The glycoprotein gp91phox is an essential component of the phagocyte NADPH oxidase and is expressed in eosinophils, neutrophils, monocytes, and B-lymphocytes. We previously suggested an eosinophil-specific mechanism of gp91phox gene expression. To elucidate the mechanism, we performed functional assays on deletion mutants of the gp91phox promoter in various types of gp91phox-expressing cells. A 10-base pair (bp) region from bp −105 to −96 of the promoter activated transcription of the gene in eosinophilic cells, but not in neutrophilic, monocytic, or B-lymphocytic cells. A 2-bp mutation introduced into the GATA site spanning bp −101 to −96 (−98GATA site) of the fragment abolished its activity. Gel shift assays using a GATA competitor and specific antibodies demonstrated that both GATA-1 and GATA-2 specifically bound to the −98GATA site with similar affinities. Individual transfection of GATA-1 and GATA-2 into Jurkat cells, which have neither endogenous GATA-1 nor GATA-2, activated the −105/+12 construct in a −98GATA site-dependent manner. Combined transfection of GATA-1 and GATA-2 activated the promoter less than transfection of GATA-1 alone. These results suggest that GATA-1 is an activator and that GATA-2 is a relative competitive inhibitor of GATA-1 in the expression of the gp91phox gene in human eosinophils.

The large subunit of flavocytochrome b556 (gp91phox) is an essential component of the phagocyte NADPH oxidase, which produces superoxide anion to kill parasites and microorganisms. Mutations in the gp91phox gene such as deletions (1) and substitutions (2) result in X-linked chronic granulomatous disease. The gp91phox gene is expressed in terminally differentiated phagocytes and B-lymphocytes (4, 5), indicating the expression of the gene to be both lineage- and differentiation stage-specific.

Several transcription factors regulate gp91phox gene expression through cis-elements in a 450-base pair (bp) sequence in the 5′-flanking region of the gene. A C/EBP displacement protein (CDP/cut) (6, 7) suppresses the expression of the gp91phox gene by binding to four sites at bp −350, −220, −150, and −110 of the gene in immature myeloid cell lines. Overexpression of CDP inhibits the induction of the gene in myeloid differentiation (8). The down-regulation of its DNA binding activity during differentiation allows the expression of the gene (9, 10). CP1, one of the CCAAT box-binding proteins, and BID proteins (binding increased during differentiation) are activators. However, these factors may work after bound CDPs are released because their binding sites overlap with sites for CDP (10, 11). Interferon regulatory factor-2 may have dual functions as a transcriptional activator and repressor depending on binding sites in gp91phox gene expression (12, 13). TFI1phox, a component of BID-2, displaces CDP and interferon regulatory factor-2, resulting in increased expression of the gp91phox gene in the myeloid cell lines (13). We have shown that PU.1 is an essential activator for the expression of the gp91phox gene in human neutrophils, monocytes, and B-lymphocytes (14). Eklund et al. (15) have proposed a cooperative activation of the gp91phox gene by PU.1 and interferon regulatory factor-1 in myeloid cell lines.

Eosinophils have an important role in the host defense against pathogenic parasites and microorganisms and produce superoxide anion as do other phagocytes (neutrophils, monocytes, and macrophages) and B-lymphocytes by means of NADPH oxidase activity (16). It has long been thought that the expression mechanism of gp91phox in eosinophils is the same as that in other phagocytes. However, a restricted expression of gp91phox in eosinophils from our X-linked chronic granulomatous disease patients (17) implied a certain eosinophil-specific expression mechanism of the gene. No positive regulatory mechanisms of the gene have, however, been shown in eosinophils, although an eosinophil-specific GATA-3 suppression mechanism of the gene was recently suggested by us (18). In this report, we show the eosinophil-specific regulation of the gp91phox gene by transcription factors GATA-1 and GATA-2 through the GATA-binding site spanning bp −101 to −96 of the gene.

