Interleukin-15 Induces Rapid Tyrosine Phosphorylation of STAT6 and the Expression of Interleukin-4 in Mouse Mast Cells*

Received for publication, December 27, 1999, and in revised form, June 7, 2000
Published, JBC Papers in Press, July 5, 2000, DOI 10.1074/jbc.M910290199

Akio Masuda, Tetsuya Matsuguchi†, Kenichi Yamaki†, Tetsuo Hayakawa‡, Masato Kubo, William J. LaRochelle**, and Yasunobu Yoshikai§

From the †Second Department of Internal Medicine, Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya 466-8550, Japan, §Division of Immunobiology, Research Institute for Biological Sciences, Science University of Tokyo, Tokyo 278-0022, Japan, and **Laboratory of Cellular and Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland 20892

Interleukin-15 (IL-15) is a member of the cytokines that have a T cell growth promoting activity (18, 19). Many functional properties of IL-15 in T and NK cells are shared by IL-2 (20) probably because the receptor complexes for IL-15 and IL-2 share two signal-transducing subunits, IL-2 receptor (R)β and γc chains, in these cell types (18, 21). In contrast, it has recently been reported that IL-15 uses a distinct receptor system in mast cells (22). Although mast cells do not respond to IL-2 because of the lack of IL-2Rβ expression, they proliferate in response to IL-15. IL-15 seems to work on mast cells through a novel 60–65-kDa IL-15R molecule, IL-15RX (19). However, the physiological roles of IL-15 in mast cell functions have remained largely unknown.

In the present study, we have demonstrated that IL-15 induces IL-4 production from a mouse mast cell line, MC/9, and bone marrow-derived mast cells (BMMCs). IL-4 mRNA expression is induced by IL-15, suggesting that IL-15 promotes IL-4 expression at the transcriptional level. Surprisingly, STAT6, but not STAT1, -3, or -5, is rapidly tyrosine-phosphorylated in response to IL-15. In MC/9 cells, expression of a C-terminally truncated dominant negative form of STAT6 significantly suppressed the IL-4 mRNA up-regulation by IL-15, suggesting that STAT6 activation is essential for the IL-15-mediated IL-4 production. Additionally, tyrosine phosphorylation of Tyk2 was rapidly increased by IL-15 treatment in this cell line. Altogether, our results suggest that IL-15 plays an important role in stimulating early IL-4 production in mast cells that may be responsible for the initiation of Th2 response.

Interleukin (IL)-4 plays an important role in the differentiation of naive T helper (Th) cells into Th2. Mast cells can produce a significant amount of IL-4 and have been proposed to play a major role in the induction of Th2 responses. Recently, it has been reported that mast cells have a distinct IL-15 receptor system different from that of T or natural killer cells. In the present study, we demonstrated that IL-15 induced IL-4 production from a mouse mast cell line, MC/9, and bone marrow-derived mast cells. IL-4 mRNA expression was increased by IL-15, suggesting that IL-15 promotes IL-4 expression at the transcriptional level. In these mast cells, signal transducer and activator of transcription (STAT) 6 were rapidly tyrosine-phosphorylated in response to IL-15. In MC/9 cells, the expression of a C-terminally truncated dominant negative form of STAT6 suppressed the IL-4 mRNA up-regulation by IL-15, suggesting that STAT6 activation is essential for the IL-15-mediated IL-4 production. Additionally, tyrosine phosphorylation of Tyk2 was rapidly increased by IL-15 treatment in this cell line. Altogether, our results suggest that IL-15 plays an important role in stimulating early IL-4 production in mast cells that may be responsible for the initiation of Th2 response.

*This work was supported by a grant from YASUDA Medical Research Foundation, Yakult Biocience Foundation Grant JSPS-RFTF97L00703, and the Center of Excellence of the Japanese Government.

†To whom correspondence and reprint requests should be addressed: Laboratory of Host Defense and Germfree Life, Research Inst. for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: (052) 744-2447; Fax: (052) 744-2449; E-mail: tmatsgui@med.nagoya-u.ac.jp.

