Activated STAT1 suppresses proliferation of cultured rat mesangial cells

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Background. JAK-STAT signaling has been shown to promote development and proliferation in lymphopoietic and hematopoietic lineages. We investigated the effect of activated STAT1 on mesangial cell proliferation.

Methods. Rat mesangial cells of primary culture (rMCs) were used in the following experiments: (*1*) Whole cell lysates were immunoblotted against JAK1 and JAK2. (*2*) Whole cell lysates and nuclear proteins were extracted from rMCs with or without treatment with interferon-γ, and immunoblotting was performed against both STAT1 and tyrosine (701)-phosphorylated STAT1. (*3*) rMCs and rMCs electroporated with either wild-type STAT1, mutated STAT1, or antibody against STAT1 were incubated with interferon-γ for 20 hours, followed by a further incubation with [³H]-thymidine for four hours.

Results. JAK1, JAK2, and STAT1 were detected in whole cell lysates, suggesting that JAK-STAT signaling could be activated by interferon-γ (INF-γ). Using an antibody specific for tyrosine-phosphorylated STAT1, we detected signal in the INF-γ–treated nuclear extracts, which showed translocation of phosphorylated STAT1 to the nucleus. [³H]-thymidine incorporation in the presence of INF-γ was significantly lower than that of control in a dose-dependent manner. The introduction of wild-type STAT1 enhanced the effect of interferon-γ and decreased [³H]-thymidine incorporation, whereas tyrosine-mutated (Y701F) STAT1 and SH2 domain (R602T)-mutated STAT1 reversed INF-γ–induced suppression of [³H]-thymidine incorporation. Electroinjected antibody against STAT1 increased [³H]-thymidine incorporation upon stimulation with INF-γ.

Conclusion. STAT1 activated by interferon-γ suppresses mesangial cell proliferation.

There is growing evidence that Janus kinases (JAKs), especially JAK2 [1, 2], and signal transducers and activators of transcription (STAT) pathways are closely associ-

ated with proliferation of lymphopoietic and hematopoietic lineages by themselves or in concert with other signal transduction pathways [3–6]. Erythropoietin, a cytokine that stimulates erythropoiesis, induces association of JAK2 with erythropoietin receptor [7–10]. Platelet-derived growth factor (PDGF) induces proliferation of vascular smooth muscle cells via activated JAK2 in concert with mitogen-activated protein (MAP) kinase cascades [11]. PDGF receptor stimulates tyrosine phosphorylation of JAK1 [12], and in addition to this pathway, the receptor itself can also directly phosphorylate and activate STAT1 in a JAK-independent way [13, 14]. Angiotensin II stimulates tyrosine phosphorylation of JAK2 and Tyk2 via type I angiotensin II receptor, which, in turn, activates STAT1, STAT2, and STAT3 and induces a cellular proliferative response in vascular smooth muscle cells [15]. The inactivation of intracellular STAT1 or STAT3 abolishes the proliferative effect of angiotensin II [11, 16, 17].

On the other hand, growth inhibition induced by epidermal growth factor, interferon-α (IFN-α), and IFN-γ requires STAT1 activation [18–20], and antiproliferative activity of IFN-γ is enhanced by prolonged activation of STAT1 [21]. Thus, it remains to be determined whether activated STAT1 has a proliferative or an antiproliferative effect. Mesangial cell proliferation causes the destruction of glomerular architecture in chronic glomerulonephritis, thereby leading to a loss of renal function. PDGF is well known to be a major candidate for mesangial cell proliferation in chronic glomerulonephritis [22], and angiotensin II may be associated with benign nephrosclerosis. In both cases, the signaling converges to STAT1 activation. PDGF activates not only STAT1, but also the MAP kinase cascade at the same time [11]. Thus, it is not clear whether STAT1 further accelerates mesangial cell proliferation in concert with MAP kinases, or STAT1 modulates and counteracts the proliferative effect of MAP kinases. The need to better understand the molecular basis for mesangial proliferative glomeru-

lonephritis is strong motivation for us to determine the precise role of STAT1.

Interferon-γ activation of JAK1 and JAK2 induces phosphorylation of STAT1 tyrosine residue (701) and formation of active homodimer, leading to nuclear translocation and DNA binding [23, 24]. Since the IFN-γ–activated signaling pathway involving STAT1 is simpler and more straightforward than other cytokines, and the signaling pathways of several growth factors converge to STAT1 in combination with the other signalings, we employed IFN-γ to stimulate the JAK-STAT pathway in this study. We investigated the effect of STAT1 activated by IFN-γ on mesangial cell proliferation and found that IFN-γ–suppressed [^{3}H]-thymidine incorporation was enhanced in the presence of transfected wild-type STAT1 and reversed by dominant-negative STAT1 (Y701F or R602T). Electroinjected antibody against STAT1 increased IFN-γ–suppressed [^{3}H]-thymidine incorporation. Our data suggest the growth-inhibitory role of activated STAT1 in mesangial proliferative glomerulonephritis.

