The Role of Activating Protein 1 in the Transcriptional Regulation of the Human FCGR2B Promoter Mediated by the $-343 \text{ G} \rightarrow \text{C}$ Polymorphism Associated with Systemic Lupus Erythematosus*

Mikhail Olferev, Emi Masuda, Shizuko Tanaka, Marissa C. Blank, and Luminita Pricop

The inhibitory receptor FcγRIIb is a negative regulator of antibody production and inflammatory responses. The $-343 \text{ G} \rightarrow \text{C}$ polymorphism in the human FCGR2B promoter is associated with systemic lupus erythematosus. The $-343 \text{ C}$ mutant promoter has decreased transcriptional activity. In the present study, we show that the transcriptional change correlates with quantitative differences in the interaction of the activating protein 1 complex with the mutant FCGR2B promoter. Promoter pulldown and chromatin immunoprecipitation assays demonstrated binding of c-Jun to the FCGR2B promoter. Phosphorylation of c-Jun was accompanied by transactivation of both FCGR2B promoter variants, whereas dephosphorylation of c-Jun by an inhibitor of c-Jun N-terminal kinase, markedly decreased the promoter activities. The $-343 \text{ G} \rightarrow \text{C}$ substitution enabled the specific interaction of the transcription factor Yin-Yang 1 with the mutant FCGR2B promoter. Yin-Yang 1 competed with activating protein 1 for binding at the $-343$ site, and contributed to the repression of the mutant FCGR2B promoter activity. This mechanism could be responsible for the decreased expression of FcγRIIb associated with the $-343 \text{ C/C}$ homozygous FCGR2B genotype in lupus patients. These findings provide a rationale for the transcriptional defects mediated by the $-343 \text{ C/C}$ FCGR2B promoter polymorphism associated with systemic lupus erythematosus, and add to our understanding of the complex transcriptional regulation of the human FCGR2B promoter.

Fc $\gamma$-receptors (FcγR) bind IgG-containing immune complexes and mediate important immune functions such as phagocytosis, degranulation, antibody-dependent cellular cytotoxicity, and production of inflammatory mediators. Human hematopoetic cells express several FcγR isofoms encoded by seven separate genes. Unlike activating FcγR, FcγRIIb is unique in that it contains an immunoreceptor tyrosine-based inhibitory motif in the intracellular domain. A specific amino acid sequence in the immunoreceptor tyrosine-based inhibitory motif domain of FcγRIIb allows the recruitment of phosphatases and the initiation of inhibitory signaling. Cross-linking of FcγRIIb with activating receptors on B cells and mononuclear phagocytes leads to down-regulation of antibody production, phagocytosis, and cytokine secretion. Several lines of evidence demonstrate that FcγRIIb is important in the maintenance of self-tolerance. FcγRIIb deficiency is associated with spontaneous development of autoimmune manifestations in several mouse genetic backgrounds (1). Autoimmune prone mouse strains share an $\text{Fcgr2}$ promoter haplotype containing deletions and polymorphisms associated with reduced expression of FcγRIIb on the surface of activated B cells and macrophages (2–4). Whereas the engineered deletion and the natural deficiency in FcγRIIb confer susceptibility to development of lupus-like disease, transplantation of bone marrow cells transduced with FcγRIIb-expressing retrovirus restored the healthy phenotype (5). This body of data created the impetus for the study of FcγRIIb regulation and function in humans.