‡The abbreviations used are: IL, interleukin; Th, T helper; JAK, Janus kinase; STAT, signal transducer and activation of transcription; BMMC, bone marrow derived mast cell; PCS, fetal calf serum; DNP, dinitrophenyl; PCR, polymerase chain reaction; MOPS, morpholino propane sulfonic acid; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; R, receptor.

**Laboratory of Cellular and Molecular Biology, NCI, National Institutes of Health, Bethesda, Maryland 20892.

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phosphorylated by IL-15 treatment in this cell line. Altogether, our results suggest that IL-15 may play an important role in the initiation of the Th2 response by stimulating IL-4 expression in mast cells.

MATERIALS AND METHODS

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 dominant negative IL-2Rβ chain antibody (rat IgG1, 11B11) was purchased from PharMingen. The anti-phospho-tyrosine monoclonal antibody (4G10) and the polyclonal anti-Jak2 antibody were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). The anti-phospho-c-Jun N terminal kinase and the anti-phosphoextracellular signal-regulated kinase monoclonal antibodies were obtained from New England Biolabs (Beverly, MA). The anti-Jak1 polyclonal antibody, the anti-JAK3 monoclonal antibody, the polyclonal antibodies against STAT5b (which recognizes both STAT5a and STAT5b of mice), STAT3, and two polyclonal STAT6 antibodies raised against the C-terminal domain (amino acids 805–823) or the DNA binding domain (amino acid 280–480) of STAT6 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

The anti-Tyk2 monoclonal antibody was obtained from Transduction Laboratories (Lexington, KY). The rat monoclonal anti-mouse IL-2Rβ and γc antibodies were purchased from PharMingen (San Diego, CA).

The phycocerythrin-conjugated goat anti-rat IgG was purchased from CALTAG Laboratories (Burlingame, CA). The mouse monoclonal anti-dinitrophenyl (DNP) antibody and the DNP-human serum albumin serum albumin were purchased from Sigma.

Cell Lines—All cell lines were grown in tissue culture flasks at 37 °C in 5% CO2, 95% air and passed every two or three days to maintain logarithmic growth. The MC/9 mouse mast cell line was obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in RPMI 1640 containing 10% FCS, 20 μM 2-mercaptoethanol, 10% Walter and Eliza Hall Institute (WEHI)-3-conditioned medium as a source for IL-3, and 10% mouse spleen-conditioned medium with concanavalin A. BMMCs were derived from femoral bone marrow 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 more than 98% mast cells assessed by toluidine blue staining.

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

Reverse Transcriptase PCR Analysis—MC/9 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.) and incubated with recombinant Tsg (Takara Shuzou, Osaka, Japan) in a total volume of 50 μI reaction buffer consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, and 0.2 mM dNTP. The amplification procedure performed in a temperature controller was as follows: after an initial denaturation step at 95 °C for 5 min, 30 cycles were performed at 95 °C for 1 min followed by 54 °C for 1 min, and 72 °C for 1 min. 5 μl of each PCR product was run on a 2% agarose gel (Life Technologies, Inc.) for UV visualization.

Primer sequences were as follows: IL-4 sense, 5'-CGAGGAACACC-ACAGAGACTGAGCTTAC-3'; antisense, 5'-GACTCATTTGATCGACCAAGCTATCC-3'; IL-13 sense, 5'-ATGAGTCTGGATCCAGTGCGG-3'; antisense, 5'-CCCTGGACAGAAGCTTTCTAC-3'; tumor necrosis factor-α, 5'-GGGAGATGTTTGGGATCCAGGT-3'; antisense, 5'-AGCTTTATGTTGCTCAGTAC-3'; STAT3 sense, 5'-TGCAGTGTCTGTCCTCGTACAG-3'; antisense, 5'-CAAGGTGCCTCTCGAGAAAAT-3'; STAT6 sense, 5'-CCAGTGAATTCGGAAGCTCCT-3'; antisense, 5'-GCTTTGACAGCTGCCTGTTTCTG-3'; STAT5b sense, 5'-CCAGTGAATTCGG-3'; antisense, 5'-CCAGTGAATTCGG-3'; toll-like receptor 4, 5'-ATGAGTCTGCAGTATCCCG-3'; antisense, 5'-ACATTCGAGGCT-3'; tumor necrosis factor-

Northern Blot Analysis—Cells were incubated for 6 h at 37 °C in serum-free medium and stimulated under various conditions. Total cellular RNA was isolated as described above. 20 μg aliquots of the total RNAs were fractionated on a 1% agarose gel containing 20 mM 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 in 2× SSC, 0.1% SDS for 10 min twice at 65 °C, 0.1× SSC, 0.1% SDS for 10 min at 50 °C and were exposed to Fuji RX-U films (Fujifilm, Tokyo, Japan). cDNA fragments of the coding regions of mouse IL-4 and β-actin were used as specific probes.