METHODS

Reagents

Mouse recombinant IFN-γ was purchased from Sigma Chemical (St. Louis, MO, USA). Rabbit polyclonal antibody directed against phosphorylated tyrosine (Tyr) (701)-specific STAT1 was from New England Biolabs (Beverly, MA, USA). Mouse monoclonal antibody against STAT1 exclusively for electroinjection experiment and rabbit polyclonal antibodies against JAK1, JAK2, and STAT1 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Blocking peptides for JAK1, JAK2, and STAT1 were from Santa Cruz Biotechnology. [^{3}H]-labeled thymidine and the ECL kit for Western blotting detection reagents were purchased from Amersham (Buckinghamshire, UK). The β-galactosidase Enzyme Assay System and horseradish peroxidase-conjugated goat antimouse IgG and antirabbit IgG were from Promega (Madison, WI, USA).

Cell culture

Rat mesangial cells of primary culture (rMCs) were derived from isolated rat glomeruli as previously described [25]. Briefly, glomeruli obtained from renal cortices of Sprague-Dawley rats by sequential sieving were resuspended in RPMI 1640 medium buffered with 20 mmol/L HEPES and were supplemented with 20% fetal calf serum (FCS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The glomeruli were cultured at 37°C with 5% CO_2. rMCs used were in early passage.

Preparation of whole cell lysates

Rat mesangial cells were harvested, pelleted by centrifugation, and resuspended in 100 μL of lysis buffer [20 mmol/L HEPES, pH 7.5, 250 mmol/L KCl, 0.1 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.1 mmol/L egtazic acid (EGTA), 10% glycerol, 0.1% NP-40, 0.5 mmol/L phenylmethylsulfonyl fluoride (PMSF), 0.5 mmol/L dithiothreitol (DTT), 1 μg/mL aprotinin, and 1 μg/mL pepstatin A]. The cell suspension was vortexed vigorously on a platform with medium rotation for 30 minutes and centrifuged at 15000 r.p.m. for 30 minutes. The protein concentration of the supernatants was determined by colorimetric assay [26].

Preparation of nuclear extracts

Nuclear extracts were prepared as described previously [27] with minor modification. Briefly, rMCs were harvested with trypsin and spun down. After resuspending the cell pellets in 400 μL of ice-cold Buffer A (10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 1 mmol/L DTT, and 0.5 mmol/L PMSF), 25 μL of 10% Nonidet P-40 solution were added, and the tube was vigorously shaken on an orbital shaker for 30 seconds and centrifuged for 30 seconds in a microfuge. Nuclear pellets were resuspended in 100 μL of ice-cold Buffer C (20 mmol/L HEPES, pH 7.9, 400 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L DTT, 1 mmol/L PMSF, 1 μg/mL aprotinin, and 1 μg/mL pepstatin A) and vigorously rocked at 4°C for 15 minutes on a shaking platform. After centrifugation for 10 minutes at 4°C, supernatants were collected. The protein concentration was determined by colorimetric assay [26].

Western blotting

Whole cell lysates or nuclear extracts (10 μg of each) were electrophoresed on the polyacrylamide gel. After transfer to a polyvinylidene difluoride (PVDF) membrane, the blocking procedure was performed with 5% nonfat milk, and the membrane was incubated with antibody directed against either JAK1, JAK2, STAT1, or tyrosine (701)-phosphorylated STAT1. Blocking peptides for JAK1, JAK2, and STAT1 were incubated with respective antibodies for two hours at 4°C. After washing the membranes, peroxidase-conjugated secondary antibody was applied, and the protein of interest was identified by enhanced chemiluminescence (ECL kit) according to manufacturer's instructions.

Construction of expression vectors

cDNA for human STAT1 (a gift from Dr. James N. Ihle) was subcloned into the pXM expression vector. To generate point mutants for SH2 domain or tyrosine residue, the arginine residue at 602 or the tyrosine residue at 701 of STAT1 was replaced by threonine or phenylalanine, respectively, using polymerase chain reaction (PCR) with primers containing the appropriate mutations, and then the mutants were confirmed by sequencing using autosequencer (Applied Biosystems, Chiba,

Japan). These mutants were named R602T and Y701F, respectively, according the amino acid number.