**MATERIALS AND METHODS**

**Cell Culture**—To prepare a differentiated eosinophilic cell line (HL-60-C15E), HL-60-C15 cells (a kind gift from Dr. Y. Yamaguchi, Kumamoto University, Kumamoto, Japan), the eosinophil-committed subline of the promyelocytic cell line HL-60, were maintained for >3 months at pH 7.7 in RPMI 1640 medium supplemented with 10% fetal calf serum and 25 mM EPPS (Sigma). They were then treated with 5% methyl-x-butyrate (Sigma) for 5 days for differentiation into eosinophils (19). HL-60 (Riken Cell Bank, Tsukuba, Japan), B-lymphocytic HS-1-12-4 Sakamoto, Nagasaki 852-8523 and the Center of Tsukuba Advanced Research Alliance, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8577, Japan

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The abbreviations used are: bp, base pair(s); CDP, CCAAT displacement protein; MBP, major basic protein; EMSA, electrophoretic mobility gel shift assay; C/EBP, CCAAT/enhancer-binding protein; EPPS, 4-(2-hydroxyethyl)-1-piperazinene-2-propanesulfonic acid; CBF, cAMP responsive element-binding protein-binding protein.
Regulation of gp91phox Gene Expression by GATA-1 and GATA-2

Sultan (Japanese Cancer Research Resources Bank, Tokyo), and T-lymphocytic Jurkat (given by Dr. K. Furukawa, Nagoya University) cells were cultured at pH 7.2 in RPMI 1640 medium with 10% fetal calf serum. HL-60 cells were differentiated into neutrophils and monocytes by treatment with 1 μM all-trans-retinoic acid (Sigma) and 1 ng/ml recombinant human transforming growth factor-β (Roche Molecular Biochemicals, Tokyo) in combination with 0.1 μM vitamin D₃ (1,25-dihyroxvitamin D; a gift from Chugai Pharmaceutical Co., Ltd., Tokyo), respectively, for 3 days (20, 41). COS-7 cells were maintained in Eagle’s minimal essential medium supplemented with 10% fetal calf serum.

Northern Blot Analysis—Total RNA was prepared from cells with Trizol LS reagent (Life Technologies, Inc.) according to the manufacturer’s protocol. The RNA (10 μg/lane) was electrophoresed on formaldehyde-containing 0.9% agarose gels, transferred to Hybond-N* nylon membranes (Amersham Pharmacia Biotech, Tokyo), and fixed by ultraviolet light. Messenger RNAs were detected by hybridization with probes labeled with [32P]dCTP using a random primer labeling kit (Amersham Pharmacia Biotech). The following DNAs were used as probes for defining cell lineage: a 780-bp full-length cDNA of human major basic protein (MBP; a gift from Dr. I. Nagaoka, Juntendo University), a 285-bp cDNA for defensin (human neutrophil peptide-3; kindly supplied by Dr. I. Nagaoka, Juntendo University), and a 258-bp cDNA for defensin (human neutrophil peptide-3; kindly supplied by Dr. I. Nagaoka). After hybridization patterns by these probes were analyzed on a FACScan (Becton Dickinson, La Jolla, CA),

Promoter Activity Assays—For HL-60-C15E and other differentiated HL-60 cells, cells (5 × 10⁵) were incubated with 20 μg of gp91⁹⁄₈₀ promoter/firefly luciferase plasmid, 3 μg of herpes simplex virus thymidine kinase promoter/ Renilla luciferase plasmid in 0.25 ml of 20 mM HEPES/RPMI 1640 medium (pH 7.2) at room temperature for 15 min and electroporated at 200 V for 70 ms on an ElectroSpherorator T820 (BTX, Inc., San Diego, CA). HS-Sultan cells (5 × 10⁴) were incubated with 10 μg of gp91⁹⁄₈₀ promoter/luciferase plasmid and 50 ng of cytomegalovirus promoter/Renilla luciferase plasmid as described above, but electroporated with a Gene Pulser II (Bio-Rad) at 950 microfarads and 310 V. Jurkat cells (5 × 10⁴) were electroporated as described for HS-Sultan cells, but in the presence of 5 μg of reporter plasmid and 20 ng of Renilla luciferase plasmid at 950 microfarads and 280 V. After HL-60-C15E, HL-60, and HS-Sultan, Jurkat cells were incubated at 37°C under 5% CO₂ and 95% air for 6, 10, and 24 h, respectively, their reporter activities were measured as described previously (14). In the case of cotransfection experiments, various amounts of human GATA expression plasmids were transfected with 5 μg of reporter plasmid into 5 × 10⁴ Jurkat cells.

