IL-2 Rescues Antigen-Specific T Cells from Radiation or Dexamethasone-Induced Apoptosis

Correlation with Induction of Bcl-2[1]

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Most studies of apoptosis on T lymphocytes have examined the effects of various stimuli on immature T cells from the thymus. Previous work has indicated that apoptosis of mature memory T cells may be an important pathophysiologic mechanism in diseases such as AIDS, cancer, and autoimmunity. The effect of IL-2 on apoptosis of T cells is not clear. Therefore, we studied the ability of IL-2 to rescue Ag-specific T cells from apoptosis. We found that IL-2, in a dose-dependent manner, prevented T cells from entering apoptosis induced by γ-irradiation, mitomycin C, or dexamethasone. This effect was specific for IL-2; IL-1β, IL-6, or IFN-γ could not reproduce it. In contrast to Ag-specific T cells, immature T cells and naive mature peripheral T cells could not be rescued by IL-2 from radiation-induced apoptosis. Apoptosis rescue by IL-2 was associated with the induction of *bcl*-2 mRNA and protein. This induction could not be attributed to the effects of IL-2 on the cell cycle, as T cells that were prevented from cell cycle progression by irradiation showed a similar induction of *bcl*-2. Rescued cells retained their Ag-specific proliferative capacity and in vivo functions. These findings demonstrate that the apoptotic death of Ag-specific T cell lines, cells which can be regarded as a model for memory T cells, can be prevented with IL-2. This effect may have important therapeutic implications for patients receiving chemotherapy or radiotherapy, and for patients with AIDS who develop immunodeficiency primarily as a result of loss of Ag-specific memory T cells. ***The Journal of Immunology,*** **1996, 156: 515–522.**

Apoptosis is a process that is imprinted in the repertoire of responses of a cell to various physiologic and pathologic stimuli (1); it is characterized by distinct morphologic features and results in the death of the cell and its removal by scavenger cells (2). As one of the prominent cell populations undergoing apoptosis, T cells are the focus of much research (3–5). Most of these studies have examined thymocytes that undergo apoptosis as a physiologic step in maturation (6). These studies have revealed the role of genes that participate in the apoptotic process, such as p53 (7, 8), APO-1/*fas* (9), *bax* (10), *bcl*-x (11), and genes that protect cells from death, such as *bcl*-2 (12). However, the control of apoptosis in immature thymocytes and mature T cells may differ (13). Thus, although thymocytes from p53 knockout mice are resistant to radiation, proliferating mature T cells are sensitive (14). Moreover, within the mature T cell population, there is evidence to indicate that memory T cells have less *bcl*-2 and are more prone to apoptosis than are naive CD45RA cells (15).

The role of IL-2 in regulation of T cell apoptosis is not clear. Some investigators report that IL-2 receptor stimulation can induce apoptosis in thymocytes (16) and program mature T cells for apoptosis (17), and others report a protective effect of IL-2 on thymocytes stimulated with anti-CD3 (18) and on dexamethasone-induced apoptosis in T cell clones (19, 20). In our studies of T cells that proliferate in response to an irradiated T cell clone, we noticed that irradiated T cells seeded in IL-2-containing medium maintained their viability, whereas T cells in cultures without IL-2 underwent typical apoptosis. We pursued this study in view of the conflicting reports regarding the effect of IL-2 on apoptosis and the potential clinical benefits of having a pharmacologic means to control apoptosis. To examine the IL-2 rescue phenomenon, we performed cell cycle analyses of Ag-specific T cell lines after γ-irradiation, dexamethasone (DEX),[3] or mitomycin C treatment. Ag-specific T cell lines were used as a model for mature memory T cells. We found that IL-2 can rescue these T cells from radiation or DEX-induced cell death probably by inducing *bcl*-2 mRNA and protein.

Materials and Methods

Rats

Inbred female Lewis rats were supplied monthly by Harlan Olac (Bicester, UK) and were used at 2 to 3 mo of age.

Ags and Abs

Myelin basic protein (MBP) from the spinal cords of guinea pigs was prepared as described (21). *Mycobacterium tuberculosis* H37Ra was purchased from Difco (Detroit, MI). Peptide p277 of the human 60-kDa heat shock protein (sequence: VLGGGCALLRCPALDSLTPANED (22)) and peptide MBP 71–90 (sequence: SLPQKSQRSQDENPVVH from the guinea-pig MBP) were synthesized using the 9-fluorenylmethoxycarbonyl technique with an automatic multiple peptide synthesizer (AMS 422; ABIMED, Langenfeld, Germany). The purity of the peptides was analyzed by HPLC and amino-acid composition. mAbs, hamster anti-mouse Bcl-2 (clone 3F11) and hamster isotype control (clone UC8–4B3, anti-TNP),

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[1] The primers used in this study were designed from the GeneBank sequence of rat *bcl*-2 (accession number L14680).

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[3] Abbreviations used in this paper: DEX, dexamethasone; MBP, myelin basic protein; BP, basic protein; PI, propidium iodide; EAE, experimental autoimmune encephalomyelitis; PE, phycoerythrin; RT, reverse transcriptase; MCF, mean peak channel fluorescence.

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were purchased from PharMingen (San Diego, CA). Phycoerythrin (PE)- and FITC-conjugated goat anti-hamster Abs were obtained from Jackson ImmunoResearch (West Grove, PA).

Primary lymphocytes

Rats were killed and single-cell suspensions were prepared from lymph nodes or thymuses by pressing the organs through a fine wire mesh as described (21).

T cell lines

Ag-specific T cell lines were established by regular cycles of stimulation with Ag for 3 days, followed by propagation in IL-2-containing medium for 5 to 10 days, as described (21). The T cell lines were used for experiments at cycles 3 to 7 (1 to 2 mo in culture). The specific lines were anti-hsp-p277, anti-BP-p71–90, and Fisher-anti-BP obtained from Fisher rats immunized with guinea pig basic protein (BP) in CFA. The Fisher anti-BP line is encephalitogenic for Lewis (see below) and Fisher rats that share the same MHC class II haplotype (RT.1^l).

