

Interleukin 7-dependent B Lymphocyte Precursor Cells Are Ultrasensitive to Apoptosis

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Summary

We have compared the sensitivity of clonogenic interleukin 7 (IL-7)-dependent murine B cell precursors with that of clonogenic mature B cells and myeloid precursors to α -particles from plutonium-238 and X radiation. All three populations are relatively sensitive, but B cell precursors are ultrasensitive. This differential sensitivity is also observed with corticosteroid, etoposide, and cisplatin, all apoptosis-inducing drugs used in the treatment of leukemia and other cancers. Further, we show that x-rays and drugs induce the bulk of the B cell precursor population to undergo rapid apoptosis, despite the continued presence of IL-7. B cell precursors were found to express very low levels of BCL-2 protein compared with mature splenic B cells and their resistance to x-rays and corticosteroid could be enhanced by expression of a BCL-2 transgene. These data have important implications for normal lymphopoiesis and for the behavior of leukemic lymphoid precursor cells.

Epidemiological (1) and experimental (2, 3) studies have focused attention on the possible leukemogenic effects of low level, high density ionizing radiation such as α -particles from various sources including plutonium-239 and radon. In model experiments designed to test this possibility, we attempted to calculate the mutation frequency in a marker gene (HPRT; hypoxanthine-guanine phosphoribosyl transferase) in clonogenic IL-7-dependent murine B cell precursors exposed to high linear energy transfer (LET)¹ α -particles emitted from plutonium-238. The surprising outcome was that B cell precursors (as compared with other more primitive hemopoietic or mature B cells) were very sensitive to the lethal effects of α -particle irradiation. We also calculated that for most B cell precursors a single α -particle induced such a low probability of survival, that the chance of producing mutagenic damage was very small (Griffiths, S. D., S. J. Marsden, E. G. Wright, M. F. Greaves, and D. T. Goodhead, manuscript submitted for publication.).

A plausible interpretation of these observations with

α -particles was that B cell precursors might be ultrasensitive to programmed cell death or apoptosis, after DNA damage. Thymocytes and T cells are known to be very sensitive to γ -irradiation and corticosteroid-induced apoptosis (4, 5) and IL-3-activated myeloid progenitors from bone marrow die by apoptosis in the absence of growth factor (6, 7).

The sensitivity of normal B cell precursors to apoptosis has not been rigorously examined but is likely to be of relevance both to the regulation of lymphopoiesis and aspects of leukemic cell development, pathology, and clinical response. B cell (and T cell) precursors may be particularly susceptible to, or programmed for, apoptosis to counteract a high risk of acquiring mutagenic damage during lymphopoiesis (Griffiths, S. D. et al., manuscript submitted for publication). Apoptosis may also act to remove clones that are either autoreactive or those that have failed to competently rearrange their immunoglobulin (or T cell receptor) genes (8). Finally, the leukemic equivalents of normal B cell precursors in children with common acute lymphoblastic leukemia (ALL) are in most cases very susceptible to therapy with apoptosis-inducing agents (9). This might reflect the intrinsic sensitivity of normal B cell precursors to apoptosis, which is also expressed in leukemic cells (10, 11).

To assess the susceptibility of B cell precursors to apoptosis following DNA damage, we have compared clonogenic

¹ Abbreviations used in this paper: ALL, acute lymphoblastic leukemia; BM, bone marrow; D₃₇, dose of radiation (Gy) giving inhibition of colony growth to 37% of unirradiated controls; LET, linear energy transfer.

IL-7-dependent B cell precursors from bone marrow and fetal liver with bone marrow-derived myeloid precursors cloned in the presence of GM-CSF and splenic B cells cloned with LPS for their differential sensitivity to ionizing radiation and apoptotic drugs. The relationship between sensitivity to apoptosis and expression of the antiapoptotic BCL-2 protein has also been examined.

Materials and Methods

Source of Murine Hemopoietic Cells

Bone Marrow. Femoral bone marrow was obtained from groups of three 8–12-wk-old BALB/c mice or individual (Che × C57Bl/6)F₁ mice. The latter strain was used to generate animals that were transgenic for a human BCL2-Ig transgene as previously described (12).

B Cell Precursors. Bone marrow (BM) cells (2×10^6 cells/ml) were cultured for 7 d at 37°C plus 7% CO₂ in McCoy's complete medium containing McCoy's 5a, 15% FCS, pyruvate, MEM vitamin, 2-ME (5×10^{-5} M), amino acid supplements (GIBCO BRL, Gaithersburg, MD) (13) and recombinant human IL-7 (500 U/ml; a gift from Dr. S. Gillis, Immunex, Seattle, WA and Sterling Winthrop Inc., Collegeville, PA) (14). The cultures were fed at 4 d with an equal volume of fresh medium containing IL-7. After culture, the nonadherent cell viability was >98% by trypan blue exclusion, and analysis by fluorescent microscopy showed that >90% of cells expressed the pre-B cell markers B220 and BP-1 (15). A variable minority (5–40%) of cells expressed cytoplasmic IgM (C μ) and 2–7% were cell surface Ig positive mature B cells. The latter were removed using anti-Ig-coated magnetic beads (Dynabeads; DYNAL, Oslo, Norway). Fetal liver was obtained from 15–16 d BALB/c fetuses (10 pooled), and were cultured in McCoy's complete medium plus IL-7 as above for 9 d. The resulting nonadherent cells were >98% viable, and 98 and 87% expressed B220 and BP-1, respectively; <1% of cells were C μ positive.

