Bruton’s Tyrosine Kinase-mediated Interleukin-2 Gene Activation in Mast Cells

DEPENDENCE ON THE c-Jun N-TERMINAL KINASE ACTIVATION PATHWAY

Daisuke Hata, Jiro Kitaura, Stephen E. Hartman, Yuko Kawakami, Takashi Yokota, and Toshiaki Kawakami

From the Division of Allergy, La Jolla Institute for Allergy and Immunology, San Diego, California 92121 and the Department of Stem Cell Regulation, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan

Cross-linking of the high affinity IgE receptor (FceRI) on mast cells induces secretion of cytokines, including interleukin (IL)-2, through transcriptional activation of cytokine genes. Previously, defects in the gene coding for Bruton’s tyrosine kinase (Btk) were shown to result in defective cytokine production in mast cells, and thereby mice carrying btk mutations exhibited diminished anaphylactic reactions in response to IgE and antigen. In this study, we provide evidence that the transcription factors involved in the IL-2 gene expression in T cells are also required for maximal activation of the IL-2 gene in FceRI-stimulated mast cells. Among them, AP-1 (Jun/Fos) and NF-AT were identified as candidate transcription factors that are regulated by Btk. Consistent with our previous data indicating that Btk regulates stress-activated protein kinases, c-Jun N-terminal kinase (JNK), c-Jun and other JNK-regulatable transcription factors are activated by FceRI cross-linking in a Btk-dependent manner. Further, FceRI-induced IL-2 gene activation is dependent on c-Jun and a component, SEK1, of its upstream activation pathway. Collectively, these data demonstrate that Btk regulates the transcription of the IL-2 gene through the JNK-regulatable transcription factors in FceRI-stimulated mast cells.

Cross-linking of antibody-bound high affinity IgE receptor (FceRI)1 with multivalent antigen initiates allergic reactions by inducing degranulation, lipid mediator release, and cytokine secretion in mast cells (1, 2). FceRI is a heterotetrameric receptor composed of an IgE-binding α-subunit, a β-subunit with four transmembrane domains, and two disulfide-bonded γ-subunits (3). Several protein-tyrosine kinases were shown to be activated following receptor cross-linking; Lyn associated with FceRI β-subunit is believed to phosphorylate β- and γ-subunits (4, 5). Phosphorylated immunoreceptor tyrosine-based activation motifs on β- and γ-subunits recruit more Lyn and Syk, respectively (5–8). These receptor-bound protein-tyrosine kinases are activated and phosphorylate target proteins such as phospholipase C-γ. Activated phospholipase C-γ hydrolyzes phosphatidylinositol 4,5-bisphosphate into two second messengers; inositol 1,4,5-trisphosphate mobilizes Ca2+ from intracellular storage sites, and diacylglycerol activates protein kinase C (9). Both protein kinase C and Ca2+1 are required for degranulation (10).

Bruton’s tyrosine kinase (Btk) plays an indispensable function in B cell development, as exemplified in human (X-linked agammaglobulinemia) and mouse (xid) immunodeficiencies (11–14). Btk is also implicated in signal transduction for several immune cell and cytokine receptors (15, 16). In contrast with Lyn and Syk, Btk and Emt (also known as Itk and Tsk) do not physically associate with FceRI. However, these Tec family protein-tyrosine kinases are activated by FceRI cross-linking (17, 18). Recently, Btk was shown to play an important role in the FceRI signaling system.2 Thus, xid (with a substitution of Arg-28 in Btk with Cys) and btk null mice exhibited diminished anaphylactic reactions. Correspondingly, FceRI-induced cytokine production was severely impaired in mast cells derived from these mutant mice. Defects in the FceRI-induced production of interleukin (IL)-2 and tumor necrosis factor α (TNF-α) in the mutant cells were found in the transcription of these cytokine genes.

