Eicosanoid-Activated PPARα Inhibits NFκB-Dependent Bacterial Clearance During Post-Influenza Superinfection

Ronald Lucarelli1, Norma Gorrochotegui-Escalante1, Jessica Taddeo1, Bettina Buttaro1,2, Joris Beld3 and Vincent Tam1*

1 Center for Microbiology and Immunology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, United States, 2 Sol Sherry Thrombosis Research Center, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, United States, 3 Department of Microbiology and Immunology, Center for Advanced Microbial Processing, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, United States

Keywords: influenza, Staphylococcus aureus, eicosanoid, Cytochrome P450, lipidomic, innate immunity, Superinfection

INTRODUCTION

Influenza virus, an enveloped, negative-sense, single-stranded RNA virus, is an important human pathogen. Influenza infection predisposes the host to secondary bacterial infection. This superinfection is a clinically significant problem and a major cause of mortality and morbidity. Superinfection with Staphylococcus aureus following influenza leads to severe disease with approximately 41% mortality (Hageman et al., 2006). S. aureus is a Gram-positive bacterium estimated to be carried by 20% of the population (Kluymans et al., 1997). The emergence and prevalence of MRSA (methicillin-resistant S. aureus) and VRSA (vancomycin-resistant S. aureus) have significantly increased the threat posed by these bacteria (Kobayashi et al., 2012).

Secondary bacterial infection occurs as the immune system is resolving the influenza-induced inflammation. While the induction of inflammation has been the subject of active research, the...
mechanisms underlying the resolution of inflammation have remained elusive. Induction of inflammatory response ensures successful pathogen clearance. Resolution of inflammation, on the other hand, returns the immune system to homeostasis thus avoiding excessive tissue damage (Serhan et al., 2015). Eicosanoids are bioactive lipids that play critical roles in both the induction and resolution of inflammation (Dennis and Norris, 2015). During microbial insult or cellular injuries, arachidonic acids and other related polyunsaturated fatty acids, like eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA), are metabolized via three major metabolic pathways, Cyclooxygenase (COX), Lipooxygenase (COX), and CYP450, to produce hundreds of lipid species with diverse physiological activities.

We have previously characterized the lipidomic landscape during the induction and resolution of inflammation in mice and human patients who were infected with influenza (Tam et al., 2013). Eicosanoids are bioactive lipids acting as signaling molecules that play a major role in both the induction and resolution of inflammation (Tam, 2013; Dennis and Norris, 2015). Eicosanoid metabolism pathways have provided highly successful targets for pharmaceutical interventions: non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenase pathway (COX) (Simmons et al., 2004), while asthma and COPD drugs inhibit the lipooxygenase pathway (LOX) (Scow et al., 2007; Bruno et al., 2018). Subsequently, we have applied systems biology approaches to define the transcriptional and lipidomic responses in a mouse model of influenza/S. aureus superinfection (Tam et al., 2020). We identified an anti-inflammatory eicosanoid response (CYP450 lipid mediators) that was highly induced during superinfection. CYP450 lipid mediators activate the nuclear receptor and transcription factor PPARα which can affect the regulatory networks of other transcription factors via protein-protein interactions. During influenza single infection, a moderate induction of CYP450 during the resolution phase which may allow for an appropriate anti-inflammatory response to promote the return to homeostasis. In contrast, during S. aureus single infection, a minimal level of CYP450 metabolites was produced. Therefore, transcription factors mediating pro-inflammatory signaling ensure successful pathogen clearance. However, excessive induction of CYP450 during superinfection leads to the suppression of innate immune response thus inhibiting efficient bacterial clearance. As S. aureus persists, the lipidomic response amplifies the infiltration of inflammatory cells, which eventually causes excessive tissue damage and increased mortality. Interestingly, excessive CYP450 lipid mediators have been observed in COVID patients with severe disease (Schwarz et al., 2021). The pathological production of these lipid mediators may dysregulate the physiological process of resolving inflammation and exacerbate morbidity and mortality during microbial infections.

Macrophages play an essential role in both immunity lipid homeostasis through their scavenger ability to phagocytose microbes or lipids in their resident tissues (Rigamonti et al., 2008). When macrophages are exposed to specific lipids, activated receptors can change the pathological states associated with the local environment (Rigamonti et al., 2008). Some of the common nuclear receptors (NR) include glucocorticoid receptors (GR) or estrogen receptors (ER), but macrophages also have a retinoid-x receptor (RXR) called the peroxisome proliferator activated receptor (PPAR) (Rigamonti et al., 2008). PPAR has three isoforms- PPARα, PPARδ, and PPARγ, which are ligand dependent transcription factors that bind to peroxisome proliferator response elements (PPRE) that are in enhancer sites of specific genes (Berger and Moller, 2002). Among immune cells, PPARα is specifically present and highly expressed in peripheral mononuclear immune cells like macrophages (Rakhshandehroo et al., 2010).

PPARα has been shown to play a critical role during microbial infections. In a mouse Mycobacterium abscessus infection model, Ppara−/− knockout mice show notably higher bacterial loads and increased cytokine expression of pro-inflammatory genes, including Il6, Il1b, and Cxcl10 (Kim et al., 2020). Furthermore, macrophage polarization profiles can be influenced by PPAR activation. Infection of macrophages with the obligate intracellular parasite, Trypanosoma cruzi, increased classically activated (M1) markers (e.g. NOS2) and increased proinflammatory cytokine signaling (Penas et al., 2015). Activation of PPARγ with 15dPGJ2 or PPARα with WY14463 showed increased Arginase-1 (M2 marker) and decreased pro-inflammatory cytokine expression (Penas et al., 2015). These studies demonstrate that activation of PPAR promote an anti-inflammatory phenotype.

While we demonstrated the role of CYP450 during superinfection in vivo, the mechanisms by which the lipid mediators affect the molecular signaling and cellular function on inflammation and bacterial clearance are not well understood. In this study we investigated the impact of CYP450-PPARα axis on the inflammatory signaling in macrophages since they are the dominant cell types in the broncho-alveolar lavage during superinfection. Using Nanostring nCounter Technology, we determined that the CYP450 lipid metabolites dampened the inflammatory transcriptional responses in macrophages. We demonstrated that the activation of PPARα inhibits NFκB, hinders antibacterial activities and modulates macrophage polarization. Lastly, using liquid chromatography-Mass spectrometry (LC-MS), we determined the lipidomic profiles in wild type and Ppara−/− mice during superinfection. The increased eicosanoid metabolism in Ppara−/− mice may contribute to increased survival in during superinfection.

