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Journal
Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 30(3)

ISSN
1058-4838

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Publication Date
2000-03-01

DOI
10.1086/313714

Reversal of Human Immunodeficiency Virus Type 1–Associated Hematosuppression by Effective Antiretroviral Therapy

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The immunodeficiency of human immunodeficiency virus type 1 (HIV-1) disease may be due to accelerated destruction of mature CD4$^+$ T cells and/or impaired differentiation of progenitors of CD4$^+$ T cells. HIV-1 infection may also inhibit the production of other hematopoietic lineages, by directly or indirectly suppressing the maturation of multilineage and/or lineage-restricted hematopoietic progenitor cells. To test this hypothesis, the effects of durable viral suppression on multilineage hematopoiesis in 66 HIV-1–seropositive patients were evaluated. Administration of effective antiretroviral therapy resulted in an increase in circulating CD4$^+$ T cell counts and statistically significant increases in circulating levels of other hematopoietic lineages, including total white blood cells, lymphocytes, polymorphonuclear leukocytes, and platelets. These results suggest that a significant lesion in untreated HIV-1 disease may lie at the level of cell production from hematopoietic progenitors.

Infection with HIV type 1 (HIV-1) is associated with a decrease in the number of circulating CD4$^+$ T cells. This effect is generally attributed to the destruction of mature CD4$^+$ T cells in the peripheral lymphoid system. Alternatively, HIV-1 may decrease production of CD4$^+$ T cells by preventing maturation of lymphoid precursors (e.g., multilineage hematopoietic progenitor cells in the bone marrow, lymphoid-restricted progenitors of T cells in the thymus, and/or clonally distributed memory T cells in peripheral lymphoid organs) [1]. In support of this hypothesis of impaired production, multilineage cytopenias have been noted in many patients with HIV-1 disease, including anemia in 70%–90%, neutropenia in 40%–50%, and thrombocytopenia in 40%–60% [2, 3]. Because mature cells in each of these lineages do not appear to be infected and/or destroyed by HIV-1 [2, 3], the occurrence of such cytopenias suggests that viral infection interferes with the function of hematopoietic progenitor cells that are not lymphoid-restricted. If so, then inhibition of viral replication should be associated with improved production of all cell lineages.

It has been difficult to address this prediction in previous studies in which various antiretroviral regimens were used. To the contrary, administration of some drugs (e.g., zidovudine) is associated with anemia, lymphopenia, and neutropenia in up to 20% of patients, which is probably the result of direct marrow toxicity [4]. In the case of other monotherapies (e.g., didanosine and stavudine) [5–7], increases in hematologic parameters were observed to be transient, presumably because resistant isolates emerged during therapy. Twelve weeks after the initiation of combination antiretroviral therapy with protease inhibitor–containing regimens, increases were noted in the number of circulating hematopoietic progenitor cells and CD4$^+$ T cells but not in the circulating levels of other hematopoietic lineages (e.g., granulocytes or monocytes) [8].

Now that potent antiretroviral combination therapy has been used for a longer period, it is possible to evaluate the effect of durable viral suppression on multilineage hematopoiesis. If HIV-1 infection has significant adverse effects on multilineage hematopoiesis, we reason that suppression of viral replication should be associated with sustained increases not only in the number of circulating CD4$^+$ T cells but also in the number of other circulating cells. To address this hypothesis, we reviewed the medical records of 66 HIV-1–seropositive patients treated with antiretrovirals who had sustained undetectable HIV-1 loads for at least 1 year and analyzed the effects of such therapy on all peripheral hematologic parameters.

Received 7 July 1999; revised 4 November 1999; electronically published 9 March 2000.

Financial support: National Institute for Allergy and Infectious Diseases (R01 AI40312) and Elizabeth Glaser Pediatric AIDS Foundation (J.M.M.); UCSF/Gladstone Institute of Virology and Immunology Center for AIDS Research (S.S.H.); UCSF/Macy's Center for Creative Therapy (J.D.B.).

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Methods

Study population. By using an administrative database that records outpatient visits, we identified all patients seen at least 3 times by the same clinician from March 1996 through September 1997 at our center. Medical records (including those from interim hospitalizations, if any) for each patient were then reviewed to identify those who received ⩾48 weeks of continuous antiretroviral therapy, including at least 1 protease inhibitor. Further analysis was done for those patients who met the eligibility criteria outlined below and were followed up by the 6 physicians who had the largest practices.