FceRI cross-linking induces activation of three major mitogen-activated protein (MAP) kinases, i.e. ERK1/2, JNK1/2, and p38 (20–25). Activities of MAP kinases are regulated through a unique set of protein kinases in a cascade, e.g. Raf-1 → MEK1/2 → ERK1/2 and MEKK1 → SEK1 (also known as MKK4, MEK4, or JNKK) → JNK1/2 (26–28). Phosphorylation targets of MAP kinases include transcription factors. Typically, JNK phosphorylates c-Jun, a component of the AP-1 transcription factor, at the critical serine residues (Ser-63 and Ser-73) in the activation domain of c-Jun (29, 30), causing increased c-Jun transcriptional activity (31–33). More recently, similar phosphorylation and transcriptional activation by JNK were shown for other transcription factors such as ATF2 (34, 35), Elk-1 (36), and Sap-1α (37). xid and btk null mutations affect JNK activities severely and, to a lesser extent, p38 in FceRI-stimulated mast cells, while ERK activities are not significantly changed (25).

Transcriptional regulation of IL-2 and TNF-α genes has been
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extensively characterized in T cells. IL-2 promoter activity depends on the 300-base pair region upstream of the transcription initiation site (38–40). Several cis-acting elements that are binding sites for the nuclear factor of activated T cells (NF-AT), nuclear factor-κB (NF-κB), CD28RC, AP-1, and Oct have been identified (41, 42). Cooperation of these transcription factors is required for maximal activation of the IL-2 promoter. NF-AT, a family of related proteins, exists in the cytoplasm before activation and translocates to the nucleus in response to an increase in intracellular concentrations of Ca2+(43). This translocation depends on dephosphorylation of NF-AT by a Ca2+- and calmodulin-dependent phosphatase, calcineurin, which is a major target of the immunosuppressive drugs cyclosporin A and FK506. Translocated NF-AT forms a complex with a ubiquitous nuclear factor AP-1 to exert its transcriptional action (reviewed in Ref. 44). NF-AT by a Ca2+-dependent mechanism is inhibited in IL-2 gene transcription (45). The IL-2 promoter region contains two conventional and two noncanonical AP-1 binding sites. The latter AP-1 sites exist in a close apposition to the distal NF-AT and proximal Oct sites. AP-1 sites are bound by Jun/Fos or Jun/Jun dimers. The binding activity for the proximal Oct (Octp) site is constitutive, although the Octp site-dependent transcription requires T cell stimulation (40). Similar to the IL-2 gene, transcriptional activation of the TNF-α gene induced by T cell receptor stimulation involves NF-ATp and ATF-2/Jun heterodimer proteins bound to the κs site and a cyclic AMP response element, respectively, in the 200-base pair TNF-α promoter (47–49).

In this study, we determined which cis-regulatory elements in the IL-2 gene promoter are involved in FceRI-induced activation of the IL-2 gene. Among the transcription factors involved in activation of the IL-2 gene, c-Jun and other JNK-activable transcription factors are shown to be regulated by Btk. Since Btk regulates JNK activity, we investigated the possible involvement of the MEKK1 → SEK1 → JNK pathway in the IL-2 gene activation in mast cells.

EXPERIMENTAL PROCEDURES

Reagents—B6/129 F2 mice were purchased from Jackson Laboratory. btk null mice (50) originally provided by Drs. Wasif N. Khan and Frederick W. Alt were bred in an animal facility in La Jolla Institute for Allergy and Immunology. Recombinant rat stem cell factor was kindly donated by Amgen. Anti-Btk antibody was described previously (51). Antibodies against MEK-4 (SEK1) and MEKK1 were from Santa Cruz Biotechnology, Inc. Anti-phospho-c-Jun(Ser-63) and anti-c-Jun antibodies were kindly provided by Drs. Michael Karin, Richard Treisman, and John M. Kyriakis, and Arpad Molnar.