RESULTS

Activated PPARα Inhibits NFκB Activity and Pro-Inflammatory Gene Expression

We have previously determined that increased production of CYP450 metabolites during superinfection have reduced pro-inflammatory genes induction in cells isolated from Broncho-alveolar lavage or whole lung lysates (Tam et al., 2020). Since a majority of the BAL cell population consisted of inflammatory
monocytes, macrophages and neutrophils, we determined to dissect
the molecular mechanism by which CYP450 lipid metabolites
hinder the proinflammatory response in macrophages. We
determined the targeted transcriptional response of bone marrow-
derived macrophages after polynosinic-polycytidylic acid (poly:IC)
stimulation by Nanostring nCounter Technology. We determined
that after the addition of 14, 15 DHET (dihydroxy-eicosatrienoic
acid, a CYP450 lipid metabolite), genes related to inflammatory
response are induced to significant lower levels than poly:IC
stimulated macrophages (Figure 1A; Supplementary Figure 1A).
The genes with blunted response to the TLR3 ligand
(Supplementary Figure 1B, cluster 1) include cytokines and
chemokines (Ccl5, Ccl7, Cxcl3, Cxcl10, Il6, Il12b), type I interferon
regulated genes (Mx1, Mx2, Ifit1, Ifit2, Ifit3, Oasl1) (Figures 1A, B).
Moreover, the effects of 14,15 DHET is Ppara-dependent in which
the induction of Il12b from poly:IC stimulation was not affected by

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1** | PPARα activation dampens pro-inflammatory gene expression in macrophages. (A) Heat map depicts fold changes of transcript levels in C57BL/6 macrophages stimulated with 14,15 DHET, poly:IC (TLR3 agonist), or 14,15 DHET with poly:IC normalized to unstimulated for 3h. RNA was extracted and analyzed using Nanostring nCounter Technology (Inflammation panel of 254 mouse genes including 15 internal reference genes). n=3 per group. Data was analyzed using nSolver software. Genes displayed have P value < 0.01, FDR<0.05. (B) Bar graphs depict the log2 expression fold change of Cxcl3, Il6, and Il12b, (cytokines/chemokines) and Ifit1 (interferon regulated gene) relative to unstimulated cells for the indicated conditions. (C) Bar graph depicts transcript levels (mean +/- SEM) of Il12b levels as measured by RT-PCR from Hoxb8 macrophages (C57BL/6, black or Ppara<–<–, purple) stimulated with 14,15-DHET, poly:IC, or 14,15 DHET with poly:IC normalized to Ef1a. Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).
the CYP450 lipid metabolite in $Ppara^{-/-}$ cells (Figure 1C). From these data, we concluded that activation of CYP450 lipid metabolite directly hinders the TLR signaling of macrophages. Next, we performed promoter enrichment analysis (HOMER v4.11) to determine candidate transcription factor mediating the suppression of inflammatory response. Using hierarchical clustering to identify genes that are induced during $S. aureus$ infection and significantly suppressed during superinfection, we determined that the top enriched motif to be that of the transcription factor, NF-$kappaB$.

Using automated digital microscopy and immunoblotting to determine the localization and abundance of NF-$kappaB$, we performed promoter enrichment analysis (HOMER v4.11) to determine how activation of PPAR$alpha$ alters NF-$kappaB$ localization and phosphorylation. To further determine the mechanism by which activation of PPAR$alpha$ affects NF-$kappaB$, we used automated digital microscopy and immunoblotting to determine the localization and abundance of NF-$kappaB$ with or without PPAR$alpha$ activation. While TLR stimulation significantly increased NF-$kappaB$ activities, activation of PPAR$alpha$ hampered the activities in wild type C57BL/6, compared to $Ppara^{-/-}$ (Supplementary Figures 2A, B). Unexpectedly, $Ppara^{-/-}$ did not induce significant NF-$kappaB$ activity upon poly:IC or LPS (TLR4 ligand) stimulations (Supplementary Figures 2A, B). Since PPAR$alpha$ plays additional roles including fatty acid metabolism, the lack of PPAR$alpha$ in $Ppara^{-/-}$ macrophages may alter the threshold for immune response. These data suggest that activation of PPAR$alpha$ with either CYP450 lipid metabolite or synthetic ligand WY14643, the chemical agonist with comparable activity to 14,15 DHET (Fang et al., 2006), suppresses NF-$kappaB$ activities and hence the inflammatory response in macrophages. We have used WY14643 in lieu of CYP450 lipid metabolites for its specificity and feasibility, and we have selected concentration by activation efficacy and ensuring cytotoxicity does not occur (Supplementary Figure 2C).

Activated PPAR$alpha$ Alters NF-$kappaB$ Localization and Phosphorylation

To further determine the mechanism by which activation of PPAR$alpha$ affects NF-$kappaB$, we used automated digital microscopy and immunoblotting to determine the localization and abundance of NF-$kappaB$ p65. NF-$kappaB$ is a master transcription factor involved in inflammation and cell death (Huang et al., 2010). Upon stimulation via TLR by microbial infection or specific TLR ligands, NF-$kappaB$ p65 is phosphorylated, which promotes nuclear translocation (Oeckinghaus and Ghosh, 2009). To assess how activation of PPAR$alpha$ suppresses NF-$kappaB$ activities, we determined the phosphorylation status of NF-$kappaB$ during TLR stimulation with or without PPAR$alpha$ synthetic agonist. While the abundance of NF-$kappaB$ was similar between conditions, abundance of phosphorylated NF-$kappaB$ p65 was decreased during TLR stimulation with WY14643 in wildtype macrophages (Figures 2A, B, Supplementary Figure 3A). Moreover, the phosphorylation status of macrophages generated from $Ppara^{-/-}$ animals did not differ with the PPAR$alpha$ agonist. Using automated digital microscopy, we performed immunofluorescent microscopy to determine the nuclear translocation of both NF-$kappaB$ and PPAR$alpha$. Upon stimulation with TLR ligand (LPS) with or without WY14643, macrophages were immediately fixed and permeated, followed by immunofluorescent staining for NF-$kappaB$ and PPAR$alpha$. We observed a significant decrease in NF-$kappaB$ and PPAR$alpha$ nuclear localization upon TLR stimulation with WY14643 in wildtype macrophages.

