Constraints on CD4 Recovery Postchemotherapy in Adults: Thymic Insufficiency and Apoptotic Decline of Expanded Peripheral CD4 Cells

By Frances T. Hakim, Rosemarie Cepeda, Song Kaimei, Crystal L. Mackall, Nanette McAtee, JoAnne Zujewski, Kenneth Cowan, and Ronald E. Gress

To examine the mechanisms of CD4 reconstitution in an adult population, lymphocyte repopulation was assessed following dose-intense chemotherapy in 25 breast cancer patients, ages 33 to 69 years. Chemotherapy resulted in a greater than 60% reduction in total CD4 T cells and, in particular, a greater than 90% loss of the CD45RA+ CD4 cells. CD4 recovery was protracted, achieving less than 50% of pretreatment levels after 12 to 14 months. Two facets of the CD4 recovery were notable. First, generation of CD45RA+ CD4 cells played only a minor role in the first year, suggesting that thymic production was not the main route of CD4 regeneration. Indeed, recovery of CD45RA+ CD4 cell levels remained limited in half of the patients even after 2 years. Second, expansion of the mature peripheral CD4 cells (CD45RO+) remaining after chemotherapy was the main source of early CD4 repopulation, peaking at 3 to 6 months postchemotherapy. This expansion was limited in duration, however, and was followed by a secondary decline, such that the total CD45RO+ CD4 levels at 9 to 12 months were lower than at 6 months. When stimulated by mitogens, an increased susceptibility to apoptosis was observed in post-chemotherapy CD4 cells as compared with those from normal donors. The elevated expression of markers such as HLA-DR during chemotherapy and for several months post-chemotherapy is consistent with the presence of an activated T-cell population. CD4 apoptotic frequency correlated with the frequency of HLA-DR expression on T cells. Thus, CD4 recovery is constrained in adults by a limited thymic regenerative capacity and by an increased susceptibility to apoptosis within the expanding peripheral CD4 population.

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MATERIALS AND METHODS

Patients. Between June 1993 and December 1994, 53 breast cancer patients (stage III to IV) entered dose intense chemotherapy (5-fluorouracil, leucovorin, adriamycin, cytoxan [FLAC]/Taxol) with cytokine support. The stage II and III patients had received no prior chemotherapy; the stage IV patients could have received prior adjuvant chemotherapy, but were previously untreated with chemotherapy for metastatic disease. All had a normal peripheral blood cell count. Chemotherapy consisted of five cycles, each lasting 3 weeks, of FLAC therapy: (5-fluorouracil (300 mg/m² intravenous [IV] daily days 1 to 3), leucovorin calcium, 500 mg/m² daily days 1 to 3), doxorubicin (17 mg/m² IV daily days 1 to 3), and cyclophosphamide (500 mg/m² IV days 1 to 3). Patients were randomized to receive either granulocyte-macrophage colony-stimulating factor (GM-CSF) (sargramostim, Leukine [Immunex, Seattle, WA], 250 µg/m² subcutaneously daily) or PIXY321 (375 µg/m² subcutaneously twice a day) from days 4 through 19. Patients were required to have recovered their blood counts to an absolute neutrophil count (ANC) >1,500/µL and platelets >90,000/µL to be treated with the next cycle of chemotherapy. Patients then received five cycles, each lasting 21 days, of paclitaxel (140 mg/m² by continuous IV infusion [CIVH] over 96 hours). Peripheral blood lymphocyte populations were assessed by flow cytometry and functional assays before the start of treatment, post-FLAC, postpaclitaxel, and during routine follow-up visits at 1, 3, 6, 9, 12, and 15 months following chemotherapy. As of June 1996, 25 patients had completed chemotherapy and decline in CD4 populations, the contribution of apoptosis to restricting the recovery of CD4 populations was investigated.
6 to 24 months of follow-up study. Data from patients who relapsed or who underwent further treatment were included only up to the follow-up visit before detection of relapse. These patients formed the basis for the lymphocyte population analysis.

