***Corresponding author:**

CSF-dependent macrophages (day 9 of culture) (Figure 1a). Protein bands also differed between GM-CSF-dependent macrophages incubated with or without human neutrophil elastase (HNE) and harvested on day 9 of culture (Figure 1b). Protease-activated receptor-2

The protease-activated receptors (PARs) are a family of G protein-coupled receptors that undergo activation following proteolytic cleavage of the amino terminal by extracellular proteases [10]. PAR-2 is found in many tissues of the body and may be an important player in inflammation. Western blotting showed that GM-CSF stimulation increased PAR-2 expression by macrophages (Figure 1c), with upregulation of PAR-2 protein over time (Figure 1d). It has been reported that PAR-2 is activated in macrophages by various serine proteases [11], including HNE [12]. When GM-CSF-dependent human macrophages were stimulated with HNE (50 μM) for 6 h on day 9 of

Figure 1: (a) and (b) Coomassie Brilliant Blue (CBB) staining. (c) Western blotting for PAR-2. (d) Time-dependent changes of PAR-2 expression. (e) Production of IL-13 after stimulation with HNE. (f) Effect of MAPK inhibitors and TMB-8 on IL-13 production. (g) Western blotting for α-SMA after human pancreatic stellate cells were stimulated with IL-13. Data were obtained using macrophages from three individuals in each group and represent the mean + SE. *P<.05; **P< .01 (with Bonferroni's correction); N.S. not significant.

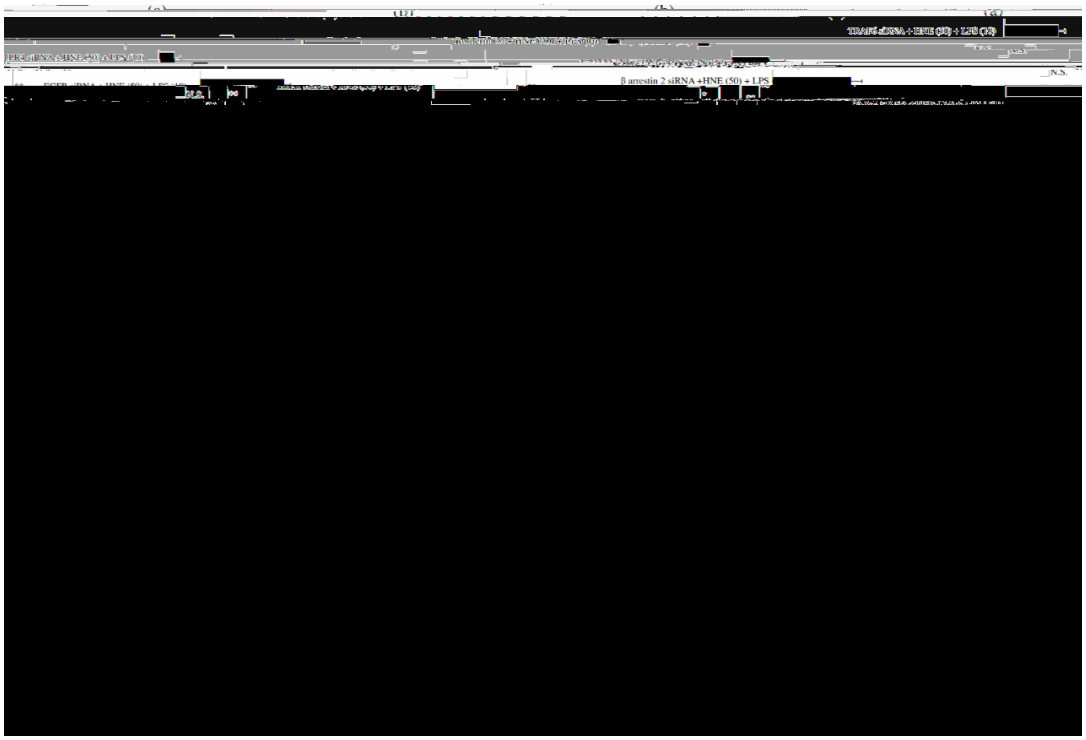
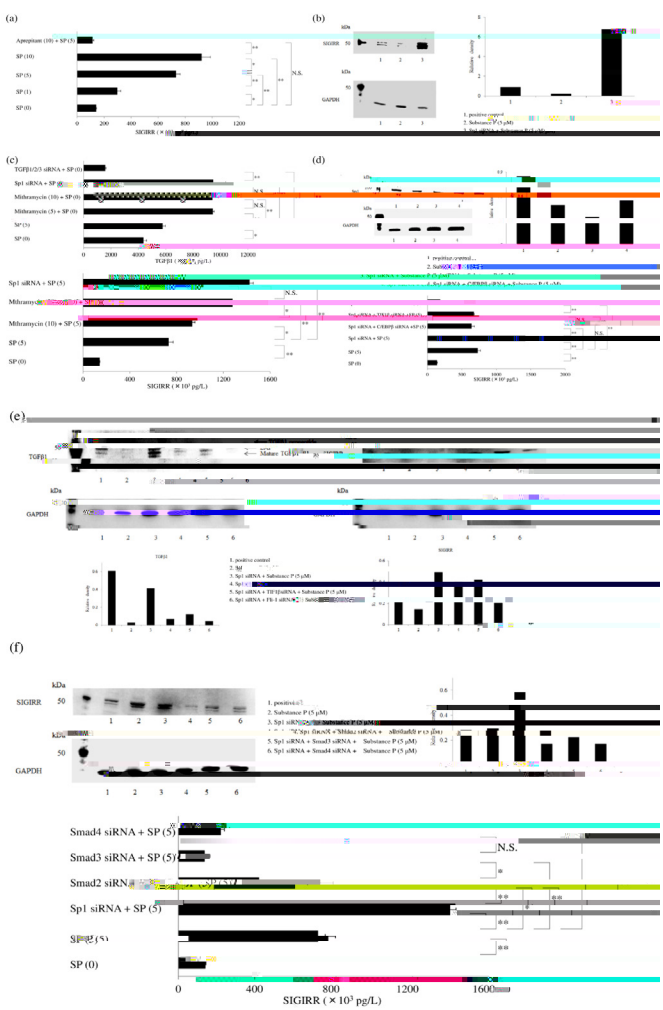


