received more than one therapy. Consequently, 1 patient may contribute to the response rate of more than 1 agent. Overall, response rates (CR and PR) to cyclophosphamide, antithymocyte globulin (ATG), and cyclosporine were 6 of 15 (40%), 8 of 13 (62%), and 2 of 3 (67%), respectively. Two patients responded to IgG therapy (one was viremic with HPV B19 and one was not). Sustained low-dose methotrexate (10 to 15 kg/week) resulted in remissions in 2 patients. One patient had a CR following thymectomy and another had a CR following thymectomy and prednisone therapy. Other forms of therapy including vincristine, splenectomy, and androgens were attempted without benefit in a few patients failing prednisone, ATG, or cytotoxic agents.

To consider possible selection bias, response rates to various agents were independently analyzed in the group evaluated before specific therapies for PRCA. Of these 21 patients, 17 received prednisone alone as their initial treatment and 8 patients (47%) obtained a complete remission. In the patients failing prednisone, 78% responded to either ATG, cyclo-
## Table 1. Characteristics of PRCA Patients

<table>
<thead>
<tr>
<th>Patient No./Age/Sex</th>
<th>Initial Study</th>
<th>Associated Conditions and Remarks</th>
<th>BFU-E</th>
<th>CFU-E</th>
<th>Excess Pro-E</th>
<th>T-Cell/ Aebt</th>
<th>Therapeutic Response</th>
<th>Clinical Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/57/F</td>
<td>Hx of Hashimoto’s thyroiditis and sarcoidosis, LGL leukemia</td>
<td>Y N N T</td>
<td>P → NR, ANDR → NR, ATG → PR</td>
<td>CR → 12 yr</td>
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<tr>
<td>2/12/F</td>
<td>HPV B19</td>
<td>Y Y Y T</td>
<td>P → NR, ATG → PR</td>
<td>CR → 12 yr</td>
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<tr>
<td>3/4/F</td>
<td></td>
<td>Y N N T</td>
<td>P → NR, spontaneous remission, relapse after 1 mo, ATG → CR</td>
<td>CR → 1 yr</td>
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<tr>
<td>4/34/F</td>
<td>P</td>
<td>Y Y N T</td>
<td>P → NR, ATG → CR</td>
<td>CR → 5 yr</td>
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<tr>
<td>5/63/M</td>
<td></td>
<td>N N N</td>
<td>P → NR, Azathioprine → NR</td>
<td>NR → 2 yr, died of ANLL</td>
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<tr>
<td>6/77/M</td>
<td>Myelofibrosis</td>
<td>Y Y N</td>
<td>P → NR, IgG → NR</td>
<td>NR → 9 mo, died of MI</td>
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<tr>
<td>7/72/M</td>
<td></td>
<td>N N N</td>
<td>Azathioprine → NR</td>
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<td>8/46/M</td>
<td>Hx of ITP and recurrent pericarditis</td>
<td>Y N N</td>
<td>P → NR, CY → CR</td>
<td>CR → 5 yr</td>
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<tr>
<td>9/50/M</td>
<td>Chronic EBV infection</td>
<td>Y Y N T</td>
<td>Plasmapheresis → NR, ATG → PR</td>
<td>PR → 10 yr, died of cardiovascular DX</td>
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<tr>
<td>10/38/F</td>
<td>Rheumatoid arthritis</td>
<td>Y N N T</td>
<td>P → CR, relapse after taper, ATG + P → CR, relapse after taper, AZA + P → CR, MTX → CR</td>
<td>CR → 5 yr on methotrexate</td>
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<tr>
<td>11/58/M</td>
<td>Thymoma</td>
<td>Y Y N T</td>
<td>P → NR, CY → NR, VIN → NR, AZA → NR, thymectomy → NR, P → CR</td>
