Hematologic Engraftment and Immune Reconstitution Posttransplantation With Anti-B1 Purged Autologous Bone Marrow


Hematologic engraftment and immune reconstitution were examined in patients who received cyclophosphamide and total body irradiation therapy followed by infusion of autologous bone marrow purged with anti-B1 monoclonal antibody (MoAb) and complement as therapy for non-Hodgkin’s lymphoma. Hematologic engraftment was prompt with return of $\geq 0.5 \times 10^9/\mu L$ granulocytes and $\geq 2 \times 10^9/\mu L$ platelets at a median of 26 and 29 days posttransplant, respectively. Immunologic reconstitution, in contrast, was prolonged. Normal numbers of circulating B cells were consistently noted by five months posttransplant, whereas return of normal immunoglobulin levels in some patients did not occur for one year. Normal numbers of T cells were evident within the first month posttransplant, but a reversed T4:T8 ratio persisted in some patients up to three years. In vitro responses of either B cells to triggers of activation or of T cells to mitogens and antigens were not normal for at least three months posttransplant. Natural killer (NK) cells predominated early after transplant and may demonstrate cytotoxicity against tumor cells. Our studies demonstrate that transplantation with anti-B1 purged autologous bone marrow results in complete hematologic and delayed immunologic engraftment. No significant acute or chronic clinical toxicities have been observed.

© 1987 by Grune & Stratton, Inc.

Materials and Methods

Hematologic Engraftment

Peripheral blood samples obtained from 24 patients who underwent autologous bone marrow transplantation therapy are included in this analysis: 23 patients with NHL and a single patient with chronic lymphocytic leukemia. Prior to in vitro marrow purging, patients were treated with conventional chemotherapy, radiation therapy, and/or surgery to a minimal tumor burden and a histologically uninvolved bone marrow with $<5\%$ cells expressing B1. To date all patients have attained a complete clinical response and have achieved complete hematologic engraftment with minimal acute and chronic clinical toxicity. The day posttransplant, when circulating white blood cells appeared, as well as the interval to achievement of $\geq 0.5 \times 10^9$ granulocytes/µL and a stable untransfused platelet count of at least $2 \times 10^9/\mu L$ served as monitors of hematologic engraftment.

From the Division of Tumor Immunology and the Blood Component Laboratory, Division of Medicine, Dana-Farber Cancer Institute, and the Department of Medicine, Harvard Medical School, Boston.

Submitted June 10, 1986; accepted September 12, 1986.

Supported in part by National Institutes of Health grant CA34183 and PHS grant 1RO1CA01105-01 awarded by the National Cancer Institute, DHHS.

K.C. Anderson is a Fellow of the Medical Foundation, Boston.

Address reprint requests to Dr K.C. Anderson, Division of Tumor Immunology, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/6902-0034$3.00/0

engraftment. The duration of hospitalization posttransplant was also recorded.

Patient Samples

After appropriate human protection validation and informed consent, patient samples were obtained for study. Peripheral blood samples were obtained prior to and at various intervals posttransplantation. Mononuclear cells were obtained from peripheral blood (PBMCs) by Ficoll-Hypaque density sedimentation for phenotypic and functional analysis. Sufficient numbers of cells were not available for every time point; in particular, detailed data were not collected for the earliest patients undergoing this treatment. In addition, no analysis was performed beyond the time of relapse for nine patients.

Phenotypic Analysis

A panel of MoAbs specific for B and T cells, monocytes, and natural killer (NK) cells was used in this study (Table I). PBMCs were assayed for reactivity with the MoAbs by indirect immunofluorescence staining and flow cytometric analysis as described previously. Antibody-coated cells were enumerated by flow cytometric analysis using an EPICS V cell sorter (Coulter Electronics, Hialeah, FL). For each sample 10,000 cells were analyzed using a log amplifier.