Transfection and Luciferase Assay—The luciferase reporter vector containing the IL-4 promoter (pIL-4 (−766) Lu) was described previously (15). MC/9 cells were transiently transfected with 3 μg of pIL-4 (−766) Lu and 0.5 μg of pRLSV40 (an internal control) by 1,2-dimyristoyl-sn-glycero-3-phosphocholine-C Reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. 24 h after the transfection, the transfected cells were stimulated with IL-15 (10 ng/ml) or left untreated. After a 12-h incubation with IL-15, the cells were lysed, and the luciferase activity was measured by using the dual-luciferase reporter assay system (Toyo Ink Co., Tokyo, Japan) according to the manufacturer’s instructions. The data were presented as the mean ± S.D. of triplicate samples.

Immunoprecipitation and Western Blotting Analysis—Cells were incubated for 6 h at 37 °C in serum-free medium and stimulated under various conditions; they were lysed in ice-cold lysis buffer (50 mM HEPES, pH 7.4, 150 mM KCl, 1% Triton X-100, 1.5 mM MgCl2, 1 mm EGTA, 100 mM NaF, 10 mM NaPP, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride with aprotenin and leupeptin at 10 μg/ml) and incubated on ice for 20 min. Samples were centrifuged (15000 rpm, 5 min), and the supernatants were stored at −80 °C for further experiments.

For immunoprecipitation, 100 μl of cell lysate (from 1 × 107 cells) was rotated with a primary antibody overnight at 4 °C, added with 20 μl of protein A-Sepharose bead slurry (1:1, beads:lysis buffer), and incubated further for 1 h at 4 °C. In all cases, beads were washed three times with lysis buffer and boiled in the loading buffer before electrophoresis.

Lysates or immunoprecipitates were analyzed on a 7.5, 10, or 12% SDS-polyacrylamide gel, transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were blocked with 1% bovine serum albumin in Tris-buffersed saline containing 0.05% Tween-20 (TBST) for 1 h, and Western blot analysis was performed as described previously (24) followed by detection using enhanced chemiluminescence system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Flow Cytometry—MC/9 cells were stained with anti-mouse IL-2Rβ, γc chain antibody, or isotype-matched control rat IgG, followed by the phycocerythrin-conjugated goat anti-rat IgG. After washing, the cells were resuspended and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA).

Surface Expression of IL2R Chains on MC/9 Cells—The dominant negative STAT6 expression plasmid was generated by cloning the DNA encoding C-terminally truncated human STAT6 (containing amino acid 1–662) into pCEV29 vector. MC/9 cells were transfected with an electroporator using 20 μg of plasmid DNA at the condition of 800 microfarad and 300 V. Transfectants were selected with G418 (1 mg/ml). Resistant clones were screened for the right sized protein expression by Western blotting using an anti-STAT6 antibody (directed against amino acid 280–480).

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

RESULTS

Surface Expression of IL2R Chains on MC/9 Cells—It is generally accepted that the IL-15 receptor is composed of three subunits, two of which (IL-2Rβ and γc) are shared by IL-2 (20, 21). However, in a previous report, it has been reported that the IL-2Rγc chain is not expressed on mast cells, and IL-15 seems to utilize a novel receptor, IL-15Rγ (22). To examine the surface expression of IL-2Rβ and γc chains on MC/9, a well established
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 mast cell line used in the experiments, flow cytometry analyses were performed using specific monoclonal antibodies (mAb). As shown in Fig. 1, there was no detectable surface expression of IL-2Rβ on MC/9 cells, although they expressed the γc chain as previously reported (22). To confirm that the expression profile is not specific to this cell line, we also examined mouse BMMCs for IL-2R expression. They also expressed γc but not IL-2Rβ on the surface as reported earlier (22) (data not shown).