Medical records were reviewed for demographic data, dates of all prior antiretroviral therapy, medication history, and medical history. Ninety-three patients met the following inclusion criteria: documented start date of effective antiretroviral therapy; documented viral load within the 3-month period before beginning effective antiretroviral therapy; documented suppression in circulating viral load to <500 HIV RNA copies/mL within 3 months of effective antiretroviral therapy; and documented maintenance of this level of suppression for at least 1 year. Effective antiretroviral therapy was defined as a regimen of antiretroviral drugs (including at least 1 protease inhibitor) that suppressed the blood plasma viral load below detectable limits for a period of at least 1 year. Twenty-seven of the 93 patients were excluded from analysis for the following reasons: transfusion of blood products during or within 1 year of the study period (5 patients); use of agents known to have effects on circulating levels of various hematopoietic lineages (e.g., prednisone [5], erythropoietin [4], granulocyte colony-stimulating factor [7], IFN-α [1], IL-3 [1], and hydroxyurea [2]) within 6 months of the start of or during the study period; treatment with chemotherapeutic agents for various malignancies (e.g., Kaposi's sarcoma or lymphoma) at any time (7); disseminated acid-fast bacillus infection (2); drug-induced cytopenias (1); or inadequate information (4). Patients were also excluded from analysis if they had opportunistic infections or other signs of infectious illness (e.g., diarrhea, fever, or rash) within 3 months of baseline. Some individuals were excluded for multiple reasons. The exclusion criteria were selected before collection and analysis of the data. The exclusion periods were chosen to be longer than the observed or expected effects of intercurrent illness and therapy on hematopoiesis.

Sixty-six HIV-1–seropositive individuals met the study criteria and were evaluated for changes in hematologic parameters after the initiation of effective antiretroviral therapy.

Data analysis. Start dates for effective antiretroviral therapy were obtained from the medical records and were correlated with observed changes in circulating viral loads. Individual data schedules were constructed based on 5 3-month intervals: a 3-month baseline period just before initiation of therapy, and 4 3-month periods corresponding to months 0–3, 4–6, 7–9, and 10–12 after initiation of therapy. For each patient, all available hematologic values determined from peripheral blood analysis were retrieved from individual medical records and were pooled for each interval to obtain mean values for viral load, $CD4^+$ T cell counts, and levels of other circulating blood cells. On average, peripheral blood analysis was performed for each patient every 1–2 months.

Peripheral blood analyses and determination of differential cell counts were performed by use of an automated counter (Technicon H*3; Bayer, Tarrytown, NY). Measured parameters included total WBC count, total RBC count, mean corpuscular volume (MCV), hemoglobin level, and total platelet count. Automated differential cell counts were expressed as the percentage of total WBCs and the absolute numbers of polymorphonuclear leukocytes (PMNs) and lymphocytes. HIV-1 loads (viral loads) were determined by use of the Quantiplex version 2.0 assay (branched DNA assay; Chiron, Emeryville, CA), with a lower limit of detection of 500 HIV RNA copies/mL. Peripheral blood $CD4^+$ T cell counts were determined by use of a direct whole blood imaging system (Imagn 2000; Biometrics Imaging, Mountain View, CA) with use of fluorescent monoclonal antibodies. This method, which generates an absolute $CD4^+$ T cell count without having to obtain a simultaneous lymphocyte count, has been extensively tested, and it has been determined that it provides results equivalent to those of standard flow cytometry. Reference ranges for all measured or calculated values were determined with use of blood samples from at least 120 healthy individuals.

Differences in mean cell counts between baseline and each 3-month interval were calculated for each patient and were termed "δ values." Individual baseline mean cell counts and δ values from months 3, 6, 9, and 12 were then used collectively as data points to analyze group trends with regard to hematopoietic recovery.

To account for differences in increases in hematologic parameters as a result of differences in baseline cell counts, data were further analyzed in subsets based on normal or abnormal baseline cell counts relative to the reference ranges. Patients were divided into 2 groups, based on whether their mean baseline WBC, lymphocyte, PMN, platelet, and RBC counts were within or lower than the reference range. No baseline cell counts were higher than the reference range. We then determined whether values for individual patients in these groups differed significantly between the 2 subsets. Data were also analyzed to account for possible association of stavudine and/or zidovudine use with lymphopenia, neutropenia, anemia, and macrocytosis.

