Interleukin-6 Induces Expression of *Ifi202*, an Interferon-inducible Candidate Gene for Lupus Susceptibility*

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Systemic lupus erythematosus (SLE) is a prototype autoimmune disease. In human SLE patients, as well as in mouse models of SLE, the development of disease is associated with increased levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6). However, IL-6 target genes contributing to the development of disease remain to be identified. Our previous studies of one mouse model of SLE identified an interferon-inducible gene, *Ifi202*, as a major contributor to the disease. We now report that IL-6 induces expression of the *Ifi202* gene. We found that IL-6 treatment of mouse splenocytes increased levels of *Ifi202* mRNA and p202 protein. Furthermore, IL-6 treatment of NIH 3T3 cells or expression of a constitutively active form of STAT3, a known mediator of IL-6 signaling, stimulated the activity of a 202-luc-reporter through a potential STAT3 DNA-binding site (the 202-SBS) present in the 5′-regulatory region of the *Ifi202* gene. Moreover, treatment of cells with IL-6 stimulated binding of the transcription factor STAT3 to an oligonucleotide containing the 202-SBSs in gel-mobility shift assays and to the 5′-regulatory region of the *Ifi202* gene in chromatin immunoprecipitation assays. Importantly, site-directed mutagenesis of 202-SBS or expression of a dominant negative form of STAT3 significantly reduced constitutive as well as IL-6-stimulated activity of the 202-luc-reporter. Together, our observations support the idea that IL-6 stimulates transcription of the *Ifi202* gene through STAT3 activation and predict that increased levels of IL-6 in lupus contribute to up-regulation of p202.

 oncogenes in human SLE patients and in mouse models of SLE, there is considerable evidence that SLE is a polygenic disease, with contributions from both major histocompatibility complex and multiple non-major histocompatibility complex genes (1, 4, 6, 7). Importantly, our recent studies in a mouse model of SLE have identified an interferon (IFN)-inducible gene, *Ifi202*, as a major genetic contribution to the development of SLE (8).

Elevated levels of endogenous cytokines, such as interleukin-6 (IL-6), have been found in human SLE patients (5). Moreover, the spontaneous production of IgG by peripheral blood mononuclear cells (PBMC) derived from SLE patients could be enhanced by exogenous IL-6 treatment (9). These observations suggest that IL-6 plays an important role in the pathogenesis of SLE. Consistent with this notion, increased levels of IL-6 enhance autoimmunity in well known mouse models of SLE, such as (NZB x NZWF1) mice (10–12). Furthermore, blockade of IL-6 signaling inhibits the onset of autoimmune kidney disease in (NZB x NZWF1) mice (13). The identity of IL-6 target genes contributing to the development of lupus disease remains unknown.

IL-6 was identified as a B-cell differentiation factor and also as a hybridoma-plasmacytoma growth factor (14, 15). It is a member of a cytokine family, which also includes IL-11, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotrophin-1, neurotrophin-1/B, cell stimulatory factor-3, and oncostatin M. The membrane receptor for these cytokines shares a common subunit, glycoprotein 130. IL-6 affects a variety of biological functions, such as immunoglobulin production, the acute phase inflammatory response, and plasmacytoma genesis by regulating cell growth, differentiation, and survival (14).

The transcription factor STAT3 (signal transducer and activator of transcription 3) is known to play a critical role in IL-6 signaling, resulting in transcriptional activation of a number of genes (14). Binding of IL-6 to its receptor, which is coupled to two glycoprotein 130 subunits, results in phosphorylation of STAT3 and its activation (14, 16). Activated STAT3 is known to form a homodimer or heterodimer with STAT1 and translocates into the nucleus, where it binds to the target sequence (STAT-binding site or SBS) in the promoter of various genes. STAT3 target genes encode c-Myc, c-Myb, JunB, p21 WAF1, interferon-regulated factor-1, and STAT3 itself (14, 15). Importantly, activated STAT3 is detected in PBMCs from patients with active SLE (17).

