Prednisone-Responsive Aplastic Anemia: A Mechanism of Glucocorticoid Action

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We performed serial agar cultures (CFU-C) using marrow cells from a patient with prednisone-responsive aplastic anemia and from five patients with prednisone-resistant aplasia. Colony growth was decreased in all patients. Cortisol (10^-7 to 10^-4 M) significantly enhanced colony growth in the prednisone-responsive patient but failed to enhance colony growth in the remaining five patients. Further studies in the responsive patient indicated that (1) colony growth was enhanced by depleting T lymphocytes from the marrow cells, (2) colony growth of T-depleted marrow cells was inhibited by autologous peripheral blood lymphocytes (PBL), (3) cortisol failed to enhance colony growth of T-depleted marrow cells, (4) PBL and PBL-conditioned medium inhibited colony growth of both autologous and allogeneic marrow cells, but neither cortisol-treated PBL nor T-depleted PBL were inhibitory. Serial cultures in the responsive patient showed that colony growth normalized during remission when "suppressor" cells were absent and that colony growth was subnormal during a later relapse when cortisol-resistant "suppressor" cells were present. Therefore, in this prednisone-responsive patient, cortisol-sensitive T lymphocytes suppressed granulopoiesis in vitro. Our observations suggest that aplastic anemia in this patient is immunologically mediated and that prednisone therapy enhanced hemopoiesis in vivo by inhibiting the "suppressor" T lymphocytes.

GLUCOCORTICOID THERAPY is sometimes effective in adults with the syndrome of pure red cell aplasia^1-4 and in children with congenital hypoplastic (Diamond-Blackfan) anemia.5 However, the role of glucocorticoids in the management of patients with idiopathic acquired aplastic anemia is poorly defined. The side effects of glucocorticoid therapy, particularly those of immunosuppression and phagocyte dysfunction,^9,10 are potentially dangerous in patients with this disorder. Favorable therapeutic responses are uncommon and when responses do occur it is sometimes difficult to attribute them specifically to glucocorticoid therapy.10 This difficulty arises largely because the mechanism of glucocorticoid action in aplastic patients who respond to therapy is unknown. A number of potential mechanisms exist. For example, glucocorticoids could directly stimulate proliferation of hemopoietic precursor cells, effect changes in the hemopoietic microenvironment or in regulator cells, or functionally suppress cells that inhibit hemopoiesis.

To evaluate the mechanisms of glucocorticoid action in a patient with prednisone-responsive aplastic anemia, we have studied granulopoiesis in vitro using...
marrow cells obtained from six patients with aplastic anemia, one of whom was responsive to prednisone therapy. We report the results of these studies, which provide evidence that in this patient hemopoietic failure was immunologically mediated and that the beneficial effect of prednisone on hemopoiesis was a result of immunosuppression.

MATERIALS AND METHODS

Prednisone-Responsive Patient

A 49-yr-old woman was referred to the University of Oregon Health Sciences Center 2 mo after a diagnosis of aplastic anemia. Her clinical course is outlined in Fig. 1. On admission the packed cell volume (PCV) was 0.20, reticulocytes <0.1%, neutrophil count 0.4 x 10^9/liter, and platelet count 15 x 10^9/liter. Antinuclear antibodies, rheumatoid factor, cryoglobulin, cold agglutinin, direct Coombs, and anti-DNA antibody studies and acid hemolysis tests were negative. No histocompatible siblings or offspring were identified.

Therapy with 5000 mg goat anti-human thymocyte globulin (29.5 mg protein/ml, Department of Surgery, University of Oregon Health Sciences Center) failed to induce hematologic improvement by the 30th posttreatment day, when steroid therapy began. Improvement did occur 14 days following the initiation of prednisone and oxymethalone therapy and was characterized by reticulocytosis, resolution of neutropenia, and an increase in marrow cellularity on marrow biopsy (Fig. 2). Discontinuation of steroid therapy was followed by a relapse (reticulocytopenia, neutropenia, and severe marrow hypoplasia). Reinstatement of prednisone therapy alone reinduced a complete hematologic remission but, after 3.5 mo, a prednisone-resistant relapse occurred (see Fig. 1). Combination chemotherapy was begun with intravenous cyclophosphamide, 600 mg/sq m on days 1 and 8 of a 28-day cycle; vincristine, 2 mg on days 1 and 8; and prednisone, 40 mg/sq m/day for 14 days (COP). Progressive hematologic improvement occurred.