<td>CR → 9 yr</td>
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<tr>
<td>12/60/M</td>
<td>P</td>
<td>Y Y N</td>
<td>P → NR, CY → PR (d/c because of neutropenia)</td>
<td>PR → 3 mo; transfusion supported, died 1 yr later of UGIB &amp; COPD</td>
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<tr>
<td>13/42/M</td>
<td>P</td>
<td>Y Y N</td>
<td>D/c marijuana → CR</td>
<td>CR → 7 yr</td>
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<tr>
<td>14/59/M</td>
<td>Sweet’s syndrome, HPV B19</td>
<td>N N N</td>
<td>P &amp; Danazol → NR</td>
<td>NR → 9 mo, died of aortic dissection</td>
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<tr>
<td>15/83/M</td>
<td>P</td>
<td>Y N N A</td>
<td>P → CR</td>
<td>CR → 5 mo</td>
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<tr>
<td>16/62/F</td>
<td>Rheumatoid arthritis</td>
<td>Y Y Y T, A</td>
<td>P → NR, CY → CR</td>
<td>CR → 8 yr</td>
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<tr>
<td>17/49/F</td>
<td>5q-cytogenetics</td>
<td>N N N</td>
<td>P → NR, CY → NR, ATG → NR</td>
<td>NR → 5 yr, died of ANLL</td>
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<tr>
<td>18/67/F</td>
<td>Thymoma</td>
<td>Y ND N</td>
<td>Thymectomy → CR</td>
<td>CR → 1 mo, died of MI</td>
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<tr>
<td>19/63/M</td>
<td>B-cell CLL</td>
<td>Y Y N A</td>
<td>P &amp; Chlorambucil → NR, CY → NR, splenectomy → NR, ATG → PR</td>
<td>PR → 1 yr, died of DLL</td>
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<tr>
<td>20/75/M</td>
<td>5q-cytogenetics</td>
<td>N N N</td>
<td>P → NR, CY → NR, ATG → NR</td>
<td>NR → 7 yr, died of ANLL</td>
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<td>21/25/F</td>
<td>ANA 1:40</td>
<td>Y Y Y T</td>
<td>P → NR, ANDR → NR, CY → NR</td>
<td>CR → 5 yrs</td>
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<tr>
<td>22/69/M</td>
<td>P</td>
<td>Y Y Y T</td>
<td>P → CR</td>
<td>CR → 10 yr</td>
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<tr>
<td>23/37/M</td>
<td>P</td>
<td>Y Y N</td>
<td>P → CR</td>
<td>CR → 1 mo</td>
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<tr>
<td>24/35/M</td>
<td>Dilantin × 1 mo</td>
<td>Y Y Y</td>
<td>D/c Dilantin → CR</td>
<td>CR → 8 yr</td>
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<tr>
<td>25/78/M</td>
<td>P</td>
<td>Y ND N</td>
<td>P → NR, CY → NR, IgG → PR</td>
<td>CR → 2 yr on methotrexate, LGL leukemia diagnosed</td>
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<tr>
<td>26/26/M</td>
<td>HIV</td>
<td>N N N</td>
<td>P → NR, IgG → CR, relapse, IgG → CR</td>
<td>CR → 6 mo, died of mycobacterial infection &amp; AIDS</td>
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<tr>
<td>27/68/F</td>
<td>LGL leukemia</td>
<td>Y N N</td>
<td>P → NR, CY → NR, ATG → NR, IgG → NR, CyA → CR</td>
<td>CR → 5 yr</td>
<td></td>
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<tr>
<td>28/68/M</td>
<td>Thymoma resection 3 mo before onset of PRCA</td>
<td>Y N N</td>
<td>P → CR, CY → CR, relapse with taper, P → CR</td>
<td>CR → 5 yr on prednisone</td>
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<tr>
<td>29/63/M</td>
<td>Rheumatoid arthritis</td>
<td>Y N N</td>
<td>P → PR, IgG → NR, CY → PR</td>
<td>PR → 1 yr, died of COPD</td>
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</tbody>
</table>