The *Ifi202* gene (encoding the protein p202) is the best characterized member of the interferon (IFN)-inducible 200-gene family (18, 19). The family in mice includes *Ifi202a*, *Ifi202b*, *Ifi203*, *Ifi204*, and *D3* and in humans *IFI16*, *MND4*, and *AIM2* (20, 21). Murine genes form a tight cluster on distal chromosome 1 (21), which is syntenic with a region on human chromosome 1q (22). In addition to the genetic evidence for a role of *Ifi202* in mouse lupus, studies in human lupus have mapped a
susceptibility locus in this region of human chromosome 1 (3, 7). Proteins encoded by the 200-gene family share at least one repeat of a partially conserved 200-amino acid domain (20, 21). Interestingly, autoantibodies to the IFI16 protein were detected in sera of SLE patients (23), and increased expression of myeloid nuclear differentiation antigen was detected in PB-MCs of arthritis patients (24), raising the possibility that increased expression of the p200 family proteins contributes to the development of autoimmune diseases, including SLE.

The protein p202 is a 52-kDa phosphoprotein, and its overexpression in a variety of transfected cell lines retards cell proliferation and increases cell survival (18, 19). Additionally, increased expression of p202 in splenic cells of the B6.Nba2 congenic strain of mice (congenic for the Nba2 locus derived from the NZB lupus-prone strain) is correlated with increased production of IgG antibodies, splenomegaly, and defects in apoptosis of B cells in vitro (8). Surprisingly, generation of NZB mice that are deficient for the IFN-α/β receptor did not result in a significant reduction in p202 levels in the spleens of old mice (25). These observations are consistent with the possibility that expression of Ifi202 gene can be regulated independent of type I interferon signaling. Because IL-6 has been implicated in the up-regulation of p202 expression.

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IL-6 Induces Ifi202 Expression

Materials and Methods

Cells and Reagents—Mouse splenocytes were derived from the B6.Nba2 strain of mice, maintained in the animal facilities of the University of Colorado Health Sciences Center, Denver, CO. Mouse NIH 3T3 fibroblasts were purchased from the American Type Culture Collection. AKR-2B embryonic fibroblasts have been described previously (26). NIH 3T3 and AKR-2B fibroblasts were maintained at low density in Dulbecco's modified Eagle's medium containing high glucose, supplemented with 10% calf serum and antibiotics in an incubator with 5% CO2. Recombinant mouse IL-6 was from Roche Applied Science supplemented with 10% calf serum and antibiotics in an incubator with 5% CO2. Recombinant mouse IL-6 was from Roche Applied Science supplemented with 10% calf serum and antibiotics in an incubator with 5% CO2.

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NIH 3T3 fibroblasts were purchased from the American Type Culture Collection. Mouse splenocytes were derived from the B6.Nba2 strain of mice, maintained in the animal facilities of the University of Colorado Health Sciences Center, Denver, CO. Mouse NIH 3T3 fibroblasts were purchased from the American Type Culture Collection.

We sought to determine whether IL-6 could regulate interferon signaling. Because IL-6 has been implicated in the regulation of STAT3, we used the Superscript one-step RT-PCR system from Invitrogen (Grand Island, NY). For Northern blotting, 25 μg of total cell RNA was loaded on a 1% agarose gel, electrophoresed, and transferred onto a nylon membrane. The ratio of Ifi202 to actin mRNA was calculated in units, with one unit being the ratio of Ifi202 mRNA to Actin mRNA in spleens of NZB female mice (age, 16 weeks).

RT-PCR and Northern Blotting—Total RNA was isolated using TRIzol reagent (Invitrogen), digested with DNAse I (to remove any DNA in the preparation), and 0.5 μg of RNA was used for RT-PCR reaction using a pair of Ifi202a-specific primers (forward primer: 5′-gtgtacctacatccagat-3′; reverse primer: 5′-cttagttgagctttgg-3′). For RT-PCR, we used the Superscript one-step RT-PCR system from Invitrogen (Grand Island, NY). For Northern blotting, 25 μg of total cell RNA was loaded on a 1% agarose gel, electrophoresed, and transferred onto a nylon membrane. The ratio of Ifi202 to actin mRNA was calculated in units, with one unit being the ratio of Ifi202 mRNA to Actin mRNA in spleens of NZB female mice (age, 16 weeks).