Fig. 1. Clinical course of the prednisone-responsive patient. Therapy with antithymocyte globulin, oxymethalone and prednisone was followed by hematologic remission. After discontinuation of steroid therapy, relapse occurred but prednisone therapy reinduced a second remission. After the second relapse, the disease was resistant to prednisone therapy, but therapy with cyclophosphamide, vincristine, and prednisone (COP) was followed by improved marrow cellularity, reticulocytosis, increased neutrophil counts, and decreased platelet transfusion requirements.
Aplastic Controls

Five patients with severe aplastic anemia (classified according to previously published criteria\textsuperscript{1}) were studied after having given their signed informed consent. All patients had negative acid hemolysis tests, and none had rheumatic diseases or hepatitis. All five had received red cell transfusions, and four had received platelet transfusions prior to the study. All patients received trials of prednisone therapy (30–50
mg/sq m/day) for from 4 to 8 wk. None of these patients exhibited favorable hematologic responses to prednisone therapy.

Volunteers

After signed informed consent was obtained, peripheral venous blood and/or bone marrow cells (posterior iliac crest) were obtained from 14 paid normal volunteers.

Preparation of Marrow and Peripheral Blood Cells

Marrow cells were aspirated directly into heparinized syringes. In the prednisone-responsive patient, 4 ml iliac crest marrow was aspirated from each of 3 sites on 4 separate occasions. Single-cell suspensions were prepared as previously described. Peripheral blood mononuclear leukocytes (PBML) and light-density bone marrow cells (LDBMC) were obtained by Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) separation of heparinized whole blood or marrow diluted 1:2 with complete medium. Complete medium for marrow cell suspensions and agar cultures was McCoy's 5A medium with 15% fetal calf serum (FCS), amino acids, and antibiotics (Grand Island Biological Co., N.Y.). Complete medium for peripheral blood cells was RPMI-1640 with amino acids, antibiotics, and 20% FCS (GIBCO).

Separation of Adherent and Phagocytic Cells

Adherent cells were removed using nylon-fiber columns as previously described. Phagocytic cells were removed from mononuclear cell suspensions using an iron-magnet technique.

Removal of T Lymphocytes

A 1% (v/v) solution of washed sheep erythrocytes (SRBC, Prepared Media Laboratories, Tualatin, Ore.) was mixed with an equal volume (0.2–0.5 ml) of PBML or phagocyte-depleted LDBMC in a 15-ml centrifuge tube. The mixture was incubated for 15 min at 37°C in 7.5% CO2 in air, centrifuged for 5 min at 100g, reincubated at 22°C for 60 min, and gently resuspended. Rosette-forming cells were removed by Ficoll-Paque centrifugation. Purification of T lymphocytes from the blood of the responsive patient was accomplished by dissociating the rosettes by vortex mixing followed by removal of SRBC on another Ficoll-Paque gradient. In normal volunteers and in the prednisone-responsive patient, T-depleted cells were mixed again with SRBC to detect residual E-rosetting lymphocytes. In bone marrow cell suspensions, the nonrosetting (I-depleted) cells were removed from the interface, were washed twice, and were resuspended in a volume of complete medium equal to the original (prerosette) volume; this was done to avoid stem cell enrichment. In each experiment, control cells (not T-depleted) were also subjected to Ficoll-Paque centrifugation without prior SRBC mixing.

Colony-Stimulating Activity (CSA)

CSA for all but two studies was prepared as follows. PBML for normal volunteers were cultured (3 x 10⁶ PBML/ml) for 3 days (37°C, 7.5% CO2) in McCoy's 5A medium with 15% FCS to which 2.0 μl/ml phytohemagglutinin (PHA; Difco) had been added. Conditioned medium was decanted and stored unfiltered at −20°C. In the responsive patient, a single source of CSA was used for the first 3 of 4 studies (Fig. 3). In studies of two aplastic controls, CSA was prepared according to the method of Iscove.

