STAT3 Negatively Regulates Type I IFN-Mediated Antiviral Response

Wei-Bei Wang, David E. Levy and Chien-Kuo Lee

J Immunol published online 1 August 2011
http://www.jimmunol.org/content/early/2011/07/31/jimmunol.1004128

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/08/02/jimmunol.1004128.DC1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Errata
An erratum has been published regarding this article. Please see next page or:
/content/187/12/6583.full.pdf
STAT3 Negatively Regulates Type I IFN-Mediated Antiviral Response

Wei-Bei Wang,* David E. Levy, † and Chien-Kuo Lee*

Type I IFNs are crucial cytokines of innate immunity for combating viral infections. Signaling through type I IFN receptors triggers the activation of STAT proteins, including STAT1, STAT2, and STAT3. Although an essential role of STAT1 and STAT2 for type I IFN-induced antiviral response has been well established by studies of gene-targeted mice and human mutations, the role of STAT3 for this response remains unclear. Using gain-of-function and loss-of-function approaches, we demonstrated that STAT3 negatively regulates type I IFN-mediated response. STAT3 knockdown or knockout cells displayed enhanced gene expression and antiviral activity in response to IFN-α/β. Restoration of STAT3 to STAT3KO cells resulted in attenuation of the response. Upon viral infection, increased type I IFN production in STAT3KO cells resulted in enhanced STAT activation and ISG expression. One mechanism for the enhanced IFN production and response in the absence of STAT3 might operate through an MDA5-dependent manner. STAT3 also appeared to suppress IFN response directly in a manner dependent on its N-terminal domain and independent of its function as a transcriptional factor. Taken together, these results define STAT3 as a negative regulator of type I IFN response and provide a therapeutic target for viral infections.

The Journal of Immunology, 2011, 187: 000–000.

*Graduate Institute of Immunology, National Taiwan University College of Medicine, Taipei 100, Taiwan; and †Department of Pathology, New York University School of Medicine, New York, NY 10016

Received for publication December 21, 2010. Accepted for publication June 21, 2011.

This work was supported by Grant NSC 99-2320-B-002-010-MY3 from the National Science Council, Taiwan.

Address correspondence and reprint requests to Prof. Chien-Kuo Lee, No. 1, Section 1, Jen-Ai Road, Room 513, Taipei 100, Taiwan. E-mail address: leekc@ntu.edu.tw

The online version of this article contains supplemental material.

Abbreviations used in this article: BMM, bone marrow-derived macrophage; CCD, coiled-coil domain; CHX, cycloheximide; DBD, DNA-binding domain; EMVC, encephalomyocarditis virus; HA, hemagglutinin; iNOS, inducible NO synthase; IFN, IFN regulatory factor; ISG, IFN-stimulated gene; ISRE, IFN-stimulated response element; MEF, mouse embryonic fibroblast; MOI, multiplicity of infection; NTD, N-terminal domain; OAS, 2′-5′-oligoadenylate synthetase; PKR, protein kinase R; QPCR, quantitative PCR; shMDA5, short hairpin MDA5; shSTAT3, short hairpin STAT3; shRNA, short hairpin RNA; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/S16/00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1004128

The Journal of Immunology
maintained and housed in specific germ-free conditions in the Animal Core Facility at the National Taiwan University College of Medicine. Procedures and use of these animals were reviewed and approved by the Institutional Animal Care and Use Committee at National Taiwan University College of Medicine. WT, STAT1KO, and STAT3KO MEFs were generated, as described previously (21, 22).

**Abs, cytokines, and DNA construct**

The sources of the cytokines are as follows: recombinant human IFN-α (Roche), recombinant murine IFN-β (ProSpec). The sources of Abs are as follows: anti-β-actin (Chemicon), anti-STAT1 (Signet/Aldrich), anti-phospho-STAT1(Y701) (Invitrogen), anti-STAT1 (homemade), anti-phospho-STAT2(Y689) (Millipore), anti-STAT2 (homemade), anti-phospho-STAT3(Y705), anti-STAT3 (Cell Signaling Technology), anti-MDA5 (Axxora), anti-IFNAR1 Ab (eBioscience), and anti-hemagglutinin (HA; 12CA5, mouse). Mouse IFN-α ELISA kit was purchased from PBL, WT STAT3 and DBD mutant carrying 5′-ACCACTAGTGAGTT3′ (Gibco, Invitrogen) and the plaques were enumerated.

