

Suppressive effect of Elf-1 on FcεRI α-chain expression in primary mast cells

Qing-hui Wang · Chiharu Nishiyama ·
Nobuhiro Nakano · Naomi Shimokawa ·
Mutsuko Hara · Shunsuke Kanada · Hideoki Ogawa ·
Ko Okumura

Received: 21 April 2008 / Accepted: 25 June 2008
© Springer-Verlag 2008

Abstract The high-affinity receptor for immunoglobulin E (IgE), FcεRI, is specifically expressed in mast cells and basophils and plays a key role in IgE-mediated allergic reactions. The transcription factor Elf-1 has been previously identified to bind to the promoter of the human FcεRI α-chain, which is essential for the function and expression of FcεRI. In the present study, Elf-1 siRNA was conducted to evaluate the effects of Elf-1 on FcεRI α-chain expression in the primary mouse mast cells, bone marrow-derived mast cells (BMMC). Introduction of Elf-1 siRNA effectively reduced expression levels of Elf-1 mRNA and protein in BMMC. Transient reporter assay showed that the knock-down of Elf-1 by siRNA resulted in increased FcεRI α-chain promoter activity, while overexpression of Elf-1 suppressed α-chain promoter activity in BMMC. Elf-1 siRNA-treated BMMC exhibited marked upregulation of FcεRI α-chain transcription, whereas β-chain mRNA was not affected by Elf-1 siRNA. Chromatin immunoprecipitation assay showed that the amount of transcription factor PU.1, recognizing the *cis*-element close to the Elf-1-site on

the FcεRI α-chain promoter, was significantly increased by introduction of Elf-1 siRNA. These results indicate that Elf-1 negatively regulates FcεRI α-chain expression by suppressing PU.1-mediated transcription of the α-chain in BMMC.

Keywords Elf-1 · FcεRI · mast cells · PU.1

Abbreviations

BMMC bone marrow-derived mast cells
ChIP chromatin immunoprecipitation
Ct cycle threshold
EMSA electrophoretic mobility shift assay

Introduction

Elf-1 is a transcription factor belonging to the Ets family, which possesses a highly conserved DNA-binding region termed the Ets domain. Elf-1 cDNA was identified from human T cells as a cell-type specific transcription factor that enhanced the HIV-2 promoter (Leiden et al. 1992). Subsequently, Elf-1 was shown to be involved in the expression of various immunorelated genes, including interleukin (IL)-2 (Thompson et al. 1992), GM-CSF (Nimer et al. 1996b; Wang et al. 1994), IL-5 (Siegel et al. 1995), IL-2RA (John et al. 1996), and CD4 (Wurster et al. 1994) in T cells; immunoglobulin heavy chain (Grant et al. 1995), blk, lyn (Oettgen et al. 1996), and CD1D1 (Geng et al. 2005) in B cells; IL-3 in megakaryocytes (Nimer et al. 1996a); and stem cell leukemia gene in mast cells (Bockamp et al. 1998).

The high-affinity receptor for immunoglobulin E (IgE), FcεRI, is critical for IgE-mediated allergic reactions. Cross-linking of the IgE/FcεRI complex by allergens rapidly

Q.-h. Wang · C. Nishiyama (✉) · N. Nakano · N. Shimokawa ·
M. Hara · S. Kanada · H. Ogawa · K. Okumura
Atopy Research Center, Juntendo University School of Medicine,
2-1-1 Hongo Bunkyo-ku, Tokyo 113-8421, Japan
e-mail: chinishi@med.juntendo.ac.jp

S. Kanada · K. Okumura
Department of Immunology,
Juntendo University School of Medicine,
2-1-1 Hongo,
Bunkyo-ku, Tokyo 113-8421, Japan

Q.-h. Wang
Department of Immunology, College of Basic Medical Sciences,
China Medical University,
No. 92 Beier Road, Heping District,
Shenyang, China

induces activation of FcεRI-expressing effector cells, such as mast cells and basophils, resulting in the secretion of allergic mediators and induction of cytokine gene transcription. Deficiencies of the α-chain, which is one of the three subunits of FcεRI, abolishes the IgE-mediated allergic reaction in mice (Dombrowicz et al. 1993), thus suggesting that elucidation of the mechanisms for FcεRI α-chain expression may provide important information regarding the prevention of allergic diseases.

In our previous studies, Elf-1 was found to recognize the nucleotide sequence around the *cis*-element of the FcεRI α-chain promoter in vitro (Nishiyama et al. 1999), and overexpressed Elf-1 suppressed the FcεRI α-chain promoter in reporter assay using cell lines (Nishiyama et al. 2002). To investigate the role of Elf-1 on FcεRI α-chain expression in primary mast cells, Elf-1 was knocked down by siRNA in the present study. Knockdown of Elf-1 up regulated α-chain promoter activity and mRNA levels, and the amount of PU.1 binding to α-chain promoter region of chromosome increased, thus suggesting that Elf-1 suppresses α-chain transcription by inhibiting recruitment of PU.1 to the α-chain promoter.