Chromatin Immunoprecipitation Assay—NIH 3T3 cells, grown to 60–80% confluence, were serum-starved for 1 day to decrease basal DNA-binding activity of STAT3 as described previously (28). After stimulation (6 h, IL-6 20 ng/ml), cells were washed and then cross-linked with 1% formaldehyde in phosphate-buffered saline at room temperature for 10 min. Cells were washed sequentially two times with 1 ml of ice-cold PBS, centrifuged, resuspended in 0.5 ml of lysis buffer (1% SDS, 10 mm EDTA, 50 mm Tris-HCl pH 8.0, 1 mm phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, and 50 μg/ml aprotinin) and lysed on ice for 30 s at the maximum setting. Supernatants were then recovered by centrifugation at 12,000 rpm for 10 min at 4 °C, diluted two times in dilution buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.0). Immunoprecipitations were performed for 2 h with no antibodies added (a negative control) or specific antibodies (sc-452x, Santa Cruz Biotechnology) to STAT3, and then 2 μg of sheared salmon sperm DNA and 20 μl of protein A/G-Sepharose (of 50% slurry) were further added for 1 h at 4 °C. Immunoprecipitates were washed sequentially for 10 min each in TSE I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl), and TSE III (0.5% LiCl, 1% EDTA, 0.5% NaCl, 0.5% sodium deoxycholate, 1% deoxycholate, 0.1% Triton X-100, 10 mM Tris-HCl, pH 8.0). Bead precipitates were then washed three times with TE buffer and eluted three times with 1% SDS, 0.1 M NaHCO3. Eluates were pooled and heated at 65 °C overnight (to reverse the formaldehyde cross-linking). Supernatants were then incubated for 1 h at 45 °C with proteinase K (80 μg/ml), and DNA was precipitated.

For PCR, 5 μl from a 20-μl DNA preparation was used for 40 cycles of amplifications. The following primers were used: region 383–402 forward primer (5′-gtgtacctgcgtccggtc-3′) and region 804–784 reverse primer (5′-acattacagctcttgattttg-3′) (see Fig. 7 in ref. 29).

Immunoblotting—Splenocytes or NIH 3T3 cells were collected in PBS and resuspended in a modified radioimmunoprecipitation assay (RIPA) buffer (8.0 mM Tris-HCl, pH 8.0, 0.5% sodium deoxycholate, 0.1% SDs), supplemented with protease inhibitors (leupeptin, 50 μg/ml; pepstatin A, 50 μg/ml; and phenylmethylsulfonyl fluoride, 1 mM) and incubated at 4 °C for 30 min. Cell lysates were sonicated briefly before centrifugation at 14,000 rpm in a microcentrifuge for 10 min at 4 °C. The supernatants were collected, and the concentration was determined by precipitation with Bio-Rad dye. Equal amounts of protein were processed for immunoblotting as described previously (26).

Gel-mobility Shift Assay—Nuclear extracts were prepared from NIH 3T3 cells as described previously (30). The protein concentration was measured with the Bio-Rad protein assay reagents. An oligonucleotide (9 nucleotides long) containing the potential STAT3 DNA-binding site present in the 5′-regulatory region of the Ifi202 gene binding site, or containing a STAT-binding site consensus sequence (purchased from Santa Cruz Biotechnology), was end-labeled with [γ-32P]ATP and polynucleotide kinase. Gel-purified labeled oligonucleotide (probe, 1

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IL-6 Induces Expression of p202—To determine whether IL-6 could regulate expression of p202, we incubated total splenocytes, derived from the B6.Nba2 congenic strain of mice (age 9–15 weeks), with increasing concentrations of IL-6 (for 6 h) and analyzed levels of Ifi202 mRNA by real-time quantitative PCR. Initial time-course analyses revealed maximum induction of Ifi202 mRNA at 6 h (data not shown). The treatment of splenocytes with IL-6 increased already constitutively expressed (8) levels of Ifi202 mRNA by real-time quantitative PCR. When treated with 20 ng/ml IL-6 for 6 h, total cell extracts were prepared from mouse splenocytes after their incubation with IL-6 as described in A above, and extracts were subjected to immunoblotting using antibodies to the indicated proteins as described under "Materials and Methods."