**Transfection and retroviral transduction of MEFs**

Transfection of vector encoding different STAT3 into MEFs was done using TurboFect (Fermentas), according to manufacturer’s instruction. Retroviral transduction of MEFs was conducted, as described (28). Briefly, a retroviral vector Pallino encoding WT STAT3 and GFP, respectively, was cotransfected with a helper plasmid into HEK293T cells for 2 d before collecting the culture supernatant containing pseudotyped virus. MEFs were incubated with the viral supernatant in the presence of 8 μg/ml polybrene and spun at 1100 x g for 90 min at 30℃. Two days after the infection, GFP-positive cells were further purified up to 90–95% using the FACSaria cell sorting system (BD Biosciences).

**Reporter assay**

Luciferase assays for pISRE-luc (Strategene) containing 5′ ISRE from Hif4 gene were performed in duplicate by transfection into HEK293-TLR3 cells (a gift of K. Fitzgerald, University of Massachusetts) and STAT3KO MEFs in conjunction with different STAT3-expressing constructs using jetPEI (Polyplus-transfection) and TurboFect (Fermentas), respectively, according to manufacturer’s instruction. After transfection for 24–48 h, the cells were treated with or without various doses of human IFN-α/2a (Roche) or mouse IFN-α (Merck) for 8 and 3 h, respectively, followed by measuring luciferase activities. Luciferase activities were measured according to manufacturer’s instructions (Promega) using Orion II microplate luminoimeter (Berthold).

**Results**

To study the role of STAT3 in type I IFN response, STAT3 was knocked down in WT MEF using lentivirus carrying shRNA to STAT3, shRNA to firefly luciferase was used as a control. As shown in Fig. 1A, following short hairpin STAT3 (shSTAT3) treatment, STAT3 protein levels were reduced to ~40% to that of

**Western blot**

Total cell lysates were prepared by lysing cells in lysis buffer (300 mM NaCl, 50 mM HEPES [pH 7.6], 1.5 mM MgCl₂, 10% glycerol, 1% Triton X-100, 10 mM NaF, 20 mM Na₃ EDTA, 0.1 mM EDTA, 0.1 mM DTT, 1 mM PMSF, and 1 mM Na₃VO₄) at 4℃ for 15 min. Lysates were first clarified by centrifugation at 12,000 x g for 20 min. Equal amounts of samples were resolved in 7–10% SDS-PAGE, followed by transfer to nitrocellulose or polyvinylidene difluoride membranes (Millipore) and blotting with indicated Abs.
shLuc control. Moreover, basal and IFN-induced STAT3 phosphorylation at Y705 was also greatly reduced as compared with that of control, whereas the levels of pSTAT1 (pY701) and pSTAT2 (pY689) remained comparable. We next examined the effect of STAT3 knockdown on expression of ISGs. As shown in Fig. 1B–D, following stimulation, the expression of PKR, OAS, and IRF1 was increased in cells treated with shSTAT3 as compared with that of shLuc control. We next investigated whether IFN-mediated antiviral activity was affected or not by the treatment. As shown in Fig. 1E, antiviral response in STAT3 knockdown cells was greater than that of control cells, revealed by the increased resistance of lytic activity of EMCV following infection. Together, these results suggest that STAT3 may have a negative effect on IFN response.

STAT3KO MEFs and macrophages display enhanced type I IFN response

We next confirmed the suppressive effect of STAT3 using MEFs lacking STAT3. As shown in Fig. 2A and 2B, following IFN-α treatment, STAT3KO MEFs expressed higher basal and induced levels of PKR and OAS than did WT MEFs. A similar phenomenon was also observed in other IFN-α downstream genes, such as RNase L, IRF7, IRF1, and IP-10 (data not shown). We next employed DNA microarray to conduct a genome-wide expression profiling for WT and STAT3KO MEFs in response to IFN-α. Expression of a variety of ISGs was also increased in STAT3KO MEFs as compared with WT MEFs (Supplemental Table 1; GEO GSE25044, http://www.ncbi.nlm.nih.gov/gds/?term=GSE25044), suggesting that the hyperresponsiveness of IFN-α in STAT3KO

FIGURE 1. STAT3 knockdown enhances type I IFN response. A, WT MEFs infected with lentivirus carrying shLuc or shSTAT3 were stimulated with or without 1000 U/ml IFN-α for 15 min. Total cell lysates were subjected to immunoblotting using Abs to pSTAT1, pSTAT2, pSTAT3, STAT3, and tubulin. Total RNA from the cells received the same treatments, as described in A, for different times was subjected to RT-QPCR using primers for PKR (B), OAS (C), IRF1 (D), and β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. E, WT MEFs infected with lentivirus-shLuc (upper) or lentivirus-shSTAT3 (lower) were pretreated with or without 2-fold serial dilution of IFN-α starting from 125 U/ml. Following the treatment, the cells were infected with or without EMCV at a MOI of 0.1 for 16 h, followed by visualizing the viable cells with crystal violet.

