INTRODUCTION

Tumour necrosis factor (TNF) is predominantly produced by macrophages and T lymphocytes in response to invasive stimuli such as bacterial and viral infections. While originally described as an oncolytic agent that caused tumour necrosis and regression, TNF has since been recognized as a multifunctional cytokine that modulates the growth, differentiation, and viability of transformed and normal cells.

STAT3 regulates inflammatory cytokine production downstream of TNFR1 by inducing expression of TNFAIP3/A20

Ricardo J. Antonia1,2 | Eveliina Karelehto1,2 | Kan Toriguchi1,2 | Mary Matli1,2 | Robert S. Warren1,2 | Lawrence M. Pfeffer3 | David B. Donner1,2

1Department of Surgery, University of California, San Francisco, San Francisco, California, USA
2UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, California, USA
3Department of Pathology and Laboratory Medicine (College of Medicine), and the Center for Cancer Research, University of Tennessee Health Science Center, Memphis, Tennessee, USA

Correspondence
Lawrence M. Pfeffer, Department of Pathology and Laboratory Medicine (College of Medicine), and the Center for Cancer Research, University of Tennessee Health Science Center, 19 S. Manassas St., Memphis, TN 38163, USA.
Email: lpfeffer@uthsc.edu

Funding information
Edmund Wallis Littlefield Foundation

Abstract
Tumour Necrosis Factor (TNF) potently induces a transient inflammatory response that must be downregulated once any invasive stimulus has resolved. Yet, how TNF-induced inflammation is shut down in normal cells is incompletely understood. The present study shows that STAT3 was activated in mouse embryo fibroblasts (MEFs) by treatment with TNF or an agonist antibody to TNFR1. STAT3 activation was inhibited by pharmacological inhibition of the Jak2 tyrosine kinase that associates with TNFR1. To identify STAT3 target genes, global transcriptome analysis by RNA sequencing was performed in wild-type MEFs and MEFs from STAT3 knockout (STAT3KO) mice that were stimulated with TNF, and the results were validated at the protein level by using multiplex cytokine assays and immunoblotting. After TNF stimulation, STAT3KO MEFs showed greater gene and protein induction of the inflammatory chemokines Ccl2, Cxcl1 and Cxcl10 than WT MEFs. These observations show that, by activating STAT3, TNF selectively modulates expression of a cohort of chemokines that promote inflammation. The greater induction by TNF of chemokines in STAT3KO than WT MEFs suggested that TNF induced an inhibitory protein in WT MEFs. Consistent with this possibility, STAT3 activation by TNFR1 increased the expression of Tnfaip3/A20, a ubiquitin modifying enzyme that inhibits inflammation, in WT MEFs but not in STAT3KO MEFs. Moreover, enforced expression of Tnfaip3/A20 in STAT3KO MEFs suppressed proinflammatory chemokine expression induced by TNF. Our observations identify Tnfaip3/A20 as a new downstream target for STAT3 which limits the induction of Ccl2, Cxcl1 and Cxcl10 and inflammation induced by TNF.

KEYWORDS
chemokines, NF-κB, STAT3, TNF
non-transformed cells and plays a key role in promoting inflammation. The pro-inflammatory activity of TNF has been most convincingly shown by the positive results from agents that block TNF action in the treatment of a range of inflammatory conditions, including rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, and psoriasis.

Cellular responses to TNF are initiated by its interaction with the type 1 TNF receptor (TNFR1) and the type 2 TNFR. Most TNF actions are elicited by TNFR1, which contains a death domain that fosters protein–protein interactions with other death-domain containing proteins. For example, the TNFR-associated death-domain protein (TRADD), bifurcates the TNF signal by recruiting the Fas-associated death-domain protein and procaspase 8 into a complex that initiates an apoptotic caspase cascade. TRADD also binds and uses the receptor-interacting protein (RIP) and TNFR-associated factor 2 (TRAF-2) to activate NF-κB, which induces genes that promote immunity and cell viability.

