Suppression of Murine Hematopoiesis *In Vivo* after Chronic Administration of Zidovudine: Evidence that Zidovudine-Induced Anemia Is the Result of Decreased Bone Marrow-Derived, Erythropoietin-Responsive Progenitor Cells (43381)

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Abstract. **We studied the long-term effect of continued zidovudine exposure in mice on hematopoiesis, as determined by peripheral blood indices, assays of erythroid (colony-forming unit-erythroid [CFU-E] and burst-forming unit-erythroid [BFU-E]), myeloid (CFU-granulocyte-macrophage [GM]), megakaryocyte (CFU-Meg), and plasma titers of erythropoietin, granulocyte-macrophage colony-stimulating factor, megakaryocyte colony-stimulating factor, and tumor necrosis factor-α. Dose-escalation of zidovudine (0.1, 1.0, and 2.5 mg/ml) induced a dose-dependent decrease in hematocrit, white blood cells, and platelets. High-dose drug, i.e., >1.0 mg/ml, reduced marrow CFU-E; splenic CFU-E was increased after 1 week, then declined. BFU-E was increased at Weeks 1 and 2, then declined to control levels. Splenic BFU-E rose during the examination period that was dose-dependent. Femoral CFU-GM was cyclic, i.e., low-dose drug, 0.1 mg/ml, was increased gradually, then declined; higher doses of 1.0 and 2.5 mg/ml were lower until Week 5, then were above controls. Splenic CFU-GM was increased initially at Week 2 (1.0 mg/ml), then declined; the higher dose (2.5 mg/ml) increased initially, then declined below controls (Week 6). Femoral CFU-Meg was increased after low-dose drug and inhibited after high dose (2.5 mg/ml). Splenic CFU-Meg was reduced initially, followed by an increase at Week 4. Plasma titer of erythropoietin was elevated, proportional to dose escalation of drug, and inversely proportional to the hematocrit. No difference was observed in plasma levels of granulocyte-macrophage colony-stimulating factor, megakaryocyte colony-stimulating factor, or tumor necrosis factor-α. This study demonstrates that zidovudine-induced anemia results from: (i) inadequate numbers of bone marrow-derived, erythropoietin-dependent hematopoietic progenitors, i.e., CFU-E; and (ii) a shift in erythropoietin-responsive progenitors from bone marrow to spleen capable of responding to obligatory growth factors.** [P.S.E.B.M. 1992, Vol 199]

The drug zidovudine, 3′-azido-3′-deoxythymidine (AZT), a synthetic thymidine analog, has been used clinically in the management of acquired immune deficiency syndrome (AIDS) (1). The drug is an effective inhibitor of viral reverse transcriptase activity due to its altered chemical structure having an azido group in place of a hydroxyl group at the 3′-carbon position in the basic sugar molecule (2). This alteration results in the inability of newly synthesized nucleotides, e.g., zidovudine triphosphate, to be effective in attachment to continue the 5′- to 3′-phosphodiester linkage necessary to construct DNA chain synthesis.

Although zidovudine has been demonstrated clinically to induce immunologic improvement, to decrease the incidence of opportunistic infections, and to reduce AIDS mortality (3), has been the induction of hematopoietic suppression manifested by anemia, neutropenia, and overall bone marrow failure (4–10) has been associated with the use of zidovudine. Although the hematopoietic toxicity associated with zidovudine has

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Received October 9, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted November 26, 1991.

0037-9727/92/1994-0459$3.00/0

been identified, its mechanism is poorly understood. Numerous *in vitro* co-culture studies have identified zidovudine-induced marrow toxicity as the result of inhibition of hematopoietic progenitors, e.g., human colony-forming unit (CFU)-granulocyte-macrophage (GM) (11, 12), CFU-erythroid (E) and burst-forming unit (BFU)-E, and multipotential CFU-GEMM (12), and murine CFU-GM, BFU-E, and CFU-megakaryocytes (Meg) (13), in addition an inhibitor of the number of marrow- and/or spleen-derived CFU-S, CFU-GM, and BFU-E following *in vivo* administration to mice (14, 15). The mechanism of zidovudine induce bone marrow toxicity has been suggested to be the result of its ability to produce pyrimidine starvation as the result of depletion of both intracellular thymidine triphosphate and deoxycytidine triphosphate pools (3, 16).

Although these *in vitro* and, in particular, *in vivo* studies have demonstrated that zidovudine exposure can be toxic to hematopoietic cells, analysis of long-term zidovudine exposure in animals has been limited (15, 17). Although it is a well-recognized fact that human immunodeficiency virus-infected patients may indeed be required to receive zidovudine for months and even years, the long-term consequences of zidovudine exposure on hematopoiesis have not been studied completely to date. In this report, we describe the results of dose-escalation studies that were designed to evaluate the effects of continuous administration of zidovudine on hematopoiesis in mice for a period of 60 days. Animals were examined for changes in erythropoiesis, granulopoiesis, megakaryocytopoiesis, and circulating plasma levels of several hematopoietic growth factors-cytokines that can influence normal, hematopoietic progenitor stem cell proliferation and differentiation.

