Elimination of Clonogenic Burkitt's Lymphoma Cells From Human Bone Marrow Using 4-Hydroperoxycyclophosphamide in Combination With Monoclonal Antibodies and Complement

By P. De Fabritiis, M. Bregni, J. Lipton, J. Greenberger, L. Nadler, L. Rothstein, M. Korbling, J. Ritz, and R.C. Bast, Jr

One requirement for autologous bone marrow transplantation is the selective removal of malignant cells from normal marrow precursors. Development of a clonogenic assay that detects elimination of up to 5 logs of Burkitt's lymphoma cells in the presence of a 20-fold excess of human bone marrow has permitted the evaluation of two different methods for the selective removal of malignant cells. Treatment with 4-hydroperoxycyclophosphamide (4-HC) (60 to 100 μg/mL) eliminated 2.0 to 3.5 logs of clonogenic cells. Antitumor activity depended upon the concentration of 4-HC and the length of incubation, but not upon the concentration of normal bone marrow cells. Comparable removal of clonogenic Burkitt's cells was achieved by treatment with rabbit complement (C') and a combination of J5 anti-common acute lymphoblastic leukemia antigen (J5 anti-CALLA), J2 anti-gp 26, and the B1 anti-B1 murine monoclonal antibodies. A combination of 4-HC and monoclonal antibodies proved slightly but significantly more effective than either single agent in eliminating clonogenic tumor cells. Although treatment with 4-HC markedly reduced granulocyte-macrophage colony-forming units-C (GM-CFU-C) content of human bone marrow, neither treatment with 4-HC nor treatment with monoclonal antibodies and C' eliminated precursor cells that could generate new GM-CFU-C after growth in continuous bone marrow cultures. Our data suggest that treatment with 4-HC in combination with multiple monoclonal antibody reagents could be a safe and effective method of eliminating clonogenic tumor cells from human bone marrow.

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ONE OF THE MAJOR obstacles to effective autologous bone marrow transplantation in acute leukemia is the continued presence of tumor cells in remission marrow. To achieve results comparable to those obtained with syngeneic or allogeneic bone marrow grafts, autologous marrow must be cleansed of contaminating tumor cells without destroying hematopoietic stem cells. Previous studies in animal models have indicated that several logs of tumor cells can be eliminated from syngeneic bone marrow using polyclonal heteroantiserum and C'. Recently, monoclonal reagents have been applied to the elimination of tumor cells from human bone marrow. Autologous bone marrow transplantation has been undertaken in patients with acute lymphoblastic leukemia using marrow treated with the monoclonal anti-common acute lymphoblastic leukemia antigen (anti-CALLA) antibody (J5) and rabbit C'. Development of a clonogenic assay that measures elimination of Burkitt's lymphoma cells from an excess of irradiated human bone marrow has permitted the definition of requirements for removing more than 2 logs of malignant cells. Multiple treatments with monoclonal antibodies and C' have proven more effective than a single treatment and treatment with multiple antibodies has proven more effective than treatment with a single reagent. In addition, elimination of malignant cells has depended upon the concentration of normal bone marrow cells in the reaction mixture.

Alternative methods have been proposed for eliminating malignant cells from human bone marrow that do not depend upon antigenic differences between tumor cells and normal hematopoietic progenitors. The activated cyclophosphamide intermediate, 4-hydroperoxycyclophosphamide (4-HC) is able to eliminate nonlymphocytic leukemia cells from rat bone marrow in vitro without destroying the ability of the marrow to reconstitute the lethally irradiated host. In a phase I clinical study, patients have experienced successful reconstitution following ablative therapy when given autologous marrow that has been treated with 40 to 100 μg/mL of 4-HC. Reconstitution occurred despite the destruction of all measurable granulocyte-macrophage colony-forming units-C (GM-CFU-C). From studies performed to date, it has not been possible to compare the relative ability of 4-HC and monoclonal antibody treatment to eliminate malignant cells from human bone marrow.

In this paper, we have defined concentrations of 4-HC and lengths of incubation that are required to eliminate clonogenic Burkitt's lymphoma cells from an...
excess of irradiated human bone marrow. Multiple monoclonal antibodies and C' have been used to remove malignant cells from the same system. Both techniques have proven effective and a combination of these agents has resulted in more complete elimination of clonogenic Burkitt's lymphoma from human bone marrow than could be attained with either single treatment, under conditions that do not damage early hematopoietic progenitor cells or cells comprising the hematopoietic microenvironment.