Apoptosis induction

Thymocytes, lymph node cells, or T cell lines were irradiated in the intensity indicated with a ^{60}Co machine (Gamma Beam 150; Nordion, Canada) and then incubated in 24-well plates (Falcon; Becton Dickinson, Lincoln Park, NJ) at 10^6 cells/ml for 20 h in supplemented DMEM (containing glutamine, sodium pyruvate, nonessential amino acids, penicillin, streptomycin, and mercaptoethanol) with various concentrations of human rIL-2 (Cetus Corp., Emeryville, CA), IL-1β (Genzyme, Cambridge, MA), IL-6 (Pharmacia-Farmitalia, Bioscience Center, Milan, Italy), or IFN-γ (PharMingen). In some experiments, DEX (1 μM; Teva Pharmaceutical Industries, Petach-Tiqva, Israel) or mitomycin C (2 μg/ml; Sigma Chemical Co., St. Louis, MO) was added to the cells for 20 h.

Cell cycle analysis

After the incubation of thymocytes, lymph node cells, or T cell lines with various concentrations of apoptosis-inducing stimuli, the cells were permeabilized with Triton X-100 (Sigma) at a final concentration of 0.2% for 20 min and then propidium iodide (PI; Sigma) was added (50 μg/ml) for 20 min at 4°C. When activated T cells and primary lymphocytes were analyzed, RNase A (0.2 mg/ml; Sigma) was added to the cells for 30 min at 37°C. The cell cycle was determined using a FACSort analyzer (Becton Dickinson, Mountain View, CA) with the Cell Quest program. The percentage of cells in apoptosis was determined from the pre-G1 peak on the PI histogram (23). This method was previously shown to yield results similar to the DNA fragmentation assay (24). The advantage of using the FACS for studying apoptosis is its capacity to quantitate the apoptotic fraction on a per cell basis (23, 24). When cell death was caused by necrosis (incubation for 20 h at 41°C), there was no appearance of the pre-G1 peak on PI DNA histograms. Cell debris was excluded by adjusting the forward scatter threshold, and cell clumps were excluded by defining a region on a FL3 area vs FL3 width dot plot (23).

Each cell sample was also analyzed for cell survival by the number of cells (counted using the trypan blue exclusion method) divided by the number of cells seeded at the beginning of the experiment, and by thymidine incorporation (incubation for 4 h with 1 μCi of [^{3}H]thymidine (sp. act. 10 Ci/mmol; Nuclear Research, Negev, Israel) followed by harvesting with a MicroMate 196 Cell Harvester). The cpm were measured with a Matrix 96 direct beta counter using avalanche gas (98.7% helium, 1.3% C_4H_{10}) ionization detectors (Packard Instrument Co., Meriden, CT).

T cell proliferation assay

To examine Ag-specific proliferation, 5×10^4 line cells were seeded in 96-well round-bottom microtiter plates (Greiner, Nurtingen, Germany) with 5×10^5 irradiated (2500 rad) thymocytes as accessory cells (21). Whole MBP or peptide p71–90 was added as indicated in dose-response experiments. The proliferation was performed in stimulation medium as described above. The cultures were incubated in quadruplicate for 72 h at 37°C in humidified air containing 7% CO_2. Each well was pulsed with 1 μCi of [^{3}H]thymidine for the final 18 h. The cultures were then harvested as described above.

Induction of experimental autoimmune encephalomyelitis

Groups of rats were injected i.p. with 5×10^6 Fisher-anti-BP line cells that had been stimulated for 48 h with 10 μg/ml guinea pig BP. Recipient rats were scored daily for clinical signs of experimental autoimmune encephalomyelitis (EAE). Clinical scoring was as follows: +1, paralysis of tail; +1.5, paresis of posterior paws and ataxia; +2, paraplegia; +3, paralysis extending to thoracic spine; +4, a moribund state (21).

Flow cytometry

Line cells were permeabilized with saponin (Aldrich, Milwaukee, WI), 0.3% in PBS without Ca^{2+} and Mg^{2+} (25) and containing 0.25% BSA (Sigma) and 0.02% sodium azide. Cells were incubated at 4°C for 45 min with a hamster anti-Bcl-2 mAb. Control cells were stained with hamster control Ab (anti-TNP catalog number 11091D; PharMingen). Both Abs were used at a final concentration of 5 μg/ml. PE-conjugated goat anti-hamster Ab was incubated at 4°C for 45 min. The cells were then washed and fluorescence was measured with the FACSort (Becton Dickinson). Analysis of the results was done with Cell Quest software.

Reverse-transcription PCR

Total cellular RNA was isolated by the single-step method using the TRI REAGENT (Molecular Reasearch Center, Cincinnati, OH). Five micrograms total RNA was used for the reverse transcriptase (RT) reaction. RNA was incubated with oligo(dT)$_{(12-18)}$ (200 ng) for 5 min at 65°C and left to cool to 42°C. The RT reaction contained dNTPs (0.25 mM each), RNasin (3 U), DTT (10 mM), sodium pyrophosphate (4 mM), avian myeloblastosis virus (AMV) RT (12 U; Promega, Madison, WI), and RT buffer. The mixture was incubated for 2 h at 42°C and then heat-inactivated for 5 min at 95°C. The primers used for the PCR were designed with PRIMER software (Version 0.5; Whitehead Institute for Biomedical Research, Rockville, MD) from the GeneBank sequence of rat *bcl*-2 (accession number L14680).

