Interleukin 3 Protects Murine Bone Marrow Cells from Apoptosis Induced by DNA Damaging Agents

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Summary

Murine bone marrow-derived cells, dependent on interleukin 3 (IL-3) for their growth in culture, undergo programmed cell, or apoptosis, upon cytokine withdrawal. Here it is reported that a variety of DNA damaging agents cause a more rapid onset of apoptosis in a factor-dependent cell line, BAF3, deprived of IL-3. In contrast, when cultured in the presence of IL-3, or other growth promoting factors, BAF3 cells are highly resistant to X-irradiation and the cytotoxic drugs etoposide and cisplatin. Overexpression of the *bcl2* gene product also protects BAF3 cells from DNA damage. The presence of IL-3 is not required during the initial events of DNA damage or its repair. In the absence of IL-3, cells still complete the repair of DNA breaks within 15 min, and continue to cycle for 5 h. At this time, IL-3 is necessary to prevent the accelerated onset of DNA cleavage from a G2 arrest point.

The development of haematopoietic cells can be controlled by regulating the rate of programmed cell death, or apoptosis, as well as the rate of cell proliferation. For example, those thymocytes that are triggered by antigen within the thymus die by apoptosis (1), which provides a mechanism for the elimination of self-reactive T lymphocytes. In contrast, B lymphocytes from the germinal centers of lymph nodes apoptose if they are not antigen triggered (2), allowing the selection of cells producing high affinity antibodies. Expansion and maturation of the immature progenitor cells of bone marrow also appear to be controlled by a balance between proliferation and apoptosis. The proliferation of such cells is stimulated by cytokines, including IL-3 and GM-CSF, which act on the earliest precursor cells (3, 4). Upon withdrawal of IL-3 or GM-CSF from progenitor cell lines (5, 6) or primary IL-3–dependent cells from bone marrow (6), a program of events typical of apoptosis is observed, which includes the digestion of cellular chromatin to oligonucleosome-length fragments by an endogenous nuclease. This provides a model for the regulation of haematopoiesis in mouse bone marrow where the least mature progenitor cells are not cycling (7), but after the stimulation of haematopoiesis, activated progenitor cells proliferating in response to cytokines could be induced to apoptose when cytokines are no longer available, rapidly terminating their expansion.

The mechanism by which cytokines such as IL-3 protect cytokine-dependent cells from the onset of apoptosis remains unclear. The initial interaction of IL-3 with its receptor stimulates rapid events such as the tyrosine phosphorylation of cellular proteins (8). However, such signals decay rapidly upon IL-3 removal (8), whereas the initiation of DNA cleavage does not occur until 6 h after IL-3 deprivation (6). The protective action of IL-3 can be mimicked either by other growth promoting factors that interact with homologous receptors, such as GM-CSF (5) and IL-4 (9), or by the overexpression of the product of the *bcl2* oncogene (10, 11). However, unlike cytokines, bcl2 inhibits the onset of apoptosis but does not cause cells to proliferate.

In addition to its role in the control of haematopoietic cell development and immune repertoire selection, the induction of apoptosis is involved in the mechanism of killing of tumor cells by a number of therapeutic regimes. A variety of agents used for some time in human tumor therapies, such as antagonists of steroid hormones (12), glucocorticoids (13), DNA damaging drugs (14, 15), or radiation (16) have recently been demonstrated to kill cells by inducing apoptosis. Again, the mechanism by which initial DNA damage leads to subsequent cleavage of DNA remains unclear. Among normal cells, those of the haematopoietic system are the most sensitive when exposed in vivo to DNA damaging agents. Because IL-3 will protect bone marrow-derived haemopoietic cells from apoptosis, we investigated whether cytokines will protect against apoptosis induced by DNA damaging agents. We show that IL-3, other growth promoting factors, and the bcl2 protein inhibit the rapid apoptosis induced by DNA damaging agents in murine bone marrow-derived IL-3–dependent cells. The extent of DNA damage and its repair were not affected by IL-3. The cytokine acted 6 h after these initial events to allow cells to pass a restriction point at which DNA damage is detected.

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Materials and Methods

Cell Culture and Reagents. BAF3 cells (17; obtained from Dr. Ronald Palacios, Basel Institute for Immunology, Basel, Switzerland) were maintained in DMEM containing 10% fetal bovine serum (FBS)[1] and 5% WEHI 3B cell-conditioned medium which was used throughout as a source of IL-3, at a density of 5×10^4–5×10^5 cells/ml. Primary IL-3–dependent cultures of murine bone marrow cells were prepared as previously described (6). They were characterized largely as mast cells by the release of β-glucosaminidase upon calcium ionophore stimulation. To remove IL-3, cells were washed twice in DMEM/10% FBS. Cell viability and viable cell density were determined by counting cells able to exclude trypan blue on a haemocytometer. Duplicate samples of at least 50 cells were analyzed. Murine IL-4 was obtained from Genzyme Corp. (Boston, MA), insulin-like growth factor (IGF-1) from Bachem (Bubbendorf, Switzerland), A23187 from Calbiochem Corp. (Boston, MA), etoposide from Bristol-Myers (Slough, UK), and cisplatin from David Bull Laboratories (Warwick, UK). X-irradiation was performed using a Pantac x-ray machine (Chester Beatty Laboratories), with an output of 24 0 keV at a dose rate of 250–300 rad/min.

