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The Interaction between GATA Proteins and Activator Protein-1 Promotes the Transcription of *IL-13* in Mast Cells[1]

Akio Masuda,[2]* Yasunobu Yoshikai,[‡] Hiroaki Kume,[†] and Tetsuya Matsuguchi[§]

IL-13 is considered to be a key modulator in the pathogenesis of Th2-induced allergic inflammation, although little is known about the regulation of *IL-13* transcription in mast cells. In T cells, involvement of GATA-3 in cell type-specific expression of the *IL-13* gene has been reported. However, the mechanisms that induce rapid transactivation of the *IL-13* gene in response to various types of stimulation have hitherto remained unknown. In this report, we describe our investigation of the promoter region necessary for *IL-13* transcription; we have found that both AP-1 and GATA proteins are indispensable for *IL-13* transcription in mouse mast cells. In our investigation, we focused on the functional interaction between GATA and AP-1 in the *IL-13* promoter context. Transfection experiments have revealed that GATA-1 and GATA-2 proteins are able to associate with AP-1 proteins. We have also shown that overexpression of GATA-1 induced excess AP-1 binding to the *IL-13* promoter as well as a significant increase in IL-13 production in mast cells. The results of the present study have shown that direct interaction between AP-1 and GATA proteins plays an important role in *IL-13* transcription in mast cells. *The Journal of Immunology,* **2004, 173: 5564–5573.**

Interleukin 13 is a pleiotropic 12-kDa product of a gene on chromosome 5 at q31, which was originally described as being produced by activated Th 2 cells, but its expression by activated mast cells has also been reported (1–4). By using the IL-4Rα chain and STAT6 for signaling, IL-13 shares some biological activities with IL-4, such as the promotion of human B cell growth and the switching of B cells to the IgE isotype (5). Furthermore, it has been shown that IL-13 is a critical mediator in experimental models of allergic asthma (6, 7). Thus, IL-13 is considered to be the key modulator in the pathogenesis of Th2-induced allergic inflammation (8).

Although the mechanisms that regulate IL-13 production in T cells or mast cells remain largely unknown, it has been reported that GATA-3 plays an important role in IL-13 production in T cells (9). GATA-3 has been reported to be involved in the chromatin remodeling of Th2 cytokine genes and the improvement in the accessibility of transcriptional factors to promoter regions of Th2 cytokines (10). Furthermore, it has recently been reported that GATA-3 binding sites in the proximal *IL-13* promoter are necessary for cell type-specific expression of IL-13 (11). However, the mechanisms that activate *IL-13* transcription immediately in response to stimulation are still underexplored. Although rapid phosphorylation of GATA proteins after various types of stimulation has been reported, the role of these phosphorylations in transcriptional regulation is still controversial (12, 13). Furthermore, almost nothing is known about the regulation of *IL-13* gene in mast cells. Mast cells have high expression levels of GATA-1 and GATA-2 but little expression of GATA-3, and the roles of GATA-1 and GATA-2 in *IL-13* transcription have not been explored (14, 15).

GATA proteins are tissue-restricted transcription factors that bind a WGATAR DNA motif through a zinc-finger DNA-binding domain. Based on sequence homology and expression patterns, GATA proteins have been divided into two subfamilies, GATA-1–3 and GATA-4–6 (16). The former family is prominently expressed in hemopoietic stem cells, and the latter family is expressed in various mesoderm- and endoderm-derived tissues (17). GATA-1 and GATA-2 are expressed in mast cells, erythroblasts, and megakaryocytes (14, 18), and are instrumental for tissue-specific gene expression. Genes that are selectively expressed in mast cells and whose expression depends on GATA proteins include those encoding carboxypeptidase A, several mast cell-specific proteases and the IgE receptor α- and β-chains (14, 19–21). Furthermore, GATA-1 and -2 play important roles in the transcription of Th2 cytokine genes such as IL-4 and IL-5 in mast cells (22, 23).

Several observations suggest that AP-1, which is a dimeric complex of a Jun family protein and a Fos family protein, may functionally interact with GATA proteins. First, GATA and AP-1 sites are found in close association in the core hypersensitivity sites of the globin locus control region and in a number of erythroid promoters (24, 25). Second, a number of experiments have indicated that GATA sites in conjunction with an AP-1 site constitute an active promoter, whereas GATA sites in conjunction with a TATA box alone do not form an active promoter (25, 26). In the promoter of the endothelin-1 gene, the Jun/Fos heterodimer was found to cooperate with GATA-2 in the activation of transcription (27). Moreover, the IL-5 promoter has been shown to require both AP-1 and GATA elements for its activity in T cells (28).

In our present study, we have investigated the promoter region necessary for IL-13 transcription and found that AP-1 and GATA binding sites are indispensable for IL-13 transcription. We focused on the functional interaction between GATA and AP-1 in the *IL-13* promoter context. Transfection experiments revealed that GATA-1 and GATA-2 proteins are able to associate with AP-1 proteins. We also found that overexpression of GATA-1 induced excess binding

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of AP-1 to the *IL-13* promoter as well as a significant increase in IL-13 production.

Materials and Methods

Reagents and Abs

RPMI 1640 medium and FCS were purchased from Sigma-Aldrich (St. Louis, MO). LPS from *Escherichia coli* serotype 055:B5, the mouse monoclonal anti-DNP Ab, the DNP-human serum albumin, ionomycin, and PMA were obtained from Sigma-Aldrich. Anti-histone H3 Ab, anti-Myc mAb, anti-Flag M2 mAb, anti-Jun polyclonal Ab, anti-*fos* polyclonal Ab, anti-GATA-1 mAb, and anti-GATA-2 mAb were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cells

The MC/9 mouse mast cell line was cultured as previously described (29). The PT18 mouse mast cell line was kindly provided from Dr. K. Okumura (Juntendo University, Tokyo, Japan). The P815 mouse mastcytoma cells were obtained from Cell Resource Center for Biomedical Research (Tohoku University, Sendai, Japan). These cells were grown in RPMI 1640 with 10% FCS. Bone marrow-derived mast cells (BMMCs)[3] were derived from femoral bone marrow cells of 6-wk-old BALB/c mice. After 3 wk of culture with 10% WEHI-3-conditioned medium, the cells were harvested for the experiments and consisted of >98% mast cells assessed by toluidine blue staining.

A human embryonic kidney cell line, HEK 293T, was maintained in DMEM with 10% FCS. Cells were washed twice and incubated in fresh culture medium for 6 h and stimulated with PMA (10 ng/ml) plus ionomycin (1 μg/ml) or LPS (1 μg/ml) unless otherwise indicated. For the cross-linking of FcϵRI on mast cells, cells were sensitized by incubation for 2 h with 1 μg/ml anti-DNP IgE in RPMI 1640 containing 10% FCS, washed, incubated (2×10^6 cells/ml) for 6 h in RPMI 1640 containing 10% FCS, and stimulated with 10 ng/ml DNP-human serum albumin.

IL-13 promoter constructs

DNA fragments of the murine IL-13 promoter were amplified by PCR using the primers: senses CGGGGTACCGGGGTAGGGGAGCTGAATAG (pGL3–2024), CGGGGTACCGCTGTCATGTTTTCCCCAGT (pGL3–1253), CGGGGTACCGAGTTCAAGCTGCCCACTG (pGL3–705), CGGGGTACCATAGTGTGGTTGGAAGTGGC (pGL3–560), CGGGGTACCGAAAGGAAGGAAGGGAGGAA (pGL3–428), CGGGGTACCGTGTCAAGGGGTCAGCATT (pGL3–322), CGGGGTACCTCTTTCCTTTAGCGGCCAC (pGL3–156), CGGGGTACCAAGATGAGTAAAGATGTGG (pGL3–106), CGGGGTACCGATGTGGTTTTCAGATA (pGL3–94), CGGGGTACCGTGAGGCGTCATCACTTTGG (pGL3–51), and an antisense TCCGCTCGAGTGAGAGAACCAGGGAGCTGT. All PCR products were then cloned into a pGEMTeasy vector (Promega, Madison, WI) and digested with *Xho*I and *Kpn*I, and the inserts were subcloned into a pGL3-basic vector (Promega). The mutations of the promoter region between −106 and −94 were prepared by ligating two PCR products amplified by primers: sense CGGGGTACCGAGTTCAAGCTGCCCACTG, antisense GCTAGCATTTTTTTTTTTCTTTTTTGG (Mut1) or GCTAGCATCTTGAATTTTTTTTTTTCTTTTTTGG (Mut2), and sense GCTAGCTGAGTAAAGATGTGGTTTTC (Mut1) or GCTAGCAGATGTGGTTTTCAGATAATGCCC (Mut2) and antisense TCCGCTCGAGTGAGAGAACCAGGGAGCTGT; after *Nhe*I digestion. The ligated DNA fragment was cloned into a pGL3 luciferase vector.