Figure 2: (a) Effect of silencing PAR-2 or β-arrestin 2 and rottlerin (a PKC inhibitor) or U73122 (a phospholipase C inhibitor) on IL-12p40 production by macrophages

Figure 3:

Citation:

Human Autoimmune and Inflammatory Responses. J Cytokine Biol 4: 128.



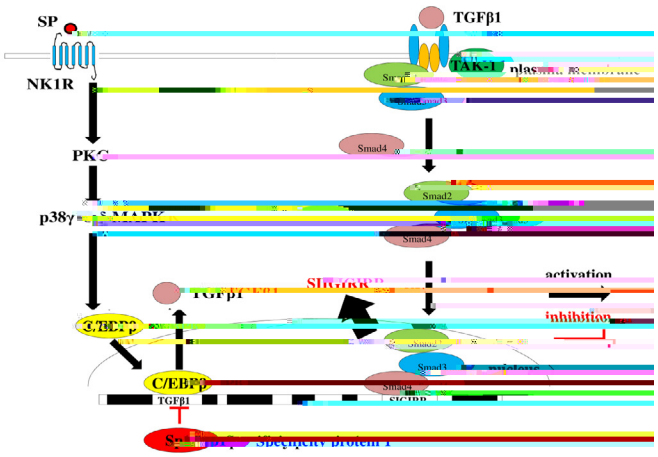


Figure 4: (a) Production of single immunoglobulin IL-1-related receptor (SIGIRR) by macrophages after stimulation with substance P (SP). (b) Effect of silencing specific protein 1 (Sp1) on SIGIRR production after stimulation with SP. (c) Effect of silencing TGFβ1/2/3 or Sp1 on TGFβ1 production after stimulation with SP and effect of Sp1 siRNA or mithramycin (an Sp1 inhibitor) on SIGIRR production. (d) Influence of cross-talk between Sp1 and Fli-1, TIF1β or C/EBPβ on SIGIRR production after