Mast Cells Increased IL-4 mRNA Expression in Response to IL-15 Stimulation—Although it has been reported that IL-15 supports the growth of mast cells (22), other effects of IL-15 on mast cells have not been fully explored. As mast cells are able to produce a variety of cytokines including IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor in response to various stimulations (25–29), we sought to examine whether exogenous IL-15 affects cytokine mRNA expression in MC/9 cells. Cells were incubated with IL-15 for 0, 1, 2, or 4 h and harvested for RNA preparation. Total RNAs from these cells were reverse-transcribed and then used for PCR amplification with pairs of specific primers for IL-4, IL-13, and tumor necrosis factor-α. Reverse transcriptase-PCR products for β-actin were measured to verify the integrity of the RNA. As shown in Fig. 2A, factor-derived MC/9 cells expressed a low level of mRNA for IL-4. IL-4 mRNA was significantly increased after 2- and 4-h stimulations by IL-15. In contrast, either IL-13 or tumor necrosis factor-α mRNA was not altered by the IL-15 stimulation. To know the concentration of IL-15 needed to induce IL-4 mRNA, MC/9 cells were stimulated with various concentration of IL-15 for 4 h and harvested for Northern blot analysis. As shown in Fig. 2B, IL-15 induced a weak IL-4 mRNA increase at 100 pg/ml, whereas 1000 and 10,000 pg/ml IL-15 induced more evident increase. IL-4 mRNA was also significantly increased by IL-15 treatment in BMMCs (Fig. 2C).

We next examined IL-4 promoter activity in IL-15-treated mast cells. MC/9 cells were transiently transfected with a luciferase reporter construct that contained an IL-4 promoter region (IL-4 (–760) Lu), and luciferase activity was measured after a 12-h stimulation by IL-15. As shown in Fig. 2D, IL-15 significantly increased the promoter activity of the IL-4 gene. To confirm whether the increase of IL-4 induced by IL-15 stimulation might contribute to the increase of IL-4 itself, we utilize a neutralizing antibody against mouse IL-4. MC/9 cells were incubated with IL-15 in the presence of the anti-IL-4 mAb for 6 h, and reverse transcriptase-PCR analysis was performed. The effectiveness of the neutralization was confirmed by the observation that the same concentration of this antibody could abrogate the IL-4-induced tyrosine phosphorylation of STAT6 (data not shown). As shown in Fig. 2E, the existence of Anti-IL-4 mAb did not affect the increase of IL-4 mRNA.

IL-15 Induced Rapid STAT6 Tyrosine Phosphorylation in Mast Cells—The JAK/STAT pathway is an important cytokine-induced signal transduction pathway that directly transfers signals from cell surface cytokine receptor to the nucleus. As it had been known that STAT6 played an important role in the regulation of IL-4 gene expression by IL-4 in helper T cells (30), we sought to examine STAT6 tyrosine phosphorylation after IL-15 stimulation in MC/9 cells. Factor-deprived MC/9 cells were stimulated with various concentration of IL-15 for 30 min before lysis. STAT6 was immunoprecipitated from the cell lysates, and subsequent Western blotting analysis was performed using an antiphosphotyrosine antibody. To compare the degree of tyrosine phosphorylation, MC/9 cells were also stimulated for 30 min with IL-3, a major growth factor for mast cells. As shown in Fig. 3A, IL-15 clearly induced STAT6 tyrosine phosphorylation within 30 min. At 100 or 1000 pg/ml, IL-15 induced moderate STAT6 tyrosine phosphorylation, whereas 10,000 pg/ml IL-15 induced it more evidently. In the time course analysis, the maximum induction of STAT6 tyrosine phosphorylation was found to occur between 15 and 30 min of IL-15 stimulation (Fig. 4B). Although it has been reported that IL-15 stimulates STAT5 tyrosine phosphorylation in another mast cell line (PT-18) (22), we could not detect any STAT5 tyrosine phosphorylation in MC/9 cells after IL-15 stimulation (Fig. 4C). Additionally, IL-15 did not induce any detectable tyrosine phosphorylation of other members of STAT, STAT1, STAT3, or STAT4 (data not shown). Additionally, IL-15 also stimulated STAT6 tyrosine phosphorylation in BMMCs (Fig. 4D). To confirm STAT6 tyrosine phosphorylation that we observed was directly regulated by IL-15 and not because of the autocrine effect by IL-4, we examined STAT6 tyrosine phosphorylation under an IL-4 depletion condition. As shown Fig. 4E, IL-4 depletion from the culture supernatant by the neutralizing anti-IL-4 mAb did not affect the STAT6 tyrosine phosphorylation by IL-15 stimulation (Fig. 4E).