Preparation of Nuclear Extracts, Electrophoretic Mobility Shift Assays (EMSAs), and Scatchard Plots—Prior to nuclear extraction, COS-7 cells (10⁶) were transfected with 4 μg of GATA-1 or GATA-2 plasmid in 5 ml of Eagle’s minimal essential medium by 20 n mole of L1-Liposome (kindly supplied by Dai-iichi Pharmaceutical Co., Ltd., Tokyo) for 8 h and cultured in 15 ml of 10% fetal calf serum/Eagle’s minimal essential medium for 40 h. Preparation of nuclear protein extracts, labeling of the double-stranded oligonucleotides from bp –115 to –90 of the human gp91phox gene for the probe, and EMSAs were performed as described previously (14). Each reaction mixture (20 μl) containing 0.5–14 μg of nuclear protein extracts, 10,000 cpm of each radiolabeled probe equivalent to 1.16 fmol, and 1 μg of poly(dI-dC)poly(dI-dC) (Amersham Pharmacia Biotech) in 20 mM HEPES (pH 7.9), 50 mM KCl, 1 mM MgCl₂, 1 mM dithiothreitol, 0.2 mM EDTA, 0.01% Triton X-100, 5% glycerol, and 0.5 mM spermidine was incubated on ice for 15 min. In competition assays, a 3000-molar excess of unlabeled competitor oligonucleotides was added prior to the addition of the probe to the mixture, which was then preincubated on ice for 15 min. For the inhibition assay with antibodies, an aliquot of nuclear extracts was incubated on ice with 2–4 μg of goat IgG against human GATA-1, murine IgG against human GATA-2, control goat IgG, or control murine IgG for 1 h before the addition of the probe. Both anti-GATA antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Electrophoresis was performed on a 6% acrylamide gel in 0.4× TBE buffer containing 36 mM Tris, 36 mM borate, and 8 mM EDTA (pH 8.3).

Upper-strand sequences for the probe and of competitors are as follows: sequence for the probe and of the wild-type competitor (bp –115 to –90 of the normal fragment of the gp91phox gene), 5'-TATTAGCTTTATTACGAGAAAGAAA-3'; GATA-binding site competitor of the human T-cell receptor α gene (25), 5'-TTATTATGGTCTTCTGCTATACCA-3'; GATA site mutant of the wild-type competitor (–105M), 5'-AATTTCATTCTCTTATAAGAAAAAGAAA-3'; and a heterologous competitor, 5'-CAACACAGATCCCTGCATACACC-3'. The underlined sequence represents mutated bases.

Apparent dissociation constants (Kₐ) were determined according to the methods described by Merika and Orkin (26). The binding and running conditions were same as those described above. Each fixed

Fig. 1. An eosinophilic phenotype of HL-60-C15E cells. Total RNAs for Northern blot analysis were extracted from parental HL-60 cells and from HL-60-C15E cells differentiated from HL-60-C15 cells by a combination of alkaline and butyrate treatments (see “Materials and Methods”). Probes used were cDNAs for MBP, eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO). After removing the first probe from each filter, it was hybridized with the probe for glyceraldehyde-3-phosphate dehydrogenase mRNA (GPD).

A. Yamauchi, unpublished data.
amount of nuclear protein extract from COS-7 cells expressing GATA-1 (0.5 μg/20 μl) or GATA-2 (14 μg/20 μl) was incubated on ice with serially diluted labeled probe and incubated for 15 min, which was confirmed to be long enough to bring the reaction to equilibrium. Quantitation of free and bound DNAs was performed with a Molecular Imager FX. Scatchard plots for GATA-1 and GATA-2 were accomplished assuming front counts and specifically retarded counts to be proportional to free and bound concentrations, respectively, and also assuming their binding to one DNA duplex to be 1.

