Effects of Monoclonal Antibody and Complement Treatment of Human Marrow on Hematopoiesis in Continuous Bone Marrow Culture

Joel S. Greenberger, Lisa Rothstein, Paolo DeFabritiis, Marco Bregni, Robert Bast, Jr., Jerome Ritz, Lee M. Nadler, Jeffrey M. Lipton, and Mary Ann Sakakeeny

ABSTRACT

Long-term bone marrow cultures were established from single-cell suspensions of human bone marrow that had been treated with monoclonal antibodies and complement. Each treated cell suspension was evaluated for production of hematopoietic stem cells over 20 weeks. Treatment with antibody to HLA-DR (Ia), B1, J2, or J5 did not remove adherent cells including those differentiating to adipocytes in 17-hydroxycorticosteroid. In contrast, treatment with monoclonal antibody directed against human \( \beta_2 \)-microglobulin reduced adipocyte numbers by 100-fold and reduced the total adherent cell density over 70%. Cumulative total nonadherent cell and granulocyte-macrophage colony-forming units (GM-CFUc) production over 20 weeks was not significantly altered by one cycle of anti-Ia plus complement or up to three cycles of treatment with complement and anti-J2, -J5, or -B1. However, one cycle of treatment with anti-\( \beta_2 \)-micro-globulin depressed production of both GM-CFUc and nonadherent cells by over 100-fold compared to other treatment groups. While one cycle of treatment of anti-Ia and complement killed all detectable cells forming GM-CFUc, blast-forming units (erythroid), and GM-CFUc, GM cluster-forming cells survived. Treatment of marrow with three cycles of anti-Ia and complement removed all detectable GM colony- and GM cluster-forming cells; however, this marrow produced fewer cumulative Ia-positive GM-CFUc. Long-term bone marrow cultures may prove to be an interesting system for in vitro analysis of the effects of new immunotherapeutic agents including other monoclonal antibodies prior to clinical use.

INTRODUCTION

The association of specific cell surface antigenic determinants with defined stages of differentiation of the pluripotential hematopoietic stem cell is well established (1-3, 12, 21, 24-26, 28, 50). Antigenic determinants have been described specific for the B-lymphocyte (25, 28), T-lymphocyte (3, 50, 52), monocyte-macrophage (21, 22), or granulocyte pathway (21). Some antigenic determinants are shared between cells committed to a single lineage and CFU-GEMM\(^{4}\) hematopoietic progenitor cells (21, 22). Other antigenic determinants may be associated with terminally differentiated cells of the neutrophile, granulocyte, eosinophile, or basophile mast cell lineage (2, 22).

The ease of correlating the distribution of specific antigenic determinants with the diverse hematopoietic cell types in the marrow has been aided by use of specific functional (34) and histochemical (18) assays. Since leukemias and lymphomas can express cell surface antigens that are not found on the majority of normal progenitor cells (1, 3, 7, 21, 22, 24-26, 28, 36, 50, 52) antibodies have been used to eliminate malignant cells from human marrow (3, 50). Monoclonal antibody reagents (3, 12, 36, 50) have made available adequate quantities of reagent for treating marrow in vitro prior to use in autologous marrow transplantation (5, 29).

In contrast, the nonhematopoietic cellular elements of the bone marrow (6, 35, 43, 49, 54), have not been as well characterized. There is uncertainty regarding the number of such cell lineages that are morphologically identified as fibroblasts, preadipocytes, reticular adventitial cells, endothelial cells, sinus-lining cells, and macrophages (6-8, 10, 13, 14, 16, 20, 27, 35, 43, 46, 49, 54). Furthermore, there are no generally accepted restrictive criteria for defining each nonhematopoietic cell category (6-8, 10, 13, 14, 16, 20, 27, 35, 43, 46, 49, 54). Controversy exists concerning the existence of a common progenitor cell for the pluripotential hematopoietic stem cell and the nonhematopoietic lineages of endothelial cell and bone marrow fibroblast (45).