Materials and Methods

Mice. C57BL/6 female mice, 10–12 weeks of age, were purchased from Jackson Laboratories, Bar Harbor, ME. Animals were housed in plastic cages and fed Purina Lab Chow and water *ad libitum*. After sacrifice by cervical dislocation, femurs and spleens were removed and single-cell suspensions were prepared using McCoy's 5A medium (Gibco, Grand Island, NY) following repeated passage through a 25-gauge needle. After centrifugation (400*g*, 10 min, 4°C), the cells were resuspended in McCoy's medium for routine cell counting using a Coulter counter (model ZBI; Coulter Electronics, Hialeah, FL). Cell viability (>95%) was confirmed via use of the trypan blue exclusion test before plating.

Treatment of Animals with Zidovudine. In order to analyze the effect of continuous long-term zidovudine treatment on murine hematopoiesis, the following studies were conducted. Normal mice were to receive zidovudine treatment in a dose-escalation fashion, i.e., 0.1 mg/ml, 1.0 mg/ml, and 2.5 mg/ml placed in sterile drinking water. Zidovudine in the form of the pure powder was kindly supplied by Dr. Philip Furman, Burroughs-Wellcome, Research Triangle Park, NC. These zidovudine concentrations corresponded to a suboptimal, optimal, and pharmacologic dose of drug with respect to its antiviral potency. Zidovudine levels were confirmed by high-performance liquid chromatography analysis. Normal control animals received only sterile water. Animals were serially sacrificed on a weekly basis for the duration of the 6-week period, with a minimum of five animals per group analyzed.

Assessment of Hematologic Parameters. Prior to sacrifice, blood was collected by cardiac puncture via 3-ml syringes containing a total of 1 mg of Na_2 EDTA. Femurs and spleens were removed and processed as described above. Hematocrits were measured after microcapillary centrifugation. White blood count values were determined via use of a Coulter counter (model ZBI), with differentials determined following staining of peripheral smears with Wright-Giemsa stain. Platelets were determined via use of the ammonium oxalate procedure. Femoral marrow and spleen cells were evaluated for their progenitor cell content of erythroid (CFU-E and BFU-E), granulocyte (CFU-GM), and megakaryocyte (CFU-Meg) using semisolid clonal assays *in vitro* as performed routinely in the laboratory (18).

Measurement of Hematopoietic Growth Factors and Cytokines. *Assay for erythropoietin.* The erythropoietin enzyme immunoassay kit from AMGEN (Thousand Oaks, CA) was used. Briefly, standards and samples of murine plasma were incubated with erythropoietin antibodies attached to microtiter wells in 96-well plates. After 3 hr of incubation at 20°C, the samples were discarded. Horseradish peroxidase conjugated with erythropoietin antibodies was added to the wells, which were further incubated at 37°C for an additional 2 hr. The plates were then washed with phosphate-buffered saline, and a mixture of tetraphenyldiamine and hydrogen peroxide was added to each well. The color reaction was stopped with 2 *N* sulphuric acid after 30 min at 20°C. The optical density in each well was read at 450 nm, with a reference at 630 nm, using a Dynatech enzyme immunoassay plate reader.

Assay for GM-CSF. Plasma derived GM-colony-stimulating factor (CSF) was determined by use of the GM-CSF E-Trac enzyme-linked immunosorbent assay (INCSTAR Corp., Stillwater, MN; catalog no. 23800). Briefly, standards and samples of murine plasma were incubated with GM-CSF-biotinylated monoclonal antiserum attached to 96-well microtiter plates. After incubation for 1 hr at room temperature, the samples were discarded and horseradish peroxidase conjugated with GM-CSF antibodies was added to the wells and further incubated for 30 min at room temperature. The plates were then exposed to chromogen for an additional 30 min in order to allow for optimum color

development. The color reaction was stopped with the addition of stopping solution, which resulted in a change in color development from blue to yellow. The optical density of each well was determined at 450 nm using a Dynatech enzyme immunoassay plate reader.