Functional Assays

Peripheral blood samples (60 mL) were obtained from patients and at least one normal donor at the time of each functional assay.

Stimulation of B Cells

B cell proliferation assays were performed as previously described. B cell-enriched preparations were made from PBMCs by E rosette depletion whenever numbers of cells permitted. Because it is extremely difficult to purify B cells from peripheral blood to homogeneity using both depletion as well as enrichment techniques, we chose anti-u, which is a stimulus for B cell but not for T cell activation.

Cells were resuspended at 5 x 10^4 cells/mL in RPMI/10% fetal calf serum, and 100-µL aliquots were dispensed in 96-well round-bottomed tissue culture plates (Costar, Cambridge, MA). Cells were cultured in the presence of either media alone or stimuli at the following final concentrations: phytohemagglutinin (PHA) 1:5 and 1:10; concanavalin A (Con A) 1:20 and 1:40; mumps 1:20; tetanus toxoid 1:10; and Candida albicans 1:50. For allogeneic mixed-lymphocyte reactions, Epstein-Barr virus (EBV)-transformed Lazz 388 cells were irradiated to 5,000 R, resuspended at 1 x 10^5/mL, and 100-µL aliquots dispensed into 96-well round-bottomed plates containing equal numbers of patient PBMCs. Twenty microliters of T cell growth factor containing media (TCGF-CM) was also added to wells already containing patient cells and/or media, PHA, or Lazz 388 cells. In all cases cells were cultured at 37 °C in 5% CO₂ and pulsed with 0.2 µCi/well (1 Ci = 3.7 x 10^10 Bq) of tritiated thymidine ([³H]TdR), harvested 18 hours later, and counted as described above. The increment in proliferation noted to the individual stimulus compared to media was calculated for both the patient and for the normal control examined on the same day. The patient response to any given stimulus was judged to be significant if it was 40% of proliferation of normal cells cultured with the identical stimulus. Finally, proliferation noted to PHA or Lazz 388 was compared to that noted when cells were cultured with these stimuli supplemented with TCGF-CM. The addition of TCGF-CM to PHA was judged to significantly enhance proliferation if proliferation to TCGF-CM and PHA/ proliferation to PHA ≥ 1.5. The TCGF-CM used in these studies was obtained as previously described.

Cytotoxicity (Natural Killer Cell) Assays

Cytotoxicity assays were performed according to a standard chromium release method previously described. Assays were performed at various effector:target ratios using 5,000 K562 target cells/well. Significant NK cell function was > 5% killing of K562, as evidenced by chromium release assay.

RESULTS

Hematologic Engraftment

A median of 5.1 (range 2.0 to 8.5 x 10^7) autologous bone marrow cells/kg were reinfused. Marrow engraftment occurred in all patients with the first evidence of white blood cell recovery generally by days 10 to 12 and 0.5 x 10^9 granulocytes/µL at a median of 26 (range 12 to 45) days after transplant. A stable nontransfused platelet count of at least 2 x 10^9/µL was attained at a median of 29 (range 14 to 137) days. Finally, duration of hospitalization was a median of 33 (range 20 to 58) days posttransplant. Eighty-four percent, 10%, and 6% of deglycerolized frozen red blood cells were transfused in the first, second, and third months posttransplant, respectively. Eighty percent of platelets were transfused in the first month, 19.5% in the second month, and only 0.5% after eight weeks posttransplant.
Immunologic Engraftment

**Phenotypic Analysis**

A panel of MoAbs directed at B and T cells, monocytes, and natural killer (NK) cells (Table 1) was utilized in an indirect immunofluorescence assay to monitor the phenotype of PBMCs as bone marrow engraftment and reconstitution of peripheral blood leukocytes proceeded (Table 2).

