PROTECTION OF 3'-AZIDO-3'-DEOXYTHYMIDINE INDUCED TOXICITY TO MURINE HEMATOPOIETIC PROGENITORS (CFU-GM, BFU-E AND CFU-MEG) WITH INTERLEUKIN-1

Vincent S. Gallicchio[1,2,3], M.A. Doukas[1], B.C. Hulette[1], N.K. Hughes[1,3], and C. Gass[1]

Hematology/Oncology Division[1], Departments of Medicine, Microbiology and Immunology[2], and Clinical Sciences[3], University of Kentucky Medical Center, Lucille P. Markey Cancer Center, 800 Rose Street, Lexington, KY 40536-0084

ABSTRACT. 3'-Azido-3'-deoxythymidine (AZT) has attained wide clinical utility in the treatment of acquired immunodeficiency syndrome (AIDS). Unfortunately, associated with AZT use, is the development of severe hematopoietic toxicity as manifested by anemia, neutropenia and overall bone marrow suppression. Interleukin-1 (IL-1), a cytokine, primarily produced by activated macrophages, has been involved in the control of hematopoiesis by acting synergistically with other hematopoietic growth factors, and has been demonstrated to be an effective agent in reducing the myelosuppression associated with the therapy for malignant disease. We report here the ability of recombinant human IL-1 alpha to protect normal murine hematopoietic progenitors (CFU-GM, BFU-E, and CFU-Meg) from the toxic effects of AZT. Following the determination of the LD_{50} dose for each progenitor, IL-1 was added in co-culture studies (10 - 1000 units; 0.001 - 1.0 µg/ml protein) with adherent cell depleted marrow. Marrow progenitors expressed differences in AZT sensitivity, e.g., BFU-E, LD_{50} 5×10^{-9}M; CFU-Meg, LD_{50} 10^{-7}M; CFU-GM, 5×10^{-5}M respectively. IL-1 inhibited AZT induced toxicity. The maximum IL-1 dose effect was observed for CFU-GM and CFU-Meg at 300 units, 0.3 µg protein; however BFU-E required a dose of 600 units, 0.6 µg/ml protein to reverse the effects of AZT. These results demonstrate marrow progenitors respond differently to AZT and identifies the potential efficacy of IL-1 to minimize the hematopoietic toxicity associated with AZT treatment.

INTRODUCTION. The drug 3'-azido-3'deoxythymidine (AZT), a synthetic thymidine analogue, 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 results in the inability of newly synthesized nucleotides to be effective in attachment to continue the 5' to 3' phosphodiester linkage necessary to construct DNA chain synthesis. This AZT activity has initiated investigations to explore the feasibility of its use as an effective agent in antiviral chemotherapy.

Clinical studies employing AZT, at different doses, have observed the induction of hematopoietic toxicity such as anemia, neutropenia and severe bone marrow depression characterized by hypoplasia of immature erythroid elements (3). AZT has also been demonstrated to inhibit normal human hematopoietic progenitors CFU-GM (4); CFU-E and BFU-E; and multipotential CFU-GEMM (5); in addition to inhibiting the number of bone marrow derived CFU-S, CFU-GM and BFU-E following administration in mice (6).

The cytokine interleukin-1 (IL-1), the mediator of the inflammatory response to both acute and immune challenges (7), has been demonstrated to be effective in preventing the hematopoietic toxicit associated with either chemotherapeutic drugs or radiation exposure (8-11). We report here the results of studies designed to investigate the ability of IL-1 to ameliorate AZT toxicity on murine marrow derived hematopoietic progenitors following combination in vitro.

MATERIALS AND METHODS. Mice. Female C3H/HeJ mice, endotoxin resistant, (Jackson Laboratories, Bar Harbor, ME) provided the bone marrow used in all in vitro studies. Animals were housed in a temperature-humidity-controlled environment and fed Purina Lab Chow and acidified water (pH 2.4) ad libitum. Mice were sacrificed by cervical dislocation. Femoral marrow was flushed using a 25-gauge needle. Following centrifugation (400 g, 10 min, 4° C), the cells were suspended in Iscove's modified Dulbecco's medium (GIBCO, Grand Island, NY) for cell counting utilizing a Coulter Counter, Model ZBI (Coulter Electronics, Hialeah, FL). Cell viability was confirmed by the use of the trypan blue exclusion procedure (>95% viability). Following preparation of a single cell suspension, the cell mixture was layered over an equal volume of Ficoll-Paque (specific gravity 1.077 g/cm^3, Pharmacia Fine Chemicals, Piscataway, NJ). Density centrifugation was performed using a centrifuge (model TJ-6R; Beckman Instruments, Inc., Palo Alto, CA) at 500 g for 25 min at 4° C, and the interface layer of low density mononuclear cells were collected, washed and resuspended in fresh media before they were adherent cell depleted.