Statistical Analyses—Each value shown as the mean ± S.D. was
from bp 2 were taken to be statistically significant.

The rel- lished into HL-60-C15E cells as described under "Materials and Methods." The rel- reflect into HL-60-C15E cells.

RESULTS

Eosinophil-specific Transcriptional Activation of the gp91phox Gene by the Gene Promoter Region from bp −105 to −90—To analyze an eosinophil-specific cis-element of the gp91phox promoter, we differentiated HL-60-C15 cells, an eosinophil-comm- mitted subline of the promyelocytic HL-60 leukemia cell line, by treatment with butyrate under alkaline pH. As shown in Fig. 1, these treated cells, but not parental HL-60 cells, expressed mRNAs for eosinophil lineage-specific MBP, eosinophil cationic protein, and eosinophil peroxidase. These results support that the cells are phenotypically eosinophilic. We named the cells HL-60-C15E and used them in further experiments as differentiated human eosinophilic cells.

Next, we transiently transfected the p−986/Luc construct, which contains the gp91phox gene fragment from bp −986 to bp +12 in front of a luciferase reporter gene, into HL-60-C15E cells. As shown in Fig. 2, the p−986/Luc construct had a sig- significantly higher reporter activity than the promoterless pXP2N construct. This result indicates that the bp −986 fragment contains positive regulatory elements for gp91phox gene expression in eosinophilic cells. To clarify the elements, a series of progressive deletion mutants was generated, and the pro- motion contains positive regulatory elements for gp91phox gene expression in eosinophilic cells. To clarify the elements, a series of progressive deletion mutants was generated, and the pro- moter activity of each was examined (Fig. 2). Deletions of bp −96 and bp −115/Luc and p2986/Luc). An additional 11-bp dele- tion resulted in a further decrease in activity (p2105/Luc). How- ever, a further 10-bp deletion from bp −105 to −96 significantly decreased promoter activity by 40% (p<0.01; compare p2105/Luc and p295/Luc). An additional 11-bp deletion resulted in a further decrease in activity (p<0.01; compare p295/Luc and p284/Luc). These results suggest that positive regulatory elements exist in the regions from bp −105 to −96 and bp −95 to −85 of the gp91phox promoter. To determine the lineage specificity of the first region from bp −105 to −96, we examined the relative promoter activity of p2105/Luc versus p295/Luc in non-eosinophilic gp91phox-ex-
pressing cells as well as eosinophilic HL-60-C15E cells (Fig. 3A). The ratio of the promoter activity of p-105/Luc to that of p-95/Luc was significantly higher than 1.0 in HL-60-C15E cells (1.7 ± 0.19; p < 0.001), but not in neutrophilic HL-60, monocytic HL-60, or B-lymphocytic HS-Sultan cells. Therefore, at least one eosinophil-specific positive cis-element should exist in the region from bp -105 to -96 of the gp91phox promoter. On the other hand, the -95/-84 region had no specificity for eosinophilic cells (data not shown). The lineage specificities of these gp91phox-expressing cells were confirmed by particular expressions of CD14, CD19, and the interleukin-5 receptor on the surfaces of transforming growth factor-β1/vitamin D3-treated HL-60, HS-Sultan, and HL-60-C15E cells, respectively, and the only significant expression of defensin mRNA in all-trans-retinoic acid-treated HL-60 cells (Fig. 3B).