Mammalian expression plasmids. The expression plasmids for the Myc-tagged mouse GATA-1 and GATA-2 were prepared by cloning the mouse GATA-1 or GATA-2 cDNAs inserted into pBluescript KS (+) vectors. A series of mouse GATA-1 deletion mutants was prepared by PCR from pEFBOS-Myc GATA-1 as the template using the sense primers: ACGCG TATGGAGGGAATTCCTGGGG (Δ1–86), ACGCGTCCATTGGCCCC TTGTGAG (ΔN and ΔN + C), ACGCGTCCCATGGATTTTCCTGGTC (ΔC), GGCGCGCCTTGTCAGCAAACGGGCAGGC (ΔN + C + NF) and antisense primers: ACGCGTTCAAGAACTGAGTGGGGC (Δ1–86 and ΔN), GGCGCGCCCCCCGCTTCTTTTTCCCTTT (ΔC, ΔN + C, and ΔN + C + NF); after *Mlu*I or *Asc*I digestion, PCR products were cloned into the pEFBOS-Flag or pEFBOS-Myc vector. ΔNF and ΔCF plasmids were prepared by ligating two PCR products amplified by primers: sense ACGCGTCCCATGGATTTTCCTGGTC, antisense CTCGAGACAAGG GGCCAATGGCAGGC (ΔNF) CTCGAGGCTGCCCGTTTGCTGACAAT (ΔCF), and sense CTCGAGATTGTCAGCAAACGGGCAGG (ΔN) CTC GAGAAGCTCCATCAGGTGAACCG (ΔC), antisense ACGCGTTCAAG AACTGAGTGGGGC; after *Xho*I digestion. The ligated DNA fragment was cloned into pEFBOS-Myc vector. The expression plasmid for mouse c-*jun* (pEFBOS-Flag-c-*jun*) and N-terminal deletion mutants (pEFBOS-Myc-DN-c-*jun*) were prepared by RT-PCR using the sense primer: ACGCGTATGAC TGCAAAGATGGAAAC (c-*jun*), ACGCGTAGCCAGAACACGCTTCCC AGT (DN-c-*jun*), and antisense primer: ACGCGTTCAAAACGTTTGCA ACTGCT. PCR products, digested with *Mlu*I, were subcloned into the pEFBOS-Flag or pEFBOS-Myc vector. All plasmid constructs were verified by restriction enzyme mapping and nucleotide sequencing. pAP-1 Luc, which is designed for monitoring induction of AP-1, were purchased from BD Clontech (Palo Alto, CA).

Transfection and luciferase assay

Cells were transiently transfected with 3.5 μg of *IL-13* promoter/luciferase plasmid and 0.1 μg of pRL/SV40 (an internal control) by DMRIE-C Reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. When indicated, cells were cotransfected with the indicated amount of GATA or c-*jun* expression vector or an empty vector with 1 μg of the *IL-13* promoter/luciferase plasmid. Forty-eight hours after the transfection, the cells were stimulated for 12 h. The luciferase activity was measured by using the Dual-Luciferase Reporter Assay System (Toyo Ink, Tokyo, Japan) according to the manufacturer's instructions. HEK293T cells were transiently transfected with a combination of expression plasmids (2 μg total/plate) by LipofectAMINE (Invitrogen Life Technologies) according to the manufacturer's instructions.

Immunoblotting, immunoprecipitation, and DNA-binding protein purification

Nuclear and cytoplasmic lysate preparation, immunoblotting, and immunoprecipitation were performed as previously described (30, 31). AP-1 and associating proteins were harvested using a DNA-binding protein purification kit (Roche, Basel, Switzerland) according to the manufacturer's instruction. The kit contains magnetic particles coupled to a double-stranded oligonucleotide. To this tethered oligonucleotide, double-stranded oligonucleotides containing the sequence for AP-1-binding were ligated. The sequences of oligonucleotides are as follows: sense CTAGTGATGAGTCAGCCGGAT (AP-1), GCTTACAATGAAGGCTTACAATGAAG (scramble), and antisense ATC CGGCTGACTCATCACTAG (AP-1), CTTCATTGTAAGCCTTCATTG TAAGC (scramble). Nuclear extracts were applied to the magnetic particles, and the oligo-particle complex captured the AP-1 protein. Separation of the bound protein from the supernatant was performed with a magnetic separator and washing steps. The purified proteins were eluted with a buffer of high ionic strength.

EMSA

Nuclear extracts were prepared as previously described (30). The double-strand DNA fragment carrying the region between −108 and −94 of the mouse *IL-13* gene 5′ upstream region was prepared by annealing two oligonucleotides: sense TTCAAGATGAGTAAA and antisense TTTACT CATCTTGAA, followed by ^{32}P labeling by T4 nucleotide kinase. The sequences of mutated version were as follows: sense TGCTAGCTGAG TAAA (Mut1), TTCAAGATGGCTAGC (Mut2), and antisense TTTACT CAGCTAGCA (Mut1), GCTAGCCATCTTGAA (Mut2). Nuclear extracts (5 μg of total protein) were incubated with the ^{32}P-labeled double-stranded probe. For competition assays, nuclear extracts were incubated with a 50-fold molar excess of the unlabeled probe before the addition of the ^{32}P-labeled probe. For the supershift experiment, 1 μg of the indicated Ab was incubated with nuclear extracts for 2 h before the binding reaction. Samples were run on a 5% nondenaturing polyacrylamide gel in Tris/glycine/EDTA buffer. The gel was dried and visualized by autoradiography.

Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed as previously described (32). The chromatin, sheared by sonication was immunoprecipitated with anti-*fos* Ab overnight at 4°C. Harvested DNAs were PCR amplified in a final volume of 50 μl containing 2.5 mmol/L magnesium dichloride, 2.5 U (AmpliTaq; PerkinElmer, Wellesley, MA), and 1 μmol/L-specific primers. The *IL-13* promoter-specific primers are as follows (−156 to +52): sense, AAGAT GAGTAAAGATGTGG; antisense, TGAGAGAACCAGGGAGCTGT. The downstream of *IL-13* promoter-specific primers are as follows (+2943 to +3138): sense, ATGAGTCTGTCAGTGTCCCG; antisense, CCGTGGCA GACAGGAGTGTT. PCR conditions are as follows: 94°C for 5 min; 94°C for 45 s; 60°C for 45 s; 72°C for 45 s; and 72°C for 7 min. PCR was done for 27 cycles. Five microliters of each PCR product were run on a 2% agarose gel for

[3] Abbreviations used in this paper: BMMC, bone marrow-derived mast cell; ChIP, chromatin immunoprecipitation.

UV visualization. For each reaction, 1% of cross-link released chromatin was saved and used as input control.

Cytokine ELISA

The cell-free culture supernatants were measured for the concentration of IL-13 (BD Pharmingen, San Diego, CA) by ELISA according to the manufacturer's instructions.

Generation of stable transfectants

P815 cells were transfected with 3 μg of pEFBOS-Myc-GATA-1 vector along with 1 μg of pCDNA3 plasmid (Invitrogen Life Technologies). Transfectants were selected with G418 (0.8 μg/ml). After 4 wk, resistant clones were screened for the right-sized protein expression by Western blotting.

Statistical analysis

The statistical significance of the data was determined by the Student's *t* test. A p value of <0.05 was taken as significant.

Results

Structural and functional analysis of the 5′-upstream region of the murine IL-13 *gene in mast cell lines*

To investigate the mechanism of IL-13 transcription in mast cells, we examined three well-established mast cell lines, MC/9 cells, PT18 cells, and P815 cells. These cell lines were stimulated with LPS, IgE cross-linking, or PMA/ionomycin, and we confirmed that MC/9 cells, as well as BMMCs, produced IL-13 in response to all of these three stimulations, and that PT18 and P815 cells responded only to stimulation by PMA/ionomycin (Fig. 1*A*).

A series of 5′-deletion constructs of the murine *IL-13* promoter region was initially generated to analyze *IL-13* promoter activity and to identify functional *cis*-acting elements required for *IL-13* gene expression. These 5′-deleted DNAs were cloned into the promoterless luciferase reporter vector pGL3-basic. The PT18 cells, transfected with the generated plasmids, were stimulated with PMA/ionomycin for 12 h, and luciferase activities were measured.

As shown in Fig. 1*B*, the highest induction was obtained with pGL3–2024 for PMA/ionomycin treatment. Four shorter constructs (1253, 705, 560, and 428) showed similar induction levels for PMA/ionomycin treatment. However, further deletion down to 322 caused a reduction in activity, and responsiveness was abrogated when the construct was deleted down to −51. Next, we made two constructs (−106 and −94) and examined the promoter activities of constructs shorter than −428 after PMA/ionomycin stimulation in PT18 cells. As shown in Fig. 1*C*, responsiveness was abrogated when the construct was deleted down to −94 (pGL3–94).

These constructs were also transfected into MC/9 cells and P815 cells. As shown in Fig. 1*D*, the results of MC/9 cells stimulated with PMA/ionomycin were similar to those of PT18 cells. The degree of response to this treatment was reduced with the reduction in the length of the construct, and was abrogated when the construct was shortened down to −94. In the case of stimulation by IgE cross-linking and LPS, the responsiveness of luciferase activity was maintained until the construct was shortened to −106 but was abrogated at −94. The high degree of inducibility through PMA/ionomycin stimulations and low degree of inducibility through IgE cross-linking and LPS stimulations were considered to reflect the production levels of IL-13, as shown in Fig. 1*A*. The luciferase activities of P815 cells were similar to those of PT18 cells and MC/9 cells. As shown in Fig. 1*E*, responsiveness was abrogated when the construct was deleted down to −94 (pGL3–94). These findings suggest that the fragment from −106 to the +62 fragment contains the minimal murine *IL-13* promoter, and that the promoter region between −106 and −94 plays an important role in the induction of *IL-13* promoter activity in mast cells.