DNA Fragmentation. 10^7 cells were lysed in 0.75 ml 10 mM Tris (pH 8.0), 150 mM NaCl, 0.1 mM EDTA, 1% SDS, and incubated for 15 h with 200 μg/ml proteinase K at 37°C. After extraction with 50% phenol/50% chloroform, total DNA was precipitated by addition of 1/10 vol 3 M NaAcetate (pH 5.0) and 2.5 vol ethanol and incubation for 15 h at −20°C. The DNA pellet was resuspended in 50 μl 10 mM Tris (pH 8.0), 0.1 mM EDTA, and the nucleic acid concentration determined by measurement of OD_{260}. 15 μg of nucleic acid was incubated for 1 h at 37°C with 10 U/ml DNAse-free RNAse, then electrophoresed on a 1% agarose gel in the presence of ethidium bromide. A 1-kb DNA ladder size marker (Gibco-BRL, Gaithersburg, MD) was included in the last lane of each gel.

Detection of Single-stranded DNA Breaks. The differential rates of elution of test sample DNA and an internal standard DNA, at alkaline pH, were used to detect the presence of single-stranded DNA breaks in the test samples. DNA was labeled by culture of test cells for 15 h with 0.5 μCi/ml [^{14}C]thymidine (Amersham International, Amersham, Bucks, UK) and culture of standard cells with 0.5 μCi/ml [^{3}H]thymidine (Amersham International). Immediately before irradiation, test cells were washed twice in DMEM/10% FBS, suspended in DMEM/10% FBS containing 50 mM Hepes pH 7.0, placed on ice, and irradiated. A nonirradiated and an irradiated control test cell sample were maintained on ice and other test samples were incubated at 37°C, with IL-3 if indicated, for the time shown. An equivalent number (10^6) of each test sample and standard cells were then admixed on ice, pelleted at 4°C, resuspended in PBS, and loaded onto polycarbonate filters at 4°C. The rate of elution of test and standard DNA in each sample by 2% tetrapropylammoniumhydroxide (pH 12.1) was then measured, after cell lysis and protease digestion, as previously described (18).

Cell Cycle Analysis. 5×10^6 cells were pelleted, resuspended in 0.2 ml PBS, and fixed by the addition of 2 ml of ice-cold 75% ethanol/25% PBS. Fixed cells were pelleted, vigorously resuspended in PBS, and incubated for 30 min at 37°C with 100 μg/ml RNase and 40 μg/ml propidium iodide. The fluorescence of the stained cells was analyzed using a FACScan® (Becton Dickinson & Co., Mountain View, CA).

Generation of bcl2-expressing Cells. A 1.9-kb human bcl2 cDNA fragment was inserted in the EcoRI restriction site of the retroviral pM5Gneo (from C. Stocking, Heinrich-Petter Institut, Hamburg, Germany). After transfection of this vector into the packaging cell line ψ2 (19) and selection in G418 a producer cell clone, which transferred G418, resistance to National Institutes of Health (NIH) 3T3 cells at a frequency of 10^6 resistant colonies/ml producer cell supernatant, was isolated. BAF3 cells were infected by cocultivation with this producer cell clone, and a cell population which was 100% positive for the expression of human bcl2, assessed by fluorescence activated cell analysis using an anti-human bcl2 antibody (20), was used for further experiments.

Results

IL-3 Inhibits Apoptosis Induced by DNA Damaging Agents in a Murine Bone Marrow-derived Cell Line and in Primary Cultures of Murine Bone Marrow. The murine bone marrow-derived cell line BAF3 has been shown to undergo apoptosis, characterized by an initial cleavage of cellular chromatin and a subsequent loss of plasma membrane integrity, upon withdrawal of IL-3 (6). Fig. 1 *A* demonstrates that such IL-3–deprived BAF3 cells began to lose viability 13 h after factor removal, and death was asynchronous within the cell population and complete only after 28 h. When BAF3 cells were exposed to X-irradiation (400 rad) immediately after IL-3 withdrawal, this loss of viability was more rapid and synchronous, being first observed after 8 h and essentially complete after 18 h (Fig. 1 *A*). In the presence of IL-3, BAF3 cells were completely resistant to this dose of radiation for 36 h (Fig. 1 *A*). Some loss of cell viability was observed over the following 36 h, and is shown in Fig. 1 *C*. During these first 72 h after X-irradiation, the viable cells showed little [^{3}H]thymidine incorporation (data not shown) and failed to increase in number (Fig. 1 *C*). However, exponential growth was observed after this initial lag period (Fig. 1 *C*). The viable cells in the culture were therefore able to divide normally. Indeed, 72 h after X-irradiation, such cells showed a cloning efficiency assessed by limiting dilution of 83%, compared with 87% of control cells. We have previously shown that primary murine bone marrow cells, cultured in the presence of IL-3, also apoptose upon cytokine removal (6). Such primary IL-3–dependent cultures, which were largely mast cells, also lost viability rapidly upon exposure to 400 rad of X-irradiation and could be protected from death by the presence of IL-3 (Fig. 1 *D*).

The main effect of X-irradiation at a dose of 400 rad is the induction of single-stranded DNA breaks (18). Therefore, to investigate whether DNA damage was responsible for the rapid induction of death in BAF3 cells, other agents known to induce DNA damage were used to treat BAF3 cells deprived of IL-3. Fig. 1 *B* demonstrates that etoposide, a topisomerase II inhibitor that also induces both single-stranded DNA and double-stranded breaks (21), and cisplatin, which causes DNA strand crosslinks (22), both induced cell death with similar kinetics to that observed after X-irradiation. IL-3 inhibited the action of these agents for the initial 24 h of culture (Fig. 1 *B*). At this time, removal of the drugs by

[1] *Abbreviations used in this paper:* FBS, fetal bovine seum; IGF-1, insulin-like growth factor 1.