TNFR1 does not contain endogenous tyrosine kinase activity, although various TNF-induced tyrosine phosphorylation events are necessary for its biological effects. Such phosphorylation events correlate with alterations of cellular sensitivity to TNF-mediated cytotoxicity and inhibitors of protein tyrosine kinases suppress TNF-stimulated DNA fragmentation, activation of NF-κB, and expression of endothelial cell adhesion molecules. The priming of neutrophils by TNF is also accompanied by tyrosine phosphorylation events that participate in the transduction of signals that direct the cells to undergo a respiratory burst. TNFR1/TRADD signalling does not provide an obvious mechanism through which such an array of tyrosine phosphorylations can be induced. However, cytokine receptors without tyrosine kinase activity associate with nonreceptor tyrosine kinases to initiate signalling. We previously showed that Jak2 and c-Src tyrosine kinases associate with TNFR1 and can activate STAT proteins, including STAT3.

STAT3 promotes diverse physiological activities, including embryonic development, the acute phase response, wound healing, cell growth, mitochondrial function, and inflammation. STAT3 dimerization and nuclear translocation are induced by cytokine and growth factor receptors that utilize Jak and Src tyrosine kinases to induce STAT3 phosphorylation of tyrosine residue 705 (Y705). We previously showed that TNFR1 forms a complex with Jak2, which mediates STAT3 Y705 phosphorylation. While the functions of other signalling molecules downstream of TNFR1, such as NF-κB and Jun, are well characterized, the role of STAT3 in TNFR1 signalling is less understood. To shed light on the role of STAT3 in TNFR1 action, we characterized gene and protein expression changes that occurred in response to TNFR1 stimulation in the wild type (WT) mouse embryo fibroblasts (MEFs) and embryo fibroblasts from mice in which the STAT3 gene had been knocked out. The results described herein show that by acting through STAT3, TNFR1 induces signalling events that culminate in changes in gene and inflammatory chemokine expression that play an important role in the cellular response to TNF.

2 | MATERIALS AND METHODS

2.1 | Cell culture

WT and STAT3 knockout (STAT3KO) MEFs, a kind gift from Dr. Albert Baldwin (Lineberger Comprehensive Cancer Center, North Carolina), were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco) and 1x Penicillin–Streptomycin–Glutamine. Another WT MEF line was used in the validation experiments, which were a kind gift from the National Institutes of Health (NIH, Zhenggan Liu at the Cell and Cancer Biology Branch, National Cancer Institute). HEK293T cells, a kind gift from Dr. Hassan Alaoui (Department of Surgery, UCSF), were maintained in DMEM supplemented with 10% fetal bovine serum and 1x Penicillin–Streptomycin–Glutamine.

2.2 | RNA sequencing

Total RNA was isolated using a NucleoSpin mini-RNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s protocols. RNA quality control, library preparation and Illumina sequencing were performed by Novogene Corporation Inc. (Sacramento, CA, USA). Raw sequencing data preprocessing, mapping to the reference genome (mm10), and gene expression quantification were performed by Novogene. Differential gene expression analysis was performed by using the iDep tool. ENRICHR tool was used for pathway analysis. For transcription factor enrichment analysis, the ChEA3 tool was used.

2.3 | Plasmid constructs and viral transduction

Stable A20 overexpression in STAT3KO MEFs was achieved by lentiviral transduction. Transfer plasmid containing the A20 insert was obtained from Origene (MR210582L4, Tnfaip3 NM_009397, OriGene Technologies, Inc., MD, USA). Packaging (psPAX2) and envelope plasmids (pMD2.G) were a gift from Dr. Hassan Alaoui. Transfer and packaging vectors were transfected into HEK 293T cells to produce lentiviruses using Lipofectamine 3000 reagent (ThermoFisher Scientific, MA, USA). Lentiviruses were harvested 48 h post transfection, concentrated using Lenti-X concentrator (Takara Bio Inc, Japan), and used to infect STAT3KO MEFs in the presence of TransduX Max (System Biosciences, Palo Alto, CA, USA) reagent. Fresh medium containing puromycin (Invitrogen, MA, USA) was added 24 h later and the cells were maintained and selected for 2 weeks. A20/Tnfaip3 overexpression in STAT3KO MEFs was confirmed by immunoblotting (data not shown).