Agar Cultures

Double-layer agar cultures were performed according to a modification of the techniques of Pike and Robinson. Underlayer agar contained CSA (0.1 ml/plate) with and without cortisol (hydrocortisone sodium succinate 10⁻⁶–10⁻⁴M). Overlayers contained LDBMC or T-depleted LDBMC. The responsive patient was studied on four separate occasions: prior to and during successful prednisone therapy, during relapse after the development of clinical steroid resistance, and after two cycles of COP therapy. Colonies (> 40 cells) and clusters (> 8 < 40 cells) were counted after 8 days of culture at 37.5°C in a humidified atmosphere of 7.5% CO2 in air.
Phagocyte-depleted light density bone marrow cells (LDBMC)

LDBMC depleted of E-rosette forming cells

Cortisol (10^{-5}M)-treated LDBMC

Cortisol (10^{-7}M)-treated T-depleted LDBMC

LDBMC plus 0.1 ml of lymphocyte-conditioned medium

Fig. 3. Serial agar cultures using marrow cells from patient 6. Bars and vertical lines represent means ± SD for 4–8 replicate plates. Prior to prednisone therapy (April 1, 1978), colony growth of LDBMC was decreased (normal range in our laboratory is 25–200 colonies/10^8 LDBMC). Colony growth increased significantly (p < 0.05) after removal of T cells (51% LDBMC were T cells) or after cortisol treatment. However, cortisol treatment of T-depleted LDBMC inhibited colony growth (a normal response) when compared to colony growth of T-depleted LDBMC not treated with cortisol. During prednisone therapy (April 26, 1978), baseline colony growth increased to normal and no evidence of “suppressor” T-lymphocyte activity was found (21% LDBMC were T cells). In addition, colony growth was inhibited by cortisol and by medium conditioned by autologous T lymphocytes obtained prior to prednisone therapy. The study on August 16, during the second relapse, showed subnormal colony growth and the reappearance of “suppressor” lymphocytes (39% LDBMC were T cells), but in contrast to the original study, cortisol failed to enhance colony growth in vitro; the patient subsequently failed to respond to prednisone therapy. The fourth study was performed after the completion of two cycles of COP and shows patterns of colony growth similar to those during the remission of April 26, 1978. At this time, 32% LDBMC were T cells.

Cortisol Treatment of Bone Marrow and Peripheral Blood Cells

Stock (10x) solutions of cortisol (Upjohn Pharmaceuticals, Kalamazoo, Mich.) were made in double strength McCoy's 5A medium. In all studies, LDBMC and T-depleted LDBMC were cultured in agar over underlayers containing both CSA and cortisol. The final concentration of cortisol in underlayers ranged from 10^{-4} to 10^{-7}M. Protein binding of cortisol in vitro ranged from 17% (10^{-4}M) to 41% (10^{-7}M). Methylprednisolone sodium succinate was used in agar cultures of LDBMC from the responsive patient on two occasions, once during relapse and once during prednisone-induced remission.

In the responsive patient, PBML (2 x 10^6/ml) and fractions thereof (phagocyte-depleted PBML and T-depleted PBML) were cultured (2 ml aliquots in RPMI 1640 with 15% FCS) for 3 days with and without cortisol (10^{-7}M) and with and without phytohemagglutinin (Difco, 0.1% v/v). Phagocyte-depleted PBML (2-4 x 10^6/ml) from 9 normal volunteers were similarly treated. The cells were harvested and washed three times, then were used in coculture experiments. The conditioned media were stored at −20°C for use in soluble inhibitor assays.

Coculture

The coculture experiments consisted of phagocyte-depleted LDBMC, which served as “target” cells, and PBML, which served as “effector” cells. PBML from nine normal volunteers (none had been previously transfused) were tested against LDBMC targets obtained from two normal volunteers. PBML from the responsive patient were used in two coculture experiments, one prior to and the other during prednisone therapy.

Lymphocyte: LDBMC ratios were 1:2. After mixing LDBMC and PBML, the mixture was placed in
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agar medium so that the resulting suspension contained $2 \times 10^5$ LDBMC/ml agar medium. Five replicate plates were allowed to gel and were cultured for 8 days under the conditions described above.

Soluble Inhibitor Assay

In order to detect serum inhibitors of granulopoiesis, serum stored at $-20^\circ$C from each patient was thawed and placed in underlayer agar (0.1 ml serum/plate) with and without standard CSA. LDBMC ($2 \times 10^5$/plate) from normal volunteers served as target cells. In addition, LDBMC from the prednisone-responsive patient were incubated for 60 min with both fresh and frozen (10%) autologous serum, after which the cells were washed and plated in agar.

