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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.

Type I IFNs, composed of an IFN-β and several IFN-α species, are critical cytokines of innate immunity for triggering antiviral response. Antiviral functions of type I IFNs are mediated by induction of IFN-stimulated genes (ISGs), including protein kinase R (PKR), 2′,5′-oligoadenylate synthetase (OAS), RNase L, inducible NO synthase (iNOS), and IFN regulatory factors (IRFs), which interfere with virus replication or trigger cell apoptosis to avoid viral spread (1). The signaling pathways resulting in induction of the ISGs involve activation of STAT family members, including STAT1, STAT2, and STAT3, by tyrosine and serine phosphorylation (2). Activated STAT1, STAT2, and IRF9 form the ISGF3 complex, binding to the IFN-stimulated response element (ISRE) in the promoters of ISGs (3). Activated STAT1 and STAT3 form a homodimer, or heterodimer, and bind to IFN-γ-activated site. Whereas the homodimer of STAT1 or STAT3 and the heterodimer of STAT1–STAT3 can bind to similar cognate sites in vitro, the in vivo binding sites for these dimers are most likely to be different because the downstream targets of STAT1 and STAT3 and the phenotypes of STAT1- and STAT3-deficient mice are quite distinct (4–7).

STAT3 is a signaling mediator of IL-6 and IL-10 family members and other cytokines such as leptin and G-CSF (6, 8). Structurally, STAT3, similar to other STAT family members, consists of an N-terminal domain (NTD) for dimerization and tetramerization, a coiled-coil domain (CCD) for protein–protein interaction, a DNA-binding domain (DBD) for specific binding to ISG-γ-activated site element, a Src homology 2 domain for receptor recruitment and STAT dimerization, and a transactivation domain at the C terminus (6). Conventional knockout of STAT3 results in embryonic lethality, underscoring a critical role of STAT3 in embryonic development (9). Conditional targeting of STAT3 in distinct tissues or organs reveals versatile roles of STAT3 in vivo, including cell survival and apoptosis, migration, development, and differentiation (6). STAT3 also plays a key role in promoting tumor formation (10). Constitutive activation of STAT3 is found in tumors and immune cells in the tumor environment, serving as a mediator for crosstalk between tumors and immunological environment, leading to tumor-induced immunosuppression (11, 12).

Targeted deletion of STAT1 or STAT2 gene in mice (5, 7, 13) or mutation-associated STAT1 deficiency in humans (14) reveals a pivotal role of either molecule in antiviral response. Although a limited number of groups have studied the role of STAT3 in type I IFN-mediated biological response, its function has remained controversial (15–18). Using gain-of-function and loss-of-function approaches, we demonstrated that STAT3 inhibits antiviral activity of type I IFNs. Whereas knockdown or knockout of STAT3 resulted in enhanced antiviral response, restoration of STAT3 in STAT3KO mouse embryonic fibroblasts (MEFs) or hyperactivation of STAT3 in wild-type (WT) MEFs attenuated the response. Overexpression of WT or mutant STAT3 lacking DNA-binding or transactivation ability suppressed IFN-driven reporter activity. Interestingly, STAT3 NTD is sufficient to confer the suppressive effect. Therefore, these results support a negative role of STAT3 in type I IFN-mediated response.

Materials and Methods

Animals and cells

Generation and mating of MxCre-STAT3fl mice and induction of STAT3 deletion have been described previously (19, 20). These animals were...
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-α2a (Roche), recombinant murine IFN-α (Merck), recombinant murine IFN-β (Progen). The following are sources for anti-β-actin (Chemicon), anti-α-tubulin (Sigma-Aldrich), anti-phospho-STAT1 (Y701) (Invivogen), 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 (eBio-science), and anti-hemagglutinin (HA; 12CA5, homemade). Mouse IFN-α ELISA kit was purchased from PBL, WT STAT3 and DBD mutant carrying 5 aa substitutions in the DBD were reported previously (23). IRES-STAT3β was a gift of H. Yu (Department of Cancer Immunotechnology & Tumor Immunology, Beckman Research Institute of City of Hope) (24). MDA5 was a gift of T. Fujita (Institute for Virus Research, Kyoto University) (25). Flag-MDA5 was a gift of T. Fujita (Institute for Virus Research, Kyoto University) (26).

