Interleukin-15 Prevents Mouse Mast Cell Apoptosis through STAT6-mediated Bcl-xL Expression*

Received for publication, December 20, 2000, and in revised form, May 17, 2001
Published, JBC Papers in Press, May 21, 2001, DOI 10.1074/jbc.M011475200

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Interleukin (IL)-15 is a member of the cytokine family with T and natural killer (NK) cell growth-promoting activity. In mast cells, however, IL-15 uses a distinct receptor system different from that used in T and NK cells. We recently reported that IL-15 induces STAT6 activation and IL-4 production in a mouse mast cell line (MC/9) and bone marrow-derived mast cells. In the present study, we have demonstrated that IL-15 prevents MC/9 and bone marrow-derived mast cell apoptosis induced by factor withdrawal or anti-Fas antibody treatment. IL-15 increased mRNA and protein levels of an anti-apoptotic protein (Bcl-xL) in these cells, whereas bcl-2 mRNA remained unchanged. In addition, the transcriptional activity of the bcl-xL promoter was increased by IL-15 in MC/9 cells. In an electrophoretic mobility shift assay, IL-15 induced STAT6 binding to the STAT recognition site in the bcl-xL gene promoter. Furthermore, the expression of a dominant-negative form of STAT6 abrogated the effects of IL-15 on both bcl-xL mRNA up-regulation and prevention of apoptosis in mast cells. Altogether, our results suggest that IL-15 plays an important role in maintaining the number of mast cells through Bcl-xL expression mediated by STAT6.

Mast cells, which originate from bone marrow but maturate mostly in peripheral connective tissues, play a major role in the initiation of the acute allergic reaction. Thus, the number of mast cells needs to be tightly controlled by cell proliferation, development, and death. Several factors have been described to induce mast cell proliferation and maturation (1–5). As for cell death mechanisms, deprivation of interleukin (IL)1-3 or stem cell factor has been demonstrated to induce apoptosis in mast cells (3, 6, 7). Furthermore, mast cells express Fas antigen and variably undergo apoptosis when stimulated with some anti-Fas antibodies (8), indicating that mast cell numbers in vivo may be regulated not only by growth factors, but also by cell death in the Fas-dependent pathway (8).

IL-15 is a member of the cytokine family with T and NK cell growth-promoting activity (9, 10). Among a variety of cytokines involved in this activity, IL-2 plays a pivotal role through a receptor system composed of at least three polypeptide chains known as the IL-2 receptor (IL-2R) α, β, and γc chains for their signal transduction (11). In T and NK cells, IL-15 and IL-2 share IL-2Rβ and IL-2Rγc (9, 12). The IL-2R α-subunit is a private receptor used by IL-2, but not by IL-15. IL-15, on the other hand, utilizes the IL-15-specific receptor subunit, IL-15Rα (13). As anticipated by this receptor sharing, IL-2 and IL-15 have similar functional activities such as growth-promoting effects on T cells and activation of NK cells into killer cells when added to T and NK cells, respectively (14–16).

It has been reported that IL-15 uses a distinct receptor system in mast cells. Although mast cells lack IL-2Rβ and do not respond to IL-2, they proliferate in response to IL-15. Mast cells express a novel 60–65-kDa IL-15R molecule, designated IL-15RX (17). IL-15 activates Jak2 and STAT5 or Tyk2 and STAT6 in mast cell lines, instead of Jak1/3 and STAT3/5, which are activated by the IL-2R/IL-15R system in T cells (17, 18). Although we have reported that IL-15 induces IL-4 production in mast cells (18), other effects of IL-15 on mast cell functions remain largely unknown.

Among STAT proteins, STAT6 was first isolated as a protein tyrosine-phosphorylated in response to IL-4 stimulation (19). STAT6 has been demonstrated to play a critical role in the IL-4-mediated Th2 response based on analyses of STAT6-deficient mouse models (20, 21). IL-4 also inhibits apoptosis of T cells at least partly due to the induction of Bcl-xL, an anti-apoptotic member of the Bcl-2 protein family. STAT6, however, seemed unnecessary for the anti-apoptotic effects of IL-4 (22). Although Bcl-xL is induced by the activation of some STAT proteins (STAT1, STAT3, and STAT5) by direct binding to a consensus sequence in the bcl-x gene promoter (23–28), the role of STAT6 in expression of Bcl-x has not been well defined.