The GATA-binding Site Is Essential for Activation of the gp91phox Promoter by the -105/-96 Region in HL-60-C15E Cells—To determine cis-elements in the -105/-96 region of the gp91phox promoter, we searched transcription factor-binding sites in the region. A sequence between bp -101 and -96 (5′-TGATAA-3′) perfectly matched with the GATA consensus sequence ((A/T)GATA(A/G)) (27). To determine whether this putative GATA-binding site (−98GATA site) actually contributed to activation of the gp91phox promoter by the −105−96 region, we examined the effect of a GATA site mutation (GA to CT) that abolishes the ability to bind GATA proteins from a GATA site (26) on activation in HL-60-C15E cells. As shown in Fig. 4, the mutation completely abolished all the activity dependent on the region, clearly demonstrating that the −98GATA site is the essential cis-element for the eosinophil-specific activation of the gp91phox promoter by the −105−96 region.

GATA-1 and GATA-2 Bind to the −98GATA Site in the gp91phox Promoter—To identify nuclear protein complexes that specifically bind to the −98GATA site in HL-60-C15E cells, we performed EMSAs with the probe from bp −115 to −90 encompassing the −98GATA site (Fig. 5). Two nuclear protein complexes (C1 and C2) specifically bound to the probe because binding was abolished by a 300-fold molar excess of wild-type competitor with sequence identical to that of the probe, but not by an excess of the heterologous sequence oligonucleotide (lanes 1–3). Binding was abolished to a similar extent even by a 120-fold molar excess of wild-type competitor (data not shown). An excess of GATA site-mutated oligonucleotide spanning bp −105 to −85 of the promoter (Mt), which has the same mutation as p-105M/Luc in Fig. 4, failed to abolish the binding of the C1 and C2 complexes (lane 4), indicating that these protein complexes bind to the −98GATA site. An excess of a GATA-binding sequence derived from the T-cell receptor δ gene completely abolished the binding of these complexes to the probe (lane 5), as did the wild-type competitor, suggesting that the C1 and C2 complexes include members of the GATA family. To further characterize these complexes, we performed gel shift immunoassays with anti-GATA-1 and anti-GATA-2 antibodies because mRNAs of GATA-1 and GATA-2 are abundantly expressed in HL-60-C15E cells as well as in human peripheral eosinophils (28). C2 and the lower part of C2 (L) disappeared when anti-GATA-1 antibody was added (lane 6). The upper part of C2 (U), but not others, was abolished by the addition of anti-GATA-2 antibody (lane 7). The formation of both C1 and C2 bands was mostly inhibited by a combination of both antibodies (lane 8). Neither control goat IgG (lane 9) nor control murine IgG (lane 10) disrupted the complexes. These results suggest that C1 and C2 include GATA-1 alone and GATA-1 and GATA-2, respectively. Although a small amount of mRNA for GATA-3 was expressed in HL-60-C15E cells as well as in peripheral eosinophils, an antibody against GATA-3 exhibited no effects on C1 or C2 (data not shown). These results demonstrate that GATA-1 and GATA-2 specifically bind to the −98GATA site of the gp91phox promoter in HL-60-C15E cells.

The different mobilities of GATA-1 and GATA-2 shown above are consistent with those reported by Briegel et al. (29) and Yamaguchi et al. (30).

To characterize the individual binding of GATA-1 and GATA-2 to the −98GATA site in the gp91phox promoter, EMSA was performed with nuclear extracts from COS-7 cells that transiently expressed either human GATA-1 or GATA-2 cDNA and the probe spanning bp −115 to −90 of the gp91phox promoter (Fig. 6). The binding of GATA-1 (G1) and GATA-2 (G2) was abolished by the unlabeled wild-type competitor (Wt; lanes 3 and 10) and the GATA-binding site of the T-cell receptor δ gene (Gα; lanes 5 and 12), but not by a heterologous sequence (He; lanes 4 and 11) or the GATA site-mutated competitor (Mt; lanes 6 and 13). Furthermore, the GATA-1 complexes were supershifted by anti-GATA-1 antibody (SS; lane 8), and the GATA-2 complexes were abolished by anti-GATA-2 antibody (lane 15), contrasting with no effects by corresponding control antibodies (lanes 7 and 14). Nuclear extracts from untransfected COS-7 cells made no such complexes (lane 1). These results confirm that GATA-1 and GATA-2 specifically bind to the −98GATA site and support the use of these nuclear extracts in the kinetic analysis of the interaction between the site and these GATA proteins.