**FIGURE 2** | PPAR$alpha$ activation hinders NF-$kappaB$ phosphorylation and nuclear translocation during TLR stimulation. Bar graphs depict protein quantifications (mean +/- SEM) from immunoblotting of wild type C57BL/6 or $Ppara^{-/-}$ Hoxb8 macrophages stimulated with LPS (TLR4 agonist) for 30 minutes with or without WY14643 (PPAR$alpha$ agonist) against NF-$kappaB$ (A) and phosphorylated NF-$kappaB$ p65 (B). Bar graphs depict nuclear localization (mean +/- SEM) of NF-$kappaB$ (C) and PPAR$alpha$ (D) in wild type C57BL/6 or $Ppara^{-/-}$ Hoxb8 macrophages stimulated with LPS (TLR4 agonist) for 30 or 60 minutes with or without WY14643 (PPAR$alpha$ agonist). Images were analyzed by HCS software for nuclear localization. Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001; ns not significant), n=3 and are representative of 3 experiments.
by stained with DAPI, anti-NFκB p65 (CY-5), and anti-PPARα (AlexaFluor 488) antibodies. Nuclear localization of p65 was significantly decreased when WY14643 was administered with LPS compared to TLR ligands alone (Figure 2C, Supplementary Figures 4, 5). While wildtype macrophages stimulated with PPARα agonist present with distinct nuclear localization of PPARα, knockout macrophages showed a faint fluorescence outside the nucleus (Figure 2D, Supplementary Figures 4, 5). The residual signal in the \textit{Ppara}−/− macrophages can be explained by the non-specific binding of anti-PPARα antibodies in both wildtype and \textit{Ppara}−/− cells (Supplementary Figure 3B). The protein analysis and localization studies suggest that the activation of PPARα decreased the phosphorylation and nuclear localization of NFκB.

**PPARα Activation Dampens Phagocytosis and Bacterial Clearance**

Since we observed persistent bacterial colonization in the lungs of influenza/\textit{S. aureus} super-infected animals, we hypothesized that CYP450-PPARα axis hinders the phagocytic or bactericidal activities of macrophages. To understand the physiological role of PPARα activation in phagocytosis and bacterial clearance, we infected C57BL/6 and \textit{Ppara}−/− macrophages derived from murine bone marrow or Hoxb8 macrophages of each genotype (data not shown) with \textit{S. aureus} for 30 and 60 minutes, washed the macrophages to isolate the intracellular populations, and determined the colony forming units. Activation of PPARα by WY14643 resulted in lowered bacterial burden in macrophages (Figure 3A). While the CFU assay can assess the intracellular bacterial loads at the given time points, the dynamic host-pathogen interactions cannot be discerned. To address whether the difference in CFU was due to differences in phagocytosis or bacterial killing, we used digital and confocal microscopy to determine uptake and bactericidal activities. We introduced heat-killed CFP-expressing \textit{S. aureus} and used automated digital microscopy and High Content Screening analysis software to determine phagocytosis by macrophages with or without PPARα activation by WY14643. While wild type C57BL/6 and \textit{Ppara}−/− macrophages were able to phagocytose similar level of heat killed bacteria at 30 minutes and 1 hour, activation of PPARα by WY14643 inhibited phagocytosis in wild type but not \textit{Ppara}−/− macrophages (Figure 3B). To determine bacterial killing activities of macrophages, we infected macrophages with CFP-expressing live \textit{S. aureus} and stained the bacteria with Sytox green (Figures 3C, D). Live bacteria will not retain the fluorescence from Sytox Green while killed bacteria, due to the loss of its membrane integrity, will be

![FIGURE 3](image-url)
stained. Using CellMask orange and DAPI to counterstain the plasma membrane and nucleus, respectively, we conducted both confocal microscopy and automated fluorescent microscopy. We observed significant bacterial killing by wild type macrophages at 30 minutes post-infection while activation of PPARγ by WY14643 significantly decreased the ability of macrophages to kill *S. aureus* (Figures 3C, D). This killing inhibition by WY14643 was also not observed in *Ppara*−/− macrophages. These data suggest that activation of PPARα in macrophages hinders the phagocytic and bactericidal activities. Furthermore, the phagocytosis and clearance capabilities are not affected in *Ppara*−/− macrophages by PPARα agonist.

**PPARα Influences Macrophage Polarization and Lipidomic Responses During Superinfection**

Macrophages play an important role in the induction and resolution of inflammation. The activities of macrophages depend on the microenvironment and autocrine/paracrine signaling. Macrophages can polarize into M1 (classical), M2a (alternatively activated), M2b (anti-inflammatory), and M2c (wound healing) subsets (Sica and Mantovani, 2012; Arora et al., 2018; Wang et al., 2018). PPAR has been shown to modulate M1 M2 macrophage polarization. While PPARγ has been shown to affect macrophage polarization (Nelson et al., 2018; Yao et al., 2018), the role of PPARα remains elusive (Penas et al., 2015). While wild type animals all succumb to superinfection, *Ppara*−/− animals were partially protected (Tam et al., 2020). Cellularity studies of collected broncho-alveolar lavage (BAL) post-infection (on day 8) determined that there were significantly fewer M2 macrophages in Ppara−/− mice during superinfection compared to wild type animals (Figure 4A; Supplementary Figure 6). Additionally, as there were comparable populations of inflammatory monocytes, DC, T cells, B cells and NK cells between wild type and *Ppara*−/− mice, there was an increase of neutrophils within the Ppara−/− mice (Supplementary Figures 6, 7). To understand how PPARα affects macrophage polarization, we skew bone marrow-derived macrophages to M1 with LPS and IFNγ for 24 to 48 h, M2a with IL4/IL13, M2b with Immune Complex (Ova and anti-Ova) and LPS, and M2c with IL10/TGFβ. When we polarized macrophages by subtype stimuli and activated PPARα with

![FIGURE 4](image_url)