Primer sequences were the following: *bcl*-2 sense CGACTTTGCAGA GATGTCCA (555–574 in the sequence of rat mRNA of *bcl*-2); antisense AAACAGAGGTCGCATGCTG (855–837) with an expected product length of 301 bp; rat β-actin sense CCTTCAACACCCCAGCCATGTA CG (418–441); and rat β-actin antisense TGCCACCAGACAGCACTGT GTTGGC (948–924) with an expected product length of 531 bp. The mouse *bcl*-2 cDNA used for positive control was a gift from Dr. M. Oren (Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel). The *bcl*-2 clone, TCM, contained the coding region for mouse *bcl*-2. The PCR reaction contained cDNA (2 μl), primers (0.3 μg), dNTPs (75 μM each), and DNA polymerase (DyNAZyme II, F501; Finnzymes Oy, Espoo, Finland) 1 U per reaction. PCR reactions were performed with the Techne thermal cycler (Cambridge, UK) and consisted of heating to 94°C for 5 min, followed by 30 cycles (denaturation 94°C for 1 min, annealing 60°C for 1 min, and extension 72°C for 1 min), followed by 10 min at 72°C. PCR products were electrophoresed in 1.7% agarose gel (FMC BioProducts, Rockland, ME) containing ethidium bromide (0.2 μg/ml).

Results

Dose response of the IL-2 effect on survival following irradiation, DEX, or mitomycin C treatment

In experiments with T cells that proliferate in response to other T cell clones, we noticed that adding IL-2 to the medium could prevent apoptosis of the irradiated stimulator T cells. To quantitate and characterize the rescue effect of IL-2, we subjected line T cells in the rest phase to 200 rad irradiation, 1 μM DEX, or 2 μg/ml mitomycin C. The cells were incubated for 16 h with graded amounts of IL-2. The time interval from irradiation to cell cycle analysis was determined in preliminary experiments that indicated apoptosis could be detected as early as 4 h postirradiation and peaked at 16 to 24 h. From each well, a sample was taken for cell count, another for determination of thymidine incorporation, and a third sample for cell cycle analysis. Figure 1 shows the results of the survival and thymidine incorporation assays. In the absence of IL-2, exposure to radiation, DEX, or mitomycin C resulted in >95% mortality of the cells after 16 h. The survival of cells increased stepwise with the increments in the IL-2 concentration. Thymidine incorporation was markedly diminished following irradiation, which is known to cause cell cycle arrest (26), despite high concentrations of IL-2. In DEX-treated cells, both survival and thymidine incorporation increased in response to IL-2. It is

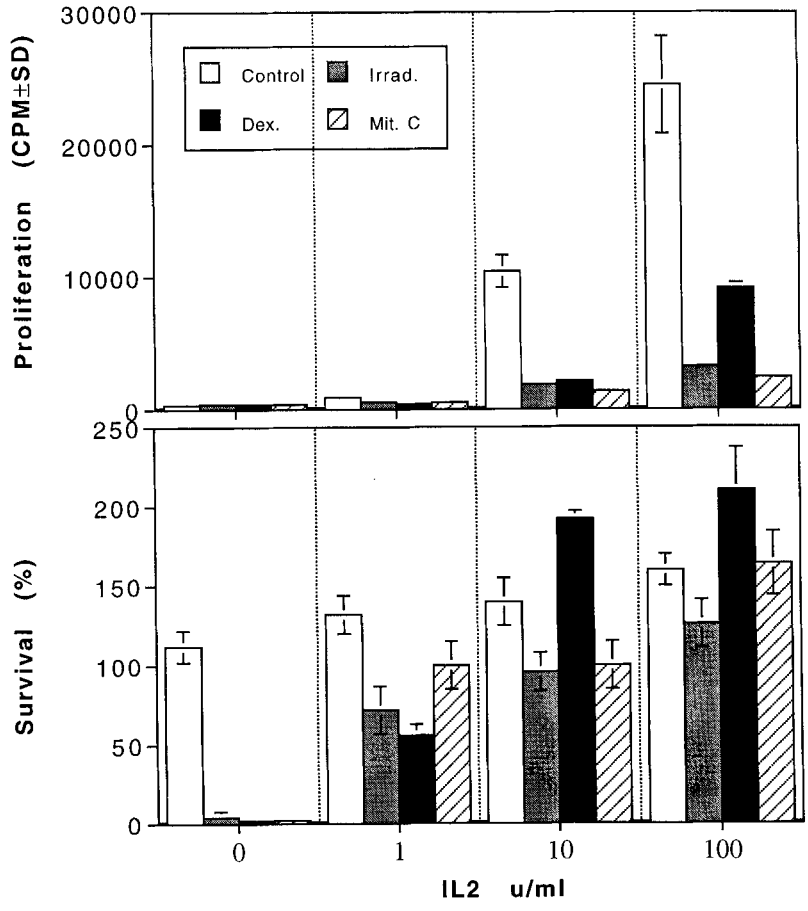

FIGURE 1. Survival and thymidine incorporation following exposure of resting anti-p277 line cells to 200 rad irradiation (Irrad.), 1 μ M DEX (Dex.), or 2 μg/ml mitomycin C (Mit. C). Survival was measured by trypan blue exclusion and thymidine incorporation was determined 4 h after adding radioactive thymidine to cultures. Cell survival progressively increases with increasing IL-2 concentrations.

important to note that although irradiation constituted a momentary insult, DEX and mitomycin C were continuously present in the cultures; thus, the T cells were actually rescued from a prolonged insult in culture.

DNA content analysis of the 12 experimental samples (Fig. 2, *E* to *P*) revealed a similar pattern. In the absence of IL-2, 90 to 95% of cells had a PI staining pattern of a pre-G1 peak characteristic of apoptotic cells (23, 24). The apoptotic fraction decreased as the IL-2 concentration increased. Note that the T cell lines are dependent on IL-2 for survival, explaining the 22% apoptosis in the control cultures (Fig. 2*A*). In different experiments, this fraction size varied between 6 and 30% depending on the line and on the time interval from the stimulation; the longer the interval the higher the fraction of apoptotic cells, because the cells were more dependent on exogenous IL-2.