Transfection and transient expression of wild-type STAT1, tyrosine-mutated STAT1 (Y701F), and SH2 domain-mutated (R602T) cDNAs

Rat mesangial cells were harvested and centrifuged at 800 r.p.m. for five minutes. Cells were washed once in Ca- and Mg-free phosphate-buffered saline (PBS) and then resuspended in potassium-rich PBS. The cell suspension was put into an electroporation chamber with empty vector or the plasmid containing cDNA for either wild-type STAT1, tyrosine-mutated STAT1 (Y701F), or SH2 domain-mutated STAT1 (R602T; 20 μg of each) and was then cotransfected with β-galactosidase expression plasmid (5 μg) by a single brief electric pulse with a field strength of 750 V/cm. After 24 hours, the cells were stimulated with IFN-γ (100 or 500 U/mL) for the additional 20 hours and then pulsed with [³H]-thymidine for 4 hours. Tetrachloric acid (TCA)-precipitable counts were determined for each sample.

Transfer of anti-STAT1 antibody into rMCs by electroporation

We electroinjected anti-STAT1 antibody according to the method described elsewhere [16, 17] with minor modification. Briefly, rMCs were harvested and centrifuged at 800 r.p.m. for five minutes. The cells were washed once in Ca- and Mg-free PBS and then resuspended in potassium-rich PBS. The cell suspension was transferred to an electroporation chamber containing monoclonal antibody against STAT1 at a final concentration of 10 μg/mL and exposed to a single brief electric pulse with a field strength of 750 V/cm. The cells were allowed to remain in the chamber for two minutes and then plated in RPMI media supplemented with 20% fetal bovine serum (FBS). After 24 hours, the cells were stimulated with IFN-γ (100 U/mL) for 20 hours and then pulsed with [³H]-thymidine for 4 hours. TCA-precipitable counts were determined for each sample.

Statistical analysis

Experiments were performed in triplicate in each experiment (Fig. 2B) or in duplicate (Figs. 4 and 5). Comparisons were made with Fisher's Protected Least Significant Difference (Figs. 2B, 4, and 5). A P value of < 0.05 (Figs. 4 and 5) or a $P < 0.01$ (Fig. 2B) was considered significant.

RESULTS

rMCs contain JAK1, JAK2, and STAT1

We adopted IFN-γ for the stimulation of the JAK-STAT pathway. Of all of the JAK and STAT isoforms, IFN-γ is known to use JAK1 and JAK2 and to phosphor-

ylate STAT1. To determine whether this signaling pathway also applied to rMCs, immunoblottings were performed against JAK1, JAK2, and STAT1 (Fig. 1 A–C). As nonspecific and confusing bands for JAK1, JAK2, and STAT1 were present, blocking peptides were used for each blotting. Compared with the usual immunoblotting on each left panel, there was a band in the respective blotting on the right that disappeared when the antibody incubated with blocking peptides was used. Therefore, the bands for JAK1, JAK2, and STAT1 were confirmed, respectively. We found that signal intensities of JAK1 and JAK2 in control were almost the same as those in IFN-γ and, furthermore, that STAT1 was localized in cytosol under the untreated condition. In Figure 1, we intended to show the existence of JAK1, JAK2, and STAT1. The translocation of phosphorylated STAT1 induced by IFN-γ is demonstrated in Figure 2.

Interferon-γ phosphorylates STAT1 and suppresses [³H]-thymidine incorporation

Rat mesangial cells responded to IFN-γ as expected, which was shown by the appearance of phosphorylated STAT1 in whole cell lysates (Fig. 2A). Phosphorylated STAT1 was detected in IFN-γ–treated nuclear extracts that originally did not contain STAT1 (Fig. 1C). These findings suggested that STAT1 was phosphorylated in cytosol and that the phosphorylated STAT1 translocated to the nucleus (Fig. 2A). IFN-γ suppressed [³H]-thymidine incorporation in a dose-dependent manner at concentrations of 10, 100, and 500 U/mL (Fig. 2B). There were significant differences between control and IFN-γ groups (*), between IFN-γ (10 U/mL) and IFN-γ (100 U/mL; **), and between IFN-γ (10 U/mL) and IFN-γ (500 U/mL; $**P < 0.01$). We infer from these data that of all the signaling pathways activated by IFN-γ, STAT1 is associated with the IFN-γ–induced suppressive effect on rMC proliferation. The causal relationship between STAT1 activation and suppression of rMC proliferation was examined in the following experiments.