FIGURE 2. STAT3 knockout enhances IFN-α response. Total RNA prepared from WT (filled) or STAT3KO (open) MEFs that were stimulated with or without 1000 U/ml IFN-α for 2 h was subjected to RT-QPCR using primers for PKR (A), OAS (B), and β-actin. Total RNA prepared from BMMs of WT (filled) or STAT3KO (open) mice that were stimulated with or without 100 U/ml IFN-α for 4 h was subjected to RT-QPCR using primers for PKR (C) and iNOS (D). Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. E, WT (upper), STAT1KO (middle), and STAT3KO (lower) MEFs pretreated overnight with 2-fold serial dilution of IFN-α from 1000 U/ml were infected with EMCV at a MOI of 0.1 for 18 h, followed by visualizing live cells with crystal violet. WT (filled) and STAT3KO (open) MEFs pretreated with the indicated doses of IFN-α were infected with EMCV (F) or vesicular stomatitis virus (G) at a MOI of 0.1 for 12 and 18 h, respectively, followed by measuring viral titers in the culture supernatant using plaque formation assay. *p < 0.05, **p < 0.01.
cells was a general phenomenon. We next examined IFN response in primary BMMs. As shown in Fig. 2C and 2D, STAT3KO BMMs also showed enhanced expression of PKR and iNOS following IFN stimulation. We next examined STAT activation in MEFs following IFN treatment. As shown in Supplemental Fig. 1, whereas activation of STAT1 and STAT2 was marginally increased in STAT3KO MEFs as opposed to WT MEFs, STAT1 activation was, however, comparable between STAT3KO and WT BMMs.

Due to STAT3KO MEFs showing a greater IFN response, we next investigated whether IFN-mediated antiviral activity was also enhanced. WT and STAT3KO MEFs were pretreated with or without 2-fold serial dilution of IFN-α, followed by infection with EMCV. Additionally, we also included STAT1KO MEFs as a control. As shown in Fig. 2E, increased doses of IFN-α reduced the lytic activity of EMCV in WT MEFs, indicating that IFN-α conferred antiviral response in a dose-dependent manner. However, the activity was dramatically abolished in STAT1KO MEFs, confirming a pivotal role of STAT1 for IFN-mediated innate immunity to viral infection (5, 7). By contrast, STAT3KO MEFs displayed an enhanced antiviral response, as revealed by increased resistance to EMCV-induced lytic activity. We next investigated whether the increased survival of STAT3KO MEFs was accomplished by reduced viral titers. Indeed, STAT3KO MEFs reduced EMCV viral titers before and after IFN-α treatment (Fig. 2F). This result was consistent with the enhanced antiviral state in STAT3KO MEFs. In addition to EMCV, STAT3KO MEFs also exerted a greater antiviral activity against vesicular stomatitis virus infection than did their WT counterparts (Fig. 2G) in response to IFN-α. Together, these results suggest that STAT3 suppresses type I IFN-mediated antiviral response.

**Restoration of STAT3 reverses the otherwise enhanced type I IFN response in STAT3KO MEFs**

To examine whether the enhanced IFN response in STAT3KO cells was intrinsic to the loss of STAT3, we restored STAT3 into STAT3KO MEFs by retroviral transduction. The levels of restored STAT3 were slightly lower than that of WT cells (data not shown). However, whereas re-expression of STAT3 did not change the levels of IFN-β-activated pSTAT1 or pSTAT2 (Fig. 3A), it attenuated IFN-α-stimulated expression of PKR and OAS (Fig. 3B, 3C).

We next determined the effect of gain of function of STAT3 in antiviral response. Compared with that restored with the empty vector, STAT3-restored cells showed increased lytic activity and viral titers (Fig. 3D, 3E). Similar results were also observed in WT MEFs when STAT3 was hyperactivated by cotreatment of IFN-α and IL-6 (data not shown). Taken together, these results suggest that enhanced gene expression and antiviral activity of type I IFN in STAT3KO MEFs are intrinsic to the loss of STAT3.