Similar experiments were performed with activated line cells (day 2 of Ag-specific stimulation) which required higher doses of apoptosis-inducing signals (2000 rad irradiation or 5 μM DEX) because activated cells have a higher threshold for apoptosis (27). The results for survival, thymidine incorporation, and cell cycle analysis were similar to those obtained with resting cells; an IL-2 dose-dependent reduction in the apoptosis fraction was observed (data not shown).

Pretreatment of T cells with IL-2 raises their apoptosis threshold

In the previous experiments, the cells were grown in a low concentration of IL-2; after irradiation, the cells were seeded in different concentrations of IL-2. We wanted to determine whether culture in a high concentration of IL-2 would save the cells from radiation-induced apoptosis and what role the actual presence of IL-2 plays in the postirradiation period. To that end, we cultured T cells in 0, 1, 10, or 100 U/ml of IL-2 for 48 h, and then irradiated the cells (2000 rad). After irradiation, the cells were either cultured in medium containing the same concentration of IL-2 or in medium without IL-2. As can be seen in Figure 3, IL-2 had a radioprotective role even when the cells were not cultured in its presence after irradiation. Thus, IL-2 receptor stimulation before irradiation also raised the apoptosis threshold of the cells and the presence of IL-2 was not essential after irradiation. In comparison with the results in Figure 1, IL-2 concentrations of 1 and 10 U/ml were not sufficient to prevent apoptosis in these cells, probably because of the higher dose of irradiation used in these experiments (2000 rad vs 200 rad).

Naive thymocytes and lymph node cells are not rescued by IL-2

To examine the specificity of the rescue phenomenon, we designed experiments with naive thymocytes and peripheral mature T cells that were devoid of functional $\alpha\beta\gamma$ IL-2 receptors. The cells were subjected to radiation and cultured in the presence of increasing doses of IL-2. The effect of IL-2 on thymocytes appears to be controversial; IL-2 was reported to be an inducer of apoptosis (16) and, in contrast, IL-2 was also found to protect thymocytes from anti-CD3-induced apoptosis (18). Figure 4 shows that although a control T cell line exhibited the rescue effect of IL-2, neither thymocytes nor lymph node cells were saved from apoptosis by IL-2. Thus, it is likely that the protective effect of IL-2 was mediated by a signal that was provided by the interaction of IL-2 with its functional receptor.

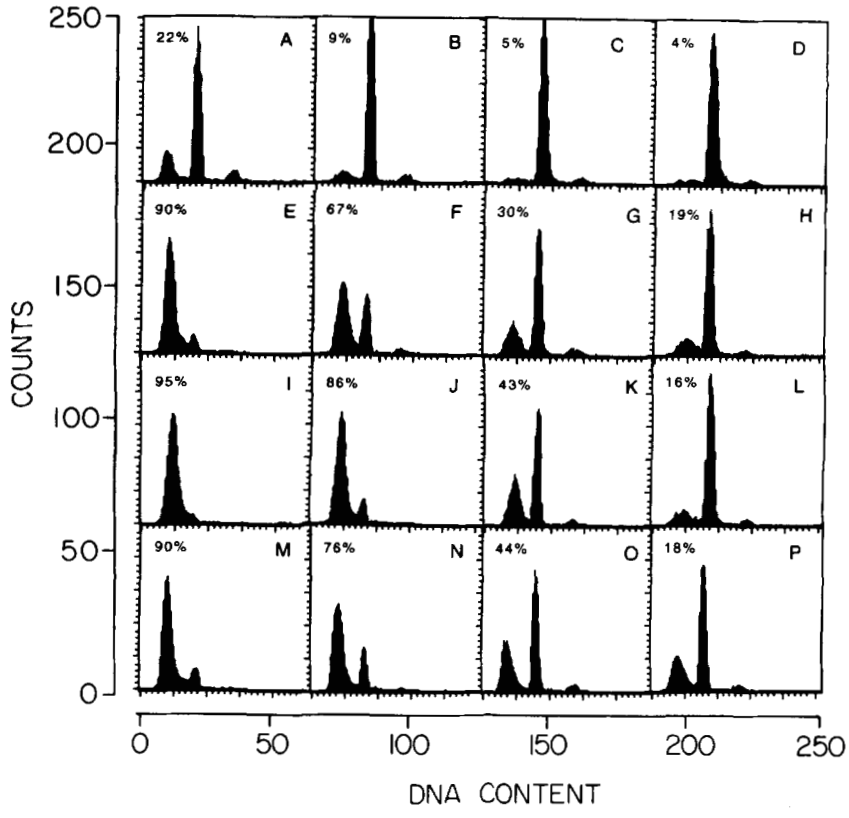

FIGURE 2. DNA content analysis of line cells (anti-p-277): control (*A* to *D*), after 200 rad irradiation (*E* to *H*), after DEX (*I* to *L*), and after mitomycin C (*M* to *P*). In each row of four figures, the first is from cells incubated without IL-2, the second with 1 U/ml IL-2, the third with 10 U/ml IL-2, and the forth with 100 U/ml IL-2. The cell cycle was determined by FACS after permeabilization with Triton X-100 and staining with PI. With increasing concentrations of IL-2 there are less cells in the pre-G1 apoptotic area (23, 24). The cell counts (y-axis) are shown as a function of DNA content (x-axis, linear fluorescence intensity; the numbers 0 to 250 refer to each of the 16 histograms). The percentage of cells in apoptosis is indicated in each histogram.