The role of the promoter region between −106 and −94 in murine IL-13 *gene expression in mast cells*

A search in the nucleotide sequence upstream of the transcriptional start sites for the potential binding sites of transcriptional factors was conducted using TRANSFAC (http://www.motif.genome-.ad.jp; cutoff score = 80), and GATA and AP-1 binding sites were found in the region between −106 and −94. To confirm that the promoter region between −106 and −94 is functionally involved in *IL-13* transcription, we made two constructs based on the pGL-705 construct, which we designated Mut1 and Mut2, and compared their activities with the activity of pGL-705. The TRANSFAC program predicted that Mut1 would show a reduced GATA binding potential as the score is reduced from 84 to 68, but would show an increased AP-1-binding potential as the score is slightly raised from 82 to 85. In the case of Mut2, the program predicted reduced AP-1-binding potential (the score was reduced from 82 to 55) and increased GATA-binding potential (the score was raised from 84 to 90). As shown in Fig. 2*B*, each mutant construct showed a significant reduction of promoter activity in response to PMA/ionomycin in both PT18 and MC/9 cells. A reduction of promoter activity was also confirmed in P815 cells (Fig. 2*B*). These findings indicate the functional involvement of GATA or AP-1 in the *IL-13* promoter region between −106 and −94.

Identification of GATA-1, GATA-2, and the AP-1 complex binding to the IL-13 *promoter in mast cells by EMSA*

EMSA was conducted to determine whether protein-DNA binding within the region between −106 and −94 is involved in the activation of the *IL-13* promoter. Nuclear extracts prepared from PT18 cells stimulated with PMA/ionomycin or not were incubated with a ^{32}P-labeled probe spanning the *IL-13* promoter region between −108 and −94. EMSA revealed the presence of two protein complexes binding to the probe (Fig. 3*A*). While the faster complex appeared constitutively, the slower one appeared after PMA/ionomycin stimulation. These two complexes were also observed in BMMCs and MC/9 cells, which were stimulated with LPS, IgE cross-linking, or PMA/ionomycin (Fig. 3*A*).

As shown in Fig. 3*B*, the introduction of mutations into the sequence of this probe (Mut1 and Mut2, which are described in Fig. 2*A*), reduced both faster and slower migrations of complexes, although Mut1 reduced faster migrations and Mut2 reduced slower migrations more efficiently.

To further confirm the components of these two protein-DNA complexes, Abs against GATA and AP-1 were added in the EMSAs. It has been reported that mast cells show high expression of GATA-1 and GATA-2, but not GATA-3 (14, 15). We also confirmed by Northern blotting that PT18 cells and MC/9 cells express GATA-1 and GATA-2 but not GATA-3 (data not shown). As shown in Fig. 3*C*, a combination of Abs against AP-1 subunits (at Fos and at Jun) efficiently abrogated or supershifted the inducible slower complexes. In contrast, a combination of Abs against GATA-1 and GATA-2 efficiently abrogated both the faster migrations and the slower migrations. These findings demonstrate that Jun/Fos complexes constitute the slower migrations and GATA-1 and GATA-2 account for both the slower and the faster migrations. Taken together, these findings indicate that GATA-1 and GATA-2 bind to the region between −106 and −94 constitutively, and AP-1 binds only after stimulation.

AP-1 activities are indispensable for the activation of the IL-13 *promoter*

To investigate the role of AP-1 in *IL-13* transcription in mast cells, we transfected an expression plasmid encoding an N-terminally

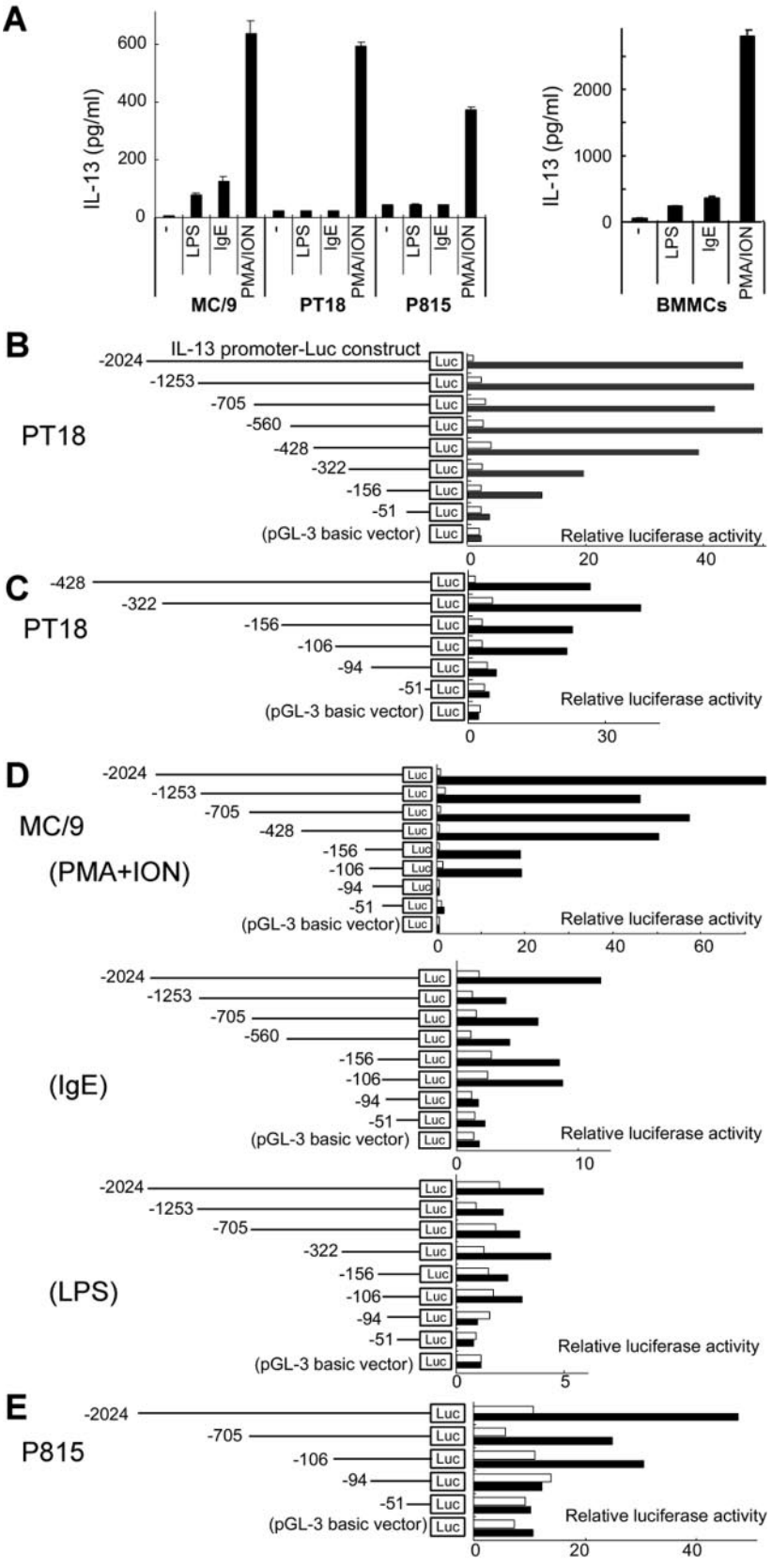

FIGURE 1. The structural and functional analysis of the 5′-upstream region of the murine *IL-13* gene. *A*, IL-13 production from mast cell lines. Cells (2×10^6 cells/ml) were stimulated with LPS, IgE cross-linking, or PMA/ionomycin. After 16 h, the cell-free culture supernatants were collected and cytokine ELISAs were performed. The error bars represent SD values. *B–D*, PT18 cells, MC/9 cells, and P815 cells were transiently transfected with 3.5 μg of *IL-13* promoter/luciferase plasmid and 0.1 μg of pRL/SV40 (an internal control). *B* and *C*, PT18 cells were stimulated with PMA/ionomycin, (*D*) MC/9 cells were stimulated with PMA/ionomycin, IgE cross-linking, or LPS, and (*E*) P815 cells were stimulated with PMA/ionomycin. After 12 h, luciferase activities were measured. Units of luciferase activity were normalized based on values of pRL/SV40 activity for transfection activity (relative luciferase activity). □, Unstimulated, and, ■, stimulated conditions. A typical result of at least three independent experiments is shown. The result in *C* was confirmed in transfections using two independent preparations of each construct.

truncated form of c-*jun* (DN-c-*jun*), which has been reported to work in a dominant-negative fashion (33). We confirmed that DN-c-*jun* inhibits AP-1 activity in PT18 cells in a dose-dependent manner (Fig. 4*A*). We then transiently transfected pGL-106 with DN-c-*jun* or control vector into PT18 cells and stimulated the cells with PMA/ionomycin for 12 h. As shown in Fig. 4*B*, the induction of luciferase activity was significantly reduced by DN-c-*jun*. These findings suggest that AP-1 activity plays a critical role in the transcriptional activation of the *IL-13* gene.