Autologous marrow transplantation is now an accepted modality in the treatment of hematopoietic cancers (3, 41) and some solid tumors (5, 48). Immunological removal of tumor cells by incubation of marrow in vitro with monoclonal antibodies and complement has been followed by reinfusion of marrow after administration of cytotoxic chemotherapeutic and/or total body irradiation (3). Successful transplant depends on the redistribution of engrafted marrow in vivo, proliferation of these cells, and their appropriate differentiation. Current monoclonal antibody and complement reagents are tested for killing of hematopoietic stem cells by assays for GM-CFUc, BFUe, and CFU-GEMM. Since the most primitive analogue of the pluripotential stem cell (CFUs) that can be tested in vitro with human marrow is the CFU-GEMM (9), a monoclonal antibody and complement treatment that kills

---

1 Supported by NIH Research Grants CA24512, CA12682, and CA28740 and United States Department of Defense Contract DMD-17-82-C-2207.

2 Present address: Dept. of Radiation Oncology, University of Massachusetts Medical School, 55 Lake Ave. N., Worcester, MA 01605. To whom requests for reprints should be addressed.

3 Scholar of the Leukemia Society of America.

4 The abbreviations used are: CFU-GEMM, multipotential erythroid-granulocyte-megakaryocyte-macrophage colony-forming unit (hematopoietic progenitor cell); B2M, \( \beta_2 \)-microglobulin; CALLA, common acute lymphoblastic leukemia antigen; GM-CFUc, granulocyte-macrophage colony-forming unit; BFUe, blast-forming unit (erythroid); CFU-F, fibroblast colonies; CFUs, pluripotential stem cell; CFU-mega, megakaryocyte colonies.

Received March 5, 1984; accepted October 23, 1984.

CANCER RESEARCH VOL. 45 FEBRUARY 1985

758
tumor cells (e.g., Burkitt's lymphoma cells) but spares CFU-GEMM (and perhaps also BFUe and GM-CFUc) is considered to be safe for clinical use.

Recent data from allogeneic transplant protocols have provided reason for renewed concern. These data indicate that nonhematopoietic as well as hematopoietic cells of the donor marrow may engraft the recipient (27, 46). Whether donor nonhematopoietic marrow cell engraftment is clinically relevant is unknown. As one approach toward understanding the effects of monoclonal antibody and complement treatment of human marrow, monoclonal antibody and complement reagents were tested for effects on the establishment of long-term bone marrow cultures that normally generate hematopoietic progenitor cells and granulocytes in vitro for several months.

MATERIALS AND METHODS

Monoclonal Antibody Reagents. Murine monoclonal antibodies tested included reagents directed against human lymphocyte antigens: J5 (CALLA) (26, 41); J2 (23); and B1 (42). A monoclonal antibody prepared against the nonpolymorphic region of the a chain of human la, HLA-DR determinant, has been described previously (33). A monoclonal antibody reagent directed against B2M has also been described (33). The spectrum of cells expressing each antigenic determinant is shown in Table 1.

Treatment of Marrow with Antibody and Complement. Single-cell suspensions of freshly removed intraoperative hip marrow were prepared for long-term bone marrow culture according to published procedures (13). Briefly, single-cell suspensions were freed of bone spicules and particulate matter by passage through a sieve. Suspensions were drawn through successively smaller-gauge needles to a 30-gauge needle in McCoy's Medium 5A supplemented according to published procedures (13) and containing 12.5% fetal calf serum and 12.5% horse serum (10, 13). The marrow was then standardized to 10^8 to 10^9 cells in 50 ml serum-free medium, washed by centrifugation, and then treated in a 5-ml volume with each monoclonal antibody (1:100 dilution). Antibody treatments were carried out at 4°C for 30 min. Following each antibody treatment, marrow preparations containing 10^6 to 10^8 cells per 5 ml were then washed in serum-free medium by centrifugation, and the cells were resuspended in 5.0 ml of rabbit complement at a 1:5 dilution at 37° for 45 min. The bone marrow preparations were then washed again and either transferred to 40-cm plastic tissue culture flasks at 2 to 4 x 10^7 cells/flask in 8.0 ml complete medium, containing McCoy's Medium 5A, supplemented as described previously (13); 12.5% horse serum, 12.5% fetal calf serum, and, in all experiments, 10^{-6} M hydrocortisone were added freshly biweekly (17, 44).