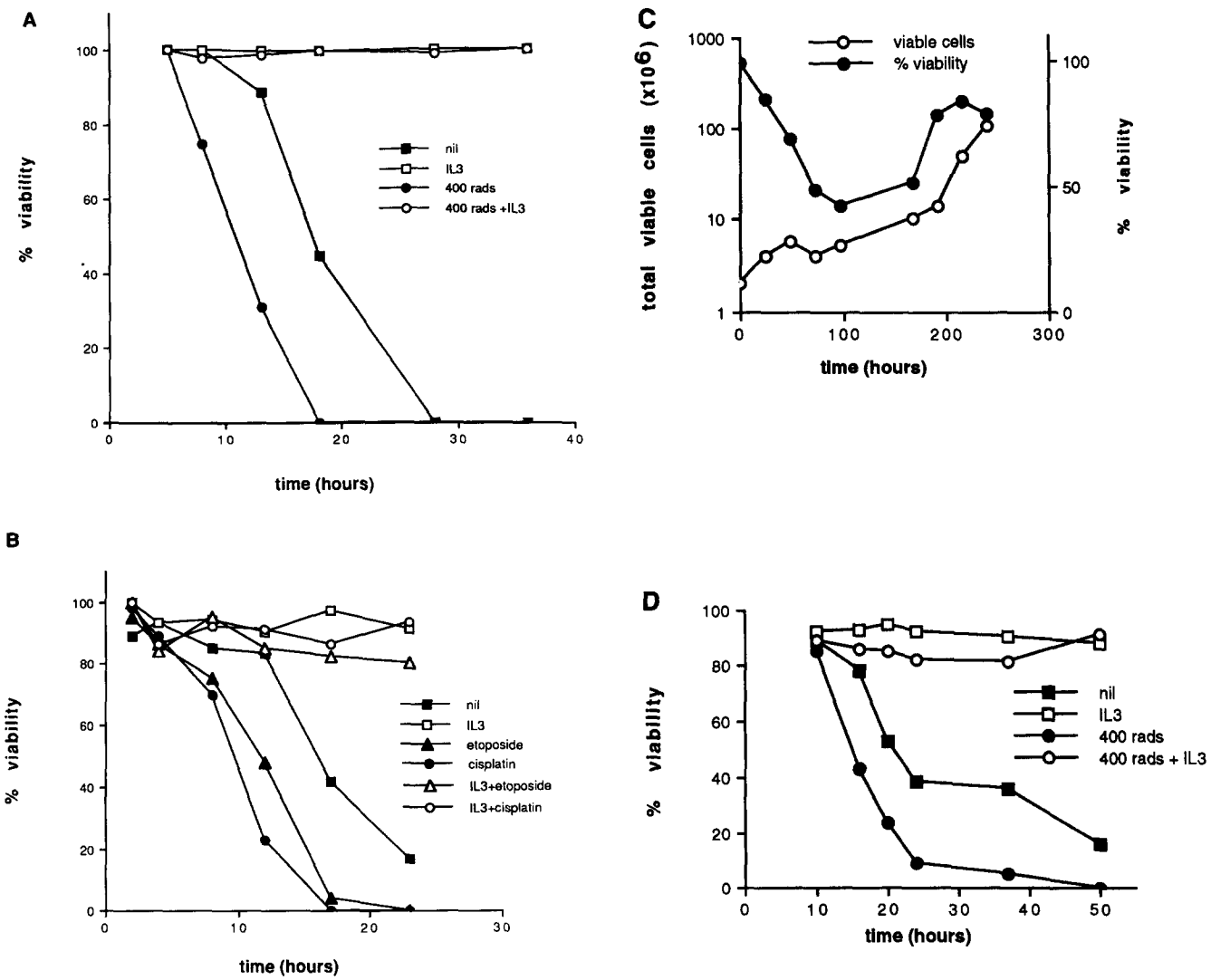

Figure 1. The induction of apoptosis by DNA damaging agents and its inhibition by IL-3. (*A*) BAF3 cells were washed to remove IL-3, exposed to 400 rad X-irradiation as indicated, then incubated, in the absence or presence of IL-3 as indicated, for the time shown. Cell viability was then determined. (*B*) BAF3 cells were incubated for the time shown with IL-3, 1 μg/ml etoposide and 4 μg/ml cisplatin as indicated, after which cell viability was determined. (*C*) 2 × 10^5 BAF3 cells were exposed to 400 rad X-irradiation, then incubated in the presence of IL-3 for the time shown, when the number and percentage of viable cells was determined. Cells were maintained at a density of <5 × 10^5 cells/ml. (*D*) Primary cultures of IL-3–dependent mast cells from murine bone marrow were treated as in *A*.

washing resulted in the recovery of cells that remained viable during subsequent culture.

The early loss of viability, observed after treatment of BAF3 cells with various DNA damaging agents, suggested that these agents might trigger the apoptotic pathway more rapidly than IL-3 removal alone. To investigate this, the time of initiation of DNA cleavage in X-irradiated, and etoposide- or cisplatin-treated BAF3 cells was observed. Fig. 2 *A* shows that oligonucleosome-length DNA fragments, characteristic of cleavage of cellular chromatin by an endogenous nuclease (13), could be detected 4 h after treatment of IL-3–deprived cells with these agents. In control cultures deprived of IL-3, such fragmentation was not observed until 8 h (reference 6 and Fig. 2 *A*). DNA cleavage was not detected in cultures treated with DNA damaging agents in the presence of IL-3 (Fig. 2 *A*). Thus, initial endogenous nuclease digestion of chromatin precedes initial loss of cell viability by 4–5 h in both DNA-damaged and control IL-3–deprived cells. Unlike this fixed time period after which cell death occurs once cleavage is initiated, it is clear that the time of onset of cleavage can be greatly shortened by DNA damaging agents.