Statistical methods. Nonparametric methods were used for hypothesis testing. To determine whether changes from baseline values were statistically significant, the data were submitted to the single-sample signed-rank test. Spearman's rank correlations were determined to measure associations among δ lineages, δ viral loads, and baseline lineage counts. To compare the magnitude of change in lineages according to the use of zidovudine or stavudine, the data were submitted to the 2-sample Wilcoxon rank-sum test. SAS system version 6.12 for Windows NT (SAS Institute, Cary, NC) was used to perform all statistical analyses.

Results

Characteristics of the 66 patients in our study population are shown in table 1. More than 50% of the patients were white, and all but 1 were male. The median age of the patients was 42.5 years (range, 24–65 years), and the median baseline $CD4^+$ T cell count before initiation of effective antiretroviral therapy was 210/μL. The antiretroviral regimens used by these patients

Table 1. Characteristics of 66 HIV-1–seropositive patients evaluated for changes in hematologic parameters after the initiation of effective antiretroviral therapy.

Characteristic	No. (%) of patients
Sex	
Male	65 (98.5)
Female	1 (1.5)
Race or ethnicity	
White	36 (54)
Black	11 (17)
Hispanic	8 (12)
Asian-American	6 (9)
Unknown	5 (8)

Table 2. Antiretroviral regimens used by 66 HIV-1–seropositive patients evaluated for changes in hematologic parameters after the initiation of effective antiretroviral therapy.

Regimen	No. (%) of patients
>2 drugs	
IDV, 3TC, d4T	16 (24)
IDV, 3TC, ZDV	10 (15)
NFV, 3TC, d4T	7 (11)
RTV/SQV, 3TC, d4T	6 (9)
RTV, 3TC, d4T	5 (7.5)
RTV, 3TC, ZDV	3 (4.5)
NFV, 3TC, ZDV	2 (3)
NFV, ddI, d4T	2 (3)
RTV/SQV, ddI, d4T	2 (3)
Other	5 (8)
Dual therapy	
IDV, ZDV	3 (4.5)
IDV, d4T	3 (4.5)
Other	1 (1.5)
IDV monotherapy	1 (1.5)

NOTE. ddI, didanosine; d4T, stavudine; IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir (hard gel capsule); 3TC, lamivudine; ZDV, zidovudine.

are summarized in table 2. Most patients (88%) were receiving therapy including ⩾1 protease inhibitor and at least 2 nucleoside analogues; 27% were receiving antiretroviral therapy including zidovudine. Median baseline $CD4^+$ T cell, total WBC, PMN, lymphocyte, monocyte, platelet, and RBC counts for the group are shown in table 3, as is the percentage of patients whose baseline cell counts fell below the reference range.

After the initiation of effective antiretroviral therapy, viral loads declined to undetectable levels from a median baseline level of 31,170 copies/mL (range, 660–561,100 copies/mL), and $CD4^+$ T cell counts increased from a median of 210/μL to a high of 350/μL at the end of 1 year, with statistically significant changes from baseline ($P < .001$) at each of the 4 3-month intervals (table 4). In addition, significant increases were observed in the circulating number of other hematopoietic lineages. These changes are detailed in table 4 and are represented graphically in figure 1. For example, the median baseline WBC count in the 3-month period before effective antiretroviral therapy was $4.5 \times 10^3/\mu L$; after initiation of effective antiretroviral therapy, increases in the median WBC count were sustained during each of the next 3-month intervals (δ values were as follows [all $\times 10^3$]: month 3, 0.35 [$P \leqslant .01$]; month 6, 0.80 [$P < .001$]; month 9, 1.30 [$P < .001$]; and month 12, 0.73 [$P < .001$]). Similarly, median PMN, monocyte, lymphocyte, $CD4^+$ T lymphocyte, and platelet counts all remained significantly above baseline values during each 3-month interval in the year following initiation of effective antiretroviral therapy. These increases were highly significant during all intervals (see table 4), with the exception of the PMN count in the first 3-month interval ($P = .23$) and the platelet count in the second 3-month interval ($P = .17$). There was no correlation observed between baseline viral loads and either baseline cell counts or subsequent changes in cell counts of any lineage (data not shown).