Expression of a Dominant Negative STAT6 Mutant Inhibited IL-15-mediated IL-4 mRNA Expression—To examine if the activation of STAT6 was responsible for the IL-4 production in response to IL-15, we transfected an expression plasmid encoding a C-terminally truncated form of STAT6 in MC/9 cells. This mutant lacks the C-terminal transactivation domain. Three clones expressing this short form of STAT6 were isolated for analyses. The Western blotting result of a typical clone was shown in Fig. 5A. To examine if this STAT6 mutant worked in

![Figure 1](image-url) 

**Fig. 1.** The surface expression of IL2R chains on MC/9 cells. MC/9 cells were stained with rat anti-mouse IL-2Rβ, γc chain, or isotype-matched control rat IgG followed by an fluorescein isothiocyanate-conjugated goat anti-rat IgG. After washing, the cells were resuspended and analyzed by flow cytometry. White areas denote staining with an isotype control antibody, whereas black areas indicate anti-IL2R antibody staining.
a dominant negative fashion, we stimulated a positive clone with IL-4 or IL-15 and analyzed the tyrosine phosphorylation of the endogenous STAT6. As shown in Fig. 5B, both IL-4- and IL-15-mediated STAT6 tyrosine phosphorylations were significantly inhibited in this clone, suggesting that this C-terminally truncated STAT6 mutant worked in a dominant negative fashion. When IL-15-induced IL-4 mRNA expression was examined by the semiquantitative reverse transcriptase-PCR, it was significantly decreased in these cells compared with that of the parental MC/9 cells (Fig. 5C). These results strongly indicated that STAT6 activation is essential for the IL-4 production mediated by IL-15.

**IL-15 Induced Tyrosine Phosphorylation of Tyk2**—To study the upstream regulators of STAT6 tyrosine phosphorylation, the activation of JAK family kinases was examined in IL-15-stimulated MC/9 cells. Factor-starved MC/9 cells were stimulated with 10 ng/ml of IL-15 for 0, 15, 30, and 60 min before cell lysis, and JAK kinases were immunoprecipitated by their specific antibodies to examine the tyrosine phosphorylation status by immunoblotting using an antiphosphotyrosine mAb (4G10). As shown in Fig. 6A, Tyk2 was rapidly tyrosine-phosphorylated by IL-15. Although IL-15-induced IL-4 mRNA expression was examined by the semiquantitative reverse transcriptase-PCR, it was significantly decreased in these cells compared with that of the parental MC/9 cells (Fig. 5C). These results strongly indicated that STAT6 activation is essential for the IL-4 production mediated by IL-15.

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**Fig. 2. A,** cytokine mRNA expression in MC/9 cells stimulated by IL-15. MC/9 cells were incubated for 6 h at 37 °C in serum-free medium and stimulated by IL-15 (10 ng/ml) for the indicated times. Total RNAs (2 μg) from MC/9 cells were reverse-transcribed and amplified by PCR as described under “Materials and Methods.” Products using primers for each cytokine were resolved on 2% agarose gels and visualized by staining with ethidium bromide. The length of each product matched that predicted from the sequence data. TNF, tumor necrosis factor. B, dose response of IL-4 mRNA expression induced by IL-15 in MC/9 cells. MC/9 cells were incubated for 6 h at 37 °C in serum-free medium and stimulated by 0, 10, 100, 1000, or 10,000 pg/ml IL-15 for 4 h. Total RNA (20 μg) from MC/9 cells was used in Northern blot analysis. The filter was hybridized first with a IL-4-specific probe and then stripped and reprobed for β-actin. C, IL-4 mRNA expression induced by IL-15 in BMMCs. BMMCs were incubated for 6 h at 37 °C in serum-free medium and stimulated by IL-15 (10 ng/ml) for 4 h. Total RNA (20 μg) from BMMCs was used in Northern blot analysis. The filter was hybridized first with a IL-4-specific probe and then stripped and reprobed for β-actin. D, transcriptional activation of a luciferase reporter construct driven by the IL-4 promoter in response to IL-15. MC/9 cells cotransfected with pIL-4 (−766) Lu and pRL/SV40 were treated with IL-15 (10 ng/ml) for 12 h or left untreated. Units of luciferase activity were normalized based on values of pRL/SV40 activity for transfection efficiency. The experiment was done in triplicate. The error bars represent S.D. values. *, p < 0.05.