The ability of IL-3 to protect BAF3 cells from X-irradiation or cytotoxic drugs could be demonstrated at very high doses of the DNA damaging agents. In the absence of IL-3, 100 rad of X-irradiation, 0.5 μg/ml cisplatin or 0.05 μg/ml etoposide were sufficient to induce the rapid onset of apoptosis

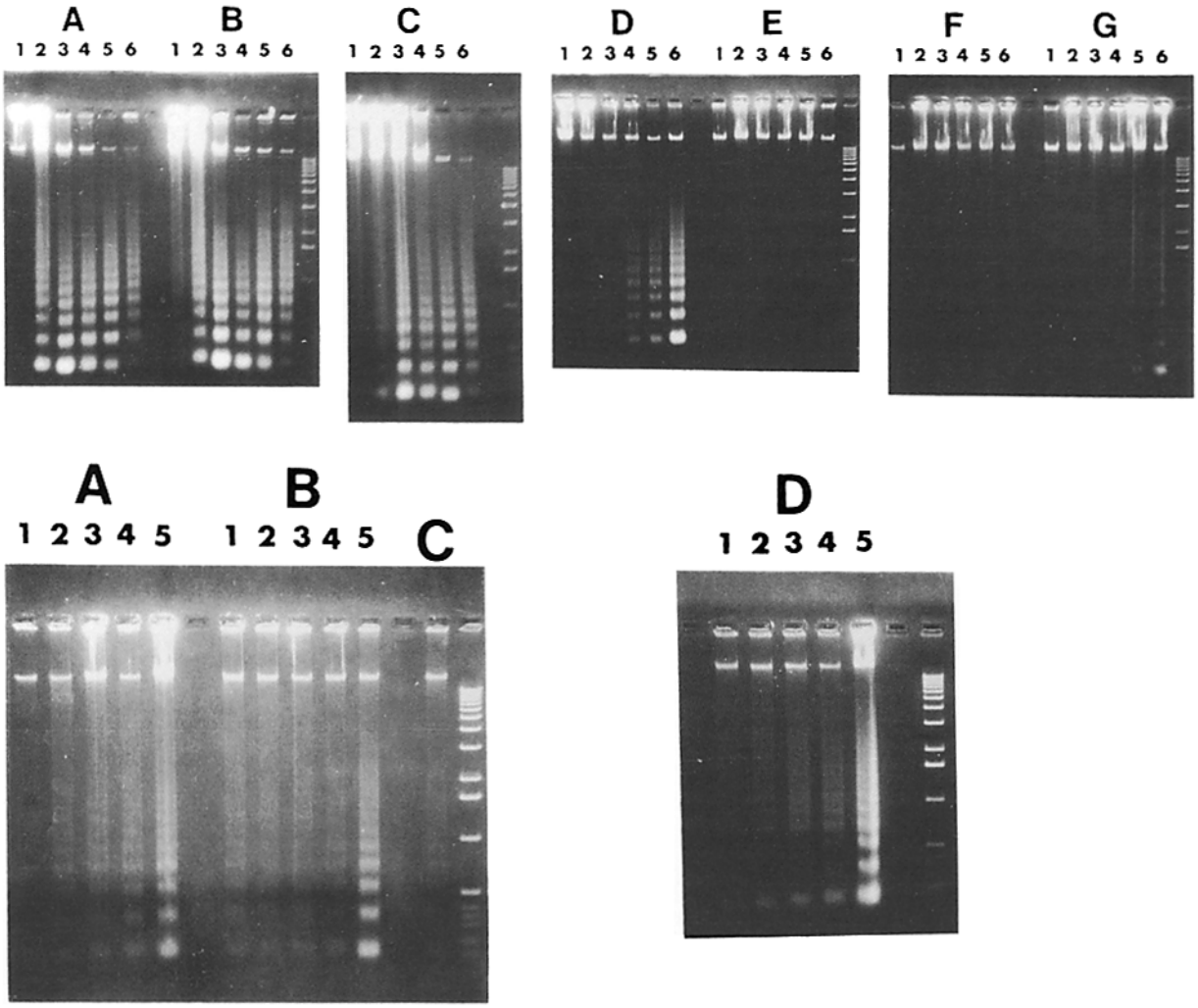

Figure 2. Effect of DNA damaging agents on DNA fragmentation. (*Upper panel*) BAF3 cells were X-irradiated (*A* and *E*), treated with 1 μg/ml etoposide (*B* and *F*) or 1 μg/ml cisplatin (*C* and *G*), then incubated in the absence (*A–C*) or presence (*E–G*) of IL-3 for 2 h (lanes *1*), 4 h (lanes *2*), 6 h (lanes *3*), 8 h (lanes *4*), 12 h (lanes *5*), and 23 h (lanes *6*). (*D*) DNA prepared from cells at the same time points, after IL-3 removal in the absence of any DNA damaging treatment. Fragmentation of cellular DNA was then analyzed. (*Lower panel*) BAF3 cells were incubated for 15 h in the presence of IL-3, either after X-irradiation: (*A*) lanes *1*. 200 rad; *2*. 400 rad; *3*. 800 rad; *4*. 1,600 rad; *5*. 3,200 rad); or in the presence of etoposide: (*B*) lanes *1*. 0.04 μg/ml; *2*. 0.1 μg/ml; *3*. 0.4 μg/ml; *4*. 1 μg/ml; *5*. 4 μg/ml); with no addition (*C*) or in the presence of cisplatin: (*D*) Lanes *1*. 1 μg/ml; *2*. 2 μg/ml; *3*. 4 μg/ml; *4*. 8 μg/ml; and *5*. 16 μg/ml). Fragmentation of cellular DNA was then analyzed.

(data not shown). In the presence of IL-3, the cells were resistant to 1,600 rad of X-irradiation. 8 μg/ml cisplatin, or 0.05 μg/ml 1 μg/ml etoposide (Fig. 2 *B*) which represent higher levels than are normally tolerated by eucaryotic cells (14, 21).