In order to detect soluble inhibitors of granulopoiesis in lymphocyte-conditioned medium, medium, conditioned by phagocyte-depleted PBML ($1 \times 10^5$/ml) from 9 volunteers and the responsive patient was placed in underlayer agar (0.1 ml conditioned medium/plate) with and without standard CSA. LDBMC ($2 \times 10^5$) from normal volunteers served as target cells in this assay. In the patient, T-depleted PBML, cortisol-treated ($10^{-3}M$ for 60 min at $37^\circ$C) PBML, and purified T cells were also used to generate conditioned medium.

Experimental Design

The granulopoietic inhibitory effects of T cells from the patient and normal volunteers were assessed in three ways. First, autologous marrow cells were cultured in agar before and after T-cell depletion. Next, T-depleted and T-enriched PBL from all patients were cultured for 3 days in RPMI 1640; the cells were used as “effectors” in coculture experiments, and the PBL conditioned media were assayed for soluble inhibitory activity.

**Fig. 4.** The effect of cortisol ($10^{-5}M$-$10^{-4}M$) on agar (CFU-C) colony growth in (A) four normal volunteers and (B) colony and (C) cluster growth in six patients with severe aplastic anemia. Points and vertical bars represent means $\pm$ SD in four replicate plates. Target cells in the patients were LDBMC. Target cells in the volunteers were phagocyte-depleted LDBMC and T-lymphocyte-depleted phagocyte-depleted LDBMC. Cortisol inhibits normal colony growth and has either no effect or an inhibitory effect on colony growth by marrow cells from five aplastic controls (patients with prednisone-resistant aplastic anemia). However, colony and cluster growth was significantly enhanced by cortisol in vitro ($p < 0.05$, Student's $t$ test) at $10^{-3}M$-$10^{-4}M$ in the prednisone-responsive patient.
RESULTS

Serum from all six patients failed to inhibit colony growth of normal LDBMC in agar. Indeed, when LDBMC were plated over underlayers containing serum alone, all sera stimulated colony growth.

Agar Culture

In the normal volunteers, cortisol (10^{-4}–10^{-6}M) inhibited colony growth of LDBMC and T-depleted LDBMC (Fig. 4A). T-depleted LDBMC contained 2%-4% E-rosetting cells.

Cortisol (10^{-4}–10^{-7}M) and methylprednisolone (10^{-4}–10^{-6}M) significantly enhanced colony and cluster growth of marrow cells from the prednisone-responsive patient, but cortisol failed to enhance colony and/or cluster growth of cells from the aplastic control patients (Fig. 4B and C).

In order to assess the mechanism by which cortisol exerted its enhancing effect in vitro, LDBMC and T-lymphocyte-depleted LDBMC from the responsive patient were plated in agar medium over underlayers containing CSA or CSA and cortisol. T-lymphocyte depletion enhanced colony growth threefold, as did cortisol treatment of LDBMC (Fig. 3, April 1, 1978). However, cortisol treatment of T-depleted LDBMC failed to enhance colony growth further (13.5 ± 5 colonies/10^5 cells) when compared to colony growth of untreated T-depleted LDBMC (16 ± 1 colony/10^5 cells). T-lymphocyte depletion of LDBMC from normal volunteers and control aplastic patients did not enhance colony growth.

![Graph](image-url)

Fig. 5. The effect of lymphocytes from the peripheral blood of patient 6 on agar colony growth of LDBMC from a normal volunteer. Bars and vertical lines represent means ± SD in 4 replicate plates. T-enriched (nonadherent PBL) cell suspensions contained 83% T lymphocytes and inhibited (p < 0.001) colony growth at lymphocyte: marrow ratios of 1:2. T-depleted and cortisol-treated PBL were also inhibitory, but to a lesser degree.
Table 1. The Effect of PBL in Coculture and of PBL-Conditioned Medium* on Granulocyte Colony Growth of Normal Marrow Cells. In No Instance Did Cells or Conditioned Medium Inhibit Colony Growth

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Coculture</th>
<th>Conditioned Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>112 ± 12 (Control)</td>
<td>112 ± 4 (Control)</td>
</tr>
<tr>
<td>1</td>
<td>111 ± 8</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>104 ± 4</td>
<td>107 ± 4</td>
</tr>
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<td>3</td>
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<tr>
<td>8</td>
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<td>116 ± 7</td>
</tr>
<tr>
<td>9</td>
<td>129 ± 2</td>
<td>124 ± 5</td>
</tr>
</tbody>
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Mean ± SD colonies/10⁶ phagocyte-depleted, nonadherent marrow cells obtained from a normal male volunteer.