Spleen Cells. Spleens were obtained from groups of two 8–12-wk-old BALB/c mice. Spleen fragments were teased through wire mesh and resuspended in McCoy's complete medium. In the majority of experiments, spleen cells (10^6 /ml) were cultured at 37°C plus 7% CO₂ for 3 d in McCoy's complete medium plus LPS (25 μ g/ml, Difco Laboratories, Inc., Detroit, MI) before irradiation, or the addition of drugs. After culture with LPS >90% of spleen cells had a lymphoblastoid morphology, expressed cytoplasmic and/or cell surface Ig. Cell cycle analysis by flow cytometry (16) showed that ~30% of blasts had entered S/G2M.

Cloning of B Cell Precursors, LPS Reactive Mature B Cell Blasts, and Myeloid Precursor Cells

B Cell Precursors. BM-derived B cell precursors were cloned in agar as described by Lee et al. (13). Briefly, 2×10^5 BM cells without culture, or B cell precursors grown in IL-7 for 7 d were cloned in three replicate 35-mm dishes containing 1.0 ml of McCoy's complete medium plus human IL-7 (500 U/ml) and 0.3% agar. B cell precursors colonies were scored after 6 d of culture at 37°C plus 7% CO₂. Colony data (mean \pm 1 SEM) after exposure to α -particles, x-rays, dexamethasone, etoposide, and cisplatin are from 3–7 replicate experiments.

Spleen B Cells. 5×10^4 spleen cells with or without LPS preactivation were cloned in replicate dishes containing 1.0 ml of McCoy's complete medium plus LPS (25 μ g/ml) and 0.3% agar. B cell colonies were scored after 4 or 6 d; with or without LPS

preactivation, respectively. Colony data (mean \pm 1 SEM) after exposure to α -particles, x-rays, dexamethasone, etoposide, and cisplatin are from four replicate experiments.

Myeloid Precursors (CFU-GM). 2×10^5 BM cells were cloned in replicate 35-mm dishes containing 1.0 ml of RPMI 1640, 10% FCS, L-glutamine, penicillin, streptomycin 0.3% agar (Difco Laboratories Inc.), and murine GM-CSF (20 U/ml; British Biotech, Oxford, UK). CFU-GM colonies were scored after 6 d of culture at 37°C plus 7% CO₂. Colony data (mean \pm 1 SEM) after exposure to either x-rays or dexamethasone, etoposide and cisplatin are from two or four experiments, respectively.

The cloning efficiency of CFU-GM, B cell precursors and LPS-reactive B cell colonies in spleen were linear with respect to the numbers of cells plated: 0.1 ± 0.01 , 0.8 ± 0.06 , and $0.5 \pm 0.06\%$ for CFU-GM, B cell precursors, and LPS reactive B cells, respectively.

Addition of Dexamethasone, Etoposide, and Cisplatin

CFU-GM, B cell precursors (cultured in the presence of IL-7 for 7 d) and LPS preactivated spleen cells were cloned in 0.3% agar as above in the presence and absence of dexamethasone (0.001 nM–10 μ M; Sigma Chemical Co., St. Louis, MO), etoposide (1.25 ng/ml–1.25 μ g/ml; Bristol Meyers Pharmaceuticals, Evansville, IN) and cisplatin (20 ng/ml–1.25 μ g/ml).

Irradiation with x-rays and α -particles

Low LET x-rays. Bone marrow (immediately after collection), fetal liver or BM cells grown in IL-7 for 9 and 7 d, respectively, and spleen cells (\pm LPS preactivation for 3 d) were exposed at $\sim 2 \times 10^6$ cells/ml in McCoy's complete medium plus IL-7 or LPS to x-rays (0–5 Gy) where the source (Pantak Ltd., Reading, UK) was set to 240 kV and 10 mA. The dose rate was 0.95–1.0 Gy/min. In some experiments, the dose rate was reduced to 0.05 Gy/min.

High LET α -particles. Bone marrow cells, 7 day IL-7-responsive B cell precursors, or spleen cells (\pm LPS preactivation) were exposed to α -particles (LET; 121 kilo-electron volts/ μ m) from a plutonium-238 source, as described elsewhere (17). After exposure, the cells were plated directly in CFU-GM, B cell precursor, or LPS colony assays.

Analysis of Apoptosis by DNA Fragmentation

Flow Cytometry. B cell precursors (cultured in IL-7 for 7 d) either exposed to x-rays (0.95–1.0 Gy/min), or dexamethasone, etoposide, and cisplatin were cultured in McCoy's complete medium plus IL-7 for up to 24 h at 37°C plus 7% CO₂. In some experiments, cyclohexamide (Sigma Chemical Co.; 0.1 μ g/ml) was present throughout the culture period. After culture, the cells were fixed in 500 μ l of 70% ethanol and resuspended in 1.0 ml of phosphate-buffered saline containing propidium iodide (20 μ g/ml) and RNase (100 μ g/ml) (both from Sigma Chemical Co.). The cell suspension was incubated at 37°C for 30 min and cell cycle analysis was performed using a FACScan® (Becton Dickinson & Co., Mountain View, CA), where the apoptotic cell fraction was detected in the red fluorescent sub-G1 peak (16). The data is expressed as a ratio between the apoptotic sub-G1 and G0/G1 peaks.

