IL-2 and IL-15 Exhibit Opposing Effects on Fas Mediated Apoptosis

Gulcin Demirci[1] and Xian Chang Li[1, 2]

It has been shown that IL-2 and IL-15 can have opposing effects on life and death of T cells. However, the role of IL-2 and IL-15 in regulating the fate of other cell types is less clear. In the present study, we examined the impact of IL-2 and IL-15 on life and death of pre-B cells using the BAF-B03 line. We showed that BAF-B03 cells constitutively expressed the private IL-2Rα chain and IL-15Rα chain, and the shared IL-2Rβ chain and γc chain. Stimulation of BAF-B03 cells *in vitro* with IL-2 and IL-15 induced vigorous cell proliferation in a dose-dependent fashion. Titration of IL-2 and IL-15 in the assay showed that the mitotic effects of IL-2 and IL-15 were remarkably similar. However, the sensitivities of BAF-B03 cells to Fas mediated apoptosis after IL-2 and IL-15 stimulation were strikingly different. Cells cultured in IL-2 readily underwent apoptotic cell death upon cross-linking of the Fas receptor whereas cells cultured in IL-15 were extremely resistant to Fas triggered cell death. The anti-apoptotic effect of IL-15 in this model was associated with increased expression of Bcl-xL. FLIP expression, however, was comparable between IL-2 and IL-15 stimulated cells. We conclude that IL-2 and IL-15 have diametrically opposite effect on the fate of BAF-B03 cells, although both cytokines share similar receptor structure and exhibit similar mitotic activities. *Cellular & Molecular Immunology*. 2004;1(2):123-128.

Key Words: cytokines, interleukins, apoptosis, lymphocytes

Introduction

IL-2 and IL-15 exhibit potent mitotic effect on several cell types including T cells, B cells, and NK cells (1). The functional high affinity receptors for IL-2 and IL-15 consist of a private α chain, which defines the binding specificity for IL-2 or IL-15, and a shared IL-2Rβ chain and IL-2Rγc chain. The γc chain is also a critical signaling component of receptors for IL-4, IL-7, and IL-9 (2). As both the β and the γc chains are required for signaling events upon ligand binding, initial efforts at mapping the proximal signaling events triggered by IL-2 and IL-15 demonstrated considerable overlaps (3). For example, both IL-2 and IL-15 stimulate rapid activation of Jak1 and Jak3 kinases, which are recruited to and associated with the β chain and the γc chain, respectively (4). Activation of Jak1 and Jak3 kinases leads to phosphorylation and activation of STAT3 and STAT5 (3, 4). Activation of the Jak/STAT pathway is one of the key signaling events in cell proliferation and cell survival.

In the T cell compartment, the roles of IL-2 and IL-15 in regulating the fate of activated T cells appear to be different (5, 6). IL-2 is clearly required for priming T cells to undergo activation induced cell death (AICD), a process that is presumably Fas mediated and likely serves as a feedback regulation of clonal expansion (7). Indeed, mice deficient for IL-2 do not have apparent immunodeficiency but rather develop profound lymphoproliferation and autoimmunity (8). Similar phenotype was observed in IL-2Rα and β chain knockout mice (9). In contrast, IL-15 is essential for cell survival and for maintenance of long-lived memory cells (10). Mice deficient for IL-15 and IL-15Rα chain develop certain degree of lymphopenia and impaired development of $CD8^+$ T cells and NK cells (11, 12). On the other hand, transgenic expression of IL-15 *in vivo* leads to marked initial expansion of $CD8^+$ T cells and NK cells with the subsequent development of lymphocytic leukemia (13). Transgenic expression of IL-2 using similar strategy, however, produces no marked phenotype. Clearly, IL-2 and IL-15 differentially affect T cell fate despite the fact that both receptors share identical signaling components.

The role of IL-2 and IL-15 in life and death of other cell types is less well characterized. Also, the precise mechanisms that can explain this enigma remain poorly defined. Attempts to study this issue using primary cells are often hampered by the heterogeneity of cells responding to IL-2 and IL-15 and the difficulties to stably maintain viable cells *in vitro*. In the present study, we used an *in vitro* approach to study the effects of IL-2 and IL-15 on cell survival and proliferation using a well-defined pre-B cell line (i.e. BAF-B03). We found that IL-2 and IL-15 stimulated vigorous cell proliferation, but the fates of

[1]Department of Medicine, Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA.

[2]Corresponding to: Dr. Xian C. Li, Department of Medicine, Harvard Medical School, Division of Immunology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA. Tel: 617-632-0176, Fax: 617-632-0160, E-mail: xli@bidmc.harvard.edu.