Stable Transfectants—Mast cells derived from btk null mice after 2 weeks of culture in IL-3 were incubated in the presence of rat stem cell factor as well as IL-3 for another 2 weeks to accelerate cell proliferation before retroviral infection. pMX-puro vectors containing no cDNAs, WT btk cDNA, or K430R btk cDNA were transduced into BOSC-23 packaging cells (64) with LipofectAMINE (Life Technologies, Inc.) according to the manufacturer's instructions. Culture supernatants were recovered 48 h after transfection. Virus titers (1–5 × 105 colony-forming units/ml) in the supernatants were measured by infecting NIH/3T3 cells and selecting in 1 μg/ml puromycin. Mast cells cultured in the presence of stem cell factor were infected with the retroviruses in the presence of 4 μg/ml polyethylene glycol and 0.8–1.2 mg/ml polybrene in puromycin (1.5 μg/ml) was used to startublish sufficient numbers of drug-resistant cells. Drug was omitted from the culture 2 days before transgenic transfected. Successful transfection by WT btk cDNA was routinely confirmed by restored secretion of IL-2 and TNF-α from immunologically stimulated transfected cells.2

Transient Transfection and Luciferase Assays—BMMC or stably transfected mast cells (1–2 × 106 cells) prepared as described above were transfected with 5–10-μg reporter plasmids together with or without the indicated combinations of expression plasmids by electroporation at 400 V and 950 microfarads using a Bio-Rad Gene Pulser II system. Twenty-four h after transfection, cells were started to be sensitized overnight with anti-dinitrophenyl monoclonal IgE antibody. Forty-one h after transfection, cells were left unstimulated or stimulated with antigen, 20 ng/ml dinitrophenyl conjugates of human serum albumin for 7 h before cell harvest. Cells were lysed in 0.2% Triton X-100 in 100 mM potassium phosphate (pH 7.8/1 mM dithiothreitol). Luminescence of cleared lysates after the addition of ATP and luciferin solutions was measured using a Monolight 2010 luminometer (Analytical Luminescence Laboratory).

Immunoblotting—Cells were lysed in 1% Nonidet P-40-containing lysis buffer (65), and cleared lysates were analyzed by SDS-polyacrylamide gel electrophoresis followed by electroblotting. Blots were blocked and incubated consecutively with primary antibody, peroxidase-conjugated secondary antibody, and enhanced chemiluminescence reagents (Amersham Pharmacia Biotech or NEN Life Science Products). Fluorescent bands were visualized by exposure to ReflectionTM autoradiography films (NEN Life Science Products).

RESULTS

AP-1 and NF-AT Elements Are Crucial for IL-2 Gene Transcription in FceRI-stimulated Mast Cells—We and others recently showed that rodent mast cells, including BMMC, secrete IL-2 in addition to numerous other cytokines upon FceRI cross-linking (66, 67). Transcriptional activation of the IL-2 gene turned out to be a major mechanism for this phenomenon.2 In the murine IL-2 promoter region, several cis-regulatory elements have been identified (41, 42). Therefore, it is interesting to determine which element is important for IL-2 gene transcription in mast cells. To this end, expression of IL-2 promoter-luciferase constructs with mutations at each cis-element was measured in transiently transfected BMMC derived from B6/129 F2 or CBA/J mice (Fig. 1). Mutations in the distal NF-AT (NF-ATd positions –285 to –281), AP-1 next to the NF-ATd site (NF-ATd/AP-1, –277 to –274), distal Oct (Octd, –253 to –251), NF-κB (–206 to –202), CD28RE (–160 to –157), proximal AP-1 (AP-1p, –151 to –149), proximal NF-AT (NF-ATp, –137 to –133), AP-1 next to the proximal Oct site (AP-1/Octp, –86 to –83), or proximal Oct (Octp, –79 to –77)
sites drastically reduced FcεRI-induced transcriptional activation of the IL-2 promoter, while the effect of mutations at the distal AP-1 site (positions -185 to -183) was relatively small (1/5 (B6/129 F2) or 2/3 (CBA/J) of the activity of the WT promoter). These results indicate that most of the known cis-regulatory elements are required for maximal activation of the IL-2 promoter in mast cells as well as in T cells. The most severe impairments in FcεRI-mediated IL-2 promoter activation were observed with the mutant promoters containing mutated NF-ATd, NF-ATd/AP-1, NF-ATp, or AP-1/Octp sites in BMMC from both mouse strains (Figs. 1, B and C), suggesting that these AP-1 and NF-AT elements are crucial for FcεRI-mediated IL-2 gene transcription in mast cells. These data are consistent with the accumulated observations on T cells that NF-AT family and