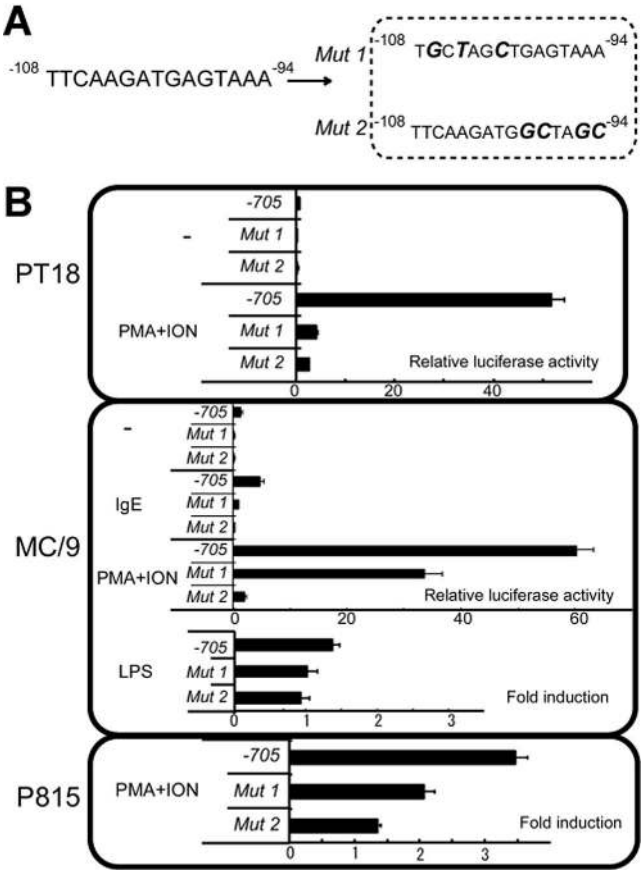

FIGURE 2. The role of the promoter region between −106 and −94 in *IL-13* gene expression in mast cells. *A*, Sequences of the promoter region between −108 and −94 in the *IL-13* gene. The altered sequences of this region in Mut1 or Mut2 are indicated in bold italic font. *B*, PT18, MC/9, and P815 cells were transiently transfected with 3.5 μg of *IL-13* promoter/luciferase plasmid and 0.1 μg of pRL/SV40. PT18 cells and P815 cells were stimulated with PMA/ionomycin. MC/9 cells were stimulated with PMA/ionomycin, IgE cross-linking, or LPS. After 12 h, luciferase activities were measured. Units of luciferase activity were normalized based on values of pRL/SV40 activity for transfection activity (relative luciferase activity). The fold inductions were calculated as follows: (relative luciferase activity of stimulated cells)/(relative luciferase activity of unstimulated cells). A typical result of at least three independent experiments is shown.

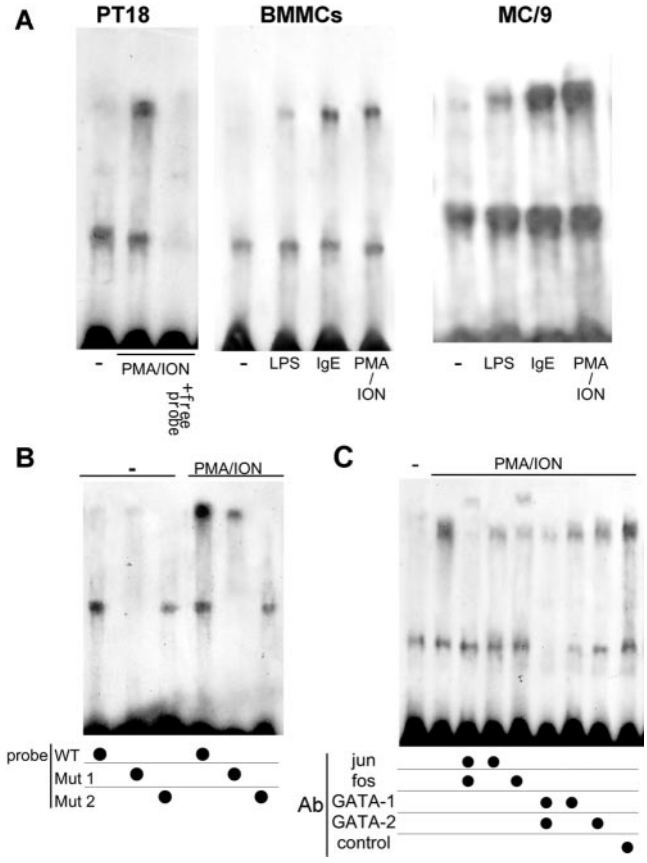

FIGURE 3. Identification of GATA-1, GATA-2, and AP-1 complex binding to the *IL-13* promoter in mast cells by EMSA. Cells were left untreated (−) or stimulated with PMA/ionomycin, IgE cross-linking, or LPS (1 μg/ml) for 20 min and nuclear extracts were prepared. *A*, EMSAs were performed by using ^{32}P-labeled probe spanning the region between −108 and −94 of the *IL-13* promoter. For competition assays, nuclear extracts were incubated with a 50-fold molar excess of the unlabeled probe before the addition of ^{32}P-labeled probe (+free probe). *B*, EMSA was performed with the nuclear extract of PT18 cells. ^{32}P-labeled probe spanning the region between −108 and −94 of the *IL-13* promoter or ^{32}P-probes that were introduced mutations (Mut1 or Mut2) were used as a probe. *C*, EMSA was performed with the nuclear extract of PT18 cells. The ^{32}P-labeled probe spanning the region between −108 and −94 of the *IL-13* promoter was used as a probe. One microgram of the indicated Abs or isotype match Abs (control) were incubated with nuclear extracts for 2 h before the binding reaction.

Both GATA-1 and GATA-2 transactivate the IL-13 *promoter through AP-1 activity*

As shown in Fig. 3, not only AP-1 but also GATA-1 and GATA-2 bind to the region between −106 and −94 in the *IL-13* promoter that is involved in transactivation of the *IL-13* gene. To examine the ability of GATA-1 and GATA-2 to transactivate the *IL-13* promoter, GATA-1 or GATA-2 expression plasmids were transfected in combination with pGL-106, and luciferase activity was measured after various stimulations. In MC/9 cells stimulated with IgE cross-linking or PMA/ionomycin, both GATA-1 and GATA-2 transactivated the *IL-13* promoter in a dose-dependent manner. Basal luciferase activity was also up-regulated by overexpression of either GATA-1 or GATA-2. Similar results were obtained with P815 cells (Fig. 4*C*).

To further confirm the role of GATA in *IL-13* transcription, PT18 cells were transfected with pGL-705 or Mut 2, which are described above and in Fig. 2, along with various amounts of GATA-1 or -2 expression plasmids and stimulated with PMA/ionomycin. Mut2 showed mild reduction of GATA-binding potential but great reduction of AP-1-binding potential to the region between −106 and −94 in the *IL-13* promoter (Fig. 3*B*). As shown in Fig. 4*D*, GATA-1 and GATA-2 promoted *IL-13* transcription in a dose-dependent manner (pGL-705), but when mutations were introduced into the region between −106 and −94 in the *IL-13* promoter (Mut2), the transactivation of the *IL-13* gene by GATA proteins was abrogated. These findings indicate the possible involvement of AP-1 in *IL-13* transactivation by GATA.

Next, we transfected DN-c-*jun* into PT18 cells and evaluated the role of AP-1 in *IL-13* transactivation by GATA. As shown in Fig. 4*E*, DN-c-*jun* clearly inhibited the transactivation of pGL-106 by GATA proteins in PT18 cells. These findings indicate that AP-1 is indispensable for the transactivation of the *IL-13* gene induced by GATA proteins.