**B cells.** Normal numbers of B lymphocytes, as reflected by reactivity with anti-B1, anti-B2, or anti-B4 MoAbs, were present in six of 14 patients examined prior to transplantation. Cells bearing these antigens were totally absent in ten patients at two weeks posttransplant, and in 2/3 of patients had multiply relapsed lymphoma, and two patients were less than 6 weeks posttransplant, the percentage of T11+ cells was as follows: 5% to 10% PBMCs reactive with anti-B1, anti-B2, or anti-B4 (B cells); 60% to 75% PBMCs reactive with anti-T3 and/or anti-T11 (T cells); 10% PBMCs reactive with anti-9O1 or anti-NH1 (NK cells); 10% to 15% PBMCs reactive with anti-Mo2 (monocytes). Normal T4:T8 ratio is 1.5 to 2.5.

**NK cells.** Natural killer (NK) cells, which express the NKH1 antigen, were as follows: 10% PBMCs in only 40% (four of 11) of patients examined prior to ABMT. However, within the first month following ABMT, the overwhelming majority of patients had increased numbers of cells (up to one third of PBMCs) expressing the NK phenotype. In some cases this increased percentage of cells with NK cell phenotype persisted to one year posttransplant.

**Monocytes.** Monocytes (Mo2+) composed of 15% PBMCs in 86% of patients tested prior to transplant, in five of 13 patients at three weeks post-ABMT, and in 2/3 of patients tested beyond one month following ABMT.

**Functional Analyses**

**B cell proliferation.** B cell-enriched populations were obtained at various intervals post-ABMT and examined for their responsiveness to anti-u, a known B cell specific mitogen (Table 3). Two of four patients tested prior to transplant demonstrated significant proliferation [stimulation index (SI) ≥ 2.0] to anti-u bound to polyacrylamide.

### Table 2. Cell Surface Phenotype of Mononuclear Cells Posttransplant

<table>
<thead>
<tr>
<th>Weeks Posttransplant</th>
<th>No. Patients Normal* No. Patients Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 2 3 4 8 12-16 20-24 28-36 40-52 56-64 68-72 76-145</td>
</tr>
<tr>
<td>B cells</td>
<td>6 1 0 4 1 6 5 7 6 5 4 5 3</td>
</tr>
<tr>
<td></td>
<td>14 10 14 15 16 9 7 6 5 4 5 3</td>
</tr>
<tr>
<td>T cells</td>
<td>3 1 0 9 10 11 5 1 4 2 2 0 2</td>
</tr>
<tr>
<td></td>
<td>14 10 14 16 16 9 7 6 5 4 5 3</td>
</tr>
<tr>
<td>T4/T8</td>
<td>4 1 0 2 2 2 0 2 0 0 0 1 1 0</td>
</tr>
<tr>
<td></td>
<td>14 10 14 16 16 9 8 6 5 4 4 3</td>
</tr>
<tr>
<td>NK cells</td>
<td>4 6 7 9 8 3 5 4 3 0 2 2</td>
</tr>
<tr>
<td></td>
<td>10 7 13 13 13 9 7 6 5 4 3 3</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1 2 1 5 11 10 7 5 6 4 4 4 3</td>
</tr>
<tr>
<td></td>
<td>14 8 13 15 15 9 6 6 5 4 4 3</td>
</tr>
</tbody>
</table>

* Normal numbers of cells were as follows: 5% to 10% PBMCs reactive with anti-B1, anti-B2, or anti-B4 (B cells); 60% to 75% PBMCs reactive with anti-T3 and/or anti-T11 (T cells); 10% PBMCs reactive with anti-9O1 or anti-NH1 (NK cells); 10% to 15% PBMCs reactive with anti-Mo2 (monocytes). Normal T4:T8 ratio is 1.5 to 2.5.

†Two of the three patients have been repeatedly tested during this interval.