Materials and methods

Cells

To generate bone marrow-derived mast cells (BMMC), BM cells prepared from BALB/c (Japan SLC, Hamamatsu, Japan) were grown in Roswell Park Memorial Institute 1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich), 100 μM 2-mercaptoethanol, 10 μM minimum essential medium nonessential amino acids solution (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% pokeweed mitogen-stimulated spleen-condition medium (Nakahata et al. 1982). More than 95% of cells were identifiable as mast cells by toluidine blue staining and as *c-kit*⁺/FcεRI⁺ by flow cytometric analysis after 4 weeks of culture.

siRNA-mediated inhibition of Elf-1 expression

The Elf-1 siRNA sequence was selected using BLOCK-iT RNAi designer (Invitrogen). Elf-1 double-stranded siRNA was prepared by annealing of the sense strand (5'-UGCAAUCAAACACUAGGUCGUU-3') and the anti-sense strand with the complementary sequence. The sense strand sequence of nonsilencing control siRNA was as follows: 5'-UGCCGAAUUAACACAAUCUGGUUC-3'. Five microliters of 20 μM double-stranded siRNA was introduced into 1×10⁶ of BMMC with Nucleofector II (Amaxa, Koeln, Germany) set at program Y-001 using a

Mouse Macrophage Nucleofector Kit (Amaxa) according to the manufacturer's instructions.

Quantitative real-time PCR

After 24 h of culture after electroporation, mRNA levels of Elf-1, FcεRI α-chain, β-chain, and γ-chain were analyzed by real-time polymerase chain reaction (PCR). Quantitative PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and a 7500 Real-Time PCR System with TaqMan Gene Expression Assays of the mouse target genes, including Elf-1 (Mm00468217_m1), the FcεRI α-chain (Mm00438867_m1), the FcεRI β-chain (Mm00442780_m1), the FcεRI γ-chain (Mm00438869_g1), and the endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Rodent GAPDH Control Reagents VIC Probe). Expression levels of Elf-1, the α-chain, the β-chain, and the γ-chain were expressed relative to those of GAPDH by calculating cycle threshold (Ct) values in amplification plots with 7500 SDS software (Applied Biosystems), as described in a previous report (Maeda et al. 2006).

Western blot analysis

Cells (1×10⁶) were collected at 48 h after electroporation, and whole cell lysates were subjected to electrophoresis on a 7.5% SDS polyacrylamide gel. Anti-Elf-1 antibody (Ab; clone #c-20, cat. #sc-631, rabbit immunoglobulin G [IgG], Santa Cruz Biotechnology) and anti-actin Ab (clone #c-2, cat. #sc-8432, mouse IgG1, Santa Cruz Biotechnology) were used as primary Abs. Alexa Fluor 680 goat anti-rabbit IgG and IR Dye 800 goat anti-mouse IgG (Molecular Probes) were used as secondary Abs against Elf-1 and actin, respectively. Infrared fluorescence on membranes was detected using the Odyssey infrared imaging system (model ODY-9201-SC; LI-COR), as described previously (Ito et al. 2005). The amount of protein was quantified by calculating band intensity using the Odyssey software.

Luciferase assay

The reporter plasmid, pGV-B2-αNN0.6, carrying the luciferase gene under control of the FcεRI α-chain promoter (−605/+29) was constructed previously (Nishiyama et al. 2002). A mutant reporter plasmid, pGV-B2-αNN0.6M5, in which three nucleotides essential for Elf-1 binding, was substituted as follows: from ATTTCC to GCGTCC at −56/−51 was also constructed in our previous study (Nishiyama et al. 2002). One microgram of pGV-B2-αNN0.6, pGV-B2-αNN0.6M5, or pGL3-Basic (promoter less control) was co-transfected with 25 ng pRL-CMV (internal control for