**Fig. 1.** Most known cis-regulatory elements are required for maximal activation of the IL-2 gene. A, a schematic representation of the known cis-regulatory elements shown in boxes (top row) and their WT nucleotide sequences (second row). Names and mutated sequences of individual mutant IL-2Luc constructs are shown below. B and C, BMMC derived from B6/129 F2 (B) or CBA/J (C) were transfected with 8 μg each of WT or mutated IL-2Luc plasmids. Twenty-four h later, cells were sensitized with IgE and left unstimulated or stimulated by antigen. Cells were lysed, and luciferase activities were determined as described under “Experimental Procedures.” Luciferase activities adjusted by protein contents are shown after normalization against that of WT IL-2Luc. Shown is a representative result out of two experiments.
AP-1 proteins play essential roles in the transcription of the IL-2 gene (44).

Btk Regulates IL-2 Gene Transcription through AP-1 and/or NF-AT Sites—We recently showed that FcεRI-induced transcriptional activation of the IL-2 as well as TNF-α genes is severely compromised in xid and btk null mast cells.2 To further confirm this observation, we measured transcriptional activities of the IL-2 promoter in btk null BMMC that were stably transfected with the empty vector, WT btk, or kinase-dead (K430R) btk cDNAs. Comparable expression of WT and K430R Btk in the transfectants was confirmed by immunoblotting (Fig. 2A). Upon FcεRI cross-linking, the transcriptional activity was strongly induced in WT btk transfected cells compared with those in vector or kinase-dead btk transfected cells (Fig. 2A). This result demonstrates that Btk regulates expression of the IL-2 gene and that the intact enzymatic activity of Btk is required for this activity. This Btk function could be ascribed to its effects on mast cell differentiation. However, this possibility is unlikely for two reasons. First, btk null BMMC is phenotypically indistinguishable from WT BMMC.2 Second, transient expression of WT Btk, but not vector or K430R Btk, in btk null BMMC restored the FcεRI-induced transcriptional activation of the IL-2 gene (Fig. 2B). In these experiments, cells were harvested for luciferase assays only 48 h after transfection. This short period of time would not be long enough for cells to differentiate. These data together with the results shown in Fig. 1 raise the possibility that Btk regulates IL-2 gene transcription by regulating the transcription factors that target the critical AP-1 and/or NF-AT sites. Therefore, we examined the transcriptional activity of individual cis-elements of the IL-2 promoter in btk null BMMC. For these transient transfection experiments, we used the basic enhancerless SV40 promoter-luciferase fusion vectors containing multiple copies of either the NF-ATd/AP-1 or AP-1/Octp sequences. Robust transcriptional activation was seen of NF-ATd/AP-1-Luc and AP-1/Octp-Luc constructs before FcεRI stimulation of WT btk-transfected cells, and FcεRI cross-linking further enhanced their transcriptional activities (Fig. 2C). In contrast, a similar luciferase construct containing eight copies of the NF-κB site exhibited a high basal transcriptional activity, but no further enhancement by FcεRI cross-linking was observed. Transcriptional activities of these reporter constructs were much smaller in vector or kinase-dead btk transfected cells. These data suggest that Btk-regulated IL-2 gene transcription is mediated by AP-1- and/or NF-AT-dependent mechanisms.

Btk Regulates c-Jun and Other Transcription Factors That Are Activated by JNK—Previous studies demonstrated that FcεRI cross-linking induces activation of all three major subfamilies of MAP kinases, i.e. ERK1/2, JNK1/2, and p38 (20-25).