Gel Electrophoresis. 10^7 B cell precursors, either exposed to x-rays or cultured in the presence of dexamethasone, etoposide, and cisplatin as above, were lysed in Tris (10 mM) EDTA (1.0 mM) proteinase-K buffer containing proteinase-K (100 μ g/ml). After incubation at 37°C for 8 h, DNA was phenol extracted as essentially described by (18). DNA fragmentation was resolved by ethidium bromide agarose (1.5%) gel electrophoresis.

Detection of BCL2 Protein

Endogenous BCL2 protein was detected in paraformaldehyde/methanol-fixed cytocentrifuge smears by indirect immunofluorescence using a polyclonal rabbit antibody raised against a BCL2 synthetic peptide based on murine BCL2 sequence information (19; Krajewsky, S., and J. C. Reed, manuscript submitted for publication). Controls consisted of either normal rabbit Ig or blocking with the relevant synthetic peptide. Expression of the human BCL2 protein derived from the *BCL2-Ig* transgene was similarly detected using a monoclonal anti-human BCL2 reagent as previously described (20). In some experiments, the expression of the *BCL2-Ig* transgene in either B cell or myeloid progenitor cells was measured by double immunofluorescence staining using BCL2 antibody with BP-1 or MAC-1 monoclonal antibodies as a marker for B cell precursors and myeloid progenitor cells respectively. Binding of BCL2, BP-1, or MAC-1 antibodies was assessed using FITC- or PE-labeled second layer antibodies, followed by confocal microscopy.

Results

The sensitivities of clonable myeloid progenitor, B cell precursor and mature B cells were assayed following in vitro exposure to either low LET x-rays, or high LET α -particles as sources of ionizing radiation. The x-ray and α -particle dose giving 37% survival (D_{37}) of the clonable fraction for the different cell types is presented in Table 1 and Fig. 1A. When these data are compared, both mature B cells (with and without LPS preactivation) from spleen and myeloid progenitor cells derived from BM had similar sensitivities to the lethal effects of x-rays. In contrast, B cell precursors present in BM or fetal liver (with and without IL-7 preculture) were ultrasensitive as reflected in very low D_{37} doses. B cell precursors were also very sensitive to x-ray damage when a reduced x-ray dose rate of 0.05 Gy min⁻¹ was employed; D_{37} , 0.22 \pm 0.02 Gy. Similarly, the D_{37} for B cell precursors exposed to high density ionizing α -particles were smaller than those obtained for mature B cells in spleen.

It seemed likely that the inhibition of clonogenicity by x-rays or α -particles was due to apoptosis. We therefore compared the same three clonogenic cell populations for sensitivity to apoptosis-inducing drugs used in the treatment of leukemias and other cancers; corticosteroid (dexamethasone), an epipodophyllotoxin (etoposide/VP16), and cisplatin. The drug dose response curves (Fig. 1, B-D) and the extrapolated drug doses resulting in either 50% (inhibitory dose [ID] 50) or 90% (ID 90) inhibition of colony growth (Table 2) demonstrated that B cell precursors were strikingly more sensitive than mature B cells or myeloid progenitors to dexamethasone and etoposide. B cell precursors were also more sensitive than mature B cells and myeloid progenitors to cisplatin but the difference was much less marked than with x-rays and etoposide (Fig. 1D, Table 2). It may be significant that, in contrast to x-rays and etoposide that induce double-stranded DNA breaks, cisplatin is a DNA cross-linker.

Apoptosis can be assayed by measuring DNA fragmentation by flow cytometry or by agarose gel electrophoresis. Cyclohexamide also inhibits apoptosis by inhibiting protein synthesis (16). To further explore the involvement of apoptosis in the sensitivity of B cell precursors cells to ionizing radiation and drugs, we next documented the response of the total IL-7 expanded B cell precursor population to x-rays, dexamethasone, etoposide, and cisplatin. B cell precursors exposed to x-rays or drugs underwent apoptosis as indicated by flow cytometry; the ratio of sub-G1 to G0/G1 DNA cell content, calculated from the areas in histogram regions 1 and 2, respectively, (shown next to the DNA profiles in Fig. 2), increased rapidly between 2 and 24 h of culture. DNA fragmentation in B cell precursors was also detected by agarose gel electrophoresis. The nucleosomal DNA ladder characteristic for apoptotic cells was observed after exposure to x-rays, or culture with either dexamethasone, etoposide or cisplatin (Fig. 3, lanes 2-5) when compared with controls (Fig. 3, lane 1). B cell precursor apoptosis induced by x-rays could also be

Table 1. Sensitivity of Myeloid Progenitor, B Cell Precursor (Pre-B), and Mature-B Clonogenic Cells to the Lethal Effects of X-rays and α -Particles

Lineage	Colony-forming cells	α -particles* (D_{37} :Gy)	x-rays* (D_{37} :Gy)
Myeloid	CFU-GM	ND	1.4 \pm 0.1
Lymphoid	BM, pre-B [‡]	0.39 \pm 0.03	0.31 \pm 0.02
	BM, pre-B + IL-7 [§]	0.22 \pm 0.03	0.24 \pm 0.01
	FL, pre-B + IL-7	0.18 \pm 0.04	0.15 \pm 0.005
	Splenic mature B	1.16 \pm 0.04	1.2 \pm 0.1
	Splenic mature B + LPS [†]	1.1 \pm 0.2	1.0 \pm 0.1

* Dose in Gray (Gy) for 37% survival (D_{37}) was calculated from a least square regression fit of data from replicate experiments to the linear equation for logarithm of clonable fraction vs. dose.

