Lymphocyte Depletion During Treatment With Intensive Chemotherapy for Cancer

By Crystal L. Mackall, Thomas A. Fleisher, Margaret R. Brown, Ian T. Magrath, Aziza T. Shad, Marc E. Horowitz, Leonard H. Waxler, Melissa A. Adde, Linda L. McClure, and Ronald E. Gress

Recently we have observed an increased incidence of opportunistic infections in patients treated with intensive chemotherapy for cancer. Because T-cell depletion is associated with similar clinical events in human immunodeficiency virus infection and after bone marrow transplantation, we have analyzed peripheral blood lymphocyte populations in a series of patients during treatment with intensive chemotherapy for cancer. Although neutrophil, monocyte, and platelet numbers consistently recovered to greater than 50% of pretreatment values after each sequential cycle of therapy, lymphocyte numbers did not recover within the same time period. B cells decreased rapidly from a mean value of 149 ± 46/mm³ before chemotherapy to 4 ± 1/mm³ during chemotherapy (P = .01). CD4⁺ T cells decreased from a mean of 588 ± 76/mm³ before chemotherapy to 105 ± 28/mm³ during chemotherapy (P = .0002) and CD8⁺ T cells decreased from a mean of 382 ± 41/mm³ before chemotherapy to 150 ± 46/mm³ during chemotherapy (P = .0009). Natural killer cell numbers did not show significant declines (171 ± 30/ mm³ before, 114 ± 24/mm³ during, P = .19). Based on the history of opportunistic complications in patients with other disorders who display similar degrees of CD4⁺ T-cell lymphopenia and preliminary observations in this population, immune incompetence could surface as a dose-limiting toxicity for highly dose-intensive chemotherapy regimens.

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Dose escalation of cytotoxic chemotherapy has been limited primarily by neutropenia and thrombocytopenia. However, improvements in the management of neutropenic fever and the widespread availability of platelet transfusions have allowed increasingly dose-intensive cytotoxic therapies to be administered without increasing treatment-related mortality. Further, new therapies have been introduced that ameliorate the duration of hematopoietic suppression induced by chemotherapy. Myeloid growth factors (granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor [GM-CSF]) have been shown in several studies to shorten the duration of chemotherapy induced neutropenia and autologous peripheral blood stem cell (PBSC) infusions have further shortened the duration of neutropenia and allowed more rapid recovery of erythroid and platelet lineages as well. Therefore, whereas chemotherapeutic dose intensity has been limited primarily by hematologic toxicity, and in particular by myelosuppression, recent therapeutic developments have allowed increasingly dose-intensive regimens to be administered with relative safety.

Dose intensity can be defined as the amount of drug administered per unit time. Therefore, the dose intensity of a particular regimen can be increased by either increasing drug dosages or by compressing cycle length. Because the greatest toxic risks in the past have been related to neutropenic infection and hemorrhage, cycle length is frequently determined by the time necessary to achieve safe values for neutrophils and platelets. As dose intensity is progressively increased using these endpoints, adequate time for lymphocyte reconstitution may not occur and could result in clinical complications related to immune incompetence. In support of this possibility is evidence of an increasing incidence of opportunistic fungal and parasitic infections in patients undergoing chemotherapy for cancer. Although in the past these complications were primarily limited to patients with leukemia or other malignancies associated with extensive bone marrow infiltration, recently there has been an increasing incidence of opportunistic complications in patients with solid tumors as well. Whereas many factors are involved in host susceptibility to these infections, the prevalence of opportunistic complications during lymphocytopenic periods in human immunodeficiency virus (HIV) infection and after bone marrow transplantation (BMT) emphasize the importance of host lymphocytes in preventing such infections.