(Continued on following page)
Table 1 (Cont’d). Characteristics of PRCA Patients

<table>
<thead>
<tr>
<th>Patient No./ Age/Sex</th>
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<th>BFU-E</th>
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<th>Excess Pro-E</th>
<th>T-Cell/ Amb</th>
<th>Therapeutic Response</th>
<th>Clinical Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/60/M</td>
<td>Stage IV non-Hodgkin's lymphoma</td>
<td>Y N N</td>
<td>M BACOD → NR, P → NR, IgG → NR</td>
<td>P → CR, Chlorambucil → NR</td>
<td>CR → 2 yr, died of lymphoma</td>
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<tr>
<td>31/78/F</td>
<td>Trisomy 8 cytogenetics, micromegakaryocytes a single unilobed neutrophil in smear</td>
<td>N N N</td>
<td>P → NR, Chlorambucil → NR</td>
<td>CR → 4 mo, died of ANLL</td>
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<tr>
<td>32/78/F</td>
<td>P</td>
<td>Y N N</td>
<td>P → CR</td>
<td>CR → 1 yr, died of CHF</td>
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<tr>
<td>33/30/M</td>
<td>P</td>
<td>Y Y N</td>
<td>P → NR</td>
<td>CR → 2 yr</td>
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<tr>
<td>34/57/F</td>
<td>P</td>
<td>Y Y N</td>
<td>D/c isoniazid → CR</td>
<td>CR → 2 yr</td>
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<tr>
<td>35/66/M</td>
<td>P</td>
<td>Y Y N</td>
<td>CVP → NR, P → NR, CY → NR</td>
<td>NR → 2 mo, died of MI</td>
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<tr>
<td>36/24/F</td>
<td>P Arthralgias</td>
<td>Y Y N</td>
<td>P → CR</td>
<td>CR → 6 yr</td>
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<tr>
<td>37/48/F</td>
<td>P Arthralgias, Hx of Hashimoto’s thyroiditis</td>
<td>Y Y Y</td>
<td>P → CR, relapse with taper, P → CR</td>
<td>CR → 3 yr on prednisone</td>
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</table>

Twenty-one patients were studied prior (P) to any drug therapies. In 16 patients, the evaluation was within 3 weeks of presentation with anemia. In 4 patients, evaluation was within 14 weeks. Patient 25 was transfused for 2 years before diagnosis and study. Fifteen patients were referred after previous treatment failure. Inverted populations of CD4+ to CD8+ cells were found in 9 patients (8, 11, 12, 14, 17, 25, 27, 28, and 29). Excess (Exc) proerythroblasts (greater than 7% of nucleated marrow cells) were present in 6 patients (2, 16, 21, 22, 24, and 37). LGL leukemia was diagnosed in two patients (1 and 27). These patients had both an absolute increase in large granular lymphocytes (>700/μL) and either an absolute increase in CD8+ lymphocytes (>1,000/μL) or NK (CD 56+ or CD 57+) cells (1,000/μL). Seven additional patients had elevated percentages of NK cells (ranging from 21% to 51%) and greater than 200 NK cells but failed to meet the diagnostic criteria for LGL leukemia (patients 10, 11, 12, 14, 16, 17, and 22). The presence of cytogenetic abnormalities was statistically associated with poor therapeutic response (P = .001 with chi square analysis). Although the sensitivity was 100%, the specificity was only 33%. The presence or absence of other associated conditions, when tested individually or when grouped together, did not correlate with clinical response (all P values > .05).

Marrow culture and coculture studies: Normal in vitro growth was defined as greater than 30 BFU-E and CFU-GM and greater than 40 CFU-E frequencies per 10^6 MMNC. Eight patients had poor BFU-E and CFU-E growth (5, 7, 14, 17, 20, 26, 31, and 35). Ten patients had poor CFU-E growth only (1, 3, 8, 10, 15, 27, 28, 29, 30, and 32). CFU-E frequencies were not determined in 2 patients (18 and 25). Thus, the level of differentiation at which erythroblast formation could be determined in 27 patients and the level of block in erythropoietic differentiation failed to predict the specific drug to which a patient might respond when evaluated with chi square analysis. CFU-GM frequencies were normal (≥30/10^6 MMNC) in all patients except patient 14 whose CFU-GM frequency was decreased. This internal control showed that cell culture methods were adequate.