RBC were removed by first allowing all cultures to remain undisturbed for 4 to 5 days, followed by removal of all nonadherent cells, ficoll-Hypaque density gradient centrifugation to remove RBC, and then return of the washed WBC fraction entirely to the culture flasks in fresh medium. This 5-day delay has been shown to be critical in facilitation of attachment of the hematopoietic stem cell islands that contain the most primitive hematopoietic cells (13).

Cultures were fed by removal of all nonadherent cells and medium, centrifugation of this medium to remove the cells for specific assays, and then refeeding of the cultures with an equivalent volume of fresh medium containing fresh hydrocortisone. Cultures were fed biweekly unless otherwise specified. Cultures were maintained at 33°C in a high-humidity incubator with 5% CO2.

Assays for Specific Hematopoietic Cell Lineages. Nonadherent cells removed from the cultures biweekly were counted by hemocytometer and transferred to assays of multipotential progenitor cells, including CFU-GEMM (9), BFUe (9), CFU-mega (9), and GM-CFUc (9, 13). For the GM-CFUc assay, the source of colony-stimulating factor included phytohemagglutinin-lymphocyte-conditioned medium prepared according to published methods (9), added in 10% volume, and 10% conditioned medium from the Mo cell line generously provided by Dr. David Golde, UCLA Medical School (11). The use of both sources of conditioned medium simultaneously allowed detection of the greatest number of granulocyte colony-forming cells in a harvest, compared to the results using Mo-cell-conditioned medium or phytohemagglutinin-lymphocyte-conditioned medium alone.

Hematological and Histological Staining. Nonadherent cells removed from long-term bone marrow cultures were stained with Wright's-Giemsa, and differential cell counts were performed according to published methods (13). Other cell smears were tested for superoxide-generating capacity by the nitroblue-tetrazolium dye reduction test (13, 18) or were tested for specific myeloid esterase (ASD-chloroacetate substrate specific) (18), nonspecific esterase (a-naphthol esterase substrate) (18, 37), myeloperoxidase (18), lysozyme (18), toluidine blue metachromasia of basophil-mast cells (19), and benzidine stain for hemoglobin (19). Hematopoietic colonies forming in agar were stained directly in the combined method for specific and nonspecific esterase, and Luxol fast blue for eosinophils (37).

In some experiments, nonadherent cells from long-term marrow cultures were tested for antigen associated with the differentiating T-
lymphocyte pathway. For these studies, the phenotype of nonadherent cells was determined by indirect immunofluorescence and flow cytometry. The percentage of cells which reacted with monoclonal antibody T4 or T8 was calculated according to published methods (23, 41, 42).

**Assays for Nonhematopoietic Cellular Components of Long-Term Bone Marrow Cultures.** Culture flasks were examined weekly for (a) the percentage of cell surface area covered by adherent cells (13), (b) the number of fat-containing adipocytes per flask (15), and (c) also the number of adipocytes per high-power field (15). Freshly treated cells prior to establishment of the above marrow cultures in several experiments were seeded onto plastic Petri dishes in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Fibroblast colonies derived from single cells were scored 7 and 14 days later (16).

The number of “cobblestone” areas (10, 13, 20) containing ≥50 cells detected per culture flask was scored each week. These areas have been associated with the adherent component of hematopoiesis including those stem cells with a high self-renewal capacity in mouse long-term marrow cultures (30) and with those hematopoietic cells that form colonies with the greatest in vitro self-renewal following replating to second-assay cultures (7).

