Signal Transducer and Activator of Transcription 3α and Specificity Protein 1 Interact to Upregulate Intercellular Adhesion Molecule-1 in Ischemic–Reperfused Myocardium and Vascular Endothelium

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Objective—Intercellular adhesion molecule-1 (ICAM-1) is upregulated rapidly on endothelial cells during ischemia–reperfusion (I-R) and mediates tissue leukocyte accumulation. The ICAM-1 proximal promoter contains a signal transducer and activator of transcription (Stat) binding motif (gamma-interferon activation site [GAS] sequence), which flanks a specificity protein 1 (Sp1) binding site. We examined the roles of Stat and Sp1 in the regulation of ICAM-1 after myocardial I-R.

Methods and Results—Open-chest anesthetized rats underwent coronary artery occlusion for 35 minutes and reperfusion for 0 to 240 minutes. Stat became activated within 15 minutes after reperfusion, primarily in vascular endothelial cells; the activated Stat protein was identified as Stat3 (α-isoform). After phosphorylation on serine 727 (p-S727), Stat3α was found in association with the transcriptional regulator Sp1, and the complex bound to an ICAM-1–GAS probe. ICAM-1 expression increased after I-R and lagged shortly behind Stat3α activation. In cultured human umbilical vein endothelial (HUVE) cells, activation of Stat3α after hypoxia–reoxygenation (H-R) was dependent on the small GTPase Rac1. Transfection of a dominant-negative Stat3 (Y705F) adenovirus or a GAS decoy oligonucleotide reduced ICAM-1 mRNA expression after H-R. Using a reporter gene transfected into HUVE cells, mutation of the GAS element in the ICAM-1 promoter resulted in reduced transcriptional activity after H-R. Sp1 coimmunoprecipitated with p-S727 Stat3 during H-R, and Sp1 or Stat3α interfering RNA markedly reduced ICAM-1 mRNA expression.

Conclusion—The Sp1–Stat3 complex appears to play an important role in the upregulation of ICAM-1 transcription after reoxygenation or reperfusion. **(*Arterioscler Thromb Vasc Biol.* 2005;25:1395-1400.)**

Key Words: adhesion molecule ■ signal transduction ■ ischemia–reperfusion ■ myocardium ■ endothelial cell

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Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein that is highly expressed in vascular endothelial cells, promotes leukocyte activity in a variety of inflammatory reactions, and plays an important role in mediating neutrophil adherence and tissue injury during reperfusion after ischemia.[1,2] The ICAM-1 proximal promoter contains a signal transducer and activator of transcription 1 (Stat1)/Stat3 binding motif (GAS sequence or palindromic interferon response element), which flanks a specificity protein 1 (Sp1) binding site and a promoter cis-acting sequence (TATAA) box. Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) and superoxide, and proinflammatory cytokines such as interleukin-6 (IL-6) and interferon-γ (IFN-γ), are known to induce transcriptional complexes that bind to the ICAM-1 GAS sequence.[3–5] Mutation or deletion of this element decreases ICAM-1 promoter activity.[4,5]

In the past 10 years, Stat proteins have been studied intensively as cellular transcriptional regulators. Various Stat proteins, including Stat1 and Stat3, have been reported to be activated in ischemia–reperfusion (I-R).[6,7] However, the role of Stats in I-R is unclear because Stats appear to mediate a broad range of seemingly conflicting processes, including apoptosis, survival pathways, proliferation, angiogenesis, immunity, and host defense.[8] Stat3 was first identified as a factor in the acute phase reaction;[9] after induction by IL-6, Stat3 acts as a transcription factor for many proinflammatory genes, including ICAM-1.[10] Previous studies have focused on nuclear factor κB (NF-κB) and activator protein-1 (Ap-1) as regulators of inflammatory gene expression after I-R[11–13] but have not addressed the possible role of Stat3. Our study reports that Stat3α,

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after phosphorylation of serine 727 (p-S727), binds to the transcriptional regulator Sp1, and that this complex appears to play an important role in ICAM-1 upregulation after I-R in the heart, or after hypoxia–reoxygenation (H-R) in vascular endothelial cells.

