Acquired amegakaryocytic thrombocytopenic purpura (AATP) is a disorder of hematopoiesis characterized by severe thrombocytopenia due to a selective reduction or total absence of megakaryocytes in an otherwise normal-appearing bone marrow. Although the development of autoantibodies directed against cells in the megakaryocyte progenitor cell pool has been implicated in the pathogenesis of this disorder, cell-mediated suppression of megakaryocytopoiesis has not been described. Accordingly, we report two cases of AATP in which in vitro suppression of megakaryocyte colony formation by autologous ancillary marrow cells was demonstrable. Light-density bone marrow mononuclear cells (MNCs) obtained from both patients were either plated directly into plasma clot cultures, or after first being depleted by adherent monocytes (MØ) or T lymphocytes using standard methodologies. In some experiments, the depleted ancillary marrow cells were recovered for autologous co-culture studies with the MNCs from which they had been depleted. Megakaryocyte colony formation was detected in the cultures using an indirect immunofluorescence assay with a rabbit anti-human platelet glycoprotein antiserum. Removal of MØ (n = 8), or T lymphocytes (n = 4) from normal marrow MNCs had no apparent effect on colony formation. In contrast, depleting T lymphocytes from the MNCs of patient 1 significantly augmented megakaryocyte colony formation; a similar effect was observed after depleting MØ from the MNCs of patient 2. This observed augmentation in colony formation could be abrogated by autologous co-culture with the putative suppressor cell at effector cell/target cell ratios of 1:10 in the case of T lymphocytes or 1:5 in the case of MØ. Neither suppression nor stimulation of megakaryocyte colony formation was observed after culturing normal MNCs with autologous T cells (n = 4) or MØ (n = 3) at similar or greater ratios. We also observed inhibition of megakaryocyte colony formation after culturing normal MNCs in the presence of tissue culture medium conditioned by the MØ of patient 2. This effect was shown to be specific for megakaryocytes since this same conditioned medium had no significant effect on BFU-E and CFU-E-derived colony formation by autologous marrow mononuclear cells. These results suggest that: (a) both T cells and MØ are capable of exerting a regulatory effect on the proliferation of human megakaryocyte progenitor cells (CFU-Meg); (b) in the case of MØ, a soluble factor elaborated by these cells may be responsible for suppressing CFU-Meg growth; and (c) aberrant ancillary cell-megakaryocyte progenitor cell interactions may lead to clinically significant disease.

MATERIALS AND METHODS

Case Reports

Patient 1. A 58-year-old woman who was seen by her physician had a 6-week history of easy bruising and spontaneous ecchymosis formation. These complaints were temporally associated with the administration of erythromycin for periodontal disease but did not abate when the drug was discontinued. Physical examination revealed only multiple skin ecchymoses. The spleen was not enlarged. A complete blood count disclosed a platelet count of 22,000/µL with no other abnormality. Blood chemistries, a serum protein electrophoresis, folic acid and B₁₂ levels, an ANA, and a nuclear scan of the spleen were normal or negative. A bone marrow aspirate and biopsy disclosed a marked reduction in bone marrow.
Megakaryocytes. Marrow cellularity was otherwise normal, maturation was orderly, and iron stores were adequate. Platelet survival, performed as previously described, was within normal limits. A diagnosis of AATP was made.

Prednisone therapy (60 mg/day) was administered for 14 days, during which time the patient’s platelet count remained <20,000/μL. The drug was therefore discontinued, and the patient was referred to Temple University Hospital for bone marrow culture studies. At the time of study, her platelet count was 44,000/μL, her WBC count was 5,700/μL, and her hematocrit was 37.3% with a mean corpuscular volume (MCV) of 93 fl. A repeat marrow aspirate confirmed the presence of only rare megakaryocytes. Celbowski.

Three weeks later, the patient was found to have an impacted wisdom tooth. Erythromycin (2 g/day) was reinstituted and continued for 16 days without reduction in her platelet count (~51,000/μL). Her condition did not improve, and she was advised to undergo an extraction. Her preoperative platelet count was 72,000/μL. The patient tolerated the procedure well without platelet infusions. Postdischarge, her platelet count continued to increase. One month later, repeat bone marrow culture studies were performed when her platelet count was 92,000/μL and rising. Six months after her initial presentation, the platelet count was normal (192,000/μL). The patient remains entirely well.