**FIGURE 4** | PPARα knockout is protective against influenza/S. aureus superinfection. (A) Bar graph depicts percentages of CD11b+ F4/80+ CD206+ M2 macrophages (mean±/SEM) from *S. aureus*, influenza, or influenza/S. aureus infected C57Bl/6 (black) or *Ppara*−/− (purple) mice on day 8 post-infection. N=3-5, representative of 3 independent experiments. (B) Bar graphs depict percentages of C57BL/6 (black [DMSO] and red [WY14643]) and Ppara−/− (purple [DMSO] and green [WY14643]) BMDM macrophages polarized to M1, M2a, and M2b phenotypes, with or without the presence of PPARα agonist, WY14643. Two way ANOVA with multiple comparisons were performed to determine statistical significance (P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001), n=3 per group and are representative of 3 experiments. (C) Lipidomic mass spectrometry measurements from broncho-alveolar lavage of immune-associated lipids (arachidonic acid, EPA, DHA, PGE2, 14,15-EET, 14,15-DHET, LTB4, 13 HODE, and 15d PGJ2). Student’s T tests were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ns not significant), n=4 per group.
WY14643, there was a significant decrease in M1 macrophages that was dependent on Ppara (Figure 4B). This effect does not show switching from M1 to M2 macrophages exclusively. M2a polarization was also slightly decreased with PPARα activation. Interestingly, M2b macrophages were significantly enhanced by WY14643 that was dependent on Ppara. We observed no difference with M2c polarization (data not shown). Using Il12b (M1), Arg-1 (M2a), and Il10 (M2b) as polarization markers, we have demonstrated that PPARα activation directly reduces the M1 and M2a populations while increasing the M2b population (Supplementary Figure 8). Moreover, as macrophage polarization results in a spectrum of phenotypes rather than strict qualitative changes, we determined that the mean fluorescent intensity of CD206, a mannose receptor expressed on M2 macrophages (Tsuchiya et al., 2019), was significantly increased in C57BL/6 macrophages with the addition of WY14643 but not during M2a polarization, or in Ppara−/− macrophages (Supplementary Figure 9). These data suggest that activation of PPARα specifically increased the M2b populations both qualitatively and quantitatively. The eicosanoid metabolic response is highly dynamic due to transcellular metabolism (a collaboration of different cell types participating in eicosanoid production) and metabolic shunt (inhibition or down-regulation of an enzyme within one pathway may “shunt” the substrate through another pathway) (Buczynski et al., 2009; Norris and Dennis, 2012). We conducted lipidomic profiling (using LC/MS/MS) in C57BL/6 and Ppara−/− mice during superinfection to determine how the genetic perturbation affects the lipidomic responses (Figure 4C). Comparative lipidomic profiling between wildtype and the Ppara−/− knockout mice illustrate an altered population of lipid mediators are produced influencing the inflammatory response during superinfection. Major precursors (including arachidonic acids and DHA), cyclooxygenase -derived PGE2, lipoxygenase-derived LTB4, CYP450 metabolite (14,15 DHET) and linoleic acid-derived metabolite (13 HODE) were significantly increased in C57BL/6 macrophages with the addition of WY14643 but not during M2a polarization, or in Ppara−/− macrophages (Supplementary Figure 8). Moreover, as macrophage polarization results in a spectrum of phenotypes rather than strict qualitative changes, we determined that the mean fluorescent intensity of CD206, a mannose receptor expressed on M2 macrophages (Tsuchiya et al., 2019), was significantly increased in C57BL/6 macrophages with the addition of WY14643 but not during M2a polarization, or in Ppara−/− macrophages (Supplementary Figure 9). These data suggest that activation of PPARα specifically increased the M2b populations both qualitatively and quantitatively. The eicosanoid metabolic response is highly dynamic due to transcellular metabolism (a collaboration of different cell types participating in eicosanoid production) and metabolic shunt (inhibition or down-regulation of an enzyme within one pathway may “shunt” the substrate through another pathway) (Buczynski et al., 2009; Norris and Dennis, 2012). We conducted lipidomic profiling (using LC/MS/MS) in C57BL/6 and Ppara−/− mice during superinfection to determine how the genetic perturbation affects the lipidomic responses (Figure 4C). Comparative lipidomic profiling between wildtype and the Ppara−/− knockout mice illustrate an altered population of lipid mediators are produced influencing the inflammatory response during superinfection. Major precursors (including arachidonic acids and DHA), cyclooxygenase -derived PGE2, lipoxygenase-derived LTB4, CYP450 metabolite (14,15 DHET) and linoleic acid-derived metabolite (13 HODE) were significantly increased in Ppara−/− mice, compared to C57BL/6. These data suggest that PPARα activation influences the macrophage polarization in vitro and in vivo. In addition, the resulting altered macrophage function and eicosanoid metabolism may contribute to the differences in mortality and morbidity in superinfection, where Ppara−/− mice are partially protected.

DISCUSSION
Influenza is an important human pathogen causing serious clinical complications. Prevalence and mortality vary greatly depending on the circulating seasonal or pandemic virus strain. Using a non-biased global systems biology approach and focusing on eicosanoids, we have identified a subset of lipid mediators that are produced during the resolution of inflammation (Tam et al., 2020). These eicosanoids are natural ligands for the nuclear receptors/transcription factors PPAR, which play critical roles in regulating macrophage polarization, inflammation, and lipid metabolism (Ng et al., 2007). To understand the mechanisms by which the CYP450-PPARα axis exacerbates mortality and morbidity during superinfection, we investigated how activation of PPARα affects the inflammatory signaling in macrophages. Using targeted transcriptional profiling with Nanostring nCounter Technology, we determined that CYP450 metabolites dampens the TLR signaling pathway via PPARα activation. We assessed the transcriptional activities of NFκB with lentiviral luciferase reporters. Activation of PPARα with CYP450 metabolite or synthetic ligand decreased NFκB activities. Moreover, activation of PPARα inhibits NFκB p65 phosphorylation and nuclear translocation.

PPAR has been shown to modulate the transcriptional networks by multiple mechanisms (Ricote and Glass, 2007; Pawlak et al., 2015). PPAR can directly interact with other transcription factors (i.e. p65) and prevent binding to NFκB response element or activating transcription. Activated PPAR can also induce 1xβ which inhibits NFκB in the cytoplasm. Additionally, activated PPAR can regulate kinase activity, compete for coactivator complex or even inhibit co-repressor clearance.

The precise mechanism by which activated PPARα inhibits NFκB will be elucidated in future studies. We used both poly:IC and LPS to highlight that regardless of signaling through TLR3-TRIF or TLR4-TRIF and MyD88, the effects of CYP450 lipid-PPARα axis acts further downstream of the signaling cascade which may be significant due to the differences in PAMPs detected in multi-pathogenic interactions with the host immunity in superinfection.

High levels of CYP450 lipid mediators were detected in influenza superinfection as well as in serum from patients with severe COVID disease (Schwarz et al., 2021). Besides hindering the anti-bacterial activities, CYP450 lipid mediators decimated the anti-viral response, including numerous Type I interferon response and regulated genes (Ifnb1, Ms1, Ms2, Oasl1, Ifit1, Ifi2, Ifit3) (Figures 1A, B). The blunted anti-viral response may allow the unrestrained replication of viral pathogens which lead to an eventual cytokine storm (Ragab et al., 2020). Concordantly, immunosuppressed adaptive and innate immune cells and early-stage immune suppression have been observed from COVID patients (Remy et al., 2020; Tian et al., 2020). In contrast, the production of CYP450 lipid mediators occur during the resolution phase of influenza (7-10 days post infection), the dampening of antiviral response would allow for the return to homeostasis while adaptive immunity continues to eradicate the virus.