Other Growth-promoting Factors and the bcl2 Oncogene Product also Protect Cells from X-irradiation. Protection of these cells was not confined to IL-3. We have previously demonstrated that the growth-promoting factors IGF-1 and IL-4 can protect BAF3 cells from apoptosis when IL-3 is removed (6, 23). Fig. 3 demonstrates that either IGF-1 or IL-4 was effective in protecting the cells from X-irradiation. We have also demonstrated that the calcium ionophore A23187 can inhibit onset of apoptosis in the absence of IL-3 (6). Unlike IGF-1 or IL-4, A23187 does not stimulate proliferation of BAF3 cells. When added alone, the ionophore maintains cells viable but not cycling, and when added in the presence of IL-3, it arrests their cell cycle (6). The observation that A23187 alone or in combination with IL-3 could protect BAF3 cells from X-irradiation (Fig. 3 *A*) therefore suggested that cells do not need to cycle to recover from its effect. Because of the difficulty of maintaining cells for more than 48 h with calcium ionophores, this conclusion was best demonstrated when the effect of overexpression of the bcl2 protein on the sensitivity of BAF3 cells to radiation was observed. Fig. 3 *B* shows the behavior of a population of BAF3 cells, infected with a recombinant retrovirus encoding human bcl2 protein, when deprived of IL-3 and exposed to X-irradiation. The cells expressing human bcl2 protein showed a normal cell cycle distribution when grown in IL-3 (Fig. 3 *B*, panel *a*) but arrested with diploid DNA content 48 h after IL-3 was removed (Fig. 3 *B*, panel

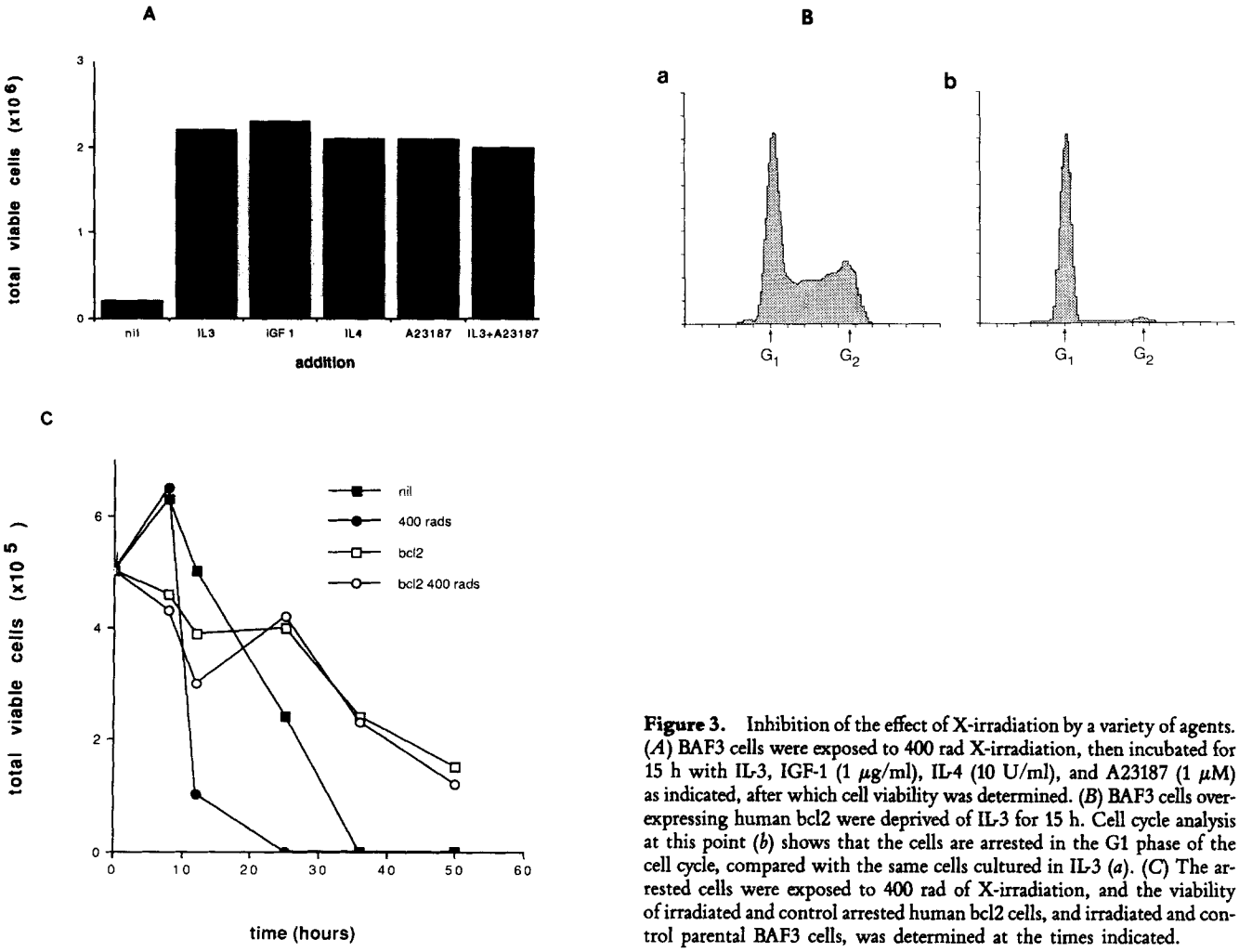

Figure 3. Inhibition of the effect of X-irradiation by a variety of agents. (*A*) BAF3 cells were exposed to 400 rad X-irradiation, then incubated for 15 h with IL-3, IGF-1 (1 μg/ml), IL-4 (10 U/ml), and A23187 (1 μM) as indicated, after which cell viability was determined. (*B*) BAF3 cells overexpressing human bcl2 were deprived of IL-3 for 15 h. Cell cycle analysis at this point (*b*) shows that the cells are arrested in the G1 phase of the cell cycle, compared with the same cells cultured in IL-3 (*a*). (*C*) The arrested cells were exposed to 400 rad of X-irradiation, and the viability of irradiated and control arrested human bcl2 cells, and irradiated and control parental BAF3 cells, was determined at the times indicated.

b). Such arrested cells lost viability after ~100 h total IL-3 deprivation (Fig. 3 *C*), and their rate of death was not accelerated by X-irradiation (Fig. 3 *C*).