Fig. 2. Btk regulates IL-2 gene transcription. A, btk null BMMC stably transfected with vector, WT btk, or K430R btk cDNAs were electroporated with 8 μg of IL-2Luc plasmid. Cell stimulation, lysis, and luciferase assays were performed as described in the Fig. 1 legend. Fold luciferase activities relative to that of unstimulated vector transfectants are shown (top). A representative result is shown out of at least 15 experiments. Expression of Btk protein in btk null BMMC stably transfected with vector, WT btk, or K430R btk cDNAs was detected by immunoblotting. Positions of molecular mass standards and Btk are indicated (bottom). B, btk null BMMC were co-transfected with 8 μg of IL-2Luc and 20 μg of pME18S, pME-btk, or pME-btk(K430R). Cell stimulation, lysis, and luciferase assays were performed as above. Fold luciferase activities relative to that of unstimulated vector transfectants are shown. A representative result out of two experiments. C, btk null BMMC were co-transfected with 8 μg of NF-ATd/AP-1-Luc, AP-1/Octp-Luc, or NFκB-Luc, together with 20 μg of pME18S, pME-btk, or pME-btk(K430R). Luciferase activities were normalized against that of vector-transfected cells without stimulation. Shown is a representative result out of two experiments.
Btk was shown to critically regulate JNK1/2 and, to a lesser extent, p38, while it did not affect ERKs significantly (25).

Targets of JNK include the transcription factors, such as c-Jun, Elk-1, and Sap-1a. Therefore, we tested whether activities of these transcription factors are under the control of Btk in mast cells. To this end, btk null-BMMC stably transfected with the empty vector, WT btk, or K430R btk cDNAs were transiently co-transfected with a luciferase reporter plasmid (pG5E1bLuc) and 0.5 μg of unfused GAL4 DNA binding domain vector (GAL4), GAL4-c-Jun, or GAL4-c-Jun(S63/73A) plasmids. Cell stimulation, lysis, and luciferase assays were performed as described in the legend for Fig. 1. Fold luciferase activities are shown relative to that in unstimulated vector transfectants. Shown is a representative result out of five experiments.

FIG. 3. Btk regulates transcriptional activities of c-Jun and other JNK-regulatable transcription factors. A, btk null-BMMC stably transfected with vector, WT btk, or K430R btk cDNAs were transiently transfected with 8 μg of a 5× (GAL4 binding site) luciferase reporter plasmid (pG5E1bLuc) and 0.5 μg of unfused GAL4 DNA binding domain vector (GAL4), GAL4-c-Jun, or GAL4-c-Jun(S63/73A) plasmids. Cell stimulation, lysis, and luciferase assays were performed as described in the legend for Fig. 1. Fold luciferase activities are shown relative to that in unstimulated vector transfectants. Shown is a representative result out of five experiments.

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A. Graph showing fold luciferase activity with different conditions.

B. Bar chart showing fold luciferase activity with various stimulations.

C. Graph showing fold luciferase activity with different constructs.

D. Bar chart showing fold luciferase activity with various stimulations.

E. Graph showing fold luciferase activity with different constructs.
tion site mutants of Elk-1 and Sap-1a constructs were suppressed in either cells. These results are consistent with the finding in other studies that these transcription factors are phosphorylated and activated by JNK. Activation of MEF2C, which was recently shown to be a target of p38 (68), in WT btk transfectants was 2.5-fold higher than activation in vector or K430R btk transfectants (data not shown), consistent with our previous finding that Btk also regulates p38, albeit to a lesser extent than JNK (25).

In contrast, Fos activity may not be regulated by Btk, since 1) ERK1/2 activity, a major component of the c-fos activating pathway, is not significantly affected by btk mutations (25) and 2) transcription of a luciferase reporter construct containing a typical AP-1 element derived from the human collagenase gene promoter was not significantly affected by btk mutations (data not shown). Transcriptional activities of c-fos-Luc were slightly enhanced by FceRI cross-linking in btk null BMMC, but they were not affected by expression of vector, WT Btk, or K430R Btk (data not shown).