**Fig. 3. IL-4 secretion from mast cells stimulated with IL-15. A,** BMMCs (2 × 10^6 cells) were incubated in RPMI + 10% FCS with 10 ng/ml IL-15 or not. The cell-free culture supernatants were collected after 24 h of culture. B, MC/9 cells (5 × 10^6 cells) were incubated in RPMI + 10% FCS with 1 mg/ml anti-DNP-human serum albumin IgE mAb for 2 h. Cells were washed twice and resuspended in medium with 100 ng/ml DNP-human serum albumin and IL-15 (10 ng/ml) or not. The cell-free culture supernatants were collected after 48 h of culture. The IL-4 contents in the culture supernatants were assayed by ELISA using a mouse IL-4 ELISA system. The experiments were done in triplicate. The error bars represent S.D. values. **, p < 0.01.
DISCUSSION

In this study, we have shown that IL-15 induces IL-4 production from a mouse mast cell line, MC/9, and BMMCs. IL-4 mRNA expression is also induced by IL-15, suggesting that the induction is regulated at the transcriptional level. We have found that Tyk2 and STAT6, but not JAK1, -2, -3, STAT1, -3, -4, or -5, are rapidly tyrosine-phosphorylated in response to IL-15 in MC/9 cells. STAT6 is also rapidly tyrosine-phosphorylated by IL-15 in BMMCs. Additionally, the expression of a C-terminally truncated dominant negative form of STAT6 significantly suppressed the IL-4 mRNA up-regulation by IL-15, suggesting that STAT6 activation is essential for the IL-15-mediated IL-4 production.

Although the exact molecular mechanisms for the tissue-specific expression of the IL-4 gene have not been clearly shown, IL-4 gene expression is primarily controlled by transcriptional factors binding to the 5′-regulatory regions of the IL-4 promoter (31). IL-4 gene expression during Th2 cell differentiation is at least partly because of c-maf and GATA-3, transcription factors expressed specifically in Th2 cells (30). Additionally, other transcription factors, nuclear factor of activation in T cells (NF-AT), NF-AT interacting protein, and activator protein-1 have also been reported to be involved in the IL-4 gene activation (30). STAT6, first identified as a transducer of IL-4 receptor signaling, binds both the promoter and the silencer of the IL-4 gene (23, 32–34). Although it is still not clear whether STAT6 directly regulates IL-4 gene transcription, the critical role of STAT6 in Th2 cell development was demonstrated by the targeted disruption of the STAT6 gene in mice. These mice were severely defective in generating the Th2 type immune responses (15, 16). It has been postulated that STAT6 may mediate the Th2-specific expression of IL-4 through an autocrine mechanism by binding and transactivat-
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It is generally accepted that bystander cells responsible for early IL-4 production play an important role in the initiation of Th2 response of the early stage of immune response. Among these cells, mast cells may play an essential role, as they are widely distributed through vascularized tissues and are able to produce a large amount of IL-4 (27). Our current data suggest that IL-15, a cytokine produced by a wide variety of cell types including macrophage and epithelial cells in the early phase of infectious diseases (19), may play a part in the initiation of Th2 type immune responses by inducing IL-4 production from mast cells.