In this study, we analyzed the effects of IL-15 on mast cell proliferation and survival. We have shown that although IL-15 alone does not induce mast cell proliferation, it synergizes with IL-3 in the increase in the number of MC/9 and bone marrow-derived mast cells (BMMCs). We have also demonstrated that IL-15 induces Bcl-xL expression and prevents apoptosis of the mast cell line MC/9 induced by either factor deprivation or Fas stimulation. Upon activation by IL-15 treatment, STAT6 bound to the STAT-binding site in the bcl-x gene promoter and seemed responsible for the transcriptional activation of the bcl-x...
promoter. Additionally, the expression of a C-terminally truncated dominant-negative form of STAT6 significantly suppressed the bcl-xL mRNA up-regulation and prevention of apoptosis mediated by IL-15, suggesting that STAT6 activation is essential for the IL-15-mediated anti-apoptotic effect.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Recombinant human IL-15 and mouse IL-3 were purchased from Peprotech Corp. (Seattle, WA). RPMI 1640 medium was from Life Technologies, Inc. Fetal calf serum (FCS) was purchased from Sigma. The anti-Bcl-xL and anti-Bcl-2 polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The anti-ERK-1 and anti-STAT6 polyclonal antibodies were purchased from American Type Culture Collection (Manassas, VA). The cells were cultured in RPMI 1640 medium containing 10% FCS, 20 μg 2-mercaptoethanol, 10% WEHI-3-conditioned medium as a source for IL-3, and 10% mouse spleen-conditioned medium with concanavalin A. BMMCs were obtained from bone marrow cells of 6-week-old BALB/c mice. After 3 weeks of culture with 10% WEHI-3-conditioned medium, the cells were harvested for the experiments and consisted of >98% mast cells as assessed by toluidine blue staining.

Cells were washed twice with serum-free medium (RPMI 1640 medium containing 1% bovine serum albumin and 20 μg 2-mercaptoethanol) and incubated in serum-free medium for 6 h before cytokine stimulation, unless otherwise indicated. Cytokine concentrations used for the stimulation experiments were as follows: IL-3, 10 ng/ml; IL-15, 10 ng/ml; and IL-3 plus IL-15, 10 ng/ml each, unless otherwise indicated.

Measurement of Proliferation—Proliferation was measured by MTS assay. Briefly, 20,000 cells were plated in 96-well microtiter plates containing a dilution series of cytokines in 100 μl of medium. After 48 h, 20 μl of freshly prepared combined MTS/phenoxybenzamine methanesulfonate solution (Promega) was added to each sample. After an additional 4 h of incubation at 37 °C, the conversion of MTS into the aqueous soluble formazan was measured by absorbance at 490 nm. Total cell counts were determined by trypan blue exclusion and by counting at least 200 cells from each individual culture.

For the measurement of apoptosis, cells were stained with the FITC-conjugated anti-annexin V antibody (CLONTECH). Flow cytometric analysis was performed using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA).

Cell Cycle Analysis—1 × 10⁶ cells were fixed in ice-cold 70% ethanol for 1 h, washed with phosphate-buffered saline twice, and stained with 250 μg/ml propidium iodide and 50 μg/ml RNase A at 4 °C for 30 min. Propidium iodide fluorescence of individual nuclei was measured using the FACSCalibur flow cytometer.