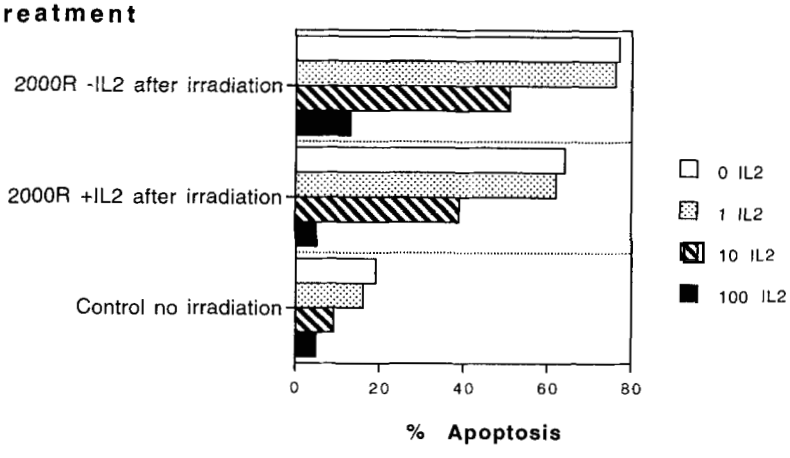

FIGURE 3. Preincubation with IL-2 raises the apoptosis threshold, and the presence of IL-2 after irradiation further augments the rescue effect. The line in this experiment was anti-BP-p71–90, reactive to MBP peptide 71–90. A decrease in the percentage of apoptosis is shown as a function of IL-2 concentration before irradiation for both cells that were cultured after irradiation with IL-2 (designated 2000 rad + IL-2 after irradiation) and without IL-2 (2000 rad − IL-2 after irradiation). Control nonirradiated cells were cultured for 48 h in the concentrations of IL-2 indicated, and apoptosis was measured after the cells were cultured in the absence of IL-2 for 16 h.

In different experiments, other cytokines were examined for their ability to rescue T cell lines from radiation-induced apoptosis. As shown in Table I, IL-1, IL-6, and IFN-γ were not able to rescue the cells from apoptosis.

Bcl-2 induction by IL-2

Although radiation-induced apoptosis in immature T cells is dependent on p53 (7, 8), mature T cells manifest p53-independent apoptosis mechanisms that are inhibited by Bcl-2 (14). Moreover, IL-2 protected the cells from DEX, which is also known to act through a p53-independent pathway (7, 8). This suggests that Bcl-2 might be important in IL-2 rescue. Therefore, we examined the effect of incubation with graded doses of IL-2 on the quantity of Bcl-2 protein detectable by FACS (28). Figure 5 shows that with increasing doses of IL-2, more cells (16 and 12% in cultures without IL-2 vs 77% in cells incubated with 50 U/ml IL-2 for 16 h) express higher amounts of Bcl-2. A similar pattern of Bcl-2 induction by IL-2 was found in cells subjected to irradiation (200 rad) and stained 16 h later with anti-Bcl-2 (Fig. 5, *D* to *F*). In addition to the increase in the fraction of cells expressing Bcl-2, the intensity of staining (as determined from the mean peak channel fluorescence (MCF)) increased in both groups (A:17, B:41, C:77, D:13, E:32, F:50). Thus, in both untreated and irradiated cells, IL-2 mediated a dose-dependent increase in the level of Bcl-2 protein, as determined by flow cytometry. Because irradiation prevents cell cycle progression, Bcl-2 induction in the irradiated cells demonstrates that this effect of IL-2 is not dependent on cell cycle progression.

A similar experiment was done with activated anti-p277 line cells (day 2 of Ag-specific stimulation, incubation for 16 h with 0, 5, 25, and 50 U/ml IL-2). The cells incubated without exogenous IL-2 were positive for Bcl-2 (30%, MCF 23); cells incubated with

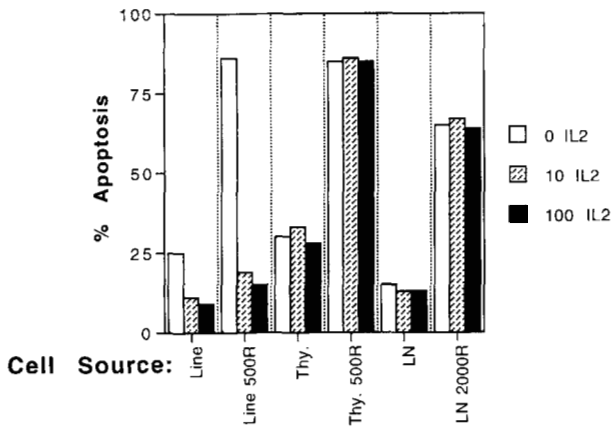

FIGURE 4. Specificity of the rescue phenomenon for line T cells. Naive thymocytes (Thy.), lymph node cells (LN), or line cells (Line) were subjected to irradiation as indicated, and the cell cycle was determined after 20 h incubation with graded doses of IL-2.

5 U/ml had a higher level of expression (36%, MCF 29); with 25 U/ml of IL-2, the expression increased further (36%, MCF 52); and with 50 U/ml IL-2, there was a small decline in expression (32%, MCF 42). These results indicate that activated T cells have a background level of Bcl-2 expression in the absence of exogenously added IL-2 (probably reflecting endogenous IL-2 secretion and autocrine IL-2 receptor stimulation) that is augmented with exogenous IL-2. The higher threshold of activated T cells for apoptosis induction could be attributed to this endogenous IL-2 secretion and concomitant Bcl-2 induction. However, it is also possible that activated cells have additional unidentified protective mechanisms against apoptosis.

Naive lymphocyte populations are devoid of functional IL-2 receptors (29). To rule out the possibility that Bcl-2 induction by IL-2 is a property peculiar to T cell lines, we examined naive lymph node cells that were stimulated with the mitogen Con A (1.2 μg/ml) and then propagated in IL-2 medium for 3 days (data not shown). These cells, in accord with results previously described on Bcl-2 protein using splenic T cells (30) and on *bcl*-2 mRNA in human lymphocytes (31), manifested an increase in Bcl-2 similar to that found in the T cell lines. Upon induction with IL-2, post-Con A lymphocytes demonstrated levels of Bcl-2 similar to that of naive lymphocytes and IL-2-stimulated line cells. Thus, acquisition of IL-2 receptors by mitogen stimulation rendered these cells responsive to the inducing effect of IL-2 on Bcl-2.