0037-9727/89/1922-0201$2.00/0

Assay for Hematopoietic Progenitors. Granulocyte-macrophage progenitors, CFU-GM, were cultured as previously reported (10). Briefly, adherent cell depleted marrow was plated at 5 x 10^4 cells/plate, in the presence of 10% pokeweed mitogen spleen cell conditioned medium as the source of GM-CSF. The plates were incubated at 37^o C in 5% CO_2. CFU-GM produced with this material following seven days incubation were a mixture of neutrophil and macrophage cell types as determined by specific histochemical staining. Colonies consisting of more than 50 cells were scored using a Nikon Diaphot (Tokyo, Japan) inverted microscope. The procedure for cloning the megakaryocyte progenitor CFU-Meg was as previously described (10). Briefly, adherent cell depleted marrow was cultured at 5 x 10^4 cells/plate in the presence of mitogen stimulated spleen cell conditioned medium as the source of Meg-CSF. Culture plates were incubated at 37^o C in 5% CO_2 for seven days. Following incubation, the culture plates were stained for the presence of acetylcholinesterase. A CFU-Meg was defined to be three or more associated acetylcholinesterase positive cells. BFU-E were cultured as previously described (10). Briefly, adherent cell depleted marrow was cultured at 5 x 10^4 cells/plate in methylcellulose in the presence of 2 IU human erythropoietin (AMGEN Biologicals, Thousand Oaks, CA, lot. no. 805, specific activity, > 1 x 10^4 units/mg). Cultures were incubated at 37^o C in 5% CO_2 in air and were scored on day 9 for BFU-E.

Treatment of Marrow Cells with 3'-Azido-3'-Deoxythymidine (AZT) and Interleukin-1. Adherent depleted marrow cells were cultured for CFU-GM, CFU-Meg and CFU-E in the presence of 3'-azido-3'-deoxythymidine (AZT, kindly supplied by Burroughs Wellcome, Research Triangle Park, NC). Two series of AZT studies were performed: 1) dose-response addition of AZT to determine the LD_{50} dose effect on hematopoietic progenitors, and 2) the LD_{50} dose in the presence of human recombinant interleukin-1 alpha (generous gift from Dr. Peter Lomedico, Hoffman-LaRoche, Nutley, NJ; specific activity 10^9 units/mg protein). The IL-1 concentration ranged from 10 - 1000 units (0.01 - 1.0 µg protein). Following co-incubation with AZT and IL-1, marrow cell cultures were evaluated for CFU-GM, CFU-Meg and BFU-E as described above compared to control cultures consisting of AZT alone. The one-tailed Student's t distribution test was used to analyze the difference between the means of experimental and control data. All studies were performed in triplicate.

RESULTS. The ability of interleukin-1 to influence the degree of AZT induced hematopoietic toxicity on clonal committed progenitors was investigated. In order to determine the LD_{50} dose effect of AZT on marrow progenitors, AZT was added in a dose-response fashion and the results are given in Fig. 1. Hematopoietic progenitors expressed different sensitivities to the toxic action of AZT. BFU-E were the most sensitive with the LD_{50} dose concentration of 5 x 10^{-9}M. The LD_{50} concentration for CFU-Meg was 10^{-7}M; CFU-GM were the least sensitive with a LD_{50} dose of 5 x 10^{-5}M. The effect of added IL-1 on AZT induced toxicity of CFU-GM, CFU-MEG and BFU-E is given in Fig. 2. For CFU-GM a dose-response effect was observed for IL-1 at all concentrations tested with a peak value observed at 250 units/ml, 0.25 µg protein ($p<0.05$), when compared to AZT added alone. A similar response was observed for CFU-Meg. IL-1 reversed AZT induced suppression from 50 - 500 units, 0.05 - 0.5 µg protein; however no protection was observed at either 10 or 1000 units, 0.01 - 1 µg protein respectively. As was observed for CFU-GM, peak stimulation occurred at 250 units, 0.25 µg protein ($p<0.05$). BFU-E were more sensitive to AZT and thus required greater amounts of IL-1 to reverse AZT toxicity. Optimal level for BFU-E stimulation was observed in the presence of 500 units of IL-1, 0.5 µg protein ($p<0.05$).