The erythroid lineage was the only one for which the measure value decreased after initiation of effective antiretroviral therapy (table 4). Notwithstanding this decrease in RBC count, the hemoglobin level and MCV increased significantly during all of these 3-month intervals except the first 3-month period. A median baseline hemoglobin level of 13.9 g/dL (range, 9.9–16.8 g/dL) increased to 14.1 g/dL at month 3 ($P =$ NS), 14.6 g/dL at month 6 ($P \leqslant .01$), 14.6 g/dL at month 9 ($P = .001$), and 14.3 g/dL at month 12 ($P \leqslant .01$). An increase in MCV also was sustained, from a baseline of 95.5 fL (range, 82–137.5 fL) to 98.9fL at month 3 ($P \leqslant .01$), 105.5 fL at month 6 ($P < .001$), 106 fL at month 9 ($P < .001$), and 102.8 fL at month 12 ($P < .001$).

We evaluated whether RBC indices were differentially influenced by the use of stavudine or zidovudine. The use of stavudine was not associated with adverse effects on RBC indices. In contrast, subjects receiving zidovudine therapy had significantly greater decreases in RBC counts than did patients receiving regimens without zidovudine ($P < .05$), although the hemoglobin level and MCV rose during zidovudine therapy (data not shown). Of note, RBC counts remained significantly lower than baseline values in all patients, regardless of zidovudine use.

Two trends emerged when changes in hematopoietic parameters were evaluated relative to baseline cell counts. First, subjects who started effective antiretroviral therapy with low WBC, lymphocyte, PMN, and platelet counts had greater improvements in production of these lineages after the initiation of effective antiretroviral therapy. These improvements were not statistically significant with the exception of that during the second 3-month interval: at this time, patients with low total WBC counts had significantly greater increases in WBC counts than did those who had normal baseline WBC counts at the initiation of effective antiretroviral therapy ($P = .03$). Second, those patients who started effective antiretroviral therapy with normal baseline RBC counts were much more likely to have decreases in the number of circulating RBCs than were those who had abnormally low RBC counts at the outset. The differences between these 2 groups were highly significant during

Table 3. Baseline values for hematopoietic lineages from 66 HIV-1–seropositive patients evaluated for changes in hematologic parameters after the initiation of effective antiretroviral therapy.

Lineage	Median cell count (range)	% of patients with cytopenia[a]
$CD4^+$ T cells	210/μL (6–637)	89.2
WBCs	$4.5 \times 10^3/\mu L$ (2.1–9.3)	32.8
Lymphocytes	$1.3 \times 10^3/\mu L$ (0.4–3.9)	31
PMNs	$2.3 \times 10^3/\mu L$ (1–5.7)	20.7
Platelets	$208 \times 10^3/\mu L$ (58–303)	17.5
RBCs	$4.5 \times 10^6/\mu L$ (2.6–5.6)	46.9

NOTE. PMNs, polymorphonuclear leukocytes.

[a] Defined as levels of circulating cells below a reference range, as follows: $CD4^+$ T cells, 420–1250/μL; WBCs, $3.9–11.7 \times 10^3/\mu L$; lymphocytes, $1–5.1 \times 10^3/\mu L$; PMNs, $1.7–8 \times 10^3/\mu L$; platelets, $150–450 \times 10^3/\mu L$; and RBCs, $4.4–5.8 \times 10^6/\mu L$ for men and $3.9–5.5 \times 10^6/\mu L$ for women.

Table 4. Baseline and δ values for hematopoietic lineages after initiation of effective antiretroviral therapy for 66 HIV-1–seropositive patients who were evaluated for changes in hematologic parameters.