MATERIALS AND METHODS

Lymphoma Cell Lines

The Namalwa, CA 46 and BJAB, BJAB 113, and JD-38 cell lines were kindly provided by Dr I. Magrath. Cells were cultured at 37 °C in an atmosphere of 5% CO2 and 95% humidified air. Cultures were prepared in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), 1 mmol/L of sodium pyruvate, 2 mmol/L of L-glutamine, 10 mmol/L of N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES), 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cultures were split 18 to 24 hours prior to each assay to assure that cells were proliferating optimally.

Bone Marrow

Using protocols approved by the Dana-Farber Cancer Institute and Children's Hospital Medical Center, bone marrow was obtained from healthy voluntary and anticoagulated with preservative-free heparin (Pan Heparin; Abbott Laboratories, N Chicago). Anticoagulated marrow was diluted 15 times with Hanks' balanced salt solution (HBSS) without calcium or magnesium (MA Bioproducts, Walkersville, Md) and layered over Ficoll-diatrizoate lymphocyte separation medium (Bionetics Laboratory Products, Kensington, Md). After centrifugation at 1,300 g for 15 minutes at 20 °C, mononuclear cells were obtained from the gradient interface and washed twice in HBSS. After an additional wash in Earle's minimal essential medium (MEM) (MA Bioproducts) containing Earle's balanced salt solution, 2 mmol/L of L-glutamine, 10 mmol/L of HEPES buffer, and 5% heat-inactivated FBS, cells were irradiated (5,000 rad). Irradiated human bone marrow cells were used both to prepare feeder layers in serial dilution assays and to prepare mixtures with nonirradiated Burkitt's lymphoma cells prior to treatment with monoclonal antibodies and/or 4-HC. Nonirradiated marrow was used in studies of GM-CFU-C and in continuous bone marrow cultures.

4-HC

Stabilized 4-HC (Astawerke, Bielefeld, FRG AZ7557) was diluted to appropriate concentrations with MEM supplemented with 5% FBS. Solutions containing 4-HC were passed through Nalgene filters (0.2 μm) prior to their addition to mixtures containing Burkitt's cells and human bone marrow. Unless otherwise specified, all the microtiter plates were plated at 80% confluence and cultured for 30 minutes at 37 °C, with frequent agitation. After incubation, cell mixtures were washed twice with MEM supplemented with 5% FBS.

Monoclonal Antibodies

All the monoclonal reagents utilized in these studies have been described previously.3 J5 is an IgG3 antibody that binds to the common acute lymphoblastic leukemia antigen (CALLA). CALLA is expressed by cells from 80% of patients with non-T cell ALL and from 30% to 50% of patients with chronic myelogenous leukemia (CML) in blast crisis. In addition, CALLA is expressed in malignant cells from approximately 9% of patients with T cell ALL. CALLA is also associated with 40% of lymphoblastic lymphomas and almost all Burkitt's and nodular poorly differentiated lymphocytic lymphomas. CALLA is not detected on GM-CFU-C, BFU-E, or bipotent granulocytic colonies in normal bone marrow.14 J2 is an IgM immunoglobulin that reacts with a 26,000-dalton cell-surface glycoprotein, expressed on tumor cells from patients with ALL and various types of lymphomas. The B1 antibody reacts with a 35,000-dalton glycoprotein found on normal B cells in peripheral blood and lymph nodes. B1 is found on the surface of Burkitt's tumor cells and almost all other B cell neoplasms. As a control, J0 has been obtained from mice inoculated with a hybridoma that produced IgG3, that did not react with CALLA, gp 26, or B1.

Rabbit C'

Rabbit C' was obtained from Pelfreeze Inc (Glen Rock, NJ) and absorbed once for one hour at 0 °C with an equal volume of human AB erythrocytes that had been washed with 0.1 mol/L of phosphate-buffered saline (PBS) pH 7.0. After absorption, C' was stored at −80 °C until use.

Treatment of Marrow Mixtures With Antibody and C'

Procedures for treatment of bone marrow with antibody and C' have been described.1 In brief, 106 Burkitt's lymphoma cells have been mixed with 2 × 106 irradiated bone marrow cells in 1 mL of MEM with 5% FBS prior to incubation with monoclonal antibodies (each at a dilution of 1:100) for 15 minutes at 4 °C. Absorbed rabbit C' was then added to a final concentration of 1:10 and the mixtures were warmed to 37 °C for 30 minutes. After incubation with C', cells were pelleted, and the procedure was repeated for a total of three cycles.