Detection of bcl-*2 mRNA following IL-2 incubation*

To test whether IL-2 could induce transcription of *bcl*-2 mRNA, we isolated total cellular RNA from resting cells that had been cultured for 24 h without IL-2 and then added IL-2 (50 U/ml) for various incubation periods. Because both the amounts of total RNA and specific RNA were low, we were unable to detect *bcl*-2 mRNA with radiolabeled probe of the 2.2 kb mouse *bcl*-2 cDNA by Northern blotting. Therefore, we decided to analyze *bcl*-2 mRNA by RT-PCR. Figure 6 shows the results of the PCR of cells after IL-2 starvation for 24 h (lane 0) and for 15, 30, and 120 min following the addition of IL-2. To control for the quantity and quality of cDNA, PCR of β-actin is shown in the lower part of the figure. Although the PCR product of *bcl*-2 is absent, as a discrete band in the ethidium bromide-stained gel in the IL-2-starved cells, the *bcl*-2 band is seen 15 min after the addition of IL-2. The amount of β-actin was similar in the cDNA from IL-2-starved cells and from cells incubated with IL-2.

Table I. *Percentage of apoptosis as determined by FACS as a function of the cytokine added to cultures of an irradiated (200 rad) T cell line*

Cytokine	Concentration (U/ml)	% Apoptosis
IL-2	0	56
	2	15
	20	7
IL-1β	10	54
	100	56
IL-6	10	69
	100	71
INF-γ	10	68
	100	69

Time course analysis of the protective effect of IL-2 on Bcl-2 levels and radiation-induced apoptosis

To explore the relationship between incubation with IL-2 and rescue of Ag-specific T cells from apoptosis, we subjected the anti-p277 T cell line to two protocols: 1) preincubation with 50 U/ml IL-2 for 24 h and then incubation without IL-2 for 6, 24, and 48 h; and 2) the same line was incubated without IL-2 for 24 h and then IL-2 was added at 5 or 50 U/ml for 6, 24, and 48 h. After the various incubation periods, Bcl-2 levels were measured by FACS analysis and the cells were irradiated (1000 rad). After irradiation, the cells were incubated in medium devoid of IL-2 for 16 h and DNA content analysis was performed to quantitate the fraction of cells in apoptosis. Longer times of exposure to IL-2 were associated with a progressive increase in Bcl-2 levels and a concomitant reduction in the percentage of cells in apoptosis after irradiation (Fig. 7). Moreover, when the cells were given a lower concentration of IL-2 (5 U/ml), the degree of Bcl-2 induction was lower and cell apoptosis was higher as a function of incubation time (data not shown). On withdrawal from 50 U/ml of IL-2, Bcl-2 levels declined and the apoptosis fraction increased in a reciprocal manner.

Ag-specific proliferation and in vivo function of rescued T cells

To examine whether the T cells rescued from apoptotic death by IL-2 were functional, these cells were compared with control cells for their proliferative capacity using a range of concentrations of peptide and MBP 5 days after 200 rad irradiation. In the absence of IL-2, all of the T cells were killed by irradiation, and therefore we could not test their proliferation or encephalitogenic potential. Figure 8 shows that the rescued cells retained their proliferative response to the specific peptide and Ag, although the proliferation was lower than that of control cells that were not irradiated. To test for the in vivo function of the cells, 5×10^6 activated T cell blasts of postirradiated and control Fisher-anti-BP line cells were injected into naive Lewis rats. All of the rats developed EAE (Fig. 8); the control cells caused lethal EAE by day 6, and the postirradiated cells caused severe EAE that peaked on day 6 and was followed by complete recovery.

Discussion

The results presented here indicate that the entry of Ag-specific memory T cells into apoptosis can be blocked by IL-2. This rescue from death can occur even when the apoptotic insult is continuously present in culture, thus underlining the potency of IL-2 in protecting T cells from an otherwise deadly insult. The protective effect was demonstrable in both resting and activated T line cells. The probable mechanism for this rescue appears to be induction of