TGFβ1 acts to downregulate NK1R gene expression [54]. Interestingly, silencing of Sp1 was found to result in signi cantly increased TGFβ1 protein production by SP-stimulated macrophages [55]. Mithramycin 0 43mframycin

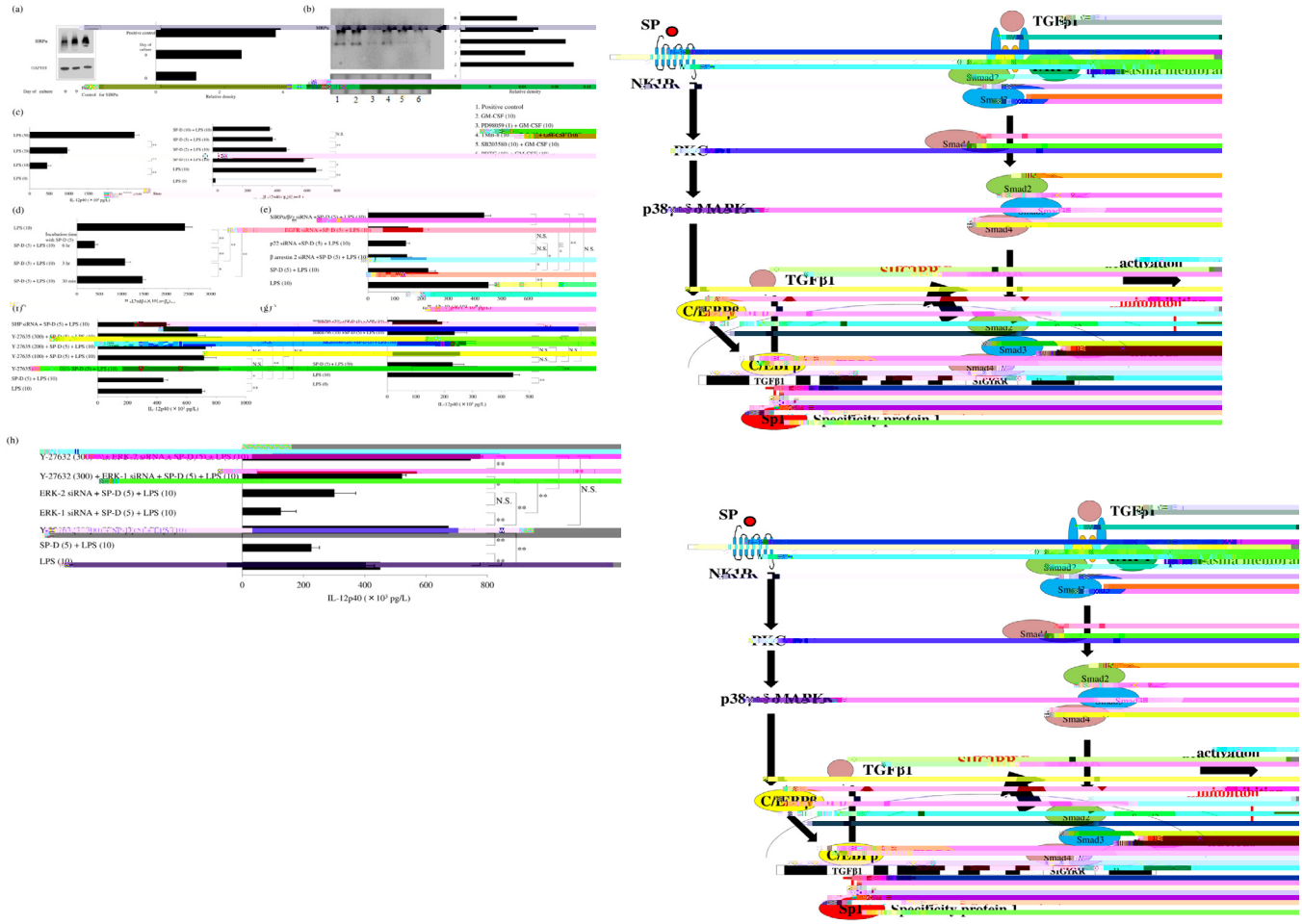


Figure 5:
(RANTES) by macrophages stimulated with substance P (SP). (c) Effects of TGFβ1/2/3 siRNA, GRK2 siRNA, β-arrestin 2 siRNA, Y-27632 (a ROCK inhibitor) or Dynasore (a dynamin inhibitor) on RANTES production after exposure to SP. (d) Effect of TGFβ1/2/3 siRNA, C/EBPβ siRNA, specific protein 1 (Sp1) siRNA or mithramycin (an Sp1 inhibitor) on production of TGFβ1 or RANTES after stimulation with SP. (e) Western blotting for TGFβ1. (f) Effects of cross-talk between Sp1 and Fli-1, TIF1β or C/EBPβ

**P< .01 (with Bonferroni's correction); N.S. not significant.

elements and forms heteromeric complexes with other transcription factors, including Sp1. e C/EBPβ promoter contains a TATA box and has binding sites for several transcription factors regulating its mRNA expression, including C/EBPβ itself [66], signal transducer and activator of transcription 3 (STAT3) [67], and Sp1 [68]. Inhibition of IL-12p40 production *via* the signal regulatory protein α (SIRPα)/surfactant protein D (SP-D) signaling pathway SIRPα is a highly glycosylated type-1 transmembrane protein comprising three immunoglobulin-like extracellular loops and a cytoplasmic tail that has three classical tyrosine-based inhibitory motifs. Western blotting showed that GM-CSF upregulates SIRPα expression by macrophages (Figure 6a). It was found that an ERK inhibitor (PD98059) signi cantly suppressed the response of SIRPα to GM-CSF, whereas this response was only partially inhibited by a p38α/βMAPK inhibitor (SB203580), an intracellular Ca^{2+} antagonist (TMB-8), or an NF-κB inhibitor (PDTC) (Figure 6b). All SIRPs possess extracellular domains with a distal immunoglobulin variable-like fold (D1) and two proximal immunoglobulin constant-like folds (D2-D3) [69]. CD47-SIRPα signaling was reported to downregulate responsiveness to IL-12 and inhibit the activation of dendritic cells [70]. e epithelium of pulmonary alveoli is largely composed of type I and type II alveolar cells, with type II cells producing GM-CSF and SP-D. It was reported that SP-D binds to the proximal domain (D3) of SIRPα, which is distant from the binding domain D1 of CD47 [71]. Binding of CD47 to SIRPα initiates signaling that inhibits phagocytosis [72] *via* several downstream molecules, including Src homology 2-containing phosphotyrosine phosphatase (SHP) and Ras homolog gene family member A (RhoA). GM-CSF was initially found in conditioned lung tissue medium a er injection of LPS into mice [73]. Recruitment of monocytes to the lungs is required for normal immune function and the in ammatory response to pulmonary injury, and resident pulmonary macrophages are reported to exist in close proximity to the respiratory epithelium [74]. e IL-12 receptor (IL-12R) has two known subunits, which are IL-12R β 1 and IL-12R β 2 [75]. In humans, IL-12R β2 is expressed by airway and parenchymal broblasts, and IL-12 signaling *via* its β2 subunit leads to the phosphorylation and activation of signal transducer and activator of transcription 4 (STAT4), promoting pulmonary brosis. IL-12 also promotes the expression of type 1α1 collagen and transforming growth factor-β1 by broblasts, which are involved in remodeling small airways, and the serum level of IL-12p40 is elevated in idiopathic

Citation:

Human Autoimmune and Inflammatory Responses. J Cytokine Biol 4: 128.