Materials and Methods

Materials

Single-stranded decoy oligonucleotides, including rat and human ICAM-1–GAS decoys and a mutant human GAS decoy (mGAS), were synthesized by the Johns Hopkins University DNA analysis facility. Small, short double-stranded interference RNAs (siRNA) for Stat3[14] and Sp1[15] and a negative control siRNA were synthesized by Ambion, Inc. The cDNAs of activated Stat3 (Stat3-C) and dominant-negative Stat3 (DN-Stat3), containing a Y705F mutation, were provided by Dr James E. Darnell, Jr. (Rockefeller University, New York, NY). The cDNAs of Stat3-C, DN-Stat3, Rac1 V12, and Rac1 N17 were incorporated into adenoviral vectors as described previously.[16,17] A 1.3-kb ICAM-1 promoter construct (pBHluc 1.3) was provided by Dr Asrar B. Malik (University of Illinois, Chicago). For decoy oligonucleotide structures and additional information about reagents, see the online supplement, available at http://atvb.ahajournals.org.

In Vivo Experiments

Healthy adult male Lewis rats (Harlan; Indianapolis, Ind) were anesthetized (thiopental sodium, 25 mg/kg IP; followed by 1.5 to 2.5% isofluorane) and underwent thoracotomy and occlusion of the anterior branch of left coronary artery for 35 minutes, followed by reperfusion for 15, 30, 60, 120, 180, or 240 minutes. Sham rats had anesthesia and thoracotomy without coronary artery occlusion. At the end of reperfusion, hearts were excised and rapidly frozen in liquid nitrogen. Samples of myocardial tissues were used for assays of mRNA and nuclear protein. All animal protocols were approved by the Johns Hopkins animal care and use committee.

Immunohistology

Myocardial samples from rats reperfused for 15 minutes were fixed in 60% methanol/10% acetic acid, embedded in paraffin, and sectioned at 7 μm. Deparaffinized sections were exposed overnight at 4°C to a 1/25 dilution of a polyclonal antibody to either phosphorylated Y705 or S727 Stat3 (Cell Signaling Technology). This was followed by exposure to biotinylated secondary antibody, immunoperoxidase staining,[12] and counterstaining with eosin.

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from myocardial samples, and EMSA assays were performed as described previously.[12,13] For details, see the online supplement.

Real-Time RT-PCR

Quantitative real-time RT-PCR was used to quantify ICAM-1 mRNA. See the online supplement for details.

Cell Culture, Transfections, and H-R

Human coronary microvascular endothelial (HCMVE) cells and human umbilical vein endothelial (HUVE) cells and cell growth media were purchased from Cambrex Bio Science. Approximately 1.5×10^5 cells were plated in 6- to 12-well plates. For experiments involving H-R, cells were incubated at 37°C with ischemia buffer[18] under a hypoxic gas mixture (<1% O_2, 5% CO_2; >94% N_2) for 1.5 hours and then reoxygenated with room air in normal culture medium at 37°C. For experiments involving Stat3 adenovirus infections, we used HCMVE cells because they exhibited less nonspecific reaction to adenoviral infection. For details of adenovirus infection, GAS decoy, and siRNA transfections, as well as ICAM-1 promoter–reporter gene experiments, see the online supplement.