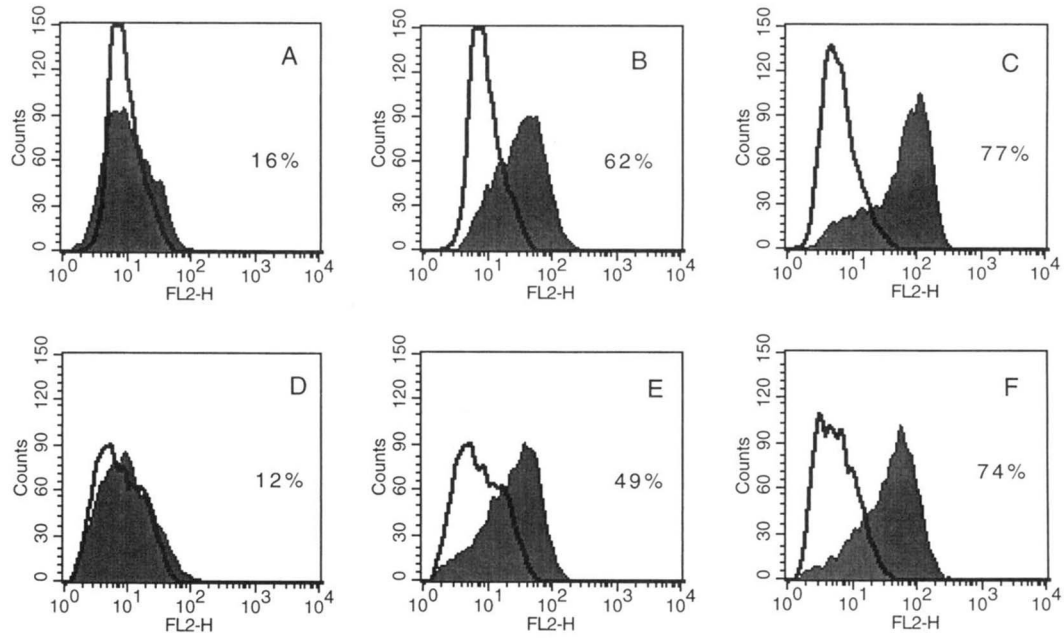

FIGURE 5. Bcl-2 induction by IL-2. Line cells (anti-p277) were incubated with graded doses of IL-2 (*A*, 0; *B*, 5 U/ml; *C*, 50 U/ml). The same experiment was done after irradiation (200 rad) of cells (*D*, 0 IL-2; *E*, 5 U/ml IL-2; *F*, 50 U/ml IL-2). One million cells were used in each sample, stained with monoclonal hamster anti-mouse Bcl-2 (clone 3F11) or with hamster isotype control (clone UC8–4B3, anti-TNP). The secondary Ab was PE-conjugated goat anti-hamster. The percentage indicates the fraction of cells expressing Bcl-2, as calculated by subtraction of the control from the experimental curve. The MCFs were *A*, 17; *B*, 41; *C*, 77; *D*, 13; *E*, 32; and *F*, 50.

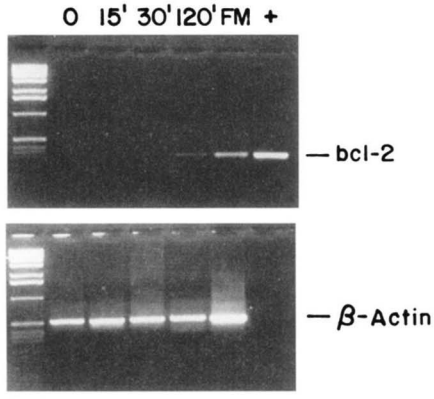

FIGURE 6. Expression of *bcl*-2 mRNA as detected by PCR. Ethidium bromide-stained gel of PCR products of cDNA from cells starved of IL-2 (24 h, lane 0) and then harvested at several time points after IL-2 (50 U/ml) was reintroduced to the cultures. The numbers at the top of the lanes indicate the time of harvesting in minutes. FM indicates the PCR product of cDNA prepared from the same cells kept in full medium (that were not starved of IL-2) and harvested at 120 min after addition of 50 U/ml of IL-2. The + indicates a PCR from the positive control plasmid containing the coding region of mouse *bcl*-2. The line used in these experiments was anti-p277.

bcl-2 gene expression and production of the potent anti-apoptosis Bcl-2 protein. Nevertheless, it is possible that the protective effect of IL-2 and the induction of *bcl*-2 are not causally related and that IL-2 exerts its anti-apoptosis effects by another mechanism. We chose to perform our experiments on Ag-specific T cell lines as a model for memory T cells, as these cells replicate in phase during the cell cycle and they have both in vitro and in vivo functions that can be monitored after experimental interventions. However, we used only lines that were in short-term culture (<2 mo) to minimize any potential artifacts developing after extended in vitro growth of the cells.

How does IL-2 control *bcl*-2 gene expression? The early genes induced by IL-2 include c-*fos*, Act-2, NFAT-1, NF-κB, and c-*myc* (32). Because the mRNA for *bcl*-2 was found to be induced as early as 15 min after IL-2 stimulation (Fig. 6), and because *bcl*-2 mRNA was reported to be suppressed after the addition of cycloheximide during mitogen stimulation of PBL (31), it is likely that one of the early genes induced by IL-2 leads to transcription of *bcl*-2. Alternatively, a previous study on the signaling pathways mediating the effects of IL-2 on T cells found *bcl*-2 to be involved directly in the response to IL-2 receptor stimulation, similar to c-*myc* and *lck* (33)

The beneficial effects of IL-2 on memory T cells exposed to apoptosis-inducing insults is analogous to effects reported with granulocyte-CSF or IL-6 on myeloid leukemia cells (34) and with IL-4 on B cells from patients with chronic lymphatic leukemia (35). Thus, some growth factors not only can protect the target cell from death caused by their deprivation, but they can also raise the cell's resistance to noxious insults, including chemotherapeutic agents (34), by increasing the amount of Bcl-2 (35).

In contrast to the results reported by Lenardo (17), our T line cells did not manifest apoptosis upon Ag stimulation after pretreatment with 50 U/ml of IL-2 for 4 days. Cell numbers, thymidine incorporation, and DNA content histograms at 50 U/ml IL-2 were similar to those at 5 U/ml IL-2 (data not shown). Similarly, Boise et al. did not document apoptosis in human cells exposed to IL-2 upon stimulation with anti-CD3 and anti-CD28 (36). In accord with our results, Zubiaga and colleagues found that IL-2 and IL-4 protected Th1 and Th2 mouse T cell clones, respectively (20). Similarly, work on peripheral lymphocyte populations isolated from human blood showed that IL-2 in high doses (200 U/ml) rescued T cells and NK cells from radiation-induced apoptosis, and this was associated with induction of Bcl-2 (37). In relation to the