RESULTS

IL-6 Induces Ifi202 Expression—To determine whether IL-6 treatment of cells of NIH 3T3 cell line, an immortalized IL-6-responsive mouse fibroblast cell line (32–34), increases expression of p202. We initially treated fibroblasts with 1% serum. After twenty-four hours of incubation, cells were treated with IL-6 (20 ng/ml) for 6 h, as shown in Fig. 2A. IL-6 treatment significantly increased the steady-state levels of Ifi202 mRNA (probably both Ifi202a and Ifi202b). Because it is not possible to distinguish between Ifi202a and Ifi202b mRNAs by Northern blotting (35), we performed a semi-quantitative RT-PCR to determine whether levels of Ifi202a mRNA (detected in abundance as compared with Ifi202b mRNA; see Ref. 35) were increased by IL-6 treatment. As shown in Fig. 2B, IL-6 treatment of NIH 3T3 cells resulted in increased levels of Ifi202a-specific mRNA. Importantly, the increase in Ifi202a mRNA levels correlated with increases in p202 levels, phosphorylation of STAT3 at Tyr-705, indicating the activation of STAT3 by IL-6. Consistent with our observation in Fig. 2A and previous studies (31), levels of STAT3 were also increased after IL-6 treatment of NIH 3T3 cells (Fig. 2C). Similarly, IL-6 treatment of AKR-2B fibroblasts also resulted in up-regulation of p202 and STAT3, albeit to a lesser extent (data not shown).

Next, to test whether the IL-6-induced Ifi202 mRNA expression in NIH 3T3 cells was due to transcriptional activation of the Ifi202 gene, we performed promoter-reporter assays. Our several initial experiments revealed that treatment of cells with IL-6 in medium supplemented with 10% serum resulted in up-regulation of Ifi202 mRNA and protein. C, cultures of NIH 3T3 cells were either treated with IL-6 (20 ng/ml) for 6 h (lane 2) or, as a control, left untreated (lane 1). Total proteins isolated from cells were subjected to immunoblotting using antisera to p202, STAT3, or beta-actin. D, cultures of NIH 3T3 cells were transfected with 202-luc-reporter plasmid as described under "Materials and Methods." Twenty-four hours after transfections, culture medium was changed to the medium containing 5% serum. After twenty-four hours of incubation, cells were either left untreated (column 1) or stimulated with 30 ng/ml (column 2) or 60 ng/ml (column 3) of IL-6 for 6 h. Cells were harvested for dual luciferase assay as described under "Materials and Methods." The value of luciferase activity in cells not treated with IL-6 is set to 1.