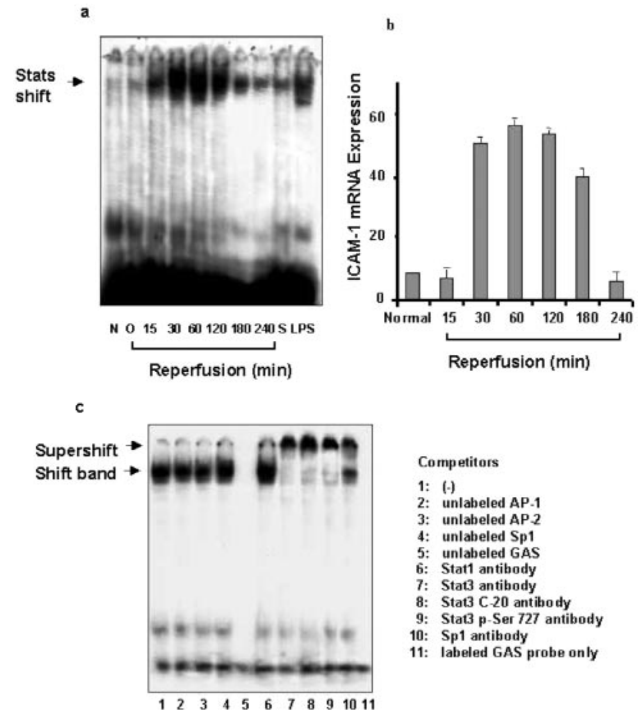

Figure 1. a, Time course of Stat3 activation in rat myocardial samples by EMSA. Nuclear extracts (NEs) were incubated with labeled ICAM-1–GAS probe. Myocardial samples were from normal rat (N), rats with ischemia and reperfusion for indicated times, sham-operated rat without occlusion (S), and rat receiving 1 mg/kg lipopolysaccharide (LPS) 3 hours before euthanasia. The arrow indicates binding of Stat protein and probe. b, Time course of ICAM-1 mRNA in myocardial samples from a normal rat and rats with ischemia and reperfusion for the indicated times (n=4 each). c, EMSA competition assays in rat myocardial samples. The NEs of samples reperfused for 30, 60, or 120 minutes were pooled. NEs were preincubated with antibodies against Stat1, Stat3, carboxyl-terminal Stat3 (Stat3-C20), phosphorylated S727 Stat3, or Sp1, or with a 100-fold excess of double-stranded DNA competitors for Sp1, Ap-1, Ap-2, or GAS. Lane 11 was run with labeled GAS probe but without NE or competitor.

Western Blotting and Coimmunoprecipitation

Please see the online supplement.

Statistics

All quantitative assays were performed in triplicate, and the results were expressed as mean±SD. For tests of significance involving 3 groups, ANOVA, followed by post hoc testing and Bonferroni correction, was used. The Student t test was used to determine the significance of differences between 2 groups.

Results

I-R Activates Stat3 and Increases ICAM-1 Transcription

Analysis of nuclear extracts from I-R rat heart samples demonstrated that Stat was activated rapidly after reperfusion. Stat binding to an ICAM-1–GAS probe occurred within 15 minutes and peaked at 60 minutes of reperfusion (Figure 1a). Lagging shortly behind Stat activation, ICAM-1 mRNA increased at 30 minutes of reperfusion, peaked at 120 minutes, and remained increased at 180 minutes (Figure 1b). Immunohistology demonstrated that activated Stat was localized primarily in vascular endothelium (Figure 2).

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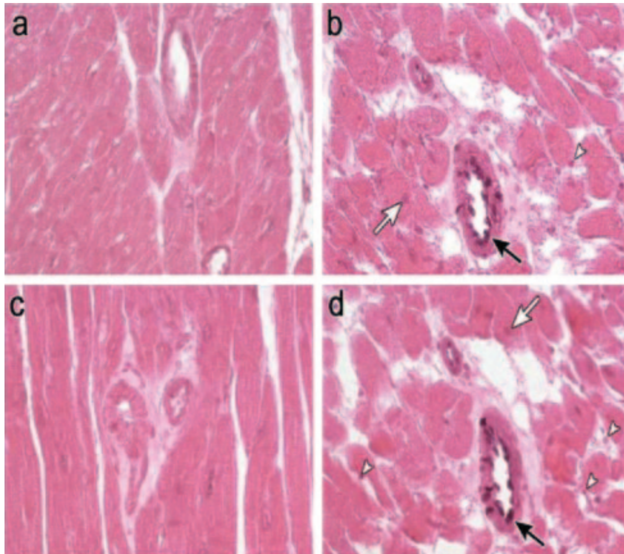

Figure 2. Activated Stat3 is localized in vascular endothelium. Immunohistology of myocardial samples from normal rats (a and c) and rat with ischemia and reperfusion for 15 minutes (b and d), stained for p-Y705 Stat3 (a and b) or p-S727 Stat3 (c and d; b and d are serial sections from I-R region). Strong brown staining, representing activated Stat3, is seen in nuclei of vascular endothelial cells in arterioles (black arrows) and venules (data not shown) and also in nuclei of vascular smooth muscle cells. Faint staining is seen in nuclei of some cardiomyocytes (white arrows) located in the border area of the infarct. Faint staining is also present in capillary endothelial cell nuclei (white arrowheads). No staining was seen in samples from normal hearts (a and c) or normally perfused regions of I-R hearts (data not shown).

