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Effects of 2′,3′-Dideoxynucleosides on Proliferation and Differentiation of Human Pluripotent Progenitors in Liquid Culture and Their Effects on Mitochondrial DNA Synthesis

ABDESSLEM FARAJ, DAVID A. FOWLER, EDWARD G. BRIDGES,† AND JEAN-PIERRE SOMMADOSSI*

Department of Pharmacology, Center for AIDS Research, The Comprehensive Cancer Center, and Division of Clinical Pharmacology, University of Alabama at Birmingham, Birmingham, Alabama 35294

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2′,3′-Dideoxynucleosides (ddNs) including 3′-azido-3′-deoxythymidine (AZT), 3′-fluoro-3′-deoxythymidine (FLT), 3′-amino-3′-deoxythymidine (AMT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-didehydro-3′-deoxythymidine (D4T) were tested for their effects on proliferation and differentiation of pluripotent progenitor cells ($CD34^+$) purified from human bone marrow cells grown in liquid cultures. These highly purified progenitor cells undergo extensive proliferation during 14 days, with a marked differentiation during the last 7 days. These differentiated cells exhibit normal morphological features in response to specific hematopoietic growth factors of both erythroid and granulocyte-macrophage lineages, as demonstrated by flow cytometry cell phenotyping. The potencies of these ddNs in inhibiting proliferation of granulocyte-macrophage lineage cells were in the order FLT > AMT = ddC > AZT ≫ D4T, and the potencies in inhibiting proliferation of erythroid lineage cultures were in the order FLT > AMT > AZT > ddC ≫ D4T. The toxic effects of ddNs assessed in these liquid cultures were in agreement with data obtained by using semisolid cultures, demonstrating the consistency of these two in vitro hematopoietic systems toward ddN toxicity. ddC was toxic to $CD34^+$ progenitor cells and/or cells in the early stages of differentiation, whereas the inhibitory effect of AZT on the erythroid lineage was predominately observed on a more mature population of erythroid progenitors during the differentiation process. Slot blot analysis of granulocyte-macrophage cultures demonstrated that exposure to ddC and FLT was associated with a decrease in total mitochondrial DNA (mtDNA) content, suggesting that these two ddNs inhibit mtDNA synthesis. In contrast, no difference in the ratio of nuclear DNA to mtDNA was observed in cells exposed to toxic concentrations of AZT and AMT, demonstrating that the bone marrow toxicity induced by AZT and its metabolite AMT is not associated with an inhibition of mtDNA synthesis. This human pluripotent progenitor liquid culture system should permit detailed investigations of the cellular and molecular events involved in ddN-induced hematological toxicity.

The thymidine analog 3′-azido-3′-deoxythymidine (AZT) was the first drug approved for the treatment of AIDS. AZT has been demonstrated to induce immunological improvement, decrease the incidence of opportunistic infections, and reduce mortality from AIDS (15, 42). The major limitation to AZT therapy is bone marrow toxicity, manifested as anemia and neutropenia (30). Our laboratory was the first to demonstrate that direct exposure of human bone marrow cells to AZT in vitro effected a dose-dependent inhibition of granulocyte-macrophage CFU (CFU-GM) and erythroid burst-forming units (BFU-E) (33). While these clonogenic assays with human bone marrow mononuclear cells in semisolid medium have been shown to be an adequate model for predicting the cytotoxicity of antiretroviral compounds to bone marrow cells (32), the semisolid nature of the assay and the low cloning efficiencies of progenitor cells make it difficult to ascertain the cellular and molecular mechanism(s) of drug-induced toxicity in a relatively pure and homogeneous bone marrow cell population.