Serial Dilution Assay

The limiting dilution assay has also been described.2 After treatment with 4-HC (one cycle) and/or antibodies and C' (three cycles), serial fivefold dilutions were prepared in RPMI 1640 with 10% FBS supplemented as described above. Aliquots (100 μL) of each dilution were plated in flat-bottomed microtiter wells (Flow Laboratories, McLean, Va) that had previously been seeded with 106 irradiated human bone marrow cells in a volume of 100 μL of clonogenic medium. Microtiter plates were incubated for 14 days at 37 °C in 5% CO2 with 95% humidified air. Wells with progressively growing tumor colonies were scored with the unaided eye and their morphology was confirmed by examination under an inverted-phase microscope. In previous studies, excellent correlation had been obtained between visual scoring and uptake of [3H]-thymidine by tumor colonies. The number of clonogenic units remaining in 106 treated tumor cells was calculated with a Spearman-Karber estimator.9 Statistical analysis was performed as previously described.

Continuous Bone Marrow Cultures

The methods for human long-term bone marrow culture have been published previously.10 In brief, 2 to 4 × 106 mononuclear cells from human bone marrow were resuspended in 9 mL of McCoy's 5A medium containing 12.5% horse serum, 12.5% FBS, 5 mmol/L of NaHCO3, 0.8% essential aminoacid mixture (MA Bioproducts), 0.4% nonessential aminoacid mixture (MA Bioproducts), 1 mmol/L of sodium pyruvate, 2 mmol/L of L-glutamine, 0.8 mmol/L of...
serine, 0.15 mmol/L of L-asparagine, 0.4% MEM vitamin solution (MA Bioproducts), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10^{-5} mol/L of hydrocortisone. Cultures were grown at 33 °C in 3% CO₂ - 97% humidified air. For each medium change, all nonadherent cells were removed and centrifuged at 1,000 g for five minutes. Half of the total cells were retained for study, and the other cells were returned to the flask in 9 mL of fresh medium. Nonadherent cells were counted weekly, and after 40 to 50 days, cumulative cell production was obtained by the weekly counts and GM-CFU-C was assayed according to published methods. Aliquots of cells at 0, 7, and 14 days were used for colony-forming cell assays, as described.

RESULTS

Optimal Concentration of 4-HC

Mixtures of Namalwa, BJAB 113, or CA 46 were prepared with a 20-fold excess of irradiated human bone marrow. These mixtures were then incubated with different concentrations of 4-HC for 30 minutes at 37 °C. Between 2 and 3 logs of tumor cells were eliminated by incubation with 60 μg/mL 4-HC (Fig 1). Slightly greater killing was observed with 80 to 100 μg/mL of 4-HC in some experiments, but there generally was very little difference over the range of 60 to 100 μg/mL. Incubation with 600 to 1,000 μg/mL of 4-HC completely eliminated clonogenic growth (data not shown).

Optimal Duration of 4-HC Treatment

With a 4-HC concentration of 60 μg/mL, mixtures of Namalwa and irradiated human bone marrow were treated for 0 to 120 minutes. Optimal lysis of Burkitt’s tumor cells was observed after 30 minutes with very little additional tumor cell elimination over the next 90 minutes (Fig 2). An incubation of 30 minutes was used in all subsequent experiments.

Concentration of Human Bone Marrow Cells

In previous studies, the concentration of human bone marrow had proven critical for the complete elimination of malignant cells using monoclonal antibodies and C'. To test the effect of marrow concentration on elimination of malignant cells with 4-HC, mixtures containing 10^6 Namalwa cells and different numbers of irradiated human bone marrow cells were prepared. These mixtures were then treated for 30 minutes with 60 μg/mL of 4-HC. In contrast to previous findings with monoclonal antibody and C' treatment, no significant difference in clonogenic tumor cell survival was observed following 4-HC treatment with bone marrow concentrations ranging from 2 x 10^8 to 2 x 10^9 cells per milliliter (Table 1). Consequently, excess marrow did not prevent the ability of 4-HC to destroy clonogenic tumor cells.
permit comparison with previous experiments using antibodies and C', a concentration of 2 \times 10^7 bone marrow cells per milliliter was chosen for further experiments. This achieved a ratio of 20 bone marrow cells to each Burkitt's lymphoma cell.