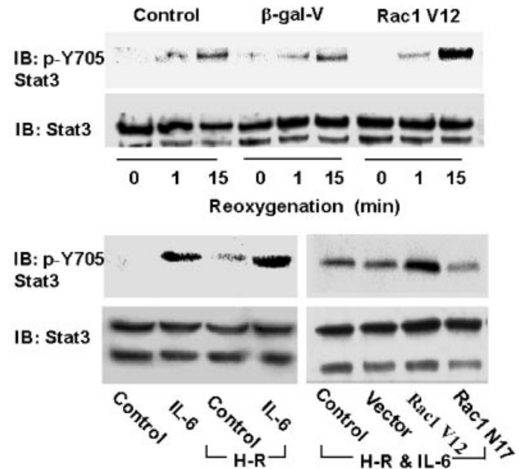

Figure 3. H-R and IL-6–induced Stat3 tyrosine 705 phosphorylation is Rac1 dependent. HUVE cells were exposed to 40 ng/mL IL-6 for 15 minutes or hypoxia for 1.5 hours, followed by reoxygenation with or without IL-6 for 15 minutes (bottom left). Other cells were infected with β-galactosidase (β-gal), activated Rac1 (Rac1 V12), or dominant-negative Rac1 (Rac1 N17) adenoviruses. After 48 hours of infection, cells were exposed to 1.5 hours of hypoxia and reoxygenation for 0 to 15 minutes, with or without IL-6. Cells were harvested for Western blotting. Top shows that H-R induced Stat3 Y705 phosphorylation, and this was enhanced by Rac1. Bottom left shows that IL-6 induced Stat3 Y705 phosphorylation, which was increased by H-R. Bottom right shows that during H-R/IL-6 stimulation, Rac1 V12 markedly enhanced Y705 phosphorylation, whereas Rac1 N17 inhibited it (p-Y705 Stat3 was 18.6% of total Stat 3 in control cells vs 10.2% with Rac1 N17). Control indicates cells under normal culture conditions.

To confirm that the nuclear protein bound specifically to the GAS probe, we mutated the GAS probe (mGAS) and demonstrated lack of binding of Stat protein (Figure I, available online at http://atvb.ahajournals.org). In addition, using a 100-fold excess of unlabeled Ap-1, Ap-2, Sp1, mGAS, and GAS probes, only the latter competed out the protein binding (Figure 1c; Figure I). Supershift assays demonstrated a complete supershift of the protein–DNA band, with antibodies against Stat3, Stat3-C20 (against the Stat3α carboxyl-terminal region without cross-reactivity to Stat3β) and p-S727 Stat3 (p-S727 Stat3) but no supershift or competition with an antibody against Stat1 (Figure 1c). These results identify the activated Stat3 isoform as Stat3α, p-S727. Addition of an antibody to Sp1 in the supershift assay formed shift and supershift bands, suggesting that the DNA–protein complex contained Sp1, which interacted with p-S727 Stat3α to bind to the ICAM-1 GAS probe. The resulting complex was too large to move into the gel. For unknown reasons, an antibody against phosphorylated Y705 Stat3 failed to compete out or supershift the DNA–protein binding band (data not shown).

H-R and IL-6 Induce Stat3 Tyrosine Phosphorylation in Vascular Endothelial Cells, and This Phosphorylation is Rac1 Dependent

HUVE cells were used to study the role of H-R in the activation of Stat3. Stat3 tyrosine 705 phosphorylation could be induced by H-R alone, but this was markedly enhanced by IL-6 (Figure 3, bottom left). The small GTPase Rac1 (a subunit of the membrane NADPH oxidase) promoted the activation of Stat3 during H-R. HUVE cells were infected with adenoviruses expressing β-galactosidase, an activated form of Rac1 (Rac1 V12), or a dominant-negative form of Rac1 (Rac1 N17). H-R induced Stat3 tyrosine 705 phosphorylation, and this phosphorylation was markedly enhanced by activated Rac1 (Figure 3, top). During H-R and IL-6 stimulation, Stat3 tyrosine phosphorylation was enhanced by activated Rac1 and inhibited by dominant-negative Rac1 (Figure 3, bottom right).