### Table 3. B Cell Stimulation

<table>
<thead>
<tr>
<th>Weeks Posttransplant</th>
<th>No. Patients Responding/No. Patients Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 0-4 4-8 12-116†</td>
</tr>
<tr>
<td>Stimulation Index to anti-u ≥ 2 (range)</td>
<td>2 (0.9–12.9) 0 (0.99–1.17) 0 (0.88–1.16) 9† (1.51–12.2)</td>
</tr>
</tbody>
</table>

* B cell-enriched populations were suspended at 5 × 10⁶ cells/mL in RPMI/10% fetal calf serum, and 100-µL aliquots were dispensed in 96-well round-bottomed tissue culture plates. Cells were cultured in the presence of either rabbit anti-u chain-conjugated polyacrylamide beads (30 µg/mL, wt/vol) or media. Cultures were pulsed 48 hours later with tritiated thymidine ([3H]Tdr) and harvested and counted 15 hours later. Counts per minute of [3H]Tdr in cultures with media alone were as follows: pre, 161–500; zero to four weeks, 320–947; four to eight weeks, 413–1126; and 12 to 116 weeks, 192–877.

†Five patients were repeatedly tested during this time interval.
beads. Of nine and eight patients tested at zero to four and four to eight weeks post-ABMT respectively, none demonstrated significant proliferation. By 12 to 116 weeks post-ABMT, B cells from nine of ten patients again proliferated to anti-U.

**Serum immunoglobulin (Ig) levels.** Serum IgG, IgA, and IgM levels were normal in the majority of patients tested prior to and at four weeks post-ABMT (≥6/13 and ≥6/10 patients, respectively) (Table 4). By eight to 20 weeks post-ABMT, a minority of patients had normal Ig levels. At 24 to 52 weeks post-ABMT, ≥50% of patients had reconstituted normal serum Ig levels, and nearly all patients tested one year post-ABMT had normal levels of IgG, IgA, and IgM.

**T cell proliferation.** PBMCs were cultured in the presence of mitogens, antigens, or growth factors known to trigger T lymphocyte proliferation. Responses were considered to be positive if the proliferation to a given T cell stimulus was ≥40% of the increment noted in a normal donor's cells cultured under identical conditions. As can be seen in Table 5, approximately 50% of patients examined prior to transplant responded to phytohemagglutinin (PHA) or concanavalin A (Con A). Responses to either mitogen were absent for a two-month period post-ABMT. In four of ten patients tested at 12 to 116 weeks posttransplant, T cell responses were again noted to either mitogen. Antigen-specific assays for the stimulation of T cell proliferation included mumps, tetanus toxoid, and Candida albicans. As was true for both PHA and Con A, only 50% of patients examined prior to ABMT responded to these antigens. Within the first month posttransplant, residual responsiveness was noted in 22% to 44% of patients tested. However, responses were largely absent by the second month posttransplant, and only two of ten patients tested at ≥12 weeks responded to these antigens.

**T cell growth factor containing medium (TCGF-CM) obtained by stimulating PBMCs with PHA, phorbol myristate acetate, and irradiated allogeneic (Laz 388) cells.** As was true for both PHA and Con A, only 50% of patients examined prior to ABMT responded to these antigens. Within the first month posttransplant, residual responsiveness was noted in 22% to 44% of patients tested. However, responses were largely absent by the second month posttransplant, and only two of ten patients tested at ≥12 weeks responded to these antigens.

**T cell growth factor-containing medium (TCGF-CM) obtained by stimulating PBMCs with PHA, phorbol myristate acetate, and irradiated allogeneic (Laz 388) cells.** As was true for both PHA and Con A, only 50% of patients examined prior to ABMT responded to these antigens. Within the first month posttransplant, residual responsiveness was noted in 22% to 44% of patients tested. However, responses were largely absent by the second month posttransplant, and only two of ten patients tested at ≥12 weeks responded to these antigens.