Patient 2. A previously well 67-year-old housewife had a 4-month history of easy bruising. Her physical examination was normal except for the presence of petechiae and scattered ecchymoses. Initial laboratory studies disclosed a platelet count of 13,000/μL. The WBC count was 13,500/μL with a normal differential. The hematocrit was 45% with a MCV of 94 fl. A reticulocyte count was 2.1%. An ANA was negative. A bone marrow aspirate and biopsy were both hypercellular with normal erythroid and granulocytic maturation. Megakaryocytes were nearly absent in each. Cytogenetic analysis of the bone marrow cells disclosed a normal female karyotype. A diagnosis of AATP was made.

Attempts were made to raise the platelet count, first with prednisone (60 mg/day for 30 days) and then with colchicine (0.6 mg/day for 60 days). Each drug was associated with a transient rise in the platelet count (80 × 10^6/μL and 220 × 10^6/μL maximum, respectively), lasting only a few weeks. No further therapeutic interventions were attempted. Twenty-four months after diagnosis, the patient maintains a platelet count which ranges between 10,000 and 50,000/μL, but she has had no serious bleeding episodes.

**Methods**

**Megakaryocyte progenitor cell assay.** Informed consent was obtained prior to aspirating bone marrow from patients and normal volunteers. Aspirated bone marrow was diluted 1:1 in α-medium without nucleosides containing 20 U/mL of preservative-free sodium heparin. A single cell suspension was prepared, layered, over an equal volume of Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) (sp gr 1.077 g/mL) and then centrifuged for 25 minutes at 1,500 rpm at 4°C. The LDMMCs were collected and washed and then cultured in plasma clot cultures at a concentration of 5 × 10^5 cells/mL as previously described. All cultures, unless otherwise indicated, were supplemented with normal human AB serum to a final concentration of 30% (vol/vol) to provide essential growth factors. In one series of experiments (Table 1), cells were also cultured in 30% autologous serum or in 20% AB serum + 10% αplastic anemia serum, a known source of MEG-CSA. A cluster of three or more fluorescent cells was scored as one megakaryocyte colony. Unless otherwise stated, all data are reported as the mean ± SEM of megakaryocyte colonies counted in quadruplicate culture dishes.

**Erythroid progenitor cell assays.** CFU-E and BFU-E were harvested, stained, and scored as previously described. The concentration of erythropoietin (Connaught, Step III, Sweetwater, Pa) in the cultures was 2 IU/mL.

**Bone marrow smear preparation.** Marrow smears were prepared at the time of initial presentation from aspirated marrow as previously described. In brief, a small aliquot of marrow was collected into EDTA and smeared directly onto clean glass slides. Specimens to be immunochemically examined for megakaryocytes were air dried, fixed in methanol/acetone (1:9), and then stored frozen until probed with anti-PGP antiserum for the presence of both morphologically and nonmorphologically recognizable megakaryocytes.

**Cell separation and recovery procedures.** Bone marrow mononuclear cells (MNCs) obtained as described above were depleted of monocyte-macrophages (MΦ) by adherence to serum-coated plastic Petri dishes using the method of Kumagi and co-workers. After being charged with the MNC cell suspension, (5 mL at 1 × 10^7 MNCs/mL) dishes were incubated at 37°C in humidified air containing 5% CO2 for 2 hours. The nonadherent cells were then removed from the culture dishes by gentle rinsing with fresh α-medium, washed once again in α-medium, and then resuspended for further culture studies.

Adherent cells were recovered for co-culture experiments by adding 0.2% EDTA in phosphate-buffered saline (PBS) to the dishes and placing them at 4°C for 30 minutes. Adherent cell-depleted mononuclear cells contained <5% contaminating nonspecific esterase-positive cells. Cells adhering to the dishes were ≥90% nonspecific esterase staining positive.

T lymphocytes (T cells) were removed from the MNCs by rosetting with neuraminidase-treated sheep RBCs. Rosettes were separated from nonsettled cells by a second Ficoll-Paque density gradient sedimentation. Nonrosetted MNCs were designated T lymphocyte-depleted and contained <1% T cells as assessed by immunofluorescence staining with OKT1 monoclonal antibody (Ortho, Raritan, NJ). Mononuclear cells in the RBC pellet were T lymphocyte enriched, as demonstrated by fluorescence staining with OKT11 (≥95% positive). They were recovered for autologous coculture studies by hypotonic lysis of the sheep RBCs with ammonium chloride (0.83% in Tris buffer).