Recent studies have demonstrated that a population of pluripotent progenitor cells, termed $CD34^+$ cells, exists in bone marrow. $CD34^+$ cells are capable of both self-renewal and differentiation into all lineages of mature hematopoietic cells (13, 31). This report describes the use of highly purified progenitor $CD34^+$ cells for the culture of GM and erythroid cell lineages. The effects of AZT, 3′-fluoro-3′-deoxythymidine (FLT), 3′-amino-3′-deoxythymidine (AMT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-didehydro-3′-deoxythymidine (D4T) were determined in these liquid culture systems and were compared with the results of soft agar and methylcellulose clonogenic assays. The mechanism(s) of AZT-induced host toxicity is multifactorial (32), including inhibition of nuclear DNA synthesis following incorporation into host DNA (17, 34, 38), inhibition of polymerases and exonucleases (3, 7, 10, 23, 29), inhibition of gene expression (20, 39, 40), and other mechanisms (14, 16, 22). In addition, some investigators have reported that inhibition of mitochondrial DNA (mtDNA) synthesis, a potential mechanism involved in the peripheral neuropathy observed in patients treated with ddC, 2′,3′-dideoxyinosine, and D4T (4, 6, 26, 32), may also be responsible for the hematological effects of AZT (24, 27). By using these newly developed liquid cultures of human pluripotent progenitor cells, the mtDNA contents of AZT-treated cells were assessed and compared with those of cells exposed to other 2′,3′-dideoxynucleosides (ddNs) including FLT, D4T, ddC, and the myelotoxic metabolite of AZT, AMT.

MATERIALS AND METHODS

Materials. FLT was a gift from R. F. Schinazi (Emory University, Atlanta, Ga.). D4T was kindly provided by Bristol-

* Corresponding author. Mailing address: University of Alabama at Birmingham, Box 600, Volker Hall 113, University Station, Birmingham, AL 35294. Phone: (205) 934-8226. Fax: (205) 975-4871.

† Present address: Department of Pharmacology, School of Medicine, Yale University, New Haven, CT 06510.

Myers Squibb Co.; and AZT, AMT, ddC, stem cell factor (SCF, or c-kit ligand), immunoglobulin G1 (IgG1)-fluorescein isothiocyanate (FITC), IgG2b-phycoerythrin (PE), and CD15-FITC were purchased from Sigma Chemical Co. (St. Louis, Mo.). Erythropoietin (EPO) and human recombinant GM–colony-stimulating factor (GM-CSF) were purchased from Connaught (Willowdale, Ontario, Canada) and Amgen Biologicals (Thousand Oaks, Calif.), respectively. Interleukin-3 (IL-3) was purchased from Upstate Biotechnologies, Inc. (Lake Placid, N.Y.). CD34-FITC, Simultest CD3-FITC–CD19-PE, and CD11c-PE were purchased from Becton Dickinson (Mountain View, Calif.). Glycophorin A-FITC was purchased from Amac, Inc. (Westbrook, Maine). Hanks' balanced salt solution, fetal bovine serum (FBS), and McCoy's 5A medium were purchased from Gibco/BRL (Grand Island, N.Y.). All other chemicals and reagents were of the highest analytical grade available.

MNC isolation. Human bone marrow cells were collected by aspiration from the posterior iliac crest of healthy human volunteers by a protocol approved by the Institutional Review Board Committee at the University of Alabama at Birmingham. Alternatively, cells were flushed from a rib obtained following thoracic surgery as described previously (31). The mononuclear cells (MNCs) were isolated by gradient centrifugation of heparinized bone marrow samples on Ficoll-Histopaque. The isolated MNCs were washed in Hanks' balanced salt solution supplemented with 10% FBS.

Progenitor cell purification. Approximately 4.0×10^7 MNCs were incubated in a T-25 flask for at least 2 h in minimum medium (McCoy's 5A medium and nutrients) at 37°C with 5% CO_2 to adhere the monocytes and macrophages. For enrichment of hematopoietic progenitor ($CD34^+$) cells, mononuclear nonadherent cells were pelleted and resuspended in 1 ml of cold phosphate-buffered saline (PBS) with 2% FBS and 0.1% sodium azide, and subsequently, 200 μl of anti-CD34 monoclonal antibody was added. After incubation for 1 h on ice and under dark conditions, the cells were washed twice with cold PBS to eliminate excess antibodies and were resuspended in 3 ml of McCoy's 5A medium plus nutrients containing 5 ng of propidium iodide per ml. $CD34^+$ cells were then positively selected by cell sorting with a FACStar instrument (Becton Dickinson). The purity of the $CD34^+$ cells was more than 99%, with a viability of more than 96%, as measured by trypan blue staining.