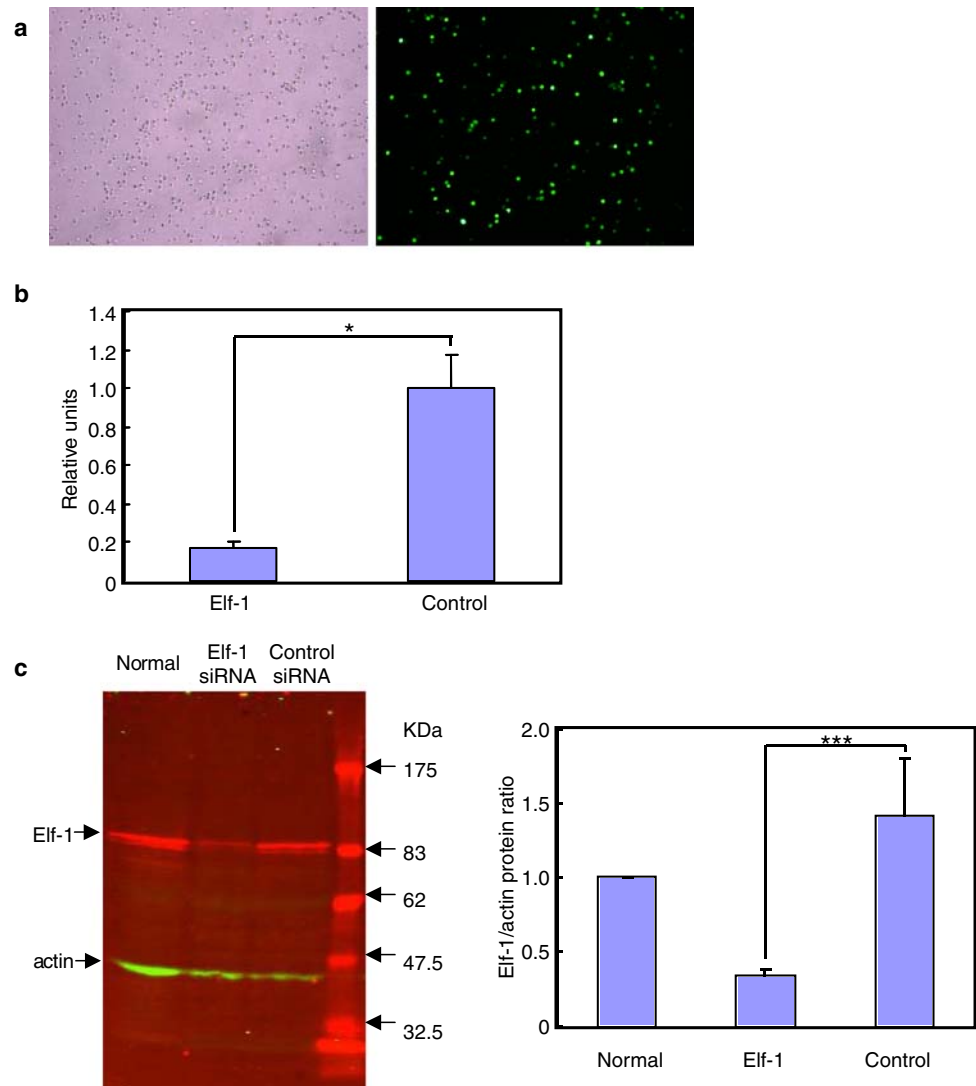
normalization of transfection efficiency) and siRNA into BMMCs by Nucleofector II as described above. For overexpression of Elf-1, 10 μ g of pCR-Elf-1 generated in a previous study (Nishiyama et al. 1999) or pCR3.1 control plasmid was introduced into BMMC with 5 μ g of reporter plasmid and 25 ng of pRL-CMV by electroporation using Bio-Rad Gene Pulser II (Bio-Rad, Hercules, CA, USA) as described previously (Nishiyama et al. 2002). Luciferase activity was determined using a luminometer as described in our previous report (Maeda et al. 2006).

ChIP assay

Chromatin immunoprecipitation (ChIP) assay was performed as in a previous report (Maeda et al. 2006). Anti-PU.1 goat IgG Ab (clone #D-19; sc-5949, Santa Cruz Biotechnologies), anti-Elf-1 rabbit IgG (clone #c-20), goat

IgG (#02-6202, Invitrogen), and rabbit IgG (#02-6102, Invitrogen) were used. Quantitative PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems) and a 7500 Real-Time PCR System (Applied Biosystems). The following primers and TaqMan probe sequences were used for this analysis: for the promoter region of the α -chain gene, -82/+1, forward primer -82/-56 (5'-GGCATAGCTGATGAGTTAACCAGATAC-3'), reverse primer +1/-22 (5'-TATGGCTTCGAAATAGGCTTGA-3'), and TaqMan probe -51/-33 (5'-FAM-CAGAAGACATTTCTTCTC-MGB-3'). The amount of target DNA bound to PU.1 was quantified from Ct value, which was determined using 7500 SDS software (Applied Biosystems). Briefly, the ratio of the amount of a specific DNA fragment in each immunoprecipitate to the amount of that fragment in the DNA before immunoprecipitation (input DNA) was calculated from each Ct value.