Received for publication Mar 25, 2004. Accepted for publication Apr 7, 2004.

proliferating BAF-B03 cells to Fas triggered apoptosis were strikingly different. In contrast to IL-2, IL-15 cultured cells were resistant to Fas mediated cell death. The survival advantage of BAF-B03 cells stimulated by IL-15 was associated with increased expression of Bcl-xL.

Materials and Methods

Cell line

BAF-B03 cells, an IL-3 dependent pre-B cell line transfected and selected for high expression of human IL-2R β chain (14), was kindly provided by Dr. T Taniguchi (Osaka, Japan). Cells were routinely maintained in RPMI-1640 medium supplemented with 4% IL-3-rich WEHI medium, 10% FCS, and 1% penicillin and streptomycin (BioWhittaker, Walkersville, MD). WEHI medium was prepared by culturing WEHI-3 cells in complete RPMI-1640 medium and the culture supernatant was collected, filtered, and stored at -80°C for usage.

Reagents

The following antibodies were purchased from BD PharMingen (San Diego, CA): hamster anti-mouse CD3ε (2C11, IgG), hamster anti-mouse Fas (Jo-2, IgG), hamster anti-mouse Bcl-2 (3F11, IgG), rat anti-mouse CD25 (PC61, IgG1), rat anti-human CD122 (Mik-β2, IgG2b), rat anti-mouse CD132 (TUGm2, IgG2b), mouse anti-p27kip (G173-524, IgG1), PE conjugated annexin V, streptoavidin-PE, streptoavidin-CyChrome.

Anti-mouse cyclin D3 (IgG2b) and anti-mouse Bcl-xL (IgG1) were obtained from Transduction Laboratories (Lexington, KY). Mouse anti-actin (JLA20, IgM) was obtained from Oncogene (Boston, MA). Polyclonal rabbit anti-human FLIP that cross-reacts with mouse FLIP was obtained from Alexis (San Diego, CA).

Western blot detection kit and HRP conjugated goat anti-rabbit IgG were purchased from Pharmacia Biotech (Piscataway, NJ). Recombinant human IL-2 and human IL-15 were obtained from R&D System (Minneapolis, MN).

Cell proliferation assay

BAF-B03 cells were starved in RPMI-1640 with only 1% FCS for at least 6h before each experiment. For cell proliferation assay, BAF-B03 cells were resuspended in complete RPMI-1640 medium with 10% FCS and 1% penicillin and streptomycin at 1×10^5 cells/ml. Cells were then plated in 96-well plates and stimulated with various doses of IL-2 (0 to 10 ng/ml) or IL-15 (0 to 10 ng/ml) in a final volume of 200 μl/well at 37°C for 48h. For the last 6h of culture, cells were pulsed with 1 μCi ^{3}H-TdR/well (Amersham, Boston, MA). Cells were harvested onto glass filter papers and ^{3}H-TdR uptake was determined by scintillation counting (Beckman Instrument, Columbia, MD). Results were plotted as mean cpm of triplicate assays.

Flow cytometry

BAF-B03 cells were resuspended in PBS/0.5% BSA solution at 1×10^7 cells/ml. Cell aliquots were stained with biotinylated anti-mouse CD25 (IL-2Rα), anti-human CD122 (IL-2Rβ), and anti-mouse CD132 (IL-2Rγ) on ice for 30 min. Cells were washed twice in PBS/0.5% BSA and further stained with PE-conjugated streptoavidin. For detection of IL-15Rα, cells were incubated with a mutant IL-15/Fc fusion protein (15), followed by staining with FITC-conjugated anti-mouse IgG. For detection of cell surface Fas expression, BAF-B03 cells were stained with PE-conjugated anti-mouse Fas mAb (Jo-2, PharMingen) on ice for 20 min. After the staining, cells were washed twice in PBS/0.5% BSA and analyzed by flow cytometry (Becton Dickinson, Mountain View, CA). Cells stained with PE-conjugated isotype control mAb were included as a control.

Induction of apoptosis

BAF-B03 cells were harvested and starved for at least 6h before IL-2 and IL-15 conditioning. Cells were resuspended in complete RPMI-1640 medium (10% FCS, 1% penicillin and streptomycin, 1% glutamine) at 1×10^4 cells/ml, plated into 24-well tissue culture plates (Corning, Boston, MA), and further stimulated with IL-2, IL-15, or WEHI medium at 2% at 37°C for 72h. For induction of apoptosis, anti-Fas mAb (Jo-2, hamster IgG) was added into the culture at 1 ug/ml, followed by the addition of protein G (1 ug/ml) to cross-link the Fc portion of the anti-Fas mAb. Cells cultured in the presence of isotype control IgG and protein G were included as a control. Apoptotic cell death was analyzed by staining with PE-conjugated annexin V 8 to 12h later.