Liquid suspension cultures. Cells were cultured in supplemented McCoy's 5A medium enriched with 15% FBS and α-mercaptoethanol (0.1 mM). For GM differentiation, purified $CD34^+$ cells were cultured in the presence of human recombinant GM-CSF (125 U/ml) and human recombinant IL-3 (200 U/ml). For erythroid cell differentiation, purified $CD34^+$ cells were cultured in the presence of human recombinant erythropoietin (2 U/ml), SCF (7 ng/ml), and human recombinant IL-3 (100 U/ml). $CD34^+$ cells (1.7×10^4 to 2.0×10^4 cells per ml) were incubated in 24-well tissue culture dishes in medium containing cytokines, with or without drugs. After 7 and 14 days of incubation, viable cells from individual wells were counted and phenotyped. All experiments were performed at least in triplicate. The viability of the cells cultured for 14 days was greater than 95%, as measured by trypan blue staining.

Cell phenotyping. Primary isolated nonadherent MNCs and cultured cells following 7 and 14 days of incubation were analyzed by flow cytometry by using six different monoclonal antibodies conjugated with FITC or PE: CD3-FITC (T-cell marker), CD19-PE (B-cell marker), CD15-FITC (granulocyte marker), CD11c-PE (monocyte marker), CD34-FITC (pluripotent progenitor marker), and glycophorin A-FITC (erythrocyte marker). IgG1-FITC and IgG2b-PE were used as negative controls to ensure accurate analysis. Cells (10^5) were incubated in 50 ml of cold PBS with 2% FBS and 0.1% sodium azide at 4°C in the dark with 5 μl of monoclonal antibodies. After 60 min of incubation, the cells were washed twice with PBS. The cells were then resuspended in PBS containing 1% paraformaldehyde and were analyzed with a FACStar instrument (Becton-Dickinson) by using the lysis program for analysis of double fluorescence.

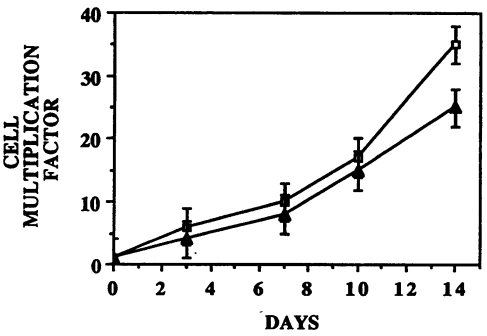

FIG. 1. Proliferation of pluripotent $CD34^+$ progenitor cells in the presence of GM-CSF and IL-3 (GM culture, □) or EPO, SCF, and IL-3 (for erythroid culture, ▲) over 14 days in liquid cultures.

mtDNA content analysis. After 14 days of incubation, in the absence and presence of drugs, approximately 1×10^5 to 2×10^5 cells were heated under alkaline conditions and blotted onto a nylon membrane (Zeta-Probe GT; Bio-Rad, Richmond, Calif.) for slot blot analysis (Bio-Rad apparatus). The mtDNA was detected by using a specific human oligonucleotide mitochondrial probe encompassing nucleotide positions 4212 to 4242 (1). A human beta-actin probe was used as a control. The beta-actin probe was a gel-purified, 625-bp fragment of a human beta-actin cDNA plasmid (40). DNA was fixed to the filters by heating for 10 min at 100°C, and slot blots were hybridized to oligonucleotide mitochondrial or beta-actin probes according to the manufacturer's procedures. Autoradiograms were scanned by using a Shimadzu CS 9000 U flying-spot densitometer.

RESULTS

Progenitor cell proliferation and differentiation. The isolated nonadherent mononuclear cells contained mostly erythrocytes (glycophorin A^+), B cells ($CD19^+$), and T cells ($CD3^+$), while only 1 to 4% of the cell population was identified as being pluripotent progenitor cells, as determined by $CD34^+$-cell binding. A yield approximating 80% was obtained after positive selection by cell sorting.