To calculate the expected number of megakaryocyte colonies after cell depletion procedures, the following formula was used: Expected colonies (in T cell or MΦ-depleted MNCs) = (Meg colonies/5 × 10^3 unseparated MNCs) times 100 divided by 100-(%T or MΦ cells in control MNC)/(% T or MΦ cells in depleted MNC).
Preparation of patient and normal control adherent cell conditioned medium. Marrow mononuclear cells (~1 x 10⁶) remaining attached to the serum-coated plastic Petri dishes after five rinses with PBS were overlaid with 5 mL of α-medium containing 10% human AB serum. The dishes were then incubated for 7 days at 37°C in an atmosphere containing 5% CO₂ at 95% humidity. At the end of that time, the medium was aspirated from the dishes, clarified by centrifugation (10,000 g for 5 minutes), and frozen at -80°C until needed.

Statistics. Statistical significance of data comparisons was tested using an unpaired Student's t test.

RESULTS

To determine where the block in megakaryocyte maturation existed in these patients,²⁴ marrow smears prepared at the time of their initial presentation were stained with anti-PGP antiserum. In patient 1, a rare fluorescein-positive cell, which under a phase-contrast observation could be morphologically identified as a mature megakaryocyte, was observed in the available study material. In patient 2, no fluorescent cells of any kind were observed. These results suggested that the perturbation in megakaryocytopoiesis in these patients existed at the progenitor cell level.

Studies were then carried out that were designed to detect either intrinsic defects in the patients' megakaryocyte progenitor cells or the presence of serum inhibitors that could have been suppressing their growth. Intrinsic defects were sought by assaying the patients' marrow mononuclear cell fraction for residual CFU-Meg cloning efficiency in response to an exogenous source of Meg-CSA in the form of aplastic anemia serum. The patients' serum was screened for humoral inhibitors by substituting fresh, autologous serum (not heat inactivated) for normal AB serum in some marrow cell cultures. The total human serum concentration (vol/vol) in all cultures was equivalent. Results obtained, along with reference data accrued from 11 normal control subjects, are shown in Table 1. When these experiments were carried out, patients 1 and 2 had platelet counts of 44,000 and 10,000, respectively, and only rare megakaryocytes in their marrow aspirates.

Patient 1 demonstrated markedly abnormal megakaryocyte colony formation in normal AB serum, autologous serum, and in the presence of aplastic anemia serum (Table 1). Her CFU-Megs were therefore either severely damaged or their development was being suppressed by as yet undefined influences.

Residual Meg-CSA responsive CFU-Megs were detected in patient 2. Furthermore, the addition of fresh autologous serum to this patient's cell cultures resulted in a 11.7-fold increase (P < .001) in megakaryocyte colony formation as compared with growth observed in AB serum alone and a 2.7-fold increase (P < .05) as compared with colony formation observed in the presence of aplastic anemia serum (Table 1). These data tended to exclude the presence of a serum inhibitor of CFU-Meg but failed to provide a reason for the aborted production of megakaryocytes in vivo.

We therefore sought to determine whether bone marrow T lymphocytes or adherent monocyte-macrophages were suppressing the patients' megakaryocytopoiesis by selectively depleting these cell types from the patients' marrow cells prior to cloning.

Depletion of adherent cells from six normal control marrows and from the marrow of patient 1 had no statistically significant effect (P > .05) on megakaryocyte colony formation (Fig 1A). In contrast, the adherent cell-depleted marrow cells of patient 2 gave rise to 121 ± 44 megakaryocyte colonies vs 25 ± 10 from unseparated marrow cells (Fig 1A). As adherent cells comprised only 12% of the unseparated marrow mononuclear cells of patient 2, we would have expected a maximum of 26 colonies to form as a result of progenitor cell concentration due to the cell depletion procedure. Sixty days later, when her platelet count was 8,000/µL and her marrow composition was unchanged, these studies were repeated and similar results were obtained. At that time unseparated marrow cells formed only 1.0 ± .48 megakaryocyte colonies/5 x 10⁶ mononuclear cells cloned, but depletion of adherent cells led to the formation of 8 ± 1 megakaryocyte colonies (P < .001).