**Treatment of Marrow Mixtures With 4-HC in Combination With Monoclonal Antibodies and C'**

Previous studies had shown that multiple treatments with monoclonal antibodies and C' were more effective than a single treatment in destroying clonogenic tumor cells. A combination of two or three antibodies was more effective than any single antibody in eliminating Burkitt's lymphoma cells from a 20-fold excess of human bone marrow. Repeated treatment with a combination of three monoclonal antibodies (J5, J2, and B1) and rabbit C' was compared to treatment with 4-HC. In addition, a combination of 4-HC with monoclonal antibodies and C' was also evaluated. Treatment, either with monoclonal antibodies and C' or with 4-HC, yielded comparable results with each of three Burkitt's lymphoma lines tested (Fig 3). Slightly but significantly more complete elimination of tumor cells was achieved in the Namalwa and BJAB 113 cell lines when both treatments were used in combination (Fig 3). Additive effects have also been observed with the EBV-negative JD-38 and parent BJAB lines (Table 2). Overall additive effects were observed with four of five cell lines studied in eight different experiments. To confirm that the additive antitumor activity was not caused by a simple loss of Burkitt's cells during processing, an additional experiment was performed in which all mixtures were incubated or washed the same number of times. Once again, combined treatment with 4-HC, monoclonal antibodies, and C' was slightly but significantly superior to treatment with 4-HC alone (Table 3).

**Clones Surviving Treatment With 4-HC**

To explore mechanisms underlying the additive effect of chemoseparation and immunoseparation, clones that survived treatment with 4-HC were tested for susceptibility to lysis with antibody and C'. Six different clones were grown after treatment of the Namalwa cell line with 40 to 60 \mu g/mL of 4-HC. All six of the clones exhibited a normal phenotype in which CALLA, gp 26, and B1 were expressed (Table 4). Each of these clones proved susceptible to treatment with multiple monoclonal antibodies and C' (Table 5). None of the clones proved resistant to a second treatment with 60 \mu g/mL of 4-HC (data not shown).
DISCUSSION

This study represents the first attempt to combine techniques of chemoseparation and immunoseparation for more effective removal of malignant cells from marrow in vitro. In earlier studies, conditions were defined for immunoseparation of $^{51}$Cr-labeled, CALLA-positive NALM 1 cells from a 100-fold excess of human bone marrow. When elimination of tumor cells was measured in vitro by use of release of radioactive compounds, no more than 2 logs of tumor cells killing could be detected. With a new clonogenic assay, optimal conditions have been defined for eliminating Burkitt’s lymphoma cell lines from a 20-fold excess of human bone marrow using monoclonal antibodies and C’. Optimal lysis was obtained when marrow was treated three times with multiple monoclonal antibodies and C’ for a total of 90 minutes. Tumor cell lysis was inhibited by high concentrations of human bone marrow cells. In the present study, a single treatment with 4-HC appeared comparable to multiple treatments with multiple monoclonal antibodies and C’ for eliminating Burkitt’s cells from an excess of normal bone marrow measured by the clonogenic assay. One advantage of 4-HC treatment, in contrast to monoclonal antibodies and C’, was that completeness of the tumor cell elimination did not depend upon the concentration of bone marrow cells. With four of the five Burkitt’s lymphoma cell lines, additive antitumor activity was observed with the combination of immunoseparation and chemoseparation, regardless of the sequence of 4-HC and antibody treatment.

Table 3. Treatment of Clonogenic Burkitt’s Cells With 4-Hydroperoxycyclophosphamide (4-HC) (60 μg/mL) in Combination With Monoclonal Antibodies and Complement

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Clonogenic Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>$3.4 \times 10^6$</td>
</tr>
<tr>
<td>J0 + C’ + 4-HC (60 μg/mL)</td>
<td>$3.1 \times 10^6$</td>
</tr>
<tr>
<td>4-HC (60 μg/mL) + J0 + C’</td>
<td>$4.1 \times 10^5$</td>
</tr>
<tr>
<td>J0, J2, B1 + C’ + medium</td>
<td>$2.4 \times 10^5$</td>
</tr>
<tr>
<td>Medium + J5, J2, B1 + C’</td>
<td>$2.4 \times 10^5$</td>
</tr>
<tr>
<td>J5, J2, B1 + C’ + 4-HC (60 μg/mL)</td>
<td>$6.3 \times 10^5$</td>
</tr>
<tr>
<td>4-HC (60 μg/mL) + J5, J2, B1 + C’</td>
<td>$6.3 \times 10^5$</td>
</tr>
</tbody>
</table>

Mixtures containing $10^6$ Namalwa Cells and $2 \times 10^7$ irradiated human bone marrow cells were treated: (1) for 30 minutes with 4-HC or diluent; (2) for 30 minutes x 3 with J5, J2, B1 and C’; or (3) with both treatments in either order.