After 14 days in liquid cultures, $CD34^+$ cells showed a pronounced growth, with GM and erythroid progenitors proliferating approximately 35- and 25-fold, respectively (Fig. 1). The results of flow cytometry analysis of purified progenitor cells incubated for 14 days in the presence of GM-CSF and IL-3 are shown in Fig. 2 (control conditions). More than 70% granulocytes ($CD15^+$) and monocytes ($CD11c^+$) were expressed in these GM cultures. Of note, no T cells, B cells, erythroid cells, or pluripotent progenitor cells were detected. Figure 3 (control conditions) shows the results of an analysis of a 14-day culture of pluripotent progenitor cells in the presence of EPO, SCF, and IL-3. These erythrocytic cultures were characterized as being 80% erythroid (glycophorin A^+), with no T cells, B cells, or pluripotent progenitor cells detected and

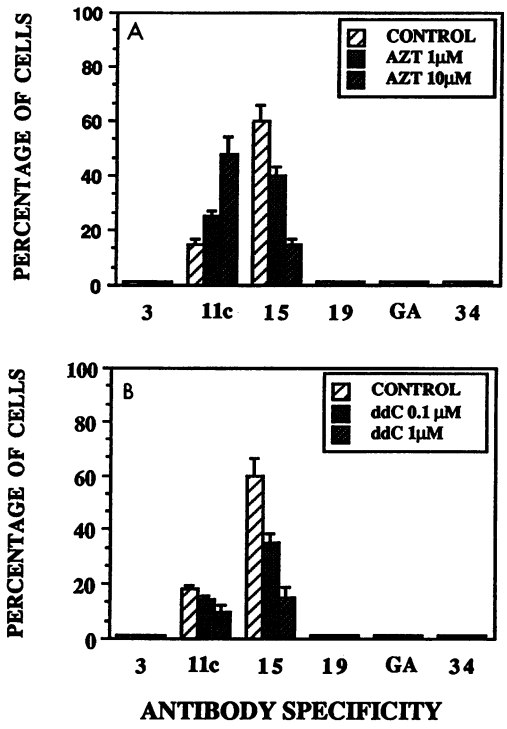

FIG. 2. Effects of AZT (A) and ddC (B) on CFU-GM liquid cultures. Columns represent the mean percentage of cells expressing different myeloid- and lymphoid-associated cell surface markers assessed after 14 days of culture of pluripotent $CD34^+$ progenitor cells with and without drugs in the presence of GM-CSF and IL-3 in at least three separate experiments performed in triplicate; bars represent standard deviations. The antibody specificity is given as CD-cell numbers with the exception of glycophorin A (GA): 3 for T cells, 11c for monocytes, 15 for granulocytes, 19 for B cells, and 34 for pluripotent progenitor cells.

low levels of granulocytes and monocytes detected, as assessed by measuring the associated cell surface markers. Phenotyping of erythrocytic cultures demonstrated that the proportion of expressed erythroid cell glycophorin A^+ increased from 30% at day 7 to more than 70% at day 14 (Fig. 3A and C versus Fig. 3B and D). Monocytes ($CD11c^+$) and granulocytes ($CD15^+$) accounted for less than 4% of the cells in erythroid lineage cultures.

Effects of ddNs on human hematopoietic progenitors. The effects of various ddNs on the proliferation of $CD34^+$ progenitor cells in liquid cultures of human CFU-GM and BFU-E lineages are shown in Table 1. The potencies of the ddNs in inhibiting the proliferation of human hematopoietic progenitors in liquid cultures were in the orders FLT > AMT = ddC > AZT ≫ D4T for GM cultures and FLT > AMT > AZT > ddC ≫ D4T for erythroid lineage cultures. FLT was the most cytotoxic compound among the ddNs tested, with a 50% inhibitory concentration (IC_{50}) of 0.14 μM in GM cultures and 0.05 μM in erythroid lineage cultures, whereas D4T was found to have no significant cytotoxicity at concentrations of up to 10 μM. In liquid cultures of human BFU-E, AZT strongly inhibited cell proliferation, with an IC_{50} of 0.18 μM, and was less inhibitory to CFU-GM, with an IC_{50} of approximately 5 μM. ddC inhibited to a similar extent erythroid and GM cell growth, with IC_{50}s of 0.5 and 0.6 μM, respectively. Toxicity data for the ddNs in the liquid cultures were compared with the IC_{50}s obtained by using semisolid cultures of human bone marrow progenitor cells previously published by our group (Table 1). Of note, the IC_{50}s of the ddNs obtained in either liquid or semisolid cultures were of the same order of magnitude, demonstrating the consistency of these two in vitro hematopoietic systems toward ddN toxicity.