Previous reports of the effects of intensive chemotherapeutic regimens on PB lymphocyte (PBL) populations are scarce. Strender et al. reported decreased PBLs in women undergoing adjuvant therapy for breast cancer with chlorambucil, methotrexate, and 5-fluorouracil; however, T-cell subset analyses and T-cell phenotyping were not performed in this report. Although the prevalence of significant lymphopenia in similarly treated patients was not reported, Brunvand et al. reported decreased CD4⁺ counts and concurrent pneumocystis pneumonia in two women treated with multiagent chemotherapy and radiation therapy for breast cancer. Because we have noted an increase in opportunistic infections in patients treated for cancer with dose-intensive regimens at our center in the past several years, we have systematically studied changes in PBL populations associated with intensive chemotherapy. Ten children and young adults treated with three separate dose-intensive protocols during the years 1990-1993 were evaluated by flow cytometric analyses of PBL populations obtained serially upon maximal hematologic recovery from successive cycles of chemotherapy. We report that although the time between cycles was adequate for granulocytic, monocytic, and platelet recovery, lymphocyte populations did not recover before the administration of successive cycles of therapy leading to severe B-cell and T-cell depletion in these patients.
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that  the total dose of cyclophosphamide varied from 3.6 to 4.5 g/
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the Institutional Review Board of the National Cancer Institute.
non-Hodgkin's lymphoma (NHL). All protocols were approved by
had detectable BM involvement with  tumor. The patients were
treated on one of three protocols: NCI PB 90-C-211 for brain tumor,
1.000/mm3,  and platelets
were rendered free of detectable disease during treat-
ment with chemotherapy. Three patients relapsed after completion
of therapy as noted in Table 1: patients no. 3, 4 and 10, approximately
15, 2, and 8 months, respectively, after completion of therapy. The
length of follow-up for those patients listed as NED in Table 1 ranges
from 14.5 to 29 months after completion of therapy.
Specimens. Total white blood cell (WBC) counts were performed
by certified hematology laboratories using a Coulter STKS
(Hialeah, FLA). Automated leukocyte differentials were used using the
Coulter STKS only when the automated histogram was within
normal limits. If there were any abnormalities noted on the auto-
mated histogram, a manual leukocyte differential was performed
using a 100-cell count. For flow cytometry, each specimen consisted
of ~2 mL of PB obtained by venipuncture and placed into 3 mL
EDTA anticoagulant tubes. Specimens were kept at room tempera-
ture for no more than 24 hours before immunofluorescence staining.
To assess the effects of chemotherapy on lymphoid populations,
we analyzed one sample obtained before the administration of any
chemotherapy and serial samples obtained upon hematologic recov-
ergy from successive cycles of chemotherapy. To allow the longest
period possible for lymphoid reconstitution after each chemotherapy
cycle studied, samples were obtained just before the ensuing cycle
of chemotherapy; in most instances, this was on the day the ensuing
chemotherapy cycle was initiated. In no circumstance was the sample
obtained greater than 48 hours before the initiation of the ensuing
chemotherapy cycle.

Immunofluorescence staining. The manufacturer’s recom-
manded volume of the appropriate monoclonal antibody (MoAb)
was aliquotted into individual tubes. One hundred microliters of PB
was added directly to each tube, vortexed, and incubated for 30
minutes at 4°C. After incubation, 2 mL of fluorescence-activated cell
sorted lysing solution (Becton Dickinson Immunocytometry Systems
(BDII S), San Jose, CA) was added to each tube and incubated for
10 minutes at room temperature in the dark. The samples were then
washed twice with phosphate-buffered saline (PBS) containing 0.1%
azide and were stored in PBS containing 1% paraformaldehyde.
They were either analyzed directly or stored at 4°C overnight before
analysis.