[‡] BM, irradiated immediately after collection.

[§] BM, cultured with IL-7 for 7 d before irradiation.

^{||} Fetal liver from a pool of eight mice; cultured with IL-7 for 9 d before irradiation.

[†] With LPS preactivation for 3 d.

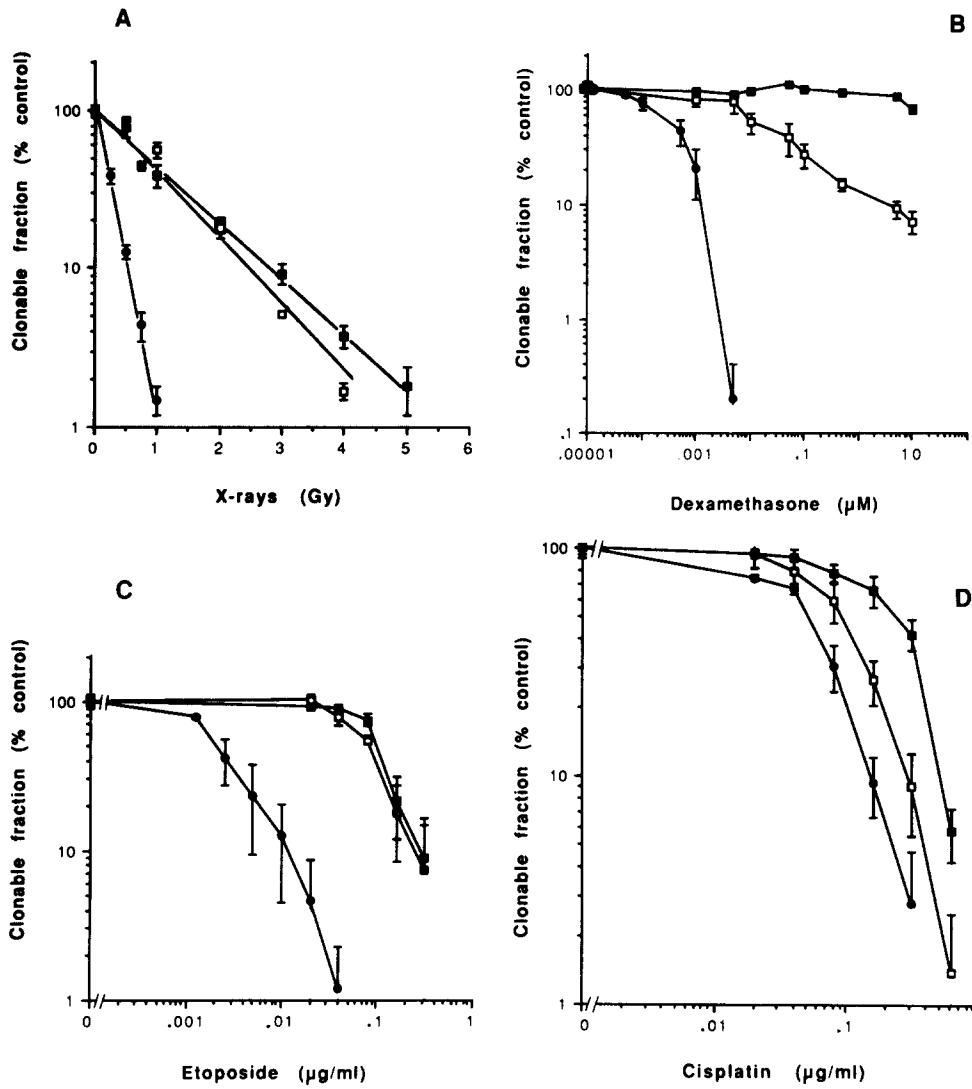


Figure 1. Survival of clonable CFU-GM, ■-■; mature B cells, □-□; and B precursors, ●-●; after exposure to: (A) x-rays; or in the presence of: (B) dexamethasone; (C) etoposide; and (D) cisplatin. Data represents the mean \pm 1 SEM. from replicate experiments (Materials and Methods), where bone marrow or spleen was obtained from pools of two or three mice. B cell precursors and spleen cells were precultured in IL-7 or LPS for 7 or 3 d, respectively, before assay.

Table 2. Sensitivity of Clonogenic Myeloid Precursor, B Cell Precursor (Pre-B) and Mature B Cells to Dexamethasone, Etoposide, and Cisplatin

Lineage	Colony-forming cells	ID (% control inhibition)	Dexamethasone*	Etoposide*	Cisplatin*
			nM	ng/ml	ng/ml
Myeloid:	CFU-GM	50	Resistant	108 \pm 8	230 \pm 45
		90	Resistant	230 \pm 30	500 \pm 37
Lymphoid:	BM, pre-B + IL-7 [†]	50	0.4 \pm 0.14	3.2 \pm 1.3	50 \pm 9
		90	1.1 \pm 0.2	8.5 \pm 5.3	160 \pm 29
	Splenic mature B + LPS [§]	50	29 \pm 21	94 \pm 10	88 \pm 10
		90	4,730 \pm 2,100	160 \pm 19	280 \pm 52

* Data represents the mean \pm 1 SEM from replicate experiments (Materials and Methods), where BM or spleen was obtained from pools of two or three mice.