Figure 2 illustrates the myeloid-associated markers of AZT- and ddC-treated cells compared with the myeloid-associated markers of control cells after 14 days of GM lineage culture. AZT inhibited granulocyte progenitor cells ($CD15^+$), whereas the monocyte cell lineage ($CD11c^+$) increased in a dose-dependent manner compared with the control cells. In contrast, both myeloid cell lineages ($CD11c^+$ and $CD15^+$) decreased as a function of the ddC concentration.

Figure 3 shows the expression of phenotypic markers of AZT-treated cells compared with those of control cells after 7 days (Fig. 3A) and 14 days (Fig. 3B) of erythroid cell culture. No effect was observed on the erythroid cell population (glycophorin A^+) after 7 days of culture, whereas a pronounced inhibitory effect by AZT was detected during the second week of culture. In contrast, ddC inhibited the erythroid cell lineage by 7 days of culture (Fig. 3C), and that effect did not increase during the second week of culture (Fig. 3D).

Effects of ddNs on mtDNA content in CFU-GM liquid culture. ddC was the most potent inhibitor of mtDNA content in human CFU-GM liquid cultures (Table 2). At 1 μM, only a weak mtDNA signal could be detected; the signal accounted for approximately 90% inhibition compared with the control, demonstrating the preferential effect of ddC on mtDNA synthesis. Of interest, FLT also substantially inhibited the mtDNA content of cells in human CFU-GM liquid cultures, with a 33% inhibition compared with the control. The IC_{50} of FLT for 33% inhibition was 0.1 μM; at an FLT concentration of 1 μM, inhibition increased to 60%. In contrast, neither AZT nor its catabolite AMT had any inhibitory effect on mtDNA synthesis at concentrations inhibitory to cell growth proliferation and differentiation. Also, AZT and AMT had no effect on the mtDNA contents of erythroid progenitor cells (data not shown). D4T at 10 μM exhibited a minor inhibitory effect on the mtDNA content; however, it should be noted that D4T was not toxic to these cells.

DISCUSSION

Bone marrow contains a heterogeneous population of cells representing at least seven lineages of hematopoietic cells in various stages of maturation. Approximately 1 to 5% of gradient-centrifuged mononuclear cells from bone marrow are $CD34^+$ cells. The CD34 antigen is expressed on myeloid precursors of the granulocyte, monocyte, erythroid, and megakaryocytic lineages. In the present study, pluripotent progenitor $CD34^+$ cells were isolated from human bone marrow, by using an anti-CD34 monoclonal antibody conjugated to FITC by cell sorting (FACStar), with a degree of purity greater than 99%. Isolation of highly purified progenitor cells by that technique was more rapid than that by the usual immunomagnetic affinity technique, which requires a double selection procedure (13, 31). In addition, the yield of $CD34^+$ progenitor cells was approximately 10- to 15-fold higher by the present methodology compared with that by the commonly used immunomagnetic bead method.

Purified $CD34^+$ progenitor cells undergo extensive proliferation during 14 days of liquid culture, with a marked increase in differentiation for the last 7 days, as demonstrated by flow cytometry. The cells differentiated in vitro exhibit normal morphological features in response to specific hematopoietic growth factors. Human bone marrow progenitor cells triggered