The MoAbs used for T-cell phenotyping included: anti-CD3 (Leu

## MATERIALS AND METHODS

### Patients and protocols.
Patient characteristics are listed in Table 1. All patients had histologic evidence of tumor at the time of presen-
tation. Informed consent was obtained from all patients or their
parents before enrollment on protocol. No patient received chem-
otherapy before enrollment on the protocols listed below. No patient
had detectable BM involvement with tumor. The patients were
treated on one of three protocols: NCI PB 90-C-211 for brain tumor,
NCI PB 86-C-169 for high risk sarcoma, or NCI PB 89-C-41 for
non-Hodgkin’s lymphoma (NHL). All protocols were approved by
the Institutional Review Board of the National Cancer Institute.

The systemic cytotoxic drugs administered and cycle length are
listed in Table 2. In protocol 90-C-211, the total dose of cyclophos-
phamide was administered on the first 2 days of each cycle. Because
this was a phase II/II protocol, dose escalation was undertaken such
that the total dose of cyclophosphamide varied from 3.6 to 4.5 g/
m² per cycle, with dose reductions for delayed hematologic recovery.
The dosage listed in Table 2 represents the dosages administered to
patient no. 1. In protocol 89-C-41, the drugs listed in Table 2 were
administered over the first 15 days of cycles 1 and 3 and over the
first 5 days of cycles 2 and 4. In protocol 86-C-169 the drugs were
administered on days 1 and 2 in the odd-numbered cycles and on
days 1 through 5 of the even-numbered cycles. GM-CSF was admin-
istered to those patients noted in Table 1 on the basis of protocol
randomizations. The dosage of GM-CSF varied from 5 to 10 µg/
kg/d depending on the protocol. Mesna was used as a uroprotectant
in each protocol. During the time of evaluation, two patients received
radiation therapy: patient no. 4 received 5,400 cGy to the pelvis and
patient no. 5 received 6,660 cGy to the pelvis. Corticosteroid therapy
was administered only to patient no. 1. During the time of evaluation
for this study, he received a maximum of 24 mg of dexamethasone
daily that was weaned to a minimum dose of 2 mg daily. Prophylaxis
for pneumocystis pneumonia with trimethoprim-sulphamethoxazole
was administered only to patients on PB 90-C-211.

All patients were rendered free of detectable disease during treat-
ment with chemotherapy. Three patients relapsed after completion
of therapy as noted in Table 1: patients no. 3, 4 and 10, approximately
15, 2, and 8 months, respectively, after completion of therapy. The
length of follow-up for those patients listed as NED in Table 1 ranges
from 14.5 to 29 months after completion of therapy.

### Table 2. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)</th>
<th>Tumor Type</th>
<th>CSF</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>Brain</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>Sarcoma</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>Sarcoma</td>
<td>none</td>
<td>Rel</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>Sarcoma</td>
<td>GM-CSF</td>
<td>Rel</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>Sarcoma</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>Sarcoma</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>NHL</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>NHL</td>
<td>none</td>
<td>NED</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>NHL</td>
<td>GM-CSF</td>
<td>NED</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>NHL</td>
<td>GM-CSF</td>
<td>Rel</td>
</tr>
</tbody>
</table>

Abbreviations: NED, no evidence of disease; Rel, relapsed.

The dosage listed in Table 2 represents the dosages administered to
patient no. 1. In protocol 89-C-41, the drugs listed in Table 2 were
administered over the first 15 days of cycles 1 and 3 and over the
first 5 days of cycles 2 and 4. In protocol 86-C-169 the drugs were
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### Table 2. Protocol Drug Regimens