**Increased expression of type I IFNs in STAT3KO cells during viral infection**

During viral infection, the binding of viral components to pattern recognition receptors triggers the production of type I IFNs, which in turn activates STAT proteins. Therefore, we next examined the role of STAT3 in viral infection in the absence of exogenous IFN-α. We first assessed STAT activation as an indicator of IFN-α signaling. As shown in Fig. 4A, at MOI of 0.1, EMCV infection failed to induce detectable tyrosine phosphorylation of STAT1 and STAT2 in WT MEFs, which might be due to an insufficient amount of type I IFN production. By contrast, the levels of pSTAT1 and pSTAT2 were increased in STAT3KO MEFs following the infection for 6 h. Concomitantly, infection-induced PKR expression was also augmented in STAT3KO MEFs as compared with WT MEFs (Fig. 4B). We next investigated whether the enhanced STAT activation in STAT3KO MEFs was due to increased production of type I IFNs during viral infection. As shown in Fig. 4C, STAT3KO MEFs expressed higher levels of IFN-β mRNA than did WT MEFs after the infection. A similar phenotype was also observed in primary BMMs. The levels of pSTAT1 (Fig. 4D) and the expression of ISGs, including PKR (Fig. 4E), OAS, iNOS, IRF1, IRF7, and TLR3 (Supplemental Fig. 2A–E), were also enhanced in STAT3KO BMMs after the infection. Additionally, STAT3KO BMMs also expressed significantly higher levels of IFN-β mRNA (Fig. 4F) and IFN-α protein (Fig. 4G) than did WT BMMs upon infection.

We next investigated whether the increased production of type I IFNs contributed to the enhanced activation of STAT1 and STAT2 in STAT3KO MEFs after the infection. STAT3KO MEFs were first pretreated with a neutralizing Ab to IFN-α/β receptor 1 (IFNAR1), followed by infection with EMCV. As shown in Supplemental Fig. 2H, the anti-IFNAR1 Ab attenuated pSTAT1 and pSTAT2 levels in STAT3KO MEFs, suggesting that virus-induced type I IFNs facilitate the enhancement of IFN-α signaling in STAT3KO cells.

**MDA5 knockdown impedes enhanced antiviral response in STAT3KO cells**

RIG-I and MDA5, two RLR family members of cytosolic sensors of viral RNA, are themselves IFN inducible (29, 30). We reasoned that STAT3 might indirectly regulate antiviral response through modulating the expression of these two molecules. As shown in Fig. 5, STAT3KO MEFs (Fig. 5A, 5B) and BMMs (Supplemental Fig. 2F, 2G) expressed higher levels of MDA5 and RIG-I than did
WT controls following EMCV infection. Although MDA5 has been shown to play a critical role in clearance of EMCV infection (31), its effector function in the absence of STAT3 remained to be verified. Therefore, we first overexpressed MDA5 in STAT3KO MEFs. As shown in Fig. 5C–E, increased MDA5 accentuated infection-induced IFN-α expression and antiviral activity. We next confirmed the role of MDA5 in STAT3KO MEFs by a knockdown approach using lentivirus carrying shRNA to MDA5. To rule out off-target effects, shRNA to luciferase was used as a control. As shown in Fig. 5F, whereas the expression of MDA5 was further induced by IFN-α treatment, short hairpin MDA5 (shMDA5), but not shLuc, greatly reduced the protein levels of MDA5. Interestingly, shMDA5 treatment resulted in decreased IFN-α expression (Fig. 5G), increased EMCV infection, as revealed by elevated viral RNA (Fig. 5H), and increased lytic activity of EMCV (Fig. 5I) in STAT3KO MEFs. These results suggest that increased MDA5 expression contributes to enhanced type I IFN response in STAT3KO MEFs.