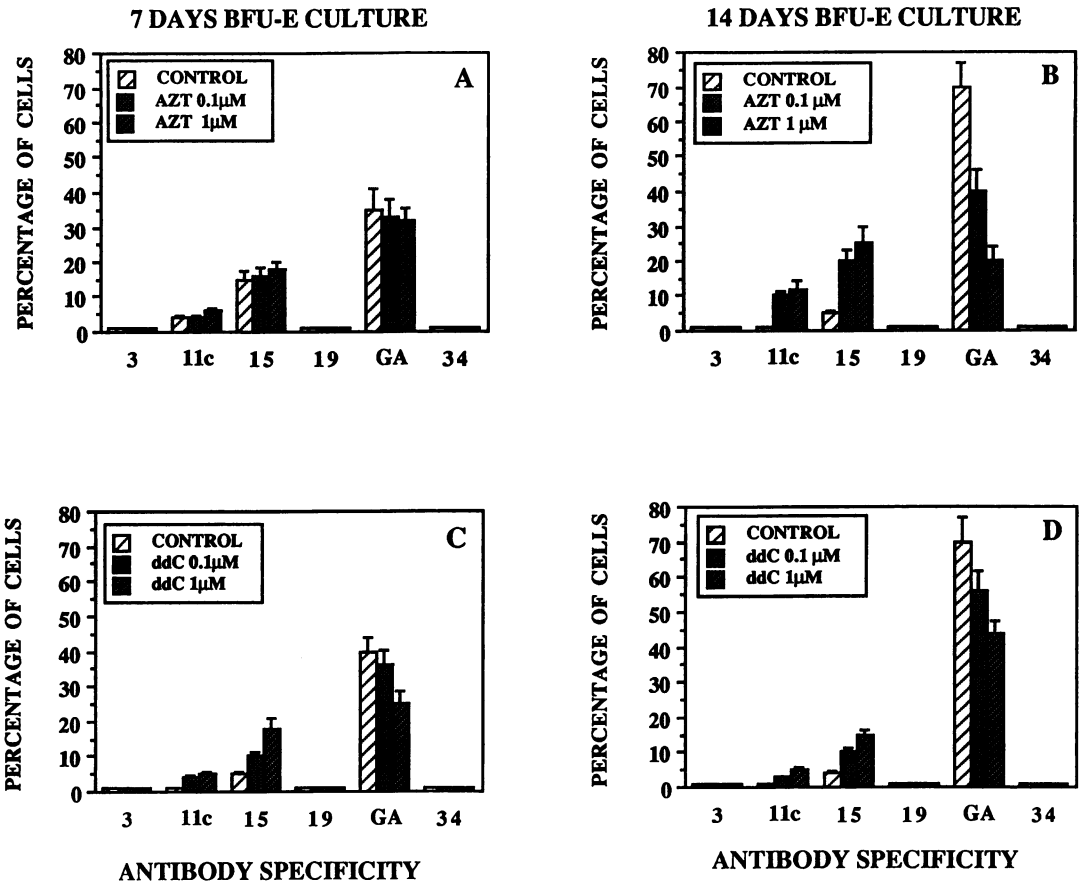

FIG. 3. Effect of AZT (A and B) and ddC (C and D) on BFU-E liquid cultures. Columns represent the mean percentage of cells expressing different myeloid- and lymphoid-associated cell surface markers after 7 and 14 days of culture of pluripotent $CD34^+$ progenitor cells with and without drugs in the presence of EPO, SCF, and IL-3 in at least three separate experiments performed in triplicate; bars represent standard deviations. Cell surface markers are as described in the legend to Fig. 2.

into cycling by hematopoietic growth factors in liquid suspension culture undergo extensive proliferation coupled with the expression of lineage-specific differentiation antigens along either the GM pathway (upon treatment with IL-3 and GM-CSF) or the erythroid pathway (upon the addition of IL-3, SCF, and EPO). Thus, these liquid cultures appear to be a potential in vitro system for assessing the toxicities of drugs to bone marrow and also permit study of their mechanism(s) of cytotoxicity by biochemical and molecular biological techniques. In contrast, while the semisolid agar system requires less sophisticated techniques and is still highly predictive of the

TABLE 1. Effects of ddNs on proliferation of $CD34^+$ progenitor cells in liquid cultures of human CFU-GM and BFU-E