These studies suggested that an adherent cell may have been suppressing megakaryocytopoiesis in patient 2. To test this hypothesis, the adherent marrow cells of patient 2 and

![Fig 1. Effect of ancillary marrow cell depletion on patients' megakaryocyte progenitor cell cloning efficiency. At the time of study, patients 1 and 2 had platelet counts of 44,000/µL and 10,000/µL, respectively.](image-url)
adherent marrow cells from three normal controls were recovered for autologous co-culture studies with the marrow mononuclear cells from which they had been removed (Table 2). At adherent cell/target cell ratios of 1:10, 1:5, and 1:2, megakaryocyte colony formation was statistically unaffected in the normal controls. In contrast, colony formation by the cells of patient 2 was inhibited ≥90% (P < .001) at effector/target cell ratios of 1:5 and above. A soluble factor elaborated by the patient’s adherent cells appeared to be responsible for this effect, since addition of an adherent cell-conditioned medium (ACCM) prepared from the patient’s marrow MØ significantly inhibited megakaryocyte colony formation by autologous marrow mononuclear cells (Fig 2, panel A). On the day this experiment was performed, the patient’s mononuclear cells gave rise to 63 ± 8 megakaryocyte colonies per 5 × 10³ cells plated. In the presence of 5% and 10% patient ACCM, this number was reduced to 16 ± 0.5 (P < .02) and 7 ± 3 (P < .01), respectively. A control ACCM assayed simultaneously against the same target cells had no statistically significant effect (P > .05) on megakaryocyte colony formation (Fig 2, panel A). The inhibitory activity was abolished by heating the ACCM for 30 minutes at 60 °C (Fig 2, panel B).

The ACCM of patient 2 also inhibited megakaryocyte colony formation by three of the four normal marrow mononuclear cells against which it was tested (Fig 2, panel C). In the experiments in which this effect was observed, inhibition averaged 74% in comparison to baseline colony formation. This result was in marked contrast to results we obtained in the past with ACCM derived from normal cells, which had no apparent effect on CFU-Meg cloning efficiency. A control ACCM assayed simultaneously with the ACCM of patient 2 in the fourth experiment also had no significant effect on megakaryocyte colony formation (baseline colony formation, 117 ± 11 [mean ± SEM/5 × 10³ cells plated]; in 5% control ACCM, 114 ± 6; in 10% control ACCM, 129 ± 26).

We also sought to determine the relative specificity of this soluble inhibitor for cells of the megakaryocyte lineage by assaying CFU-E-derived and BFU-E-derived colony formation in the presence of the autologous or normal control ACCM on two separate occasions (Fig 3). Final concentra-

![Fig 2. Effect of adherent cell conditioned medium (ACCM) of patient 2 on megakaryocyte colony formation by autologous and allogenic normal bone marrow mononuclear cells. Serum used to supplement the cultures was derived solely from normal AB donors. Results are expressed as mean ± SEM of colonies enumerated in duplicate or quadruplicate cultures. Panel A demonstrates the effect of the patient's ACCM on autologous megakaryocyte colony-forming unit (CFU-Meg) cloning efficiency. Highly significant inhibition was seen at the 5% (vol/vol) (P < .02) and 10% (P < .01) ACCM concentrations. No significant effect (P > .06) on megakaryocyte colony formation was observed in the presence of control ACCM at either the 5% or 10% concentration when assayed simultaneously against the same cells. Panel B demonstrates that CFU-Meg inhibitory activity was lost after the ACCM was heated for 30 minutes at 60 °C. The effect of the ACCM of patient 2 on four different normal donor-derived marrow mononuclear cells is shown in panel C. The number of colonies enumerated in the presence of ACCM, at both the 5% and 10% final concentration (vol/vol), differ significantly (P < .05) from control in experiments 2 through 4.](image-url)
The effect of T lymphocyte depletion on megakaryocyte colony formation by marrow cells obtained from four normal controls and each of the patients is shown in Fig 1B. In aggregate, the data derived from studying the four normal individuals disclosed no significant effect on megakaryocyte colony formation as a result of this procedure. This was not true for the patients as, in each one, significant changes in cloning efficiency were noted after their marrow cells were T lymphocyte depleted.