### Activated STAT1 caused suppression of rMC proliferation

We performed in situ β-galactosidase assay in order to ascertain the introduction of ectopic DNA into cultured rMCs by electroporation (Fig. 3A). Transcription efficiency of the plasmid coding β-galactosidase was 17%. Overexpression of STAT1 protein was ascertained by Western blotting (Fig. 3B). To demonstrate our hypothesis that STAT1 is responsible for the IFN-γ–induced suppressive effect on rMC proliferation, IFN-γ–activated (100 and 500 U/mL) STAT1 signaling was enhanced by overexpressing wild-type STAT1 cDNA, and then the STAT1 signaling was suppressed by dominant-negative STAT1 cDNA (Fig. 4). There were significant differences between control transfected with empty vec-

A

B

C
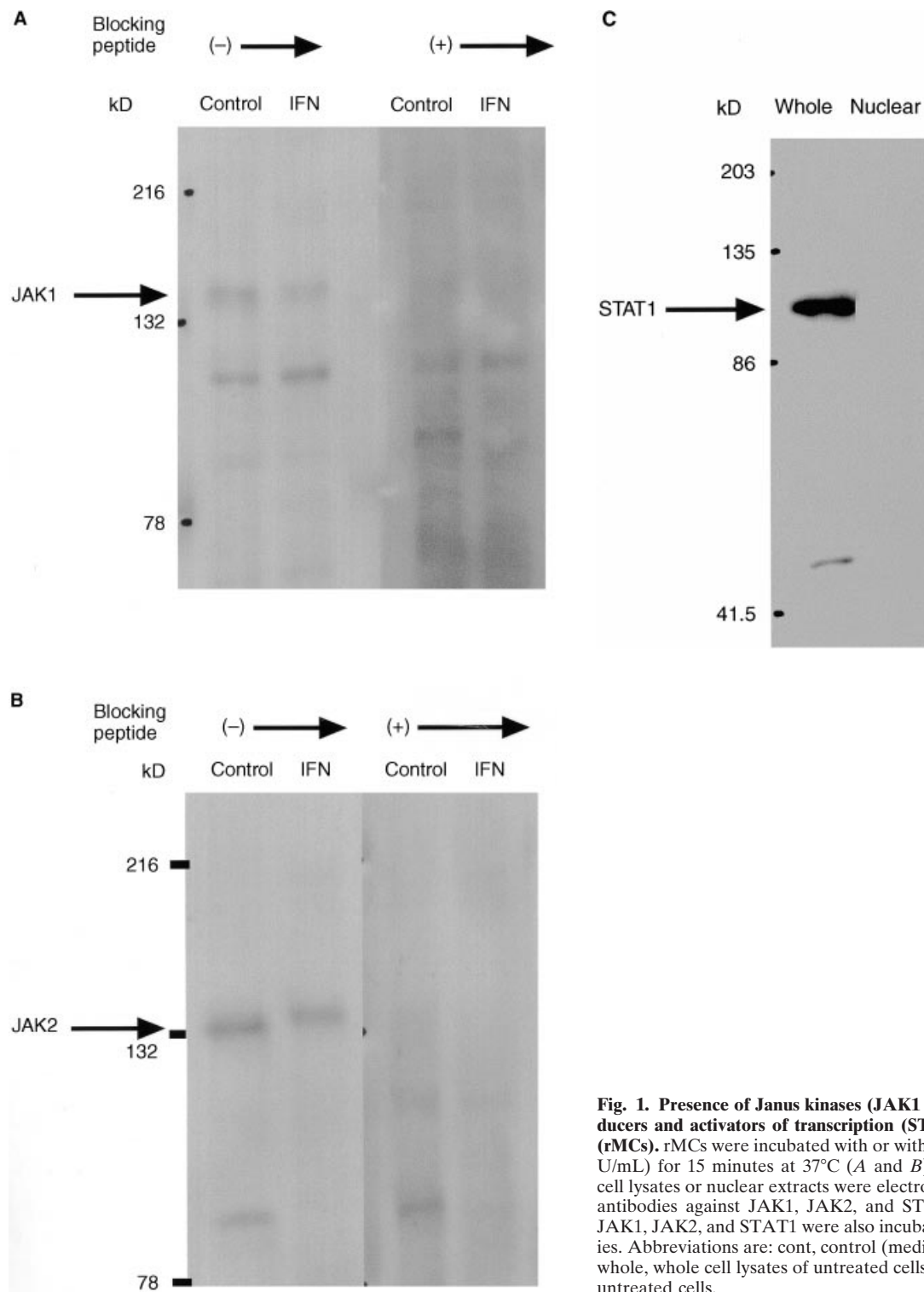
Fig. 1. Presence of Janus kinases (JAK1 and JAK2) and signal transducers and activators of transcription (STAT1) in rat mesangial cells (rMCs). rMCs were incubated with or without interferon-γ (IFN-γ; 100 U/mL) for 15 minutes at 37°C (*A* and *B*). Ten micrograms of whole cell lysates or nuclear extracts were electrophoresed and analyzed with antibodies against JAK1, JAK2, and STAT1. Blocking peptides for JAK1, JAK2, and STAT1 were also incubated with respective antibodies. Abbreviations are: cont, control (medium only); IFN, interferon-γ; whole, whole cell lysates of untreated cells; nuclear, nuclear extracts of untreated cells.