2.4 | Multiplex cytokine assays

MEFs were grown on 10 cm dishes until 80% confluent, serum-starved for 24 h and then stimulated with TNFR1aab (R&D
Sample text
false positives. To accomplish this, we used RNA sequencing data from WT MEFs derived from a different genetic background that were stimulated with either TNF or a TNFR1 agonist antibody (TNFR1aab). The overlapping group of genes induced by TNF or the TNFR1aab in each MEF genotype were included in the TNF target gene list. Of the 35 TNFR1 induced genes, 23 genes were more greatly induced in the STAT3<sup>KO</sup> MEFs (STAT3-suppressed genes), while 2 genes had lower induction in the STAT3<sup>KO</sup> MEFs (STAT3-promoted genes) and 10 genes were similarly induced in response to TNF (STAT3-independent genes) (Figure 2B). Since most of the genes were STAT3-suppressed, we focused on these genes. Enrichr pathway analysis of the STAT3 suppressed genes showed that these were characteristic of the "TNF Signaling via NF-κB" signature, and the "Binding of chemokines to chemokine receptors" signature (Figure 2C).<sup>24,25</sup> which included Ccl2/Mcp1, Cxcl1/KC, and Cxcl10/IP10. Enrichr analysis indicated that STAT3 was acting at least in part on a cohort of genes induced by NF-κB.

### 3.3 STAT3 represses the secretion of CCL2, CXCL1, and CXCL10 and promotes secretion of GM-CSF downstream of TNFR1

We next determined if alterations in gene expression was reflected in protein levels. We also confirmed that our observations were specific to TNFR1 by comparing TNF stimulation with TNFR1aab stimulation. Since many of the genes repressed by STAT3 in response to TNF encoded secreted chemokines, we performed multiplex cytokine assays that evaluated the expression of 44 murine cytokines. WT and STAT3<sup>KO</sup> MEFs were treated with TNF or with the TNFR1aab for 4 h, and culture media were collected and analysed for cytokines and chemokines using a multiplex assay. Using a cut-off of at least a two-fold change, levels of Ccl2/Mcp1, Cxcl1/KC, and Cxcl10/IP10 were greater in STAT3<sup>KO</sup> versus WT MEFs in response to treatment with TNF or the TNFR1aab (Figure 3A). Thus, RNA sequencing and assays of cytokine protein expression...
concordantly show that STAT3KO MEFs had greater cytokine gene expression and chemokine protein levels downstream of TNFR1 as compared to their WT counterparts. These observations show that STAT3 limits the production of a specific group of chemokines downstream TNFR1.

In contrast to these findings on chemokine protein secretion, GM-CSF secretion was more greatly induced by TNF in WT than in STAT3KO MEFs, indicating that its induction was STAT3-dependent (Figure 3B). GM-CSF was not detected by RNA sequencing in unstimulated cells, and hence its fold-change could not be calculated. Nonetheless, protein-based assays show that GM-CSF secretion induced by TNF is STAT3 dependent.

### 3.4 STAT3 induces expression of A20/Tnfaip3 that suppresses Ccl2, Cxcl1, and Cxcl10 expression

We next examined the mechanism whereby STAT3 suppresses TNF-induced Ccl2, Cxcl1, and Cxcl10 expression. Since the binding of STAT3 to various gene promoters has been found to change with time,30 we hypothesized that STAT3 played an obligate role in the acute induction of a negative regulator of TNFR1 in WT MEFs, which could explain excess cytokine production in STAT3KO MEFs. To identify genes rapidly induced by STAT3, we performed RNA sequencing on WT and STAT3KO MEFs that were stimulated with TNF for only 30 min. In contrast to the marked gene expression changes that were observed at 4 h after TNF treatment, the pattern of TNF-regulated gene expression in WT and STAT3KO MEFs was relatively similar at 30 min as illustrated in the Volcano plot shown in Figure 4A. However, A20/Tnfaip3 was found to be a TNF-induced gene whose expression was promoted by STAT3, since it was induced by TNF to a greater extent in WT MEFs versus STAT3KO MEFs. A20/Tnfaip3 is a well-characterized deubiquitinating enzyme that inhibits NF-κB activity.31 This observation is consistent with our hypothesis that STAT3 acutely increases the expression of a negative regulator of TNFR1 signaling in which NF-κB plays a prominent role.32–34 To validate the role of STAT3 on A20/Tnfaip3 gene expression observed in the RNA-seq data, we performed western blots on WT MEFs stimulated with the TNFR1ab, in the presence or absence of the STAT3 inhibitor Stattic.35 A20/Tnfaip3 levels were induced by treatment with TNFR1ab, and this induction was markedly reduced by the STAT3 inhibitor (Figure 4B,C). Taken together our results suggest that STAT3-dependent induction of A20/Tnfaip3 may be responsible for the role that STAT3 played in suppressing Ccl2, Cxcl1, and Cxcl10 expression.