[†] BM, cultured with IL-7 for 7 d before assay.

[§] With LPS preactivation for 3 d.

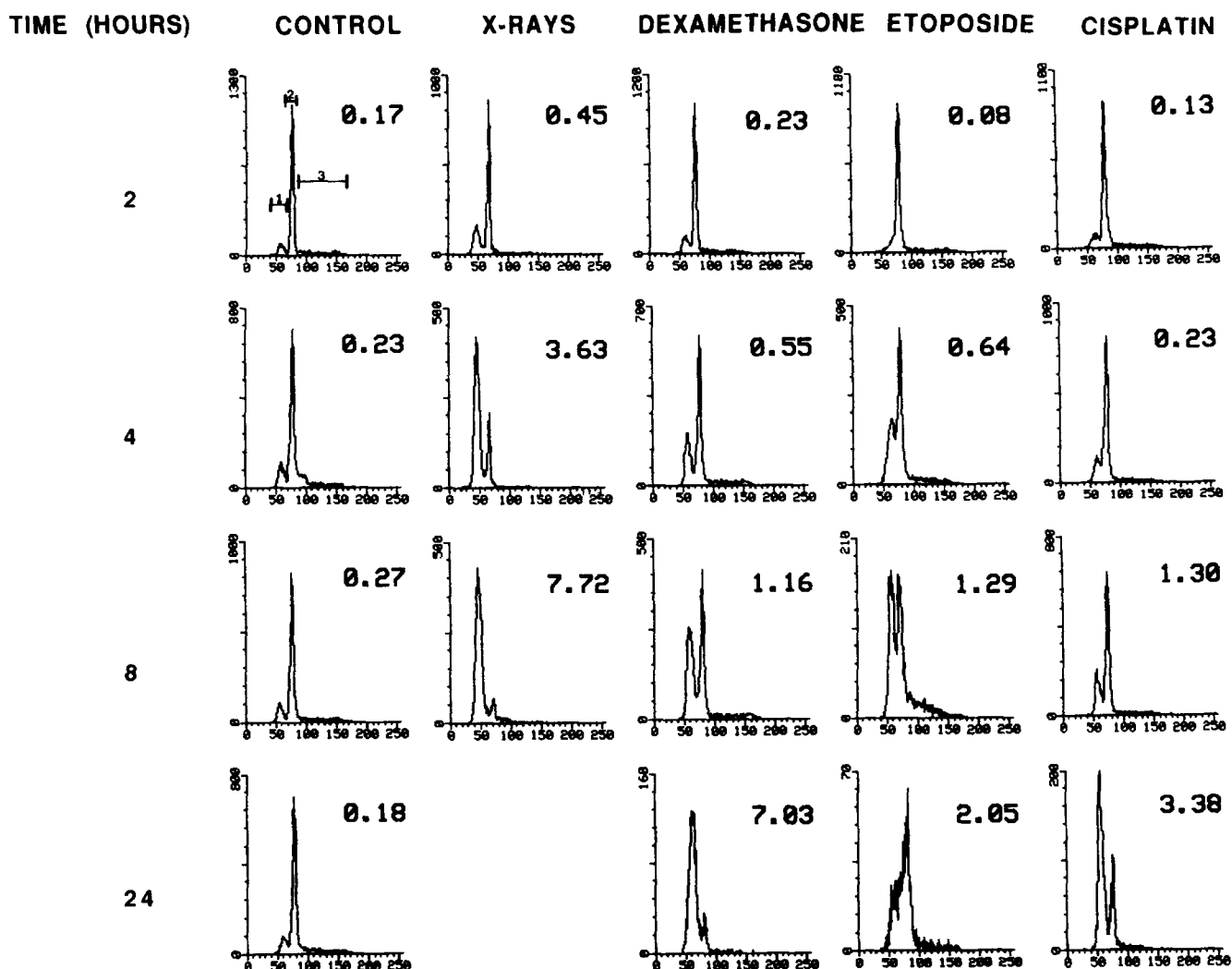


Figure 2. Time course for B precursor cell apoptosis as detected by flow cytometry after exposure to x-rays (3.0 Gy) or in the presence of: dexamethasone (0.1 μ M), etoposide (0.16 μ g/ml), and cisplatin (10 μ g/ml). Regions 1–3 represent cells with sub-G1, G0/G1, or S/G2M DNA content, respectively. Data are expressed as the ratio between the sub-G1 and G0/G1 peaks.

largely, but not completely, inhibited by cyclohexamide, as measured by flow cytometry, where rescue of cells expressing G0/G1 DNA content was observed (Fig. 4). The total, albeit heterogeneous, B cell precursor population was more sen-

1 2 3 4 5 bp

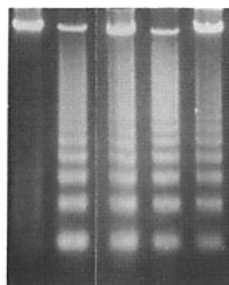


Figure 3. DNA fragmentation as detected by PAGE electrophoresis for B cell precursors exposed to x-rays and apoptotic drugs: (lane 1) control; (lane 2) x-rays (3.0 Gy) 4 h culture; (lanes 3–5) dexamethasone (0.1 μ M), etoposide (0.16 μ g/ml), and cisplatin (10 μ g/ml), respectively. 24 h culture.