While we observed a persistence of S. aureus in the lungs of influenza-infected mice (Tam et al., 2020), using an in vitro system, we determined that the anti-bacterial function of macrophages was greatly hindered due to PPARα activation (Figure 3). By utilizing CFU assays and digital microscopy, we were able to quantify a diminished phagocytic capability during PPAR activation. Digital microscopy using fluorescent markers to distinguish killed bacteria further showed that bacterial killing functions were also hampered during infection. Macrophages greatly affect the mediation of inflammation from the onset to resolution of infection. Interestingly, activation of PPARα is
While Ppara have determined that there was a significant increase in pro-inflammatory genes during bacterial infection, as similarly observed during influenza/S. aureus superinfection, Ppara−/− mice have increased bacterial loads during *M. tuberculosis* (MTB) and *P. aeruginosa* infections. While MTB and *P. aeruginosa* are restricted and controlled by autophagy, *S. aureus* induces and exploits autophagy for its survival and are restricted and controlled by autophagy, *P. aeruginosa* inhibits NFκB post-infection (Ouyang et al., 2020).

The effects of CYP450-PPARα axis on macrophage polarization, anti-bacterial activities, and abilities to recruit other immune cells (particularly neutrophils), may be exploited by the bacterial pathogens during superinfection (Supplementary Figure 4). When PPARα is activated during superinfection, macrophages have reduced immune response involving cytokines, such as Cxcl3 (Figure 1), and eicosanoids, such as LTB4 (Figure 4C). These signaling molecules play critical roles in recruitment of neutrophils (Zhang et al., 2001; Afonso et al., 2012; Wu et al., 2012; Lämmermann et al., 2013). While neutrophils are recruited in knockout mice during superinfection, the dampening of Cxcl3 and LTB4 correlates with decreased infiltration into the lungs of wildtype mice. This is problematic for the host immune system that has decreased macrophage functions in both immune response and function (namely phagocytosis and bacterial killing). Neutrophils and macrophages both play an essential role during influenza pneumonia infections as the main immune cell infiltrates during infection (Rudd et al., 2019). When uncontrolled recruitment and activation of neutrophils occur during influenza infection, the exacerbated outcomes are altered due to acute lung injury caused by excessive infiltration and generation of NETs (Rudd et al., 2019). However, the regulatory network to prevent exacerbated immune response may benefit the opportunistic secondary bacterial infection. Besides LTB4, other eicosanoids, such as PGE2, which has both pro- and anti-inflammatory activities (Sander et al., 2017), and 13-HODE (anti-inflammatory lipid mediator) (Vangaveti et al., 2010), were significantly increased during superinfection in *Ppara−/−* mice. Moreover, while 14, 15 EET (precursor to 14,15 DHET) was

![FIGURE 5](Image) | An overview of superinfection. An illustrated image depicting the progression of post-influenza secondary bacterial superinfections. The major hallmarks of infection revolve around the dampening of immune response, the subsequent immune shock, and the outcome of increased morbidity and mortality. Illustration created with BioRender.com.
produced at similar levels, 14,15 DHET was produced at a significantly higher level in Ppara−/− mice, compared to wild type. The increased lipodomic response of Ppara−/− mice may be due to the lack of anti-inflammatory signals via CYP450-PPARα axis. The increased lipod mediators produced in Ppara−/− mice suggests that the CYP450-PPARα axis limits the eicosanoid metabolism. This negative feedback loop may be signaled via the transcriptional changes, immune cell activation status, or indirectly via altered fatty acid metabolism which generates the precursors for eicosanoids. The precise mechanism by which PPARα affects eicosanoid metabolism will be investigated in future studies.

The shift in anti-bacterial activities, macrophage polarization, and lipodomic responses may explain the difference in mortality and morbidity during superinfection, where Ppara−/− mice are partially protected. When individuals are infected with influenza, resolution of inflammation occurs approximately around 7-10 days (Figure 5). The activation of PPARα promotes a systemic dampening of inflammatory response, propagating a cascade that affects macrophage function and immune cell recruitment to assure successful resolution. The resultant dampening of the immune response during resolution is sometimes exploited by a secondary bacterial infection post-influenza. Due to this anti-inflammatory immune environment, the macrophages are not activated to properly respond to the infection, nor recruit the proper immune cell response (i.e., neutrophils). The failure to control the bacterial infection ultimately leads to an immune shock that will severely impact the pathology of the lung, possibly causing death (Figure 5).

PPARα has been demonstrated in other infection and disease models to exert an anti-inflammatory or repair activity. For example, activation of PPARα can reduce the inflammatory effects of LPS-induced acute lung injury by ameliorating vascular leakage and release of cytokine and eicosanoids into the alveolar space (Schaef er et al., 2008). Activation of PPARα can also restore the mitochondrial structure and promote gut epithelial repair during SIV infection in a nonhuman primate (Crakes et al., 2019). Moreover, PPARα is a master regulator for lipid metabolism (Bougarne et al., 2018). The activation of PPARα may modulate the metabolism which alters the immune function and activity of the cells. Surprisingly, we observed a decreased NFκB activity during TLR stimulation and an absence of increased bacterial killing in Ppara−/− macrophages. The lack of PPARα in the Ppara−/− macrophages may alter the threshold for immune response. The mechanisms by which CYP-PPARα affect the immunometabolism will be elucidated in future studies. Interestingly, while fenofibrate (PPARα agonist) are prescribed for abnormal blood lipid levels, infection and pneumonia are cited as possible adverse side effects (FDA, 2008; FDA, 2010) and fenofibrate has been associated with higher risk of mortality in MTB infections (Liu et al., 2020). In contrast, in a mouse influenza model, administration of oseltamivir (antiviral) and fibrates (PPARα agonist) prolong survival time during lethal H7N9 infection (Xu et al., 2015). Similarly, administration of gemfibrozil (fibrates) also increased survival during H2N2 infection (Budd et al., 2007). From these data, fibrates had been proposed as an inexpensive treatment against severe influenza infections or influenza pandemic (Fedson, 2009). Our data may explain the seemingly contradictory concepts. PPARα agonists may be useful for successful resolution during influenza infection (Figure 5). However, during superinfection, the pathological production of DHET excessively activates PPARα, compromising the immune system’s ability to control secondary bacterial infections (Figure 3). If fibrates therapy drastically increase the activation of PPAR, patients may be at risk for superinfection. However, the use of fibrates is confounded by the fact that it also inhibits CYP2C enzymes (Gong et al., 2016) which may prevent dangerous levels of DHET. Furthermore, data is not available to assess PPAR activation in the lungs of patients under fibrates therapy. And the level of PPAR activation may not be increased at a pathological level for bacteria to cause opportunistic infections.