We next tested the hypothesis that induction of A20/Tnfaip3 inhibited chemokine expression. To accomplish this, STAT3KO MEFs transduced to overexpress A20/Tnfaip3 were stimulated with
TNFR1aab, and the levels of secreted cytokines were determined by a multiplex assay. Enforced expression of A20/Tnfaip3 in STAT3\(^{\text{KO}}\) MEFs diminished the TNFR1-induced levels of Ccl2, Cxcl1, and Cxcl10 (Figure 5A). These results show that chemokine expression in STAT3\(^{\text{KO}}\) MEFs was elevated due to the absence of A20/Tnfaip3 induction, which is dependent on STAT3, and that expression of A20/Tnfaip3 in the STAT3\(^{\text{KO}}\) MEFs repressed chemokine expression.

In contrast to the findings on Ccl2, Cxcl1, and Cxcl10, TNFR1aab augmented expression of GM-CSF in WT but not in STAT3\(^{\text{KO}}\) MEFs (Figure 5B). The different effects of STAT3 on TNF-induced expression of Ccl2, Cxcl1, and Cxcl10 versus GM-CSF in MEFs show that these inflammatory factors are regulated through distinct mechanisms.

4 | DISCUSSION

Inflammation is a self-limiting process that provides protection against infections, injury, and trauma.\(^6\) The severity and duration of the inflammatory response is important in many disease states and may determine whether the disease resolves or becomes chronic.\(^6\) TNF has a pivotal role in the initiation and amplification of the inflammatory cascade; it regulates the release of chemokines and cytokines, oxidative stress, recruitment of immune cells and adhesion molecules, apoptosis, wound healing, and tissue-specific repair mechanisms.\(^2\) Aberrant TNF production and TNF receptor signalling have been associated with several diseases in which inflammation is an underlying element, including rheumatoid arthritis, Crohn’s disease, atherosclerosis, psoriasis, sepsis, diabetes, and obesity.\(^2,\)\(^37-40\) TNF orchestrates a cytokine cascade in many inflammatory diseases and because of its role as a “master-regulator” of inflammatory cytokine production it is a therapeutic target in inflammatory diseases.\(^38\) Indeed, anti-TNF drugs are licensed for treating inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease.\(^37-40\)

Given that overactive TNF1 activity contributes to many diseases, important homeostatic mechanisms are present to ensure that TNF1 signaling is transient and to resolve the inflammatory response to prevent pathological inflammation. Thus, genes induced by TNF1 signaling encode negative feedback regulators of the inflammatory process, including NF-\(\kappa\)B dependent expression of I\(\kappa\)B\(\alpha\) and A20/Tnfaip3.\(^41,42\) Knockout of these regulators of TNF induced inflammation leads to hyperinflammatory phenotypes in mice.