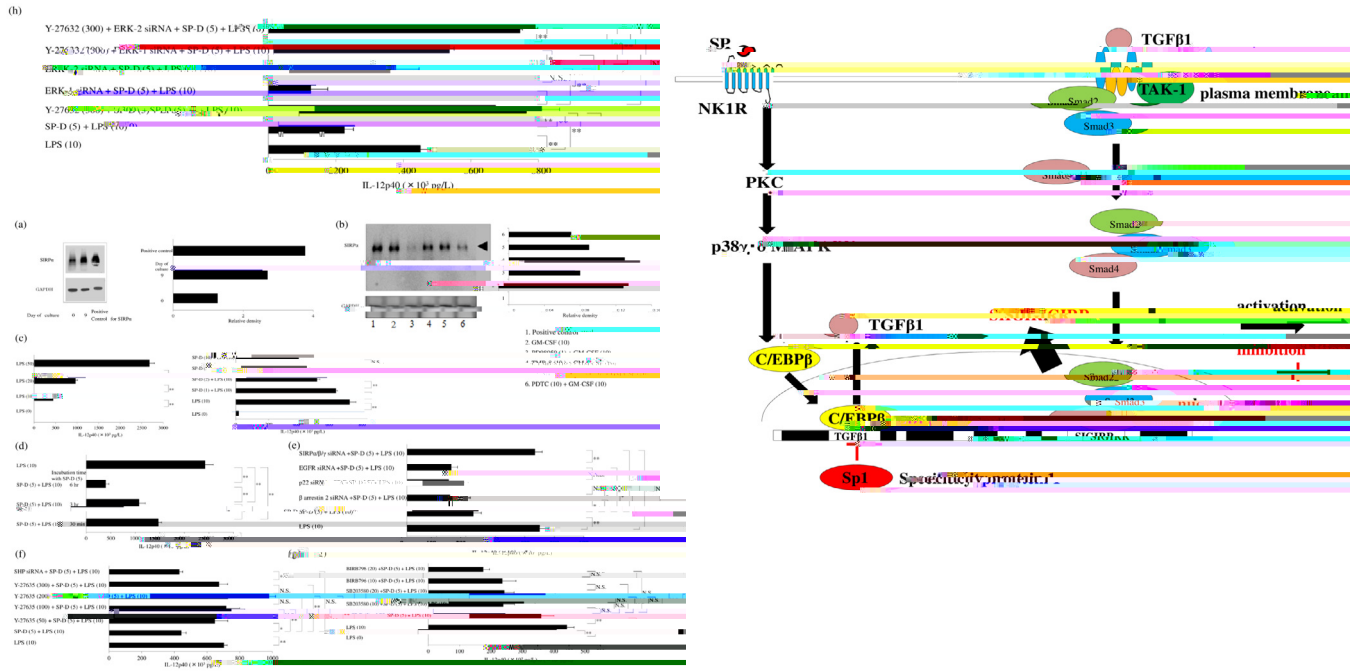


Figure 6: (a) Western blotting for signal regulatory protein α (SIRPα). (b) Effect of PD98059, TMB-8, SB203580 or PDTC on SIRPα production and western blotting for SIRPα. (c) Inhibitory effect of surfactant protein D (SP-D) on IL-12p40 production after exposure of macrophages to lipopolysaccharide (LPS). (d) Time-dependent inhibitory effect of SP-D on IL-12p40 production after exposure to LPS. (e) Effects of silencing SIRPα/β/γ, EGFR, p22phox or β-arrestin 2 on IL-12p40 production after exposure to LPS. (f) Effects of Src homology 2-containing phosphotyrosine phosphatase (SHP) siRNA or Y27632 (a ROCK inhibitor) on IL-12p40 production after pretreatment with SP-D and exposure to LPS. (g) Effects of BIRB796 or SB203580 on IL-12p40 after pretreatment with SP-D and exposure to LPS. (h) Effects of Y-27632