Assay for tumor necrosis factor-α. The L929 cell line was used to assay tumor necrosis factor (TNF)-α activity. L929 cells are efficiently killed by TNF-α, but are resistant to recombinant interleukin (IL) 1 (19). The cells were harvested by a 1-min trypsinization, washed, and resuspended in RPMI 1640 medium with 5% human antibody serum. Cells (7×10^3) were plated into each well of a 96-well Microtest II plate and incubated for 6 hr at 37°C. The well contents were aspirated, and then freshly collected, cell-free, pooled plasma samples from control and zidovudine-treated mice were added, in triplicate wells, to the adherent L929 cells. In this assay, growth inhibition induced by TNF-α was quantified by overnight labeling of growing cells in 96-well, flat-bottom plates with [^{3}H]thymidine, following a 3-day culture period. The test was calibrated against recombinant TNF-α. The growth inhibition percentage was calculated as follows:

$$\text{TNF-}\alpha\ \text{(units/ml)(\% growth inhibition)} = 100 \times \frac{\text{(cpm in target cells in medium)} - \text{(cpm in target cells in test samples)}}{\text{(cpm in target cells in medium)}}$$

Assay for Meg-CSF. A modification of a bioassay for GM-CSF, previously reported from this laboratory (20), was performed to evaluate the presence of Meg-CSF in plasma. For the analysis of Meg-CSF, WEHI-3 conditioned medium was used as the standard source of Meg-CSF. The bioassay consisted of progenitor cell assays consisting of plastic-adherent, cell-depleted, normal murine marrow as the source of target cells plated in the presence of dose escalation of either the standard (v/v WEHI-3 conditioned media added at 1%, 5%, and 10%) or an amount of unknown test sample (either 100 or 250 μl). The number of colonies generated by the standard was then compared with the number of colonies observed with the unknown test sample.

Statistical Analysis. Mean ± SE for values comparing control with experimental utilizing triplicate studies were determined and analyzed using the two-sample ranks test of Wilcoxan-White ($P < 0.05$) to determine significance.

Results

Dose-Response Effect of Zidovudine on Hematopoietic Peripheral Blood Indices. The effect of zidovudine treatment on hematocrit, white blood cells, and platelets is given in Table I. Low dose (0.1 mg/ml) drug was without effect (data not shown); however, a dose-dependent decrease in hematocrit was observed as the zidovudine dose increased, i.e., 2.5 mg/ml > 1.0 mg/ml, which reached their nadir by 4 weeks after zidovudine. A partial recovery was observed at Weeks 5 and 6, but the levels were still below normal controls. A dose-dependent decrease in white blood cells was observed that was consistently present during the 6-week period. A significant reduction in circulating platelets was observed that was dose dependent, i.e., 2.5 mg/ml > 1.0 mg/ml.

Table I. Effect of Dose Escalation of Zidovudine on Peripheral Blood Indices[a]

Week	AZT (mg/ml)	Hematocrit (%)	White blood cells ($\times 10^3$)	Platelets ($\times 10^6$)
1	0	50 ± 2	11.8 ± 0.5	0.82 ± 0.05
	1.0	46 ± 2	10.8 ± 0.5	0.80 ± 0.07
	2.5	47 ± 3	10.5 ± 0.5	0.75 ± 0.06
2	0	52 ± 2	10.5 ± 0.5	0.78 ± 0.04
	1.0	45 ± 2[b]	9.5 ± 0.5	0.72 ± 0.02
	2.5	40 ± 2[b]	6.3 ± 0.6[b]	0.62 ± 0.03[b]
3	0	48 ± 2	11.2 ± 0.3	0.85 ± 0.05
	1.0	42 ± 2[b]	8.1 ± 0.4[b]	0.70 ± 0.03[b]
	2.5	32 ± 2[b]	5.5 ± 1.0[b]	0.55 ± 0.05[b]
4	0	50 ± 3	11.8 ± 0.4	0.84 ± 0.04
	1.0	37 ± 3[b]	7.2 ± 0.6[b]	0.65 ± 0.03[b]
	2.5	25 ± 2[b]	5.4 ± 0.6[b]	0.53 ± 0.02[b]
5	0	51 ± 2	11.5 ± 0.3	0.82 ± 0.04
	1.0	43 ± 3[b]	6.2 ± 0.3[b]	0.63 ± 0.03[b]
	2.5	35 ± 2[b]	4.3 ± 0.5[b]	0.51 ± 0.03[b]
6	0	50 ± 2	10.5 ± 0.5	0.87 ± 0.03
	1.0	40 ± 3[b]	4.3 ± 0.5[b]	0.60 ± 0.03[b]
	2.5	33 ± 3[b]	3.5 ± 0.5[b]	0.50 ± 0.05[b]

[a] Values are mean ± SE from triplicate studies, nine animals per group.
[b] P-value < 0.05.