STAT3 can promote or limit inflammation depending on the stimulus.\(^43\) For example, STAT3 is pro-inflammatory downstream of IL-6, but an effector of anti-inflammatory IL-10.\(^17,43,44\) In addition, we previously showed that STAT3 negatively regulated the expression of interferon-responsive chemokines and cytokines.\(^35,46\) The present study shows that STAT3 plays an acute anti-inflammatory role downstream of TNFR1 in MEFs. We found that several TNFR1 regulated genes were more greatly induced in STAT3\(^{\text{KO}}\) MEFs than in WT MEFs at 4 h post TNF stimulation, leading us to hypothesize that STAT3 is necessary for the rapid induction of a negative regulator of the TNFR1 pathway. In agreement with this hypothesis, RNA sequencing from cells 30 min after TNFR1 stimulation showed that induction of the NF-\(\kappa\)B target
gene A20/Tnfaip3 is also STAT3-dependent. A20/Tnfaip3 acts as a feedback regulator to limit the inflammatory response by repressing chemokine expression (see model Figure 6). A20/Tnfaip3 is both an NF-κB target gene and an endogenous inhibitor of NF-κB. Under basal conditions, A20/Tnfaip3 is expressed at low levels in cells. Inflammatory cytokines, such as TNF, activate NF-κB and IKKβ, which leads to the transcription of NF-κB target genes, including A20/Tnfaip3. If IKKβ activation continues, newly translated A20/Tnfaip3 is phosphorylated by IKKβ, which subsequently inhibits NF-κB signalling. Regulation of A20 activity by gene transcription and protein phosphorylation allows NF-κB to be regulated by the strength and duration of the inflammatory signal.

Chemokines induce chemotaxis, tissue extravasation, and the differentiation of immune cells and thus play a central role in coordinating and promoting inflammation. The present study identifies specific chemokines whose expression is acutely repressed by STAT3, thereby limiting the development of inflammation. CXCL1, CXCL10, and CCL2 are all induced by TNF through activation of NF-κB in various cell types. Here, we show that in MEFs TNF represses the expression of these chemokines through TNF-induced expression of A20/Tnfaip3, and that this pathway is also highly STAT3 dependent. We suggest that NF-κB and STAT3 cooperate to regulate A20/Tnfaip3 expression, which is supported by the finding that CXCL1, CXCL10, and CCL2 production is increased by TNF in STAT3MEFs relative to WT MEFs and that enforced expression of A20/Tnfaip3 in STAT3MEFs suppresses the levels of these chemokines. NF-κB and STAT3 regulate expression of pro-survival, cell growth, and immune genes. In addition to acting independently, NF-κB and STAT3 can physically interact with each other and cooperate at gene promoters containing both NF-κB and STAT3 binding sites. For example, in immortalized human epithelial cells TNF induced 1225 genes, of which 123 were dependent on both NF-κB and STAT3.

GM-CSF has a broad range of activities in innate and adaptive immune responses. In contrast to the chemokines which are suppressed by a STAT3-dependent pathway, GM-CSF expression was increased by TNF in WT MEFs but not in STAT3MEFs, showing that GM-CSF expression is differently regulated from that of CXCL1, CXCL10, and CCL2. In some cell types, GM-CSF expression is induced by NF-κB; however, in nasopharyngeal carcinoma cells,
GM-CSF expression was transcriptionally induced by ERK. Likewise, our results suggest that a STAT3 pathway not involving NF-κB induces GM-CSF by TNF in MEFs.

We previously showed that Jak2 is constitutively associated with TNFR1.15 Oligomerization of TNFR1 in the absence of TNF binding may bring receptor associated Jak2 into proximity and permit activation by transphosphorylation.56 Aggregation of other cytokine receptors facilitates activation of receptor-associated Jak kinases, including the receptor for growth hormone.57 These observations provide a foundation for understanding the initial steps through which TNFR1 may initiate the JAK2-STAT3 signalling pathway.

Although cytokine receptors that activate STAT3 usually contain a YXXQ motif in their cytoplasmic domain that undergoes JAK-mediated tyrosine phosphorylation and interacts with the SH2 domain of STAT3,38,59 the cytoplasmic domain of murine TNFR1 does not contain this consensus STAT3 docking site. However, activation of STAT3 by growth hormone is independent of tyrosine motifs in the receptor but is accomplished through direct interaction with phosphorylated Jak2 which contains a STAT3 binding motif.60 In addition, while the cytoplasmic tyrosine residues of the IL-22 receptor are also not required for STAT3 activation, the N-terminal coiled-coil domain of STAT3 is constitutively associated with the C-terminus of the receptor.61 We suggest that TNFR1 may recruit and activate STAT3 through constitutive tyrosine independent binding or through direct binding of phosphorylated Jak2 with STAT3. A potential advantage of constitutive association of a receptor with STAT3 is that it might allow rapid or more efficient STAT3 activation in cells with low STAT3 expression. Another advantage of SH2-independent recruitment of STAT3 could be to obviate negative feedback by proteins, such as SOCS3, which compete with STAT3 for phosphotyrosine binding sites.62 Whether these putative mechanisms might account for biphasic activation of STAT3 by TNFR1 will require further study.