STAT3 suppresses type I IFN response independent of its DNA-binding and transactivation ability

To investigate whether the suppressive effect of STAT3 on IFN-α response requires de novo protein synthesis, we pretreated cells with cycloheximide (CHX). As shown in Fig. 6, CHX pre-treatment could not block the enhanced expression of PKR and OAS in STAT3KO MEFs (Fig. 6A, 6B) and elevated expression of PKR and iNOS in STAT3KO BMMs (Fig. 6C, 6D) in response to IFN-α. Although these results suggest that the suppressive effect of STAT3 is independent of its downstream effector molecules, we still cannot rule out the possibility of involvement of CHX-sensitive inhibitors of IFN-α response. Therefore, we next

WT or STAT3KO MEFs (A) or BMMs (D) were infected with EMCV at a MOI of 0.1 and 10, respectively, for the indicated times. Total cell lysates were subjected to immunoblotting using Abs to pSTAT1, STAT1, pSTAT2, STAT2, pSTAT3, STAT3, and β-actin. Total RNA prepared from EMCV-infected MEFs (B, C) or BMMs (E, F) for the indicated times was subjected to RT-QPCR using primers for PKR (B, E), IFN-β (C, F), and β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. G, Production of IFN-α by WT (filled) or STAT3KO (open) BMMs after viral infection for 48 h was measured by ELISA. *p < 0.05, **p < 0.01.

FIGURE 4. Enhanced expression of type I IFNs in STAT3KO cells after viral infection. WT or STAT3KO MEFs (A) or BMMs (D) were infected with EMCV at a MOI of 0.1 and 10, respectively, for the indicated times. Total cell lysates were subjected to immunoblotting using Abs to pSTAT1, STAT1, pSTAT2, STAT2, pSTAT3, STAT3, and β-actin. Total RNA prepared from EMCV-infected MEFs (B, C) or BMMs (E, F) for the indicated times was subjected to RT-QPCR using primers for PKR (B, E), IFN-β (C, F), and β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. G, Production of IFN-α by WT (filled) or STAT3KO (open) BMMs after viral infection for 48 h was measured by ELISA. *p < 0.05, **p < 0.01.

FIGURE 5. Gain or loss of function of MDA5 in STAT3KO MEFs during viral infection. Total RNA from WT (filled) or STAT3KO (open) MEFs that were infected with EMCV at a MOI of 0.1 for the indicated times was subjected to RT-QPCR using primers for MDA5 (A), RIG-I (B), and β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. C, Empty vector (EV) or vector encoding flag-MDA5 was transfected into STAT3KO MEFs for 24 h, followed by immunoblotting using Abs to flag and tubulin. EV- or MDA5-transfected MEFs were subjected to EMCV infection at a MOI of 0.1 for 3 and 6 h, followed by RT-QPCR using primers for IFN-α (D) and EMCV 2A-2B (E), respectively. F, STAT3KO MEFs infected with lentivirus carrying shLuc or shMDA5 were stimulated with 1000 U/ml IFN-α for the indicated times, followed by immunoblotting using Abs to MDA5 and tubulin. shLuc- or shMDA5-treated STAT3KO MEFs were infected with EMCV at a MOI of 0.1 for 9 and 4 h, followed by RT-QPCR using primers for IFN-α (G) and EMCV 2A-2B (H), respectively. Relative mRNA was calculated by normalizing the values of IFN-α to that of β-actin. I, shLuc- or shMDA5-treated STAT3KO MEFs were infected with or without indicated doses of EMCV for 24 h, followed by visualizing live cells with crystal violet. *p < 0.05, **p < 0.01.
performed reporter assays to examine whether STAT3 was able to suppress IFN-driven transactivation activity. Expression of WT STAT3 suppressed IFN-driven ISRE reporter activity in a dose-dependent manner (Fig. 6). Interestingly, both DBD mutant of STAT3 and STAT3b were also capable of suppressing the reporter activity (Fig. 6F), suggesting that DNA-binding and transactivation ability of STAT3 were not required for the suppressive effect. To dissect further the functional domains of STAT3 required for the effect, different truncation mutants of STAT3 were used. As shown in Fig. 6G, 1–134 aa (NTD only) or 1–317 aa of STAT3 (NTD and CCD) remained the inhibitory activity, whereas the activity was almost abolished in cells expressing STAT3 318–770 aa lacking NTD and CCD. These results suggest that STAT3 NTD is sufficient to confer the suppressive activity. These results are also consistent with the dispensable role of DNA-binding and transactivation ability of STAT3. Taken together, these results suggest that STAT3 may directly suppress type I IFN response, and the effect is independent of its DNA-binding and transcriptional function.