**Table 5. T Cell Proliferation**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>No. Patients Responding* / No. Patients Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weeks Posttransplant</td>
</tr>
<tr>
<td></td>
<td>Pre 0-4 4-8 12-116</td>
</tr>
<tr>
<td>Phytohemagglutinin 1:5 or 1:10</td>
<td>3 1 0 4</td>
</tr>
<tr>
<td>Concanavalin A 1:20 or 1:40</td>
<td>4 1 0 4</td>
</tr>
<tr>
<td>Mumps 1:20</td>
<td>7 8 9 10</td>
</tr>
<tr>
<td>Tetanus Toxoid 1:10</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>Candida albicans 1:50</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>TCGF-CM†</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>Laz 388‡</td>
<td>8 9 7 10</td>
</tr>
</tbody>
</table>

*The increment in 3H thymidine uptake by cells cultured with stimulus compared to cells cultured in media alone was calculated. A patient was judged to have demonstrated a response if the increment was ≥40% of the increase in proliferation noted in cells obtained from a normal donor cultured with the same stimulus under identical conditions.

†T cell growth factor-containing medium was obtained by stimulating unfractionated peripheral blood mononuclear cells with phytohemagglutinin, phorbol myristate acetate, and irradiated allogeneic (Laz 388) cells.

‡Epstein-Barr virus (EBV)-transformed Laz 388 cells were irradiated and cultured with equal numbers of patient peripheral blood mononuclear cells.

**Table 6. T Cell Proliferation**

<table>
<thead>
<tr>
<th>Patient Response</th>
<th>No. Patients Responding / No. Patients Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weeks Posttransplant</td>
</tr>
<tr>
<td></td>
<td>Pre 0-4 4-8 12-116</td>
</tr>
<tr>
<td>TCGF + PHA ≥ 1.5</td>
<td>7 3 4 9</td>
</tr>
<tr>
<td>PHA</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>TCGF + PHA ≥ 1.5</td>
<td>8 3 4 3</td>
</tr>
<tr>
<td>TCGF-CM</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>Laz 388 + TCGF ≥ 1.5</td>
<td>7 4 6 9</td>
</tr>
<tr>
<td>TCGF-CM</td>
<td>8 9 7 10</td>
</tr>
<tr>
<td>Laz 388 + TCGF ≥ 1.5</td>
<td>7 4 6 9</td>
</tr>
<tr>
<td>Laz 388</td>
<td>8 9 7 10</td>
</tr>
</tbody>
</table>

*20 μL of T cell growth factor containing media (TCGF-CM) was added to wells already containing patient cells and/or media, phytohemagglutinin (PHA), or Laz 388 cells. The addition of TCGF-CM to PHA or Laz 388 was judged to significantly enhance proliferation if it increased proliferation by 50% compared to the proliferation noted to PHA or Laz 388 alone.

---

**Table 4. Serum Immunoglobulin Levels**

<table>
<thead>
<tr>
<th>Immunoglobulin Isotype</th>
<th>Weeks Posttransplant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 4 8 12 16 20 24 28-36 40-52 56-90</td>
</tr>
<tr>
<td>IgG</td>
<td>8 7 4 2 4 2 3 5 2 3</td>
</tr>
<tr>
<td></td>
<td>13 10 13 7 11 6 8 8 4 4</td>
</tr>
<tr>
<td>IgA</td>
<td>9 7 3 0 6 2 3 4 2 4</td>
</tr>
<tr>
<td></td>
<td>13 10 13 7 11 6 8 8 4 4</td>
</tr>
<tr>
<td>IgM</td>
<td>6 6 3 2 3 2 3 5 3 3</td>
</tr>
<tr>
<td></td>
<td>13 10 13 7 11 6 8 8 4 4</td>
</tr>
</tbody>
</table>

*Normal serum levels of IgG, IgA, and IgM were 800 to 1800 mg/dL, 90 to 450 mg/dL, and 60 to 280 mg/dL, respectively.
HEMATOLOGIC AND IMMUNE RECOVERY POSTTRANSPLANT

In contrast to hematologic engraftment and immunologic reconstitution in recipients of autologous bone marrow purged with anti-B1 MoAb and complement, hematologic engraftment was prompt with return of \( \geq 0.5 \times 10^9/\mu L \) granulocytes and \( \geq 2 \times 10^4/\mu L \) platelets at a median of 26 and 29 days posttransplant, respectively. Virtually all blood product support was totally abrogated normal B cell function in vitro and in vivo.