	Baseline		3 mo		6 mo		9 mo		12 mo	
Lineage	Cell count, median (range)[a]	n	δ, median (range)	n	δ, median (range)	n	δ, median (range)	n	δ, median (range)	n
$CD4^+$ T cells/μL	210 (6 to 637)	64	54 (159 to 310)	53	85 (−123 to 346)	49	112 (−160 to 460)	51	140 (−140 to 450)	50
WBCs $\times 10^3$/μL	4.5 (2.1 to 9.3)	64	0.35[a] (−3.9 to 7)	55	0.80 (−3.1 to 5.1)	52	1.30 (−3.1 to 5.7)	51	0.73 (−3.4 to 4.1)	48
Lymphocytes $\times 10^3$/μL	1.3 (0.4 to 3.9)	59	0.30 (−0.96 to 2.1)	50	0.30 (−1.4 to 1.4)	46	0.44 (−1.1 to 1.5)	47	0.38 (−1.1 to 2.7)	40
PMNs 10^3/μL	2.3 (1 to 5.7)	59	0.15[b] (−2.4 to 5.9)	50	0.34[a] (−2.7 to 4.1)	46	0.55 (−2.5 to 4.7)	47	0.44[a] (−3.7 to 3.9)	40
Platelets $\times 10^3$/μL	208 (58 to 303)	63	18 (−68 to 231)	53	6[b] (−99 to 204)	51	26 (−149 to 155)	50	38 (−59 to 166)	48
RBCs $\times 10^6$/μL	4.5 (2.6 to 5.6)	64	−0.16[a] (−1.4 to 1.2)	55	−0.22 (−1.5 to 1)	52	−0.29 (−1.8 to 1.1)	51	−0.20[a] (−1.3 to 1.3)	48

NOTE. Data were not available for some patients during each of the 3-month intervals. PMNs, polymorphonuclear leukocytes.
[a] As derived by use of a single-sample signed-rank test, all interval changes from baseline values were statistically significant at $P < .001$, except for those for which $P \leq .01$.
[b] As derived by use of a single-sample signed-rank test, all interval changes from baseline values were statistically significant at $P < .001$, except for those for which $P > .05$.

all 4 3-month intervals after the initiation of therapy ($P < .001$).

Discussion

Many patients with advanced HIV-1 disease will develop ⩾1 cytopenias, suggesting that the virus may disrupt central multilineage hematopoiesis. The mechanisms responsible for these cytopenias are yet unclear and may involve underlying opportunistic infections (e.g., those with cytomegalovirus or *Mycobacterium avium* complex), medications (e.g., zidovudine), autoimmune reactions, or adverse effects caused by HIV-1 gene products [2, 3]. Another potentially significant mechanism is hematosuppression caused by infection and destruction of hematopoietic progenitor cells and/or their stromal supports [1–3]. If this latter mechanism is operative, hematopoietic recovery (including increases in circulating $CD4^+$ T cell counts) might occur when HIV-1 replication is suppressed, provided that hematopoietic progenitor cells are not irreversibly damaged. We show here that there are sustained increases in the circulating levels of total WBCs, lymphocytes, PMNs, platelets, and hemoglobin after the initiation of effective antiretroviral therapy. These increases occur within the first 3 months of therapy and are sustained for at least 1 year when the peripheral viral load is suppressed to undetectable levels (<500 copies/mL, determined by a branched DNA assay). We eliminated from our study population patients who had received transfusions or therapeutic agents known to enhance hematopoiesis, as well as patients with systemic diseases that might suppress hematopoiesis (e.g., neoplasms and disseminated infection with *M. avium* complex or *Mycobacterium tuberculosis*). Therefore, improved multilineage hematopoiesis observed in our cohort appears to be related to reductions in HIV-1 load.

These observations are consistent with several hypotheses. Reductions in viral load may be associated with the following: redistribution of sequestered cells into the bloodstream, leading to an apparent increase in the representation of multiple lineages; decreased destruction of mature hematopoietic cells of multiple lineages; and/or increased production from multilineage and/or lineage-restricted hematopoietic progenitor cells. Because redistribution of $CD4^+$ T cells appears to occur during the initial months of effective combination therapy [9] and increased production of new T cells is observed thereafter [10], we favor the hypothesis that the sustained recovery of other lineages (which was observed in this study) is also the result of increased production from multilineage and/or lineage-restricted progenitors (e.g., in the bone marrow, thymus, or peripheral lymphoid organs). This possibility seems even more likely since mature cells of other lineages do not appear to be targeted by HIV-1 for accelerated destruction, as can be the case for mature $CD4^+$ T cells [1–3]. Nonetheless, the current data do not permit firm discrimination between the abovementioned mechanisms, and indeed, all of the mechanisms may operate simultaneously. Ongoing analysis of cell turnover before and after effective antiretroviral therapy, particularly turnover in bone marrow, should provide additional insights into these and other possibilities.