**NDT of STAT3 is sufficient to suppress type I IFN response**

To further confirm the antagonistic effect of STAT3 1–134 aa, we restored HA-tagged WT or STAT3 1–134 aa into STAT3KO MEFs. As shown in Fig. 7A, the expression of WT or mutant STAT3 was detected by anti-HA Ab. STAT3 1–134 aa exerted almost the same inhibitory activity as did WT STAT3 in reporter

![Figure 6](https://www.jimmunol.org/) STAT3 suppresses type I IFN response independent of its DNA-binding and transactivation activity. WT (filled) or STAT3KO (open) MEFs (A, B) or BMMs (C, D) were pretreated with or without 20 μg/ml CHX, followed by 1000 U/ml IFN-α stimulation for the indicated times. Total RNA of the treated cells was then subjected to RT-QPCR using primers for PKR (A, C), OAS (B), INOS (D), and β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. E, Different doses of vector encoding WT STAT3 was cotransfected with plSRE-luc and pCMV-RL into HEK293-TLR3, followed by treating the cells with 1000 IU/ml recombinant human IFNα-2a or without (medium) for 8 h before measuring luciferase activity. F, Empty vector (EV) or vector encoding WT STAT3, DBD STAT3, or STAT3b was cotransfected with plSRE-luc and pCMV-RL into HEK293-TLR3, followed by treating the cells with or without IFN-α. G, EV or vector encoding STAT3 full-length 1–770 aa, 1–134 aa, 1–317 aa, and 318–770 aa was cotransfected with plSRE-luc and pCMV-RL into HEK293-TLR3 cells for 24 h, followed by stimulation with or without IFN-α. Relative luciferase activity was calculated by normalizing firefly luciferase activity to that of Renilla luciferase. *p < 0.05, **p < 0.01.

![Figure 7](https://www.jimmunol.org/) STAT3 N-terminal 1–134 aa is sufficient to suppress type I IFN response. A, Empty vector (EV), vector encoding HA-tagged WT or STAT3 1–134 aa was transfected into STAT3KO MEFs for 48 h. Total cell lysates were then subjected to immunoblotting using anti-HA and anti-tubulin Abs. B, EV, WT, or STAT3 1–134 aa was cotransfected with plSRE-luc and a vector carrying GFP into STAT3KO MEFs for 3 h, followed by treating the cells with IFN-α. Relative luciferase activity was calculated by normalizing firefly luciferase activity to that of GFP-positive percentage. STAT3KO MEFs transfected with EV, WT, or STAT3 1–134 aa were treated with or without IFN-α for the indicated times. Total RNA of the treated cells was subjected to RT-QPCR using primers for PKR (C), OAS (D), or β-actin. Relative mRNA was calculated by normalizing the values of the indicated genes to that of β-actin. E, STAT3KO MEFs transduced with EV or vector encoding STAT3 1–134 aa were treated with 2-fold serial dilution of IFN-α from 1000 U/ml for 24 h, followed by infection with EMCV at a MOI of 1 for 24 h. Live cells were visualized with crystal violet. *p < 0.05.
also showed a compromised antiviral response as compared with
and OAS (Fig. 7C) and ISG expression. Moreover, restoration of STAT3 1–134 aa also showed a compromised antiviral response as compared with empty vector control (Fig. 7E). Together, these results suggest that NTD of STAT3 is sufficient to trigger negative effect on type I IFN response.

Discussion
This work highlights a previously unappreciated role of STAT3 in type I IFN response. Using loss- and gain-of-function approaches, we demonstrated that STAT3 negatively regulates type I IFN-mediated responses. Whereas knockdown or knockout of STAT3 resulted in enhanced ISG induction and antiviral activity in response to type I IFNs, restoration of STAT3 in STAT3KO MEFs attenuated the response. Interestingly, viral infection of STAT3KO cells induced higher levels of type I IFN expression and activation of STAT1 and STAT2, leading to the expression of elevated levels of ISGs, including a viral sensor MDA5. The results of MDA5 overexpression and knockdown experiments suggest a positive role of MDA5 in regulating type I IFN production and antiviral response in the absence of STAT3. In addition, our truncation experiment showed that STAT3 1–134 aa is sufficient to confer antagonism of IFN response, demonstrating that the inhibitory effect of STAT3 on type I IFN response is independent of its DNA-binding and transcriptional activity. Taken together, we demonstrated that STAT3 suppresses antiviral response through directly regulating IFN response and inducing viral sensors and IRFs during viral infection.