GATA-1 and GATA-2 associate with AP-1

It has been reported that the cooperativeness between GATA-2 and AP-1 synergistically increases the transcriptional activity of the

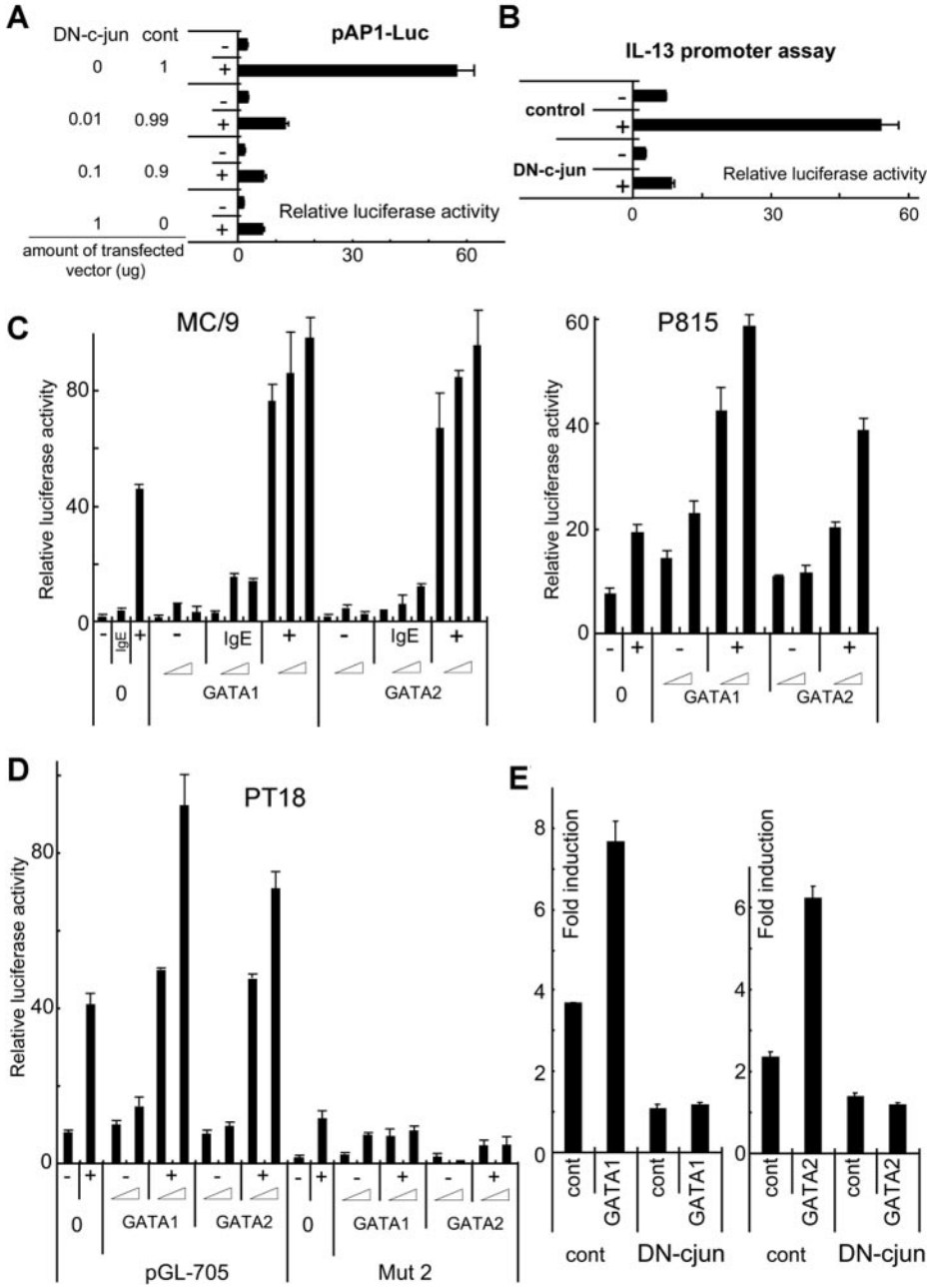

FIGURE 4. Both GATA-1 and GATA-2 transactivate the *IL-13* promoter through AP-1 activity. *A*, PT18 cells were transiently transfected with 1 μg of pAP1-luciferase plasmid, which is designed for monitoring induction of AP-1, and 0.1 μg of pRL/SV40 with the indicated dose of DN-c-*jun* expression plasmid or control plasmid (total 1 μg). Cells were stimulated with PMA/ionomycin for 12 h and luciferase activities were measured. *B*, PT18 cells were transiently transfected with 1 μg of pGL3–106 and 0.1 μg of pRL/SV40 with DN-c-*jun* expression plasmid or control plasmid (0.1 μg). Cells were stimulated with PMA/ionomycin for 12 h and luciferase activities were measured. *C*, MC/9 cells or P815 cells were transiently transfected with 1 μg of pGL3–106 and 0.1 μg of pRL/SV40 with an increasing dose of GATA-1 or -2 expression plasmid (0.01, 0.05, or 0.1 μg for MC/9 and 0.01 or 0.1 μg for P815 cells) or control plasmid. Cells were stimulated with PMA/ionomycin and luciferase activities were measured. *D*, PT18 cells were transiently transfected with 1 μg of *IL-13* promoter/luciferase plasmid (pGL3–705 or Mut2) and 0.1 μg of pRL/SV40 with an increasing dose (0.01 or 0.1 μg) of GATA-1 or -2 expression plasmid or control plasmid. Cells were stimulated PMA/ionomycin for 12 h and luciferase activities were measured. *E*, PT18 cells were transiently transfected with 1 μg of pGL3–106 and 0.1 μg of pRL/SV40 with DN-c-*jun* expression plasmid (0.1 μg), GATA expression plasmid (0.05 μg), or control plasmid. Cells were stimulated with PMA/ionomycin for 12 h and luciferase activities were measured. Units of luciferase activity were normalized based on values of pRL/SV40 activity for transfection activity (relative luciferase activity). The fold inductions were calculated as follows: (relative luciferase activity of stimulated cells)/(relative luciferase activity of unstimulated cells). A typical result of at least three independent experiments is shown.

endothelin-1 gene in endothelial cells (27). The authors demonstrated that GATA-2 and AP-1 bind into complexes in endothelial cells and HEK293 cells. However, the nature of this cooperativeness in the transcriptional activity of the *IL-13* promoter in mast cells has not been explored. Moreover, it is not clear whether GATA-1 interacts with AP-1. To elucidate these points, GATA-1 or GATA-2 N-terminally tagged with Myc, and c-*jun* N-terminally tagged with Flag were expressed in HEK293T cells, and immunoprecipitation analyses were performed. In this cell line, GATA proteins exist constitutively in the nucleus, whereas c-*jun* proteins translocate into the nucleus from the cytoplasm only after stimulation (data not shown). The cells were stimulated with PMA/ionomycin and nuclear lysates were harvested. As shown in Fig. 5*A*, we found that c-*jun* was coprecipitated with GATA-1 or GATA-2 after PMA/ionomycin stimulations.

We next examined these associations in mast cells by using a DNA-binding protein purification technique. By using magnetic particles to which oligonucleotides containing a DNA target sequence are coupled, the specific DNA-binding protein and associated proteins were harvested. In mast cells, GATA-1 and GATA-2 exist constitutively in the nucleus, whereas c-*fos* and c-*jun*, which are subunits of AP-1, translocate into the nucleus only after stimulation (Fig. 5*B*). As shown in Fig. 5*C*, by using particles coated with the oligonucleotides containing the sequence for the region between −108 and −94 in the IL-13 promoter, subunits of AP-1 and GATA-1 were efficiently purified from the nuclear lysate of PT18 cells stimulated with PMA/ionomycin. Next, we made particles coated with oligonucleotides containing the AP-1-specific binding sequence, and examined the association of GATA-1 with AP-1. As shown in Fig. 5*D*, GATA-1 was copurified with AP-1 and detected by Western blotting from the nuclear lysate of PT18 and MC/9 cells after various stimulations. Taken together, these findings clearly indicate that both GATA-1 and GATA-2 are capable of associating with AP-1.