Genetic studies revealed a significant association of a single nucleotide polymorphism (SNP) encoding the amino acid substitution I232T in the transmembrane of FcγRIIb with systemic lupus erythematosus (SLE) in several racial groups (6–8). The SLE-associated transmembrane polymorphism (FcγRIIb-Thr$^{232}$) induced loss of inhibitory function of FcγRIIb through exclusion from sphingolipid rafts (9). In addition, several SNPs present in the FCGR2B promoter correlated with differences in regulatory function and associated with autoimmunity in certain racial groups (10,11). The $-343 \text{ G} \rightarrow \text{C}$ substitution identified by us in the proximal FCGR2B promoter correlates with decreased transcriptional activity and associates with SLE in European-Americans (11).
In the present study, we investigated the regulation of the human proximal FCGR2B promoter activity, and the mechanism underlying the defect mediated by the −343 C SNP associated with SLE. The −343 G → C substitution correlated with decreased promoter activity in transiently transfected cells. Electrophoretic mobility shift assays (EMSA) and supershift experiments indicated differences in the binding of activating protein 1 (AP-1) family members to the two FCGR2B promoter sequence variants, with decreased binding of c-Jun to the mutant sequence. The −343 G → C substitution created a new binding site for the nuclear factor Yin-Yang 1 (YY1) that interacted specifically with the mutant sequence competing with c-Jun for DNA binding. The inhibition of YY1 synthesis with specific short hairpin RNA (shRNA) resulted in the up-regulation of the mutant promoter activity. We identify a shift in the binding of YY1 versus c-Jun to the mutant promoter, and this mechanism could be responsible for the repression of FCGR2B promoter activity in SLE patients bearing the −343 C/C genotype. Our study has implications for the modulation of FCGR2B gene expression in SLE and other diseases having altered expression and function of inhibitory FcγRIIB as pathogenic factor.

EXPERIMENTAL PROCEDURES

Cell Cultures—Human U937, Raji, and CL-01 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (Invitrogen) (12). Cells were stimulated with 1 ng/ml phorbol 12-myristate 13-acetate (Bt,cAMP) (Sigma). The peptide inhibitor of c-Jun NH2-terminal kinase (JNKIII) Ac-YGRKKRRQRRR-gaba-ILKQ5MTLNA-DPV-GSLKPHLRK-NH2 (Calbiochem) was added to the cell cultures at the concentration of 10 μM.

Plasmids—The −537 to +43 fragment of the human FCGR2B promoter was amplified with primers containing recognition sites for KpnI in the forward primer (5′-GCGGCGTACCCGCACTCTGTACACATCT-3′) and XhoI in the reverse primer (5′-GCGCCTCGAGCACTCCGGAAGCCGTGTC-3′). The products were directionally ligated using a rapid DNA ligation kit (Roche) into the KpnI/XhoI site of the pGL3-Enhancer vectors (Promega, Madison, WI). Constructs containing G at position −343 were used as template to create −343 C constructs by site-directed mutagenesis using the QuikChange kit (Stratagene). The c-Jun trans-Reporting System PathDetect Kit containing the pFC-MEKK plasmid expressing mitogen-activated/extracellular signal-regulated kinase kinase kinase (MEKK) and the pFA2-cJUN plasmid expressing c-Jun was obtained from OriGene. The pCMV-Y1 vector expressing full-size YY1 was obtained from OriGene.

Cell Transfection and Reporter Assays—Transient transfection of CL-01 cells was performed with Lipofectamine (Invitrogen) according to the manufacturer’s instructions. CL-01 cells (1.6 × 10⁶ cell/ml) in Opti-MEM I medium (Invitrogen) were incubated with plasmid-Lipofectamine complexes for 5 h, and then supplemented with complete medium and cultured for 48 h. Firefly and Renilla luciferase activities were measured in cell lysates by the dual luciferase assay kit (Promega) using a Wallac MicroBeta Trilux 1450 microplate luminometer. Where indicated, CL-01 cells were co-transfected with shRNA against YY1 1 day prior to the luciferase reporter assay. The TransSilent YY1 shRNA and TransSilent control vector (Panomics) were transfected into CL-01 using AMAXA nucleofector kit. Where indicated, co-transfection with pFC-MEKK, pFA2-cJUN, and pCMV-Y1 was performed.

EMSA—Nuclear extracts from CL-01 and U937 cells were prepared as previously described (11). Oligonucleotides (Operon, Inc.) were annealed and radiolabeled with [α-32P]dCTP. Specific oligonucleotide competitors against AP-1, YY1, and SP1 were purchased from Santa Cruz.