Annexin V staining

For analysis of apoptotic cell death, BAF-B03 cells were stimulated with IL-2 or IL-15 for 72h. Cells were harvested and stained with PE conjugated annexin V (PharMingen) at 4°C for 15 min, washed in labeling buffer, and analyzed by FACScan (Becton Dickinson, Mountain View, CA). In some experiments, BAF-B03 cells were subject to Fas cross-linking after IL-2 and IL-15 conditioning before PE-conjugated annexin V staining. Apoptotic cell death was analyzed by FACS as previously reported (16).

Western blotting

Cell extracts were prepared by lysing BAF-B03 cells in Brij97 cell lysis buffer (0.875% Brij97, 0.125% NP40, 0.15M NaCl, 1mM Tris-HCl (pH 7.5), and 2.5mM EDTA with protease and phosphatase inhibitor mix). The protein fraction was recovered following centrifugation (14,000 rpm for 20 min). Extracts (10 to 18 ug/lane) were separated in precasted 4 to 20% gradient Tris-Glycine gel (Novex, Carlsbad, CA) under denaturing conditions and subsequently transferred onto nitrocellulose membrane (Pharmacia). Blots were blocked in blocking buffer (0.15% Tween-20, 5% nonfat dry milk in PBS) for at least 1 hour at room temperature, followed by incubation with primary antibodies at 4°C for overnight. Blots were then washed in washing buffer (0.15% Tween-20 in PBS) for three times with 15 min interval, and incubated with secondary antibody conjugated to horseradish peroxidase (HRP) for 1 hour at 4°C. Blots were washed again and visualized using the chemiluminescent western blot detection kit (ECL Pharmacia) according to manufacturer's instructions.