treatment of cells with increasing concentrations of IL-6 (from 5–50 ng/ml) for 6 h or a fixed concentration of IL-6 (20 ng/ml) for increasing length of time (from 1 to 6 h) revealed that treatment of NIH 3T3 cells with 20 ng/ml IL-6 for 6 h was sufficient to induce the activity of the 202-luc-reporter above the constitutive basal levels (data not shown). Therefore, we treated NIH 3T3 cells with IL-6 (20 ng/ml for 6 h) and compared the levels of Ifi202 mRNA and protein with untreated cells. As shown in Fig. 2A, IL-6 treatment significantly increased the steady-state levels of Ifi202 mRNA (probably both Ifi202a and Ifi202b). Because it is not possible to distinguish between Ifi202a and Ifi202b mRNAs by Northern blotting (35), we performed a semi-quantitative RT-PCR to determine whether levels of Ifi202a mRNA (detected in abundance as compared with Ifi202b mRNA; see Ref. 35) were increased by IL-6 treatment. As shown in Fig. 2B, IL-6 treatment of NIH 3T3 cells resulted in increased levels of Ifi202a-specific mRNA. Importantly, the increase in Ifi202a mRNA levels correlated with increases in p202 levels (Fig. 2C). Consistent with our observation in Fig. 2A and previous studies (31), levels of STAT3 were also increased after IL-6 treatment of NIH 3T3 cells (Fig. 2C). Similarly, IL-6 treatment of AKR-2B fibroblasts also resulted in up-regulation of p202 and STAT3, albeit to a lesser extent (data not shown).
only moderate (35–55%) increases in the activity of 202-luc-reporter above the basal activity (data not shown). Therefore, we chose to treat cells with IL-6 in under reduced (1% serum) serum conditions to decrease the basal activity of the reporter. As shown in Fig. 2D, treatment of cells with IL-6 under reduced serum conditions resulted in significant (~2.5- to 3-fold) stimulation of the activity of 202-luc-reporter. These observations indicate that IL-6 treatment of NIH 3T3 cells stimulates transcription of the reporter gene, expression of which is driven by the 5′-regulatory region of the Ifi202 gene.

IL-6-activated STAT3 Binds to a Potential DNA Binding Site Present in the 5′-Regulatory Region of the Ifi202 Gene—It is well known that IL-6 exerts its effects, in part, through the activation of the transcription factor STAT3 (14). Therefore, we analyzed the 5′-regulatory sequence of the Ifi202 gene for the presence of STAT3 DNA-binding sites using the MatInspector 2.0 software program. The analysis revealed a potential STAT-binding site (SBS) of the N3 type (5′-TTACAGGAA), which is present in the 5′-regulatory region of the Ifi202 gene.

To determine whether this potential DNA-binding site can bind to IL-6-activated STAT3 in nuclear extracts, we chose to treat cells with IL-6 in under reduced (1% serum) serum conditions to decrease the basal activity of the reporter. As shown in Fig. 2D, treatment of cells with IL-6 under reduced serum conditions resulted in significant (~2.5- to 3-fold) stimulation of the activity of 202-luc-reporter. These observations indicate that IL-6 treatment of NIH 3T3 cells stimulates transcription of the reporter gene, expression of which is driven by the 5′-regulatory region of the Ifi202 gene.

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Underlining of the nucleotide indicates a variation from the STAT3 DNA-binding consensus sequence. See Ref. 36 for details.

| Gene/enhancer | Putative STAT-binding cis-acting element (N3 sites) |
|---------------|--------------------------------------------------|
| Consensus sequence | TTCNNNGAA |
| a/antichymotrypsin | TTACAGGAA |
| GBP-1 | TTATCTGAA |
| gp-130 | TTACGGGAA |
| MIG | TTATGTGAA |
| Stat3 | TGCCAGGAA |
| Ifi202 | TTACAGGAA |

A comparison of the STAT3 DNA-binding site in the Ifi202 gene with known STAT3 DNA-binding sites in other genes

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Table I

Underlining of the nucleotide indicates a variation from the STAT3 DNA-binding consensus sequence. See Ref. 36 for details.