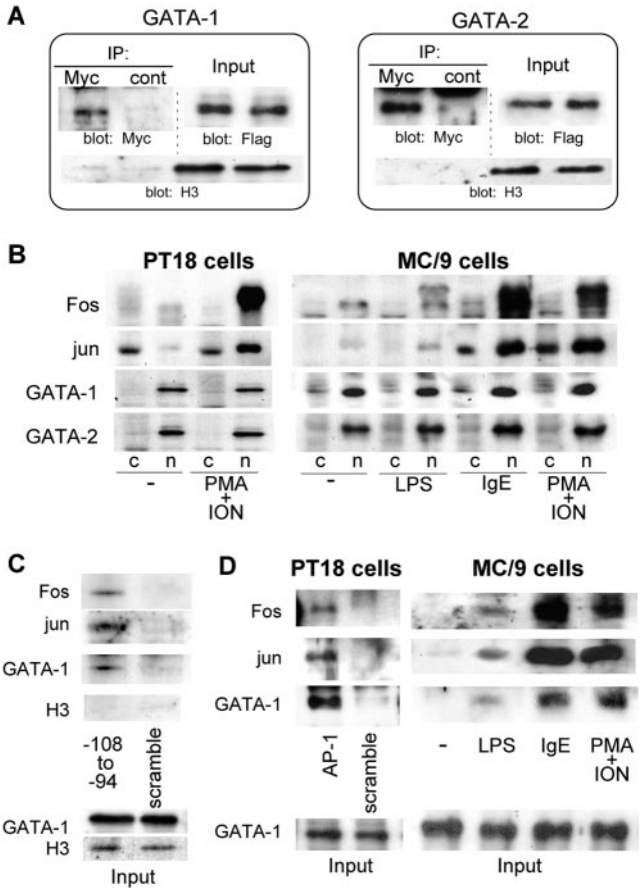

FIGURE 5. Both GATA-1 and GATA-2 associate with AP-1. *A*, HEK293T cells were transiently transfected with pEFBOS-Flag-c-*jun* (1 μg) in combination with either pEFBOS-Myc-GATA-1 or pEFBOS Myc-GATA-2 (1 μg). After 48 h, cells were stimulated with PMA/ionomycin for 15 min and nuclear extracts were prepared. Anti-Flag and control Ab immunoprecipitates were separated by SDS-PAGE, and immunoblotting was performed with anti-Flag Ab. Cell lysates were also probed with anti-Myc Ab (input) or anti-histone H3 Ab. As the m.w. of GATA-1 and GATA-2 is almost same as the Ig H chain, we could not evaluate the amount of immunoprecipitated GATA proteins. *B*, Distribution of GATA-1 and GATA-2 in mast cells. PT18 cells or MC/9 cells were stimulated with PMA/ionomycin, IgE cross-linking, or LPS for 15 min. Both nuclear extracts (n) and cytoplasmic lysates (c) were harvested, separated by SDS-PAGE, and immunoblottings were performed with the indicated Ab. *C*, PT18 cells were stimulated with PMA/ionomycin. Nuclear extracts harvested 15 min after stimulations were applied to the magnetic particle-ligated oligonucleotides containing the sequence for the region between −108 and −94 in the IL-13 promoter or scramble sequence. The purified proteins were eluted from the immobilized particle, separated by SDS-PAGE, and immunoblottings were performed with indicated Ab. Nuclear extracts were also probed with anti-GATA-1 or anti-histone H3 Ab (input). *D*, Nuclear extracts harvested 15 min after stimulations were applied to the magnetic particle-ligated oligonucleotides containing the sequence for AP-1-binding sequence (or scramble sequence in the case of PT18 cells). The purified proteins were eluted from the immobilized particle, separated by SDS-PAGE, and immunoblottings were performed with the indicated Ab. Nuclear extracts were also probed with anti-GATA-1 Ab (input).

GATA-1 interacts with AP-1 through its N-terminal region, C-terminal region, and N-finger domain

To identify the AP-1-binding domain of GATA, we expressed several deletion mutants of GATA-1 in combination with Flag-tagged c-*jun* in HEK293T cells for coprecipitation assays. Although GATA proteins interact with other proteins through their N-terminal zinc finger or C-terminal zinc finger (16, 34), it has recently been reported that physical interaction is mediated through the N-terminal region or the C-terminal region of GATA (35). As shown in Fig. 6*A*, Flag-tagged c-*jun* was coprecipitated with all of the GATA deletion mutants: Δ1–86 (87–413), ΔN (194–413), ΔNF (1–199 and 249–413), ΔCF (1–251 and 287–413), and ΔC

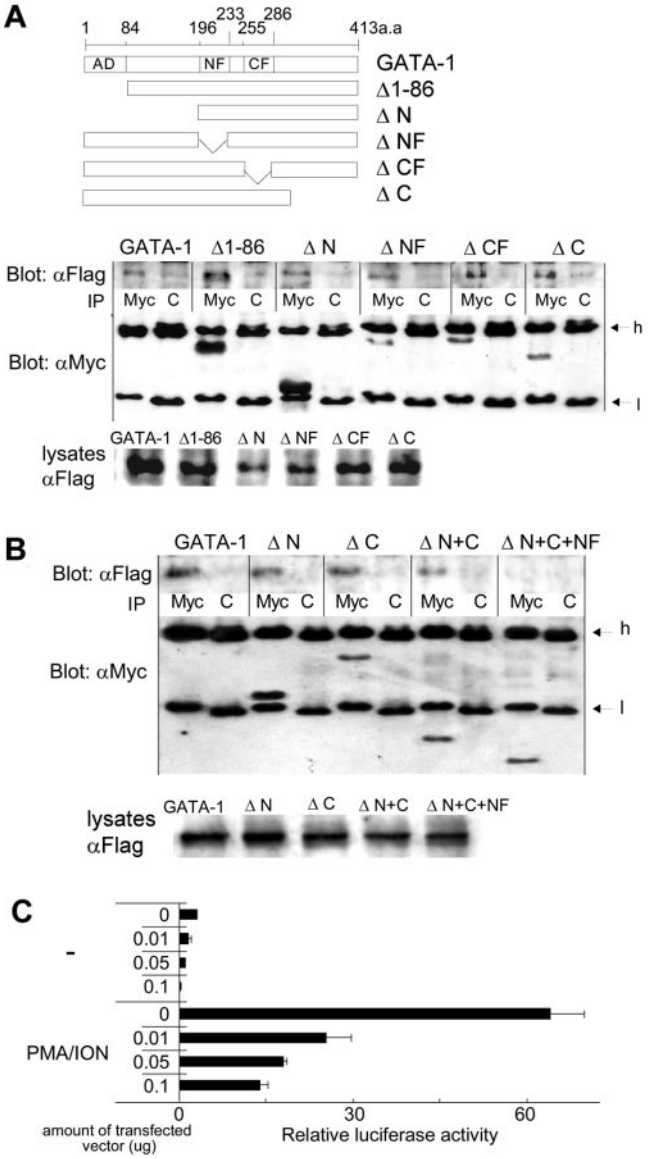

FIGURE 6. GATA-1 interacts with AP-1 through N-terminal, C-terminal, and N-finger domains. *A* and *B*, HEK 293T cells were transiently transfected with pEFBOS-Flag-c-*jun* (1 μg) in combination with the Myc-tagged GATA deletion mutant expression vector (1 μg). At 48 h after transfection, cells were stimulated with PMA/ionomycin for 15 min and nuclear extracts were prepared. Anti-Myc (Myc) and control (C) Ab immunoprecipitates were separated by SDS-PAGE, and immunoblotting was performed with anti-Flag Ab (*top panel*). The membrane was stripped and reprobed using anti-Myc Ab. Cell lysates were also probed with anti-Flag Ab. Arrows denote Ig H chain (h) or L chain (l). *C*, PT18 cells were transiently transfected with 1 μg of *IL-13* promoter/luciferase plasmid (pGL3–106) and 0.1 μg of pRL/SV40 with the indicated amount of GATA-1 deletion mutant expression plasmid (ΔC+ΔN+ΔNF). Cells were stimulated PMA/ionomycin for 12 h and luciferase activities were measured. Units of luciferase activity were normalized based on values of pRL/SV40 activity for transfection activity. A typical result of at least three independent experiments is shown.

(1–318). These results suggest that at least two domains of GATA-1 are required for the interaction with c-*jun*.

Next, we made two new deletion mutants of GATA-1, ΔN + C (194–318), and ΔN + C + NF (250–318), and expressed them in combination with the wild-type c-*jun* for coprecipitation assays in HEK293T cells. As shown in Fig. 6*B*, Flag-tagged c-*jun* was coprecipitated with ΔC, ΔN, and ΔN + C. However, no coprecipitation was detected for ΔN + C + NF, suggesting that all of these sites are essential for GATA-1 to associate with c-*jun*.

To confirm the importance of the interaction between GATA and c-*jun*, we transfected ΔN + C + NF in PT18 cells along with pGL-705 and performed luciferase assays. Transfection of this GATA deletion mutant reduced the transactivation of *IL-13* by PMA/ionomycin significantly (Fig. 6*C*). These results indicate the importance of the interaction between GATA and AP-1 in *IL-13* transcription.

Overexpression of GATA-1 promotes IL-13 production in vivo

To examine the ability of GATA-1 to promote the production of IL-13 in vivo, we transfected a Myc-tagged GATA-1 expression vector into P815 cells. Two clones stably expressing GATA-1 were isolated for analysis. As shown in Fig. 7*A*, one clone (designated 01) showed significant expression of Myc-tagged GATA-1, whereas the other clone (designated 02) showed only moderate expression. We then stimulated these cells with PMA/ionomycin and examined the production of IL-13 in culture supernatant. As shown in Fig. 7*B*, 01 cells showed significant IL-13 production and 02 cells showed moderate IL-13 production, whereas control cells produced much less IL-13.

To examine the importance of the interaction between GATA and AP-1 in vivo, ChIP experiments were performed using mast cell chromatin and Fos antisera. We confirmed that AP-1 binds to the IL-13 promoter after PMA/ionomycin stimulation in BMMCs (Fig. 7*C*). We then stimulated 01 and 02 cells with PMA/ionomycin and ChIP experiments were performed. As shown in Fig. 7*D*, 01 cells showed significant AP-1 binding to the *IL-13* promoter and control cells showed much less binding. The AP-1 binding levels exhibited the same tendency as the GATA-1 expression levels. These findings indicate the importance of GATA for the binding of AP-1 to the *IL-13* promoter. Based on these results, we concluded that the interaction between GATA and AP-1 promotes *IL-13* transcription and IL-13 production in vivo.

Discussion

Almost nothing has been known about the regulation of *IL-13* transcription in mast cells, although we have previously reported that JNK is necessary for IL-13 transcription in mast cells stimulated by LPS (36). In T cells, the involvement of GATA-3 in cell type-specific expression of the *IL-13* gene has been reported (11). Moreover, GATA-3 seemed to transactivate the *IL-13* gene through its N-terminal region and N-finger domain in T cells (37). However, the mechanism that induces the transactivation of the *IL-13* gene immediately in response to stimulation has not been elucidated. Although rapid phosphorylation of GATA proteins other than GATA-3 after stimulations has been reported, its physiological significance is still controversial (12, 13). Furthermore, as shown in Figs. 3 and 5*B*, GATA-1 and GATA-2 constitutively exist in the nucleus and stimulation did not affect their DNA binding activity in mast cells. Taken together, these facts suggest that transcription factors other than GATA induce immediate transactivation of the *IL-13* gene in response to stimulation.