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Cytotoxic Drugs</th>
<th>Cycle Length</th>
<th>No. of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>90-C-211</td>
<td>Cycles 1-3: cyclophosphamide 4,500 mg/m²</td>
<td>On hematologic recovery*</td>
<td>4</td>
</tr>
<tr>
<td>(brain tumor)</td>
<td>Cycle 4: cyclophosphamide 3,375 mg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>89-C-41 (NHL)</td>
<td>Cycles 1, 3: cyclophosphamide 1,600 mg/m², doxorubicin 40 mg/m², methotrexate 6,720 mg/m², vincristine 1.5 mg/m² x2, cycle 1, x3 cycle 3</td>
<td>On hematologic recovery†</td>
<td>4</td>
</tr>
<tr>
<td>86-C-169 (sarcoma)</td>
<td>Cycles 2, 4: ifosfamide 7,500 mg/m², etoposide 300 mg/m², cytarabine 8 g/m²</td>
<td>Every 21 days‡</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Cycles 9, 11, 13, 15: cyclophosphamide 1,200 mg/m², doxorubicin 70 mg/m², vincristine 2.0 mg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cycles 2, 4, 6-8, 10, 12, 14, 16-18: ifosfamide 9,000 mg/m², etoposide 500 mg/m²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Ensuing cycles of chemotherapy begun as soon as AGC > 1,500/mm³, and platelets > 75,000/mm³.
† Ensuing cycles of chemotherapy begun as soon as AGC > 1,000/mm³, and platelets > 75,000/mm³.
‡ Chemotherapy was administered every 21 days if the patient had an AGC > 500/mm³ and platelets > 75,000/mm³ for cycles 1-5, every 21 days if the patient had an AGC > 1,000/mm³ and platelets > 75,000/mm³.

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4, -CD2 (Leu 5), -CD5 (Leu 1), -CD4 (Leu 3), -CD8 (Leu 2), -CD45RO (UCHL1), -CD45RA (Alb1). -CD28 (Leu 28). T-cell activation antibodies included: anti-HLA-DR, -CD25 (2A3), -CD69 (Leu 23). Natural killer (NK) antibodies included: anti-CD57 (Leu 7), -CD16 (Leu 11), -CD56 (Leu 19). B-cell antibodies were: anti-CD19 (Leu 12), -CD20 (Leu 19). The Leu reagents, anti-CD25 and anti-HLA-DR were obtained from Becton-Dickinson (Mountain View, CA); the anti-CD45RO reagent was obtained from Dako, Inc (Carpinteria, CA), and anti-CD45RA from Gentak (Plymouth Meeting, PA). Irrelevant murine MoAbs of the IgG1, IgG2a, and IgG2b subclass (BDIS) were used to define background staining.

Flow cytometry. Samples were analyzed on a FACScan (BDIS, Mountain View, CA) equipped with Lysis II software. Instrument calibration was performed daily using Calibrite beads (BDIS) and QC3 beads (Flow Cytometry Standards, Research Park, NC) as per the manufacturer’s recommendations. Lymphocytes were identified by forward- and side-scatter analyses and the lymphocyte gate was checked using the Leucogate reagents (CD45/CD14, BDIS) with back gating to confirm the forward- and side-scatter characteristics of the PBLs. Analysis of 10,000 cells contained within the lymphocyte gate was performed for each tube. A control sample obtained from normal volunteers was analyzed concurrently with each experimental sample.

List mode parameters were collected for 10,000 cells and percent positive staining was calculated based on the subcell control specimens. B cells were defined as CD19+ cells, T cells as CD3+ cells, CD4+ T cells as double-positive CD4+/CD3+ cells, CD8+ T cells as double-positive CD8+/CD3+ cells and NK cells as CD3- cells that were CD16+ and/or CD56+. For data analysis, the total percentage of B cells, T cells and NK cells contained within the lymphocyte gate was normalized to 100%. The normalized percentage of positive cells for each subset was then multiplied by the absolute PBL count measured as described above on a blood sample obtained simultaneously. Normal values for B cells, CD4+ T cells, CD8+ T cells and NK cells were determined by PB flow cytometric analyses of 50 normal volunteers in our laboratory.