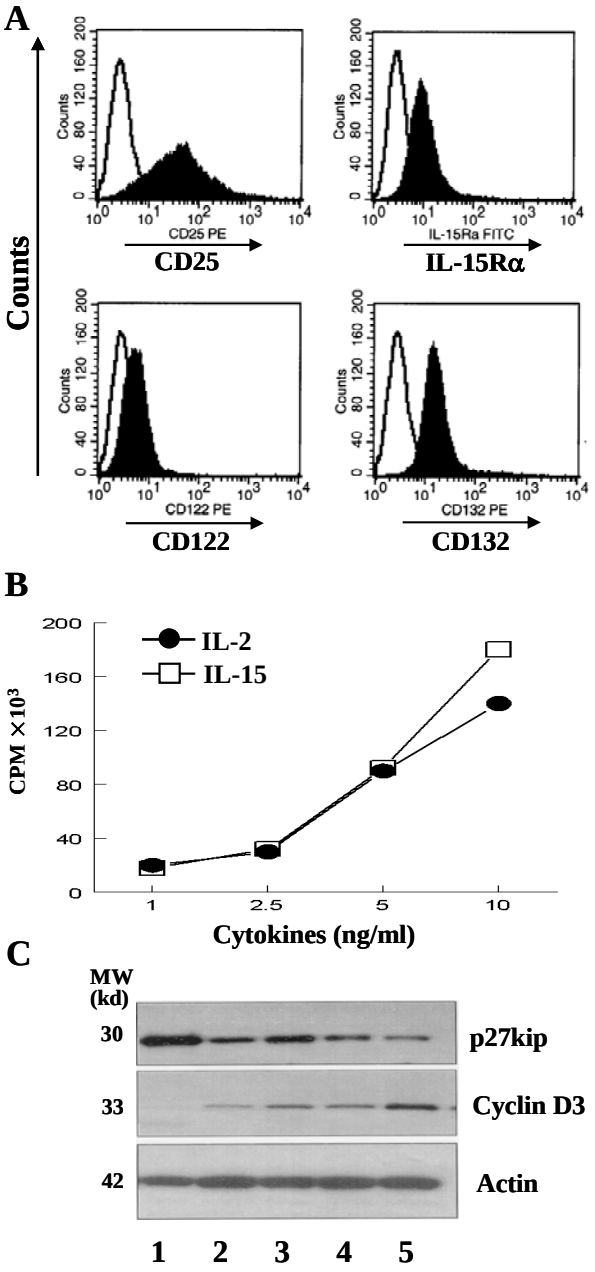

Figure 1. (A) Expression of IL-2R and IL-15R on BAF-B03 cells. BAF-B03 were stained for the expression of IL-2Rα chain, IL-15Rα chain, and the shared IL-2Rβ and the common γ chain. Cell staining was analyzed by flow cytometry. Open histograms represent isotype control antibody staining. (B) Proliferation of BAF-B03 cells in response to IL-2 and IL-15. BAF-B03 cells were stimulated with graded doses of IL-2 and IL-15 for 48h and cell proliferation was determined by ^{3}H-TdR incorporation. Results are presented as mean cpm of triplicate assays. Representative data of three experiments were shown. (C) Expression of p27kip and cyclin D3 in response to IL-2 and IL-15 stimulation. BAF-B03 cells were cultured in WEHI medium, IL-2 (5 ng/ml), or IL-15 (5 ng/ml) for 24h. Cellular protein was extracted, separated in 4-20% Tris-Glycine gel, and blotted onto nylon membranes. Expression of p27kip and cyclin D3 proteins was determined by Western blotting. Expression of actin was included to ensure equal loading amoung samples. Cellular extract from resting lymph node cells and activated T cells was included as controls. Similar result was obtained in three individual experiments. (Lane 1, LN; 2, T blast; 3, WEHI; 4, IL-2; 5, IL-15.)

Results

IL-2 and IL-15 induce similar proliferative responses

To determine if BAF-B03 cells expressed all the receptor elements required to respond to IL-2 and IL-15, freshly prepared BAF-B03 cells were stained for the expression of IL-2R and IL-15R. As shown in Figure 1A, FACS analysis showed that the IL-2Rα chain, the IL-15Rα chain, and the shared IL-2Rβ chain and the γc chain were constitutively expressed on the cell surface. To determine if BAF-B03 cells were fully responsive to IL-2 and IL-15, cells were cultured in the presence of various concentrations of IL-2 and IL-15, and cell proliferation was determined by ^{3}H-TdR uptake. As shown in Figure 1B, provision of IL-2 and IL-15 in the culture induced vigorous proliferation of BAF-B03 cells in a dose dependent fashion. Titration of IL-2 and IL-15 in this model showed that IL-2 and IL-15 could stimulate a similar magnitude of proliferation. Western blot analysis demonstrated that cell proliferation stimulated by IL-2 and IL-15 was associated with down-regulation of p27kip and marked upregulation of cyclin D3 (Figure 1C), the prerequisite for cell cycle entry (17). These data suggested that IL-2 and IL-15 exhibited similar mitotic activities *in vitro*.

Proliferation of BAF-B03 cells in response to IL-2 and IL-15 stimulation can be completely blocked by the anti-γc mAbs (18). Furthermore, IL-15, but not IL-2, stimulated proliferation is inhibited by a mutant IL-15/Fc, a fusion protein that acts as an IL-15Rα specific antagonist (15), confirming that the functional receptors for IL-2 and IL-15 in this model are γc-dependent, and proliferation of BAF-B03 cells is unlikely due to the presence of other receptors.

IL-2 and IL-15 exhibit opposing effects on Fas mediated apoptosis

FACS analysis showed that BAF-B03 cells constitutively expressed the Fas receptor on the cell surface (Figure 2A), and cross-linking of which with the anti-Fas mAb (Jo-2, hamster IgG) readily induced prominent apoptotic cell death, as determined by annexin V staining (Figure 2B), suggesting that the Fas receptor was fully functional in transducing the apoptotic signals in BAF-B03 cells.

We then examined the role of IL-2 and IL-15 in regulating the susceptibility of BAF-B03 cells to Fas-mediated apoptosis. BAF-B03 cells were conditioned in IL-2 or in IL-15 for 72h and their sensitivity to Fas mediated apoptosis was analyzed. As shown in Figure 2C, cross-linking Fas receptor induced precipitous cell death of BAF-B03 cells conditioned in IL-2, and more than 50% cells were stained positive for annexin V. In striking contrast, BAF-B03 cells conditioned in IL-15 were extremely resistant to Fas induced cell death, and only about 10% of the cells were annexin V positive regardless of the presence or absence of Fas cross-linking. Clearly, stimulation of BAF-B03 cells with IL-15, but not with IL-2, resulted in the acquisition of survival advantage.