Normal numbers of circulating B cells consistently returned by five months post-ABMT, whereas return of immunoglobulins in some patients did not occur for one year. Concomitant with the appearance of normal numbers of circulating B cells was the return of in vitro responses of B cells to triggers of activation (anti-u). Although normal numbers of T cells were evident within the first several months post-AMBT, a reversed T4:T8 ratio persisted in some patients indefinitely (up to three years). Moreover, in vitro responses to T cell mitogens and specific antigens were again not normalized for at least three months. Phenotypically and functionally defined NK cells appear to have a relative growth advantage early after transplant. These studies demonstrate that anti-B1 purged autologous bone marrow can result in complete hematologic and immunologic engraftment, although T and B cell engraftment appears to be prolonged. Most importantly, the time to hematologic and immunologic engraftment has not been associated with any significant acute or chronic clinical toxicities.

DISCUSSION

In the present report we have examined both hematologic engraftment and immunologic reconstitution in recipients of autologous bone marrow purged with anti-B1 MoAb and complement. Hematologic engraftment was prompt with return of \( \geq 0.5 \times 10^9/\mu L \) granulocytes and \( \geq 2 \times 10^4/\mu L \) platelets at a median of 26 and 29 days posttransplant, respectively. Virtually all blood product support was complete by eight weeks posttransplant. In contrast to hematologic engraftment, immunologic reconstitution was prolonged. As expected, anti-B1 and complement marrow treatment completely eradicated detectable B cells as well as totally abrogating normal B cell function in vitro and in vivo. Normal numbers of circulating B cells consistently returned by five months post-ABMT, whereas return of immunoglobulins in some patients did not occur for one year. Concomitant with the appearance of normal numbers of circulating B cells was the return of in vitro responses of B cells to triggers of activation (anti-u). Although normal numbers of T cells were evident within the first several months post-AMBT, a reversed T4:T8 ratio persisted in some patients indefinitely (up to three years). Moreover, in vitro responses to T cell mitogens and specific antigens were again not normalized for at least three months. Phenotypically and functionally defined NK cells appear to have a relative growth advantage early after transplant. These studies demonstrate that anti-B1 purged autologous bone marrow can result in complete hematologic and immunologic engraftment, although T and B cell engraftment appears to be prolonged. Most importantly, the time to hematologic and immunologic engraftment has not been associated with any significant acute or chronic clinical toxicities.

Overt or occult bone marrow involvement is very common at the time of relapse of NHL, regardless of the initial histology, and it therefore seemed important to develop methods to eradicate tumor cells from the marrow. We have attempted to purge tumor cells from the marrow using the pan-B cell MoAb anti-B1 and complement. To date, all patients have responded to the ablative therapy with a complete response and no acute toxicity, all have engrafted hematologically, and all were discharged from the hospital.

Our patients achieved \( \geq 0.5 \times 10^9 \) granulocytes/\( \mu L \) at a median of 26 (range 12 to 45) days post-ABMT, which is not significantly prolonged when compared to previous reports for nonpurged or pharmacologically purged autologous marrow. These results are slightly delayed when compared to recipients of syngeneic or allogeneic marrow grafts, but it is important to note that autologous marrow in most instances had seen significant prior cytotoxic therapy. Moreover, although most patients developed fever during neutropenic periods, no increased numbers of bacterial (n = 2) or fungal (n = 1) septic infections were noted in our series compared to previously reported trials. Stable nontransfused platelet counts of at least \( 2 \times 10^9/\mu L \) were maintained at a median of 29 (range 14 to 137) days in our series compared to recipients of syngeneic marrow who no longer required platelets at a median of 16 (range nine to 45) days2 or to recipients of allogeneic and autologous transplants who achieved sustained untransfused platelet counts of \( 5 \times 10^9/\mu L \) at a median of 20 to 34 days respectively. In our series, 80% of platelets were transfused in the first month, 19.5% in the second month, and only 0.5% beyond eight weeks. There was not a single episode of clinically