Dose-Response Effect of Zidovudine Treatment on Hematopoietic Progenitors. The influence of zidovudine on erythroid colony formation from femoral marrow and spleen is given in Figure 1. Marrow CFU-E (Fig. 1A) were increased in response to the low-dose zidovudine (0.1 mg/ml); however, following exposure to higher doses, i.e., 1.0 and 2.5 mg/ml, marrow CFU-E demonstrated a significant reduction in CFU-E which was below the normal control level that continued for the entire length of the study. Splenic CFU-E (Fig. 1B) produced an initial rise in CFU-E from all zidovudine concentrations, then declined to control levels for the duration of the study, except for a short rebound present 4 weeks after drug treatment that was prominent in the 1.0- and 2.5-mg/ml group. Marrow BFU-E (Fig. 2A) were increased early during Weeks 1 and 2 after zidovudine treatment; however, by Week 3, the values were within the normal control range. No effect was observed at the low zidovudine dose (0.1 mg/ml). In contrast, splenic BFU-E (Fig. 2B) were significantly elevated, which was dose dependent, and reached a zenith by 4 weeks after zidovudine treatment.

The effect of zidovudine on granulocyte colony formation is represented in Figure 3. Femoral CFU-GM (Fig. 3A) responded with an increase in CFU-GM;

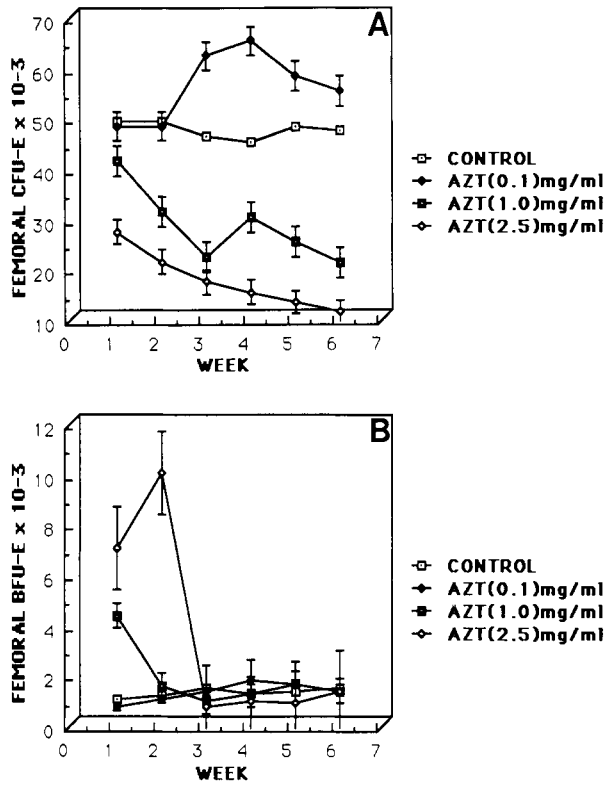

Figure 1. (A) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on femoral CFU-E. Mean ± SE, pooled samples performed in triplicate. (B) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on femoral BFU-E. Mean ± SE, pooled samples performed in triplicate.

i.e., higher doses, 1.0 and 2.5 mg/ml, produced an increase in CFU-GM, which was not evident until Weeks 5 and 6 after zidovudine treatment. Splenic CFU-GM (Fig. 3B) responded with an increase in CFU-GM that was increased at Week 2 from the 1.0-mg/ml dose group, at Week 4 from the low dose (0.1 mg/ml) group, and from Week 5 with the high dose (2.5 mg/ml) group.

The effect of zidovudine on megakaryocyte colony formation is shown in Figure 4. A slight inhibition in femoral CFU-Meg (Fig. 4A) was observed at Week 1 from the low-dose (0.1 mg/ml) zidovudine group which rebounded significantly above control by Week 2. Splenic CFU-Meg (Fig. 4B) was elevated at Week 1 from the 1.0-mg/ml dose group; however, the highest increase in CFU-Meg colony formation was observed 4 weeks after zidovudine treatment.

The ability of zidovudine to influence several hematopoietic growth factors-cytokines was examined. There was no significant increase in either GM-CSF, Meg-CSF, or TNF-α measured in plasma obtained from animals treated with escalating doses of zidovudine compared with non-zidovudine-treated controls for each week analyzed (1–6). However, the level of erythropoietin was increased, which was proportional to the dose escalation in drug and which was inversely related to the level of hematocrit, e.g., zidovudine (2.5 mg/ml), control: week 1, $< 3 \pm 2$ mU/ml; week 3, 55 ± 10 mU/ml; (that peaked at) week 4, 95 ± 15 mU/ml.

Discussion

Zidovudine continues to be the treatment of choice for patients with AIDS; however, the use of zidovudine has been associated with the development of significant hematopoietic toxicity manifested by anemia, neutropenia, and, occasionally, thrombocytopenia. Since the nature of current therapeutic strategies warrants continued zidovudine treatment, potentially for long durations, possibly for the rest of a patient's life, a thorough analysis of the long-term consequences of zidovudine exposure and its effects on hematopoiesis need to be clearly elucidated.