Fig. 1 Effects of siRNA on Elf-1 expression levels in BMMC. **a** Transfection efficiency. Fluorescence microscopy of FITC-labeled control oligonucleotide-treated BMMC. After 24 h of culture after transfection, cells were washed and visualized by phase microscopy (*left*) and by fluorescence microscopy using a green fluorescent protein filter (*right*). **b** The amount of Elf-1 mRNA in siRNA-treated BMMC were analyzed by real-time PCR. * $p < 0.05$, as determined by Student's t test ($n = 3$). **c** Elf-1 protein levels in siRNA-treated BMMC were analyzed by Western blotting. A typical profile of Western blotting is shown in the *left*. The amount of Elf-1 protein was normalized as the ratio to that of actin after quantification by using Odyssey software. The average of the four independent experiments is shown in the *right*. *** $p < 0.005$



Statistical analysis

Statistical analysis was performed using the two-tailed Student's *t* test. A *p* value less than 0.05 was considered statistically significant. Error bars in all figures mean SD.

Results

Elf-1 expression knockdown by siRNA

Elf-1 siRNA was introduced into BMMC using electroporation. Transfection efficiency was confirmed to be approximately 70% by fluorescence microscopy and phase microscopy, in which fluorescein-5-isothiocyanate (FITC)-labeled control oligonucleotides were introduced by electroporation under the same conditions as for siRNA (Fig. 1a). Expression levels of Elf-1 mRNA and protein in siRNA-treated BMMC were determined by real-time PCR and Western blot analysis, respectively. The amount of Elf-1 transcripts in Elf-1 siRNA-treated BMMC decreased to 0.17 ± 0.035 -fold compared with control BMMC, which was introduced with control siRNA (Fig. 1b). The band intensity of Elf-1 protein was markedly reduced by Elf-1 siRNA on Western blotting (Fig. 1c, lane 2) as compared with normal BMMC (Fig. 1c, lane 1) and control siRNA-treated BMMC (Fig. 1c, lane 3). These results indicate that Elf-1 siRNA generated in the present study successfully down regulated Elf-1 expression in BMMC.

Suppressive effects of Elf-1 on promoter activity of FcεRI α-chain

To evaluate the effects of Elf-1 on FcεRI α-chain promoter activity, a reporter plasmid, pGV-B2-αNN0.6, carrying the luciferase gene under the control of the FcεRI α-chain promoter (Nishiyama et al. 2002) was introduced into BMMC in addition to the above-mentioned siRNA. When Elf-1 siRNA was co-introduced into BMMC with the α-chain reporter plasmid, luciferase activity derived from BMMC (6.28 ± 0.35) was significantly increased in comparison with that of control siRNA transfectants (4.87 ± 0.22 ; Fig. 2a, left), whereas luciferase activity driven by the promoterless control plasmid, pGL3-Basic, was not markedly affected by Elf-1 siRNA (Fig. 2a, right). The mutant promoter lacking Elf-1 binding sequence exhibited higher luciferase activity compared with wild-type promoter and did not response to Elf-1 knockdown (Fig. 2a, center). In addition, overexpression of Elf-1 by co-introduction of the Elf-1 expression plasmid (Nishiyama et al. 1999) decreased FcεRI α-chain-driven luciferase activity in BMMC in Elf-1 binding sequence-dependent manner (Fig. 2b). These