Fig. 3. IL-6 activated STAT3 binds to a potential STAT DNA-binding site present in the 5′-regulatory region of the Ifi202 gene. A, nuclear extracts containing equal amounts of proteins, prepared from NIH 3T3 cells after the indicated treatments, were subjected to gel-mobility shift assays using an end-labeled oligonucleotide containing the 202-SBS. Extracts without any treatment (lane 2), extracts after IL-6 treatment (lane 3), extracts after incubation with 20-fold excess of cold oligonucleotide containing the STAT consensus sequence (lane 4), and extracts after incubation with 20-fold excess of cold oligonucleotide containing the 202-SBS (lane 5). As a control, the probe was also loaded without any incubation with nuclear extracts (lane 1). The arrow indicates increased binding of proteins to the probe after IL-6 treatment. B, nuclear extracts derived from IL-6-treated cells (lanes 2 and 3) were subjected to gel-mobility shift assays without any incubation (lane 2) or after incubation with antibodies to STAT3 (lane 3). As a control, the probe was also loaded without any incubation with nuclear extracts (lane 1). The arrow indicates the protein band, binding of which was induced by IL-6 treatment in panel A. C, nuclear extracts (used in B) containing equal amounts of proteins were subjected to gel-mobility shift assays. Extracts after IL-6 treatment (lane 2), extracts after incubation with 20-fold excess (lane 3), 40-fold excess (lane 4), 80-fold excess (lane 5), or 160-fold excess (lane 6) of cold oligonucleotide containing the STAT consensus sequence. As a control, the probe was also loaded without any incubation with nuclear extracts (lane 1). The arrow indicates a reduction in binding of proteins to the 202-SBS probe.
DNA-binding site present in the 5'-regulatory region of the *Ifi202* gene.

To confirm binding of STAT3 to the SBS in vivo, we performed chromatin immunoprecipitation assays. As shown in Fig. 4, some binding of STAT3 to the *Ifi202* regulatory region was evident in untreated cells (compare lane 5 with lane 3). However, treatment of cells with IL-6 significantly (>2.5-fold) increased binding of STAT3 to *Ifi202* regulatory region (compare lane 6 with 5). This observation is consistent with our other observations shown in Fig. 3A in which basal DNA-binding activity of STAT3 was detected in extracts derived from untreated NIH 3T3 cells.

**IL-6 Activates Transcription of the *Ifi202* Gene through a STAT3 DNA-binding Site in the Promoter**—Because the above studies indicated that STAT3 could bind to a potential STAT3 DNA-binding site present in the 5'-regulatory region of the *Ifi202* gene, we mutated this site to determine the effect on IL-6 stimulation. As shown in Fig. 5A, site-directed mutagenesis resulted in decreases in basal as well as IL-6-stimulated activity of the 202-luc-reporter, suggesting that the STAT3 DNA-binding site in *Ifi202* promoter is needed for constitutive as well as IL-6-induced transcriptional activation of the *Ifi202* gene.

To investigate the potential role of STAT3 in IL-6-mediated
The transcriptional activation of the Ifi202 gene, we tested whether expression of a dominant negative mutant of STAT3 (indicated as S3-DN (38)) in NIH 3T3 cells inhibits IL-6-induced stimulation of the 202-luc-reporter. As shown in Fig. 5D, transfection of a plasmid encoding S3-DN in NIH 3T3 cells resulted in increases in levels of STAT3 (compare lane 3 with 1), indicating that S3-DN was expressed. Importantly, expression of S3-DN in promoter-reporter assays (Fig. 5B) resulted in decreases in basal (compare column 2 with 1) as well as IL-6-stimulated (compare column 4 with 3) activity, indicating that STAT3 activity is needed for constitutive as well as IL-6-induced transcriptional activation of the Ifi202 gene.

To confirm that IL-6 stimulates the activity of the 202-luc-reporter through the activation of STAT3 DNA binding in Ifi202-promoter, we chose to compare the activity of the 202-luc-reporters (wild type and mutated) after expression of wild type STAT3 or a mutant of STAT3 (indicated as S3-C), which has been shown to be constitutively active (39). As shown in Fig. 5C, transfection of a plasmid encoding S3-C in NIH 3T3 cells resulted in increases in levels of STAT3 (compare lane 2 with 1), indicating that S3-C was expressed. Importantly, as shown in Fig. 5C, consistent with results described above, mutations in the STAT3 DNA-binding site resulted in a significant (>50%) decrease in the activity of 202-luc (compare column 2 with 1). Expression of STAT3 (column 4) or S3-C (column 6) did not result in stimulation of the activity of the mutated reporter. In contrast, expression of S3-C resulted in significant stimulation of the activity of the 202-luc-reporter (compare column 5 with column 1 or 3). These observations indicated that activation of STAT3 by IL-6 (or other cytokines) contributes to the transcriptional activation of the Ifi202 gene.