RESULTS

Reconstitution of PB subpopulations during chemotherapy. The dose intensities of the regimens studied were substantial. All patients developed absolute granulocyte counts (AGC) of less than 500/mm3 with each cycle of chemotherapy evaluated. The mean duration of neutropenia (AGC < 500/mm3) was 8.2 days, and the mean cycle length for the cycles shown in Fig 1 was 24 days. Figure 1 displays the mean percent recovery of PB subpopulations for the 10 patients studied before each cycle of therapy and at days 2-4, 6-8, 10-12, and 14-16 posttherapy for the first four cycles of therapy. As shown, despite repeated depletion of PB neutrophils, monocytes, and platelets, these populations consistently recovered to safe values before the administration of the next cycle of therapy. Monocyte populations showed the highest recovery with successive cycles. Neutrophil and platelet populations recovered fully after cycles 1 and 2 and although they decreased somewhat after cycles 3 and 4, both recovered to greater than 50% of the pretreatment value. In contrast, the lymphocyte population as determined by the leukocyte differential showed significant decreases with therapy and did not recover to pretreatment values at any time after the first cycle of therapy. Flow cytometry performed during the nadir of WBC numbers in the PB showed essentially an absence of all lymphocyte subpopulations, but during the period of hematologic reconstitution from each cycle of chemotherapy there was partial recovery of lymphocyte numbers. Because of the diversity of populations that make up PBLs, we used flow cytometry to analyze changes in specific lymphocyte subpopulations with intensive chemotherapy.

B cells. B cells were defined as CD19+ cells. Several patients were noted to have subnormal values before chemotherapy as shown in Fig 2A. After one cycle of chemotherapy, however, there was a rapid decrease in B-cell number to nearly undetectable values in the PB of all patients studied. The changes in B-cell number occurred rapidly and were evident throughout the duration of therapy. Analysis was also performed for CD20+ cells and similar results were found. Table 3 shows the mean absolute number of CD19+...
cells in the peripheral blood of patients before and during chemotherapy. Because of the possibility that the full extent of lymphocyte depletion may not have been visible until a substantial portion of the therapy was completed, the “during-chemotherapy” timepoint used in these analyses was one in which the specimen was obtained after the majority of the chemotherapy cycles had been administered. Because of the greater number of cycles administered in protocol PB 86-C-169 compared with PB 90-C-211 and PB 89-C-41, the samples reported as “during therapy” for patients with sarcoma were obtained after at least 10 cycles of chemotherapy, whereas samples reported as “during therapy” for brain tumor and NHL patients were obtained after three cycles of therapy. A detailed analysis of Ig levels was not undertaken; however, after 10 cycles of chemotherapy, Ig levels were obtained from four sarcoma patients. IgM was absent in two of the four patients studied and low-normal levels were seen in the remaining two patients (data not shown). IgG levels studied simultaneously were within the normal range. Previous reports have also shown greater decreases in IgM levels compared with other Ig classes in patients treated with chemotherapy.17

T cells. Like B-cell numbers, the absolute CD4+ and CD8+ lymphocyte count in these previously untreated pa-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before (1/mm3)</th>
<th>*During (1/mm3)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>149 ± 46</td>
<td>4 ± 1</td>
<td>.01</td>
</tr>
<tr>
<td>CD4+ cells</td>
<td>588 ± 76</td>
<td>105 ± 28</td>
<td>.0002</td>
</tr>
<tr>
<td>CD8+ cells</td>
<td>382 ± 41</td>
<td>150 ± 46</td>
<td>.0009</td>
</tr>
<tr>
<td>NK cells</td>
<td>171 ± 30</td>
<td>114 ± 24</td>
<td>.19</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.56 ± 0.17</td>
<td>0.66 ± 0.11</td>
<td>.002</td>
</tr>
</tbody>
</table>