sitive to x-ray-induced apoptosis at 8 h of culture than the total LPS-activated splenic B cell population where sub G1 to G0/G1 DNA peak ratios of 7.72 vs. 0.77 respectively were observed. Withdrawal of IL-7 from proliferating B cell precursors also prompted a rapid onset of apoptosis (not shown) as previously reported (21). Thus in common with IL-3-dependent hemopoietic progenitors, IL-7 can exert an antiapoptotic function in B cell precursors but is, in the concentration range used, incapable of preventing x-ray or drug-induced inhibition of colony formation by clonogenic B cell precursors (despite providing a proliferative stimulus), or apoptosis of the total population.

The vulnerability of B cell precursors to apoptosis could be due to the pattern of expression of gene products including BCL-2 (22), and related apoptosis-regulating genes (23–25). We found that levels of BCL-2 protein in B cell precursor populations as assessed by antibody staining and confocal mi-

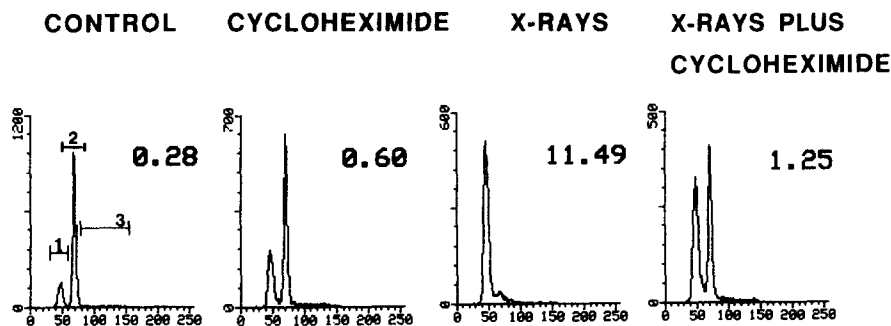


Figure 4. DNA analysis from B cell precursors exposed to x-rays (3.0 Gy) and cultured in the presence or absence of cycloheximide (0.1 $\mu\text{g}/\text{ml}$) for 8 h. Regions 1-3 represent cells with sub-G1, G0/G1, or S/G2M DNA content, respectively. Data are expressed as the ratio between sub-G1 and G0/G1 peaks.

scopy were very low as compared with lymphocytes in spleen (Fig. 5, C and A, respectively), as previously suggested when stable mRNA levels were used as a marker (26, 27). This parallels the very low levels of BCL-2 protein in thymocytes with T cell precursor phenotypes (CD4^+ , CD8^+ ; references 28, 29).

A BCL-2 transgene has been shown to protect thymocytes from steroid and radiation-induced apoptosis (30-32). Similarly, a retrovirally packaged BCL-2 gene was shown to protect IL-7-dependent B cell precursor cell lines from apoptosis after removal of IL-7 (21). We assessed the ability of a human BCL-2-Ig transgene (12) to protect murine B cell precursors. The human transgene was highly expressed in $\sim 90\%$ of BP-1-positive BM B cell precursors from transgenic mice as assessed by two-color immunofluorescent staining. Furthermore, BCL-2-Ig expression was restricted to the lymphoid lineage, since $>99\%$ immature MAC-1 positive myeloid cells present in the BM from the same animals failed to stain for human BCL-2 (data not shown). The IL-7-dependent cloning efficiency was similar in transgenic (0.14

$\pm 0.03\%$) and control age and strain (Chex C57BR/6) F_1 matched mice ($0.17 \pm 0.06\%$). As anticipated, the total B cell precursor population expressing the BCL-2-Ig transgene had markedly increased resistance to x-ray (after 8 h of culture) and dexamethasone (after 24 h of culture) induced apoptosis, where the ratio of sub G1 and G0/G1 DNA contents were small and close to control values (Fig. 6, legend). A modest increase in the survival of clonable B cell precursors to the lethal effect of x-rays was observed in cells obtained from four of the six BCL-2-Ig mice, as compared with age and strain matched controls (Fig. 7). A distinct shoulder in the x-ray survival curves from the BCL-2-Ig mice was also present, indicating that the inhibition or delay of apoptosis had allowed some DNA repair to occur. Why this effect was absent in two of the mice is unknown, but could reflect variable levels of BCL-2-Ig expression in clonable B cell precursors which may be more primitive than the majority of the B cell precursor population. Alternatively, the BCL-2 protein may fail to substantially inhibit apoptosis in the long time frame (6 d) of the clonogenic assay.