IL-2 and IL-15 do not affect Fas and cellular FLIP expression

Analysis of Fas expression on BAF-B03 cells showed that

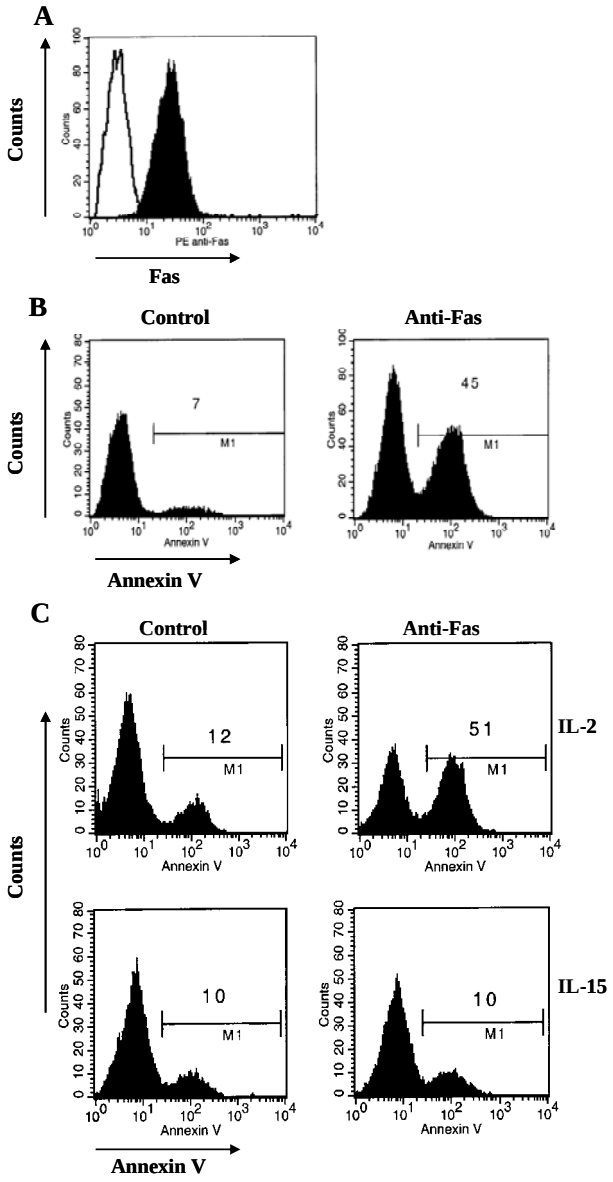

Figure 2. (A) Expression of Fas receptor on BAF-B03 cells. BAF-B03 cells were harvested and stained with PE-anti-Fas mAb and cell staining was analyzed by FACS. Cells stained with PE-isotype control mAb were included as a control. (B) Induction of Fas mediated apoptosis of BAF-B03 cells. Cells were cultured in IL-3-rich WEHI medium for 72h, and the anti-Fas mAb (Jo-2, 1 μg/ml) was added into the culture. The Fas receptor was cross-linked by the addition of protein G (1 μg/ml). Cell death was assayed 12h later by annexin V staining. Cells cultured with isotype control antibody were included as a control. Representative data of five experiments were shown. (C) Differential effect of IL-2 and IL-15 on Fas mediated apoptosis. BAF-B03 cells were conditioned in IL-2 (5 ng/ml) or IL-15 (5 ng/ml) for 72h and apoptotic cell death was induced by the addition of anti-Fas mAb (Jo-2, hamster IgG) plus protein G. Cell death was analyzed 12h later by Annexin V staining. Cells cultured with control antibody plus protein G were included as a control. Similar results were obtained in 3 experiments.