The SEK1-JNK-c-Jun Pathway Is Crucial for FceRI-induced, Btk-dependent IL-2 Gene Transcription in Mast Cells—Upstream of c-Jun, various stimuli lead to JNK activation through a cascade of protein kinases: MEKK1 \(\rightarrow\) SEK1 \(\rightarrow\) JNK (26–28). Therefore, we examined whether c-Jun and this cascade are used to activate the IL-2 gene. First, the effects of a c-Jun dominant negative mutant were tested on IL-2 promoter activity. A c-Jun mutant, Jun in 282 protein, which lacks the DNA binding ability and acts as a dominant negative protein in Bcr-Abl-induced transformation (61), was transiently expressed in WT BMMC. Jun in 282 protein was expressed 2-fold more than the endogenous c-Jun protein in the transfected WT BMMC (Fig. 4A, inset). FceRI-induced transcriptional activation of the IL-2Luc reporter in WT BMMC was suppressed by \(-60\%\) by Jun in 282 protein compared with the vector transfected cells under our conditions (Fig. 4A). btk null BMMC were also transfected with control or Jun in 282 expression vectors together with btk expression and IL-2Luc plasmids. Jun in 282 protein almost completely abolished the enhancing effect of Btk on FceRI-induced IL-2 promoter activity (Fig. 4B). These data clearly indicate that c-Jun plays an important role in IL-2 gene transcription in mast cells and that Btk regulates the activity of c-Jun.

SEK1 is a well characterized direct JNK activator, and many stimuli activate JNK via SEK1 (37). Since a kinase-dead mutant, SEK1(K/R), works as a dominant negative mutant, this mutant was transiently expressed together with the IL-2Luc plasmid in WT or btk null BMMC. Expression of glutathione S-transferase-SEK1(K/R) fusion protein was confirmed by immunoblotting (Fig. 4C, inset). Glutathione S-transferase-SEK1(K/R) suppressed IL-2 promoter activity by \(-60\%\) in FceRI-stimulated WT BMMC (Fig. 4C). In btk null BMMC, glutathione S-transferase-SEK1(K/R) also suppressed the potentiating effect of Btk on IL-2 promoter activity upon FcεRI cross-linking (Fig. 4D). These data collectively demonstrate that SEK1 is also involved in Btk-mediated IL-2 gene transcription in mast cells.

MEKK1 is a representative activator of SEK1, although recent studies identified several other protein kinases as SEK1 activators (69–76). A constitutively active MEKK1 mutant, ΔMEKK1, was co-expressed with the IL-2Luc plasmid in btk null BMMC. Compared with the vector control, ΔMEKK1 induced a very strong activation of IL-2 promoter without FcεRI stimulation, and FcεRI cross-linking further stimulated IL-2 promoter activity (Fig. 4E). Although these data do not prove that MEKK1 is involved in Btk-mediated IL-2 gene activation, they are certainly consistent with the notion that IL-2 gene activation by FcεRI cross-linking involves SEK1 and JNK, downstream of Btk and upstream of c-Jun and other transcription factors.