*1–2 × 10⁶ phagocyte-depleted PBML cultured in RPMI-1640 for 72 hr; 0.1 ml conditioned medium added to agar underlayers containing CSA.

These observations suggest that the effect of cortisol on granulopoiesis in vitro in the responsive patient related to an interaction of cortisol with T lymphocytes rather than CFU-C. In order to further test this hypothesis, PBML from this patient were cocultured with normal LDBMC. As shown in Fig. 5, T-enriched (nonadherent) PBL inhibited colony growth. This inhibition decreased significantly after removal of T lymphocytes by E-rosette formation or treatment of T-enriched PBML with cortisol (Fig. 5). Phagocyte-depleted PBML from 9 normal volunteers failed to inhibit colony growth (Table 1).

To determine the potential role of soluble factors in the inhibition of granulopoiesis by T lymphocytes, lymphocyte-conditioned media were tested against CSA-stimulated LDBMC from normal volunteers. While lymphocyte-conditioned media from 9 normal volunteers failed to inhibit colony growth (Table 1), conditioned medium from cultures of PBML obtained from the responsive patient prior to prednisone therapy significantly inhibited agar colony growth of autologous (Fig. 6).
Table 2. The Effect of Medium (LCM) Conditioned by Lymphocytes From the Prednisone-Responsive Patient on CSA-Stimulated Agar Colony Growth of Normal LDBMC. LCM Prepared at the Time of Relapse Inhibited Granulopoiesis but LCM Prepared During Prednisone-Induced Remission Did Not

<table>
<thead>
<tr>
<th>Medium Type</th>
<th>Colonies/10^9 Cells†</th>
</tr>
</thead>
<tbody>
<tr>
<td>O (Control)</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>LCM relapse</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>LCM remission</td>
<td>42 ± 6</td>
</tr>
</tbody>
</table>

*Both LCM samples were tested simultaneously.
†Mean ± SD of 5 replicate plates.

3) and normal (Fig. 6) LDBMC. Medium conditioned by T-depleted PBL and by cortisol-treated PBL failed to inhibit colony growth. Because these results suggest that T cells were responsible for the elaboration of a soluble inhibitor of granulopoiesis, we performed a similar study using semipurified T lymphocytes; T-lymphocyte-conditioned medium was markedly inhibitory (Fig. 6). However, medium conditioned by T lymphocytes obtained from this patient’s peripheral blood during remission was not inhibitory (Table 2). We attempted to enhance the production of soluble inhibitor by culturing T-enriched cells with PHA and found that colony growth of CSA-stimulated normal LDBMC was increased by PHA-stimulated lymphocyte-conditioned medium. In additional studies, PHA-stimulated lymphocyte-conditioned medium contained CSA. That is, it stimulated colony growth of phagocyte-depleted LDBMC cultured over underlayers that contained no exogenous source of CSA.

DISCUSSION

Because antilymphocyte globulin (ALG) and androgens may be effective in the treatment of some patients with severe aplastic anemia, we cannot be certain that prednisone was the critical ingredient in our patient’s initial induction therapy. We do attribute the second remission to prednisone therapy, because readministration of prednisone alone was followed by increases in blood counts (Fig. 1), increased marrow cellularity, and increased agar colony growth (Fig. 3).

We observed that cortisol in vitro enhanced agar colony growth of LDBMC obtained from this patient during relapse, whereas cortisol failed to enhance colony growth of LDBMC from five aplastic patients who were unresponsive to steroid therapy (Fig. 4). These observations are consistent with those previously reported from our laboratory, which indicate a correlation between in vitro and in vivo responses to glucocorticoids in patients with granulopoietic failure. In addition, the effectiveness of cortisol in vitro permitted further studies on the mechanism of steroid action in this patient.

Because there is considerable cellular heterogeneity in LDBMC suspensions, these studies focused on techniques of fractional depletion of certain cell populations from the LDBMC. The T-lymphocyte depletion technique was used because (1) work in our laboratory has shown that cortisol-sensitive T-lymphocytes are capable of inhibiting granulopoiesis in vitro, and (2) recent observations suggest that certain cases of aplastic anemia are immunologically mediated. For example, observations in patients with aplastic anemia of autologous hemopoietic recovery following unsuccessful marrow transplantation, immunosuppressive thera-
py, or plasmapheresis and observations in vitro of lymphocyte-mediated or immunoglobulin-mediated hemopoietic suppression have supported this suggestion. The findings in our patient also support the hypothesis that aplastic anemia can be immunologically mediated.