**RESULTS**

**Effects of Monoclonal Antibody Treatment on Hematopoiesis in Long-Term Bone Marrow Cultures.** Individual marrow specimens were treated with each monoclonal reagent using a protocol identical to that used for clinical autologous marrow transplantation as described under “Materials and Methods.”

Weekly and cumulative production of nonadherent cells, weekly and cumulative production of both 7- and 14-day GM-CFUc, and the morphological appearance of the adherent cell compartment were quantitated for each experiment. In some experiments, BFUe and CFU-GEMM were quantified. A summary of the results in a representative experiment with each monoclonal antibody is shown in Table 2. Treatment with antibody to B1, J5, or J2 did not significantly decrease CFU-GEMM, BFUe, CFU-mega, or GM-CFUc from marrow. Long-term bone marrow cultures established from these marrow samples produced CFU-GEMM, BFUe, CFU-mega, and GM-CFUc in vitro for several weeks (Table 2). Treatment of marrow with anti-la plus complement significantly reduced CFU-GEMM, BFUe, CFU-mega, and GM-CFUc, while few GM cluster-forming cells survived (Table 2). In long-term marrow culture, however, this marrow reconstituted in vitro and generated CFU-GEMM, BFUe, CFU-mega, and GM-CFUc over the next 2 to 4 weeks (Table 2). In contrast, marrow treated with antibody to B2M was devoid of detectable hematopoietic progenitor cells and did not produce any detectable progenitor cells in vitro in the next 4 weeks (Table 2).

Long-term marrow cultures were next set up with marrow that had been treated with each monoclonal antibody and complement and studied in greater detail. In one study, marrow was treated with antibody to B1 alone, B1 + complement, J5 alone, J5 + complement, or complement alone. Several of the treatments reduced after 30 days the weekly production of nonadherent cells when compared to untreated marrow (Chart 1A). There was, however, no detectable decrease in cumulative nonadherent cell production compared to control untreated marrow (Chart 1B). There was also no detectable effect on cumulative production of GM-CFUc scored as 7-day GM-CFUc colonies (Chart 2A) or 14-day GM-CFUc colonies (Chart 2B). These data with B1 and J5 confirm the data in Table 2.

In contrast, in a second experiment with another marrow specimen, treatment with monoclonal antibody to B2M and complement resulted in rapid and significant decrease in the weekly (Chart 3A) and cumulative (Chart 3B) production of nonadherent cells. The decrease was detected within 2 weeks after establishment of the cultures. Treatment with anti-B2M alone or complement alone did not alter cell production. As another control for this experiment, culture of marrow treated with antibody to B1 + complement showed no decrease in cell production (Chart 3), confirming the data in Charts 1 and 2 by a second experiment. Anti-B2M + complement treatment significantly decreased production of GM-CFUc scored as Day 7 colonies (Chart 4A) or Day 14 colonies (Chart 4B). Control cultures or anti-B1 + complement-treated cultures produced GM-CFUc for over 8 weeks (Chart 4).

**Effect of Monoclonal Antibody and Complement Treatment on CFU-F Adipocyte Colony Formation, and Adherent Cell Layer Function by Human Bone Marrow Cells in Vitro.**

**Table 2**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Immediately posttreatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Colonies/2 × 10&lt;sup&gt;6&lt;/sup&gt; nonadherent cells after long-term bone marrow culture&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU-GEMM/10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>BFUe/10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>CFU-mega/10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Anti-B1 + complement</td>
<td>3</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>J5 + complement</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Anti-la + complement</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>None</td>
<td>4</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>J2 + complement</td>
<td>3</td>
<td>NT</td>
</tr>
<tr>
<td>17</td>
<td>None</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Anti-B2M + complement</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> GM-CFUc scored on Days 7 and 14 (results presented for Day 14) (13). BFUe, CFU-mega, and CFU-GEMM scored on Day 14 (9). Nonadherent cells from long-term bone marrow cultures (13).