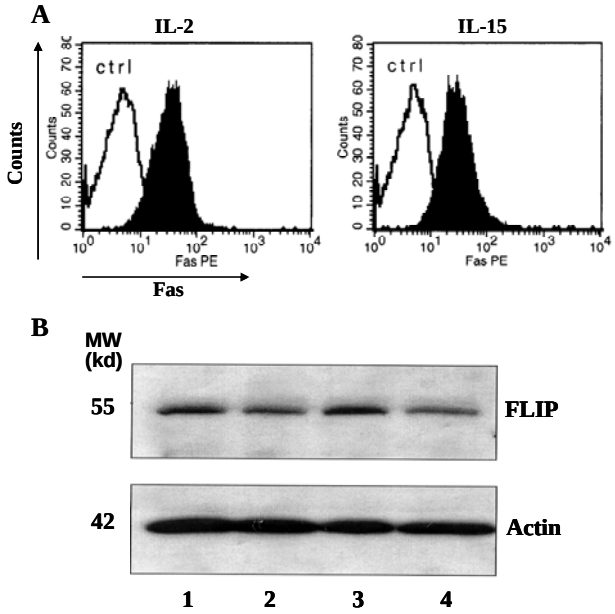

Figure 3. (A) Fas expression on BAF-B03 cells stimulated by IL-2 and IL-15. BAF-B03 cells were conditioned in IL-2 (5 ng/ml) or IL-15 (5 ng/ml) for 72h and cell surface expression of Fas was analyzed and compared by flow cytometry. Open histogram represents cells stained with isotype control antibody. (B) Expression FLIP in BAF-B03 cells in response to IL-2 and IL-15 stimulation. BAF-B03 cells were conditioned in WEHI medium, IL-2 (5 ng/ml), or IL-15 (5 ng/ml) for 24h and expression of FLIP protein was assayed by western blotting. Similar expression of FLIP was observed in cells conditioned for 48h and 72h. (Lane 1, Medium; 2, WEHI; 3, IL-2; 4, IL-15.)

Fas was expressed at comparable levels on the cell surface regardless of conditioning in IL-2, IL-15 or in IL-3-rich WEHI medium (Figure 3A). Thus, resistance of IL-15 conditioned cells to Fas triggered cell death is unlikely due to the differential expression of the Fas receptor on the cell surface, suggesting a possible signaling defect downstream of the Fas receptor.

In certain models, FLIP has been shown to control Fas mediated cell death (19) and IL-2 has been suggested to play a critical role in regulating levels of FLIP expression (20). We therefore examined whether expression of FLIP was differentially regulated by IL-2 and IL-15, thereby accounting for the opposing effects of IL-2 and IL-15 in apoptotic cell death. Western blot analysis showed that FLIP was constitutively expressed in BAF-B03 cells and the levels of FLIP expression did not show marked differences in BAF-B03 cells conditioned in IL-2, IL-15 or in IL-3-rich WEHI medium (Figure 3B). Thus, the differential effects of IL-2 and IL-15 on life and death of BAF-B03 cells are unlikely due to differential expression of FLIP in this model.

IL-15 but not IL-2 preferentially induces increased Bcl-xL expression

As the Bcl-2 family proteins also play an important role in regulating life and death of a variety of cell types, and this family of proteins consists of pro-apoptotic members (i.e. Bad, Bax, Bim) and anti-apoptotic members (i.e. Bcl-2, Bcl-xL) (21). We further analyzed the expression of Bad and Bcl-2/Bcl-xL expression in BAF-B03 cells after IL-2

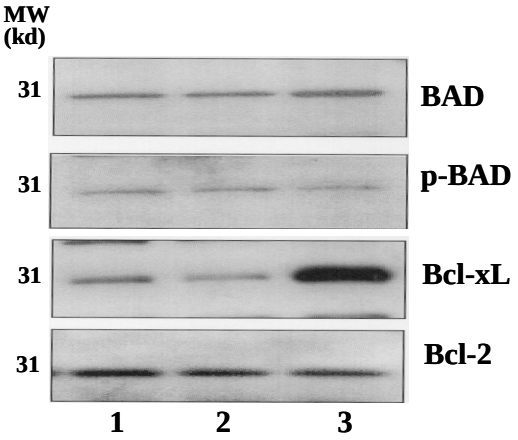

Figure 4. Differential expression of Bcl-xL in BAF-B03 cells stimulated with IL-2 and IL-15. BAF-B03 cells were conditioned in WEHI medium, IL-2 (5 ng/ml), or IL-15 (5 ng/ml) for 72h and cellular protein was extracted. Expression of BAD, phospho-BAD, Bcl-2, and Bcl-xL was determined by western blotting. Representative data of three experiments were shown. (Lane 1, WEHI; 2, IL-2; 3, IL-15.)

and IL-15 stimulation. As shown in Figure 4, Bad, phosphorylated Bad (Ser136), and Bcl-2 were expressed at comparable levels 72h after IL-2 and IL-15 stimulation. In striking contrast, cells conditioned in IL-15 expressed high levels of Bcl-xL when compared to cells conditioned in IL-2, suggesting that the survival advantage of IL-15 conditioned cells might be associated with sustained expression of Bcl-xL.

Discussion

Similar to their effect on T cells (22), IL-2 and IL-15 are redundant in stimulating proliferation and cell cycle transition of BAF-B03 cells. However, the fates of BAF-B03 cells responding to IL-2 and IL-15 are strikingly different. IL-2 sensitizes whereas IL-15 blocks Fas triggered apoptosis of BAF-B03 cells. In contrast to T cells (20, 23), however, levels of FLIP expression had no effect on the sensitivity of BAF-B03 cells to Fas mediated cell death, as FLIP is constitutively expressed in BAF-B03 cells regardless of IL-2 or IL-15 stimulation. Interestingly, the survival advantage of BAF-B03 cells conferred by IL-15 stimulation appears to be associated with the selective and sustained expression of Bcl-xL. These data suggest that IL-2 and IL-15 may exhibit similar effects in regulating life and death of different cell types, but the underlying mechanisms may be different.