H-R Increases Phosphorylation of Stat3 on S727 and Results in Binding of Sp1

To study the possible role of Stat3 and Sp1 cooperativity during H-R, cultured HUVE cells were exposed to H-R, and cell lysates were analyzed by Western blotting. Stat3 was weakly p-S727 in control cells. After hypoxia, p-S727 Stat3 increased significantly at 15 and 30 minutes of reoxygenation (Figure 4a). Because p-S727 Stat3α interacted with Sp1 in the intact I-R rat heart, we examined whether the same interaction could be demonstrated in cultured HUVE cells after H-R. Although absent in control cells, within 15 minutes of reoxygenation, an Sp1–p-S727 Stat3 complex could be detected by coimmunoprecipitation (Figure 4b). The bound complex decreased at 30 minutes of reoxygenation and was present at only low levels after 60 minutes. p-Y705 Stat3 and Stat3β did not coimmunoprecipitate with Sp1 (data not shown).

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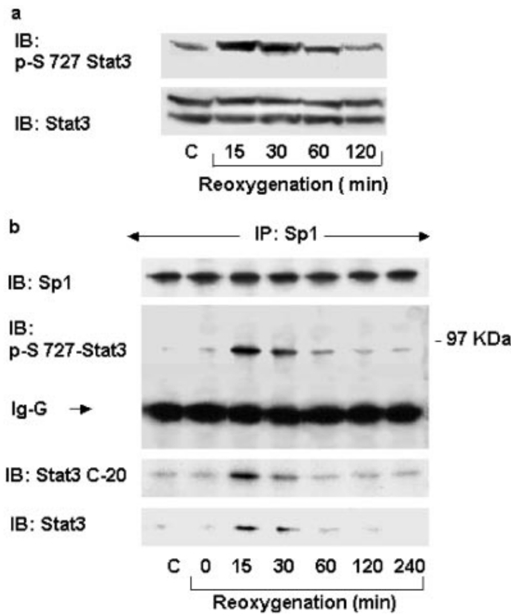

Figure 4. a, Western blots show that Stat3 S727 phosphorylation is rapidly increased in HUVE cells during H-R. After blotting with p-S727 Stat3 antibody (top panels), the membrane was stripped and reblotted with Stat3 antibody (bottom panels). C indicates control cells under normal culture conditions. b, Sp1 interacts with Stat3α containing p-S727. HUVE cells were exposed to hypoxia, reoxygenated for the times indicated, and harvested. Anti-Sp1 antibody was added to the sample to pull down the Sp1 complex. Pellets were electrophoresed, transferred to a membrane, and incubated with primary anti–p-S727 antibody. The membrane was stripped and reprobed with antibodies against Stat3 and Sp1, respectively. Arrow indicates IgG band as a molecular marker. IP, indicates immunoprecipitated with Sp1; IB, immunoblotted with primary antibodies against p-S727 Stat3, Stat3-C20, Stat3, and Sp1, respectively.

Activated Stat3 Increases ICAM-1 Transcription, Whereas a Y705F Stat3 Mutant Blocks ICAM-1 Transcription After H-R

To confirm that Stat3 upregulates ICAM-1 transcription, we infected HCMVE cells (less nonspecific reactivity than HUVE cells) with constitutively active Stat3-C or dominant-negative Y705F Stat3 adenoviruses. After 48-hour infection, cells were exposed to H-R. Compared with cells infected with control virus, cells with Stat3-C had strongly increased ICAM-1 transcription under baseline conditions (Figure 5a). In contrast, Y705F Stat3 inhibited ICAM-1 transcription in control cells, but the inhibition was much greater in cells exposed to H-R (Figure 5b).

ICAM-1 GAS Element Decoy Inhibits ICAM-1 Transcription Induced by H-R

HUVE cells transfected with a double-stranded ICAM-1 GAS decoy oligonucleotide demonstrated significantly reduced ICAM-1 mRNA expression after H-R compared with cells transfected with a scrambled oligonucleotide (Figure 5c). The amount of inhibition appeared to be greater the longer the duration of reoxygenation.