* In NHL and brain-tumor patients, samples were obtained after 3 cycles of therapy, and in sarcoma patients, samples were obtained after at least 10 cycles of therapy.
Patients presenting with cancer was below normal. Decreased T-cell number has been reported previously by Magrath et al in children with NHL. However, with successive cycles of chemotherapy, there was a progressive decrease in both CD4+ and CD8+ T-cell numbers. As shown in Fig 2B, several patients treated according to these treatment protocols developed CD4+ T-cell numbers which were less than 200/mm3. The changes in CD8+ T cells are illustrated in Fig 2C and showed a similar but less severe decrease. Patient no. 1 developed an excess of CD8+ cells after cycle 2 of therapy, but this was absent on subsequent analyses and has not been seen in other patients. The reason for this isolated and transient CD8+ T-cell rise was not clear. Statistical analyses of the changes in mean CD4+ and CD8+ T-cell numbers with chemotherapy is shown in Table 3. The mean CD4/CD8 ratio before therapy was 1.56 whereas the mean CD4/CD8 ratio during therapy was 0.66, showing a statistically significant change with a P value of .0015. Thus, whereas chemotherapy depletes both CD4+ and CD8+ T cells, there is a more dramatic effect on the CD4+ lymphocyte population. This CD4+ T-cell depletion does not appear to be a transient phenomenon related to lymphocyte trafficking because after the final cycle of chemotherapy, decreased lymphocyte counts (ie, CD4+ < 200/mm3) persisted for a minimum period of 4 months in all patients studied, and several patients have not shown complete recovery as late as 12 months after completing chemotherapy. A detailed analysis of the regeneration of lymphoid populations after completion of chemotherapy in these patients is underway and will be the focus of a subsequent report.

Further phenotypic analysis showed additional striking changes in the T-cell populations. This is illustrated in Fig 3A in which HLA-DR expression is shown on gated CD3+ lymphocytes from patient no. 1 before and during chemotherapy. An increase in HLA-DR expression was observed in all patients studied and persisted throughout the entire period of treatment. The percentage of CD3+ cells that were HLA-DR+ increased after chemotherapy from a mean value of 9.9% ± 2.79% before chemotherapy to a mean value of 41.2% ± 19.56% during chemotherapy (P = .007). Because this indicates widespread lymphocyte activation, the expression of other lymphocyte activation markers including CD25 and CD69 was also assessed; transient upregulation of these molecules was seen in some, but not all patients. CD45 isoform expression on CD4+ T cells also showed dramatic changes consistent with lymphocyte activation. CD45 isoform expression in patients at the time of presentation showed a mixture of "naive" (CD45RA+) and "memory" (CD45RO+) CD4+ cells in an age-dependent manner as has been described in healthy patients. However, after two cycles of chemotherapy, all patients showed an absence of CD45RA+CD4+ T cells, and all remaining CD4+ T cells expressed the CD45RO isoform consistent with a "memory" phenotype. This is illustrated on gated CD4+ T cells.

Fig 3. Changes in lymphocyte phenotype after chemotherapy. Lymphocytes from patient no. 1 were analyzed before therapy and after hematologic recovery from cycle 3 of therapy. Subclass controls for background staining did not vary significantly before and during therapy. (A) HLA-DR expression on gated CD3+ cells. (B) CD45 isoform expression on gated CD4+ T cells.
from patient no. 1 in Fig 3B. The mean CD45RA/RO ratio before therapy was 1.52 ± 0.43 and the mean CD45RA/RO ratio during chemotherapy was 0.04 ± 0.01. This difference was significant (P < 0.01). Like increased HLA-DR expression on PB T cells, this shift in T-cell subtype persisted throughout the entire period of chemotherapy.

**NK cells.** The absolute number of NK cells, as defined by all cells that were CD3- and CD16+ and/or CD56+, showed a high degree of interpatient variability at presentation. The response to chemotherapy was also variable as is shown in Fig 2D. Some patients showed decreases in absolute NK cell number whereas others showed increased numbers. In those patients with high NK cell numbers noted at the end of therapy, as many as 70% to 80% of the PBLs were NK' cells during the time of evaluation because of the depletion of T cells and B cells as noted above.