A negative role of STAT3 in type I IFN response is reported recently. Ho and Ivashkiv (15) showed that overexpression of STAT3 in THP-1 cells downregulated IFN-α-activated, STAT1-dependent genes such as IRF-1, CXCL9, and CXCL10, and knocking down STAT3 led to elevated expression of the same set of genes. Conversely, IFN-α-activated STAT3 supported ISRE-driven genes such as OAS and Mx2. Enhanced STAT3 expression did not affect tyrosine phosphorylation or nuclear translocation of STAT1; instead, it sequestered STAT1 and suppressed the formation of DNA-binding STAT1 homodimers. Contrary to their findings, we showed that STAT3 negatively regulated ISRE-driven genes, including OAS, PKR, and IRF7. The discrepancy between our system and theirs remains unclear. It could be that we addressed the role of STAT3 in mouse MEFs and BMMs, whereas Ho and Ivashkiv studied STAT3 in human monocyte cell line. The advantage of studying STAT3 in mouse cells is that we could apply both knockdown and knockout technologies to ensure consistent results. Concerning the suppressive mechanism of STAT3, we showed that STAT3 1–134 aa is sufficient to antagonize IFN response, suggesting that competition for dimerization with STAT1, requiring Src homology 2 domain, is not involved. Therefore, the sequestration mechanism does not seem to operate in our system.

In most biological responses, STAT3 acts as a positive regulator (6). However, we and others have shown that STAT3 also acts as a negative regulator for G-CSF–mediated granulo genesis (19, 32). As such, excessive granulocytes are produced in the bone marrow and periphery in mice lacking STAT3 in the hematopoietic system. Reduced expression of suppressor of cytokine signaling 3, a potent negative regulator, in STAT3KO bone marrow in response to G-CSF is shown to account for the hyperproliferative activity of the cells (33, 34). STAT3 also functions as a negative regulator of TLR-mediated inflammatory response (35–37). Mice devoid of STAT3 in macrophages and neutrophils are highly susceptible to LPS-induced endotoxic shock with increased production of inflammatory cytokines. This is due to the lack of suppressive effect of IL-10 on cytokine production from macrophages and neutrophils in the absence of STAT3 (38). The results of this study define a new negative role of STAT3 in IFN-mediated functions and provide another mechanism to fine-tune type I IFN response. Additionally, they underscore a potential therapeutic target of STAT3 for viral infections.

Acknowledgments
We are grateful to Drs. Lih-Hwa Hwang, Yi-Ling Lin, John Kung, Jeou-Yuan Chen, Kate Fitzgerald, Hua Yu, and Takashi Fujita for providing reagents, and Dr. Betty Wu-Hsieh for reading the manuscript. We also thank the sorting service provided by the Core Facility of Cell Sorting System at the National Taiwan University College of Medicine. siRNA reagents were obtained from the National RNAi Core Facility at the Institute of Molecular Biology/Genomic Research Center, Academia Sinica, supported by the National Research Program for Genomic Medicine.

Disclosures
The authors have no financial conflicts of interest.