A recent report by Cronkite and Bullis (15) analyzed the effect of continued zidovudine treatment administered to normal CBA/Ca mice for 7 weeks' duration. The concentrations of zidovudine evaluated were 0.5 and 1.0 mg/ml. This study reported the development of significant macrocytic anemia, neutropenia, and lymphopenia as the result of zidovudine exposure. Bone marrow- and spleen-derived spleen colony-forming cell (CFU-S) content were also evaluated and found to be significantly reduced as the result of zidovudine exposure, which recovered following cessation of drug. Stem cell function of this pool of hematopoietic progenitors was found to be normal, as CFU-S derived from zidovudine-treated recipient animals were capable of rescuing lethally irradiated animals; therefore, zidovudine treatment, at least for this duration of exposure, failed to produce a long-lasting effect in the mitotic potential of these hematopoietic progenitors.

A more recent study by Chow *et al.* (17) investigated the anemia induced by zidovudine after 15 days of treatment to either normal or MAIDS-infected C57BL/6 mice. Zidovudine was examined at doses of 1.0 and 2.5 mg/ml. Dose-dependent anemia developed in both groups. In this study, only the early-stage erythroid progenitor, i.e., BFU-E, was evaluated from bone marrow and spleen. BFU-E, from either tissue, was increased significantly (5-fold) following the 15-day treatment. The mean plasma titer of erythropoietin was evaluated and found to be elevated in both groups, which correlated with the degree of anemia observed. Because the degree of reticulocytosis produced was inappropriate for the degree of anemia following zidovudine treatment, this response suggested to these authors that the anemia produced, as the result of zidovudine exposure, was the result of inappropriate erythroid differentiation. An analysis of the late-stage erythroid progenitor cell, i.e., CFU-E, could have provided additional supportive data to substantiate this hypothesis.

The results given in this report demonstrate that continued zidovudine exposure (for a period of 6 weeks) produced a dose-dependent anemia, neutropenia, and

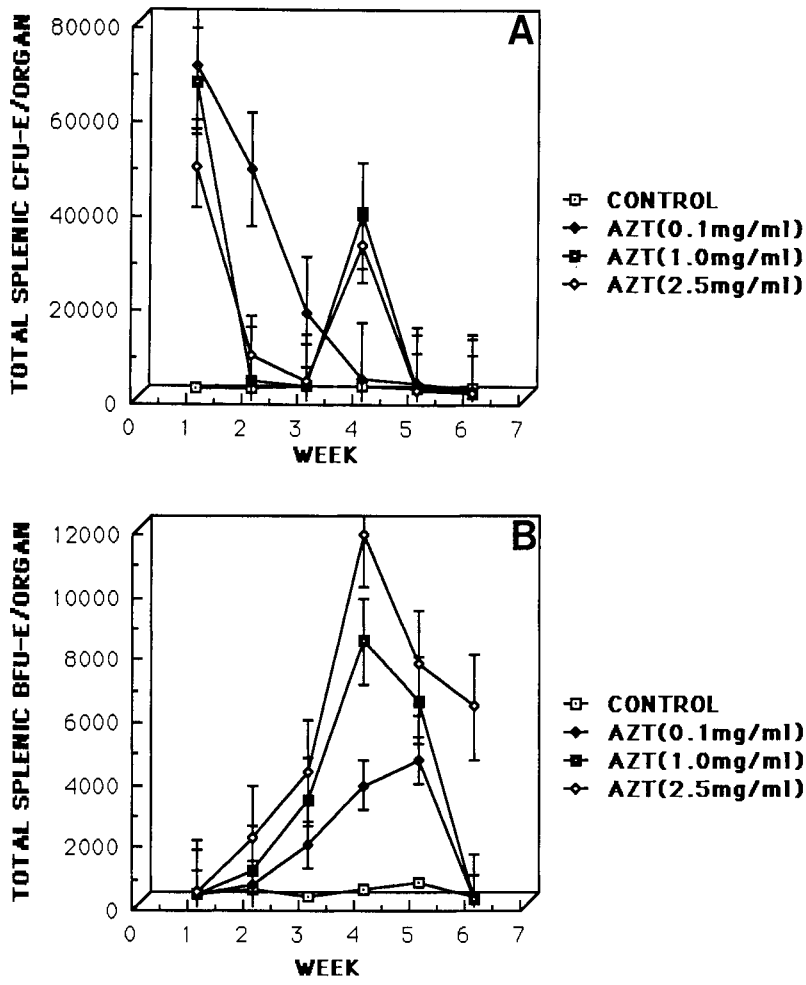

Figure 2. (A) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on splenic CFU-E. Mean ± SE, pooled samples performed in triplicate. (B) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on splenic BFU-E. Mean ± SE, pooled samples performed in triplicate.