In the apoptosis assays, some peripheral blood (PBL) samples were tested from patients who had been treated with a second chemotherapy protocol involving three successive 2-week cycles of adriamycin-cyclophosphamide (AC) therapy (consisting of cytoxan 2,000 mg/m² and doxorubicin 60 mg/m² IV on day 1 followed by 5 μg/kg granulocyte colony-stimulating factor (G-CSF) on days 2 to 13), followed by three cycles of taxol therapy (paclitaxel at 140 mg/m² by IV infusion 96 hours days 1 to 4; G-CSF days 6 to 13 5 μg/kg/d).

Flow cytometric analyses. Peripheral blood, collected in heparin, was stained at 4°C with a panel of antibodies, lysed using fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson Immunocytometry Systems, Mountain View, CA), washed with FACS buffer (Hanks’ Balanced Salt Solution [HBSS] without phenol red [GIBCO, Grand Island, NY] supplemented with 0.1% sodium azide and 0.2% bovine serum albumin) and analyzed on a FACSORT (BDIS, Mountain View, CA) equipped with a 480 nm CD4 population, harvested cells were stained with anti–CD4-FITC, anti–CD8-PE (Caltag), washed, and resuspended in FACS buffer containing 5 μg/mL Hoeschst stain (Sigma), fixed in 2% paraformaldehyde and viewed under a fluorescence microscope (Zeiss, Thornwood, NY) for changes in nuclear morphology consistent with apoptosis. 5

Induction and assay of apoptosis. Peripheral blood mononuclear cells from normal donors and from postchemotherapy patients (at 3 to 21 months of follow-up) were separated by ficoll-sodium diatrizoate gradient (Lymphocyte Separation Medium, Organon Teknika Corp, Durham, NC), washed two times in Dulbecco’s Phosphate-Buffered Saline (DPBS) (GIBCO/BRL, Gaithersburg, MD) and resuspended at 3 × 10⁷ cells/mL in RPMI 1640 (GIBCO) supplemented with penicillin/streptomycin, nonessential amino acids, sodium pyruvate and glucose, and 5% normal AB serum (Sigma, St Louis, MO). Cells were cultured at 3 × 10⁶ cells/well in 24-well plates (Costar, Cambridge, MA) or at 1 × 10⁵ cells/well (in quadruplicate wells) in 96-well round bottom plates (Costar) in either medium alone, phytohemagglutinin (PHA) (0.75% final, GIBCO), or pokeweed mitogen (PWM) (10 μg/mL final, Sigma) and staphylococcal enterotoxin B (SEB) (1 μg/mL final; Sigma). Cells were harvested after 18 to 48 hours, washed, stained for 10 minutes at 37°C in 2 μg/mL Hoeschst stain (Sigma), fixed in 2% paraformaldehyde and viewed under a fluorescence microscope (Zeiss, Thornwood, NY) for changes in nuclear morphology consistent with apoptosis. To identify the frequency of apoptosis specifically in the CD4 population, harvested cells were stained with anti–CD4-FITC or CD4-PE (Caltag), washed, and resuspended in FACS buffer containing 20 μg/mL Tamino actinomycin D (7AAD, Calbiochem, San Diego, CA), as previously described. 6 After 30 minutes at 4°C in 7AAD, cells were either analyzed immediately or were fixed in 2.0% paraformaldehyde, washed, and resuspended in 20 μg/mL actinomycin D (Sigma) in FACS buffer and analyzed within 24 hours.

RESULTS

Dose-intensive FLAC chemotherapy resulted in severe depletion of lymphocyte populations. Lymphocyte populations were assessed by flow cytometry in 25 women, ranging in age from 33 to 69 years, during the course of intensive chemotherapy for treatment of breast cancer and during a 6- to 24-month follow-up period. Although hematopoietic growth factors were used during chemotherapy, patients did not receive autologous stem cell infusions or immunomodulatory cytokines. Hence, the recovery of lymphocyte populations was dependent on lymphocytes and lymphocyte precursors remaining at the end of chemotherapy.

Five cycles of FLAC chemotherapy severely depleted all lymphocyte populations (Fig 1). The average lymphocyte population fell from 2,084 ± 161 cells/μL to only 721 ± 113 cells/μL. Peripheral blood B-cell populations were the most severely affected. Most peripheral blood B cells were lost within the first two cycles of chemotherapy. At the end of five cycles, B-cell populations had been reduced 96%, to fewer than 6 B cells/μL. Similarly FLAC chemotherapy resulted in a greater than 60% reduction in CD4 T cells in the peripheral blood and, in particular, a 95% loss in the number of CD45RA⁺ naive CD4 T cells (Table 1, Fig 1). Although not as severely affected, CD8 and NK cell populations were reduced to 46% and 62% of their pretreatment numbers (Fig 1).