tor and the other groups (*) and between wild-type STAT1 and dominant-negative STAT1s (Y701F and R602T; **$P < 0.05$). Transfection of wild-type STAT1 decreased [³H]-thymidine incorporation, whereas that of tyrosine-mutated (Y701F) or SH2 domain-mutated

(R602T) STAT1 reversed IFN-γ–induced suppression. The recovery of [³H]-thymidine incorporation attained via disruption of intrinsic STAT1 activity by dominant-negative STAT1s was observed to the same extent in the electroinjection experiment using anti-STAT1 antibody

A

B
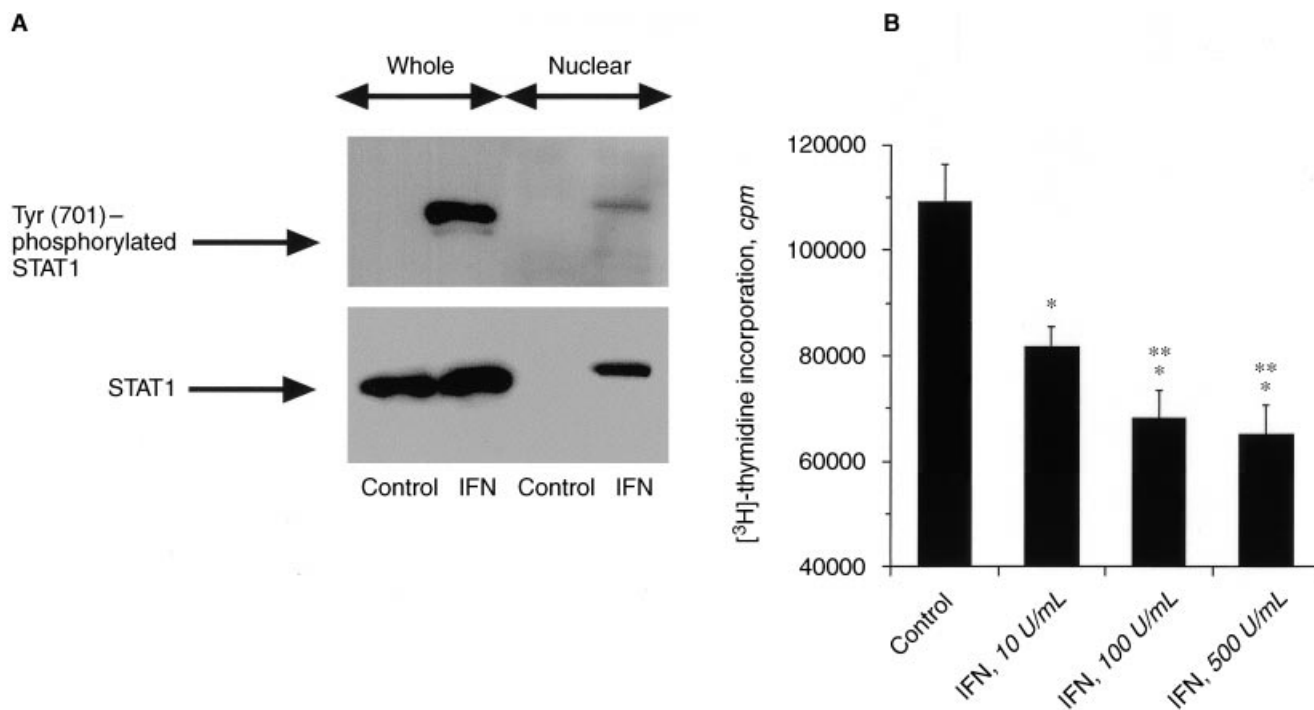
Fig. 2. (*A*) **IFN-γ phosphorylates STAT1, leading to translocation to the nucleus.** (*B*) IFN-γ suppresses mesangial cell proliferation. (A) rMCs were incubated with or without IFN-γ (100 U/mL for nuclear extracts and 500 U/mL for whole cell lysates) for 15 minutes at 37°C, and 10 μg of whole cell lysates or nuclear extracts were electrophoresed and analyzed with antibody against either STAT1 or Tyr (701)-phosphorylated STAT1. The left upper two lanes show that STAT1 is phosphorylated by IFN-γ as expected in Figure 1, and the right upper two lanes show that Tyr (701)-phosphorylated STAT1 translocates to the nucleus. The left half of the lower panel shows the presence of STAT1 under IFN-γ-stimulated and -unstimulated conditions, and the right half of the lower panel shows the appearance of STAT1 in nuclear extracts of IFN-stimulated cells. (B) rMCs cultured onto 24-well plates were incubated with increasing concentrations of IFN-γ (10, 100, and 500 U/mL, respectively) for 20 hours; thereafter, cells were subject to further incubation for four hours with [³H]-thymidine, and TCA-precipitable counts were determined. Control, 109104 ± 7296 cpm; IFN (10 U/mL) 81545 ± 3789 cpm; IFN (100 U/mL), 68256 ± 5094 cpm; IFN (500 U/mL), 64980 ± 5691 cpm. The data are expressed as mean ± SD (*N* = 4). There were significant differences between control and IFN groups (*) and between IFN (10 U/mL) and other IFN groups (**). Abbreviations are: control, medium only; IFN, interferon-γ; whole, whole cell lysates; nuclear, nuclear extracts; TCA, tetrachloric acid.