DISCUSSION. The results described in this report demonstrate IL-1 is effective in reversing the toxic effect of AZT on hematopoietic progenitors.

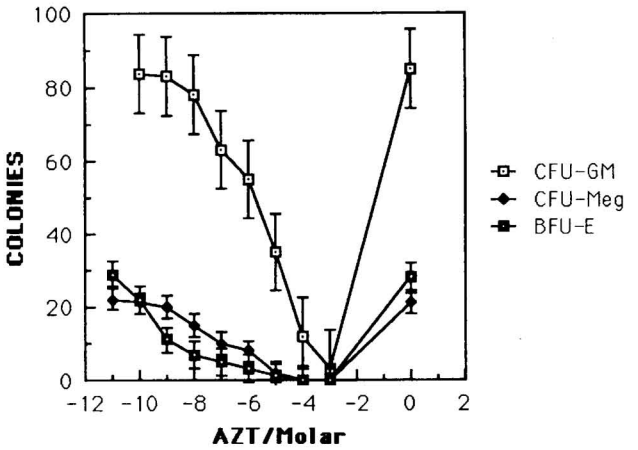

Figure 1. Dose-response effect of added AZT on normal murine bone marrow derived hematopoietic progenitors, CFU-GM, CFU-Meg, and BFU-E. Cultures were plated with 5 × 10^4 adherent cell-depleted marrow cells. Values represent ±SEM from triplicate studies.

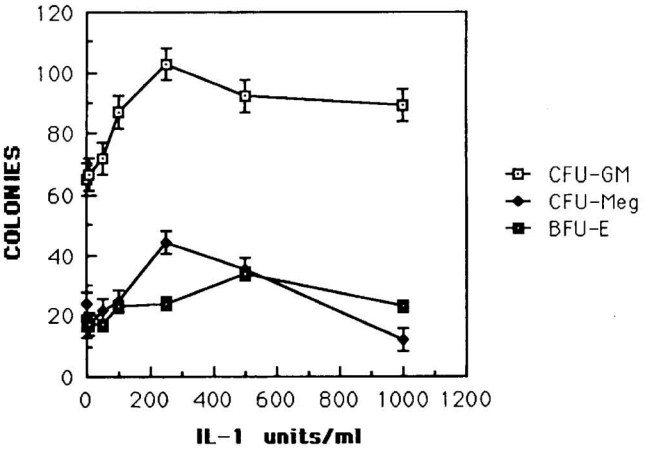

Figure 2. Dose-response effect of interleukin-1 alpha (10–1000 units; 0.01–1.0 µg protein) added in co-culture studies in the presence of the LD_{50} dose of AZT for each hematopoietic progenitor (CFU-GM, 5 × 10^{-5} *M*; CFU-Meg, 10^{-7} *M*; BFU-E, 5 × 10^{-9} *M*). Cultures were plated with 5 × 10^4 adherent cell-depleted marrow cells. Values are ±SEM from triplicate studies.

CFU-GM, CFU-Meg and BFU-E were all susceptible to AZT toxicity; however BFU-E were the most sensitive with a LD_{50} dose of 5×10^{-9}M compared to 10^{-7}M for CFU-Meg and 5×10^{-5}M for CFU-GM. This sensitivity of erythroid precursors to AZT has been described previously following AZT exposure to normal human marrow (5) or when AZT was administered in vivo in mice (6) or rats (7). This AZT effect on BFU-E indicates that this may serve as a mechanism for the anemia associated with its clinical use, although more recent data (8) indicates that the stromal microenvironment is also damaged as the result of AZT exposure and may therefore be responsible for the impaired hematopoiesis associated with AZT and that the toxic action of AZT on hematopoiesis can be partially restored with hemin. AZT induced toxicity, as reported here, appeared not to be limited to any one particular hematopoietic compartment and this observation supports previous findings (5-8). The toxic action of AZT on hematopoietic progenitors was completely abrogated following the addition of IL-1; however, the amount required to produce an optimum response differed with the progenitor cell. To reverse the toxic action of AZT on BFU-E required twice the amount of IL-1 when compared to the amount necessary to reverse the toxic action of AZT on CFU-GM or CFU-Meg. These maximum concentrations (600 units and 300 units, respectively) are higher IL-1 levels than have been reported for IL-1 to influence normal hematopoietic events in vitro (9) or for the protection of hematopoietic progenitors from radiation exposure in vitro (10). These differences may indicate the action of IL-1 to reverse AZT toxicity requires higher concentrations in order to overcome the DNA inhibitory effect of AZT on hematopoietic cells, since IL-1 has been demonstrated to influence the survival of hematopoietic progenitors and not their proliferation or differentiation (11). As was demonstrated here in this report utilizing AZT, IL-1 has been shown to prevent hematopoietic toxicity following a wide variety of chemotherapeutic agents (12-16). Although the precise mechanism of IL-1 action to prevent hematopoietic toxicity has remained elusive, more recent data from this laboratory has indicated IL-1 protects the hematopoietic microenvironment from drug induced toxicity (17). This apparently is a response very similar to that recently reported for hemin and AZT toxicity (8). Further studies are presently in progress to elucidate the relationship between IL-1, AZT exposure and the microenvironment.