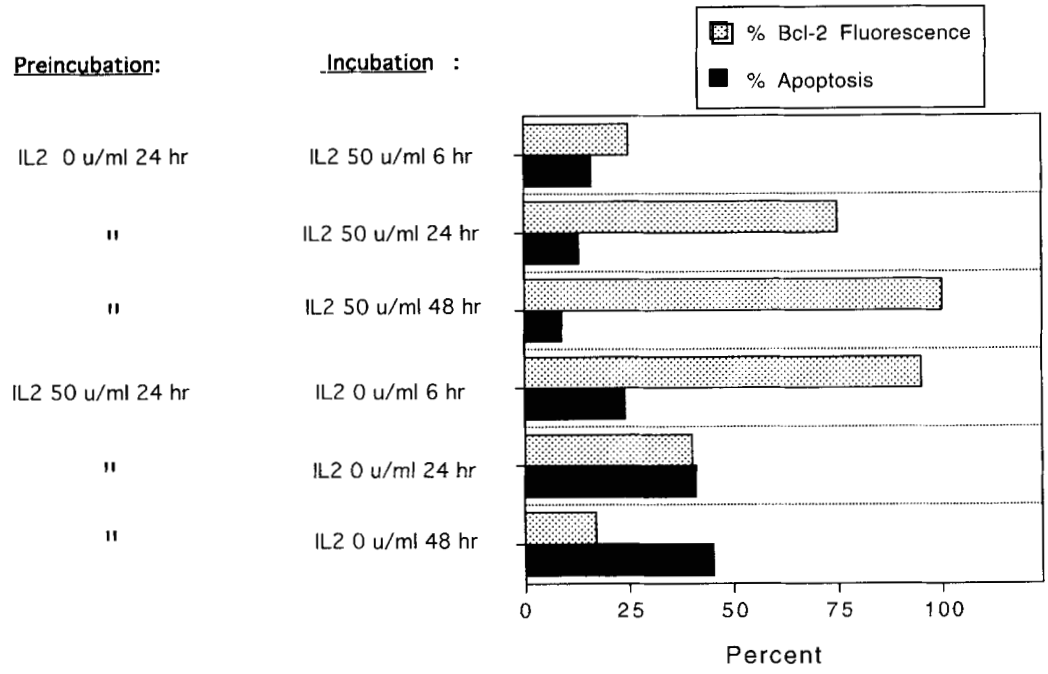

FIGURE 7. A time course study of levels of Bcl-2 expression and percentage of apoptosis in relation to incubation or withdrawal of IL-2. The line used was anti-p277 in the rest phase. The levels of Bcl-2 are expressed as the percentage of MCF of the tested cells as compared with the maximal MCF in this test (sample incubated with 50 U/ml IL-2 for 48 h, MCF = 301). Bcl-2 fluorescence was measured at the end of incubation. Apoptosis was measured from DNA histograms 16 h after 1000 rad irradiation.

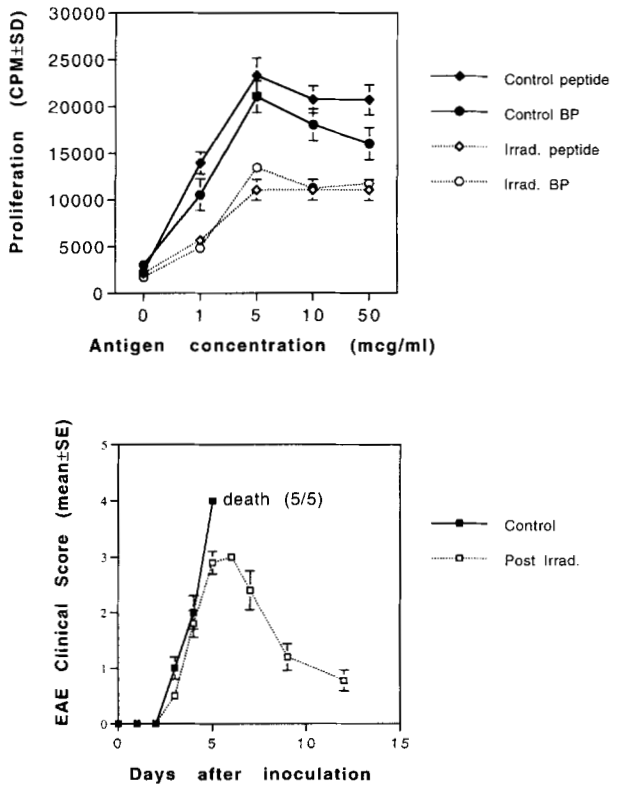

FIGURE 8. Proliferation and EAE induction with a IL-2-rescued T cell line (Fisher-anti-BP). Line cells were irradiated (200 rad) on the fourth day after the end of stimulation, rescued with IL-2-containing medium, and tested in proliferation to both peptide (71–90) and guinea pig BP 5 days after irradiation. The same cells were stimulated and injected (5×10^6 per rat) into five naive Lewis rats (Post Irrad. designates postirradiation T cells). Control cells were not irradiated.

mechanism of radioprotection of resting T cells by IL-2, Boise et al. presented evidence that the rescue phenomenon is independent of the growth-promoting effects of IL-2 (36). These two reports demonstrate a radioprotective activity of IL-2 on resting human peripheral blood T cells that lack IL-2 receptor α-chain. Indeed, the rescue effect has been ascribed to the engagement of $\beta\gamma$ units of the IL-2 receptor (36). Because we did not find that naive lymph node cells and thymocytes were rescued from radiation-induced apoptosis by IL-2, it is possible that, unlike human PBL, rat lymph node cells and thymocytes do not express the β-chain of IL-2 receptor, or that we did not see this effect because we did not examine the high concentrations of IL-2 (200 U/ml) similar to those used in the human studies. In addition, it is possible that the PBL compartment in humans contains many Ag-specific memory T cells that respond to IL-2, unlike thymocytes and naive lymph node cells that are probably dominated by virgin unprimed T cells.

The fact that memory T cells have less *bcl*-2 (15) and are more prone to apoptosis points to an important therapeutic potential of IL-2 in several clinical settings. Patients treated with irradiation, chemotherapy, and high-dose corticosteroids probably lose a large fraction of their apoptosis-prone memory T cell pool. A short course of IL-2 either before the ablative treatment or during such therapy could conceivably raise the apoptosis threshold of these cells, and so reduce the detrimental effect of infectious complications arising from a reduction in the memory T cell repertoire. Moreover, as patients with AIDS lose their memory T cell function, partly through apoptosis caused by TCR or CD4 cross-linking (38), it is possible that long-term, low-dose IL-2 therapy may raise the apoptosis threshold in these cells and reduce the frequency or severity of opportunistic infections. One study on the efficacy of intermittent IL-2 therapy in patients with AIDS reports a significant increase of $CD4^+$ cells in treated patients after 1 year of

treatment (39). Our results suggest that *bcl*-2 expression by memory T cells might be a useful marker of adequate IL-2 therapy to use to avoid the side effects of excessive IL-2.

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