### Table 7. Natural Killer Cell Function

<table>
<thead>
<tr>
<th>Weeks Posttransplant</th>
<th>Pre</th>
<th>0-4</th>
<th>4-8</th>
<th>12-116</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Patients Demonstrating Cytotoxicity &gt;/ No. Patients Tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*Assays were performed at effector:target ratios of 7.5 to 60:1 using 5,000 K562 target cells/well. Greater than 5% maximal chromium release was judged as significant killing of target cells.
significant bleeding. These results suggest that hematologic engraftment following anti-B1 purging is delayed but has not led to significant acute clinical toxicities.

The question of whether anti-B1 purging will become a more generalized treatment modality for patients with NHL will depend on the answers to two questions: first, and most important, is whether this purging technique can effectively deplete the clonogenic tumor cell; and second is whether the marrow purging affects engraftment sufficiently to produce clinical toxicity. The first question is still unanswered. Our results suggest that purging does not appear to produce significant clinical toxicity, which is surprising considering (1) the time to B and T cell engraftment observed in this study; and (2) the fact that many of our patients had abnormal numbers of B and T cells, NK cells, and T4:T8 ratio pretransplant attributable to their underlying disease and its chemotherapeutic treatment as well as to multiple transfusions. Less than 50% of recipients of anti-B1 purged autologous bone marrow have normal numbers of B cells either pretransplant or within the first two months post-ABMT, but all patients examined at ≥20 weeks post-ABMT have achieved normal numbers of phenotypically identified B cells. Fifty percent and 100% of our patients examined at ≥six months and one year post-ABMT, respectively, achieved normal serum Ig levels, confirming the return of in vivo B cell function. Moreover, in vitro examination of B cells at ≥12 weeks post anti-B1 marrow purging also confirmed normal B cell responses to triggers of activation. Since the B1 antigen is expressed on 50% of pre-B cells in the marrow and on virtually all mature B cells excluding the plasma cell, the delayed time to B cell engraftment in our patients suggests that many pre-B cells and most mature B cells have been eradicated by the in vitro purging and the in vivo ablative therapy. The present data do not permit us to determine whether the time to B cell engraftment is simply due to repopulation of B cell progenitors or whether it is also due to the defects in T cell reconstitution. Future studies of B cell reconstitution in the bone marrow may provide insight into this question.

Although return to normal B cell function in vivo requires approximately six months, it is important to note that infections associated with agammaglobulinemia have not been seen post-ABMT. B cell phenotype and function have been examined postallogeneic transplantation for leukemia; however, there have been few studies detailing B cell reconstitution post syngeneic or post-ABMT for lymphoma. It is therefore difficult to determine whether B cell engraftment in our hands is significantly prolonged. Moreover, it is not possible to compare B cell engraftment post-ABMT with that noted after allogeneic transplant because of the immune disregulation of graft-vs-host disease (GVHD) and prolonged immune suppression by drugs in the majority of allogeneic studies as well as the immune defects following T cell depletion in more recent studies in recipients of allogeneic transplants. We have studied one patient who underwent an identical twin transplant for relapsed B cell lymphoma. In this case B cells returned between six and eight weeks and normal Ig levels were present by four months. This suggests that anti-B1 purging does prolong engraftment, although it is important to note that the autologous bone marrow had seen significant prior cytotoxic therapy. Further studies of B cell reconstitution post-ABMT in patients who receive nonpurged marrows will be necessary to determine whether anti-B1 MoAb and complement treatment significantly impairs B cell function.