In patient 1, T lymphocyte depletion led to the formation of 9 ± 0 (mean ± SEM of duplicate cultures) megakaryocyte colonies whereas essentially none were formed by unseparated mononuclear cells (P < .001). Furthermore, the unfractionated bone marrow cells of patient 1 did not increase colony formation in the presence of aplastic anemia serum (data not shown). Addition of the same amount of this same source of Meg-CSF to T depleted marrow mononuclear cells caused colony formation to double from 9 ± 0 to 18.5 ± 1.5 (P < .001). Because the number of T lymphocytes in this patient’s unseparated mononuclear cell preparation was ~35%, removal of this cell type would have been expected to lead to the formation of 1.5 colonies/5 x 10^5 cells cloned. It is therefore unlikely that this observed increase in cloning efficiency was an artifact of the depletion procedure.

Removal of T cells from the marrow mononuclear cells of patient 2 totally abrogated megakaryocyte colony formation (Fig 1B). This effect could not be overcome by the addition of aplastic anemia serum to the cultures. It was not due to loss of CFUs, since culturing the T lymphocyte-rich cell fraction at 5 x 10^5 cells/mL never resulted in the formation of more than two megakaryocyte colonies.

Thus, the T cell depletion studies suggested that these cells might exert either a net suppressive (patient 1) or trophic (patient 2) influence on in vitro megakaryocytopoiesis. This hypothesis was tested by co-culturing recovered T lymphocytes with autologous T lymphocyte-depleted marrow mononuclear cells (Table 3). At a target cell/T cell ratio of 20:1, megakaryocyte colony formation by the marrow cells of patient 1 was decreased, but statistically unchanged, from colony formation observed in her T-depleted control cells. At ratios of 10:1 and 5:1, however, colony formation was suppressed 94% and 100%, respectively (P < .01). The opposite effect was noted on both occasions that patient 2 was studied. At target cell/T cell ratios of ≥10:1, a significant augmentation in megakaryocyte cloning efficiency was observed (Table 3). In contrast to these results, addition of

Table 3. Co-Culture Experiments With Autologous T Lymphocytes

<table>
<thead>
<tr>
<th>T Lymphocyte/</th>
<th>Target Cell*</th>
<th>No. of</th>
<th>Effect on Colony Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>Colonies Formed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Cells alone</td>
<td>0.0 ± 0.0†</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Target cells alone</td>
<td>9.0 ± 0.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td>2.0 ± 1.4</td>
<td>—</td>
<td>8-fold (P &lt; .05)</td>
</tr>
<tr>
<td>1:10</td>
<td>0.5 ± 0.5</td>
<td>↓ 94%</td>
<td>(P &lt; .01)</td>
</tr>
<tr>
<td>1:5</td>
<td>0.0 ± 0.0</td>
<td>↓ 100%</td>
<td>(P &lt; .01)</td>
</tr>
<tr>
<td>1:2</td>
<td>—</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Cells alone</td>
<td>1.5 ± 0.7‡</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Target cells alone</td>
<td>0.0 ± 0.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1:10</td>
<td>8.3 ± 1.5</td>
<td>↑ 8-fold (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>1:5</td>
<td>—</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>9.0 ± 1.1</td>
<td>↑ 9-fold (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>6.8 ± 1.6</td>
<td>↑ 7-fold (P &lt; .02)</td>
<td></td>
</tr>
<tr>
<td>Controls§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Cells alone</td>
<td>0.5 ± 0.3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Target cells alone</td>
<td>39 ± 12</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1:10</td>
<td>27 ± 8</td>
<td>—</td>
<td>8-fold (P &lt; .05)</td>
</tr>
<tr>
<td>1:5</td>
<td>19 ± 4</td>
<td>—</td>
<td>8-fold (P &lt; .05)</td>
</tr>
<tr>
<td>1:2</td>
<td>26 ± 7</td>
<td>—</td>
<td>8-fold (P &lt; .05)</td>
</tr>
<tr>
<td>1:1</td>
<td>35 ± 13</td>
<td>—</td>
<td>8-fold (P &lt; .05)</td>
</tr>
</tbody>
</table>

NT, Not tested.
* T Lymphocyte-depleted marrow mononuclear cells.
† Mean ± SEM of duplicate cultures.
‡ Mean ± SEM of quadruplicate cultures.
§ Aggregate results from four normal donors.
autologous T cells to four normal control marrows effected no significant \( (P > 0.05) \) change in colony formation.