Our study suggests that control of Bcl-xL expression may be a key process by which IL-2 and IL-15 differentially affect Fas induced apoptosis in this model. Fas is clearly one of the key pathways in mediating apoptotic cell death, as mutations of Fas or Fas ligand often lead to marked expansion of activated T cells in the periphery (24). The Fas/Fas ligand pathway is regulated by several means. One of the most interesting regulators in this regard is FLIP (20, 23). It has been shown that FLIP can bind to caspase 8, thus, preventing it from binding to the death inducing and signaling complex (DISC) downstream of Fas receptor (19). In the T cell compartment, IL-2 can transcriptionally shut down FLIP expression, thereby rendering activated T cells susceptible to Fas triggered cell death (20). Similarly, retroviral mediated over-expression of FLIP in activated T cells often blocks Fas induced cell death (23). Thus, FLIP is a key player at the interface of the IL-2/IL-2R pathway and Fas/Fas ligand pathway, and the critical role of FLIP in regulating the sensitivity of Fas mediated apoptosis provides a molecular basis for the pro-apoptotic effect of IL-2 in certain models. However, in the BAF-B03 cells, FLIP appears to have minimal effect on Fas triggered cell death, as levels of FLIP expression did not show marked differences regardless of IL-2 or IL-15 stimulation. This finding suggests that the role of FLIP in regulating Fas mediated apoptosis is not a uniform process and is likely cell type dependent (25). Our data also suggest that other molecules besides FLIP can also play an important role in regulating the Fas pathway.

In several models, Bcl-2 family molecules can exhibit significant impact on life and death of different cell types (26). For example, over-expression of Bcl-xL can block Fas induced cell death in certain models. Also, peripheral deletion of activated T cells *in vivo* can be markedly reduced in Bcl-xL transgenic mice in which the Fas/FasL pathway remains intact (27). Compelling evidence suggests that the impact of Bcl-2/Bcl-xL on Fas mediated cell death is cell type dependent (25). While Bcl-2/Bcl-xL exhibit minimal impact on Fas triggered cell death in type I cells (e.g. activated T cells), expression of Bcl-2/Bcl-xL can drastically inhibit Fas mediated cell death in type II cells (25). The molecular difference between type I cells and type II cells seems to be the amount of caspase 8 activity recruited to the DISC. In type II cells, engagement of Fas receptor leads to minimal caspase 8 activation, and execution of apoptosis then depends on cytochrome c release from the mitochondria to amplify the death signals, which can be modulated by Bcl-2/Bcl-xL (26). In this regard, certain characteristics of BAF-B03 cells in Fas triggered apoptosis resemble those of type II cells. It is tempting to speculate that the Fas signaling pathway may be differentially regulated in T cells and B cells. While FLIP is clearly critical in regulating Fas induced apoptosis of activated T cells, Bcl-xL may play a particularly important role in B cells.

Despite shared receptor components, the IL-2/IL-2R and IL-15/IL-15R systems clearly have certain inherent distinctions, which may be the basis for such strikingly different biological outcomes. For example, IL-2 is a T cell product whereas IL-15 is produced by other cell types but not T cells (28). Furthermore, the private IL-15Rα chain, which is constitutively expressed on a variety of cell types, can bind IL-15 with incredibly high affinity, albeit signaling events require the presence of the β chain and the γ chain (2). In contrast, IL-2Rα chain is expressed only on activated T cells and by itself exhibits extremely low binding affinity for IL-2 (2). Thus, ligand availability and accessibility are probably different between the two receptor systems. Whether such a difference is responsible for the functional dichotomy observed in the present study

is uncertain, as BAF-B03 cells constitutively express all the receptor components required to respond to IL-2 and IL-15. Also, the doses of exogenous IL-2 and IL-15 used in this model are carefully titrated.

Our data reinforce the notion that Bcl-xL can regulate Fas triggered death signaling pathway, and importantly, IL-2 and IL-15 play a key role in regulating this process by controlling the levels of Bcl-xL expression. Thus, the cytokine milieu during immune activation may have a critical impact on the fate of activated lymphocytes.

Acknowledgement

We gratefully acknowledge the technical assistance of Azine Ima.