<sup>b</sup> Numbers in parentheses, week tested.

<sup>c</sup> NT, not tested.
HEMATOPOIETIC EFFECTS OF MONOCLONAL ANTIBODIES

Chart 1. A, weekly production of nonadherent cells by flasks of a single specimen of marrow prepared as described under "Materials and Methods" and treated with monoclonal antibody to B1, anti-B1 + complement (C'), complement alone, anti-J5 alone, anti-J5 + complement, or untreated control. Results are the mean nonadherent cell harvest per flask for at least 4 flasks per group, standardized to 4 x 10^7 cells per flask after treatment. All treatments were one cycle. B, cumulative nonadherent cell production of experiment shown in A over first 85 days of experiment. Results are as described in legend to A.

Analysis was next made of the effect of each monoclonal antibody and complement treatment on the number of ≥10 cell adipocyte colonies in the adherent layer of the cultures at 4, 8, and 12 weeks; the percentage of surface area of the flask covered by adherent cells at 4, 8, and 12 weeks; and the formation of adherent fibroblast-like colonies of ≥50 cells by serial dilutions of single-cell marrow suspensions at 1 x 10^6, 1 x 10^5, or 1 x 10^4 cells/4.0-ml Petri dish scoring at Days 7 or 14.

In control cultures, we scored 50 ± 7 adipocyte colonies at Week 4, 73 ± 9 at Week 8, and 115 ± 18 at Week 12 (mean ± S.E. of at least 3 flasks at each point). Cultures derived from marrow treated with antibody to B1, J5, J2, or Ia with or without complement treatment showed no detectable decrease in this parameter. In contrast, cultures derived from marrow treated with anti-B2M + complement (but not B2M alone) showed 3 ± 1 adipocyte colonies/flask at 4 weeks and no detectable adipocyte colonies at Week 8 or 12, a significant decrease.

In control cultures, we scored 7 ± 1 CFU-F/10^6 cells plated to Petri dishes in 4.0 ml complete medium, scoring at 7 days; and 18 ± 3 colonies/dish scoring at Day 14. Only colonies of ≥50 cells/colony were counted. In contrast, cultures derived from marrow treated with anti-B2M + complement showed a detectable decrease at Day 7 (3 ± 1 per 10^6 cells plated) and Day 14 (1, 0, 0 colonies detected in triplicate plates).

The percentage of surface area covered by adherent cells was 100% at 4 weeks for control and all monoclonal antibody groups except those treated with anti-B2M + complement. Here, 8% of the surface was covered.

Effect of Monoclonal Antibody and Complement Treatment on "Cobblestone Island" Formation by Human Marrow in Continuous Marrow Cultures. The adherent cell layer of cultures derived from marrow treated with antibody to B2M and complement showed significant decrease in "cobblestone" areas by 6 weeks (<1/flask; control, 50 to 63/flask) and no detectable production of hematopoietic cells past 6 weeks. In other monoclonal antibody-treated cultures with or without complement treatment, there was no decrease compared to control cultures in the number of "cobblestone" areas and cultures continued to

CANCER RESEARCH VOL. 45 FEBRUARY 1985
761

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 1985 American Association for Cancer Research.
HEMATOPOIETIC EFFECTS OF MONOCLONAL ANTIBODIES

Chart 3. A, weekly production of nonadherent cells from flasks of a single specimen of marrow prepared as described under "Materials and Methods" treated with monoclonal antibody to B1 + complement (C'), complement alone, antibody to B2M alone, or anti-B2M + complement. Results are presented as described in the legend to Chart 1A. In this experiment, there were 2 x 10^7 cells/flask after treatment. The line for O stops early due to the absence of detectable cells in the flask at that time. B, cumulative nonadherent cell production for experiment shown in A over first 58 days of experiment. Results are as described in the legend to Chart 1B.