To determine the relative binding affinities of GATA-1 and GATA-2 for the −98GATA site, we determined the apparent Kd values from Scatchard plots obtained from EMSA data (Fig. 7). The amount of bound probe increased with increases in the concentration of the probe (panels A1 and B1). Scatchard plots for GATA-1 (panel A2) and GATA-2 (panel B2) revealed their
apparent $K_d$ values to be 0.13 and 0.10 nm, respectively, suggesting that both GATA proteins have similar affinities for the −98GATA site. Accordingly, GATA-1 and GATA-2 may efficiently compete with each other for binding to the site.

**GATA-1 and GATA-2 Individually Transactivate the gp91^phox Promoter through the −98GATA Site**—The above results indicate that both GATA-1 and GATA-2 similarly bind to the −98GATA site of the gp91^phox promoter. To determine whether either one or both of the GATA proteins transactivate the gp91^phox promoter, we individually cotransfected various amounts of their expression vectors with wild-type p−105/Luc into Jurkat cells, which express neither of the endogenous GATA proteins (Fig. 8). Up to 4 μg cotransfected GATA-1 and GATA-2 cDNAs dose-dependently and significantly ($p < 0.01$ and 0.05, respectively) increased gp91^phox promoter activity. At doses higher than 6 μg, the activity decreased similarly in both plasmids (panel A). The GA-to-CT mutation at the −98GATA site of p−105/Luc significantly decreased the GATA-1- and GATA-2-mediated promoter activities, to 30.5 ± 13.6 and 16.4 ± 16.2%, respectively (panels B and C). These results indicate that individually expressed GATA-1 and GATA-2 transactivate the gp91^phox promoter mostly through the −98GATA site.

**GATA-2 Is a Relative Competitive Inhibitor of GATA-1 for Transactivation of the gp91^phox Promoter**—To clarify each role of GATA-1 and GATA-2 in cells expressing both GATA proteins as eosinophils, GATA-1 and GATA-2 plasmids were cotransfected individually or in combination with the wild-type p−105/Luc reporter construct into Jurkat cells (Fig. 9). The amount of GATA-1 plasmid and that of GATA-2 were fixed to 2 μg, which gave 85 and 80%, respectively, of their maximal activities (Fig. 8A). Transiently expressed GATA-1 (Fig. 9A, second bar) produced a (15.5 ± 1.5)-fold increase above vehicle activity ($p < 0.001$ versus the first bar), and transiently expressed GATA-2 (third bar) produced a (1.0 ± 0.2)-fold increase ($p < 0.001$ versus the first bar). The combined expression of the two GATA proteins (fourth bar) exhibited an (8.3 ± 0.6)-fold increase, which is significantly lower than the increase obtained by GATA-1 alone ($p < 0.005$), suggesting that GATA-2 inhibits the transactivation of the gp91^phox promoter by GATA-1. This was confirmed in Fig. 9B. The amount of GATA-1 plasmid was also fixed here to 2 μg, but the amount of GATA-2 plasmid was increased from 0 to 4 μg to reach its maximal activity (Fig. 8A). Total amounts of GATA plasmids were 6 μg or less for avoiding their inhibitory effects. The combined expression of increased amounts of GATA-2 along with a fixed amount of GATA-1 impaired the ability of GATA-1 to transactivate the gp91^phox promoter in a dose-dependent fashion. Therefore, GATA-1 is the principal activator for the gp91^phox promoter, and GATA-2 is a relative competitive inhibitor in the presence of both GATA proteins, as in eosinophils.