Finally, we repeated the above studies on patient 1 when she was in the midst of a spontaneous recovery, and her platelet count was 92,000/\( \mu \text{L} \). At the time, cloning efficiency of her unseparated marrow cells was still poor but, in contrast to the first study, T lymphocyte depletion had no discernible effect on megakaryocyte colony formation \([3.8 \pm 2.2 v. 2.5 \pm 2 \text{ colonies, respectively, per } 5 \times 10^3 \text{ mononuclear cells cloned } (P > .05)]\).

**DISCUSSION**

Various bone marrow failure states, including aplastic anemia,17–20 acquired pure RBC aplasia,22,24 and selective neutropenia25,26 have been associated with either T lymphocyte-mediated17,19,24–27 or monocyte-macrophage-mediated20,21 suppression of in vitro hematopoiesis. Our data implicating marrow accessory cells of both types in the suppression of in vitro megakaryocytosis in some patients with AATP appears to be the first of its kind.

T lymphocytes of undetermined subtype appeared to be the suppressor-effector cells in the bone marrow of patient 1. This conclusion is based on a number of observations. First, T lymphocyte depletion augmented megakaryocyte colony formation by the patient's marrow mononuclear cells (Fig 1B). It is unlikely that this effect was due to concentration of megakaryocyte progenitor cells by the T cell depletion procedure, since the number of colonies formed was still significantly more than would have been expected based on the percentage of T lymphocytes present in the patient's unseparated marrow mononuclear cells. It is possible, however, that the observed increase in CFU-Meg cloning efficiency was due to the effect of concentration an unknown marrow accessory cell. Such a possibility is difficult to exclude but is also unlikely, since no increase in colony formation was noted after performing the same procedure in patient 2, who had a similar illness or in the normal controls (Fig 1B). In addition, autologous co-culture studies with the recovered T lymphocytes and T cell-depleted target cells resulted in suppression of colony formation at physiologic ratios (Table 3). Finally, during the recovery phase of the patient's illness, this in vitro phenomenon could no longer be demonstrated.

Unfortunately, we were unable to obtain sufficient marrow cells from patient 1 to demonstrate that the T cells involved suppressed only cells of the megakaryocyte lineage. Nevertheless, we still believe it is reasonable to assume that the suppression observed was specific since: (a) T cell-mediated suppression of erythropoiesis and granulopoiesis is well documented22–26; and (b) the patient's erythroid and granulocyte compartments, in distinct contrast to her megakaryocyte compartment, were intact. Had suppression not been specific, we would have expected to see aplasia of another line as well.

It is unclear why megakaryocyte colony formation by the recovering marrow cells of patient 1 remained so low, but persistent subnormal progenitor cell cloning efficiency in spite of clinical hematologic recovery has been reported in patients with aplastic anemia21 and pure RBC aplasia.22 Possible explanations include an absolute decrease in the number of marrow megakaryocyte progenitor cells or a more subtle form of damage than these assays can detect.

Quite different results were obtained in the second patient. In this case, suppression of megakaryocytosis appeared to be due to the effect of adherent cells of the monocyte-macrophage lineage. This conclusion is based on reasoning similar to that applied to the case of patient 1. Megakaryocyte colony formation was augmented by depletion of adherent marrow cells (Fig 1A) and could not be attributed to nonspecific concentration of megakaryocyte progenitor cells by the depletion procedure. Again, the possibility that the observed increase in megakaryocyte colony formation was due to the effect of concentrating an unidentified marrow accessory cell must be considered but would appear unlikely in this case as well since colony formation was inhibited by co-culturing recovered adherent cells with the marrow mononuclear cells from which they had been removed (Table 2).