Northern Blot Analysis—MC9 cells were incubated for 6 h at 37 °C in serum-free medium and stimulated under various conditions. Total cellular RNA was isolated using Trizol™ reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. 20-μg aliquots of the total RNAs were fractionated on a 1% agarose gel containing 20 μM MOPS, 5 mM sodium acetate, 1 mM EDTA (pH 7.0), and 6% (v/v) formaldehyde and transferred to a nylon membrane. After UV cross-linking, membranes were soaked in prehybridization solution (6× SSC, 5× Denhardt’s reagent, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA, and 50% formamide) for 3 h at 65 °C, followed by incubation with 32P-labeled probe in hybridization solution (6× SSC, 0.5% SDS, 100 mg/ml denatured salmon sperm DNA, and 50% formamide) for 14 h at 65 °C. The membranes were washed with 2× SSC and 0.1% SDS for 10 min at room temperature and with 0.1× SSC and 0.1% SDS for 10 min twice at 50 °C and exposed to Fuji RX-U film. DNA fragments of the coding regions of mouse bcl-xL, bcl-2, and β-actin were used as specific probes.

Western Blot Analysis—Cells incubated for 6 h at 37 °C in serum-free medium and stimulated under various conditions were lysed in ice-cold lysis buffer (50 mM Hepes (pH 7.0), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EGTA, 100 mM NaF, 10 mM sodium inorganic pyrophosphate, 1 mM Na₃VO₄, and 1 mM phenylmethylsulfonyl fluoride with 10 μM each aprotinin and leupeptin) and incubated on ice for 20 min. Samples were centrifuged (15,000 rpm, 5 min); the supernatants were analyzed on a 12% SDS-polyacrylamide gel, and the proteins were transferred to Immobilon polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA). The membranes were blocked with 1% bovine serum albumin in Tris-buffered saline containing 0.05% Tween 20 for 1 h, and Western blot analysis was performed as described previously (29), followed by detection using an enhanced chemiluminescence system (ECL Western Blotting Reagents, Pharmacia Biotech) according to the manufacturer’s instructions.

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as previously described (30). The double-strand DNA fragment carrying the STAT-binding site in the mouse bcl-xL gene 5′-upstream region was prepared by annealing two oligonucleotides (sense, 5′- GAGATGCCTTTCTCCAAAAGT-3′; and antisense, 5′-CCAC- CCCCTTTTCTCCAGAATG-3′), followed by 3P labeling by T4 polynucleotide kinase. As described elsewhere (30), nuclear extracts (5 μg of total protein) were incubated with the 32P-labeled double-stranded probe. For the supershift experiment, 1 μg of the anti-STAT6 antibody or isotype-matched control antibody was added to the binding reaction. For competition assays, nuclear extracts containing equal amounts of total protein were preincubated with a 100-fold molar excess of the unlabeled bcl-xL probe. Samples were run on a 5% non-denaturing polyacrylamide gel in Tris/glycine/EDTA buffer. The gel was dried and visualized by autoradiography.

Plasmids—The 5′-upstream region of the murine bcl-xL gene was obtained by polymerase chain reaction using two primers (5′-GCT-CACCACTGCATTTGTC-3′ and 5′-CTAAAACCATCTTCAGGGG-3′) that annealed to the mouse genomic Bcl-xL sequence. The restriction enzyme digestion analysis showed that the PCR product was the expected size. The restriction enzyme digestion of this PCR product was performed to confirm the authenticity of the restriction enzyme recognition sites. The 5′-upstream region of the mouse bcl-xL gene was perfectly cloned into the Pgl II site of the pGL3 luciferase vector (Promega) to create the bcl-xL promoter/luciferase construct. The STAT-binding site deletion mutant was prepared by ligating two polymerase chain reaction products amplified by primers 5′-GCTCAAACGCTCCATTTG-3′ (sense) and 5′-CCTGC-GAGACTGCTTTCTCAGAAGT-3′ (antisense) and primers 5′-GGCTC-GAGAAGGTTGTTGGTGCCTGC-3′ (sense) and 5′-CTAAAACCACTTCAGGGG-3′ (antisense) after Xho I digestion. This procedure replaced the STAT-binding site with an Xho I recognition site. The ligated DNA fragment was cloned into the Pgl3-luciferase vector. The expression plasmid of dominant-negative STAT6 lacking the C-terminal transactivation domain was previously described (18).