(Fig. 5). These data demonstrate that the IFN-γ–induced suppression of rMC proliferation is mediated, at least in part, through the activated STAT1.

DISCUSSION

Many cytokines that are associated with cellular proliferation, such as PDGF, angiotensin II, erythropoietin, IFN-α, and IFN-γ, activate JAKs, and STATs [9, 11, 12, 15, 23, 28–34]. PDGF in particular induces mesangial cell proliferation and is believed to play an important role for progression of renal dysfunction in chronic glomerulonephritis [22, 35–40]. Angiotensin II has a profound effect on proliferation of vascular smooth muscle cells [41] and may be a cause of benign nephrosclerosis [42], in concert with high blood pressure itself. However, the precise role of STAT1 in mesangial cell proliferation in chronic glomerulonephritis remains unclear, because activated STAT1 induces proliferation in some cell types and suppresses proliferation in others [11, 18, 19, 21]. These differences in the effects of activated STAT1 are probably caused by cell-type specificity, ligand specificity, and the complexity of signal integrity.

As for cell-type specificity and ligand specificity, the inhibition of STAT1 or STAT3 abolishes the proliferative effect of angiotensin II in vascular smooth muscle cells [11]. On the other hand, antiproliferative activity of IFN-γ is enhanced in NIH 3T3 cells by the specific inhibition of tyrosine dephosphorylation of STAT1, ultimately resulting in the constitutive phosphorylation on Tyr (701) residue and the prolonged activation of STAT1 [21]. Furthermore, the growth inhibition induced by epidermal growth factor, IFN-α, and IFN-γ requires STAT1 activation in U3A cells, mouse embryonic fibroblasts, and A431 cells [18, 19].

As for the complexity of signal integrity, PDGF activates STAT1 and stimulates proliferation of vascular smooth muscle cells in concert with MAP kinase cascades [11].