**DISCUSSION**

In a previous report, we suggested the existence of an eosinophil-specific mechanism of gp91^phox gene expression (17). In this study, we have shown that the −98GATA site works as a positive cis-element for the gp91^phox promoter in an eosinophil lineage, but not in other lineages expressing gp91^phox. EMSAs have shown that GATA-1 and GATA-2 are the only specific DNA-binding proteins for the gp91^phox gene in eosinophils. These results suggest that expression of the gp91^phox gene is regulated by the combination of GATA-1 and GATA-2 through the −98GATA site, particularly in eosinophils. We also found that GATA-1 and GATA-2 individually bind to the −98GATA site with similar apparent dissociation constants and can individually work as positive regulators for the gp91^phox gene. However, GATA-2 inhibits the transactivation of the gene by GATA-1 in coexisting conditions, as in eosinophils. Taken together, our findings suggest that GATA-1 works as a principal activator and that GATA-2 works as a competitive inhibitor of GATA-1 for the expression of the gp91^phox gene in eosinophils. The negative regulation of the gene by GATA-2 can be done simply by competitive binding to the −98GATA site with GATA-1 with an assumption that GATA-2 is one order less effective than GATA-1 for the transactivation of the gene. However, inhibition of the interaction between GATA-1 and some protein factors by GATA-2 may also contribute to decreasing the GATA-1-dependent promoter activity of the gene.

At least six members have been identified in the GATA transcription factor family, which recognizes the consensus motif ((A/T)GATA(A/G)) through a highly conserved zinc finger. These results demonstrate that among them, GATA-1 and GATA-2 have overlapping functions, but their gene expression is regulated by the opposing activities of these factors.
DNA-binding domain (27). In hematopoietic tissues, GATA-1, GATA-2, and GATA-3 are expressed in distinct but overlapping patterns (31): GATA-1 in erythrocytes, megakaryocytes, mast cells, basophils, and eosinophils; GATA-2 in all these cells and neutrophils; and GATA-3 in T-lymphocytes, mast cells, and eosinophils. Only eosinophils among gp91(phox)-expressing cells express the gp91(phox) gene (32) and the human MBP gene (30). The MBP gene is, in particular, regulated by a combination of GATA-1 and GATA-2 (33). Some critical factor(s) for the expression of the MBP gene, but not that of the gp91(phox) gene, might have disappeared in the late stage of terminal differentiation to peripheral eosinophils. The expression of the gp91(phox) gene is regulated by various endogenous and exogenous mediators, including interferon-γ, tumor necrosis factor-α, and lipopolysaccharide (35). Some factors related to allergy and parasitic infections may specifically regulate the gp91(phox) gene in eosinophils through the GATA-1 system.

How does GATA-1 activate the gp91(phox) gene? GATA-1 directly interacts with transcription factors such as Sp1, EKLF (36, 42), and POG-1 (37). An acetylase of p300/CREBβ (38) also directly associates with GATA-1 as a coactivator. The p300/CREBβ acetylates GATA-1 and increases its binding to DNA, resulting in stimulation of GATA-1-dependent transcription (39–40). This mechanism of the ubiquitous cofactor may participate in the activation of the gp91(phox) promoter by GATA-1.

Expression of myeloid-specific genes is regulated by a combination of transcription factors generally expressed in hematopoietic cells (c-Myb, AML-1/CREBβ, and Ets-1) and lineage-specific or -restricted factors (GATA-1, PU.1, and C/EBP) (23). Recently, Yamaguchi et al. (30) demonstrated the transactivation of the MBP P2 promoter by C/EBPs, especially by C/EBPβ. C/EBPβ may also play a role in the activation of the gp91(phox) promoter in eosinophils because possible binding sites of C/EBPs are found in the promoter. Previously, we reported the deficient expression of gp91phox in the neutrophils, monocytes, and B-lymphocytes, but not in the eosinophils, of an X-linked chronic granulomatous disease patient (17). Further analyses suggested that the deficient binding of PU.1 to the gp91(phox) promoter due to the point mutation (C85T) at its PU.1 motif results in the deficient expression of the gp91(phox) gene in the patient (14). These findings suggest that eosinophils have their own gp91(phox) gene expression mechanism independent of PU.1. The GATA-mediated mechanism presented here is a strong candidate for this because the transactivation of the gp91(phox) gene by a combination of GATA-1 and GATA-2 has occurred in Jurkat cells, which have no PU.1.

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