Transfection and Luciferase Assay—MC9 cells were transiently transfected with 3.5 μg of bcl-xL promoter/luciferase plasmid and 0.5 μg of pRL/SV40 (an internal control) by DMRIE-C reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. When indicated, cells were cotransfected with 1.8 μg of dominant-negative STAT6 or an empty vector and 1.8 μg of bcl-xL promoter. 24 h after transfection, the cells were stimulated with IL-15 (10 ng/ml) or left untreated. After 12 h of incubation, the cells were harvested and the luciferase activity was measured using the dual-luciferase reporter assay system (Tuo Ink Co., Tokyo, Japan) according to the manufacturer’s instructions. The data are presented as the means ± S.D. of triplicate samples.

Statistical Analysis—The statistical significance of the data was determined by Student’s t test. A p value of <0.05 was taken as significant.

RESULTS

IL-15 Synergizes with IL-3 in MC9 Cell Growth—It has previously been shown that IL-15 weakly stimulates the proliferation of mast cells (17). To examine the efficiency of IL-15 as a mast cell growth factor, we measured the number of MC9 cells stimulated by IL-15, IL-3, or both using the MTS assay. As shown in Fig. 1A, IL-15 weakly increased the number of MC9 cells compared with IL-3, a potent growth factor of mast cells. Although it has been reported that IL-15 is a growth factor of mast cells (17), a high concentration of IL-15 (>1000 pM) was needed to promote significant growth, and the concentration we used (10 ng/ml) promoted little growth. Surprisingly, when IL-15 was used in combination with IL-3, it significantly promoted MC9 cell growth stimulated by IL-3. Furthermore, this synergistic effect of IL-15 and IL-3 was also observed when BMMCs were used, as shown Fig. 1B. To confirm the results of the MTS assay, we also counted the MC9 cell numbers. Similar to the results in Fig. 1A, the addition of IL-15 further promoted the cell number increase by IL-3 for at least up to 48 h (Fig. 1C).

IL-15 Protects MC9 Cells from Apoptosis Induced by IL-3 Withdrawal—To determine whether the growth of mast cells...
stimulated by IL-15 or IL-3 was due to increased cell division or inhibition of cell death, the cell cycle status was analyzed. MC/9 cells were incubated with IL-3, IL-15, or both for 48 h, and the cell cycle status was examined by flow cytometric analysis using propidium iodide staining. As shown in Fig. 2A, IL-3 stimulation moderately reduced the number of cells in the sub-G0 phase and increased the number of cells in S/G2/M compared with the unstimulated control. In contrast, there was only a slight increase in the proportion of S/G2/M cells in the presence of IL-15, consistent with the previously reported weak proliferative activity of IL-15 in mast cells. On the other hand, a moderate reduction was observed in the number of cells in the sub-G0 phase. The combination of IL-3 and IL-15 reduced the number of cells in the sub-G0 phase more significantly. The anti-apoptotic effect of IL-15 was observed as soon as 18 h after IL-3 withdrawal (data not shown). To confirm whether these cytokines could prevent mast cell apoptosis, we examined apoptotic cell death by flow cytometric staining with FITC-conjugated annexin V. As shown in Fig. 2B, IL-15 significantly inhibited cell death induced by growth factor deprivation. MC/9 cells were incubated for 48 h in RPMI 1640 medium containing 10% FCS with IL-3, IL-15, or IL-3 plus IL-15. The concentration of each cytokine was 10 ng/ml. The cells were stained with propidium iodide and analyzed by flow cytometry. A typical result of at least three independent experiments is shown. B, inhibition of apoptosis by IL-15 during growth factor deprivation. MC/9 cells were incubated for 48 h in RPMI 1640 medium containing 10% FCS with IL-3, IL-15, or IL-3 plus IL-15. The concentration of each cytokine was 10 ng/ml. The cells were stained with FITC-conjugated annexin V and propidium iodide and analyzed by flow cytometry. **, p < 0.01.