The tyrosine phosphorylation of STAT6 by IL-15 and the suppression of IL-15-mediated IL-4 mRNA expression by a dominant negative form of STAT6 strongly suggest that STAT6 is essential in the process (Figs. 4 and 5). Additionally, IL-15 induced the activation of IL-4 promoter, which contains three STAT-responsive elements (23, 33). This is not a cell line-specific phenomenon, because mouse BMMCs also showed increased IL-4 mRNA and STAT6 tyrosine phosphorylation in response to IL-15 (Figs. 2C and 4D). In contrast to our present findings, it has been reported that BMMCs from STAT6/fl− mice can express IL-4 comparable to those of wild-type mast cells, and mutation of the consensus STAT6 sites does not diminish IL-4 promoter activity (35). However, in this study, mast cells were stimulated by ionomycin, a strong inducer of IL-4 production from mast cells (27), and the IL-15-mediated IL-4 expression has never been explored.

In T cells, it is still uncertain if the activated STAT6 directly induces IL-4 production. As multimers of STAT6-corresponding elements were inducible by IL-4 when linked to heterologous promoters and transfected into STAT6-expressing B-cell lines (32), it seemed reasonable to conclude that STAT6 would directly enhance IL-4 transcription in T cells. However, a recent study using STAT6-deficient Jurkat T cells has suggested that although cotransfected STAT6 strongly enhanced transcription from the multimerized STAT6 response elements, the human IL-4 promoter was significantly repressed under similar conditions (33), suggesting that STAT6 might directly inhibit the IL-4 promoter function in some types of cells. In mast cells, mechanisms of IL-4 production have not been well understood but seem to be different from those in T cells. Another evidence for this hypothesis in addition to our present findings is a recent report that IL-4 production by mast cells does not require c-maf (36).

It is of note that a novel STAT6 isoform has been reported to be present in mast cells (37). In the report, this STAT6 isoform is also tyrosine-phosphorylated by IL-4, 65 kDa in size, and lacks reactivity with an anti-STAT6 antibody directed toward the C terminus, suggesting that it is a C-terminally truncated form. We sought to examine the existence of this short form of STAT6 in the MC/9 cell line we used. Although we utilized the same antibody used in the previous report, which was against the middle portion of STAT6, we could not detect a molecule around 65 kDa in MC/9 cells (data not shown). This is probably not because of the problem with our MC/9 cells, because the same antibody did not detect this short STAT6 isoform in mouse BMMCs (data not shown). Currently, we consider that the different antibody batches could cause the different Western blotting results.

Because mast cells constitutively produce a small amount of IL-4 (38) and IL-15 induces the IL-4 secretion (Fig. 3), STAT6 tyrosine phosphorylation that we observed might not be directly regulated by IL-15 and may be because of the autocrine effect by IL-4. However, we consider this possibility unlikely by the following reasons. First, Tyk2 and STAT6 were very rapidly tyrosine-phosphorylated as early as 15 min after stimulation with IL-15. Second, IL-4 depletion from the culture supernatant by neutralizing anti-IL-4 mAb did not affect the STAT6 tyrosine phosphorylation by IL-15 (Fig. 4E).

In our study, we have demonstrated that exogenous IL-15 induces IL-4 mRNA synthesis, a phenomenon that has not been reported in T cells. Recently it has been reported that IL-15 uses a distinct receptor system in mast cells, which does not utilize IL-2Rβ and is designated as IL-15 RX (22). Therefore, it is suggested that the mast cell-specific downstream signals from IL-15RX may play a role in the induction of IL-4 expression.

Our current findings suggest that the IL-15-mediated IL-4 production from mast cells may play an important part in some types of infectious diseases. For example, IL-15 is produced early in the intracellular parasitic infections (39, 40). In a mouse model, it has been reported that IL-15-IgG2b fusion protein accelerates and enhances Th2 type response in vivo (41). This fusion protein seems to prolong its half-life in vivo and enhances IL-15 effects. This report is consistent with our current findings and suggests that at least in some cases IL-15 is responsible for the induction of the Th2 type immune responses. Also epithelial cells are known to produce an appreciable level of IL-15 following infection (42, 43). Thus, it appears that IL-15 produced locally stimulates mast cells to secret IL-4 and in turn serves to alert a host defense system to local infection. In conclusion, our present data have suggested that IL-15 may play a role in the initiation of the Th2 type immune responses by inducing IL-4 production from mast cells through its unique receptor.

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