**Opportunistic infectious complications.** Three patients in this series developed four opportunistic infections. Patient no. 1 developed a severe adenovirus pneumonia requiring mechanical ventilation approximately 1 month after chemotherapy was completed. Patient no. 9 developed pulmonary histoplasmosis approximately 2 months after chemotherapy was completed, and subsequently developed herpes zoster approximately 12 months after completion of chemotherapy. Patient no. 10 developed herpes zoster approximately 5 months after chemotherapy was completed. In all of these instances, there was persistent CD4' T-lymphocyte lymphopenia at the time of opportunistic infection.

**DISCUSSION**

The most important finding from this study is that high-dose sequential chemotherapy, given to relatively young patients without BM involvement from tumor, consistently induced severe lymphocyte depletion. The mean value for CD4' T cells during chemotherapy in this study was 105/ mm³. Previous reports in HIV infection have shown that susceptibility to opportunistic infection increases dramatically with CD4' T-cell numbers less than 200/mm³. Although this study involved too few patients to ascertain whether opportunistic complications directly correlate with the degree of lymphopenia in patients with cancer, there is evidence suggesting that this could be the case. With regard to the patients prospectively evaluated in this study, the mean CD4' T-lymphocyte count after 10 cycles of therapy in patients treated on protocol 86-C-169 was 120 ± 38/mm³; the incidence of opportunistic infection was 0%. In protocol 89-C-41, the mean CD4' T-lymphocyte count after four cycles of therapy was 68 ± 16/mm³ and opportunistic infection occurred in approximately one third of patients. Although only one patient was available for prospective evaluation from protocol 90-C-211, after four cycles of therapy, this patient's CD4' T-lymphocyte count was 9/mm³. Approximately two thirds of patients treated on this protocol developed opportunistic complications. Review of the absolute lymphocyte counts of 36 cycles from retrospectively evaluated patients treated on protocol 90-C-211 showed that in all cycles after the first, the absolute lymphocyte count was less than 1,000/mm³ and in nine of these cycles, the absolute lymphocyte count was less than 500/mm³. Therefore, lymphocyte depletion was a common feature of this protocol and was not unique to the patient evaluated as part of this report. These results suggest that although each regimen of intensive chemotherapy was capable of inducing severe lymphocyte depletion, the degree of depletion may have varied with the specific protocol used and the extent of depletion may have influenced the rate of opportunistic complications seen.

As noted above, retrospectively evaluated patients who underwent treatment according to protocols 89-C-41 and 90-C-211 had a relatively high incidence of opportunistic infections. On PB 90-C-211, there were 13 opportunistic complications observed in 23 patients with 1 case of pneumocystis pneumonia, 4 cases of viral pneumonia (3 of which required mechanical ventilation), 3 cases of herpes zoster, 4 cases of herpes simplex, and 1 case of fatal transfusion-associated graft-versus-host disease (GVHD) observed. Although the use of corticosteroids in these patients could have contributed to their immunosuppression, the possibility remains that lymphocyte depletion contributed as an independent variable as well. The patient who died from transfusion-associated GVHD was not receiving corticosteroid therapy. On PB 89-C-41, there were 11 opportunistic infections in 33 patients with 9 cases of herpes zoster, 4 cases of pneumocystis pneumonia, and 1 case of histoplasmosis observed. No patient on this protocol received corticosteroid therapy.

Other evidence which suggests that chemotherapy-induced lymphocyte depletion may play a significant role in predisposing cancer patients to opportunistic complications is reports of cancer patients treated elsewhere who have developed opportunistic infections during chemotherapy, at which time their CD4' T-cell numbers were less than 200/ mm³. Further, there has been an overall increase in the incidence of opportunistic fungal and parasitic infections observed in cancer patients in the last several years, which has concurred temporally with the use of increasingly dose-intensive chemotherapeutic regimens. Although other variables have changed during the same time period, there is at least one report of patients treated for lymphoma wherein the dose intensity of the treatment regimen had a significant influence on the incidence of opportunistic complications, which was independent of disease histology and stage at presentation.