Mutation of the GAS Element Decreases ICAM-1 Promoter Activity in H-R

HUVE cells were transfected with ICAM-1 proximal promoter constructs containing either a wild-type GAS or mutated GAS

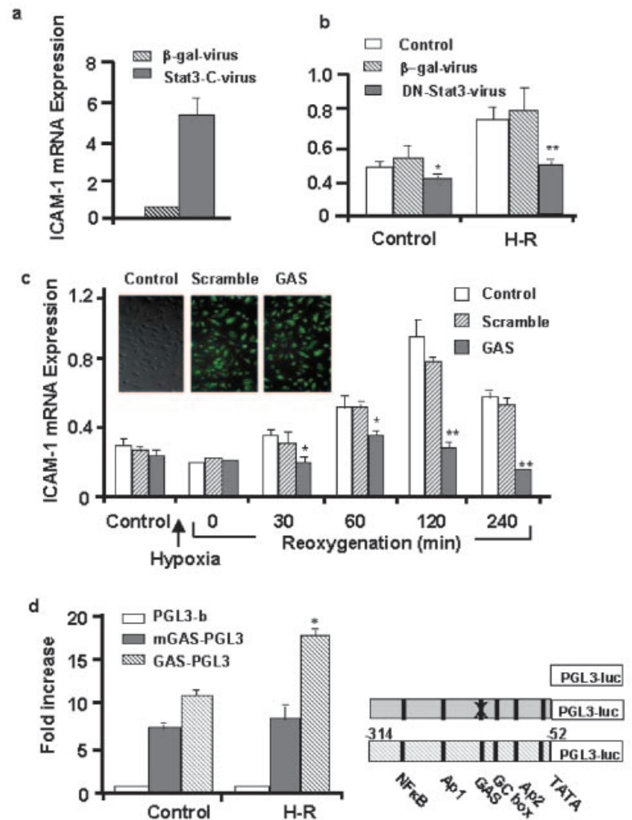

Figure 5. Activated Stat3 regulates ICAM-1 in endothelial cells. HCMVE cells were infected with adenoviruses carrying β-galactosidase (β-gal) or activated Stat3-C (a) or dominant-negative (Y705F) Stat 3 (b). Cells were then exposed to H-R. Activated Stat3 increased ICAM-1 transcription, whereas dominant-negative Stat3 inhibited transcription (*$P<0.05$ by ANOVA; DN virus vs β-gal virus or normal cells). This inhibition was much stronger after H-R (**$P<0.001$ vs β-gal virus or normal cells). c, HUVE cells were transfected with scrambled oligonucleotide (negative control) or GAS decoy, exposed to hypoxia, and reoxygenated for indicated times. The GAS decoy reduced ICAM-1 transcription at each reoxygenation time compared with the scrambled oligonucleotide or control (*$P<0.05$; **$P<0.001$ by ANOVA). Inset shows immunofluorescence of fluorescein isothiocyanate (FITC)–labeled scrambled and GAS oligonucleotides to verify similar transfection efficiencies. d, Using reporter constructs ligated into PGL3 vector and transfected into HUVE cells, mutation of GAS element (mGAS–PGL3) eliminated the H-R–induced increase in ICAM-1 promoter activity that was found for normal GAS (GAS–PGL3; H-R vs control, *$P<0.05$ for GAS–PGL3; P=NS for mGAS–PGL3 by t tests).

element in a luciferase basic vector and exposed to H-R. Mutation of the GAS element resulted in a significant decrease in transcriptional activity induced by H-R (Figure 5d).