Our observations identify a TNFR1-STAT3/NF-κB-A20/Tnfaip3 signalling pathway in MEFs. Most cells express low levels of A20/Tnfaip3, which is induced by TNF within 30 min by NF-κB in concert with STAT3. A20 inhibits NF-κB signalling by interfering with the ubiquitination of multiple proteins that promote NF-κB activation, including RIPK1, NEMO, and even TNFR1.64 In addition, A20 is an NF-κB target gene and its induction forms a negative feedback loop that inhibits NF-κB activity. A20 suppresses cytokine expression thus imposing an early brake on inflammatory processes mediated by TNF.38,39 Thus, acute exposure of cells to TNF induces an autoregulatory program that suppresses inflammation through the coordinated activities of STAT3 and NF-κB. Chronic TNF signalling from unresolved insults or infections can override the acute temporal restraints described herein and ultimately results in pathological conditions.

**AUTHOR CONTRIBUTIONS**

**Ricardo Antonia:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); writing – original draft (equal); writing – review and editing (equal).

**Eveliina Karelehto:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal).
(equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). Kan Toriguchi: Investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). Mary Matli: Investigation (equal); methodology (equal); writing – review and editing (equal). Lawrence Pfeffer: Formal analysis (equal); resources (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). David Donner: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS
This work was supported by the Edmund Wallis Littlefield Foundation.

CONFLICT OF INTEREST
The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study and the STAT3-KO and mutant expressing GBM cells generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID
Lawrence M. Pfeffer https://orcid.org/0000-0003-2809-1234