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The relationship between the presence of erythroid bursts in culture and whether the patient obtained a therapeutic remission is central to this study (Fig 2). Of 29 patients who had normal numbers of erythroid bursts in culture, 27 obtained a remission. In contrast, of 8 patients from whom BFU-E failed to mature in culture (erythroid bursts <6/10^5 MMNC), 7 failed to respond to any treatment. Therefore, BFU-E maturation in vitro was a superb predictor of clinical response. Its sensitivity was 96%, its specificity was 78% and its predictive value was 93% (P = .0001 with 2-tailed chi-square analysis).

Coculture studies showed T-cell mediated suppression of erythropoiesis in 10 patients and antibody mediated inhibition in 3 patients (one patient had evidence of both T-cell and antibody mediated inhibition). Although positive coculture studies were an excellent predictor of ATG response (CR or PR) (P = .0001), it was not predictive of responses to other agents (P > .6 cyclophosphamide or prednisone). All 12 patients who had remissions (10 CR, 2 PR) following therapy, with transfusion free survivals extending 7 years at present.

Among the 7 patients with poor BFU-E growth in vitro and no response to therapies, patient 14 had chronic HPV B19 infection, and patient 35 had extensive B-cell CLL. Five patients had or developed some evidence of myelodysplasia. Cytogenetic abnormalities and a unilobed neutrophil were seen in patient 31, cytogenetic abnormalities alone were seen in patients 17 and 20, and myelofibrosis in patient 7. Patient 5 had marrow morphology indistinguishable from the other PRCA patients and normal cytogenetics but later died of acute nonlymphocytic leukemia (ANLL). Patients 7, 17, 20, and 31 also died with ANLL.

Instructive cases. Several other cases were instructive clinically. Patient 27, a 68-year-old female presented with a hematocrit of 19% and reticulocyte index of 0. Immunophenotype studies showed 63% of lymphocytes were CD8+ (absolute CD8 count of 3,995 cells/µL) and 35% were NK cells (absolute count 1,645 NK cells/µL). T-cell receptor gene rearrangement studies confirmed the diagnosis of LGL leukemia. Her marrow aspirate showed no excess proerythroblasts. Neither T-cell nor antibody mediated inhibition was shown with coculture studies. Cell culture results revealed normal BFU-E and CFU-GM growth. Only 6 CFU-E colonies were detected.

The patient failed prednisone, cyclophosphamide, ATG and IgG therapy. At the time when cyclosporine was initiated, her transfused hematocrit was 26% and reticulocyte index was 0. Two months after beginning cyclosporine, her hematocrit normalized at 41.6% (hgb 14.3 g/dL). She has remained in complete remission without relapse or maintenance therapy 5 years at present. This case illustrates that in patients with normal BFU-E growth, one should persist with trials of drug therapies until a CR is obtained.

Patient 25 presented with a hematocrit of 17% and reticulocyte index of 0.1. His age of 79 years and high MCV of 107 raised the concern of myelodysplasia. Marrow culture studies were obtained when he was referred to the University of Washington Medical Center after 2 years of red cell transfusions and showed normal BFU-E maturation suggesting immunologically mediated PRCA. For this reason he was started on prednisone and cyclophosphamide, both without changing his transfusion requirement. He next received a 5 day course of IgG therapy with a subsequent reticulocytosis and a rising hematocrit to 32. This partial response continued for one year while he remained transfusion and symptom free (Hct = 29 to 32). However, following a viral illness, his PRCA relapsed. He did not respond to repeated IgG therapy. Cyclosporine then resulted in a partial remission (Hct = 26 to 29). At this time, 4 years after his initial diagnosis of PRCA, a large granular lymphocytosis (LGL's >1,200/µL) developed. Repeated T-cell immunophenotype studies revealed increased populations of CD8+ (3,265/µL) and NK (826/µL) cells and gene rearrangement studies revealed a clonal T-cell population confirming LGL leukemia. This patient has subsequently responded to methotrexate with a complete response (stable Hct = 35) currently extending 24 months on maintenance therapy of 5 mg methotrexate orally per week. This case shows that individual patients can respond to different therapies at different times.