The paclitaxel chemotherapy regimen used in this trial was much less toxic to lymphocytes than FLAC therapy. Although lymphocyte numbers were reduced during the course of each cycle of paclitaxel therapy, cumulatively the lymphocyte populations recovered from the nadir reached during FLAC therapy. B-cell levels increased 10-fold to an average of 66 B cells/μL during therapy with paclitaxel.
CD8 and NK levels increased to 86% and 100% of pretreatment levels. The recovery of CD4 populations was less extensive, but nonetheless, CD4 populations increased to 59% of pretreatment levels, to an average of 391 CD4 cells/μL.

**CD4 recovery was protracted in adults compared with other lymphocyte populations.** During the course of the first year postchemotherapy, all lymphocyte populations except CD4 cells recovered to pretreatment levels. B-cell populations recovered to pretreatment levels within the first 3 months (Fig 1). During the remainder of the first year, B-cell numbers in the peripheral blood were higher than pretreatment levels (Fig 1). Similarly, CD8 and NK populations recovered to levels not significantly different from pretreatment numbers during most of the first year. CD4 populations, in contrast, remained low in most patients. After an early recovery to approximately 69% of pretreatment levels (448 ± 62 cells/μL), CD4 numbers declined, falling to only 45% of the pretreatment levels (358 ± 48 cells/μL) in the peripheral blood after 1 year (Fig 1 and 2). CD4 levels remained below 400 in six of 14 patients even at the end of 2 years (Fig 3A).

The generation of new CD4 cells through the thymus, as indicated by expression of CD45RA, contributed little to CD4 repopulation in the first year postchemotherapy. The CD4+ T-cell population consists of two subpopulations as defined by the mutually exclusive expression of low and high molecular weight isoforms of the leukocyte common antigen CD45 (CD45RO and CD45RA, respectively). CD4 cells emerging from the thymus express the CD45RA+/CD45RO− phenotype, whereas activated or memory CD4 cells express the CD45RA−CD45RO+ phenotype. Naive CD4 cells, which have not been activated following release from the thymus, however, may continue to express CD45RA; hence in normal donors, the maturation of new CD4 cells cannot be readily distinguished from existing naive CD4 cells. During chemotherapy, however, 95% of naive CD45RA+CD45RO−CD4 cells were lost. Therefore, increases in the number of CD45RA−CD45RO+ cells could be used to approximate new generation of CD4 cells through the thymic maturation pathway.

During the first year postchemotherapy, the proportion of CD45RA+ cells in the CD4 population increased gradually (Fig 2). At pretreatment, CD45RA+ cells constituted on average 26% of the total CD4 cells in the patient population; only after 18 months had patients returned to the pretreatment frequency of CD45RA+ cells. Similarly, the number of CD45RA−CD45RO−CD4 cells increased steadily, but very gradually, from the nadir following FLAC treatment (Figs
Table 1. Depletion and Recovery of CD4 Populations

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* Deceased, off study, or lost to follow-up. ² 15 months postchemotherapy. ³ 9 months postchemotherapy.

2 and 4; Table 1). No rapid increase in CD45RA+ CD4 cells was observed, as has been found in young children following chemotherapy or transplantation.1-3 After 1 year, the number of CD45RA+ CD4 cells remained less than one third of pretreatment levels (76 ± 20 cells/µL compared with 225 ± 30 cells/µL). Thus, generation of new CD45RA+ CD4 cells supplied a steadily increasing, but numerically small, component of CD4 cells in the first year after chemotherapy.