thrombocytopenia. Analysis of the committed stem cell compartment for erythropoiesis, granulopoiesis, and megakaryocytopoiesis, from bone marrow and spleen, revealed that not only were marrow and splenic BFU-E elevated following zidovudine treatment, but the CFU-E compartment was also influenced following drug treatment. Marrow CFU-E were increased only after exposure to the lowest dose of zidovudine, i.e., 0.1 mg/ml, a concentration that did not produce anemia; however, increased concentrations of zidovudine, 1.0 and 2.5 mg/ml, produced significant reductions in marrow CFU-E that were dose-dependent and correlated with the degree of anemia, as measured by the reduction in hematocrit. In contrast, splenic CFU-E was initially elevated at Week 1 of drug treatment, and then declined, only to increase again at Week 4; however, this secondary response was only observed from the two high-drug groups, i.e., 1.0 and 2.5 mg/ml. The erythropoietin response following zidovudine treatment corresponded to the degree and level of anemia produced. These stem cell and erythropoietin data suggest that the induction of anemia, as a consequence of zidovudine exposure, results from the inability of the bone marrow to produce adequate numbers of the erythropoietin-responsive precursors, i.e., CFU-E, possibly as the result of a blockage in differentiation between BFU-E and CFU-E, although studies by Lutton *et al.* (21) have identified that AZT-induced toxicity may be due in part to a depression in the pool of available heme, since α-aminolevulinic acid synthase activity was significantly reduced in bone marrow cells from AZT-treated animals. These studies demonstrate that both bone marrow and spleen accumulate increased numbers of early erythroid progenitors, BFU-E. These data provide intriguing information for designing strategies to overcome this blockage in erythroid differentiation. More recent data from our laboratory have indicated that AZT-induced anemia and reduction in bone marrow-derived erythroid progenitors *in vivo* can be reversed with combination treatment with erythropoietin and IL-3 (manuscript in preparation). As was concluded in the study of Chow *et al.* (17), the erythropoietin-responsive mechanism is intact and fully capable of responding to inadequate production of erythropoiesis

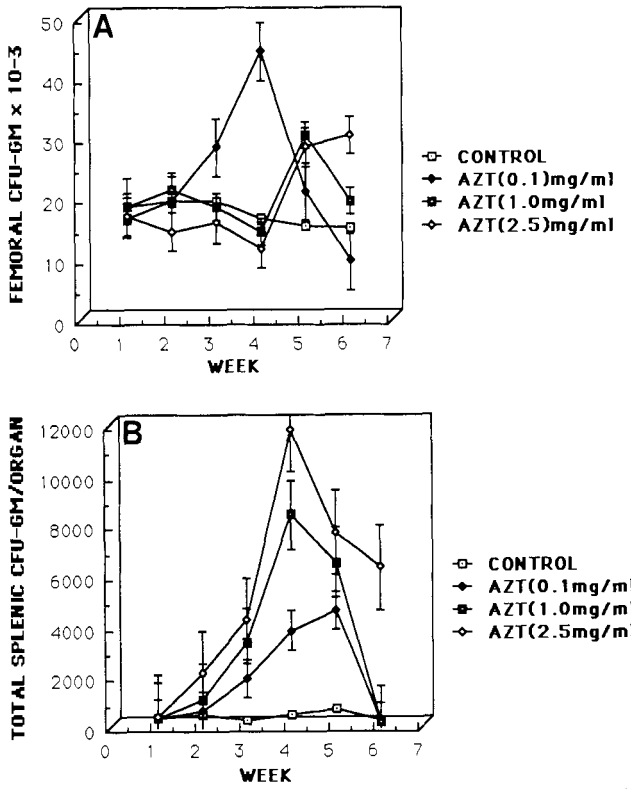

Figure 3. (A) dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on femoral CFU-GM. Mean ± SE, pooled samples performed in triplicate. (B) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on splenic CFU-GM. Mean ± SE, pooled samples, performed in triplicate.