The complexity of the superinfection stems from a triad of interacting players: the host immune response, influenza virus, and the bacterium. Successful pathogen clearance and resolution of inflammation ensure physiological return to homeostasis. In contrast, dysregulated functions resulting from aberrant PPAR activation will hinder pathogen control and eventually amplify inflammation. Combining systems approaches, targeted molecular methods, and high throughput cell imaging, we have determined how the CYP450-PPARα axis potentiates the increase of morbidity and mortality during superinfection. Understanding the molecular mechanism by which resolution of inflammation affects our immune response will yield therapeutic targets for sever microbial infections and inflammation-mediated diseases.

**MATERIALS AND METHODS**

**Mouse Influenza and Staphylococcus aureus Infection**

C57BL/6j and Ppara−/− (Stock No: 008154) mice were obtained from Jackson Laboratory (Bar Harbor, ME). Experiments were approved by the Temple University IACUC. Infection groups were 10 animals each, 5 male and 5 female randomly selected among 8-12 week age group from our holding colony. Mice were either infected with influenza, S. aureus, or both viral and bacterial infection (superinfection). Animals were anesthetized with a ketamine/xylazine mixture and infected intranasally with 100 PFU of influenza virus strain PR8 in 30µl sterile PBS. Mock-infected animals were inoculated with 30µl sterile PBS. Animals were weighed and monitored daily. S. aureus (Newman) at 1x10⁷ CFU/20µl was inoculated via non-invasive intratracheal inoculation (DuPage et al., 2009). Eight (8) days post-infection, mice were euthanized and both lungs and broncho-alveolar lavage using sterile PBS was collected for further analysis.

**Animal Husbandry**

Animals are kept under the veterinary care of the Temple University Laboratory and Animal Resources (ULAR) department. Mice are assessed for health and safety each day.
and are provided fresh food and water by animal husbandry staff. The facility undergoes a 12-hour daylight, 12-hour nighttime cycle. The Temple University Institutional Animal Care and Use Committee (IACUC) has approved our experimental and care approaches for bone marrow harvesting (5002) and our superinfection model (5000).

**Hoxb8 Macrophages Stimulation**

Macrophages were stimulated with poly:IC (6µg/ml, LMW, In vivogen) or LPS (10µg/ml, *Salmonella minnesota* R595, List Biological Laboratories). Cells were pretreated with vehicle (DMSO) or WY14643 (100µM, Sigma Aldrich), or 14, 15-DiHETeR (10µM, Cayman Chemical). WY14643 is a specific PPARα chemical agonist and is a feasible substitute used instead of the purified lipids.

**RNA Extraction and qRT-PCR**

After stimulation of macrophages, RNA was extracted by TRIzol (Invitrogen) and Direct-zol 96 RNA Preps (Zymo Research). cDNA was synthesized using random hexamer and TaqMan Reverse Transcription Reagents (Applied Biosystems). TaqMan Fast Advance Master mix and TaqMan Primer/Probe sets were used for qRT-PCR in ABI StepOne System (Applied Biosystems).

**Hoxb8 Macrophages Transduced With Luciferase Reporter**

NFκB luciferase reporter, pHAGE NFκB-TA-LUC-UBC-GFP-W, was a gift from Darrell Kotton (Addgene plasmid # 49343; http://n2t.net/addgene/49343; RRID: Addgene_49343). NFκB reporter construct, Δ8.9, and pCMV-VSVG were transfected into 293T (ATCC) using TransIT-RF-Lenti Transfection Reagent (Mirus Bio LLC). Supernatant containing lentivirus was harvested 48 hours post transfection and incubated at 4°C overnight after diluting with 40% PEG8000, 2M NaCl pH7.2. Lentivirus was concentrated by ultracentrifugation at 16,000rpm for 30’. Pellet was resuspended transduced into Hoxb8 progenitors using polybrene. Transduced cells were FACS sorted with Influx (BD Biosciences).

**Luciferase Assay**

After stimulation of Hoxb8 macrophages, cell lysates were analyzed using Luciferase 1000 Assay System according to manufacturer (Promega). Plates were analyzed using BMG Labtech Omega Plate reader.

**Automated Digital Microscopy**

Cells were seeded into 384 or 96 well #1.5 glass bottom plate (Nunc or Cellvis). Images were captured using EVOS 2 FL (Invitrogen) and analyzed using HCS Studio Cell Analysis Software (ThermoFisher).

**NFκB and PPARα Nuclear Translocation**

C57Bl/6 and *Ppara*−/− macrophages were first stimulated with WY14643 for one hour to activate PPARα, followed by TLR stimulation via LPS for 30 or 60 minutes to induce the NFκB cascade. Cells were fixed with 2% PFA, then permeated with 0.1% Triton-X for 10 minutes for intracellular staining. Cells were blocked with blocking buffer (PBST, 1%BSA, 22mg/mL glycine) for 30 minutes, then stained with primary antibodies for NFκB p65 (Santa Cruz Biotechnology, SC-8008, 1:100) and PPARα (Novus Biologicals, NR1C1 (pSer12), 1:100). Secondary staining goat anti-mouse IgG Cy5 (ThermoFisher, A10524, 1:1000) and goat anti-rabbit Alexa Fluor™ 488 (ThermoFisher, A11054, 1:1000) were used. DAPI (Cayman Chemical, 14285, 300nM) was used for nuclear staining. Cells were imaged using EVOS 2 FL (Invitrogen) and analyzed using HCS Cell Studio Analysis Software (ThermoFisher).

**Protein Analysis**

Protein lysates were separated by electrophoresis (Tris-Glycine SDS PAGE). Transferred PVDF membranes (LI-COR) were stained with anti-NFκB p65 (F-6, Santa Cruz), anti-PPARα (MA1-822, ThermoFisher), or anti-actin (C4, Santa Cruz). Secondary antibodies IRDye 800CW and IRDye 680RD were used and protein bands were detected using LI-COR Odyssey and analyzed using ImageStudio (LI-COR).