References

1. Tagaya Y, Bamford RN, DeFillippis AP, Waldmann TA. IL-15, a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. Immunity. 1996;4:329-336.
2. Sugamura K, Asao H, Kondo M, et al. The interleukin-2 receptor γ chain: its role in multiple cytokine receptor complexes and T cell development in XSCID. Annu Rev Immunol. 1996;14:179-205.
3. Johnston JA, Bacon CM, Finbloom DS, et al. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by IL-2 and IL-15. Proc Natl Acad Sci U S A. 1995; 92:8705-8709.
4. Russell SM, Johnston JA, Noguchi M, et al. Interaction of IL-2R β and γ chains with Jak1 and Jak3: implications for XSCID and XCID. Science. 1994;266:1042-1045.
5. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. Science. 2000;288:675-678.
6. Li XC, Demirci G, Lacraz SF, et al. IL-15 and IL-2: a matter of life and death for T cells *in vivo*. Nat Med. 2001;7:114-118.
7. Lenardo MJ. Interleukin-2 programs mouse T lymphocytes for apoptosis. Nature. 1991;353:858-861.
8. Schorle H, Holtschke T, Hunig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature. 1991;352: 621-624.
9. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW. Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. Immunity. 1995;3:521-530.
10. Zheng X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory phenotype $CD8^+$ T cells *in vivo* by IL-15. Immunity. 1998;8:591-599.
11. Lodolce JP, Boone DL, Chai S, et al. IL-15 receptor maintains lymphoid honeostasis by supporting lymphocyte homing and proliferation. Immunity. 1998;9:669-676.
12. Kennedy MK, Glaccum M, Brown SN, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin-15 deficient mice. J Exp Med. 2000;191:771-780.
13. Fehniger TA, Suzuki K, Ponnappan A, et al. Fatal leukemia in IL-15 trangenic mice follows early expansions in natural killer and memory phenotype $CD8^+$ T cells. J Exp Med. 2001; 193:219-231.
14. Miyazaki T, Lin ZJ, Kawahara A, et al. Three distinct IL-2 signaling pathways mediated by bcl-2, c-myc, and lck cooperate in hematopoietic cell proliferation. Cell. 1995;81: 223-231.
15. Kim YS, Maslinski W, Zheng XX, et al. Targeting the IL-15 receptor with an antagonist IL-15 mutant/Fc protein blocks delayed-type hypersensitivity. J Immunol. 1998;161:5742-5748.
16. Ishaque A, Al-Rubeai M. Use of intracellualr pH and annexin V flow cytometric assays to monitor apoptosis and its suppression by bcl-2 overexpression in hybridoma cell culture. J Immunol Methods. 1998;221:43-57.
17. Brown EJ, Schreiber SL. A signaling pathway to translational control. Cell. 1996;86:517-520.
18. Li XC, Ima A, Li Y, Zheng XX, Malek TR, Strom TB. Blocking the common γ chain of cytokine receptors induces T cell apoptosis and long term islet allograft survival. J Immunol. 2000;164:1193-1199.
19. Tschopp J, Irmler M, Thome M. Inhibition of Fas death signals by FLIPs. Curr Opin Immunol. 1998;10:552-558.
20. Refaeli Y, Parijs LV, London CA, Tschopp J, Abbas AK. Biochemical mechanisms of IL-2 regulated Fas mediated T cell apoptosis. Immunity. 1998;8:615-623.
21. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell survival. Science. 1998;281:1321-1326.
22. Li XC, Roy-Chaudhury P, Hancock WW, et al. IL-2 and IL-4 double knockout mice reject islet allografts: A role for novel T cell growth factors in allograft rejection. J Immunol. 1998; 161:890-896.
23. Parijs LV, Refaeli Y, Abbas AK, Baltimore D. Autoimmunity as a consequence of retroviral-mediated expression of c-FLIP in lymphocytes. Immunity. 1999;11:763-770.
24. Nagata S. Apoptosis by death factor. Cell. 1997;88:355-365.
25. Scaffidi C, Schmitz I, Krammer PH, Peter ME. The role of c-FLIP in modulation of CD95-induced apoptosis. J Bio Chem. 1999;274:1541-1548.
26. Roy S, Nicholson DW. Cross-talk in cell death signaling. J Exp Med. 2000;192:21-25.
27. Wekerle T, Kurtz J, Sayegh MH, et al. Peripheral deletion after bone marrow transplantation with costimulatory blockade has features of both activation-induced cell death and passive cell death. J Immunol. 2001;166:2311-2316.
28. Fehniger TA, Caligiuri MA. Interleukin-15: biology and relevance to human disease. Blood. 2001;97:14-32.