Sp1 Inhibition With iRNA Decreases ICAM-1 mRNA Expression

Because we found that Sp1 interacts with Stat3 in the nucleus and binds as a complex to the ICAM-1 promoter GAS sequence, we examined the roles of Stat3 and Sp1 in ICAM-1 transcription using the RNA interference method. Compared with control cells, cells transfected with Stat3 or Sp1 siRNA demonstrated an approximate 70% to 80% decrease in the respective Stat3 or Sp1 proteins (Figure 6a). Compared with normal cells, there was significant inhibition of ICAM-1 transcription in the Stat3, Sp1, or double knockdown cells

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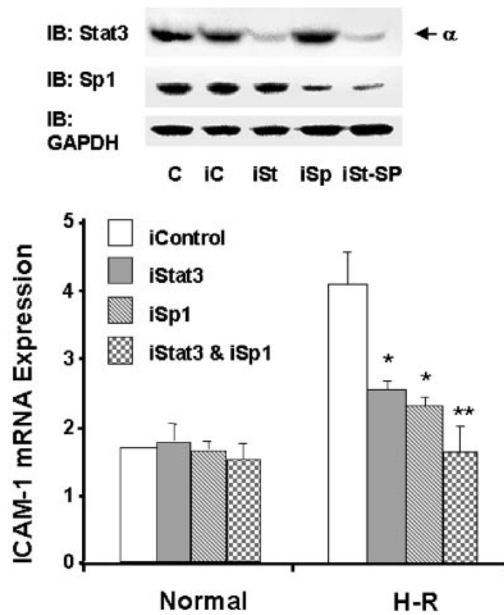

Figure 6. Stat3 and Sp1 siRNA decrease ICAM-1 expression in HUVE cells after H-R. Transfection with Sp1 siRNA (iSp), Stat3 siRNA (iSt), or both resulted in a decrease in respective Sp1 or Stat3α proteins compared with no transfection (C) or cells transfected with an iRNA-negative control (iC; top). After hypoxia and reoxygenation for 2 hours, cells transfected with iSp, iSt, or both demonstrated reduced transcription of ICAM-1. Cells maintained under normal culture conditions were not affected, maintaining low levels of ICAM-1 expression after Stat3 and Sp1 siRNA transfection (compared with iC; *$P<0.05$; **$P<0.01$). IB indicates immunoblot.

exposed to H-R. Stat3 or Sp1 knockdown resulted in an approximate 50% decrease in ICAM-1 mRNA, whereas the Stat3/Sp1 double knockdown had an approximate 70% decrease after H-R (Figure 6b). These results suggest that Stat3 and Sp1 interact together in a cooperative fashion to upregulate ICAM-1 transcription in H-R.

Discussion

Previous studies have shown that ICAM-1 increases and mediates neutrophil adhesion in myocardium after I-R.[1–3,12,13] Our study clearly shows that activated Stat3 is an important regulator of ICAM-1 in vascular endothelium after I-R. On the basis of the structure of the ICAM-1 promoter,[4] upregulation of ICAM-1 could occur through stimulation of tumor necrosis factor-α (TNF-α), IL-6, or IFN-γ, all of which are increased in the postischemic myocardium.[10,17,18] TNF-α activates NF-κB, whereas IL-6 and IFN-γ activate the janus kinase (Jak)–Stat pathway.[4,5] In addition, I-R generates a burst of ROS, which could activate the Jak–Stat pathway[3] independently of IL-6 or IFN-γ, and provide an important additional mechanism for upregulation of ICAM-1 after I-R. The importance of activated Stat in ICAM-1 regulation is supported by our finding that mutation of the GAS element, its binding site in the ICAM-1 promoter, markedly limits transcriptional activity in endothelial cells after H-R (Figure 5d).

The small GTPase Rac1, a component of the membrane NADPH oxidase, is a key regulator for ROS generation in many cell types, including vascular endothelial cells.[17,19] Stat3 activation has been shown to be Rac1 dependent in epidermal growth factor–stimulated COS-1 cells,[20] attributed to direct binding of Rac1 to Stat3 or indirect enhancement of ROS production by Rac1. Rac1 could also mediate Stat3 activation by autocrine production of IL-6.[21] In any event, our study is the first to show that Stat3 activation is Rac1 dependent in endothelial cells and that this mechanism is important in the context of H-R.

Of the various Stat isoforms activated during I-R,[6,7] only Stat1 and Stat3 can bind the GAS element in the ICAM-1 promoter.[5,22] EMSA competition assays demonstrated that the activated Stat isoform was Stat3α without evidence of Stat1 or Stat3β activation (Figure 1c). Cultured rat fibroblasts exposed to H_2O_2 demonstrated strong activation of Stat3, but only weak activation of Stat1.[3] An intact rat model of cardiac I-R exhibited Stat3 but not Stat1 activation.[7] Our results are consistent with these studies and suggest that ROS and IL-6 are strong activators of the Stat3 protein. Our experiments producing Stat3 inhibition using a GAS decoy, Stat3 iRNA, or a dominant-negative Stat3 adenovirus provide strong support for ICAM-1 being a target gene for activated Stat3 in I-R or H-R (Figures 5 and 6).