Compound	Mean ± SD IC_{50} (μM)[a]			
	CFU-GM culture		BFU-E culture	
	Liquid	Semisolid	Liquid	Semisolid
AZT	4.90 ± 0.35	1.90 ± 1.20[b]	0.18 ± 0.03	0.60 ± 0.50[b]
ddC	0.60 ± 0.05	0.70 ± 0.20[c]	0.50 ± 0.05	0.05 ± 0.04[c]
FLT	0.14 ± 0.02	0.55 ± 0.37[d]	0.05 ± 0.02	0.04 ± 0.01[d]
AMT	0.64 ± 0.04	0.40 ± 0.20[b]	0.06 ± 0.01	0.09 ± 0.05[b]
D4T	>10	20–100[e]	>10	10[e]

[a] Each set of data represents the arithmetic mean value ± standard deviation of at least three independent experiments performed in triplicate. IC_{50}s were obtained by using least-squares linear regression analysis of the logarithm of drug concentration versus CFU-GM or BFU-E survival fractions.
[b] Taken from reference 11.
[c] Taken from reference 21.
[d] Taken from reference 35.
[e] Taken from reference 32.

TABLE 2. Effects of ddNs on mtDNA levels of human CFU-GM in liquid cultures

Compound (concn [μM])	Ratio of mtDNA/actin[a]	% decrease in mtDNA level
Control	1.50 ± 0.20	0
ddC (0.1)	0.80 ± 0.05	47
ddC (1.0)	0.15 ± 0.05	90
FLT (0.1)	1.00 ± 0.10	33
FLT (1.0)	0.60 ± 0.05	60
AZT (1.0)	1.65 ± 0.20	NM[b]
AZT (10.0)	1.55 ± 0.25	NM
AMT (0.1)	1.70 ± 0.15	NM
AMT (1.0)	1.60 ± 0.15	NM
D4T (1.0)	1.45 ± 0.10	NM
D4T (10.0)	1.28 ± 0.05	15

[a] Each set of data represents the arithmetic mean value ± standard deviation of at least three independent experiments.
[b] NM, not measurable.

in vivo bone marrow toxicity of antiviral drugs (32), the limited number of progenitor cells available with the agar system does not allow investigative pharmalogical techniques to be performed with a homogeneous cell population.

Among the tested ddNs, FLT was the most toxic compound, inhibiting the proliferation of $CD34^+$ cells induced toward both lineages. This severe inhibition of human progenitor cells is consistent with the pronounced hematotoxicity observed in patients (18), which has led to the discontinuation of clinical studies with this anti-human immunodeficiency virus drug.

As reported previously in clonogenic assays (32), D4T did not exhibit any cytotoxicity at concentrations of up to 10 μM in human CFU-GM liquid cultures. AMT, a metabolite of AZT detected both in vitro (11) and in vivo (36), was shown to be more toxic than AZT to either GM or erythroid lineages by using liquid cultures. These results are in agreement with data obtained with semisolid cultures of human CFU-GM and BFU-E clonogenic assays (Table 1) and confirm the validity of the novel assay described here. In particular, inhibition of the erythroid cell phenotype by AZT occurred during the second week of culture, as determined by decreased glycophorin A expression. Therefore, these data suggest that AZT inhibits differentiation of more mature BFU-E progenitor cells. Consistent with these data, our group has previously demonstrated that AZT inhibits hemoglobin synthesis through down-regulation of globin gene transcription (39, 40), and in vivo studies have reported a blockage in the maturation of erythroid cells in a mouse AIDS model after AZT administration (8). Therefore, a blockage of erythroid differentiation may also play a potential role in the AZT-induced anemia observed in patients.