In a population ranging in age from 33 to 69 years, no correlation was found between age and recovery of total CD4 cells or CD45RA+ CD4 cells. The recovery of total CD4 cells or CD45RA+ CD4 cells postchemotherapy or transplantation has previously been found to be correlated with the age of the host.1-3 These studies, however, contained a significant number of pediatric or adolescent cases. Because this study included patients ranging in age from 33 to 69 years at the start of chemotherapy, the effect of age on CD4 recovery in a strictly adult population was examined. At 6 months postchemotherapy, the same time point used in the earlier pediatric studies, no significant correlation was observed between age and total CD4 recovery or recovery of CD45RA+ CD4 cells (Fig 3A and B). Because of the concern that the 6-month time point might reflect residual CD45RA+ cell survival postchemotherapy rather than new maturation, CD4 recovery was assessed for as long as 2 years after chemotherapy (Fig 3A and B). The CD45RA+ population gradually increased in essentially all patients, but age was not a determinant in CD45RA+ CD4 levels even after 2 years. Overall, recovery of total CD4 cells (or of CD45RA+ CD4 cells) was quite variable in the adult population studied, with some achieving normal numbers within 1 year and others still at low CD4 levels (<400/µL) after 2 years.

CD45RO+ peripheral CD4 cell expanded rapidly following chemotherapy, but this expansion was transient, with a secondary fall in CD4 numbers at 3 to 6 months postchemotherapy. Although the changes in CD45RA+ populations were incremental, the overall CD4 population, evaluated either as a percentage of individual pretreatment levels or as absolute counts/µL (Figs 1 and 2), was observed to follow a more complex pattern. CD4 levels initially increased concurrent with the expansion of other lymphocyte populations, but declined in the latter half of the first year. Both this early increase in total CD4 levels and the later decline were primarily due to changes in CD45RO+ CD4 cell levels (Figs 2 and 4). CD45RO+ CD4 cell numbers initially increased, recovering to 81% of pretreatment levels by 3 to 6 months postchemotherapy. This increase was short-lived, however, as CD45RO+CD4 numbers subsequently declined to less than 50% of pretreatment levels by 12 months. But a concern arises in using summary data, in that not all patients were assessed at each time point and the early peak in CD4 recovery occurred at different times in different patients (Fig 4). Hence, depending on which patients were tested at a particu-
at that early peak constituted only 55 cells/μL; this is a significant ($P = .023$) change from the nadir, but it is quantitatively much less than the CD45RO$^+$ contribution to total CD4 regeneration.

When this early peak was then compared with the lowest point in CD4 cells during the later follow-up period (between 6 months and 1 year), the level of CD45RO$^+$ cells consistently declined. CD45RO$^+$ CD4 numbers fell to 260 ± 27 cells/μL, little difference from the original chemotherapy nadir levels (Fig 5B). Comparison of CD45RA$^+$ CD4 subpopulations at the same time points in each patient showed no significant change ($P = .60$). Thus, the CD45RO$^+$ CD4 cells remaining after chemotherapy undergo a rapid early expansion, but then decline.

Lymphocytes are susceptible to apoptosis for a prolonged

Fig 2. Time course of loss and recovery of CD4 cells, subdivided into the CD45RA$^-$CD45RO$^+$ and the CD45RA$^+$CD45RO$^-$ subpopulations, during chemotherapy and 1 year of follow-up. CD45RA$^+$ CD4 cells are severely depleted by FLAC chemotherapy and recover slowly during the first year postchemotherapy. CD45RO$^+$ CD4 cells are not depleted as severely, recover rapidly in the first 3 months postchemotherapy, and then decline secondarily. Data are calculated as the mean ± SE of the CD4 cell numbers/μL; these data were calculated based on the WBC, the percentage of CD4 cells in the lymphocyte population (FACS acquisition gate), and the percentage of lymphocytes in the leukocyte population. When an individual was assayed more than once in the 3-month follow-up periods, only that assay closest to the 3-month interval was used in the calculation. All time points during chemotherapy and follow-up are significantly different from pretreatment. Comparison between time points is difficult because data from different patients were included at different time points.

Fig 3. Recovery of either (A) total CD4 or (B) CD45RA$^+$ CD4 cells does not correlate with patient age at 6, 9, or 12 months postchemotherapy. Within an adult age range from 33 to 69 years, the overall pattern of slow steady recovery of CD45RA$^+$ CD4 cells within the first year postchemotherapy is consistent.
in vitro, many are induced to undergo programmed cell death. Recent work in human immunodeficiency virus (HIV)-infected individuals has shown that susceptibility to apoptosis is correlated not with disease state or viral load, but rather, with the activation state of T cells, specifically CD45RO and HLA-DR expression. The chronically stimulated T cells from HIV patients require no priming step, as do normal lymphocytes, but undergo apoptosis on initial stimulation.