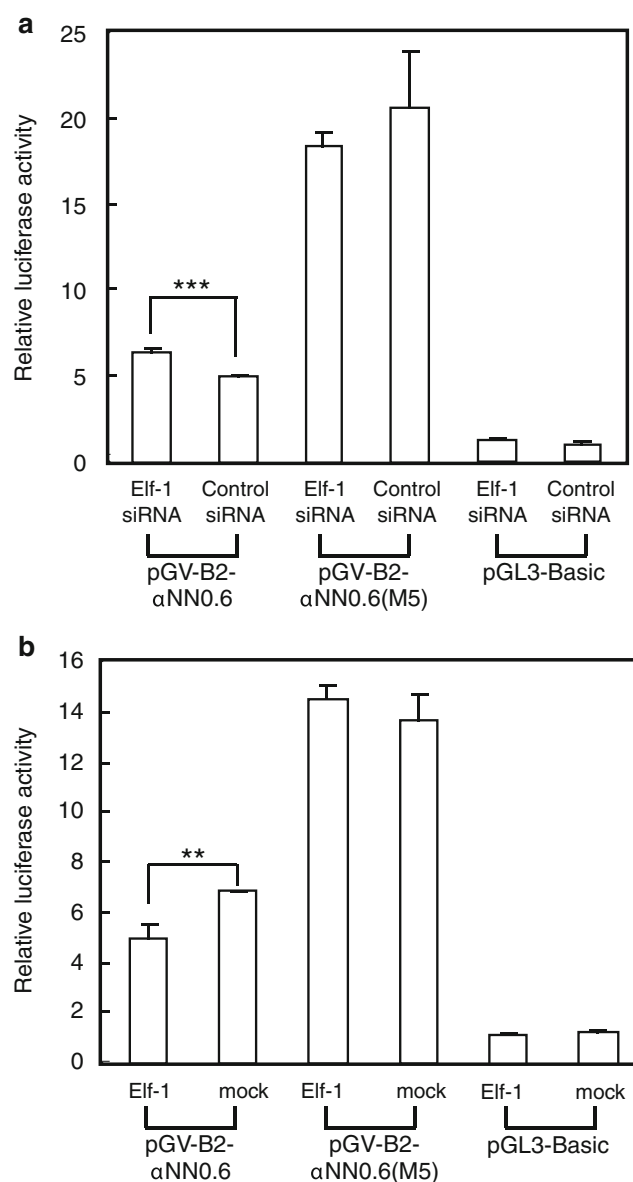


Fig. 2 Effects of suppressed or enforced expression of Elf-1 on FcεRI α-chain promoter activity. **a** FcεRI α-chain promoter activity in Elf-1 siRNA-introduced BMMC. **b** FcεRI α-chain promoter activity in Elf-1 expression plasmid-introduced BMMC. Relative luciferase activity is represented as the ratio of activity to that of pGL3-basic. Data represent the average±SD of triplicate samples. Representative results of three independent experiments are shown. B2-αNN0.6 wild-type α-chain promoter, B2-αNN0.6M5 α-chain mutant promoter lacking Elf-1 binding sequence, Basic promoter less; ****p*<0.005 in (a) (*n*=3) and ***p*<0.01 in (b) (*n*=3)

results suggest that Elf-1 exhibits suppressive effect on the FcεRI α-chain promoter in BMMC.

Effects of Elf-1 knockdown on FcεRI α-, β-, and γ-chain transcription

To evaluate the effects of Elf-1 on FcεRI α-chain transcription in BMMC, α-chain mRNA in Elf-1-knocked-down

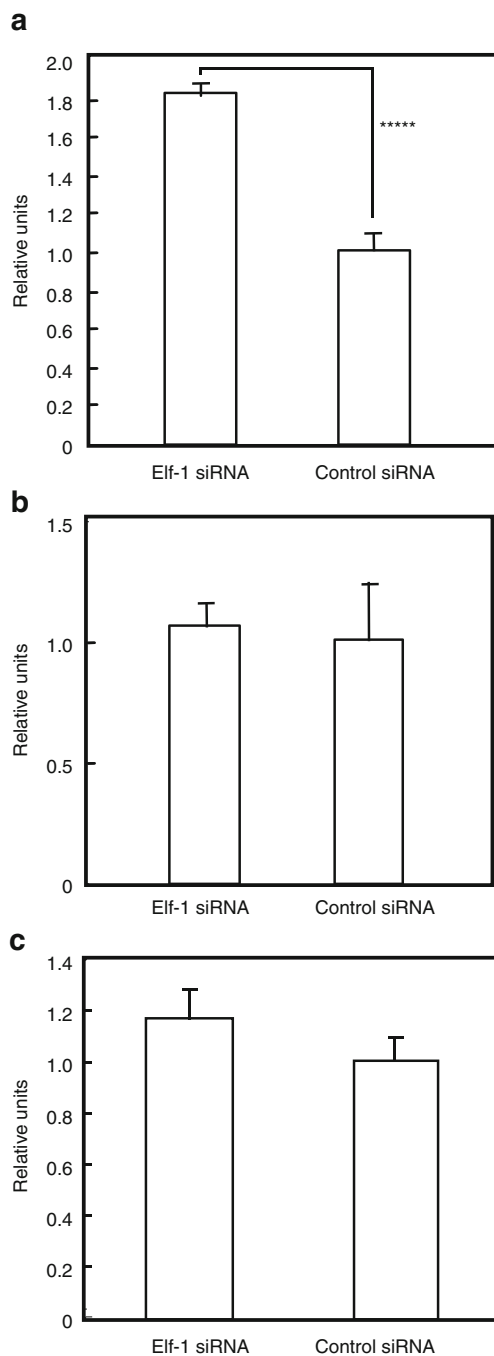


Fig. 3 Transcription levels of FcεRI α-, β-, and γ-chains in siRNA-introduced BMMCs. The amounts of mRNA for FcεRI α-chain (a), β-chain (b), and γ-chain (c) in BMMC transfectants were measured by real-time PCR ($n=2$). **** $p<0.0005$

BMMC was measured by real-time PCR. The amount of α-chain mRNA in Elf-1 siRNA-treated BMMC was 1.82 ± 0.055 -fold higher than that of control siRNA-treated BMMC (Fig. 3a). In contrast, significant differences were not observed in β-chain mRNA levels between BMMC transfectants with Elf-1 or control siRNA ($p=0.72$; Fig. 3b).