Another limitation of this study is that it was not possible to analyze a control group of untreated HIV-1–seropositive individuals with detectable levels of viremia. Previous studies indicate, however, that increases in hematologic parameters over time would not likely occur for such individuals [2, 3]. It would of interest to determine whether increases in multiple hematologic parameters are also observed for individuals whose viral loads are only partially suppressed during combination antiretroviral therapy. For some such patients, for example, persistent increases in $CD4^+$ T cell counts are noted [11]. We are currently evaluating the possibility that there may be improvements in production of other hematopoietic lineages in such cases.

A striking and discrepant observation was that circulating RBC counts decreased significantly after the initiation of effective antiretroviral therapy, despite sustained increases in the hemoglobin level and MCV. This effect was observed for the entire cohort and was most pronounced for patients receiving zidovudine treatment. The clinical significance of this anomaly

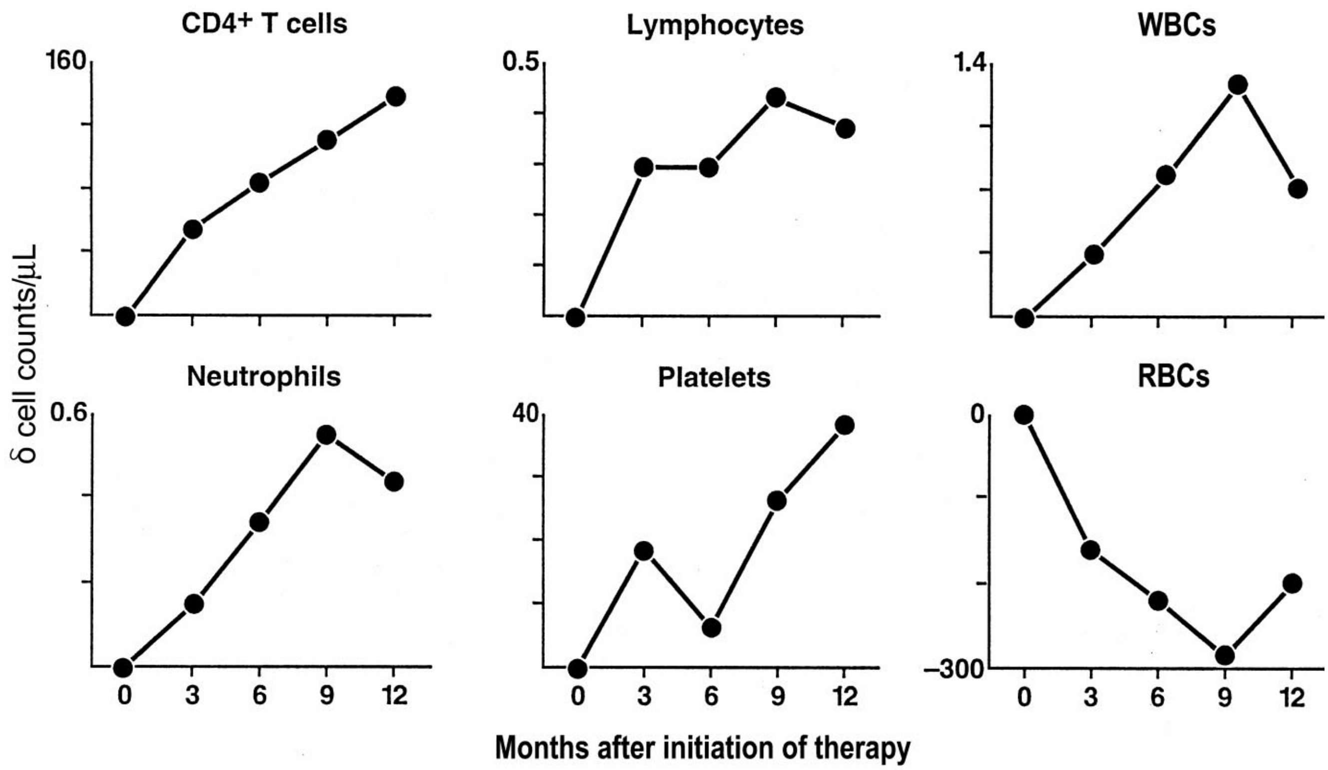

Figure 1. Changes in the circulating numbers of peripheral blood cells for 66 HIV type 1–seropositive patients during the year after initiation of effective antiretroviral therapy. On each graph, data points represent the δ cell counts (*y* axis) plotted against baseline and 3-month-interval median values (*x* axis) for the cohort. The ranges for these values are detailed in table 4. Note that the *y* axis varies according to the cell type: $CD4^+$ T cells, /μL; lymphocytes, $\times 10^3/\mu L$; WBCs, $\times 10^3/\mu L$; neutrophils, $\times 10^3/\mu L$; platelets, $\times 10^3/\mu L$; and RBCs, $\times 10^6/\mu L$.

is at present unclear. However, the use of effective antiretroviral therapy (and, most especially, zidovudine) may be associated with defects in the production of erythrocytes from erythroid progenitor cells, leading to the generation of fewer but larger cells.

Several mechanisms may underlie the cytopenias associated with HIV-1 disease. First, HIV-1 may directly infect pluripotent and lineage-restricted hematopoietic progenitor cells, resulting in their destruction or dysregulation. Although studies suggest that pluripotent progenitors are only rarely infected by HIV-1 [2, 3, 12, 13], infection of lineage-restricted progenitors is well described for megakaryocytes [14], eosinophils [15], and T lymphocytes [16]. Furthermore, HIV-1 proteins (e.g., gp120) can induce apoptosis in these cells [17, 18], accounting for reduced numbers of burst- and colony-forming cells seen in the peripheral blood of HIV-1–seropositive patients. Second, HIV-1 may infect marrow stromal cells [19–22], indirectly