**DISCUSSION**

Production of cytokines is regulated at various levels, e.g. mRNA stability, posttranslational modification, and processing of precursor proteins. However, transcriptional activation is a major regulatory mechanism generally adopted by numerous cytokine genes. Similar to T cells, mast cells seem to use this strategy in response to FcεRI cross-linking. As shown in Fig. 1, most of the known cis-elements that are binding sites for transcription factors are required for maximal activation of the IL-2 gene. Since mutations in any one of these elements lead to drastic decreases in IL-2 gene expression, individual transcription factors cooperate for maximal gene activation, a finding also consistent with previous studies on T cells. Among the cis-elements tested, mutations in the two AP-1 (NF-AT/AD-AP-1 and AP-1/Octp) and two NFAT (NF-ATd and NF-ATp) sites affected the gene expression most severely in mast cells. On the other hand, transcriptional activities of the cis-elements containing the NF-ATd/AD-AP-1 and AP-1/Octp regions were shown to be regulated by FceRI cross-linking in a Btk-dependent manner. These results suggest that Btk regulates activities of AP-1 and/or NF-AT transcription factors. AP-1 is a complex of Jun and Fos family members. Our present studies have demonstrated that activities of c-Jun and other JNK-activatable transcription factors, i.e. JunB, JunD, Elk-1, and Sap-1a, are regulated by Btk. Jun and Fos family members associate in various combinations and with other transcription factors, including NF-AT (reviewed in Ref. 77). Therefore, one possible explanation for the Btk dependence of transcriptional activities of NF-ATd/AD-AP-1 and AP-1/Octp-Luc in mast cells is that the activity of the transcription factors bound to the AP-1 sites, but not those of the factors bound to the NF-ATd or Octp sites, is dependent on Btk. Although we have not studied NF-AT or Oct as a candidate Btk-regulated transcription factor in further detail, this possibility certainly warrants further investigation.

**Fig. 4.** Btk-mediated IL-2 gene transcription is dependent on JNK activation pathway. A, WT BMMC were co-transfected with 8 μg of IL-2Luc and 40 μg of either empty vector (pSRoMSVtkneo) or Jun in 282 expression vector. Standard luciferase assays were performed on unstimulated or immunologically stimulated cells. Fold luciferase activities are shown after normalization against that in unstimulated vector-transfected cells. Expression of c-Jun was detected by immunoblotting and quantification of total cell lysates with anti-c-Jun (inset). The protein encoded by the Jun in 282 vector construct was superimposed on the band of endogenous c-Jun. B, btk null BMMC were co-transfected with the indicated combinations of 8 μg of IL-2Luc, 20 μg of either pME18S or pME-btk, and 40 μg of either pSRoMSVtkneo or Jun in 282 expression vector. Luciferase activities are shown relative to that in unstimulated vector (pME18S and pSRoMSVtkneo)-transfected cells. C, WT BMMC were co-transfected with 8 μg of IL-2Luc and 40 μg of either the empty vector (pEBG) or pEBG-SEK1(K/R)-transfected cells. Fold luciferase activities are shown after normalization against that in unstimulated vector-transfected cells. Expression of endogenous SEK1 and glutathione S-transferase-SEK1 was detected by immunoblotting and quantification of total cell lysates with anti-SEK1 (inset). D, btk null BMMC were co-transfected with the indicated combinations of 8 μg of IL-2Luc, 20 μg of either pME18S or pME-btk, and 40 μg of either pEBG or pEBG-SEK1(K/R)-transfected vector. Luciferase activities are shown relative to that in unstimulated (pME18S and pEBG)-transfected cells. E, btk null BMMC were co-transfected with 8 μg of IL-2Luc and 40 μg of either the empty vector (pCMV5) or ΔMEKK1 expression vector. Luciferase activities are shown relative to that in unstimulated vector (pCMV5)-transfected cells. ΔMEKK1 was detected by immunoblotting with anti-EE antibody (inset). A–E, representative results are shown out of two identical experiments.
Btk regulates the enzymatic activities of JNK and p38, but not ERK (25). At least some stimuli use the cascade of protein kinases, MEKK1 → SEK1 → JNK2/1 (28). Mast cells seem to use this pathway to transcriptionally activate the IL-2 gene upon FceRI stimulation. Thus, dominant negative mutants of SEK1 and c-Jun inhibited the FceRI-induced IL-2 gene expression, while a constitutively active MEKK1 mutant activated the transcription of the IL-2 gene. However, our study does not rule out the possibility that a protein kinase(s) other than MEKK1 might be activated by FceRI cross-linking to activate SEK1 and JNK. There are several reports on other protein kinases with SEK1-activating ability (83–85). On the other hand, the enzymatic activity of Btk is negatively regulated by phospho-

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