Fig. 1. Growth of mast cells in response to IL-3 or IL-15. A, response of MC/9 cells to IL-3, IL-15, or IL-3 plus IL-15. MC/9 cells (2 × 10^4 cells) were washed two times and incubated with RPMI 1640 medium containing 10% FCS in 96-well microplates for 48 h with increasing concentrations of cytokines. The number of MC/9 cells was measured by MTS assay. Results are representative of at least five independent experiments. The error bars represent S.D. **, p < 0.01 (compared with the results obtained with MC/9 cells cultured with the same concentration of IL-3 alone). Corrected absorbance at 490 nm was calculated by subtracting the background absorbance (medium alone). B, response of BMMCs to the combination of IL-3 and IL-15. BMMCs (2 × 10^4 cells) were washed two times and incubated with RPMI 1640 medium containing 10% FCS in 96-well microplates for 48 h with IL-3, IL-15, or IL-3 plus IL-15. The concentration of each cytokine was 10 ng/ml. Total cell counts were determined by trypan blue exclusion. Results are representative of three independent experiments. The error bars represent S.D. *, p < 0.05; **, p < 0.01 (compared with the number of MC/9 cells cultured with the same concentration of IL-3 alone).

Fig. 2. Effect of IL-15 on apoptosis of mast cells. A, cell cycle status for cytokine stimulation. MC/9 cells (5 × 10^5 cells) were incubated for 48 h in RPMI 1640 medium containing 10% FCS with IL-3, IL-15, or IL-3 plus IL-15. The concentration of each cytokine was 10 ng/ml. The cells were stained with propidium iodide and analyzed by flow cytometry. A typical result of at least three independent experiments is shown. B, inhibition of apoptosis by IL-15 during growth factor deprivation. MC/9 cells were incubated for 48 h in RPMI 1640 medium containing 10% FCS with IL-3, IL-15, or IL-3 plus IL-15. The concentration of each cytokine was 10 ng/ml. The cells were stained with FITC-conjugated annexin V and propidium iodide and analyzed by flow cytometry. **, p < 0.01.
**IL-15 Increases Bcl-x<sub>L</sub> Expression at Both the mRNA and Protein Levels**—Members of the Bcl-2 family of proteins have central roles in the regulation of apoptosis (32–34). In T- and B-lymphoid cells, cytokines have been reported to regulate the expression of Bcl-2, Bcl-x<sub>L</sub>, Bcl-x<sub>A</sub>, A-1, and Mc-1 and the activity of pro-apoptotic family members such as Bad (42). To investigate the effects of IL-15 on the gene expression involved in the intrinsic resistance of MC/9 cells to apoptosis, bcl-2 and bcl-x<sub>L</sub> mRNA levels were examined during deprivation and cytokine stimulation. As shown in Fig. 4A, only IL-3, but not IL-15, increased bcl-2 mRNA expression. In contrast, only IL-15, but not IL-3, increased bcl-x<sub>L</sub> mRNA. The up-regulation of bcl-x<sub>L</sub> mRNA was also observed when BMMCs were stimulated with IL-15 (Fig. 4A). As shown in Fig. 4B, 100 or 1000 pg/ml IL-15 induced moderate increases in bcl-x<sub>L</sub> mRNA, whereas 10,000 pg/ml IL-15 showed a more evident effect. In the time course analysis, the increase in bcl-x<sub>L</sub> mRNA was found to occur as fast as 4 h and lasted for at least 24 h after IL-15 stimulation (Fig. 4B). Since it has been reported that the levels of bcl-2 mRNA do not necessarily correlate well with the levels of Bcl-2 protein (43), we sought to analyze the protein expression of Bcl-2 and Bcl-x<sub>L</sub>. MC/9 cells were cultured in IL-3 or IL-15 for 16 h, and Western blot analyses were performed. The levels of Bcl-2 protein did not vary significantly during factor deprivation or IL-3 or IL-15 stimulation (Fig. 4C). However, the levels of Bcl-x<sub>L</sub> protein were increased when the cells were stimulated with IL-15 (Fig. 4C).