Recently, Elf-1 was reported to suppress γ-chain transcription in human T cell line and basophilic cell line (Juang et al. 2007). Therefore, we also measured γ-chain mRNA level in siRNA-treated BMMC and found that γ-chain mRNA was slightly but not significantly ($p=0.18$) up regulated by Elf-1 knockdown (Fig. 3c). The upregulation degree of γ-chain (1.16 ± 0.12) was similar to that in human basophilic cell line KU812 in the previous report (Fig. 5a in the report by Juang et al.), although the effect of Elf-1 siRNA on γ-chain has been significant in KU812. Regardless, the α-chain promoter may be strongly suppressed by Elf-1 compared with the γ-chain promoter in BMMC. Based on these results, the reduced expression of Elf-1 apparently leads to specific upregulation of FcεRI α-chain transcription.

Elf-1 knockdown enhances recruitment of transactivator PU.1 toward FcεRI α-chain promoter

The Elf-1 binding sequence in the α-chain promoter is located near binding sites of another Ets family transcription factor, PU.1, which artificially transactivates the human α-chain promoter in transient reporter assay (Nishiyama et al. 2002). Therefore, one possible mechanism is that decrease of Elf-1 binding to the α-chain promoter allows increased PU.1 binding to *cis*-enhancing elements. To confirm this hypothesis, ChIP assay was performed (Fig. 4). The amount of *cis*-enhancing region of the α-chain promoter immunoprecipitated with anti-Elf-1 Ab or anti-PU.1 Ab was significantly higher than that with each control Ab in control siRNA-treated BMMC ($p=0.0498$ for Elf-1 and $p=0.00040$ for PU.1, respectively), thus indicating the constitutive binding of Elf-1 and PU.1

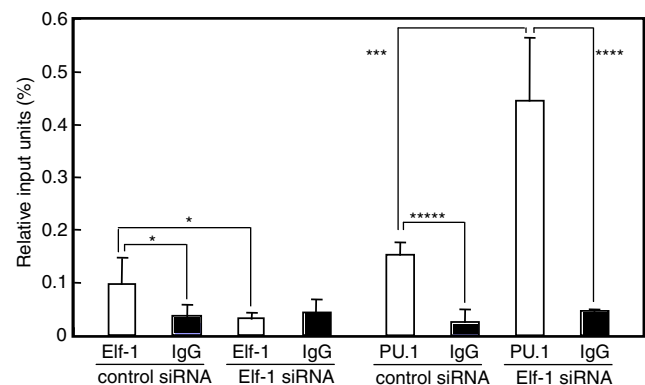


Fig. 4 In vivo binding of Elf-1 and PU.1 to FcεRI α-chain promoter region of chromosome DNA in BMMC. Quantitative analysis of Elf-1 or PU.1 binding to the promoter region (−82/+1) of the α-chain gene by ChIP assay. Results are expressed as means±SD for two or three PCRs with duplicate samples for each of three independent ChIPs. Open bars specific Ab, closed bars control Ab. * $p<0.05$, *** $p<0.005$, **** $p<0.001$, ***** $p<0.0005$

on the α -chain promoter of chromosomes in normal mast cells. In addition, the amount of α -chain promoter region bound with PU.1 increased in Elf-1 siRNA-treated BMMC ($p=0.0032$), whereas the amount of Elf-1 binding to the α -chain promoter was markedly reduced to basal level, suggesting that Elf-1 siRNA effectively reduced Elf-1 protein binding to the α -chain promoter resulting in the increase of PU.1 binding. In contrast, specific binding of PU.1 against the β -chain promoter was not observed with or without Elf-1 siRNA (data not shown). These results indicate that Elf-1 specifically inhibits recruitment of transactivator PU.1 toward the α -chain promoter.

Discussion

A transcription factor Elf-1 has been previously found to bind -55/-40 region of the human Fc ϵ RI α -chain gene, which is overlapping with the *cis*-enhancing element located at -52/-45 of this promoter (Nishiyama et al. 1999, 2002). However, a detailed analysis to clarify the role of Elf-1 on Fc ϵ RI α -chain expression has not yet been reported. In the present study, Elf-1 siRNA was conducted to evaluate the effects of Elf-1 knockdown on α -chain expression in primary mast cells. We found that Elf-1 siRNA was introduced into BMMC for sufficient knockdown of endogenous Elf-1 expression and preferentially reduced expression of the Fc ϵ RI α -chain by transcription suppression.