References


Corrections


In *Materials and Methods*, the primer sequences of IFN-β provided in the *Quantitative RT-PCR* section should read “forward 5’-ATGAGTTGGTGGTCAGGC-3’, reverse 5’-TGACCTTTCAAATGCAGTAGATTCA-3’”.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1190075
<table>
<thead>
<tr>
<th>Gene name</th>
<th>Fold change (ST3KO/IFN vs ST3KO)</th>
<th>Fold change (WT/IFN vs WT)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIPERIN</td>
<td>102.6</td>
<td>16.8</td>
<td>IFN-inducible antiviral protein</td>
</tr>
<tr>
<td>TYKI</td>
<td>44.9</td>
<td>8.1</td>
<td>Thymidine kinase family LPS-inducible member</td>
</tr>
<tr>
<td>USP18</td>
<td>37.5</td>
<td>9.6</td>
<td>ISG15-specific protease</td>
</tr>
<tr>
<td>MX2</td>
<td>30.3</td>
<td>3.2</td>
<td>Antiviral GTPase</td>
</tr>
<tr>
<td>IRGM2</td>
<td>23.3</td>
<td>9.3</td>
<td>IFN-inducible GTPase</td>
</tr>
<tr>
<td>G1P2</td>
<td>22.4</td>
<td>4.8</td>
<td>ISG15</td>
</tr>
<tr>
<td>IGTP</td>
<td>18.7</td>
<td>8.6</td>
<td>IFNγ-inducible GTP binding protein</td>
</tr>
<tr>
<td>IFI1</td>
<td>17.1</td>
<td>9.9</td>
<td>IFN-induced protein with tetraticopeptide repeats 3</td>
</tr>
<tr>
<td>CXCL10</td>
<td>14.7</td>
<td>9.2</td>
<td>Chemokine</td>
</tr>
<tr>
<td>OCIL</td>
<td>10.5</td>
<td>2.7</td>
<td>Osteoclast inhibitory lectin</td>
</tr>
<tr>
<td>TOR3A</td>
<td>10.3</td>
<td>8.0</td>
<td>ATP-dependant IFN response protein</td>
</tr>
<tr>
<td>STAT1</td>
<td>9.2</td>
<td>4.0</td>
<td>STAT</td>
</tr>
<tr>
<td>GBP4</td>
<td>8.7</td>
<td>3.7</td>
<td>GTPase</td>
</tr>
<tr>
<td>IFIT3</td>
<td>8.6</td>
<td>3.5</td>
<td>IFN-inducible protein</td>
</tr>
<tr>
<td>IFI47</td>
<td>7.4</td>
<td>2.8</td>
<td>IFN-inducible protein</td>
</tr>
<tr>
<td>PRKR</td>
<td>7.3</td>
<td>6.7</td>
<td>Ser/Thr protein kinase</td>
</tr>
<tr>
<td>ISGF3G</td>
<td>6.7</td>
<td>2.0</td>
<td>IFN regulatory factor</td>
</tr>
<tr>
<td>STAT2</td>
<td>6.5</td>
<td>4.3</td>
<td>STAT</td>
</tr>
<tr>
<td>OASL1</td>
<td>6.3</td>
<td>2.6</td>
<td>2',5'-oligoadenylate synthetase</td>
</tr>
<tr>
<td>Protein</td>
<td>Log2Ratio</td>
<td>FC</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>-----</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>ADAR</td>
<td>6</td>
<td>2.2</td>
<td>adenosine deaminase, RNA-specific</td>
</tr>
<tr>
<td>TREX1</td>
<td>5.5</td>
<td>3.3</td>
<td>DNase III</td>
</tr>
<tr>
<td>BST2</td>
<td>4.7</td>
<td>2.3</td>
<td>CD317, tetherin</td>
</tr>
<tr>
<td>IFI205</td>
<td>4.1</td>
<td>4.3</td>
<td>IFN-inducible protein</td>
</tr>
<tr>
<td>IRF1</td>
<td>4</td>
<td>2.9</td>
<td>IFN regulatory factor</td>
</tr>
<tr>
<td>ZC3HAV1</td>
<td>4</td>
<td>2.8</td>
<td>Zinc finger antiviral protein</td>
</tr>
<tr>
<td>DAXX</td>
<td>3.8</td>
<td>2.5</td>
<td>Death-associated protein</td>
</tr>
<tr>
<td>PML</td>
<td>3.5</td>
<td>3</td>
<td>Ring finger protein, TRIM19</td>
</tr>
<tr>
<td>DDX58</td>
<td>3.2</td>
<td>2.4</td>
<td>RIG-I</td>
</tr>
</tbody>
</table>

* RNA prepared from WT or STAT3KO MEFs that were treated without or with IFNα 1000 U/ml for 2 h was subjected to expression microarray analysis using Illumina MouseWG-6 v1.1 Expression BeadChip.
The microarray data has been deposited to GEO accession # GSE25044.
Fig. S1 Activation of STATs in WT and STAT3KO cells in response to IFN. WT (left) or STAT3KO (right) MEFs (A) or BMMs (B) were treated with IFNα 1000 U/ml and 100 U/ml, respectively, for the indicated times. Total cell lysates were subjected to immunoblotting using antibodies to pSTAT1, pSTAT2, pSTAT3, STAT1, STAT2, STAT3 and tubulin for MEFs and pSTAT1, STAT1 and tubulin for BMMs.
Fig. S2 Increased expression of IFN downstream genes in STAT3KO BMMs after EMCV infection. WT (solid) or STAT3KO (empty) BMMs were infected with EMCV at an MOI of 10 for the indicated times. RNA prepared from the cells was subjected to RT-QPCR using primers for OAS(A), iNOS(B), IRF1 (C), IRF7(D), TLR3(E), MDA5(F), RIG-I(G) and β-actin. Relative mRNA was calculated by normalizing the values of specific genes to that of β-actin. (H) STAT3KO (right) MEFs were pretreated without or with anti-IFNAR1 antibody 1 μg/ml, followed by infection with EMCV at an MOI of 0.1 for the indicated times. Total cell lysates were then subjected to immunoblotting using antibodies to pSTAT1, pSTAT2 and tubulin. h.p.i. hour post-infection