We also examined other signaling pathways known to regulate cell apoptosis. Although Akt is a known target of phosphatidylinositol 3-kinase and has been implicated in the survival of mast cells (44), no activation of Akt was observed in MC/9 cells after stimulation with IL-15 (data not shown). Bad becomes phosphorylated and inactivated by several cytokines (42, 45–49). However, we did not detect any Bad phosphorylation in MC/9 cells upon IL-15 stimulation (data not shown).

**STAT6 Binds to the STAT-binding Site in the bcl-x Gene Promoter**—The role of various STAT proteins in the regulation of bcl-x<sub>L</sub> gene expression has recently been described. Bcl-x<sub>L</sub> is induced by activation of STAT proteins STAT1, STAT3, and STAT5 by their direct binding to the STAT consensus sequence in the bcl-x gene promoter (23–28). We have recently reported that IL-15 directly activates STAT6 in MC/9 cells and BMMCs (18). Thus, to determine if IL-15-activated STAT6 could bind to the STAT-binding site in the bcl-x gene in MC/9 cells, we carried out DNA-protein binding analyses. MC/9 cells were stimulated with IL-15 for 30 min, and the nuclear lysates were isolated for electrophoretic mobility shift assays using the specific competitor (±x), with an antibody specific for STAT6 (+αSTAT6), or with an isotype-matched control antibody (+cont). Results are representative of four independent experiments.
bcl-xL, the efficiency. **, 12 h or left untreated. A typical result of at least three independent experiments is shown. **, 24 h after transfection, the cells were stimulated with the indicated cytokines for 8 h after stimulation with IL-15 (10 ng/ml), and Northern blot analysis was performed using the bcl-xL cDNA probe. The filter was stripped and reprobed for β-actin. Results are representative of three independent experiments.

IL-15 Activates Transcription of the bcl-x Promoter through STAT6—We next analyzed the ability of STAT6 to transactivate the bcl-x promoter. To directly test the contribution of the STAT-binding site in the bcl-x gene promoter, we cotransfected with pRL/SV40 (an internal control) into MC/9 cells. 24 h after transfection, the cells were stimulated with the indicated cytokines for 12 h, and luciferase assays were performed. A typical result of at least three independent experiments is shown. **, p < 0.01. B, the bcl-x promoter/luciferase construct was transfected into MC/9 cells together with a pCEV-neo vector or pCEV-dominant-negative STAT6 in combination with pRL/SV40. Cells were incubated with IL-15 for 12 h or left untreated. A typical result of at least three independent experiments is shown. Units of luciferase activity were normalized based on values of pRL/SV40 activity to control for transfection efficiency. **, p < 0.01. C, shown is bcl-xL mRNA expression under IL-4-depleted conditions in MC/9 cells stimulated with IL-15. MC/9 cells were or were not pretreated with the anti-IL-4 antibody (10 μg/ml) for 30 min, followed by 8 h of IL-15 stimulation (10 ng/ml). Total RNA was prepared from MC/9 cells just before cytokine stimulation (Control) or 8 h after stimulation with IL-15 (10 ng/ml), and Northern blot analysis was performed using the bcl-xL cDNA probe. The filter was stripped and reprobed for β-actin. Results are representative of three independent experiments.

FIG. 6. Transcriptional activation of the bcl-x gene promoter in response to IL-15. A, the bcl-x promoter/luciferase construct or its mutant version in which the STAT-binding element has been mutated was cotransfected with pRL/SV40 (an internal control) into MC/9 cells. 24 h after transfection, the cells were stimulated with the indicated cytokines for 12 h, and luciferase assays were performed. A typical result of at least three independent experiments is shown. **, p < 0.01. B, the bcl-x promoter/luciferase construct was transfected into MC/9 cells together with a pCEV-neo vector or pCEV-dominant-negative STAT6 in combination with pRL/SV40. Cells were incubated with IL-15 for 12 h or left untreated. A typical result of at least three independent experiments is shown. Units of luciferase activity were normalized based on values of pRL/SV40 activity to control for transfection efficiency. **, p < 0.01. C, shown is bcl-xL mRNA expression under IL-4-depleted conditions in MC/9 cells stimulated with IL-15. MC/9 cells were or were not pretreated with the anti-IL-4 antibody (10 μg/ml) for 30 min, followed by 8 h of IL-15 stimulation (10 ng/ml). Total RNA was prepared from MC/9 cells just before cytokine stimulation (Control) or 8 h after stimulation with IL-15 (10 ng/ml), and Northern blot analysis was performed using the bcl-xL cDNA probe. The filter was stripped and reprobed for β-actin. Results are representative of three independent experiments.