REFERENCES
1. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol. 2001;11(9):372-377. doi:10.1016/s0962-8924(01)02064-5
2. Bradley JR. TNF-mediated inflammatory disease. J Pathol. 2008;214(2):149-160. doi:10.1002/path.2287
3. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 2005;115(1):1-20. doi:10.1111/j.1365-2567.2005.01243.x
4. Fuortes M, Jin WW, Nathan C. Adhesion-dependent protein tyrosine phosphorylation in neutrophils treated with tumor necrosis factor. J Cell Biol. 1993;120(3):777-784. doi:10.1083/jcb.120.3.777
5. Fuortes M, Jin WW, Nathan C. Beta 2 integrin-dependent tyrosine phosphorylation of paxillin in human neutrophils treated with tumor necrosis factor. J Cell Biol. 1994;127(5):1477-1483. doi:10.1083/jcb.127.5.1477
6. Fuortes M, Melchior M, Han H, Lyon GJ, Nathan C. Role of the tyrosine kinase pyk2 in the integrin-dependent activation of human neutrophils by TNF. J Clin Invest. 1999;104(3):327-335. doi:10.1172/JCI6018
7. Ji L, Zhang G, Hirabayashi Y. Inhibition of tumor necrosis factor alpha- and ceramide-induced intracellular DNA fragmentation by herbimycin a in U937 cells. Biochem Biophys Res Commun. 1995;212(2):640-647. doi:10.1016/bbr.1995.0217
8. Mishra S, Mathur R, Hamburger AW. Modulation of the cytotoxic activity of tumor necrosis factor by protein tyrosine kinase and protein tyrosine phosphatase inhibitors. Lymphokine Cytokine Res. 1994;13(2):77-83.
9. Sasaki CY, Patek PQ. The involvement of protein tyrosine kinase activity in a tumor necrosis factor resistance mechanism. Proc Soc Exp Biol Med. 1995;210(1):25-32. doi:10.3181/00377927-210-43920
10. Takada Y, Aggarwal BB. TNF activates Syk protein tyrosine kinase leading to TNF-induced MAPK activation, NF-kappaB activation, and apoptosis. J Immunol. 2004;173(2):1066-1077. doi:10.4049/jimmunol.173.2.1066
11. Reddy SA, Chaturvedi MM, Darnay BG, Chan H, Higuchi M, Aggarwal BB. Reconstitution of nuclear factor kappa B activation induced by tumor necrosis factor requires membrane-associated components. Comparison with pathway activated by ceramide. J Biol Chem. 1994;269(41):25369-25372.
12. Weber C, Negrescu E, Er, L. et al. Inhibitors of protein tyrosine kinase suppress TNF-stimulated induction of endothelial cell adhesion molecules. J Immunol. 1995;155(1):445-451.
13. Ingleby E, Klinken SP. Cross-regulation of Jak and Src kinases. Growth Factors. 2006;24(1):89-95. doi:10.1080/08977190500368031
14. Guo D, Dunbar JD, Yang CH, Pfeffer LM, Donner DB. Induction of Jak/STAT signaling by activation of the type 1 TNF receptor. J Immunol. 1998;160(6):2742-2750.
15. Pincheira R, Castro AF, Ozes ON, Idumalla PS, Donner DB. Type 1 TNF receptor forms a complex with and uses Jak2 and c-Src to selectively engage signaling pathways that regulate transcription factor activity. J Immunol. 2008;181(2):1288-1298. doi:10.4049/jimmunol.181.2.1288
16. Leaman DW, Leung S, Li X, Stark GR. Regulation of STAT-dependent pathways by growth factors and cytokines. FASEB J. 1996;10(14):1578-1588.
17. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009;9(11):798-809. doi:10.1038/nrc2734
18. Zouein FA, Duhe RJ, Arany I, et al. Loss of STAT3 in mouse embryonic fibroblasts reveals its Janus-like actions on mitochondrial function and cell viability. Cytokine. 2014;66(1):7-16. doi:10.1016/j.cyt.2013.12.006
19. Silva CM. Role of STATs as downstream signal transducers in Src family kinase-mediated tumorigenesis. Oncogene. 2004;23(48):8017-8023. doi:10.1038/sj.onc.1208159
20. Hayden MS, Ghosh S. Regulation of NF-kappaB by TNF family cytokines. Semin Immunol. 2004;26(3):253-266. doi:10.1016/j.smim.2004.05.004
21. Schutz S, Wiegmann K, Machleidt T, Kronke M. TNF-induced activation of NF-kappa B. Immunobiology. 1995;193(2-4):193-203. 10.1016/s0171-2985(95)80543-7.
22. Xia Y, Makris C, Su B, et al. MEK kinase 1 is critically required for c-Jun N-terminal kinase activation by proinflammatory stimuli and growth factor-induced cell migration. Proc Natl Acad Sci U S A. 2000;97(10):5243-5248. doi:10.1073/pnas.97.10.5243
23. Ge SX, Son EW, Yao R. IDEP: an integrated web application for differential expression and pathway analysis of RNA-seq data. BMC Bioinformatics. 2018;19(1):534. doi:10.1186/s12859-018-2486-6
24. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set knowledge discovery tool. Nucleic Acids Res. 2016;44(W1):W212-W224. doi:10.1093/nar/gkw377
25. Xie Z, Bailey A, Kuleshov MV, et al. Gene set knowledge discovery and pathway analysis of RNA-Seq data. BMC Genomics. 2019;20(1):534. doi:10.1186/s12864-019-5026-6
26. Keenan AB, Torre D, Lachmann A, et al. ChEA3: transcription factor activity of tumor necrosis factor by protein tyrosine kinase and growth factor-induced cell migration. Proc Natl Acad Sci U S A. 2000;97(10):5243-5248. doi:10.1073/pnas.97.10.5243
of human breast carcinoma cells. Oncogene. 2001;20(20):2499-2513. doi:10.1038/sj.onc.1204349

29. Rane SG, Reddy EP. JAKs, STATs and Src kinases in hematopoiesis. Oncogene. 2002;21(21):3334-3358. doi:10.1038/sj.onc.1205398