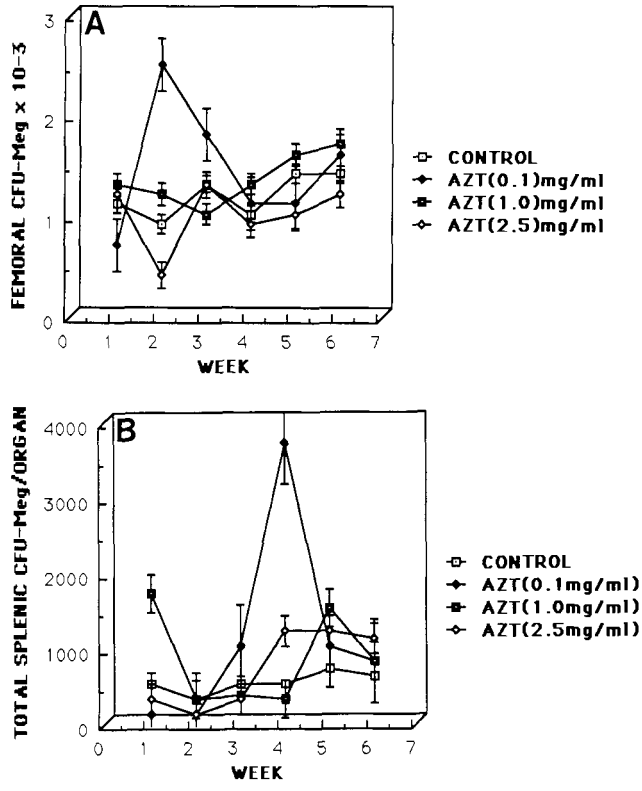

Figure 4. (A) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on femoral CFU-Meg. Mean ± SE, pooled samples, performed in triplicate. (B) Dose-response effect of continuous exposure to zidovudine (0.1, 1.0, and 2.5 mg/ml) on splenic CFU-Meg. Mean ± SE, pooled samples, performed in triplicate.

following zidovudine treatment. However, the role of erythropoietin remains controversial, since the addition of erythropoietin alone *in vitro* (2–10 units/ml) fails to reduce the magnitude of AZT toxicity on human bone marrow-derived erythroid progenitors; but when it was added in the presence of either IL-1 or IL-6, a reduction of AZT suppression of erythroid progenitors was demonstrated (22). Additional studies are in progress to elucidate how best to overcome this blockage in erythroid progenitor cell differentiation.

CFU-GM and CFU-Meg responses from bone marrow and spleen following zidovudine produced a similar response. In general, although both neutropenia and thrombocytopenia were demonstrated, we did not observe an acute suppression of either CFU-GM or CFU-Meg from either bone marrow or spleen, with the exception of a reduced CFU-Meg following 2 weeks of exposure of zidovudine at 2.5 mg/ml. This response was corrected by Week 3, since the CFU-Meg were within the control range. The appearance of decreased neutrophils and platelets without the appearance of an acute depression in progenitor cells cannot explain the reduction in peripheral cells observed. This may be the result of the higher sensitivity of erythroid progenitors to the toxic action of zidovudine. *In vitro* erythroid progenitors demonstrate a 2- and 4-log difference in sensitivity to zidovudine when comparing the effects of zidovudine to megakaryocyte and granulocyte-macrophage progenitors, respectively (13).

Furthermore, we were unable to detect any difference, compared with normal controls, in plasma titers of GM-CSF, Meg-CSF, and TNF-α. TNF-α was analyzed to evaluate whether the increased presence of a cytokine known to inhibit hematopoietic-committed progenitor cell colony formation could be responsible for the decrease in either erythropoiesis, granulopoiesis, or megakaryocytopoiesis. Our results suggest that these factors are not increased following dose escalation of zidovudine; however, as described above, this reaction may be the result of differences in the dose-response curve of zidovudine to committed hematopoietic progenitors. Additional studies are presently in progress to evaluate the relationships between circulating growth factors-cytokines and the extent of zidovudine use in the treatment of human immunodeficiency virus-infection. Such information may prove to be useful in designing more effective treatment strategies incorporating antiviral drugs in the treatment of AIDS.

These studies were supported in part by grants from the Department of Health and Human Services, Grant CA-46509 from the National Cancer Institute, and a grant from the Veterans Adminis-

tration. The authors acknowledge the assistance of Betty Miller in the performance of the TNF-α assays.