**CFU Assays**

BMDM or Hoxb8 macrophages were plated stimulated with vehicle DMSO (Sigma Aldrich, D2650) or WY14643 (Sigma-Aldrich C7081) for 1 hour, then biological triplicates were infected with *S. aureus* Newman-mCherry for 30, 60, 90, or 120min. 24 well plates were immediately centrifuged at 200 x g for 5 minutes. Macrophages were washed 3x with PBS, then lysed with 0.1% Triton-X and plated in serial dilutions on TSA plates. Colony formation was manually counted and analyzed using Graphpad Prism (San Diego, CA).

**Phagocytosis- Microscopy**

Macrophages were stimulated with vehicle DMSO (Sigma Aldrich, D2650) or WY14643 for 60 minutes, then biological triplicates were infected at an MOI of 10, 20, or 50 with heat-killed *Staphylococcus aureus* Newman-CFP for 30, 60, 90, or 120 minutes. 24 well plates were immediately centrifuged at 200 x g for 5 minutes. Macrophages were fixed at end of experimental trial and stained with DAPI (Cayman Chemical, 14285, 300nM), then imaged using the EVOS 2 FL (Invitrogen) and analyzed with HCS Cell Studio Analysis Software (ThermoFisher) to detect fluorescent bacteria within stained macrophages.

**Anti-Bacterial Killing- Microscopy**

Macrophages were stimulated with vehicle DMSO (Sigma Aldrich, D2650) or WY14643 for 60 minutes, then biological triplicates were infected at an MOI of 20 with live *Staphylococcus aureus* Newman-CFP for 30, 60, 90, or 120 minutes. 24 well plates were immediately centrifuged at 200 x g for 5 minutes. Macrophages were stained with CellMask™ Orange (#C10045) just prior to infection, and Sytox™ Green (S7020, 0.4 mM) to detect bacterial killing. Imaging was done on EVOS 2 FL (Invitrogen) and analyzed using HCS Cell Studio Analysis Software (ThermoFisher).

**Time-Lapsed Confocal Microscopy**

Cells were seeded in Nunc or Cellvis 96 well #1.5 glass bottom plates. Macrophages were stained with CellMask™ Orange (#C10045), and *S. aureus* Newman-CFP bacteria were stained with Sytox™ Green
(S7020) for bacterial killing assays. Leica (TCS SP5 spectral confocal microscope) confocal microscope with a thermoregulated chamber at 37°C was used for imaging, and ImageJ was used for analysis.

**Cellularity by FACS**
Murine lungs and bronchoalveolar lavage (BAL) were collected on day eight (8) of infection trials. BAL was collected using 2mL PBS via trachea. Lungs were minced with surgical scissors, then digested in Hank’s Balanced Salt Solution (HBSS) containing liberase (8ug/mL) and DNase I (40ug/mL) for 30-minute incubation. 0.5M EDTA was used to inactivate enzymes. Digested lungs were strained through 40µM cell strainer, then treated with ACK Lysis Buffer for 1 min. Cells were counted and stained with the following panels: A- CD11b FITC, Lys6C PE, Ly6G APC, Fixable Viability Dye eFluor780, B- CD11b FITC, F4/80 PE, CD206 Alexa Fluor 647, CD80 PerCP-Cy 5.5, Fixable Viability Dye eFluor780, C- CD11b FITC, CD11c PE, I-A APC, Fixable Viability Dye eFluor780, D- CD49b FITC, CD19 PE, CD3 APC, Fixable Viability Dye eFluor780.

**Macrophage Polarization**
BMDM were stimulated with the following conditions to induce respective M1 and M2 polarizations: M1- IFNg (Pepro Tech, #315-05) and LPS, M2a- IL4 (Pepro Tech, #214-14/IL13 (Pepro Tech, #210-13), M2b- Immune complex (Polysciences, #23744-5) and LPS, and M2c- IL10 (Pepro Tech, #210-10/Tgib (Pepro Tech, #100-21). Cells were induced for 48 hours, then washed and stained. Macrophages were stained with the following: CD86 FITC (Fisher Scientific, BDB553691), Anti-IA PE (Fisher Scientific, 501129471), Anti-CD80 PerCPCy5.5 (Fisher Scientific, BDB560526), Anti-CD206 APC (Fisher Scientific, BDB565250), and fixable viability dye eFluor 780 (eBioscience). Flow Analysis was done on BD LSR II (Franklin Lakes, NJ) and FlowJo (Ashland, OR).

**Lipidomic Profiling by Liquid Chromatography Mass Spectrometry**
Lipid mediators were examined by LCMS essentially as described previously (Quehenberger et al., 2011; Tam et al., 2013; Tam et al., 2020). Before lipid metabolite isolation by solid phase extraction (SPE), deuterated standards (Cayman Chemical) were added to 0.9 mL of BAL Methanol was evaporates and the samples reconstituted in a minimal volume of water/acetonitrile (60/40) containing 0.02% v/v acetic acid. Eicosanoids were separated using a Waters Acquity UPLC BEH 1.7 µm 2.1 × 50 mm column using a 4 minute gradient of 99.9% A/B to 75/25 A/B followed by washing and reconditioning. Solvent A is 50/50 water/acetonitrile containing 0.02% acetic acid and solvent B is 50/50 acetonitrile/isopropanol. Eicosanoids were analyzed by a Waters Synapt G2Si QTOF operated in negative-ionization mode via MS². Data analysis was performed using UNIFI 1.6 (Waters), MS-DIAL4 (Tsugawa et al., 2020), and Mzmine 2.53 (Pluskal et al., 2010).

**DATA AVAILABILITY STATEMENT**
The data presented in the study are deposited in the MetaboLights repository, accession number MTBLS2927.

**ETHICS STATEMENT**
The animal study was reviewed and approved by IACUC Temple University.

**AUTHOR CONTRIBUTIONS**
VT, RL, BB, and JB contributed to conception and design of the study. RL, JT, and NG-E performed the experiments. BB provided expertise on confocal microscopy. JB performed mass spectrometry analysis. RL and VT wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

**FUNDING**
This work was supported by National Institute of Allergy and Infectious Disease R21 AI142278 and R01 AI168550 (VT).

**ACKNOWLEDGMENTS**
We thank Çağla Tükel, Stefania Gallucci, and Laurie Kilpatrick for their helpful advice.

**SUPPLEMENTARY MATERIAL**
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.881462/full/supplementary-material

**Supplementary Figure 1 |** Modulating immune response during superinfection. (A) Heatmap depicts the hierarchical clustering of transcripts detected (by Nanoscript) in macrophages during unstimulated, DHET (14,15-DHET), poly:C, or poly:IC and DHET stimulation. (B) Cluster analysis of co-regulated genes during stimulations described in (A). Red lines represent the mean transcript levels of the clusters. (C) Motif enrichment analysis showing promoter sites that have been repressed during superinfection.