Charts. A, weekly production of nonadherent cells from flasks of a single specimen of marrow prepared as described under "Materials and Methods" treated with monoclonal antibody to B1 + complement (C'), complement alone, antibody to B2M alone, or anti-B2M + complement. Results are presented as described in the legend to Chart 1A. In this experiment, there were 2 x 10^7 cells/flask after treatment. The line for O stops early due to the absence of detectable cells in the flask at that time. B, cumulative nonadherent cell production for experiment shown in A over first 58 days of experiment. Results are as described in the legend to Chart 1B.

produce over 10^4 cells/flask after 6 weeks (Charts 3 and 4). No significant toxic effect on cumulative cell production or GM-CFUc production was detected using one cycle treatment with antibody to J2 (Chart 5) or HLA-DR (la) (Chart 6) (Table 2).

Effects on Hematopoiesis in Long-Term Marrow Cultures of Multiple Cycles of Treatment with One Monoclonal Antibody or Sequential Treatment Cycles with Different Monoclonal Antibodies. To determine whether sequential treatment with 3 different monoclonal antibodies would produce a toxic effect on production of hematopoietic stem cells in long-term marrow cultures that was not observed using one antibody alone, a single specimen of >10^10 human marrow cells was treated sequentially with each of 3 monoclonal antibodies and complement (B1, J2, and J5) prior to establishment of long-term marrow cultures. As shown in Table 3, this treatment did not detectably depress cumulative cell production or GM-CFUc production by cultures initiated with 2.5 x 10^7 nucleated cells/flask adjusted at the last treatment compared to control cultures. The lack of toxicity was comparable to that of cultures treated with one monoclonal antibody and complement cycle as shown above. These data indicate that 3 treatments are not necessarily more toxic than one treatment using monoclonal antibodies to antigens that are not on normal hematopoietic stem cells.

To determine whether 3 treatments with a monoclonal antibody directed toward an antigen that is present on normal hematopoietic stem cells would have a greater toxic effect compared to one treatment, several experiments were carried out with monoclonal antibody to HLA-DR (la). First, 3 successive treatments with monoclonal antibody and complement were carried out, and the cultures were established. As shown in Table 3, there were no detectable GM-CFUc surviving this treatment regimen compared to some survival of GM cluster-forming...
HEMATOPOIETIC EFFECTS OF MONOCLONAL ANTIBODIES

Charts A and B show the weekly production of nonadherent cells by flasks of a single specimen of human marrow prepared as described under "Materials and Methods," treated with monoclonal antibody to J2 + complement (C'), complement alone, or untreated control. Results are presented as described in the legend to Chart 1A. Here, control cultures had 5 x 10^7 cells while anti-J2 + complement-treated cultures started with 3 x 10^7 cells. B, cumulative nonadherent cell production per flask by the cell harvest shown in A over the first 85 days of the experiment. Results are as described in the legend to Chart 1B. Control cultures had twice as many starting cells in this experiment; therefore, the line comparison is between A and B.

Nonadherent cells after one cycle of treatment (Tables 2 and 4). Thus, a 3-cycle treatment was more effective at killing GM cluster-forming cells than was one treatment cycle. The inoculum used to establish the cultures after 3 successive monoclonal antibody treatments was standardized to 2.5 x 10^7 nucleated cells/flask and contained no detectable GM-CFUc (Tables 3 and 4). Nonadherent cells removed after 1 week from long-term marrow cultures established from this marrow contained 3 to 5 per 10^5 GM-CFUc and 14 to 15 GM cluster-forming cells per 10^5 cells assayed (Table 3). At 2 weeks, these GM-CFUc were inhibited by antibody to la (Table 3). The appearance of the adherent compartment in anti-la-treated cultures was quantitated weekly. As shown in Fig. 1, numerous cobblestone areas (50 to 76/flask) were scored at Week 8 in cultures derived from marrow that was treated with 3 sequential cycles of antibody to la and complement in the experiment in Table 3. These data establish that the cells forming cobblestone areas were not removed by up to 3 cycles of anti-HLA-DR monoclonal antibody and complement treatment. Cell proliferation continued in these cultures although at a decreased level compared to untreated controls (Chart 7; Fig. 1). In 2 separate experiments (Tables 3 and 4), there was a cumulative decrease in GM-CFUc production and total cell production in long-term marrow cultures following 3 cycles of treatment with monoclonal antibody to la and complement.