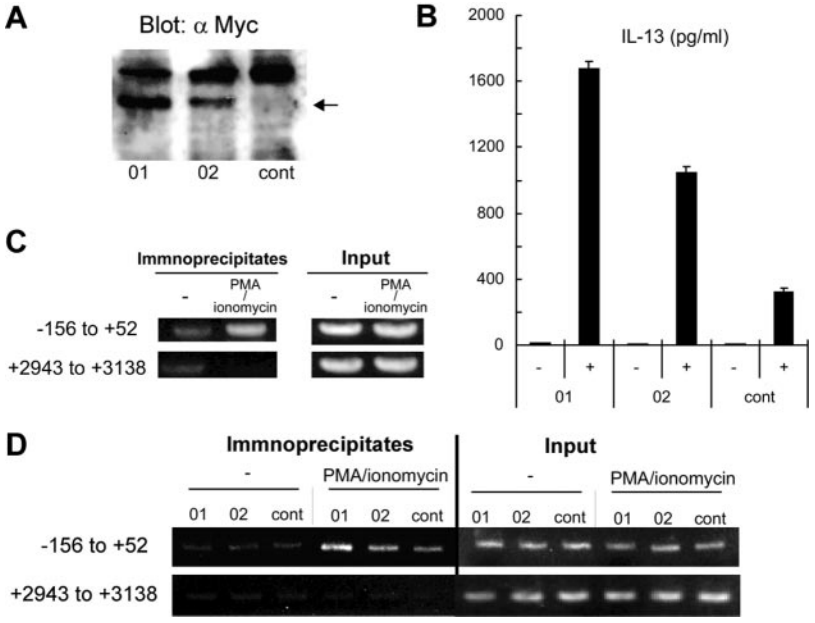

FIGURE 7. Overexpression of GATA-1 promotes IL-13 production in vivo. *A*, Expression of the Myc-tagged GATA-1 in P815 cells. Western blot analysis was performed with nuclear extracts from the G418-resistant clones transfected with the pEFBOS-Myc-GATA-1 vector along with pCDNA3 plasmid (01 or 02), or pCDNA3 alone (cont). An arrow denotes the band of Myc-tagged GATA-1. *B*, Cells (2×10^6 cells/ml) were incubated in RPMI 1640 + 10% FCS with PMA/ionomycin (+) or not (−). The cell-free culture supernatants were collected after 24 h of culture. The IL-13 contents in the culture supernatants were assayed by ELISA using a mouse IL-13 ELISA system. The experiments were done in triplicate. The error bars represent SD values. *C*, Binding of AP-1 to the IL-13 promoter after PMA/ionomycin stimulation in BMMCs. *D*, The ability of AP-1 to bind to the *IL-13* promoter through interaction with GATA-1 in vivo. Cells were untreated or treated with PMA/ionomycin for 15 min. After treatment, chromatin was extracted and immunoprecipitated with anti-Fos Ab. PCR analyses of DNA products from immunoprecipitation reactions were conducted as described in *Materials and Methods*. PCR analyses were performed against the *IL-13* promoter region including the AP-1 binding site (−156 to +52) or downstream of the *IL-13* promoter (+2943 to +3138).

In the present study, we have shown that both AP-1 and GATA account for the transactivation of the *IL-13* gene and that AP-1 is indispensable for the transactivation of the *IL-13* gene induced by GATA proteins (Fig. 4). Furthermore, overexpression of GATA-1 induced a significant amount of IL-13 production and significant binding of AP-1 to the *IL-13* promoter (Fig. 7). These results indicate that activation of AP-1 is the key regulator of *IL-13* transcription and that GATA proteins promote the binding of AP-1 to the *IL-13* promoter in mast cells.

We have shown that GATA-1 and GATA-2 bind into complexes with AP-1 and that transfection of the deletion mutant of GATA-1 which results in the loss of association with c-*jun* ($\Delta N + C + NF$) significantly reduced the transactivation of the *IL-13* gene in mast cells (Figs. 5 and 6). These results indicate the importance of physical interaction between GATA and AP-1 in *IL-13* transcription. However, recently it has been reported that both GATA-1 and GATA-2 regulate the histone acetylation pattern of chromatin, which is thought to improve the accessibility of other transcriptional factors (38, 39). Although the DNase hypersensitivity pattern of the *IL-13* gene in mast cells is unknown, it is possible that GATA proteins improve the binding of AP-1 to the *IL-13* promoter through modifying chromatin, in addition to direct interaction.

Various GATA-interacting proteins, including DNA-binding factors and general transcription activators and repressors, have been described (16). Most physical interactions are mediated by either the N-terminal zinc finger or the C-terminal zinc finger (16, 34). However, it has recently been reported that the N-terminal region (between residues 146 and 215) of GATA-3 interacts with smad-3 (35). Furthermore, it has quite recently been reported that the N-terminal region of GATA-4 interacts with c-*fos* (40). We have shown that GATA-1 interacts through its N-terminal, C-terminal, and N-finger domains with c-*jun* and that disruption of any single domain of GATA-1 dose not result in loss of the interaction with c-*jun*. Thus, GATA proteins interact with AP-1 through multiple domains. AP-1 consists of two subunits formed either by the heterodimerization of a Fos family protein with a Jun family protein or the homodimerization of Jun family members (41, 42). In the present study, although GATA and c-*jun* were overexpressed in HEK293T cells for the examination of the interaction between AP-1 and GATA, some overexpressed c-*jun* proteins probably bound endogenous Fos family proteins and acted as AP-1. In addition to direct binding, c-*jun* might bind to GATA proteins through Fos family proteins or other unknown proteins, and this might be the reason why GATA proteins interact with AP-1 through multiple domains.

The roles of AP-1 and GATA in Th2 cytokine production in T cells have been described in detail. P1, an element of the IL-4 promoter that binds AP-1, is important for Th2-restricted IL-4 expression (43). The IL-5 promoter has been shown to require both AP-1 and GATA elements for its activity in T cells (28). Among the GATA family proteins, GATA-3 plays a central role in Th2 cytokine production in T cells (9). Similarly to GATA-3, JunB, a component of AP-1, stimulates the expression of multiple Th2 cytokine genes (44). In mast cells, GATA-1 and GATA-2 play important roles in the transcription of both IL-4 and IL-5 (22, 23). Furthermore, the activation of JNK plays an important role in Th2 cytokine production in mast cells (36, 45), and it is well known that JNK activates AP-1 through the phosphorylation of c-*jun* (46). Similarly to GATA and AP-1, the NF-AT has been reported to play an important role in Th2 cytokine productions in mast cells. The transcription of IL-4 in mast cells is dependent on an NF-AT site in the murine IL-4 gene (47). The transcription of IL-5 in mast cells is also dependent on NF-AT (23). In the murine IL-13 promoter, there is an NF-AT-like binding site between −216 and −227 bp and, as shown in Fig. 1, a reduction of *IL-13* promoter activity was observed between pGL-322 and pGL-156. Furthermore, it has been reported that NF-AT acts in collaboration with both AP-1 and GATA (23, 48). In the present study, we have demonstrated that the interaction between AP-1 and GATA has a critical role in the production of IL-13 in mast cells. This interaction might play an essential role in Th2 cytokine production.