Treatment with IFN-γ results in recruitment of JAK2 into IFN-γ receptor-JAK1 complex [43] and activates
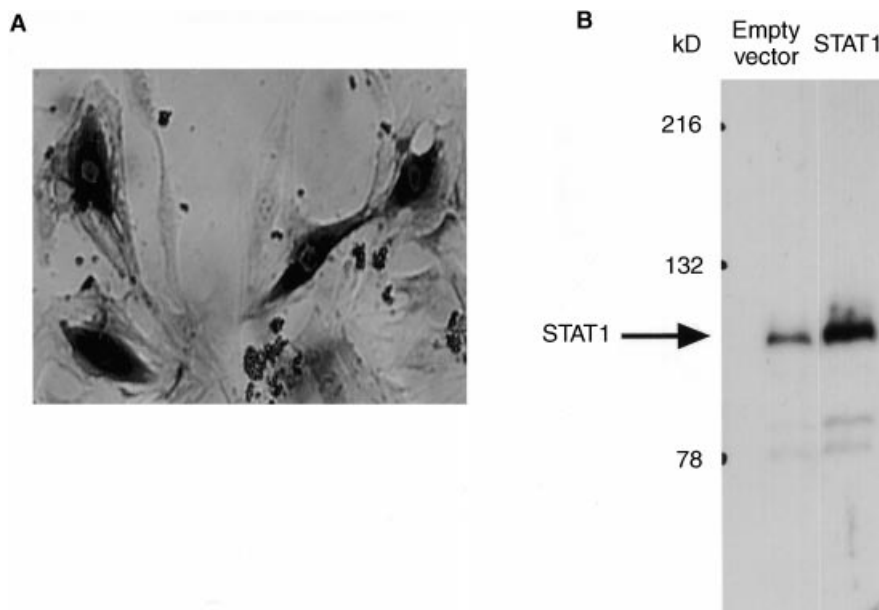
Fig. 3. (A) Introduction of β-galactosidase expression plasmid into rMCs by electroporation. (*B*) The presence of overexpressed STAT1 protein in rMCs electroporated with wild-type STAT1. (A) The β-galactosidase expression plasmid (30 μg) was electroporated into rMCs. After 24 hours, the cells were subject to in situ staining for β-galactosidase. The figure shown is representative of three independent experiments. (B) Wild-type STAT1 plasmid was electroporated into rMCs. Whole cell lysates were made after 24 hours. Twenty micrograms of the lysates were analyzed by Western blotting against STAT1.
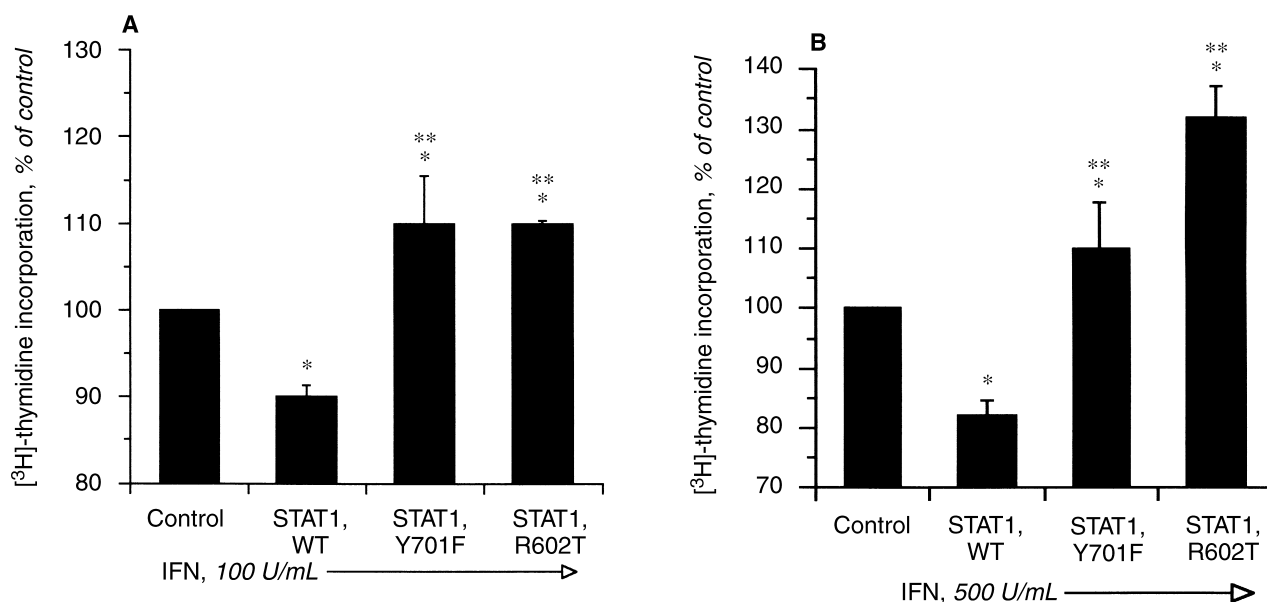
Fig. 4. (A) [³H]-thymidine incorporation is enhanced by electroporated wild-type STAT1 and reversed by Tyr-mutated (Y701F) STAT1 or SH2-mutated (R602T) STAT1 in the presence of 100 U/mL (A) or 500 U/mL (B) of IFN-γ. (A and B) The plasmids containing cDNA for wild-type STAT1, Tyr-mutated STAT1 (Y701F), or SH2-mutated (R602T) STAT1 (20 μg of each) were cotransfected with β-galactosidase expression plasmid (5 μg) into rMCs by electroporation. In control cells, the same amount of empty vector as that of STAT1 was cotransfected with β-galactosidase plasmid. After 24 hours, cells were stimulated with IFN-γ (100 or 500 U/mL) for 20 hours, followed by further incubation with [³H]-thymidine for 4 hours. TCA-precipitable counts were determined for each sample: control, 100%; STAT1, WT, 90 ± 2.3%; STAT1, Y701F, 110 ± 9.6%; STAT1, R602T, 110 ± 0.58% for IFN-γ (100 U/mL, $N = 3$) and cont, 100%; STAT1, WT, 82 ± 5.2%; STAT1, Y701F, 110 ± 15%; STAT1, R602T, 132 ± 11% for IFN-γ (500 U/mL, $N = 4$). The data are shown as mean ± SEM and are expressed as a percentage of control. Abbreviations are: control, medium only; IFN, interferon-γ; TCA, tetrachloric acid.
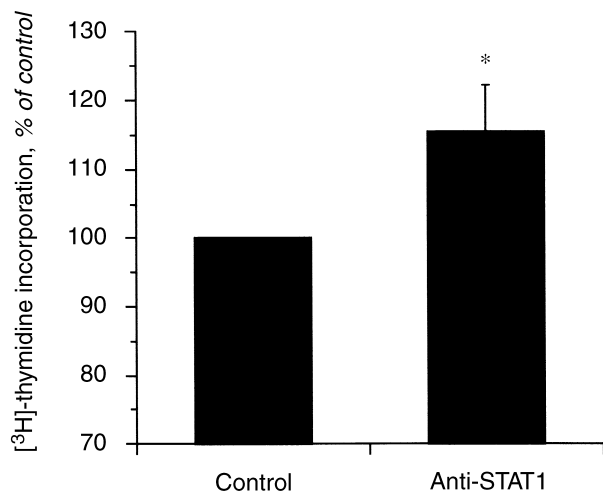
Fig. 5. Electroinjected anti-STAT1 antibody inhibits antiproliferative effect of IFN-γ on mesangial cell proliferation. The cell suspension was put into an electroporation chamber in the presence of either monoclonal antibody against STAT1 (10 µg/mL) or nonimmunized mouse IgG for control (10 µg/mL) and was exposed to a single brief electric pulse. Control mesangial cells or cells electroinjected with anti-STAT1 antibody were incubated with IFN-γ (100 U/mL) for 20 hours, followed by further incubation with [³H]-thymidine for 4 hours. TCA-precipitable counts were determined for each sample: cont, 100%; anti-STAT1, 115.6 ± 6.7% ($N = 5$). The data are shown as mean ± SD and are expressed as percentage of cont. There was significant difference between cont and anti-STAT1 (*$P < 0.05$). Abbreviations are: cont, control (culture media with nonimmunized mouse IgG); IFN, interferon-γ (100 U/mL); anti-STAT1, electroporated antibody against STAT1; TCA, tetrachloric acid.