Nuclear extracts from resting and activated CL-01 and U937 cells were incubated on ice for 10 min in EMSA binding buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.5 mM dithiothreitol, 1 mM EDTA, 0.05% Nonidet P-40, 4% glycerol) with 0.5 μg of poly(dI-dC), followed by addition of labeled probe. In EMSA competition studies, nuclear extracts were incubated with 200-fold excess of unlabeled competitor. Anti-c-Jun (number 06–225, Upstate Biolabs), anti-c-Fos, anti-JunB, anti-YY1, and anti-SP1 antibodies (Santa Cruz Biotechnology) were used in supershift assays.

Promoter Pulldown Assay—Biotinylated WT, MUT, and AP-1 oligonucleotides (Operon, Inc.) were annealed and coupled to streptavidin-agarose CL-4B (Sigma). Nuclear extracts from activated CL-01 cells were incubated overnight with agarose-coupled oligonucleotides. Agarose suspensions containing bound nuclear factors were applied to minicolumns, washed, and the bound nuclear proteins were eluted with 400 mM NaCl and separated in 10% SDS-PAGE. Immunoblotting was carried out with anti-YY1, -c-Fos, and –phospho-c-JunP73 antibodies.

Chromatin Immunoprecipitation (ChiP) Assay—ChiP assay was performed according to a published procedure, with several modifications (13). Briefly, CL-01 cells, harvested at baseline or after activation, were treated with 1% formaldehyde for 15 min. The cells were lysed and sonicated with SONICATOR 3000 (MISONIX). Supernatants were incubated overnight at 4 °C with anti-c-Jun antibodies (sc-45X, Santa Cruz Biotechnology) and anti-XBP (sc-7160X, Santa Cruz Biotechnology), followed by incubation with protein A-agarose in the presence of salmon sperm DNA. The chromatin fraction was eluted, treated with proteinase K, and precipitated. The DNA fragments were amplified by PCR using primers spanning regions −495 to −472 and −278 to −258 of the FCGR2B promoter.

RESULTS

We investigated the binding of nuclear factors to the nucleotide sequence (−369 to −330) containing the −343 G → C substitution (dbSNP number rs3219018) identified by us in the human FCGR2B promoter (11). Two FcyRIIB-expressing cell types, the B lymphoma cell line CL-01 and the myelomonocytic leukemia cell line U937, were used as sources for nuclear extracts. EMSA experiments were carried out with double-stranded oligonucleotide probes labeled with [α-32P]dCTP. The WT probe corresponded to the sequence of the common FCGR2B promoter that contained −343 G, whereas the MUT probe contained −343 C (sequences are shown in Table 1). Analysis of the EMSA pattern revealed that the WT and MUT
probes formed distinct DNA-protein complexes (Fig. 1, A and B). The WT probe formed complex b, and the MUT probe formed complexes b and c with nuclear extract from resting CL-01 cells (Fig. 1A, lanes 2 and 3). Nuclear extracts from U937 incubated with the MUT probe allowed the formation of complex c (Fig. 1B, lanes 2 and 3). Complex b was not evident with nuclear extracts from U937 cells, suggesting cell-specific differences in the binding of nuclear factors to the FCGR2B promoter probes.

To test the effect of cell activation on the binding of nuclear factors to the WT and MUT probes, we treated CL-01 cells and U937 cells with PMA:ION. Two slow migrating DNA-protein complexes, a1 and a2, were formed when the WT probe was incubated with nuclear extracts from PMA:ION-treated CL-01 and U937 cells (Fig. 1, A and B, lane 5). The formation of complex a1 and a2 with the MUT probe was weak in activated CL-01 and U937 cells (Fig. 1, A and B, lane 6).
Computer-based analysis of the FCGR2B sequence containing the −343 site indicated similarity with the core consensus binding sequence for AP-1 (TGACTCTA) (Fig. 1C). We compared the migration pattern of complex a1 and a2 with that of AP-1 by generating a probe (AP-1 probe) containing the canonical AP-1 binding motif (TGACTCTA) within the sequence of the FCGR2B promoter (Fig. 1C). The AP-1 probe formed bands with similar mobility with complexes a1 and a2 in activated CL-01 and U937 cells (Fig. 1, A and B, lane 7). These results suggested that AP-1 participated in the formation of activation-induced complex a1 and a2. The expression level of the AP-1 family members, c-Jun, JunB, and c-Fos in nuclear extracts was analyzed in Western blots. The activation of CL-01 and U937 cells with PMA:ION induced the phosphorylation and nuclear translocation of c-Jun, JunB, and c-Fos (Fig. 1D).