Acknowledgments

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References

1. Cherwinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann. 1987. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med. 166:1229*.
2. Brown, K. D., S. M. Zurawski, T. R. Mosmann, and G. Zurawski. 1989. A family of small inducible proteins secreted by leukocytes are members of a new superfamily that includes leukocyte and fibroblast-derived inflammatory agents, growth factors, and indicators of various activation processes. *J. Immunol. 142:679*.
3. McKenzie, A. N., J. A. Culpepper, R. de Waal Malefyt, F. Briere, J. Punnonen, G. Aversa, A. Sato, W. Dang, B. G. Cocks, S. Menon, et al. 1993. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. *Proc. Natl. Acad. Sci. USA 90:3735*.
4. Burd, P. R., W. C. Thompson, E. E. Max, and F. C. Mills. 1995. Activated mast cells produce interleukin 13. *J. Exp. Med. 181:1373*.
5. Zurawski, G., and J. E. de Vries. 1994. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today 15:19*.
6. Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp, and D. D. Donaldson. 1998. Interleukin-13: central mediator of allergic asthma. *Science 282:2258*.
7. Grunig, G., M. Warnock, A. E. Wakil, R. Venkayya, F. Brombacher, D. M. Rennick, D. Sheppard, M. Mohrs, D. D. Donaldson, R. M. Locksley, and D. B. Corry. 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science 282:2261*.
8. Hershey, G. K. 2003. IL-13 receptors and signaling pathways: an evolving web. *J. Allergy Clin. Immunol. 111:677*.
9. Zhou, M., and W. Ouyang. 2003. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol. Res. 28:25*.
10. Takemoto, N., Y. Kamogawa, H. Jun Lee, H. Kurata, K. I. Arai, A. O'Garra, N. Arai, and S. Miyatake. 2000. Cutting edge: chromatin remodeling at the IL-4/IL-13 intergenic regulatory region for Th2-specific cytokine gene cluster. *J. Immunol. 165:6687*.
11. Kishikawa, H., J. Sun, A. Choi, S. C. Miaw, and I. C. Ho. 2001. The cell type-specific expression of the murine IL-13 gene is regulated by GATA-3. *J. Immunol. 167:4414*.
12. Crossley, M., and S. H. Orkin. 1994. Phosphorylation of the erythroid transcription factor GATA-1. *J. Biol. Chem. 269:16589*.
13. Tremblay, J. J., and R. S. Viger. 2003. Transcription factor GATA-4 is activated by phosphorylation of serine 261 via the cAMP/protein kinase a signaling pathway in gonadal cells. *J. Biol. Chem. 278:22128*.
14. Zon, L. I., M. F. Gurish, R. L. Stevens, C. Mather, D. S. Reynolds, K. F. Austen, and S. H. Orkin. 1991. GATA-binding transcription factors in mast cells regulate the promoter of the mast cell carboxypeptidase A gene. *J. Biol. Chem. 266:22948*.
15. Harigae, H., S. Takahashi, N. Suwabe, H. Ohtsu, L. Gu, Z. Yang, F. Y. Tsai, Y. Kitamura, J. D. Engel, and M. Yamamoto. 1998. Differential roles of GATA-1 and GATA-2 in growth and differentiation of mast cells. *Genes Cells 3:39*.
16. Molkentin, J. D. 2000. The zinc finger-containing transcription factors GATA-4, -5, and -6: ubiquitously expressed regulators of tissue-specific gene expression. *J. Biol. Chem. 275:38949*.
17. Orkin, S. H. 1998. Embryonic stem cells and transgenic mice in the study of hematopoiesis. *Int. J. Dev. Biol. 42:927*.
18. Martin, D. I., L. I. Zon, G. Mutter, and S. H. Orkin. 1990. Expression of an erythroid transcription factor in megakaryocytic and mast cell lineages. *Nature 344:444*.
19. Bocek, P., Jr., M. D. Guthmann, and I. Pecht. 1997. Analysis of the genes encoding the mast cell function-associated antigen and its alternatively spliced transcripts. *J. Immunol. 158:3235*.
20. Maeda, K., C. Nishiyama, T. Tokura, Y. Akizawa, M. Nishiyama, H. Ogawa, K. Okumura, and C. Ra. 2003. Regulation of cell type-specific mouse FcεRI β-chain gene expression by GATA-1 via four GATA motifs in the promoter. *J. Immunol. 170:334*.
21. Nishiyama, C., T. Yokota, K. Okumura, and C. Ra. 1999. The transcription factors Elf-1 and GATA-1 bind to cell-specific enhancer elements of human high-affinity IgE receptor α-chain gene. *J. Immunol. 163:623*.

22. Hural, J. A., M. Kwan, G. Henkel, M. B. Hock, and M. A. Brown. 2000. An intron transcriptional enhancer element regulates IL-4 gene locus accessibility in mast cells. *J. Immunol. 165:3239*.
23. Prieschl, E. E., V. Gouilleux-Gruart, C. Walker, N. E. Harrer, and T. Baumruker. 1995. A nuclear factor of activated T cell-like transcription factor in mast cells is involved in IL-5 gene regulation after IgE plus antigen stimulation. *J. Immunol. 154:6112*.
24. Ney, P. A., B. P. Sorrentino, K. T. McDonagh, and A. W. Nienhuis. 1990. Tandem AP-1-binding sites within the human β-globin dominant control region function as an inducible enhancer in erythroid cells. *Genes Dev. 4:993*.
25. Walters, M., and D. I. Martin. 1992. Functional erythroid promoters created by interaction of the transcription factor GATA-1 with CACCC and AP-1/NFE-2 elements. *Proc. Natl. Acad. Sci. USA 89:10444*.
26. Lee, M. E., M. S. Dhadly, D. H. Temizer, J. A. Clifford, M. Yoshizumi, and T. Quertermous. 1991. Regulation of endothelin-1 gene expression by Fos and Jun. *J. Biol. Chem. 266:19034*.
27. Kawana, M., M. E. Lee, E. E. Quertermous, and T. Quertermous. 1995. Cooperative interaction of GATA-2 and AP1 regulates transcription of the endothelin-1 gene. *Mol. Cell. Biol. 15:4225*.
28. Yamagata, T., J. Nishida, R. Sakai, T. Tanaka, H. Honda, N. Hirano, H. Mano, Y. Yazaki, and H. Hirai. 1995. Of the GATA-binding proteins, only GATA-4 selectively regulates the human interleukin-5 gene promoter in interleukin-5-producing cells which express multiple GATA-binding proteins. *Mol. Cell. Biol. 15:3830*.
29. Masuda, A., T. Matsuguchi, K. Yamaki, T. Hayakawa, M. Kubo, W. J. LaRochelle, and Y. Yoshikai. 2000. Interleukin-15 induces rapid tyrosine phosphorylation of STAT6 and the expression of interleukin-4 in mouse mast cells. *J. Biol. Chem. 275: 29331*.
30. Adachi, O., T. Kawai, K. Takeda, M. Matsumoto, H. Tsutsui, M. Sakagami, K. Nakanishi, and S. Akira. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity 9:143*.
31. Matsuguchi, T., T. Musikacharoen, T. R. Johnson, A. S. Kraft, and Y. Yoshikai. 2001. A novel mitogen-activated protein kinase phosphatase is an important negative regulator of lipopolysaccharide-mediated c-Jun N-terminal kinase activation in mouse macrophage cell lines. *Mol. Cell. Biol. 21:6999*.
32. Musikacharoen, T., Y. Yoshikai, and T. Matsuguchi. 2003. Histone acetylation and activation of cAMP-response element-binding protein regulate transcriptional activation of MKP-M in lipopolysaccharide-stimulated macrophages. *J. Biol. Chem. 278:9167*.
33. Peakman, M. C., C. Colby, L. I. Perrotti, P. Tekumalla, T. Carle, P. Ulery, J. Chao, C. Duman, C. Steffen, L. Monteggia, et al. 2003. Inducible, brain region-specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain Res. 970:73*.
34. Crispino, J. D., M. B. Lodish, J. P. MacKay, and S. H. Orkin. 1999. Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG complex. *Mol. Cell 3:219*.
35. Blokzijl, A., P. ten Dijke, and C. F. Ibanez. 2002. Physical and functional interaction between GATA-3 and Smad3 allows TGF-β regulation of GATA target genes. *Curr. Biol. 12:35*.
36. Masuda, A., Y. Yoshikai, K. Aiba, and T. Matsuguchi. 2002. Th2 cytokine production from mast cells is directly induced by lipopolysaccharide and distinctly regulated by c-Jun N-terminal kinase and p38 pathways. *J. Immunol. 169:3801*.
37. Takemoto, N., K. Arai, and S. Miyatake. 2002. Cutting edge: the differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during Th2 development. *J. Immunol. 169:4103*.
38. Letting, D. L., C. Rakowski, M. J. Weiss, and G. A. Blobel. 2003. Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1. *Mol. Cell. Biol. 23:1334*.
39. Ozawa, Y., M. Towatari, S. Tsuzuki, F. Hayakawa, T. Maeda, Y. Miyata, M. Tanimoto, and H. Saito. 2001. Histone deacetylase 3 associates with and represses the transcription factor GATA-2. *Blood 98:2116*.
40. McBride, K., F. Charron, C. Lefebvre, and M. Nemer. 2003. Interaction with GATA transcription factors provides a mechanism for cell-specific effects of c-Fos. *Oncogene 22:8403*.
41. Angel, P., and M. Karin. 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim. Biophys. Acta 1072:129*.
42. D'Alonzo, R. C., N. Selvamurugan, G. Karsenty, and N. C. Partridge. 2002. Physical interaction of the activator protein-1 factors c-Fos and c-Jun with Cbfa1 for collagenase-3 promoter activation. *J. Biol. Chem. 277:816*.
43. Rooney, J. W., T. Hoey, and L. H. Glimcher. 1995. Coordinate and cooperative roles for NF-AT and AP-1 in the regulation of the murine IL-4 gene. *Immunity 2:473*.
44. Li, B., C. Tournier, R. J. Davis, and R. A. Flavell. 1999. Regulation of IL-4 expression by the transcription factor JunB during T helper cell differentiation. *EMBO J. 18:420*.
45. Garrington, T. P., T. Ishizuka, P. J. Papst, K. Chayama, S. Webb, T. Yujiri, W. Sun, S. Sather, D. M. Russell, S. B. Gibson, et al. 2000. MEKK2 gene disruption causes loss of cytokine production in response to IgE and c-Kit ligand stimulation of ES cell-derived mast cells. *EMBO J. 19:5387*.
46. Johnson, G. L., and R. Lapadat. 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science 298:1911*.
47. Weiss, D. L., J. Hural, D. Tara, L. A. Timmerman, G. Henkel, and M. A. Brown. 1996. Nuclear factor of activated T cells is associated with a mast cell interleukin 4 transcription complex. *Mol. Cell. Biol. 16:228*.
48. Macian, F., C. Lopez-Rodriguez, and A. Rao. 2001. Partners in transcription: NFAT and AP-1. *Oncogene 20:2476*.