**DISCUSSION**

Our observations suggest that IL-6 can activate transcription of the Ifi202 gene in splenocytes or mouse fibroblast lines through activation of the transcription factor STAT3. Additionally, our observations provide a mechanism, independent of IFNs, by which levels of p202 could be up-regulated in cells of lupus-prone strains of mice. Increased levels of p202 in splenocytes derived from lupus-prone strains of mice, such as B6.Nra2 mice, are correlated with production of IgG autoantibodies, splenomegaly, and defects in apoptosis of B cells in vitro (8). In other lupus-prone strains of mice, these traits have been linked with defects in cell cycle progression and cell survival pathways (40–42). Importantly, forced expression of p202 in cultured cells results in retardation of cell proliferation (18, 19). Additionally, increased levels of p202 in a variety of cultured cells correlate with increased resistance to apoptosis (18, 19). The ability of p202 to retard cell cycle progression and increase cell survival may correlate with its ability to bind several transcription factors and inhibit their transcriptional activity (19). Thus, stimulation of expression of p202 by IL-6 could result in altered expression of numerous genes that are implicated in the development of autoimmunity.

Activation of STAT3 by IL-6 is also known to result in cell cycle arrest and increased cell survival in a variety of cell systems (14, 15). For example, treatment of M1 cells with IL-6 causes cell growth arrest, which is correlated with up-regulation of p21^{WAF1/CIP1} (43–45). Because up-regulation of p202 in a variety of cells correlates with retardation of cell proliferation and increased resistance to apoptosis (18, 19), our observations raise the possibility that IL-6-induced levels of p202 in IL-6-responsive immune cells contribute to inhibition of cell growth and increased cell survival.

Regulation of cell cycle plays an important role in lymphocyte differentiation, effector function and memory acquisition, tolerance induction, and apoptosis (42). The ability of p202 to up-regulate p21^{WAF1/CIP1} levels (46) suggests that IL-6 induction of p202 in lupus-prone strains of mice may contribute to the loss of self-tolerance by retardation cell proliferation. IL-6 is known to inhibit p53-induced apoptosis (47). Because p202 inhibits p53-induced apoptosis (27), it is conceivable that IL-6-induced increases in p202 mediate this inhibition. Moreover, increased levels of p202 inhibit p53-mediated transcription of its target genes (48). Because p53 inhibits transcription of the IL-6 gene (49, 50), inhibition of p53 activity by p202 in IL-6-producing cells could result in increased expression of IL-6. This could contribute to the increased levels of IL-6 in lupus-prone strains of mice (10–12).

The molecular mechanisms by which increased levels of p202 enhance the development of autoimmunity are currently unknown. Homozygous deletion of the Gadd45 gene, a transcriptional target of p53, in mice results in development of a lupus-like disease (51). Therefore, it is conceivable that increased levels of p202 inhibit p53-mediated transcription of the Gadd45 gene, thus, contributing to a lupus phenotype. The activity of E2F2 is needed for suppression of T cell proliferation and immunologic self-tolerance (52), and mice null for E2F2 also develop a lupus-like disease (41). Importantly, E2F2 appears to repress the transcription of the E2F2, which is required for normal S phase entry (41). On the contrary, E2F1 positively regulates the expression of E2F2. The protein p202 inhibits the transcriptional activity of a subset of E2F family members (19). Therefore, it is also possible that increased levels of IL-6 in lupus-prone strains of mice, by up-regulating p202 and inhibiting the activity of E2F2 (E2F1 and E2F2), impairs immune tolerance. Further work is in progress to test this possibility.

In summary, our observations support the possibility that increased levels of IL-6 in lupus-prone strains of mice activate transcription of the Ifi202 gene in responsive cells. Importantly, we predict that IL-6-induced up-regulation of p202 in cells of the immune system, by retarding cell cycle progression and inhibiting apoptosis, may contribute to increased susceptibility of mice to the development of lupus.

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