Finally, these studies indicate the necessity to measure the levels of circulating cytokines in AIDS patients and also those currently receiving AZT therapy, and therefore suggest the potential therapeutic efficacy of IL-1 administered as adjuvant therapy with AZT to AIDS patients.

REFERENCES

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 i:575, 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 cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus in vitro. PNAS, USA 82:7096-7100, 1985.

3. Walker R, Parker R, Kovacs JA, Masur H, Grainick H, Lane HC, Fauci AS. 3'-azido-3'-deoxythymidine causes red cell aplasia and hypoplasia in AIDS patients with Kaposi's sarcoma. Clinical Research 35:435A, 1987.

4. Sommadossi JP, Zhu Z, Carlisle 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.

5. Daniak N, Worthington M, Riordon MA, Kreczko S, Goldman L. 3'-azido-3'-deoxythymidine (AZT) inhibits proliferation in vitro of human haematopoietic progenitor cells. Brit J Haematol 69:299-304, 1988.

6. Bogliolo G, Lerza R, Memcoboni M, Saviane A, Pannacciulli I. Azidothymidine-induced depression of murine hemopoietic progenitor cells. Exper Hematol 16:938-940, 1988.

7. Lutton JD, Brown AC, Niranjan U, Mittelman A, Distenfeld A, Abraham NG. Mechanism of AZT induced hemopoietic toxicity. Exper Hematol 16:74A, 1988.

8. Abraham NG, Bucher D, Niranjan AL, Brown AC, Lutton JD, Distenfeld A, Ahmed T, Levere RD. Microenvironmental toxicity of azidothymidine: partial sparing with hemin. Blood 74:139-144, 1989.

9. Bagby GC Jr., Dinarello CA, Wallace P, Wagner C, Hefeneider S, McCall E. Interleukin-1 stimulates granulocyte-macrophage colony stimulating activity release by vascular endothelial cells. J Clin Invest 78:1316-1323, 1986.

10. Gallicchio VS, Hulette BC, Messino MJ, Gass C, Bieschke MW, Doukas MA. Effect of various interleukins (IL-1, IL-2, and IL-3) on the in vitro radioprotection of bone marrow progenitors (CFU-GM and CFU-Meg). J Biol Resp Modif, in press 1989.

11. Williams DE, Broxmeyer HE. Interleukin-1 α enhances the in vitro survival of purified murine granulocyte-macrophage progenitor cells in the absence of colony-stimulating factors. Blood 72:1608-1615, 1988.

12. Kampschmidt R. The numerous postulated biological manifestations of interleukin-1. J Leuko Biol 26:341-355, 1984.

13. Neta R, Douches S, Oppenheim J. Interleukin-1 is a radioprotector. J Immunol 36:2483-2485, 1986.

14. Moore MAS, Warren DJ. Synergy of interleukin-1 and granulocyte colony stimulating factor: In vivo stimulation of stem-cell recovery and hematopoietic regeneration following 5-fluorouracil treatment of mice. PNAS, USA 84:7134-7138, 1987.

15. Gallicchio VS. Accelerated recovery of hematopoiesis following sub-lethal whole body irradiation with recombinant murine interleukin-1 (IL-1). J Leuko Biol 43:211-216, 1988.

16. Johnson CS, Keckler DJ, Topper MI, Branschweiger PG, Furmanski P. In vivo hematopoietic effects of recombinant interleukin-1 alpha in mice: Stimulation of granulocytic, monocytic, megakaryocytic, and early erythroid progenitors, suppression of late-stage erythropoiesis, and reversal of erythroid suppression with erythropoietin. Blood 73:678-683, 1989.

17. Gallicchio VS, Hulette BC, Hughes NK, Messino MJ, Friedman D, Doukas MA. Interleukin-1 accelerate the recovery of hematopoiesis following cyclophosphamide induced injury: evidence for an effect mediated by stromal cells (submitted).

Received June 20, 1989
Accepted September 11, 1989