solely STAT1 of all the STAT isoforms [23]. Therefore, STAT1 is the most important candidate to assess the role of STATs in our settings. In the present study, we found that under the switch-on condition of IFN-γ signaling, the effect of STAT1 was enhanced by transfection of wild-type STAT1 and was suppressed by tyrosine-mutated (Y701F) STAT1, SH2 domain-mutated (R602T) STAT1, and electroinjected anti-STAT1 antibody. These three STAT1 plasmids and anti-STAT1 antibody fluctuated only the activity of STAT1 under the constant activation of JAK1 and JAK2. This strategy enables us to assess the specific effect of STAT1 on cellular proliferation by separating STAT1 signaling from JAK1 and JAK2.

Tyrosine-mutated STAT1, SH2 domain-mutated STAT1, and electroinjected anti-STAT1 antibody were expected to inhibit the suppressive effect of IFN-γ to theoretically almost the same extent, because all three procedures caused the same effect. First, tyrosine-mutated STAT1 could not be phosphorylated upon stimulation with IFN-γ, which made it impossible to dimerize STAT1. Second, mutation of the SH2 domain in STAT1 interfered with the interaction between tyrosine-phosphorylated STAT1s, and this also inhibited dimerization. Third, electroinjected anti-STAT1 antibody bound to

intracellular STAT1 and blocked dimerization. Our results met this expectation and thus supported the authenticity of the data.

Although there is a possibility that endogenous STAT1 plays a rate-limiting role in the suppressive effect of IFN-γ, our data show that overexpressed STAT1 strengthens the effect. This suggests that the STAT1-binding promoter region (γ-activated site) is not saturated with phosphorylated STAT1 caused either by partial phosphorylation of all the existing STAT1 or to inadequate amounts of STAT1 in cytosol. If there are inadequate amounts of STAT1 in cytosol, then increased amounts of STAT1 as a substrate of JAK1 and JAK2 give rise to more phosphorylated STAT1, thereby resulting in the enhanced effect of IFN-γ, which is consistent with our data.

We first demonstrated that activated STAT1 suppressed mesangial cell proliferation. Together with findings of previous reports, our findings suggest that mesangial cell proliferation is positively regulated by MAP kinase cascade [11] and negatively regulated by STAT1 signaling, and furthermore, that the predominant effect of PDGF and angiotensin II is the activation of MAP kinase cascade, which results in a net effect of proliferation. Our hypothesis on the dual regulation of proliferation is supported by bifunctional effects of IFN-γ observed in KG-1a cells, a cell line of acute myelocytic leukemia, and primary cultures of BM CD34^{+} cells [44].

Marra, Choudhury, and Abboud found that IFN-γ by itself had very little effect on DNA synthesis in mesangial cells and that pretreatment with IFN-γ markedly potentiated the DNA-binding activity of STAT1 in response to PDGF or epidermal growth factor [45]. This discrepancy between our results and Marra's may be attributable to the different concentrations of IFN-γ, the species difference of mesangial cells, differences in the mesangial cell character, which may reflect distinct basal [³H]-thymidine incorporation, and different concentrations of endotoxin that might be contained in the serum supplemented to culture media.

In conclusion, STAT1 activated by IFN-γ inhibits proliferation of cultured rMCs.

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