IL-3 Does Not Affect the Extent of DNA Damage or the Rate of Its Repair. To investigate the mechanism by which IL-3 protected BAF3 cells from X-irradiation, the extent of repair of DNA damage was measured in cells X-irradiated at 4°C in the absence of IL-3, then incubated in the absence or presence of IL-3 at 37°C. Single-stranded DNA breaks after a dose of 400 rad were detected by alkaline filter elution (18). No double-stranded DNA breaks could be detected by neutral filter elution (24). Fig. 4 *A* demonstrates that the initial single-stranded DNA breaks were undetectable after a 1-h incubation in either the absence or presence of IL-3. This suggested that DNA damage did not lead directly to the onset of DNA cleavage in cells deprived of IL-3. However, the sensitivity of this measurement of single-stranded DNA breaks did not exclude the possibility that a small number of breaks remained unrepaired in the cells deprived of IL-3. The rate of DNA repair was therefore measured in cells deprived of IL-3 for 6 h. After 5 min, the extent of DNA repair was identical in IL-3–deprived (Fig. 4 *C*) and control cells (Fig. 4 *B*) and no breaks could be detected after 15′ in either culture (data not shown). Thus, as the rate of repair was not impaired in cells deprived of IL-3 for 6 h, a failure to repair a small number of breaks seemed unlikely to be responsible for the damage sensitivity of IL-3–deprived cells.

IL-3 Allows Cells To Pass a G2 Restriction Point at which DNA Repair Is Monitored. Because IL-3 did not affect the initial repair of DNA damage, it was then determined at which point during induction of the apoptotic process the presence of IL-3 was necessary. Fig. 5 shows that IL-3 could be removed from control BAF3 cells for 9 h, then readded with no subsequent loss in cell viability. Further experiments demonstrated that this period of IL-3 deprivation could be extended to 12 h (data not shown). X-irradiated cells could be deprived of IL-3 for the first 2 h after irradiation with no loss in viability upon IL-3 readdition. Even at 6 h after irradiation, 70% of the cells could be rescued by IL-3 (Fig. 5). These data demonstrate that the first irreversible event in the onset of apoptosis occurred close to the time of initiation of DNA cleavage in both control and X-irradiated cells.

As the point at which IL-3 protected cells from X-irradiation was several hours after the initial damage and repair, the cy-

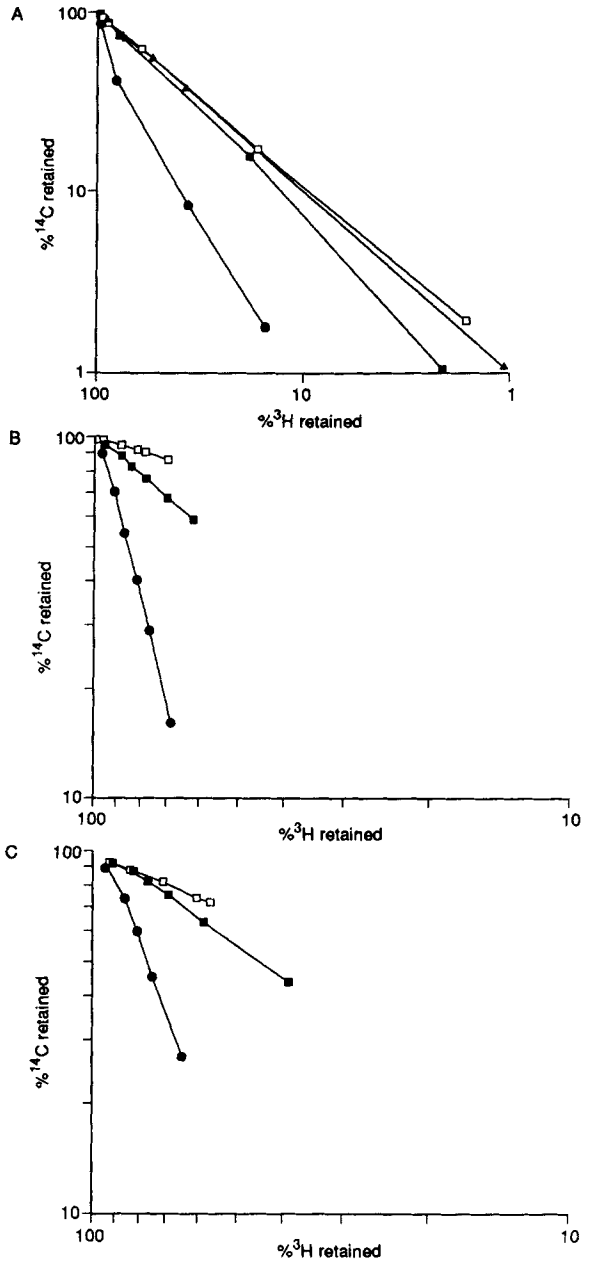

Figure 4. Measurement of single-stranded DNA breaks and their repair. (*A*) Comparative rates of elution of test (^{14}C-labeled) and internal standard (^{3}H-labeled) DNA. Test samples were prepared from BAF3 cells that were washed to remove IL-3, then analyzed (□), given 400 rad X-irradiation, and analyzed without further treatment (●), or irradiated and then incubated for 1 h at 37°C in the absence (▲) or presence (■) of IL-3. (*B*) Test samples were prepared from control BAF3 cells (□), cells given 400 rad X-irradiation in the presence of IL-3 (●), and irradiated cells subsequently incubated for 5′ at 37°C in the presence of IL-3 (■). (*C*) Test samples were prepared from BAF3 cells deprived of IL-3 for 6 h (□), these cells were given 400 rad X-irradiation in the absence of IL-3 (●), and these irradiated cells subsequently incubated for 5′ at 37°C in the absence of IL-3 (■).