To verify the interaction of complexes a1 and a2 with the AP-1 binding sequence, we created an additional probe AP-1neg bearing a mutated sequence (TAAGAC) of the AP-1 core consensus binding motif (Table 1). The AP-1neg probe did not form activation-induced complexes a1 and a2 (Fig. 2A, lanes 1 and 4). The nuclear factors that participate in the formation of complex b interacted with the AP-1neg probe, indicating that complex b bound outside the −343 to −340 nucleotide sequence (Fig. 2A, lane 4).

To localize the interaction of nuclear proteins with the WT and MUT promoter sequences, we synthesized a competitor probe corresponding to the FCGR2B promoter left flanking sequence excluding the −343 site (C1). In addition, we synthesized competitor probes corresponding to the FCGR2B promoter right flanking sequence containing −343 G (C WT) and −343 C (C MUT) (Table 1). We performed EMSA with 200-fold excess of each unlabeled competitor probe. The addition of competitor C1 abolished the formation of complex b with the WT and MUT probes (Fig. 2A, lanes 5 and 6), suggesting that the interaction of complex b with the FCGR2B promoter sequence was upstream and outside of the −343 G/C polymorphic region. Competitor C1 added in excess did not abolish the formation of complexes a1 and c (Fig. 2A, lanes 5 and 6). Competitors C WT and C MUT did not influence the formation of complex b (Fig. 2A, lanes 7–10). C WT eliminated complex a1, but did not affect the formation of the MUT-specific complex c (Fig. 2A, lanes 7 and 8). C MUT eliminated the formation of complex a1 with the WT, and the formation of complex c with the MUT probe (Fig. 2A, lanes 9 and 10). Complex a1 was eliminated by 200-fold excess of AP-1 oligonucleotide competitor, confirming the interaction of AP-1 factors with the WT promoter sequence at that site (Fig. 2A, lanes 13 and 15). The excess SP1 competitor did not affect the formation of complex a1 (Fig. 2A, lanes 14 and 17).

We sought to identify the nuclear factors participating in the formation of the AP-1 transcriptional complex. Antibodies against SP1, used as control, did not form supershifts and did not eliminate the formation of the activation-induced complexes a1 and a2 (Fig. 2B, lane 1). Antibodies specific for c-Fos, c-Jun, and Jun-B enabled the formation of supershift bands with the WT probe (Fig. 2B, lanes 2–4). The incubation of nuclear extract from CL-01 cells with anti-c-Jun antibodies eliminated complex a1 (Fig. 2B, lane 3), whereas anti-c-Fos antibodies eliminated complex a2 (Fig. 2B, lane 2). These results suggested that in CL-01 cells, complex a1 is formed preferentially by c-Jun, and that c-Fos participates mainly in the formation of complex a2.

We investigated the direct interaction of c-Jun with the FCGR2B promoter by ChIP assay. Nuclear extracts were prepared from resting CL-01 cells (0 h) and CL-01 cells activated with PMA:ION for 3, 7, and 30 h (Fig. 2C). Immunoprecipitation with anti-c-Jun antibodies (sc-45X, Santa Cruz Biotechnology) generated a PCR product with specific primers for the FCGR2B promoter (Fig. 2C). We did not detect PCR products by amplification with FCGR2B-specific primers following chromatin immunoprecipitation with control antibody (anti-XBP...
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sc-7160X, Santa Cruz Biotechnology). These results demonstrate the direct interaction of c-Jun with the FCGR2B promoter and suggest that c-Jun could act as transcriptional regulator of the FCGR2B promoter activity at this site.