Whereas B cell immunosuppression did not result in clinical toxicity, T cell immunosuppression appears to result in increased episodes of Herpes Zoster. In our series approximately one third of patients who are disease free at ≥six months have developed Zoster without a significant increase in other viral or fungal infections. Other investigators have noted interstitial pneumonitis and cytomegalovirus (CMV) infections in recipients of nonpurged autologous grafts that we have not observed to date. These specific immune defects in our patients are thus far unresolved. Although normal numbers of T cells were observed by two months post-ABMT, reversed T4:T8 ratio persists for most patients past one year. These phenotypic abnormalities correlated with abnormal T cell responses to mitogens and antigens in vitro. Responsiveness of T cells to mitogens was largely absent in all patients within the first two months and was present in <50% at 12 to 116 weeks post-ABMT, reflecting a relative decrease in T4+ cells. In contrast, responsiveness to either TCGF-CM or allogeneic cells was noted in the majority of patients at ≥12 weeks post-ABMT. It is interesting to note that the addition of TCGF-CM to cells stimulated with PHA-LCM or allogeneic cells led to greater proliferation than the proliferation induced by either stimulus alone. This suggests that there may be a relative excess of activated T cells expressing TCGF receptors but that there may be a deficiency of interleukin 2 (IL 2) post-ABMT. A relative deficiency of IL 2 has been previously reported early following allogeneic transplant. Since the provision of external IL 2 containing media led to augmentation of T cell proliferation these T8+ cells appear to respond in a normal fashion to TCGF. Again these T cell defects observed in vitro do not convert into significant clinical toxicities.

It is of great interest to note that 10% to 15% of PBMCs during the first three weeks post-ABMT express the NKHI antigen. These cells have both the phenotypic and functional characteristics of NK cells. Large numbers of these cells (≥10% marrow PBMCs) are infused in the graft and may have a selective growth advantage early following ABMT. Previous studies have demonstrated that several NKHI positive clones isolated from a patient post-allogeneic transplant had demonstrated marked NK activity not only against K562 but also against cryopreserved autologous tumor cells. The demonstration of in vitro cytotoxicity of these NK cells noted post-B1 purging against cryopreserved tumor cells further suggests that these cells may have an in vivo role in killing small numbers of residual tumor cells within the host. Delineation of this antitumor effect of NK cells post-ABMT coupled with definition of the factors regulating growth of these cells may offer important insights into the treatment of human tumors.

Our studies suggest that purging autologous bone marrow with anti-B1 MoAb and complement does not inhibit hematologic and immunologic engraftment post-ABMT. More importantly, minimal acute and chronic clinical toxicities are
observed following this treatment modality. The next step in these studies is to improve in vitro ablation by first using multiple antibodies and then comparing complement-mediated lysis to other forms of in vitro ablation. With each of these future studies we must carefully examine not only the in vitro tumor ablation but also the effects of hematologic and immunologic engraftment. Moreover, it is hoped that a better understanding of the growth and regulation of B and T cells will permit us to modulate their growth in vivo and thereby to hasten engraftment.

REFERENCES

on hematopoiesis in continuous bone marrow culture. Cancer Res 45:758, 1985
62. Verdonk LF, DeGast GC: Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone marrow transplantation? Lancet i:932, 1984
65. Verdonk LF, DeGast GC: Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone marrow transplantation? Lancet 1:932, 1984
68. Verdonk LF, DeGast GC: Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone marrow transplantation? Lancet 1:932, 1984
Hematologic engraftment and immune reconstitution posttransplantation with anti-B1 purged autologous bone marrow

KC Anderson, J Ritz, T Takvorian, F Coral, H Daley, BC Gorgone, AS Freedman, GP Canellos, SF Schlossman and LM Nadler