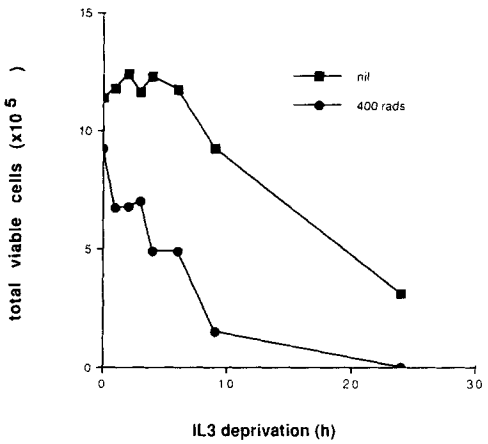

Figure 5. Determination of the time at which IL-3 is required to prevent cell death. BAF3 cells were washed to remove IL-3 and irradiated with 400 rad X-rays as indicated. They were then incubated at 37°C for the time shown in the absence of IL-3. IL-3 was then readded and cell viability was determined 24 h after the initial IL-3 deprivation.

cling of the cells during this period in the absence or presence of IL-3 was observed by cytofluorimetry of propidium iodide-stained cells. The presence of a subdiploid DNA content population, with light scatter characteristics identical to the rest of the cells, can be used as a measure of the number of cells undergoing apoptosis (6). In control cells deprived of IL-3, such a population was <5% of the total 9 h after factor removal. At this time the cells were still distributed throughout the cell cycle very much as were cells cultured in IL-3 (Fig. 6). Thus removal of IL-3 did not result in any rapid cessation of cell cycle progression in these cells. X-irradiation of the cells resulted in an accumulation in the G2 phase of the cell cycle, which was similar in the absence or presence of IL-3 (Fig. 6). This is characteristic behavior of eucaryotic cells exposed to DNA-damaging agents (25). However, in the absence of IL-3, apoptotic cells began to appear in the culture after 6 h. This appearance of apoptotic

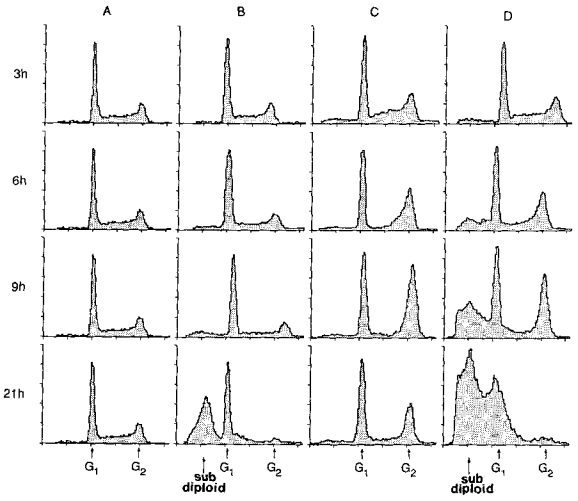

Figure 6. Cell cycle analysis of X-irradiated cells. The cell cycle distribution of BAF3 cells cultured in the presence of IL-3 (*A*), in the absence of IL-3 (*B*), X-irradiated with 400 rad, then cultured in the presence of IL-3 (*C*), or X-irradiated with 400 rad, then cultured in the absence of IL-3 (*D*), for the time shown, was determined.