Quantitative RT-PCR

Total RNA was prepared from MEFS or primary bone marrow-derived macrophages (BMMs) using a TRizol reagent (Invitrogen). A total of 1–3 μg RNA was subjected to reverse-transcriptase reaction, and cDNA was then subjected to quantitative PCR (QPCR) by iCyq-IQ (Bio-Rad) using the following primer sets. Each sample was performed in duplicate. OAS, forward 5′-GCTCAGCTGTCGCTACT-3′, reverse 5′-CTCCTGGCATCCGAGGCTTC-3′; PKR, forward 5′-CGCCCGACATATGGTTATGCT-3′, reverse 5′-ATGTCGAAACATGAGGATG-3′; IRF7, forward 5′-GAGTTGGCGCATTTTTCTGTC-3′, reverse 5′-ATCCTCTCTGTGCACTATGG-3′; IFN-α, forward 5′-GTAAAATACTTCTCGATAGTGGCA-3′, reverse 5′-TTTGGTCCCATCTGCAACTGTC-3′; IFN-β, forward 5′-CTCGATGTTGATGACGACC-3′, reverse 5′-TCACCTCCGACAGCACAGA-3′; MDA5, forward 5′-GACGCAGATGGACTGAGA-3′, reverse 5′-TGCTATGGWCACTACCTCGTC-3′; RIG-I, forward 5′-GCATATTGAACTGACGTGG-3′, reverse 5′-CAGTCGTTGCGTCTGCTGAC-3′; IFN-γ, forward 5′-CTGAGCACGACTGCCCCAC-3′, reverse 5′-TTTGGGGCGCATGAGCAAG-3′; and β-actin, forward 5′-ATGCTGAGTTTGGCTGTTACT-3′, reverse 5′-ATGTCGAAACATGAGGATG-3′. Relative mRNA was calculated by normalizing the values of indicated genes to that of β-actin.

Microarray analysis

Total RNA from WT or STAT3KO MEFS stimulated with or without 1000 U/ml IFN-α for 2 h was obtained using the Trizol reagent (Life Technologies), followed by cleanup and DNase I treatment with QIAGEN RNeasy mini kit, according to the protocol provided by the kit. Quality control was performed by Agilent Bioanalyzer. The RNA was then subjected to expression microarray analysis using Illumina MouseWG-6 v1.1 Expression BeadChip. The complete dataset was available at National Center for Biotechnology Information Gene Expression GSEA accession GSE25044 (http://www.ncbi.nlm.nih.gov/geo/?term=GSE25044).

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 Na3VO4, 1 mM EDTA, 1 mM DTT, 1 mM PMSE, and 1 mM Na3VO4) at 4°C for 15 min. Lysates were first clarified by centrifugation at 12,000 × g for 20 min. Equal amounts of samples were resolved in 7–10% SDS-PAGE, followed by transferring to nitrocellulose or polyvinylidene difluoride membranes (Millipore) and blotted with indicated Abs.

In vitro antiviral assay and plaque formation assay

MEFS were pretreated with or without 2-fold serial dilution of IFN-α starting from 1000 to 1.8 U/ml for 24 h. EMCV at a multiplicity of infection (MOI) of 0.1 was added in the cells using serum-free DMEM for 45 min at 37°C. Viral supernatant was then removed, and the cells were refreshed with complete medium. Sixteen to 18 h postinfection, the medium was removed and cells were fixed with 10% formaldehyde solution for 20 min at room temperature. After fixation, cells were visualized with crystal violet. The excessive dye was then removed by immersing the plate in water. Each treatment was performed in duplicate. For plaque formation assay, different dilutions of supernatant from virus-infected cells (typically MEFS 16–18 h postinfection and BMMs 48 h postinfection) were used to infect Vero cells in serum-free DMEM for 1 h, followed by overlaid with 2% FBS in DMEM containing 1% SeaPlaque agarose (Lonza) to immobilize the virus. After 24 h, cells were fixed and visualized with crystal violet, 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 Packino delivering 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 × g for 90 min at 30°C. 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 pSRE-luc (Stratagene) containing 5× ISRE from Iki54 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-α2a (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 luminometer (Berthold).
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 MEFs and macrophages display enhanced type I IFN response.
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 1 IFN-mediated antiviral response.

Restoration of STAT3 reverses the otherwise enhanced type 1 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 to 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 1 IFN in STAT3KO MEFs are intrinsic to the loss of STAT3.

Increased expression of type 1 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 1 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 1 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 MEFs 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 1 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 1 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...
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. 6E). Interestingly, both DBD mutant of STAT3 and STAT3β 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.

FIGURE 7. 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 pISRE-luc and a vector carrying GFP into STAT3KO MEFs for 3 h, followed by treating the cells 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.
assay (Fig. 7B) and IFN-stimulated expression of PKR (Fig. 7C) and OAS (Fig. 7D). 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 monocytic 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 dimer formation 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 granulopoiesis (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.

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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’-ATGAGTGGTGGTGCAGGC-3’, reverse 5’-TGACCTTCAAATGCAGTAGATTCA-3’”.

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