30. Tripathi SK, Chen Z, Larjo A, et al. Genome-wide analysis of

32. Arlt A, Kruse ML, Breitenbroich M, et al. The early response

35. Schust J, Sperl B, Hollis A, Mayer TU, Berg T. Stattic: a small-

34. Qiu LQ, Lai WS, Bradbury A, Zeldin DC, Blackshear PJ.

40. Patsalos O, Dalton B, Leppanen J, Ibrahim MAA, Himmerich H.

39. Keystone EC, Ware CF. Tumor necrosis factor and anti-

42. Renner F, Schmitz ML. Autoregulatory feedback loops terminating

38. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and

49. Singh S, Anshita D, Ravichandiran V. MCP-1: function, regulation, and involvement in disease. Int Immunopharmacol. 2021;101(Pt B):107598. doi:10.1016/j.intimp.2021.107598

50. Tokunaga R, Zhang W, Naseem M, et al. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy. Cancer Treat Rev. 2018;63:40-47. doi:10.1016/j.ctrv.2017.11.007

51. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-κB in cancer therapy. J Rheumatol Suppl. 2010;85:27-39.

52. Yang J, Liao X, Agarwal MK, Barnes L, Auron PE, Stark GR. Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFκB. Genes Dev. 2007;21(11):1396-1408. doi:10.1101/gad.155370

53. Wicks IP, Roberts AW. Targeting GM-CSF in inflammatory diseases. Nat Rev Rheumatol Jan 2016;12(1):37–48. 10.1038/nrrheum.2015.161

54. Schreck R, Baueerle PA. NF-kappa B as inducible transcriptional activator of the granulocyte-macrophage colony-stimulating factor gene. Mol Cell Biol. 1990;10(3):1281-1286. doi:10.1128/mcb.10.3.1281-1286.1990

55. Zhao W, Xiang Y, Zhang Z, et al. Pharmacological inhibition of GSK3 promotes TNFalpha-induced GM-CSF via up-regulation of ERK signaling in nasopharyngeal carcinoma (NPC). Int Immunopharmacol. 2020;83:106447. doi:10.1016/j.intimp.2020.106447

56. Chan FK, Chun HJ, Zheng L, Siegel RM, Bui KL, Lenardo MJ. A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science. 2000;288(5475):2351-2354. doi:10.1126/science.288.5475.2351

57. Argetsinger LS, Campbell GS, Yang X, et al. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell. 1993;74(2):237-244. doi:10.1016/0092-8674(93)90415-m

58. Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE Jr, Yancopoulos GD. Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science. 1995;267(5202):1349-1353. doi:10.1126/science.7871433

59. Yang CH, Shi W, Basu L, et al. Direct association of STAT3 with the IFNAR1 signal transducing chain of the type I IFN receptor. J Biol Chem. 1996;271:8057-8061.

60. Fujitani Y, Hibi M, Fukuda T, et al. An alternative pathway for STAT activation that is mediated by the direct interaction between JAK and STAT. Oncogene. 1997;14(7):751-761. doi:10.1038/sj.onc.1200970

61. Dumoutier L, de Meester C, Tavernier J, Renaud JC. New activation modus of STAT3: a tyrosine-less region of the interleukin-22 receptor recruits STAT3 by interacting with its coiled-coil domain. J Biol Chem. 2009;284(39):26377-26384. doi:10.1074/jbc.M109.007955

62. Ilangumaran S, Ramanathan S, Rottapel R. Regulation of the immune system by SOCS family adaptor proteins. Semin Immunol. 2004;16(6):351-365. doi:10.1016/j.smim.2004.08.015

63. Krikos A, Laherty CD, Dixit VM. Transcriptional activation of the tumor necrosis factor alpha-inducible zinc finger
protein, A20, is mediated by kappa B elements. J Biol Chem. 1992;267(25):17971-17976.

64. Shembade N, Ma A, Harhaj EW. Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. Science. 2010;327(5969):1135-1139. doi:10.1126/science.1182364

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Antonia RJ, Karelehto E, Toriguchi K, et al. STAT3 regulates inflammatory cytokine production downstream of TNFR1 by inducing expression of TNFAIP3/A20. J Cell Mol Med. 2022;26:4591-4601. doi:10.1111/jcmm.17489