T lymphocytes in the marrow and peripheral blood of this patient suppressed in vitro granulopoiesis of autologous and allogeneic marrow cells (Figs. 3 and 5). Granulopoiesis was as effectively enhanced by cortisol treatment of LDBMC as it was by T-lymphocyte depletion. That cortisol failed to enhance colony growth of T-depleted LDBMC (Fig. 3) suggests that cortisol's effect was on T lymphocytes and not CFU-C. Although cortisol treatment and T depletion of the PBML sample decreased the inhibition seen in coculture, colony growth was not restored to control levels by virtue of these maneuvers. There are a number of potential explanations for this lack of complete correction. For example, our SRBC-rosetting technique was carried out at room temperature and we avoided pretreatment of SRBC in order to facilitate disruption of the rosettes for T-cell cultures. Because pretreatment techniques facilitate SRBC-T-cell binding, T depletion may not have been complete. Alternatively, the inhibition of granulopoiesis seen in coculture may have resulted from either T-cell/non-T-cell cooperation, or from two separate inhibitory cell populations; the latter is a mechanism suggested by coculture data obtained using effector cells from patients with Felty's syndrome. Finally, the results of coculture experiments using effector cells from the blood of multiply transfused animals and humans with aplastic anemia are at risk of revealing suppression of colony growth due to alloimmunization rather than suppression of primary etiologic significance. Thus, in this multiply transfused patient, inhibition of granulopoiesis in cocultures may reflect either a pathophysiologically relevant event or a transfusion-related phenomenon, or both.

As such, our coculture data must be interpreted in the light of the data from (1) autologous marrow cultures where T-cell depletion enhanced colony growth (Fig. 3) and (2) conditioned medium experiments that showed that cultured lymphocytes (83% T-lymphocytes) and T-lymphocyte suspensions (95% T-lymphocytes) from this patient generated a soluble inhibitor of granulopoiesis but that T-depleted PBML and cortisol-treated PBML did not (Fig. 6). Furthermore, the soluble inhibitor was not only effective against normal LDBMC but inhibited colony growth of autologous LDBMC obtained during the second remission (Fig. 3).

T-cell suppression of granulopoiesis in vitro was mediated, at least in part, by a soluble factor. The production and/or release of this activity in vitro was independent of the patient's serum, mononuclear phagocytes, and complement. Additional studies indicated that the T-enriched PBML from this patient, which produced a potent inhibitory activity in culture, were stimulated to produce/release CSA under the influence of PHA. We do not know whether the CSA-producing and inhibitor-producing cells are identical, but the observation documents functional heterogeneity in our T-enriched cell populations.

While the steroid/T-cell interaction was responsible for the effect of cortisol on granulopoiesis in vitro, the mechanisms of prednisone's effect in this patient are difficult to assess because the in vivo effects of glucocorticoids on human blood and reticuloendothelial cells are numerous and poorly defined. Nonetheless, the serial in vitro observations in this patient suggest that prednisone in vivo functioned primarily as a T-lymphocyte inhibitor. For example, at the time of relapse, colony
growth was abnormally low and cortisol-sensitive T lymphocytes were present in the marrow cell suspensions. However, during prednisone-induced remission, baseline colony growth increased to within our normal range (the source of CSA and the volume of marrow aspirated were identical) and suppression by T lymphocytes was not detectable. Finally, the second relapse was characterized by reappearance of "suppressor" T cells that were cortisol-resistant; the disease was also steroid-resistant.

Thus, although we recognize the inherent limitations of in vitro/in vivo associations, our observations are consistent with the hypothesis that aplastic anemia in this woman was mediated, at least in part, by cortisol-sensitive T lymphocytes, that the initial effect of prednisone therapy and the later effect of combination chemotherapy was to functionally suppress the activity of these cells in vivo, and that the second relapse resulted from the development of glucocorticoid resistance in these T lymphocytes. The future application of similar techniques may aid in the identification of those aplastic patients in whom immunosuppressive therapy will be beneficial. A much larger series will be required, but the data should be rewarding considering the dangers of immunosuppressive therapy in such patients.

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