To test whether susceptibility to programmed cell death is increased in postchemotherapy lymphocytes, mononuclear cells from normal controls and postchemotherapy patients were cultured for 18 to 48 hours in PHA, stained with Hoechst stain, and assessed for apoptosis by nuclear morphology. In patients ranging from 3 months to 15 months postchemotherapy, the frequency of apoptotic nuclei was twofold to threefold higher than in normal control donors (Fig 6).

Because CD4 populations were the limiting population postchemotherapy, apoptotic cell death was then assessed by flow cytometry using 7AAD uptake to discriminate living from apoptotic CD4 populations. This recently developed technique permits the assessment of apoptosis concurrent with identification of lymphocyte subpopulations by surface markers. Furthermore, this technique permits larger numbers of cells to be assessed than can be done by examination of nuclear morphology; live/apoptotic data was typically collected on 5,000 CD4 cells. The technique was validated by sorting CD4 cell populations identified as living or apoptotic by 7AAD uptake, staining with Hoechst stain, and examining nuclear morphology. This analysis corroborated the flow cytometric data.

CD4 cells from postchemotherapy patients were found to undergo apoptosis at an elevated frequency when peripheral blood mononuclear cells were cultured with PHA (34.6% ± 2.8%, mean ± SE) or PWM/SEB (27.6% ± 2.9%), as compared with CD4 cells from normal controls (18.1% ± 1.8% and 18.5% ± 2.6%) (Fig 7). Patients were tested at periods from 3 to 21 months postchemotherapy. Although CD4 cells from some of the patients at later time points had apoptotic frequencies similar to those from normal donors, others continued to have elevated apoptotic frequencies. This evidence is consistent with a protracted period of increased susceptibility to apoptosis following chemotherapy.

Given the apparent increased susceptibility to activation-induced apoptosis, the question arises as to whether the postchemotherapy T cells are in a chronically activated state, as has been described for HIV individuals. As noted above, the CD45RA–CD45RO+ CD4 cells largely disappeared during chemotherapy, either dead or converted into CD45RA+CD45RO+ cells. The FLAC and taxol chemotherapy regimen also resulted in elevated expression of CD25, CD38, and in particular HLA-DR on CD3+ T cells (Fig 8). Both the percentage of T cells expressing these surface markers and the level of expression per cell (mean fluorescent intensity) were increased. Because of the elevated expression of these markers, the term activated is used in preference to memory in regard to the CD45RO population postchemotherapy; the overall phenotype is consistent with a broadly
based activation of T-cell populations, rather than a conversion into resting memory cells. Furthermore, the elevated expression of activation markers is not a transient event. The percentage of T cells expressing HLA-DR did not return to pretreatment levels until after 9 months (Fig 8), that is, after the period of flush of CD45RO+ CD4 cell levels.

The final consideration was whether the frequency of apoptosis following stimulation in CD4 cells correlated with expression of activation markers. In 15 patients tested for apoptosis and lymphocyte activation markers, a strong correlation was observed ($P = .007$) between the percentage of T cells expressing HLA-DR antigen and the percentage of CD4 cells undergoing apoptosis (Fig 9). Expression of HLA-DR was only assessed on the CD3+ T cells (not on the CD4+ T-cell subset) during the longitudinal study of FLAC/taxol patients, but ongoing studies of postchemotherapy patients have shown a strong correlation between the percentage of CD4 cells expressing HLA-DR and that of CD3+ cells (data not shown). Furthermore the CD4:CD8 ratio was not reduced below an average of 1.5 during the follow-up period in this study. Hence, the elevated DR was not disproportionately attributable to CD8 cells. Thus, the T-cell population was apparently activated during chemotherapy or during the early expansion phase. This activated population declined subsequently, concurrent with a period of decline of the activated/memory CD45RO+ CD4 population. CD4 cells from postchemotherapy patients showed an increased susceptibility to apoptosis on stimulation that correlated with the overall expression of HLA-DR on T cells.