The formation of complex c was specific for the MUT probe in both CL-01 and U937 cells (Fig. 1, A and B, lanes 3 and 6). The nuclear factor YY1, also known as NF-E1 and UCRBP, has the core consensus binding sequence CCATNTT (14, 15). The −343 G → C mutation created a possible YY1 binding site (CCATCAC) to the FCGR2B promoter sequence (Fig. 1C). The MUT-specific complex c was eliminated by an excess of CYY1 competitor, used as control (Fig. 2A, lane 17). Supershift formation with YY1 antibodies, but not with SP1 antibodies, established the identity of complex c formed with the MUT probe as being YY1 (Fig. 2B, lanes 5 and 6). Western blot experiments did not show changes in the level of YY1 expression in resting or activated cells (Fig. 2B, right panel). These results suggested that the −343 G to C nucleotide substitution in the FCGR2B promoter created a new binding site for YY1 that may alter the transcriptional activity of the MUT promoter.

Complex a1 and a2 had the highest intensity with the AP-1 probe (Figs. 1, A and B, lane 7, and 2A, lane 3). The WT probe formed more intense a1 and a2 complexes compared with the MUT probe (Figs. 1, A and B, lanes 5 and 6, and 2A, lanes 1 and 2). These results pointed out a lower ability of the MUT sequence to bind AP-1 compared with the WT sequence.

To confirm that the MUT probe interacted less efficiently with the AP-1 complex, we performed EMSA experiments with nuclear extract from activated CL-01 cells in which 32P-labeled AP-1-specific probes were preincubated with the competitor oligonucleotides CWT and CMUT (Fig. 3A). The concentration of unlabeled CWT or CMUT ranged from 62.5– to 1000-fold excess. Analysis of the intensity of the AP-1 band indicated that 4 times more CMUT was necessary to attain 50% inhibition of AP-1 complex formation compared with using CWT (Fig. 3A, CWT lanes 2–6, and CMUT, lanes 7–11). The graphic representation of the inhibition of optical density of the AP-1 band by the increasing concentrations of CWT and CMUT is depicted in Fig. 3B.

We tested the ability of AP-1 and YY1 nuclear factors to bind to the WT, MUT, and AP-1 probes in pull-down assays. Biotinylated probes coupled to streptavidin-agarose beads were incubated with nuclear extract from activated CL-01 cells. Bound nuclear factors were eluted and analyzed by Western blotting using anti-c-Jun P73, anti-c-Fos, and anti-YY1 antibodies (sc-7160X, Santa Cruz Biotechnology). These results demonstrated the direct interaction of c-Jun with the FCGR2B promoter and suggest that c-Jun could act as transcriptional regulator of the FCGR2B promoter activity at this site.

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We tested whether the −343 G/C polymorphic region participates in transcriptional regulation of the FCGR2B promoter in luciferase reporter assays. WT and MUT promoter constructs (−537 to +43) were inserted into the pGL3-Enhancer vector upstream of the luciferase gene, and transfected into CL-01 cells. The −343 C promoter construct (MUT) had reduced promoter activity (0.6 ± 0.1, p < 0.01, n = 8) compared with the −343 G promoter construct (WT) in CL-01 cells (Fig. 4A).

As c-Jun was the major component of the AP-1 complex in activated CL-01 cells, we tested whether c-Jun participates in
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The cell permeable compound Bt2cAMP is a synthetic analog of cAMP. Bt2cAMP triggers intracellular signaling leading to the phosphorylation and activation of c-Jun by a mechanism involving activation of protein kinase A. We evaluated the role of Bt2cAMP in the transcriptional regulation of the FCGR2B promoter activity (Fig. 4D). Treatment of CL-01 cells with 1 mM Bt2cAMP for 48 h increased the luciferase activity mediated by the WT and MUT promoter constructs (1.8 ± 0.2, p < 0.01, n = 8 and 1.3 ± 0.2, p < 0.01, n = 8). The difference in relative luciferase activity between the two promoter constructs was evident before and after activation with Bt2cAMP (Fig. 4D). In Bt2cAMP-activated CL-01 cells, treatment with JNKIII decreased the luciferase activity of the WT and MUT reporter constructs to the basal level (0.8 ± 0.1, p < 0.01, n = 8), similar to the effect observed with PMA:ION-activated cells.