The nucleotide sequences of the Fc ϵ RI α -chain proximal promoter are highly conserved among rodents and humans (Nishiyama et al. 1999), whereas the distal promoter has been found in the human α -chain gene but not in those of rodents (Hasegawa et al. 2003; Nishiyama et al. 2001). The proximal promoter is constitutively and strongly transactivated in a mast cell- and basophil-specific manner. In contrast, the distal promoter is induced by IL-4 stimulation in monocytes. Therefore, mouse cells are suitable to examine the effects of Elf-1 on mast cell-specific α -chain promoter function. Reduced expression of Elf-1 markedly increased mouse Fc ϵ RI α -chain mRNA levels, indicating that Elf-1 plays a repressive role in the Fc ϵ RI α -chain proximal promoter by binding to the Elf-1 motif in the mouse α -chain genomic DNA, which is conserved between mice and humans (Nishiyama et al. 1999, 2002). Recruitment of transactivator PU.1 recognizing the critical *cis*-enhancing element near the Elf-1-binding region was enhanced in Elf-1-knocked-down BMMC (Fig. 4), in contrast to decreased Elf-1 binding to the α -chain promoter (Fig. 4). This indicates that inhibition of binding between PU.1 and the α -chain promoter is one of the mechanisms of Elf-1-mediated suppression of α -chain promoter activity. Electrophoretic mobility shift assay (EMSA) using *in vitro*

translated Elf-1 and PU.1 has indicated that Elf-1 binding region (-55/-40) is overlapping with PU.1 binding region (-52/-45; Nishiyama et al. 2002). The ChIP assay data in the present study are coincident with this previous observation. Because further study including EMSA with recombinant Elf-1 and PU.1 would be useful to evaluate direct inhibitory effect, we try to establish the method for preparation of recombinant transcription factors.

Transcription of the third subunit of Fc ϵ RI, the γ -chain, was recently reported to be negatively regulated by Elf-1 (Juang et al. 2007), and we also found similar but slight suppressive effect of Elf-1 on γ -chain in BMMC. The mouse β -chain promoter is regulated by a transcription factor GATA-1 and a cofactor FOG-1, but the binding of Elf-1 against the β -chain promoter has not been found so far (Maeda et al. 2003, 2006). Considering that Elf-1 siRNA did not affect β -chain mRNA level in the present study, Elf-1 may not be involved in the mouse β -chain promoter function. Although Elf-1 is a suppressor for α - and γ -chain expression, cell surface expression of Fc ϵ RI was not increased by Elf-1 siRNA on fluorescence-activated cell sorting analysis (data not shown). Considering that β -chain mRNA levels were comparable between Elf-1 siRNA-treated BMMC and control-treated BMMC, increases in β -chain protein levels may be required to detect the effect of Elf-1 on cell surface expression of Fc ϵ RI.

As we also believe that overexpression of Elf-1 is useful in clarifying the role of Elf-1 on Fc ϵ RI expression, retrovirus infection experiments were performed to overexpress Elf-1 in BMMC. Unfortunately, few Elf-1 transfectants were obtained, as the cell number of transfectants is rapidly and dramatically decreased by transfection of Elf-1-expressing retrovirus vectors when compared with mock transfectants (data not shown). This suggests that Elf-1 plays an important role in the growth, proliferation, development, and/or survival of primary mast cells. Although there are α -chain studies (Hasegawa et al. 2003; Nishiyama et al. 1999, 2002) and a study in which Elf-1 was confirmed to be involved in stem cell leukemia gene expression in mast cells (Bockamp et al. 1998), the roles of Elf-1 in the regulation of other genes expressed in mast cells remains unclear. Further detailed analyses are required to reveal the mechanisms by which Elf-1 is involved in the generation of mast cells.

Acknowledgments We would like to thank members of the Atopy (Allergy) Research Center and the Department of Immunology for helpful discussions. We are grateful for Drs. Y. Niwa, T. Fukai, and D. Potaczek for their technical assistance and Ms. M. Matsumoto for secretarial assistance. This work was supported in part by a grant-in-aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to C. N.).