1. Yarchoan R, Klecker RW, Weinhold KJ, Markham PD, Lyerly HK, Durack DT, Gelmann E, Nusinoff-Lehrman S, Blum RM, Barry DW, Shearer GM, Fischl MA, Mitsuya H, Gallo RC, Collins JM, Bolognesi DP, Myers CE, Broder S. Administration of 3′-azido-3′-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet **1**:575–577, 1986.
2. Mitsuya H, Weinhold KJ, Furman PA, St Clair MH, Nusinoff-Lehrman S, Gallo RC, Bolognesi D, Barry DW, Broder S. 3′-azido-3′-Deoxythymidine (BW-A509), an anti viral agent that inhibits the infectivity and cytopathetic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus *in vitro*. Proc Natl Acad Sci USA **82**:7096–7100, 1985.
3. DeVita VT, Broder S, Fauci AS, Kovacs JA, Chabner BA. Developmental therapeutics and the acquired immunodeficiency syndrome. Ann Int Med **106**:568–581, 1987.
4. Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volverding PA, Laskin GL, Leedom JM, Groopman JE, Mildvan D, Schooley RT, Jackson GG, Durack DT, King D. The efficacy of azidothymidine (AZT) in the treatment of AIDS and the AIDS related complex. N Engl J Med **317**:185–191, 1987.
5. Forester G. Profound cytopenia secondary to azidothymidine. N Engl J Med **317**:772, 1987.
6. Gill PS, Raick M, Brynes RK, Causey D, Loureiro C, Levine AM. Azidothymidine associated with bone marrow failure in the acquired immunodeficiency syndrome (AIDS). Ann Int Med **107**:502–505, 1987.
7. Gottlieb MS, Wolfe PR, Chafey S. Response of AIDS-related thrombocytopenia to intravenous and oral azidothymidine (3′-azido-3′-deoxythymidine). AIDS Res Hum Retroviruses **3**:109–112, 1987.
8. Groopman JE, Mitsuyasu RT, deLeo MJ, Oette DH, Golde DW. Effect of recombinant human granulocyte-macrophage colony stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. N Engl J Med **317**:593–598, 1987.
9. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildran D, Hirsch MS, Jackson GG, Durack DT, Nusinoff-Lehrman S, AZT Collaborative Working Group. The toxicity of azidothymidine (AZT) in the treatment of AIDS and AIDS-related complex: A double-blind, placebo-controlled trial. N Engl J Med **317**:192–197, 1987.
10. Dournon E, Rozenbaum W, Michon C, Penonne C, DeTruchis P, Bouvet E, Leevachen M, Matheron S, Gharakhanian S, Girard PM, Salmon D, Ceport C, Dazza MC, Regnier B. Effects of zidovudine in 365 consecutive patients with AIDS or AIDS-related complex. Lancet **2**:1297, 1988.
11. Sommadossi JP, Zhu Z, Carisle R. Mechanism of toxicity of 3′-azido-3′-deoxythymidine in normal hematopoietic progenitor cells: Evidence of inhibition of the de novo pyrimidine pathway. Proc Am Assoc Cancer Res **28**:329A, 1987.
12. Dainiak N, Worthington M, Riordon MA, Kreczko S, Goldman L. 3′-Azido-3′-deoxythymidine (AZT) inhibits proliferation *in vitro* of human haematopoietic progenitor cells. Br J Haematol **69**:299–304, 1988.
13. Gallicchio VS, Doukas MA, Hulette BC, Hughes NK, Gass C. Protection of 3′-azido-3′-deoxythymidine induced toxicity to murine hematopoietic progenitors (CFU-GM, BFU-E, and CFU-Meg) with interleukin-1. Proc Soc Exp Biol Med **192**:201–204, 1989.
14. Bogliolo G, Lerza R, Mercoboni M, Saviane A, Pannacciulli I. Azidothymidine-induced depression of murine hemopoietic progenitor cells. Exp Hematol **16**:938–940, 1989.
15. Cronkite EP, Bullis J. *In vivo* toxicity of 3′-azido-3′-deoxythymidine (AZT) on CBA/Ca mice. Int J Cell Cloning **8**:332–345, 1990.
16. Ruprecht RM, O'Brien LG, Rossini LD, Nusinoff-Lehrman S. Suppression of mouse viremia and retroviral disease by 3′-azido-3′-deoxythymidine. Nature **323**:467–469, 1986.
17. Chow FPC, Sutton PA, Hamburger AW. Sensitivity of erythroid progenitor colonies to erythropoietin in azidothymidine treated immunodeficient mice. Br J Haematol **77**:139–144, 1991.
18. Gallicchio VS, Shedlofsky SI, Swim AT, Robinson JM, Hulette BC, Messino MJ, Doukas MA. Modulation of murine hematopoiesis *in vivo* with recombinant murine interleukin-1. J Biol Response Mod **8**:422–439, 1989.
19. Bertoglio J, Rimsky L, Kleinerman ES, Lachman LB. Cell line-derived interleukin-1 is cytotoxic for melanoma cells and promotes the proliferation of an astrocytoma cell line. Lymphokine Res **6**:83–87, 1987.
20. Gallicchio VS, Chen MG, Watts TD. Lithium stimulated recovery of granulopoiesis after sublethal irradiation is not mediated via increased levels of colony stimulating factor (GM-CSF). Int J Radiat Biol **47**:581–590, 1985.
21. Lutton JD, Mathew A, Levere RD, Abraham NG. Role of heme metabolism in AZT-induced bone marrow toxicity. Am J Hematol **35**:1–5, 1990.
22. Gallicchio VS, Hughes NK, Hulette BC, Noblitt LN. Effect of IL-1, IL-6, GM-CSF and erythropoietin on the in vitro toxicity associated with AZT on human marrow haematopoietic progenitor stem cells: CFU-GM and BFU-E. Antiviral Chem Chemother **2**:75–82, 1991.