causing suppressed proliferation of noninfected progenitor cells [23, 24]. Third, HIV-1 infection may disrupt the extensive cytokine network that governs the differentiation and proliferation of hematopoietic cells, not only by infection and dysregulation of progenitor and stromal cells but also by effects on mature cells. For example, infection of mononuclear phagocytes suppresses release of granulocyte-macrophage colony-stimulating factor [25], macrophage colony-stimulating factor, and IL-1, all of which are potent inducers of bone marrow proliferation [26]. Inhibitory cytokines also may be upregulated in HIV-1–seropositive patients [27–30], suppressing proliferation and/or inducing programmed cell death in noninfected hematopoietic cells [31–33]. Finally, the HIV-1 Tat protein can induce the release of potent marrow suppressive factors (e.g., IFN-γ and TNF-α) in $CD8^+$ cells without cellular infection by HIV-1 [34, 35].

The observations reported here suggest that the suppressive effects of HIV-1 on multilineage hematopoiesis may be largely indirect. No correlation was observed between baseline viral loads and cell counts or between changes in viral load and changes in cell counts after the initiation of therapy. Perhaps most strikingly, increases in cell counts were sustained for at least 6 months after viral replication had been suppressed below detectable limits. Possibly, indirect adverse effects of virus persisted for long periods after such suppression. If so, and if such effects can be identified and reversed, it may be possible to accelerate recovery of the hematopoietic system in HIV-1 disease.

Irrespective of the exact mechanism, our data underscore the fact that HIV-1 disease is associated with suppression of multiple hematopoietic lineages. All hematopoietic cell lines, with the exception of RBCs, had rapid and persistent recovery after initiation of effective antiretroviral therapy. The CD4+ T cell deficiency characteristic of HIV-1 infection may thus be largely due to decreased production rather than to increased destruction of mature CD4+ T cells. Reciprocally, increases in blood cell counts (including CD4+ T cell counts) that are associated with effective antiretroviral therapy may result in part from increased production of blood cells from hematopoietic progenitors in central compartments (e.g., the bone marrow and thymus). Further studies of impaired cell production in HIV-1 disease may point to therapeutic approaches that facilitate immunologic recovery after viremia is suppressed.

Acknowledgments

We gratefully acknowledge the assistance of Roy Colombe, Joe Palace, and Lidia Moy of the Laboratory Information Systems, Clinical Laboratories, San Francisco General Hospital.

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