**BFU-E MATURATION**

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**Fig 2.** The clinical value of in vitro culture. The relationship of normal BFU-E growth (>30 bursts/10^5 MMNC) and remission (CR and PR) is shown. BFU-E maturation in vitro was a superb predictor of clinical response. Its sensitivity was 96%, its specificity was 78% and its predictive value was 93% (P = .0001 with 2-tailed chi-square analysis).
It also shows that associated diseases can become clinically apparent years after the presentation of PRCA.

**DISCUSSION**

In this study, we prospectively followed 37 PRCA patients at the University of Washington. Overall, the clinical disorders associated with their PRCA were compatible to those previously described by Clark et al. We also report a low incidence of thymoma (8%) compared to case reports in the literature of up to 50%. This discrepancy probably represents the reporting partiality of thymoma associated PRCA in the earlier literature. Our results are consistent with the thymoma incidences of 5 to 13% reported more recently. One of the patients in our study developed PRCA 3 months after thymoma resection; similar patients have been reported previously.

Recently, considerable attention has been focused on the association between various lymphoproliferative disorders, particularly LGL leukemia, and PRCA. Evidence suggests that NK cells may directly inhibit erythropoiesis. Although 9 patients had elevated percentages (>20%) of NK cells (see Table I), only 2 had an increase in their absolute numbers of NK cells and thus fulfilled the criteria for LGL leukemia at the time of their initial evaluation. A third patient (patient 25) developed LGL leukemia 4 years later. As T-cell gene rearrangement studies were performed infrequently, the actual incidence of a clonal T-cell or NK-cell process may have been higher than the 8% we report.

To define physiologies of PRCA and correlate this with therapeutic responses, marrow BFU-E, CFU-E, and CFU-GM frequencies were determined and coculture studies were obtained. Our results indicate that PRCA involves three mechanisms: immunologically mediated disease including T-cell or antibody mediated inhibition of erythropoiesis, HPV B19 infection, and an intrinsic stem or multipotent progenitor cell defect (ie, myelodysplasia).

**Immunologically mediated PRCA.** In patients with immunologically mediated PRCA, BFU-E differentiate normally in culture. We interpret this data to imply that BFU-E are present in vivo, and when moved from the immunologic milieu of the patient, are able to fully differentiate to hemoglobinized cells. Presumably within the patient there is a T-cell or antibody mediated inhibition of erythropoiesis, although the in vitro coculture studies are significantly sensitive to confirm these mechanisms in only a fraction of studies.

Normal frequencies of BFU-E in vitro were associated with therapeutic remission in 27 of 29 patients (sensitivity 0.93, specificity 0.78, predictive value 0.96, P = .0001). There were only 2 patients with normal BFU-E growth but no clinical response (patients 6 and 30). Patient 6, without any associated conditions, was referred following prednisone failure. After failing subsequent azathioprine treatment, further agents were not tried because of his poor health status resulting from severe congestive heart failure. It is possible that further immunomodulating therapy may have been successful. Patient 30 presented with PRCA secondary to stage IVA non-Hodgkin’s lymphoma of intermediate grade that was unresponsive to chemotherapy. Response of PRCA in this setting is reported to depend on a remission of the lymphoma.

These results are compatible with data previously reported by Lacombe et al who retrospectively analyzed 22 PRCA patients and found a positive correlation between in vitro erythroid growth and patient response rates to immunosuppressive therapies.