Although quantitative analyses have been the primary focus of this report, the implications of the changes in lymphocyte phenotype may be significant. This "activated phenotype," consisting of HLA-DR positivity and low molecular weight CD45 isofrom expression on CD4' T cells, has also been described on PBLs from patients after BMT. Although this could reflect preferential depletion of CD4' cells bearing high molecular weight CD45 isofroms by chemotherapy, studies in mice have suggested that this phenotype is typical of T cells that undergo regeneration via thymic-independent pathways during immune reconstitution. Further, it has been suggested by Miller et al. that the persistent abnormalities in T-cell function seen in BMT recipients may be related to a relative deficiency of "naïve" type T cells after BMT. Preliminary studies in patients who are receiving chemotherapy have also shown functional deficits in T-cell populations (C.L. Mackall, unpublished observations, December 1993) suggesting that like BMT recipi-
ents, patients undergoing dose-intensive chemotherapy may have both qualitative and quantitative deficiencies in cellular immunity.

Based on these data, it may be important to analyze new chemo therapeutic regimens not only for hematopoietic toxicity, but also for effects on lymphocyte populations in vivo as there may be significant differences among regimens. One example of relatively selective lymphotoxicity was seen with purine antimetabolites (2'-deoxycoformycin and fludarabine), which have been shown to have a propensity for T-cell lymphotoxicity in vivo.\(^1\) It should be noted that because these drugs are primarily used as therapeutic agents in patients with chronic lymphocytic leukemia and hairy cell leukemia, disease-specific factors may play a role in the lymphocytotoxicity observed. However, as cyclophosphamide is known to be immunosuppressive,\(^1\) it may have a more dramatic effect on lymphocyte populations than other agents. Conversely, other chemotherapeutic regimens might induce much less immunotoxicity. Knowledge of these effects could lead to selective prophylaxis of patients treated with particular regimens for pneumocystis, fungal, or varicella infection. Furthermore, it would be important to analyze the effects of various growth factors and PBSC infusions not only on hematopoietic recovery, but on their ability to enhance lymphocyte regeneration as well. We have analyzed the absolute lymphocyte counts during chemotherapy for 17 patients treated with GM-CSF and 18 patients treated without GM-CSF in protocol 86-C-169 and have found no differences in the absolute lymphocyte counts observed during therapy (data not shown). This suggests that the addition of GM-CSF to a complex chemotherapy regimen does not alter the degree of lymphocyte depletion induced.

Despite an increased incidence of opportunistic infections compared with historical controls, the overall incidence and severity of opportunistic infections in these cancer patients receiving intensive chemotherapy still appears to be less than in patients with similarly decreased CD4\(^+\) counts in other circumstances (ie, HIV infection, post-BMT). This suggests that other factors may play a role in defending cancer patients from overwhelming infection. Possibilities include NK cells that appear to be relatively spared with the regimens used in this report and which have been shown to play a role in the host response to fungal infection.\(^32\) Furthermore, monocyte populations that have been described as impaired in HIV infection\(^33\) may help to protect patients with cancer from infection. Studies that focus on secondary levels of defense will be important in dissecting the various pathways by which the immune system can protect against opportunists.

Finally, despite significant morbidity and mortality caused by defective lymphocyte regeneration in HIV infection and post-BMT, very little is known about the processes by which T cells regenerate in vivo,\(^25\) or regarding therapeutic interventions by which this can be enhanced. The study of lymphocyte depletion and reconstitution in patients receiving chemotherapy may not only improve the care of patients with cancer, but could serve as a model for the study of T-cell generation in vivo. Information learned could result in opportunities to enhance T-cell regeneration in other states of compromised T-cell number and function, such as in HIV infection and in the post-BMT setting.

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