**DISCUSSION**

As chemotherapy regimens have become more intensive, the depletion of lymphocytes and the associated immune deficiency have become more severe. In this study, we have shown that CD4 recovery is delayed for more than 1 year following dose-intensive chemotherapy in an adult population. This delay was produced by defects in both thymic-dependent and thymic-independent pathways of CD4 regeneration. First, the classic route of thymic maturation of new naive CD45RA+ CD4 cells from pre-T cells provided a steadily increasing, but very limited, number of new T cells in the first year. Second, expansion of peripheral CD45RO+ CD4 cells predominated in early recovery, but this expansion was limited in size and duration.
Higher frequencies of apoptotic CD4 cells were found in unstimulated, PWM/SEB, or PHA stimulated culture from postchemotherapy patients (PC) than in normal control cultures. Cultures of PMN were cultured for 18 to 48 hours with medium alone or mitogens. Apoptosis was determined by flow cytometry using 7AAD uptake in CD4 cells. Data include assays on patients ranging from 3 to 21 months postchemotherapy from the FLAC/paclitaxel study and the ACT study. Bar ( ■ ) represents the mean and ( □ ) SE of the assayed populations. Number of assays (n) and P value of the comparison between normal donors and postchemotherapy donors are indicated at the top.

Generation of naive CD45RA+ CD4 cells by thymic maturation apparently contributed little to CD4 recovery in the first year. Several concerns should be addressed regarding the use of the CD45RA frequency to approximate new thymic-dependent CD4 generation. Peripheral expansion of residual or newly matured CD45RA+ CD4 cells without conversion to the activated/memory phenotype or conversion of dividing cells back to the naive form could of course increase the level of CD45RA+ cells without new generation by the thymus. Both murine and human data, however, suggest that the main route of CD45RA+ CD4 cell generation is thymic maturation. Transplants into thymectomized mice do not result in expansion of residual or transferred CD4 cells expressing the high molecular weight isoforms of CD45 (CD45RB in mice); peripherally expanded cells convert to the low molecular weight CD45 isoform. Furthermore, expansion of CD45RA+ CD4 cells in pediatric patients postchemotherapy correlated with thymic rebound, an expansion of the thymus to a size greater than that observed prechemotherapy. Young adult patients lacking this thymic rebound showed minimal CD45RA recovery for several months. Thus, nonthymic dependent pathways make at most a minor contribution.

The frequency of apoptosis in PHA-stimulated CD4 cells correlated with the expression of HLA-DR on T cells. In 15 of the postchemotherapy patients assayed in Fig 7, data on the percentage of total CD3+ cells in the peripheral blood expressing HLA-DR were compared with the frequency of CD4 cells determined to undergo apoptosis after culture of PMN with PHA.
constraint to the generation of CD45RA CD4 cells. An alternative concern is that continued activation of CD4 cells after the end of chemotherapy could siphon newly generated naive CD4 cells into the activated/memory phenotype pool, lowering the CD45RA frequency. Elevated percentages of T cells expressed HLA-DR for several months after chemotherapy (Fig 8), but increased expression of CD69, an early activation marker, was not observed (data not shown). Furthermore, in studies of young patients, CD45RA cells accumulated to more than 60% of the total CD4 cells within 6 months, hence, conversion to CD45RO CD45RA is not automatic in CD4 cells in the postchemotherapy period. Thus, the CD45RA CD4 cell level postchemotherapy provides a useful approximation of CD4 regeneration along the thymic-dependent pathway.

The current study identified no correlation between age and CD4 recovery. Rather, newly matured naive CD4 cells were found to play a minor role in CD4 reconstitution throughout the adult age range in the first year. Other studies, which have found an inverse correlation between age and total CD4 recovery or CD45RA CD4 recovery, have included a significant pediatric or adolescent component. Examinined strictly in an adult population, age differences may not be a major factor in the level of thymopoiesis. The diminished thymic function in adults may be due to a combination of factors. The adult thymus is smaller than the prepubertal thymus and does not generally expand (show “thymic rebound”) in man in the early postchemotherapy period. Furthermore, aged thymus produce fewer CD4 cells than young thymuses following lethal irradiation and transplantation with young bone marrow (Mackall, in preparation). Indeed, the aged thymus replaces depleted CD4 cells at a lower rate than the young thymus, even when the method of depletion is not toxic to the thymus, as in aged versus young mice treated with anti-CD4. Finally, the thymus is disorganized by chemotherapy drugs and does not recover function immediately even in young mice. Hence, chemotherapy may have exacerbated the deficits of aged thymuses.