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STAT-binding site in the bcl-x gene promoter and activated bcl-x<sub>L</sub> transcription. Additionally, the expression of a dominant-negative form of STAT6 significantly suppressed the bcl-x<sub>L</sub> mRNA up-regulation by and anti-apoptotic effects of IL-15, suggesting that STAT6 activation is essential for the IL-15-mediated anti-apoptotic effect through bcl-x<sub>L</sub> mRNA induction. Interestingly, unlike IL-3, IL-15 was also effective in the inhibition of Fas-mediated mast cell apoptosis.

IL-3 and stem cell factor are the major growth factors for mast cells (1, 50, 51). These factors seem essential for the regulation of mast cell numbers (2, 3). Other co-stimulatory factor may, however, contribute to the maintenance of mast cell number, and the synergistic effects of cytokines in mast cell proliferation have been observed (4, 5). In these reports, it has been demonstrated that IL-4 or IL-10 alone promotes little growth of mast cells, but potently promotes mast cell proliferation when combined with IL-3 or stem cell factor. However, the molecular mechanisms of these synergistic effects have not been demonstrated. In this report, IL-15 alone only weakly stimulated mast cell growth. However, it significantly promoted mast cell growth when combined with IL-3. In cell cycle analyses, IL-15 had only a slight ability to stimulate DNA synthesis in comparison with IL-3. In contrast, IL-15 prevented cell death, as did IL-3; and when combined with IL-3, further prevention of apoptosis was observed (Fig. 1). Additionally, when apoptosis was induced by Fas ligation, only IL-15 (but not IL-3) prevented apoptosis (Fig. 3), suggesting that IL-15 might prevent apoptosis by a different mechanism compared with IL-3. We presume that this different regulation of apoptosis by IL-3 and IL-15 may contribute to the synergistic effect on the mast cell number increase.

The regulation of apoptosis in mast cells has not been well defined. It has been reported that Rac2 stimulates Akt activation, affecting Bad/Bcl-x<sub>L</sub> expression while mediating survival (44). Although the relationship between IL-15 and Rac2 activation has not been defined, we could not detect any phosphorylation of Akt in MC/9 cells stimulated by IL-15 (data not shown). We also could not detect a change in Bad expression or phosphorylation after IL-15 stimulation (data not shown). These results indicate that Akt or Bad is not involved in the anti-apoptotic effects of IL-15. Recently, it has been reported that co-stimulation with IL-3, IL-4, and IL-10 decreases expression of Bcl-x<sub>L</sub> and Bcl-2 and induces apoptosis of mast cells (52). However, this process requires 6 days, and it might be possible that some secondary effects such as other cytokine releases are involved.

In T- and B-lymphoid cells, cytokines have been variably reported to regulate the expression (32, 35–39) and/or cleavage (40, 41) of the anti-apoptotic family members Bcl-2, Bcl-x<sub>L</sub>, Bcl-x<sub>S</sub>, A-1, and Mcl-1 and the activity of pro-apoptotic family members such as Bad (42). On the other hand, in mast cells, it has been reported that nerve growth factor induces the expression of Bcl-2 protein (53). In this study, we have shown that IL-15, but not IL-3, promoted the expression of bcl-x<sub>L</sub> mRNA and protein (Fig. 4, A–C). We presume that the expression of Bcl-x<sub>L</sub> protein is the main cause of the anti-apoptotic activity of IL-15 because the protein expression of other factors related to apoptosis such as Bcl-2, Bad, and Akt was not affected by IL-15 stimulation. Although IL-3 promoted the expression of bcl-2 mRNA, the level of Bcl-2 protein was not affected (Fig. 4, A and C). It has been reported that bcl-2 mRNA levels do not correlate well with Bcl-2 protein levels (43). As IL-3 also stimulated the phosphorylation of phosphatidylinositol 3-kinase and Akt in MC/9 cells (data not shown), these pathways may be important in the anti-apoptotic activity of IL-3.