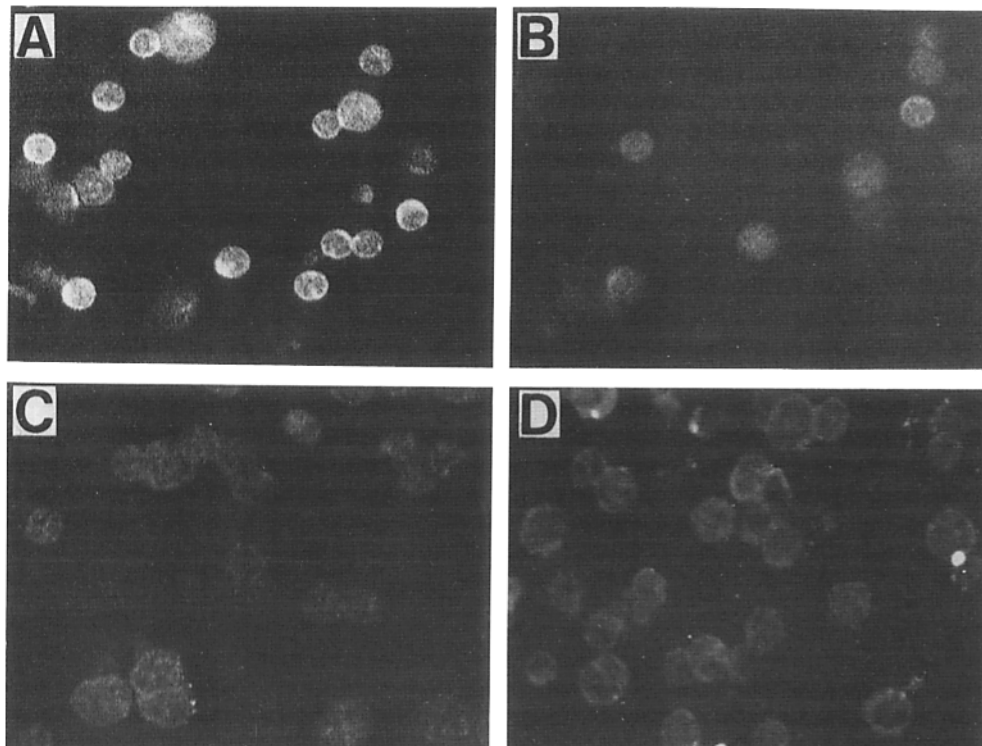


Figure 5. Spleen cells and B cell precursors stained for BCL-2 protein, (A) spleen cells plus BCL-2 antibody, (B) spleen cells plus control rabbit serum, (C) B cell precursors plus BCL-2 antibody, (D) B cell precursors plus rabbit serum.

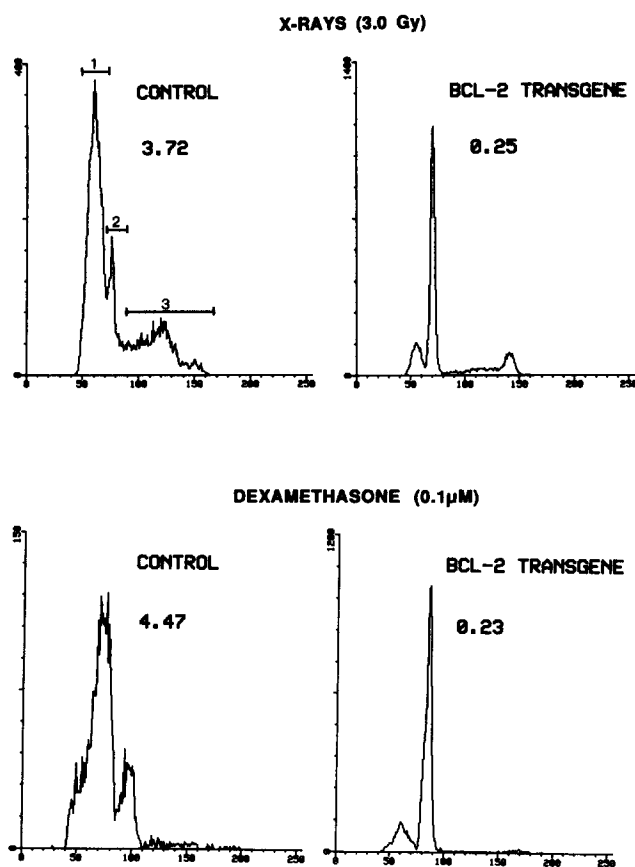


Figure 6. DNA analysis from B cell precursors (with and without expression of the *BCL2* transgene) after exposure to x-rays (3.0 Gy, 8 h culture), or in the presence of dexamethasone (0.1 μ M, 24 h culture). Regions 1–3 represent cells with sub-G1, G0/G1, and S/G2M DNA content. Data are expressed as the ratio between the sub-G1 and G0/G1 peaks. Controls (B cell precursors not exposed to x-rays or dexamethasone) gave mean \pm 1 SEM ratios of 0.06 ± 0.01 and 0.14 ± 0.03 in the presence and absence of the *BCL-2* transgene.

A minor decrease in sensitivity of clonogenic IL-7-dependent B cell precursors to dexamethasone was also observed with cell populations expressing the *BCL-2* transgene (not shown). The lack of substantial rescue of *BCL-2*-expressing cells in the clonogenic (i.e., proliferation-dependent) assay with dexamethasone, could be due to *BCL-2*-delaying apoptosis in a 6-d assay. Alternatively, we cannot exclude that *BCL-2* did provide effective protection from apoptosis but did not prevent inhibition of B cell precursor proliferation by steroid (33).