The morphological features of human CFU-GM after 14 days of continuous exposure to AZT showed a perturbation in the ratio of granulocyte to monocyte cell lineages (Fig. 2). AZT markedly decreased the granulocyte population, consistent with the AZT-induced neutropenia observed in patients (15, 30, 42). In contrast, the monocyte population increased in a dose-dependent manner, and this effect was not detected with ddC (Fig. 2) or the other ddNs tested (data not shown). Although the CD11c antibody (predominantly monocyte phenotype) also has an overlapping specificity for granulocyte lineage cells recognized by the CD15 antibody (predominantly of the granulocyte phenotype), the significance of these data is unclear, and further studies are needed to understand the underlying mechanism(s) and its consequences.

ddC was toxic to the same extent to erythroid and GM lineages, with a prominent inhibitory effect during the first week of culture. In contrast, when $CD34^+$ cells were also incubated for 7 days in the presence of AZT, FLT, or AMT, concentrations as high as 10 μM were required to achieve a 50% inhibition of cell growth (data not shown). In addition, when these drugs were added at day 8 (when no $CD34^+$ progenitor cells were detected), the IC_{50} of ddC increased severalfold, whereas no substantial changes in the IC_{50}s were detected with the thymidine derivatives. These data suggest that AZT, FLT, and AMT may interfere at a later stage than ddC in the proliferation or differentiation processes of progenitor cells, with ddC being more toxic to progenitor $CD34^+$ cells and/or to the early-stage erythroid and GM progenitor cells.

Large variabilities in the IC_{50}s of ddC (several orders of magnitude) have been reported by different groups of investigators (9, 12, 19, 25), including the investigators in our own laboratory (21, 32, 35). These apparent discrepancies may possibly result from a wide variation in the percentage of pluripotent $CD34^+$ cells present in the human bone marrow MNCs which are used in semisolid clonogenic assays.

The hematological toxicity of ddC is not usually a limiting factor in the treatment of AIDS with this antiretroviral agent, probably because of the administration of low ddC dosages, which results in exposure to concentrations less than those necessary to affect hematopoietic cell lineages. Consistent with this hypothesis, early clinical trials of ddC at higher doses led to the development of hematotoxic side effects in 40% of patients (41), which is in agreement with results of in vivo studies in animals (37), and our present study also demonstrated the susceptibilities of human $CD34^+$ pluripotent cells to ddC.

Previous studies have suggested that inhibition of mtDNA synthesis is a possible mechanism of AZT-induced hematopoietic toxicity (24, 27). In contrast, Chen et al. (6) reported that high concentrations of AZT, well above the IC_{50}s, were required to affect the mtDNA contents of cells. The same group of investigators demonstrated that ddC and, to a lesser degree, D4T preferentially inhibit mtDNA synthesis in a human lymphoblastoid cell line and that the degree of inhibition correlates with drug-induced toxicity (4, 6). To further elucidate the role of inhibition of mtDNA synthesis in ddN-induced toxicity, we investigated the potential effects of these ddNs on the mtDNA content of human CFU-GM after 14 days of continuous exposure to drugs. Consistent with previous reports (4, 6), ddC preferentially decreased the mtDNA content. In contrast, AZT and AMT, at concentrations inhibiting more than 75% of cell growth, had no detectable effect on the mtDNA content in liquid cultures of CFU-GM (Table 2) or BFU-E (data not shown), suggesting that this cellular event does not play a major role in AZT- or AMT-induced bone marrow suppression. The possible role of inhibition of mtDNA synthesis in D4T-induced host toxicity could not be ascertained in the in vitro model described here, since D4T at a concentration of up to 10 μM did not affect the growth of human CFU-GM cells in liquid cultures. Of particular interest was the substantial inhibition of mtDNA content by FLT, a close analog of AZT which has shown pronounced hematotoxicity in preclinical (2) and clinical (18) studies. As suggested previously for ddC (5), FLT is probably not a substrate for the relevant mitochondrial phosphorylating enzyme which will lead to formation of its 5′-triphosphate derivatives within mitochondria (28). That study further raises the question of the cellular mechanism(s) that leads to that selective toxicity for some ddNs.

In conclusion, this is the first report of a simple methodology that can be used to isolate homogeneous human erythroid and GM lineage cells in primary liquid cultures. This report further supports the results of previous studies demonstrating the unique and multifactorial mechanism(s) of ddN-induced toxicities on human BFU-E and CFU-GM lineages at various stages of proliferation and differentiation. This human primary cell liquid culture system provides an adequate model for investigating the toxicities of antiviral compounds on human bone marrow function and should allow detailed examination of the cellular and molecular events involved in ddN-induced hematological toxicity.

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