As evidence against Stat3β activation, the antibody directed against the C-terminal portion of Stat3α, which does not cross-react with Stat3β (because the C-terminal portion is missing from Stat3β), produced a virtually complete supershift of the Stat3 band in the EMSA assay (Figure 1c). Recently, an important study showed that Stat3α and Stat3β have distinct functions[23] and that Stat3β is not merely a dominant-negative factor, as has been generally thought. Expression of Stat3β can rescue the embryonic lethality of the Stat3α null mutation, and it can, by itself, induce the expression of specific Stat3 target genes.[23] Nevertheless, Stat3α is the isoform that mediates the cell response to cytokines such as IL-6 or IL-10.[23]

It is known that there are 2 sites of phosphorylation in Stat3: tyrosine 705 and S727. These phosphorylation sites appear to be induced by distinct signaling cascades.[8,10,24] In our study, tyrosine phosphorylation occurred strongly after IL-6 stimulation and to a lesser extent after H-R; H-R and IL-6 together produced enhancement of tyrosine phosphorylation (Figure 3). In contrast, S727 phosphorylation seemed to be more strongly related to I-R or H-R (Figure 4a). Phosphorylation of both sites is necessary for maximal activation of transcription.[25] Tyrosine 705 phosphorylation is required for Stat3 dimerization, nuclear translocation, and gene activation.[8] S727 phosphorylation is necessary for maximal transcription efficiency, although it was not known why.[25]

In our study, EMSA analysis of nuclear extracts from I-R myocardial samples demonstrated that p-S727 Stat3 and Sp1 formed a protein complex that could be supershifted by antibodies to either component. In cultured HUVE cells with H-R, p-S727 Stat3 and Sp1 coimmunoprecipitated, confirming the presence of a transcriptional protein complex. Although it was known that Stat1 can interact with Sp1 in the ICAM-1 promoter after IFN-γ stimulation[26] and that Stat3 and Sp1 can cooperate in the CCAAT/enhancer-binding protein δ gene,[27] our study is the first to demonstrate that Sp1 interacts specifically with p-S727 Stat3 to form an Sp1–Stat3 protein complex, and that inhibition of either Stat 3 or Sp1 can decrease ICAM-1 transcription in H-R.

Several different kinases have been implicated in serine phosphorylation, suggesting that there is an interaction between Stat signaling and serine kinase signaling pathways.[24] protein kinase Cδ has been identified as the kinase responsible for S727 phosphory-

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lation after IL-6 stimulation, whereas c-jun N-terminal kinases mediate p-S727 Stat3 after UV irradiation. Although p-S727 Stat3 could occur by extracellular signal regulated kinases through the stimulation of chemotactic factors and cytokines,[24] it is unknown which kinases are in fact responsible after I-R or H-R. It will be important to identify these kinases in future studies.

A number of seemingly conflicting effects of Stat3 on gene regulation have been described. Although Stat3 may be proinflammatory, it also appears to be antiapoptotic, on the basis of results of inhibitor studies.[28,29] Stat3 has also been reported to play an important role in early and late ischemic preconditioning.[6,28] Mice with myocyte-specific deletion of Stat3 exhibit increased interstitial fibrosis, heart failure, increased myocardial infarct size after I-R, and increased ischemia-induced cardiac apoptosis.[30] However, the beneficial effects of Stat3 as an antiapoptotic protein may be counterbalanced during I-R by its significant proinflammatory effects in vascular endothelium, so the net effect of Stat3 in I-R cannot be known a priori and will need to be tested in future studies in intact animal models. This paradigm is similar to that of NF-κB, which exhibits antiapoptotic effects at the same time that it upregulates proinflammatory genes.[31] In most models of cardiac I-R, inhibition of NF-κB results in net benefit, with a reduction in myocardial damage.[32]

Acknowledgments

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