In this study, we have shown that STAT6 activated by IL-15 is the regulator of Bcl-x<sub>L</sub> expression and anti-apoptotic activity. We have recently reported that IL-15 directly induces tyrosine phosphorylation of STAT6 and IL-4 mRNA increases in MC/9 cells and BMMCs (18). We have also shown that STAT6 bound to the STAT-binding site in the bcl-x promoter after IL-15 stimulation and that the expression of bcl-x<sub>L</sub> mRNA was significantly up-regulated as early as 4 h after IL-15 stimulation. Furthermore, a dominant-negative form of STAT6 significantly impaired the bcl-x<sub>L</sub> promoter activation mediated by IL-15. As IL-4 also directly activates STAT6 (19), we also examined bcl-x<sub>L</sub> mRNA expression under IL-4-depleted conditions and found that IL-4 depletion from the culture supernatant by the neutralizing anti-IL-4 mAb did not affect the bcl-x<sub>L</sub> mRNA expression mediated by IL-15 stimulation. Thus, IL-15 directly regulates bcl-x<sub>L</sub> gene expression through STAT6. It has recently been shown that bcl-x<sub>L</sub> is induced by the activation of STAT proteins STAT1, STAT3, and STAT5 through their direct binding to the STAT consensus sequence in the bcl-x gene promoter (23–28). Our present finding is the first example to show that STAT6 directly binds to the consensus sequence in the bcl-x gene and regulates bcl-x<sub>L</sub> mRNA expression. The relationship between STAT6 and Bcl-x<sub>L</sub> was reported in T cells stimulated with IL-4 using STAT6 knockout mice (22). In this report, however, STAT6 did not seem to be required for the anti-apoptotic activity of IL-4. Thus, Bcl-x<sub>L</sub> may participate differently in the regulation of apoptosis in T cells and mast cells. Additionally, we have shown that the expression of a dominant-negative form of STAT6 suppressed the bcl-x<sub>L</sub> mRNA up-regulation by and anti-apoptotic effects of IL-15. However, we cannot rule out the possibility that other anti-apoptotic factors whose expression is also controlled by STAT6 may have contributed to the pro-apoptotic effects of dominant-negative STAT6.

The anti-apoptotic activity of IL-15 has been well documented for T and NK cells (10, 54). However, as it has been reported that IL-15 uses a distinct receptor system in mast cells that does not utilize IL-2Rβ (17), the role of IL-15 in mast cells may be different from that in T and NK cells. Recently, we demonstrated that exogenous IL-15 induces IL-4 secretion from mast cells; and thus, the mast cell-specific downstream signals from IL-15 may play a role in the Th2-type response in vivo (18). Our current findings suggest that IL-15 increases the number of mast cells in combination with IL-3 and enhances their survival.

In summary, IL-15 may play an important role in some allergic diseases by increasing mast cell numbers and inducing their IL-4 secretion. Asthma, in which mast cells and Th2 cells are dominant, may be one of them. IL-15 is produced early in viral infections (55, 56) and asthma often worsens when viral infection in the bronchus occurs (57, 58). In some cases, IL-15 may be responsible for the pathogenesis of such diseases.

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Interleukin-15 Prevents Mouse Mast Cell Apoptosis through STAT6-mediated Bcl-xL Expression

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J. Biol. Chem. 2001, 276:26107-26113. doi: 10.1074/jbc.M011475200 originally published online May 21, 2001

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