DISCUSSION

Monoclonal antibody reagents currently used to prepare for autologous transplant of marrow from patients with CALLA-positive acute lymphoblastic leukemia (42), T-cell leukemia (50), or myeloid leukemia (2) have been tested in assays for killing of normal multipotential stem cells (CFU-GEMM) and committed hematopoietic progenitor cells BFUe and GM-CFUc (9, 21). Monoclonal antibodies prepared for specificity against lymphocyte-specific lineage cells have usually been found to spare cells forming CFU-GEMM and GM-CFUc in vitro (21). Thus, the successful engraftment and tumor cell reduction in several patients whose whole marrow was purged with J5 + complement might be solely attributable to selective sparing of the donor stem cells that successfully seeded the host.

The role if any in vivo of donor marrow nonhematopoietic cells that are known to facilitate self-renewal of hematopoietic progenitors in vitro (13) is not well understood. Recent data show a donor cell origin of some fibroblastic cells that appear in long-term bone marrow cultures derived from the marrow of successfully engrafted allogeneic transplant recipients (27, 46). These data have stressed the need to understand the possible phys-
HEMATOPOIETIC EFFECTS OF MONOCLONAL ANTIBODIES

Table 3
Effect of repeat cycles of treatment with one monoclonal antibody and complement or sequential cycles of different monoclonal antibody treatment on longevity of hematopoiesis in long-term marrow cultures

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment with</th>
<th>Initial posttreatment marrow GM-CFUc/GM cluster/2 × 10⁶ cells (Day 14)</th>
<th>hematopoietic longevity</th>
<th>Cumulative NA GM-CFUc/flask</th>
<th>Wk production of &gt;10⁴ NA cells/flask</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Nothing</td>
<td>J₂ + complement, J₅ + complement, anti-B₁ + complement (sequential)</td>
<td>98 ± 6/31 ± 2²</td>
<td>GM-CFUc/GM cluster/1 × 10⁶ (Day 14)</td>
<td>481 ± 6/23 ± 2</td>
<td>1407 ± 58/420 ± 2</td>
</tr>
<tr>
<td>2 Nothing</td>
<td>Anti-la + complement (3 cycles)</td>
<td>71 ± 6/8/18 ± 3</td>
<td>Anti-la + complement (1 cycle) after harvest from long-term bone marrow cultures</td>
<td>371 ± 6/4/18 ± 3</td>
<td>5210 ± 420 ± 2</td>
</tr>
</tbody>
</table>

* NA, nonadherent; NT, not tested.
+ Mean ± S.E.
+ Also <1 CFU-GEMM/2 × 10⁶; <1 CFU mega/2 × 10⁶; and <1 BFUe/2 × 10⁶.

The biological role of nonhematopoietic cells in the donor marrow. There are as yet no specific markers for identifying each of the morphological categories of these nonhematopoietic cells of the marrow.