We tested whether overexpression and activation of c-Jun was associated with changes in the promoter activity of the WT and MUT constructs. CL-01 cells transfected with pFA2-cJUN plasmid expressed increased levels of c-Jun assessed by Western blotting (Fig. 4E). The activation of MEKK leads to phosphorylation of JNK and subsequent phosphorylation and activation of c-Jun. JNKIII disrupts the formation of c-Jun-JNK complexes, and prevents the phosphorylation and activation of c-Jun (16). Treatment of non-activated CL-01 cells with 10 μM JNKIII slightly lowered the luciferase activity driven by the WT reporter construct (Fig. 4A). The relative luciferase activity of the MUT promoter construct was higher after treatment of non-activated CL-01 cells with JNKIII compared with medium-treated cells (Fig. 4A). In activated CL-01 cells, treatment with JNKIII decreased the amount of phosphorylated c-Jun in Western blots (Fig. 4B). Activation of CL-01 cells with PMA:ION for 48 h markedly increased the luciferase activity driven by the WT (3.1 ± 0.4, p < 0.01, n = 8) and MUT (2.1 ± 0.2, p < 0.01, n = 8) promoter constructs (Fig. 4C). The difference in relative luciferase activity between WT and MUT remained evident after activation. Interestingly, treatment of PMA:ION-activated CL-01 cells with JNKIII reduced the luciferase activity of the WT (0.9 ± 0.1, p < 0.01, n = 8) and MUT (0.7 ± 0.2, p < 0.01, n = 8) reporter constructs to the basal level (Fig. 4C). These results suggested that phosphorylation and activation of c-Jun are critical for regulation of the FCGR2B promoter activity.

The transcriptional regulation of the WT and MUT promoter constructs. The inhibitor of c-Jun N-terminal kinase JNKIII is a fusion-penetrating peptide containing a fragment of the human c-Jun. JNKIII disrupts the formation of c-Jun-JNK complexes, and prevents the phosphorylation and activation of c-Jun (16). Treatment of non-activated CL-01 cells with 10 μM JNKIII slightly lowered the luciferase activity driven by the WT reporter construct (Fig. 4A). The relative luciferase activity of the MUT promoter construct was higher after treatment of non-activated CL-01 cells with JNKIII compared with medium-treated cells (Fig. 4A). In activated CL-01 cells, treatment with JNKIII decreased the amount of phosphorylated c-Jun in Western blots (Fig. 4B). Activation of CL-01 cells with PMA:ION for 48 h markedly increased the luciferase activity driven by the WT (3.1 ± 0.4, p < 0.01, n = 8) and MUT (2.1 ± 0.2, p < 0.01, n = 8) promoter constructs (Fig. 4C). The difference in relative luciferase activity between WT and MUT remained evident after activation. Interestingly, treatment of PMA:ION-activated CL-01 cells with JNKIII reduced the luciferase activity of the WT (0.9 ± 0.1, p < 0.01, n = 8) and MUT (0.7 ± 0.2, p < 0.01, n = 8) reporter constructs to the basal level (Fig. 4C). These results suggested that phosphorylation and activation of c-Jun are critical for regulation of the FCGR2B promoter activity.
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9) (Fig. 4F). Treatment with JNKIII of CL-01 cells co-transfected with pFA2-cJUN plus pFC-MEKK reduced the luciferase activity of the WT reporter construct (0.8 ± 0.1, p < 0.01, n = 9) (Fig. 4F). Co-transfection of the MUT construct with pFA2-cJUN plus pFC-MEKK was not associated with significant changes in luciferase activity (Fig. 4F).