References

- Bockamp E-O, Fordham JL, Gottgens B, Murrell AM, Sanchez M-J, Green AR (1998) Transcriptional regulation of the stem cell leukemia gene by PU.1 and Elf-1. *J Biol Chem* 273:29032–29042 doi:10.1074/jbc.273.44.29032
- Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet J-P (1993) Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell* 75:969–976 doi:10.1016/0092-8674(93)90540-7
- Geng Y, Laslo P, Barton K, Wang C-R (2005) Transcriptional regulation of *CD1D* by Ets family transcription factors. *J Immunol* 175:1022–1029
- Grant PA, Thompson CB, Pettersson S (1995) IgM receptor-mediated transactivation of the IgH 3' enhancer couples a novel Elf-1-AP-1 protein complex to the development control of enhancer function. *EMBO J* 14:4501–4513
- Hasegawa M, Nishiyama C, Nishiyama M, Akizawa Y, Takahashi K, Ito T et al (2003) Regulation of the human FcepsilonRI alpha-chain distal promoter. *J Immunol* 170:3732–3738
- Ito T, Nishiyama C, Nishiyama M, Matsuda H, Maeda K, Akizawa Y et al (2005) Mast cells acquire monocyte-specific gene expression and monocyte-like morphology by overproduction of PU.1. *J Immunol* 174:376–383
- John S, Robbins CM, Leonard WJ (1996) An IL-2 response element in the human IL-2 receptor alpha chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. *EMBO J* 15:5627–5635
- Juang Y-T, Sumibcay L, Tolnay M, Wang Y, Kyttaris VC, Tsokos GC (2007) Elf-1 binds to GGAA elements on the FcRgamma promoter and repress its expression. *J Immunol* 179:4884–4889
- Leiden JM, Wang C-Y, Petryniak B, Markovitz DM, Nabel GJ, Thompson CB (1992) A novel Ets-related transcription factor, Elf-1, binds to human immunodeficiency virus type 2 regulatory elements that are required for inducible *trans* activation in T cells. *J Virol* 66:5890–5897
- Maeda K, Nishiyama C, Tokura T, Akizawa Y, Nishiyama M, Ogawa H et al (2003) Regulation of cell type-specific mouse FcepsilonRI beta-chain gene expression by GATA-1 via four GATA motifs in the promoter. *J Immunol* 170:334–340
- Maeda K, Nishiyama C, Tokura T, Nakano H, Kanada S, Nishiyama M et al (2006) FOG-1 represses GATA-1-dependent FcepsilonRI beta-chain transcription: transcriptional mechanism of mast-cell-specific gene expression in mice. *Blood* 108:262–269 doi:10.1182/blood-2005-07-2878
- Nakahata T, Spicer SS, Cantey JR, Ogawa M (1982) Clonal assay of mouse mast cell colonies in methylcellulose culture. *Blood* 60:352–361
- Nimer S, Zhang J, Avraham H, Miyazaki Y (1996a) Transcriptional regulation of interleukin-3 expression in megakaryocyte. *Blood* 88:66–74
- Nimer SD, Zhang W, Kwan K, Wang Y, Zhang J (1996b) Adjacent, cooperative elements from a strong, constitutive enhancer in the human granulocyte-macrophage colony-stimulating factor gene. *Blood* 87:3694–3703
- Nishiyama C, Yokota T, Okumura K, Ra C (1999) The transcription factors Elf-1 and GATA-1 bind to cell-specific enhancer elements of human high-affinity IgE receptor alpha-chain. *J Immunol* 163:623–630
- Nishiyama C, Hasegawa M, Nishiyama M, Takahashi K, Yokota T, Okumura K et al (2001) Cloning of full length genomic DNA encoding human FcepsilonRI alpha-chain and its transcriptional regulation. *Biochem Biophys Res Commun* 284:1056–1064 doi:10.1006/bbrc.2001.5079
- Nishiyama C, Hasegawa M, Nishiyama M, Takahashi K, Akizawa Y, Yokota T et al (2002) Regulation of human FcepsilonRI alpha-chain gene expression by multiple transcription factors. *J Immunol* 168:4546–4552
- Oettgen P, Akbarali Y, Boltax J, Best J, Kunsch C, Libermann TA (1996) Characterization of NERF, a novel transcription factor related to the Ets factor Elf-1. *Mol Cell Biol* 16:5091–5106
- Siegel MD, Zhang D-H, Ray P, Ray A (1995) Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLE0 elements. *J Biol Chem* 270:24548–24555 doi:10.1074/jbc.270.41.24548
- Thompson CB, Wang C-Y, Ho I-C, Bohjanen PR, Petryniak B, June CH et al (1992) cis-Acting sequences required for inducible interleukin-2 enhancer function bind a novel Ets-related protein, Elf-1. *Mol Cell Biol* 12:1043–1053
- Wang C-Y, Bassuk AG, Boise LH, Thompson CB, Bravo R, Leiden JM (1994) Activation of the granulocyte-macrophage colony-stimulating factor promoter in T cells requires cooperative binding of Elf-1 and AP-1 transcription factors. *Mol Cell Biol* 14:1153–1159
- Wurster A, Siu G, Leiden JM, Hedrick SM (1994) Elf-1 binds to a critical element in a second CD4 enhancer. *Mol Cell Biol* 14:6452–6463