In the present studies, we have quantified the ability of marrow to establish a functioning long-term bone marrow culture (13) after treatment with monoclonal antibody and complement. There was no detectable toxicity of monoclonal antibodies B₁, J₂, or J₅ alone or in combination to the establishment of the long-term marrow culture system. In this regard, CALLA has been found on cultured marrow fibroblasts in vitro (4); however, no CALLA was detected by immunoperoxidase staining of freshly removed human hematopoietic tissues (31). Thus, CALLA may be expressed during tissue culture on some nonhematopoietic cells that normally do not express this antigen in situ. Our data showing no detectable damage to long-term marrow cultures by prior J₅ treatment support clinical transplant data where J₅ treatment of marrow resulted in successful engraftment (42). In contrast, anti-B₂M and complement treatment was severely toxic to the establishment of long-term bone marrow cultures as evidenced by rapid decline of hematopoiesis in culture flasks standardized to control cultures for a similar number of viable cells posttreatment. The mechanism of the toxicity is not known but may be attributable to death of nonhematopoietic cells in addition to hematopoietic progenitor cells, since both populations were reduced as measured by colony assays.

A monoclonal antibody that is selectively toxic for nonhematopoietic cells was not available for the present studies and will be helpful if available in the future. The data with anti-HLA-DR (anti-la) and complement-treated marrow are of interest with regard to a previously published report claiming that human pluripotent stem cells do not contain (la) HLA-DR (32). The previous study with a 2-step culture system established long-term marrow cultures using first an inoculum of untreated marrow and then a "recharged" addition to the flasks 3 to 4 weeks later of a second inoculum of anti-la and complement-treated marrow that was free of detectable GM-CFUc or BFUe (32). Since the anti-la and complement-treated cultures generated GM-CFUc and BFUe with detectable surface la, it was concluded that primitive human multipotent stem cells had escaped antibody and complement killing and therefore were la negative (32).

It is now known that the first inoculum is itself a vast reserve of

CANCER RESEARCH VOL. 45 FEBRUARY 1985

764
Hematopoietic Effects of Monoclonal Antibodies

In the present studies, one cycle of la + complement treatment did not remove all GM cluster-forming cells, while 3 cycles of treatment that did remove all proliferative hematopoietic cells resulted in a significantly decreased cumulative hematopoietic cell production in long-term culture. Whether the decrease in hematopoiesis in vitro after 3 cycles is attributable to selective removal of other HLA-DR-positive nonhematopoietic cells such as endothelial cells (38) or to efficient removal of enough CFU-GEMM and possibly the human pluripotential progenitors is not yet known. Three cycles of treatment itself did not account for the toxicity since cultures from marrow treated with J5, J2, and anti-B1 in succession were not detectably altered. The return of la-positive GM-CFUc in long-term culture after 3 cycles of treatment with anti-la + complement suggests that la-positive cells that form GM-CFUc may evolve from la-negative treated marrow in culture. However, the possibility exists that the data are explained by: (a) very small numbers of residual la-positive true human CFUs which can repopulate the whole in vitro system (2, 40); or (b) that human la-positive CFUs could be protected from anti-la and complement killing due to residence within a nonhematopoietic protective cell membrane that is itself la negative. Adventitial reticular cells that line the marrow sinuses could be such protective cells and could function as do the murine thymic “nurse” cells described by Werkele (53) and recently identified in human thymus (51). Further studies will be required to resolve these possibilities.

ACKNOWLEDGMENTS

We thank Thomas Novak for technical assistance; Drs. Thomas Thornhill, Clement Sledge, William Thomas, and Frederick Ewald for obtaining marrow specimens; and Briana Walker for typing the manuscript.

REFERENCES

HEMATOPOIETIC EFFECTS OF MONOCLONAL ANTIBODIES

Fig. 1. Morphological appearance of “cobblestone” hematopoietic islands among a flask derived from marrow that was treated with 3 cycles of anti-la + complement. A, 8 weeks in culture, B, 12 weeks in culture. Arrows, cobblestone areas. × 1000.

CANCER RESEARCH VOL. 45 FEBRUARY 1985

766
Effects of Monoclonal Antibody and Complement Treatment of Human Marrow on Hematopoiesis in Continuous Bone Marrow Culture

Joel S. Greenberger, Lisa Rothstein, Paolo DeFabritiis, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/2/758

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.