Clones Surviving Treatment With Antibody

Clones that had resisted treatment with rabbit C’ and a combination of J5, J2, and B1 monoclonal antibodies were tested for sensitivity to 4-HC in vitro. Clones that had survived treatment with antibody and C’ exhibited no greater resistance than did the parent Namalwa line to treatment with 60 to 100 μg/mL of 4-HC for 30 minutes (Table 6).

Effect of Treatment on Normal Marrow Precursors

For effective autologous bone marrow transplantation, treatment of contaminated marrow in vitro must produce a selective deletion of malignant cells. Consequently, nonirradiated human bone marrow was treated with 4-HC, alone or in combination with C’, and monoclonal antibodies prior to measurement of GM-CFU-C or to the establishment of continuous bone marrow cultures. Consistent with earlier observations, treatment with 60 to 100 μg/mL of 4-HC markedly reduced the content of GM-CFU-C (Table 6). If, however, continuous cultures were established from treated and nontreated bone marrow (Fig 4), 4-HC did not inhibit subsequent production of GM-CFU-C (Table 6), suggesting that more primitive precursors had been spared. Treatment with C’ and a combination of J5, J2 and B1 monoclonal antibodies did not inhibit the initial growth of GM-CFU-C and had no adverse effect on subsequent growth of cells in continuous culture (Fig 4). Moreover, a combination of 4-HC with antibody and C’ had no greater effect than did 4-HC alone upon clonogenic growth of normal precursors in these culture systems (Fig 4).

Table 4. Antigenic Phenotype of Clones That Have Survived Treatment With 4-Hydroperoxycyclophosphamide (4-HC)

| Percentage of Positive Cells (Median Channel of Fluorescence Intensity) |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| Clone             | J0 (7)          | J5 (7)          | J2 (7)          | B1 (7)          |
| Namalwa           | 6.0 (7)         | 94.6 (52)       | 86.9 (92)       | 10.4 (8)        |
| 4-HC resistant clone 1 | 4.9 (7)        | 94.8 (138)      | 82.8 (84)       | 9.9 (9)         |
| 4-HC resistant clone 2 | 9.8 (8)        | 96.6 (150)      | 85.0 (97)       | 7.5 (7)         |
| 4-HC resistant clone 4 | 5.1 (6)        | 93.4 (138)      | 86.5 (100)      | 7.3 (8)         |
| 4-HC resistant clone 5 | 5.0 (6)        | 96.0 (156)      | 88.2 (99)       | 5.2 (6)         |
| 4-HC resistant clone 6 | 4.1 (6)        | 96.7 (138)      | 86.4 (88)       | 9.3 (8)         |
| 4-HC resistant clone 7 | 5.7 (6)        | 93.8 (160)      | 81.8 (86)       | 6.2 (7)         |

Clones were incubated with J0, J5, J2, or B1 and washed. After further incubation with fluoresceinated rabbit anti-mouse immunoglobulin, antibody binding was evaluated by flow cytometry.
and C' failed to affect GM-CFU-C production by
of bone marrow with multiple monoclonal antibodies
GM-CFU-C and BFU-E. As continuous marrow cul-
ture techniques may detect a more primitive marrow
precursor, we evaluated the effect of 4-HC and/or
antibody treatment on the ability of nonirradiated
human bone marrow to populate continuous long-term
marrow cultures. Concentrations of 4-HC in excess of
60 μg/mL delayed, but did not prevent, growth of cells
in continuous bone marrow cultures. By seven days in
culture, GM-CFU-C could be recovered in numbers
comparable to controls. In previous clinical studies,
treatment ofuffy coat preparations with as much as
100 μg/mL of 4-HC did not inhibit their ability to
reconstitute recipients of total body irradiation in the
experience of the Johns Hopkins transplant group. With
Ficoll-diatrizoate separated mononuclear cells,
however, the tolerance of marrow for 4-HC treatment
may be somewhat reduced. Thus, treatment of
human bone marrow with 60 to 100 μg/mL of 4-HC
permitted generation of GM-CFU-C in continuous
cultures and also permitted reconstitution of patients
who had received otherwise lethal myelotoxic chemo-
therapy or radiotherapy.