A key observation of this study was that expansion of mature peripheral T cells was the main contributor to the early rise in CD4 cell levels postchemotherapy. The CD45RO CD4 cells expanded significantly even during the rounds of paclitaxel therapy and were the dominant population throughout the first year in this study. In our previous study of pediatric and young adult patients, this peripheral expansion was not observed, perhaps because the level of T-cell depletion was more severe than in the FLAC/Taxol study. The number of mature, peripheral CD4 cells present (or administered) posttransplantation has been observed to play an important role in the rate of CD4 recovery. T-cell recovery is delayed in T-depleted transplants. Patients receiving an infusion of autologous peripheral blood stem cells (PBSC) recover lymphocyte populations faster than those receiving autologous marrow, perhaps because of the more sizeable population of lymphocytes included in the PBSC. In adult patients receiving dose-intensive chemotherapy or transplantation without such an infusion, an extended period is required for recovery of the CD4 T-cell populations critical for immune function. Hence, the early expansion of CD4 cells may be dependent on the size of the residual CD4 population.

CD4 reconstitution dependent upon mature CD45RO expansion has significant consequences for immune function postchemotherapy. When thymectomized mice were reconstituted with limited numbers of mature CD4 cells, the resultant population was limited in number and did not reach the levels found in intact mice. More important, the expansion was antigen-driven and prone to skewing and loss of portions of the T-cell repertoire. Following autologous and allogeneic transplantation, T-cell recovery dependent on expansion of existing mature cells has similarly resulted in a skewed oligoclonal repertoire. A limited repertoire could contribute to prolonged immune deficits, particularly in response to neoantigens. CD4 populations following chemotherapy or autologous transplantation have several elements in common with those in HIV-infected individuals. We have observed prolonged deficits in IL-2 production in response to mitogens in postchemotherapy patients (data not shown); immune deficits continue for the first year following autologous transplantation, and accompany even asymptomatic HIV infection. In both chemotherapy and transplantation, an early T-cell expansion is followed by rapid decline; recent evidence suggests a continuous high level of CD4 expansion in HIV patients, as well. All populations show a predominance of CD45RO CD4 cells and an increased frequency of cells bearing activation markers such as HLA-DR and CD38, CD8 ratios may decrease or even become inverted due to both loss of CD4 cells and more rapid production of CD8 cells. The T-cell receptor repertoire shows evidence of oligoclonal expansion or loss of repertoire diversity. These diverse elements may have in common the rapid expansion of depleted peripheral T-cell populations in response to antigenic stimulation. Increased susceptibility to apoptosis in these systems may therefore be a regulatory response, a consequence of the increased level of activation in the CD4 population.

The evidence that postchemotherapy lymphocytes are susceptible to apoptosis on stimulation provides a new perspective on postchemotherapy immune dysfunction. This susceptibility may be responsible for the immune deficiency that has often been observed in vitro in the first year after chemotherapy or transplantation. If a sizeable percentage of the cells were to undergo apoptosis when stimulated, then a variety of common functional assays (such as mitogen-stimulated proliferation and cytokine production) would be depressed as stimulated cells were eliminated. Furthermore, the susceptibility to apoptosis may be responsible for the decline in CD4 numbers observed in many of the patients. An immune response weighted toward apoptosis would result in T-cell decline rather than expansion when environmental pathogens were encountered. This susceptibility to apoptosis could also result in a loss of relevant T-cell repertoire. If this were to occur, then attempts at immunotherapy involving administration of tumor antigen could result in deletion from the T-cell repertoire of the very cells that were targeted for activation and expansion. Thus, the susceptibility to apoptosis should be considered in the design of immune therapies in the postchemotherapy period.
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Constraints on CD4 Recovery Postchemotherapy in Adults: Thymic Insufficiency and Apoptotic Decline of Expanded Peripheral CD4 Cells

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