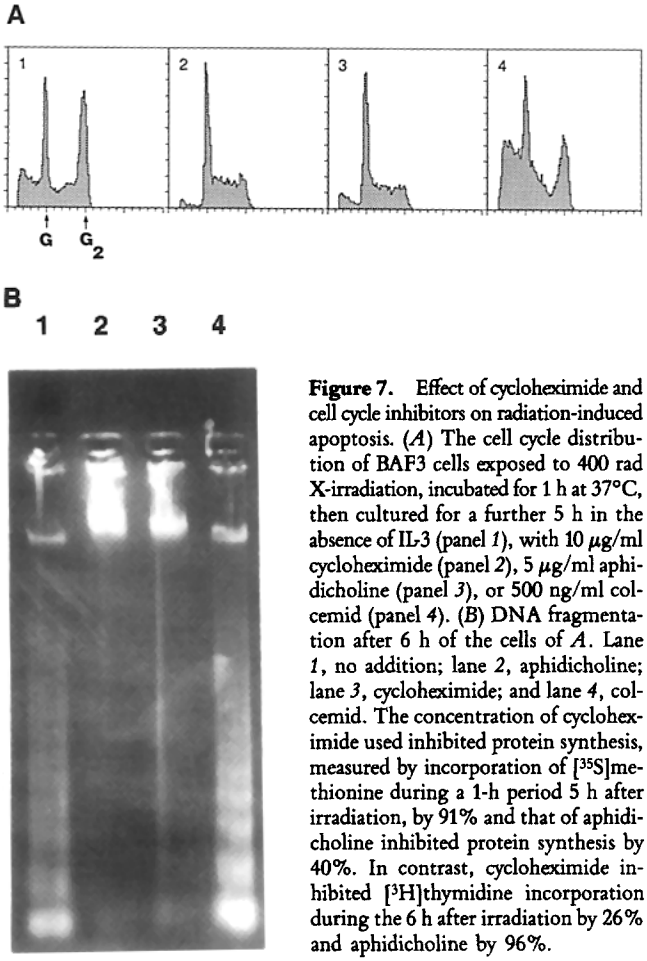

Figure 7. Effect of cycloheximide and cell cycle inhibitors on radiation-induced apoptosis. (*A*) The cell cycle distribution of BAF3 cells exposed to 400 rad X-irradiation, incubated for 1 h at 37°C, then cultured for a further 5 h in the absence of IL-3 (panel *1*), with 10 μg/ml cycloheximide (panel *2*), 5 μg/ml aphidicholine (panel *3*), or 500 ng/ml colcemid (panel *4*). (*B*) DNA fragmentation after 6 h of the cells of *A*. Lane *1*, no addition; lane *2*, aphidicholine; lane *3*, cycloheximide; and lane *4*, colcemid. The concentration of cycloheximide used inhibited protein synthesis, measured by incorporation of [^{35}S]methionine during a 1-h period 5 h after irradiation, by 91% and that of aphidicholine inhibited protein synthesis by 40%. In contrast, cycloheximide inhibited [^{3}H]thymidine incorporation during the 6 h after irradiation by 26% and aphidicholine by 96%.

cells was accompanied by a loss of cells from the G2 arrest point (Fig. 6 *B*).

To investigate whether a checkpoint associated with G2 arrest was directly responsible for the onset of apoptosis, we used agents that prevented this G2 arrest. Fig. 7 *A* demonstrates that both cycloheximide, an inhibitor of protein synthesis, and aphidicholine, which inhibits DNA polymerase α and therefore blocks DNA replication, prevented the accumulation of cells in G2 after X-irradiation in the absence of IL-3. Significantly, both of these agents were able to prevent the rapid onset of DNA cleavage observed in control cells that had accumulated in G2 (Fig. 7 *B*), whereas colcemid, which blocks the cell cycle in mitosis and thus does not affect the G2 arrest point, had no effect on the radiation-induced DNA cleavage (Fig. 7 *B*).

Discussion

The data presented here demonstrate a direct inhibition by cytokines of apoptosis triggered by DNA damage. Various cytokines have been shown to be radioprotective in vivo (for review see reference 26). They can be classified into those that can be given before radiation and afford subsequent protection, such as IL-1 (27) and TNF-α (27), and those that have been shown to be myelorestorative when given after irradiation. IL-3 belongs to the latter category (28), and under these conditions, presumably acts to stimulate the proliferation of residual undamaged progenitor cells. The direct protection of BAF3 cells by IL-3, and other growth-promoting factors, suggests that bone marrow progenitor cells might be protected by appropriate cytokines administered during chemo- or radiotherapy of nonhaematopoietic tumors. It also implies that differential sensitivity of a variety of tumors to DNA damaging drugs or radiation could be explained by their production of autocrine growth factors, or overexpression of the bcl2 protein.

The presence of IL-3 does not affect the initial extent of DNA breaks caused by irradiation, or their rate of repair. Therefore, the mechanism by which DNA damage leads to apoptosis in BAF3 cells deprived of IL-3 involves the detection of DNA repair. The arrest of eucaryotic cells in the G2 phase of the cell cycle subsequent to DNA damage and repair is a well-recognized phenomenon (25) and, although the molecular basis of this control is not understood, yeast mutants deficient in this regulation have been isolated (29). The death of BAF3 cells from such a G2 restriction point and its inhibition by preventing cells from reaching the restriction point, demonstrates that cells are particularly sensitive to the onset of apoptosis when arrested there in the absence of IL-3. However, it is clear that arrest in G2 per se is not necessary for recovery of BAF3 cells from DNA damage in the presence of IL-3. Bcl2 overexpressing cells, which remain arrested in G1 in the absence of IL-3, are also resistant to X-irradiation. Such radio-resistance has also been reported in thymocytes isolated from transgenic mice overexpressing bcl2 (30, 31), and we have also demonstrated that bcl2 overexpressing BAF3 cells are resistant to DNA damaging agents such as etoposide (Ascaso, R., M. Collins, J. Marvel, and A. Lopez-Rivas, manuscript in preparation). Whether maintenance or induction of bcl2 function represents part of the mechanism of action of IL-3 remains to be investigated. A decrease in bcl2 mRNA has been reported late after IL-3 removal from a factor-dependent cell line (10).

IL-3 removal from either control, or X-irradiated BAF3 cells does not affect the cycling of the cells. Furthermore, IL-3 is not required to protect cells from apoptosis until a time point very close to the initiation of DNA cleavage. This implies that IL-3 is not only a growth factor that is required to maintain cell proliferation of these factor-dependent cells, but also a survival factor that prevents the onset of DNA cleavage. The signaling pathway that leads to apoptosis after IL-3 removal requires at least 8 h for the initiation of this cleavage. DNA damage either induces a different pathway or the same pathway with more rapid kinetics, as cleavage in this case starts after 4 h. The ability of IL-3 to rapidly reverse the apoptotic program when added close to the time of nuclease cleavage, argues against a coordinated and irreversible program of gene expression leading to apoptosis. One possibility is that IL-3 acts to maintain the level of crucial

cellular metabolites such as ATP (32). It is known that the presence of DNA strand breaks causes the activation of poly(ADP-ribose)polymerase, which consumes cellular NAD^+ leading to a fall in ATP (33). This could explain the more rapid onset of apoptosis after DNA damage. To define the mechanism of action of IL-3, it is now necessary to identify the cellular changes that immediately precede onset of DNA cleavage, determine how they lead to an increase in nuclease activity, and demonstrate their rapid reversal by IL-3.

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