(a non-peptide agonist). It was unexpectedly found that pretreatment with AC-264613 attenuated IL-12p40 production by macrophages after LPS stimulation compared to pretreatment with HNE (Figure 7a). Tumor necrosis factor receptor associated factor 6 (TRAF6) is the key adaptor in the TLR4 signaling pathway [84]. TLR4 induces IL-12p40 expression in macrophages [85], while HNE activates both TLR4 [86,87] and PAR-2, so HNE-TLR4 interaction may influence IL-12p40 production. HNE also stimulates MyD88, IRAK, and TRAF6 signal transduction, leading to NF-κB activation and induction of various cytokines [88]. The IRF transcription factor family is a member of the winged helix-turn-helix DNA-binding domain superfamily [89]. IRF-5 is important for innate antiviral and inflammatory responses, and is activated by TLR4 [90]. Because IRF5 expression is upregulated by GM-CSF [91], it shows higher expression in GM-CSF-dependent macrophages than M2 macrophages. IRF5 directly activates transcription of genes encoding IL-12p4, IL-12p35, and IL-23p19 [33]. Treatment of macrophages with siRNA for IRF5 significantly reduced IL-12p40 production after stimulation with LPS (Figure 7b). Treating macrophages with HNE caused a concentration-dependent decrease of IRF5 protein expression (Figure 7c), while siRNA for PAR-2 or beta-arrestin 2 blunted this effect. Silencing SPAK/JNK also suppressed the effect of HNE on macrophages, but STAT3 siRNA had a weaker influence (Figure 7d). PAR-2 is involved in the regulation of apoptosis [92], and PAR-2 signaling is independently mediated *via* a β-arrestin 2-dependent pathway and a G-protein/Ca^{2+} pathway. β-arrestin interacts with mouse double minute 2 homolog

(MDM2), anE3 ubiquitin-protein ligase that ubiquitinates p53 and thus promotes its degradro). Ting

dependent like macrophage phenotype *via* HO-1 expression [97]. It was been reported that SP induces transformation of GM-CSF-dependent rat macrophages to an M-CSF-dependent like phenotype [98,99]. GM-CSF-dependent human macrophages and M-CSF-dependent human macrophages were exposed to substance P for 6 h, followed by western blotting to assess cell markers. Before stimulation with SP, GM-CSF-dependent macrophages were $CD80^{high}CD163^{low}$, while M-CSF-dependent macrophages were $CD80^{low}CD163^{high}$. Incubation with SP increased expression of both CD163 and CD80, so$CD80^{low}CD163^{high}$ M-CSF-dependent like macrophages were not induced.

Conclusion

erefore, incubation of human GM-CSF-dependent macrophages with substance P for 6 h did not result in a shi to the M-CSF-dependent like phenotype, unlike murine and rat M1 macrophages.

Acknowledgement

Role of the Funding Source

References

Wicks IP, Roberts AW (2016) Targeting GM-CSF in inflammatory diseases. Na Rev Rheumatol 12: 37-48.

Hamilton JA (2008) Colony-stimulating factors in inflammation and

El-Behi M, Ciric B, Dai H (2011) The encephalitogenicity of T(H)17 cells is

Immunol 12: 568-575.

Codarri L, Gyülvészi G, Tosevski V (2011) RORγt drives production of the

Wicks IP ב π Dai

Wicks IP ή ، ir τ Ů Ǯ ḏ 8 -5) ' ľ æony-st3 τ ᵀ ! Ů Å 7-4 ! ƠNa 2.

Citation:

Human Autoimmune and Inflammatory Responses. J Cytokine Biol 4: 128.

Cheng MK, Doumad AB, Jiang H (2004) Epoxyeicosatrienoic acids mediate *via* A2A receptors. Br J Pharmacol 141: 441-448.

Maeng YS, Min JK, Kim JH (2006) ERK is an anti-inflammatory signal that suppresses expression of NF-kappaB-dependent inflammatory genes by

human lung microvascular endothelia. J Biol Chem 287: 16132-16145.

migration. J Immunol 181: 5560-5567.

87.

via toll-like receptor 4. FEBS Lett 544: 129-132.

Benabid R, Wartelle J, Malleret L (2012) Neutrophil elastase modulates cytokine

induced pneumonia. J Biol Chem 287: 34883-34894.

epithelium. J Biol Chem 276: 35494-35499.

polarization:enabling diversity with identity. Nat Rev Immunol 11: 750-761.

via
and 2. Am J Respir Cell Mol Biol 48: 94-104.

Wang P, Gao H, Ni Y (2003) Beta-arrestin 2 functions as a G-protein-coupled receptor-activated regulator of oncoprotein Mdm2. J Biol Chem 278: 6363-6370.