Discussion

The significance of these results is twofold. First, with respect to the developmental biology of B lymphoid cells, they suggest that precursors at the stage of IL-7 dependence (13, 34, 35) are extraordinarily sensitive to both ionizing radiation and to apoptosis-inducing drugs. It seems likely that this feature is linked to the pronounced developmental loss of dividing B cell precursors in BM as deduced by kinetic studies (36). Sensitivity of dividing B cell precursors to both experimentally induced and developmentally regulated apoptosis might be dependent upon the level of expression and

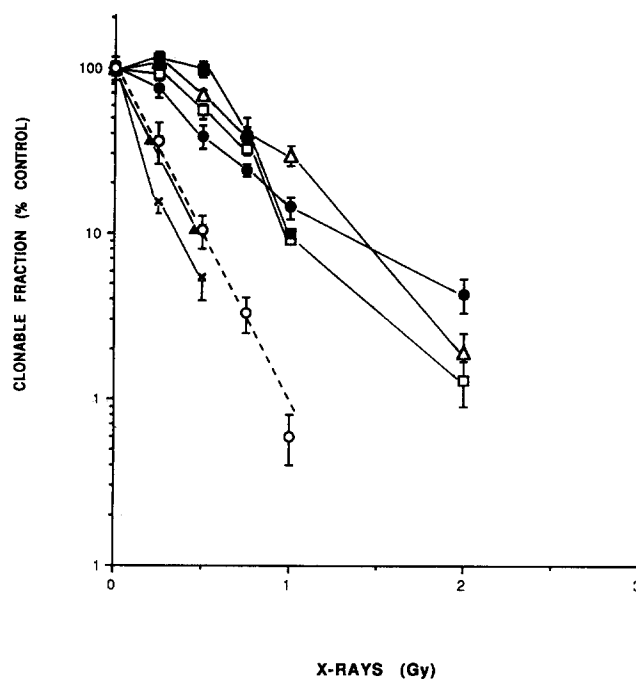


Figure 7. Survival of clonogenic IL-7-dependent B cell precursors from individual *BCL-2* transgenic mice after exposure to x rays, ■–■, □–□, ●–●, X–X, ▲–▲, Δ–Δ, or pooled data (mean \pm 1 SEM) from five control mice lacking the *Bcl-2-Ig* transgene, ○–○. B cell precursors from all transgenic mice expressed the *Bcl-2-Ig* transgene by immunofluorescence staining and confocal microscopy.

activity of a number of gene products that are known to be involved in the regulation of apoptosis including *BCL-2* (22), the *BCL-2*-related genes *BAX* (23) and *BCL-x* (24), *p53* (37), *c-MYC* (38) protein tyrosine kinases (39), as well as endonuclease (40) and DNA repair enzymes (41). Our data indicates that ultrasensitivity of IL-7-dependent B cell precursors in apoptosis is associated with very low levels of *BCL-2* protein expression. Others have recently reported low levels of *BCL-2* mRNA in murine B cell precursors (26, 27) but mRNA and protein levels for *BCL-2* may not always be concordant (42). However, as shown here with single cell staining, the great majority of BP-1⁺ IL-7-dependent B cell precursors have little or no detectable *BCL-2* protein.

Although in principle a similar apoptotic pathway is functional in most myeloid precursors and mature (activated) B cells (7, 8), they appear to have a considerably higher threshold for apoptosis induction in the presence of a proliferative signal. Immature T cells or cortical thymocytes as a population are very sensitive to apoptosis-inducing γ -radiation and steroid (4, 5, 43) and it may be that clonogenic T cell precursors are as sensitive as the B cell precursors assayed here, although this has not yet been reported. In this context, the marked sensitivity of the apoptotic response in lymphoid precursors may be linked to a stringent requirement for clonal elimination of cells with nonfunctional or self-reactive antigen receptor rearrangements; successful response to a differentiation signal would then be required to override the default cell death response. A similar selection appears to operate for a subset of mature B cells in germinal centers (44). Lymphocyte pro-

genitors are unusual but not unique in their ultrasensitivity to ionizing radiation since primordial oocytes and a subset of spermatogonial cells are equally if not more sensitive (45). Lymphocyte precursors, in common with germ cells, have the dual features of recombinatorial genetic activity with extreme longevity of clonal offspring; vulnerability to apoptosis may in this context be required by both types of cells as a sensitive and efficient mechanism for eliminating DNA damaged cells. It is interesting in this respect that B and T lymphoid cell progenitors at the earliest developmental stage before active immunoglobulin or T cell receptor gene rearrangement appear to express high levels of BCL-2 (27, 29, 46); it can be anticipated that these cells will be more resistant to apoptosis induced by ionizing radiation and drugs.

Second, we initially selected B cell precursors for these experiments as the normal murine counterparts of the likely "target" cell population for the major subtype of childhood

cancer, common, B cell precursor ALL (10, 47). This leukemia was the first disseminated and otherwise lethal cancer to be curable by chemotherapy and X radiation (9), a success repeated with very few other cancers; Wilm's tumour, Hodgkin's disease, and testicular teratoma being significant exceptions. The biological basis for what appears to be an intrinsic curability of the majority of childhood ALL and a few other cancers has been an enigma. It is now recognized that many drugs used in the therapy of cancer and leukemia operate via the induction of apoptosis (48, 49) and that clonogenic leukemic cells are likely to vary in their vulnerability to induced apoptosis (11, 50, 51). Clonogenic leukemic B cell precursors in ALL with developmentally programmed ultrasensitivity to apoptosis and in differentiation arrest, would be expected to be very sensitive to drug treatment; the essential caveat is that leukemogenic mutations should not, in most cases, override the apoptotic response (11).

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