**Human parvovirus B19 associated PRCA.** PRCA can result from chronic parvovirus infection in patients with immunodeficiency. HPV B19 specifically infects and lyses erythroid progenitor cells. When immunocompromised patients are unable to develop neutralizing antibodies, persistent infection results in PRCA. Therapy with intravenous IgG (which contains high titers of antibody to HPV B19) at 400 mg/kg/d × 5 d is indicated and universally effective. In a recent retrospective study analyzing sera from 57 PRCA patients, HPV B19 DNA was detected in 14%, suggesting chronic parvovirus infection may be a relatively common cause of PRCA.

Marrow culture of HPV B19 infected patients showed two growth patterns. Two patients had normal in vitro erythroid differentiation (2 and 13) and obtained a CR to ATG or drug (marijuana) withdrawal, currently extending 12 and 6 years, respectively. BFU-E failed to mature in marrow cultures from two additional patients. Patient 26 had a CR with IgG but died 6 months later of a mycobacterial infection secondary to AIDS. Patient 14 failed prednisone therapy and subsequently died of an aortic dissection. As the anemias responded to ATG as well as IgG, HPV B19-associated PRCA may have an immunologic component to its pathogenesis in certain circumstances.

**Intrinsic stem cell defect (myelodysplasia).** An important subset was 5 of the 8 patients whose BFU-E failed to grow in culture. Four of these patients had some evidence of myelodysplasia (eg, a cytogenetic abnormality, myelofibrosis, a single uniloculated neutrophil). None of these 5 patients responded to immunosuppressive therapies and all died secondary to ANLL. We assume that these individuals had a neoplastic hematopoietic stem multipotent progenitor cell that was unable to fully differentiate along the erythroid pathway, resulting in the morphology of PRCA. Erythroid bursts were not seen because BFU-E were absent or were unable to mature in vitro because of this genetic defect.

Interestingly, the two patients with 5q- abnormality (pt. numbers 17 and 20) had transient improvement in erythroid production concurrent with acute hepatitis (associated with red cell transfusion). This may imply that early in its course, myelodysplastic erythroid cells have some capability for erythropoiesis, but that reactive T cells may inhibit their differentiation. Later in its evolution, immunosuppressive therapies are no longer helpful because the myelodysplastic stem/progenitor cell lacks any intrinsic capacity to differentiate.

Three patients meeting standard criteria for PRCA were excluded from our study because of trilineage abnormalities (thrombocytopenia, circulating blast cells or Pelger-Huet cells in peripheral smear, small and dysmorphic megakaryocytes, megaloblastic erythropoiesis, and ringed...
sideroblasts) allowing the definitive morphologic diagnosis of myelodysplasia. Erythroid bursts were similarly absent from marrow cultures and in all three the anemia was refractory to treatment. It appears that in vitro culture is a powerful tool in predicting myelodysplasia when clinical and morphologic findings are diagnostically indistinct.

Management of PRCA. An initial work-up should include a drug and medical history, a physical examination, and a complete blood count and blood smear. Chest x-ray or computed tomography to rule out thymoma, lymphocyte immunophenotype, and/or T-cell gene rearrangement studies, marrow cytogenetic studies, and serum Southern analysis for presence of HPV B19 are generally indicated. Specific therapies (ie, drug withdrawal, IgG for chronic HPV B19, thymectomy) should be pursued where appropriate. If the work-up for concomitant disease is unhelpful, immunosuppressive therapy should be employed. Because many PRCA patients respond to corticosteroids, we suggest initial therapy with prednisone. If corticosteroids do not produce a remission within 6 weeks, a second line agent such as ATG, cyclosporine, or cyclophosphamide should be initiated depending on their relative contraindications. Low dose methotrexate therapy may be of specific benefit in patients with coexistent LGL leukemia.

We suggest in vitro erythroid culture be used in patients refractory to prednisone therapy and a second-line agent. If BFU-E mature poorly in vitro no further immunologic therapy is warranted, and the clinical diagnosis of myelodysplasia should be strongly considered. If in vitro culture shows adequate BFU-E growth, then individual therapies should be used consecutively until a sustained remission is achieved. Our results show that PRCA is amenable to therapy, and that durable remissions can be obtained in most patients.

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The pathophysiology of pure red cell aplasia: implications for therapy

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