A combination of treatment with 60 μg/mL of
4-HC with monoclonal antibody and C' did not inhibit
growth of cells in continuous cultures. This suggests
that the additive elimination of malignant cells from
mixtures with human bone marrow would not produce
a greater loss of primitive marrow precursors. Ulti-
mately, the viability of marrow that has been treated
with 4-HC and monoclonal antibodies must be evalu-
ated in a phase I clinical trial. Our in vitro data suggest,
however, that chemoseparation with 4-HC and
immunoseparation with multiple monoclonal anti-
odies and C' might provide a new alternative for
eliminating rapidly proliferating malignant cells from
bone marrow.

The Burkitt’s lines represent a model system that
may or may not reflect results with malignant cells
that actually contaminate human bone marrow. Cell
lines used in this study were chosen for high clonogenic
efficiency and proliferative activity in cell culture.
Rapidly cycling cells are likely to prove more suscepti-
ble to alkylating agents than are noncycling cells. The
proliferative potential of many acute leukemias may
not be comparable to that of Burkitt’s lymphoma cells.
If autologous transplantation is undertaken in second
or third remission, tumor cells that contaminate mar-
row may have become relatively resistant to alkylating
agents. Consequently, we may have overestimated the
ability of 4-HC to destroy tumor cells. Treatment with
monoclonal antibodies and C' eliminated clones that
had survived 4-HC treatment. Similar treatment with
monoclonal antibodies and C' should destroy cells
resistant to alkylators, but we might also encounter
tumor cells that were more difficult to lyse in the
presence of monoclonal antibodies and C' than the
Burkitt’s cells.

Given the precisely defined reactivity of monoclonal
reagents, immunoselection with antibody might prove
less toxic for normal marrow elements than chemose-
lection with 4-HC. As in previous studies, treatment
of bone marrow with multiple monoclonal antibodies
and C' failed to affect GM-CFU-C production by
normal bone marrow. In the experiments outlined
above, treatment with monoclonal antibodies and C'
did not impair the ability of normal marrow to grow in
continuous culture.

The present and previous data indicate that treat-
ment of marrow with 4-HC can remove all detectable
GM-CFU-C and BFU-E. As continuous marrow cul-
ture techniques may detect a more primitive marrow

Table 5. Clones That Have Survived Treatment With
4-Hydroperoxycyclophosphamide (4-HC) Are Susceptible to
Treatment With Monoclonal Antibodies and C'

<table>
<thead>
<tr>
<th>Clone</th>
<th>Control (60 μg/mL)</th>
<th>4-HC (60 μg/mL)</th>
<th>4-HC (100 μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namalwa</td>
<td>2.6 x 10⁸</td>
<td>5.4 x 10⁷</td>
<td>1.1 x 10⁷</td>
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<td>J5, J2, B1 RC 75</td>
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<td>1.4 x 10⁷</td>
</tr>
<tr>
<td>J5, J2, B1 RC 111</td>
<td>4.4 x 10⁸</td>
<td>7.0 x 10⁷</td>
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</tr>
</tbody>
</table>

Table 6. Clones That Have Survived Treatment With Multiple
Monoclonal Antibodies and C' Are Susceptible to Treatment With
4-Hydroperoxycyclophosphamide (4-HC) (60 to 100 μg/mL)

<table>
<thead>
<tr>
<th>Clone</th>
<th>Control (60 μg/mL)</th>
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<td>9.2 x 10⁷</td>
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</tr>
<tr>
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The present and previous data indicate that treat-
ment of marrow with 4-HC can remove all detectable
GM-CFU-C and BFU-E. As continuous marrow cul-
ture techniques may detect a more primitive marrow

Fig 4. Cumulative nonadherent cell production after treat-
ment of human bone marrow with 4-hydroperoxycyclophospham-
ide (4-HC) (60 to 100 μg/mL) and/or monoclonal antibodies and
C'.
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Elimination of clonogenic Burkitt's lymphoma cells from human bone marrow using 4-hydroperoxycyclophosphamide in combination with monoclonal antibodies and complement

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