Two sites in the proximal FCGR2B promoter sequence (−281 to −276 and −181 to −178) contain the core consensus binding sequence for YY1, thus creating putative binding sites for YY1 in both WT and MUT promoter constructs. We sought to investigate whether the interaction of YY1 and AP-1 with the −343 FCGR2B promoter sequence was associated with differences in the transcriptional activity mediated by the WT and MUT promoter constructs. We tested whether the co-transfection of YY1 with MEKK and c-Jun induced changes in the promoter activity of the WT and MUT constructs (Fig. 5A). The luciferase activity mediated by the WT promoter was down-regulated in CL-01 cells co-transfected with pFA2-cJUN plus pFC-MEKK plus pCMV-YY1 compared with cells transfected with pFA2-cJUN plus pFC-MEKK (0.9 ± 0.2 versus 1.2 ± 0.2, p < 0.001, n = 9) (Fig. 5A). Co-transfection of CL-01 cells with pFA2-cJUN plus pFC-MEKK plus pCMV-YY1 did not induce major changes in the transcriptional activity of the MUT promoter construct (Fig. 5A).

We investigated whether the inhibition of YY1 expression was associated with changes in the promoter activity of the WT and MUT constructs. Expression of YY1 was down-regulated with small hairpin RNA against YY1 expressing vector (shYY1). Partial inhibition of YY1 expression by shYY1 was observed in lysates of CL-01 cells by immunoblotting with anti-YY1 antibodies (Fig. 5B). CL-01 cells were transfected with shYY1 or with control shRNA (shContr), followed by transfection with WT and MUT reporter constructs. The WT promoter activity was not different in CL-01 cells transfected with plasmid expressing shYY1 compared with that of shContr, indicating that inhibition of YY1 did not affect the transcriptional activity of the WT promoter (Fig. 5C). The luciferase activity driven by the MUT promoter construct was significantly higher in CL-01 cells transfected with shYY1 compared with shCont (09 ± 0.3 versus 0.6 ± 0.2, p < 0.01, n = 9) (Fig. 5C). These results suggested that down-regulation of YY1 expression could restore, at least in part, the transcriptional activity of the MUT promoter construct. We believe that this effect is most likely the result of reduced binding of YY1 to the −343C promoter sequence in cells transfected with shYY1 plasmid.

DISCUSSION

FcγRIIb is widely expressed in cells of the immune system, such as B lymphocytes, monocytes, dendritic cells, neutrophils, mast cells, as well as in non-hematopoietic cells. Endowed with the ability to mediate inhibitory signaling upon binding IgG-containing immune complexes, FcγRIIb is an important regulator of antibody-mediated reactions. Generally, FcγRIIb deficiency is associated with increased susceptibility and severity to organ-specific and systemic autoimmune disease (17). Relatively small reductions in expression of FcγRIIb receptors contribute to the breakdown of self-tolerance (4, 18). Mice heterozygous for deletions in FcγRIIb exhibit only modest reductions in protein expression, but have a predisposition to autoimmunity (19). Recently, the expression of FcγRIIb was found to be up-regulated on memory B cells of healthy controls, whereas FcγRIIb was considerably decreased in memory B cells from SLE patients, reflecting inadequate inhibitory signaling in SLE memory B cells (20). These observations call for a detailed investigation of mechanisms involved in regulation of the FCGR2B gene.

Acquired and genetic factors regulate the expression of FcγRIIb and influence its inhibitory potential. Data from our group demonstrated differential modulation of FcγRIIb expression in human monocytes and neutrophils by cytokines suggesting that alterations in cytokine levels common during inflammatory and autoimmune reactions could cause changes in FcγRIIb expression (21–23). A role for hormones in the reg-
ulation of FCGR2B gene expression was revealed when dihydrotestosterone was found to inhibit FcyRIIb expression, suggesting a role for sex hormones in the onset and progression of autoimmune diseases (24).

More recently, inherited defects in FcyRIIb expression have been linked to the development of autoimmune diseases. The I232T substitution in the transmembrane domain of FcyRIIb is associated with autoimmunity in the Asian population (6–8, 25). The SLE-associated Thr232 allele does not alter membrane expression of FcyRIIb but is lacking functional inhibitory activity (9). A haplotype containing the −343 G → C SNP in the FCGR2B gene was found to associate with SLE in Caucasians (10). The association of the FCGR2B −343 C/C allele with SLE correlated with altered nuclear protein binding and with decreased transcriptional activity of the mutant FCGR2B promoter (11).