The suppressive effect of the MØ cells was noted at target/effector cell ratios similar to those reported by others to cause inhibition of erythropoiesis.28–30 In addition, studies performed with ACCM suggested that the inhibitory factor was elaborated by these cells into their environment. Initial characterization of the inhibitor showed that it was heat labile, suggesting that it was not macrophage derived PGE\(_2\),31 or PGE\(_1\), which is known to be even more heat stable. We demonstrated that its activity was not MHC restricted since it inhibited allenguin as well as autologous CFU-Meg (Fig 2, panels A and C). Finally, the effect of this inhibitor appeared to be specific for cells of the megakaryocyte lineage, since no inhibitory activity against autologous BFU-E could be demonstrated on either of two separate occasions (Fig 3). The significance of the 29% inhibition of autologous CFU-E growth by the ACCM of patient 2 at the 10% (vol/vol) concentration in the first experiment (Fig 3, panel A) is unclear. Some degree of inhibition was also noted with control ACCM in this experiment as well, and in neither case did the suppression observed approach the 50% level that some experts require before imparting biological significance to such an observed effect.32

In cases of bone marrow failure attributed to humoral inhibitors, progenitor cell cloning efficiency in vitro may be less perturbed32–34 than in those in which cellular mechanisms are implicated.19,22 With this consideration in mind, the number of residual megakaryocyte progenitors detected in the bone marrow of patient 2 was somewhat surprising. Bagby and co-workers have demonstrated that these associations are not absolute, however, by showing significant granulocyte-macrophage colony formation in some patients with apparent T cell-mediated granulopoietic failure.25,26

In addition, humoral suppression of hematopoiesis is often demonstrable by co-culturing patient marrow in fresh, autologous plasma or serum that has not been heat inactivated.33,34 Such a technique was used in the first description of antibody-mediated AATP.3 In that patient, addition of fresh autologous serum to her bone marrow cultures suppressed megakaryocye colony formation. Screening of the serum of patient 2 for potential inhibitors, using the same technique, resulted in marked stimulation of her own (Table
1) as well as other normal donors' megakaryocyte progenitor cells (data not shown). Accordingly, we feel that the existence of a co-existing humoral inhibitor in this patient was unlikely. In patient 1, this question could not be reliably addressed since her cloning efficiency was so poor.

Our results also suggest that the role of T lymphocytes in the regulation of human megakaryocytopoiesis in vitro is complex. These cells may exert either suppressive or trophic effects on megakaryocyte progenitor cells (Fig 1, Table 3). Thus, the CFU-Meg of patient 1 appeared to be inhibited by T cells, whereas in patient 2, CFU-Meg-derived colony formation fell to zero when T cells were selectively removed from her marrow mononuclear cells (Fig 1B). This result could not be attributed to procedure-induced loss of megakaryocyte progenitors and was presumably caused instead by unopposed suppressor activity of the patient's adherent marrow cells. By implication, T lymphocyte–monocyte interactions may have been important in determining net megakaryocyte colony formation in vitro and perhaps the growth of megakaryocyte progenitor cells in vivo as well. We also noted that when the T lymphocytes of patient 2 were co-cultured with autologous marrow mononuclear cells, marked stimulation of megakaryocyte colony formation occurred (Table 3). These data also support the hypothesis that T cells may exert a trophic influence on progenitor cells of the megakaryocyte compartment and may cooperate with other ancillary bone marrow cells in regulating their growth. The recent report by Geissler and co-workers35 that phytohemagglutinin–leukocyte conditioned medium (PHA-LCM)–activated T cells stimulate CFU-Meg proliferation in an agar culture system would appear to support this hypothesis.

Recent reviews36,37 have pointed out that the body of evidence implicating immune effector cells in the regulation of erythropoiesis and granulopoiesis is both large and compelling. Little is presently known regarding the cellular regulation of human megakaryocytopoiesis under pathologic or normal conditions. Further studies on the cellular interactions regulating growth and development of CFU-Meg are therefore likely to reveal important insights into the pathogenesis of as yet poorly characterized disorders of megakaryocyte and platelet production.

ACKNOWLEDGMENT

We are grateful to Dr. Scott Murphy, Cardeza Research Foundation, Thomas Jefferson University, Philadelphia, for referring one of the patients for study and to Dr. Kenneth F. Mangan, Montefiore Hospital, University of Pittsburgh, for helpful suggestions regarding the preparation of this manuscript. We also thank Dr. Wen Yu Xu, Department of Immunology, Second Shanghai Medical University, Shanghai, China, for invaluable technical assistance.

REFERENCES

Cell-mediated suppression of megakaryocytopenesis in acquired amegakaryocytic thrombocytopenic purpura

AM Gewirtz, MK Sacchetti, R Bien and WE Barry