Primer extension experiments identified two transcription initiation sites in the FCGR2B promoter, and the minimal activity was contained within the first 154 nucleotides of the 5′-untranslated region (26). Several potential regulatory elements were identified upstream of the transcription initiation sites in the human FCGR2B promoter sequence (26, 27). Two members of the Kruppel family of transcription factors, zinc finger proteins ZNF140 and ZNF91, expressed in T cells possibly bind to the human FcyRIIIB promoter and could function as transcriptional repressors (26).

In the present study, we detected binding of AP-1 family members to the FCGR2B promoter sequence in activated CL-01 and U937 cells, despite the sequence variation in the AP-1 core consensus motif. The interaction of AP-1 transcription factors with the TGGTCA sequence contained in the HLA-DR promoter in B-cell lymphoma cells provides another example that AP-1 is capable to interact with non-canonical consensus DNA sequences (28). c-Jun was one of the main components of the AP-1 complex that interacted with the WT promoter in CL-01 cells. In addition, JunB and c-Fos participated in the formation of the AP-1 complex with the WT probe, most likely by forming heterodimers with c-Jun.

Our results suggest a critical role for c-Jun in the transcriptional regulation of the FCGR2B promoter activity. Increased cell activation induced by PMA and B_{2}cAMP was shown to up-regulate FcyRIIb expression and to increase the luciferase activity driven by FCGR2B promoter constructs in U937 cells (10, 29, 30). We find that the transcriptional activity of the WT and MUT promoter constructs is potently and consistently induced by PMA/iono and B_{2}cAMP. These activators trigger independent signaling pathways, ultimately leading to the phosphorylation and activation of c-Jun. JNKIII, a specific inhibitor of c-Jun activation, decreased the level of phosphorylated c-Jun and down-regulated the FCGR2B promoter activity. By inducing the synthesis and phosphorylation of c-Jun, JNK may regulate the transactivation of the FCGR2B promoter. Increased activation of JNK in B lymphocytes from SLE patients has been reported (31). Patients with rheumatoid arthritis have constitutive activation of JNK in synovial tissue (32, 33). The relationship between the increased activation of JNK during chronic inflammatory and autoimmune responses and the regulation of the FCGR2B gene expression remains to be established.

The homozygous −343 C/C genotype in the FCGR2B promoter is more frequent in SLE patients (10, 11). Analysis of luciferase activity in transiently transfected cells indicated reduced activity of the MUT promoter in both resting and activated cells. Reduced binding of AP-1 to the sequence containing −343 C correlated with down-regulation of the MUT promoter activity. In another report, a mutant FCGR2B promoter haplotype containing −343 C/C was associated with increased luciferase activity, presumably mediated by the interaction of YY1 and GATA-4 with the mutant promoter haplotype (34). In agreement with this report, our data show binding of YY1 to the MUT promoter sequence. YY1 has been reported to have a dual effect, acting as a repressor or activator of gene transcription depending on the promoter sequence surrounding the YY1 binding site (14, 35, 36). In several genes, competition for binding sequences with an activator was identified as the main mechanism for the suppressive activity of YY1 (36). The inhibition of YY1 synthesis with specific shRNA resulted in up-regulation of the transcriptional activity of the MUT reporter construct. Our results suggest that YY1 competes with AP-1 for DNA binding at the −343 site, a mechanism that could contribute to the transcriptional repression of the mutant FCGR2B promoter activity.

In summary, we propose a mechanism for a non-coding region polymorphism affecting FCGR2B gene activity in SLE. A schematic model showing the specific binding of YY1 to the sequence containing −343 C and the impaired interaction of the AP-1 transcriptional complex with the MUT promoter leading to down-regulation of the FCGR2B promoter activity is presented in Fig. 6. Our findings may lead to the development of therapeutic approaches for induction of FCGR2B gene expression in autoimmune diseases.
Regulation of FCGR2B Promoter Activity by AP-1

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