**Supplementary Figure 2 |** Cytotoxicity and Luminescence Activity of NFκB decreases during PPARα activation. (A) Hoxb8 macrophages with a GFP inducible luciferase reporter were stimulated in combinations with poly:IC (TLR4 agonist), WWY14643 (PPARα agonist), or 14,15 DHET (metabolite). Cells were lysed after 1,4 hours, treated with luciferase reagent, and read for 10 seconds per well. n=3 per group and are representative of 3 experiments. (B) Hoxb8 macrophages with a GFP inducible luciferase reporter were stimulated in combinations with LPS (TLR4 agonist), WWY14643 (PPARα agonist), or 14,15 DHET (metabolite). Cells were lysed after 6 hours, treated with luciferase reagent, and read for 10 seconds per well, n=4-6 per group. (C) Hoxb8 macrophages were stimulated with varying concentrations of WWY14643. Cells were stained with AnnexinV and Propidium iodide to determine early (AnnexinV+ PI-) and late (AnnexinV+ PI+) apoptosis by FACS. Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).
Supplementary Figure 3 | Immunoblots of NfκB and PPARα. (A) Immunoblotting of HoxB8 macrophages (C57BL/6 and Ppara−/−) stimulated with mock (DMSO), LPS, LPS and WY14643 against NfκB P65, phospho-NFκB P65 (Ser536) and β-actin. (B) Immunoblotting of HoxB8 macrophages (C57BL/6 and Ppara−/−) against PPARα. Arrow indicates PPARα (~52kDa).

Supplementary Figure 4 | Nuclear Translocation of NfκB and PPARα. Representative images from digital microscopy of nuclear translocation of NfκB and PPARα are shown. NfκB is shown in red (CYS), PPARα is shown in green (AlexaFluor 488), and the nucleus is stained blue (DAPI).

Supplementary Figure 5 | Nuclear Translocation of NfκB and PPARα. (single channel images). Representative images from digital microscopy of nuclear translocation of NfκB and PPARα are shown. Single channel images of DAPI (nucleus), PPARα, and NfκB are shown.

Supplementary Figure 6 | Flow cytometry gating strategy for quantifying cells from bronchoalveolar lavage samples. After debris was gated out (FSC-A + SSC-A, live cells (Fixable viability dye−) and singlets (FSC-H v FSC-A) were selected. Neutrophils (CD11b+ Ly6G+), In live cells (Fixable viability dye−) and singlets (FSC-H v FSC-A) were selected. B cells, CD3+ T cells, and CD49b+ NK cells were quantified.

Supplementary Figure 7 | Cellularity of superinfection. Bar graphs depict the percentages of CD11b+ Ly6G+ inflammatory monocytes (A), neutrophils (B), DC (C) T cells (D), NK cells (E), and B cells (F) isolated from the bronchoalveolar lavage from infected lungs during influenza, S.aureus, or superinfection. Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).

Supplementary Figure 8 | Transcriptional responses of macrophage polarization change during PPARα activation. Bar graphs depict transcript levels (mean +/- SEM) as measured by RT-PCR from C57BL/6 macrophages stimulated with PnY LPS (M1), IL4/IL13 (M2a), and immune complex and LPS (M2b) with (red) or without (black) WY14643. Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001).

Supplementary Figure 9 | Increased expression of CD206 upon WY14643 stimulation during M2b polarization. Bar graph depicts the mean fluorescent intensity of CD206 (mean +/- SEM) of macrophages stimulated with mock (media), WY14643, IL4/IL13 (M2a), Immune complex (IC) + LPS (M2b). Two way ANOVA with multiple comparisons were performed to determine statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001). n=3 per group and are representative of 3 experiments.

**REFERENCES**

Afonso, P. V., Janka-Junttila, M., Lee, Y. J., McCann, C. P., Oliver, C. M., Aamer, K. A., et al. (2012). LTβ is a Signal-Relay Molecule During Neutrophil Chemotaxis. Dev. Cell 22, 1079–1091. doi: 10.1016/j.devcel.2012.02.003

Arora, S., Dev, K., Agarwal, B., Das, P., and Syed, M. A. (2018). Macrophages: Their Role, Activation and Polarization in Pulmonary Diseases. Immunol. Res. 69, 383–396. doi: 10.1007/j41849-018-0001-8

Buczynski, M., Dumlao, D., and Dennis, E. (2009). An Integrated Omics Analysis of Transcriptional Responses of Macrophage Polarization During Chronic Granulomatous Disease. Frontiers in Cellular and Infection Microbiology | www.frontiersin.org July 2022 | Volume 12 | Article 881462 12

Bougarne, N., Weyers, B., Desmet, S. J., Deckers, J., Ray, D. W., Staels, B., et al. (2009). Increased Survival After Gemfibrozil Treatment of Severe Mouse Influenza Infection. J. Virol. 83, 116, 24819–24829. doi: 10.1128/JVI.00427.2005

Budd, A., Alleva, L., Alsharifi, M., Koskinen, A., Smythe, V., Mühllacher, A., et al. (2017). Increased Survival After Gemfibrozil Treatment of Severe Mouse Influenza. Antimicrob. Agents Chemother. 51, 4265–4269. doi: 10.1128/AAC.00847-17

Clarkes, K. R., Santos Rocha, C., Grishina, I., Hirao, L. A., Napoli, E., Gaulke, C. A., et al. (2012). LTB4 is a Signal-Relay Molecule During Neutrophil Chemotaxis. J. Immunol. 188, 48. doi: 10.4049/jimmunol.1100475

Crakes, K. R., Napoli, E., Gaulke, C. A., Kluytmans, J., van Belkum, A., and Verbrugh, H. (1997). Nasal Carriage of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, and the Challenge for Immunology. Clin. Microbiol. Rev. 10, 122–145. doi: 10.1128/CMR.10.1.122-145.1997

Fedson, D. S. (2009). Meeting the Challenge of Influenza Pandemic Preparedness in Developing Countries. Emerg. Infect. Dis. 15, 365–371. doi: 10.3202/jid.201003.080857

Gong, Y., Shao, Z., Fu, Z., Edin, M. L., Sun, Y., Liegl, R. G., et al. (2016). Fenofibrate Inhibits Cytochrome P450 Epoxygenase 2c Activity to Suppress Pathological Ocular Angiogenesis. EBioMedicine 13, 201–211. doi: 10.1016/j.ebiom.2016.09.025

Gopal, R., Lee, B., McHugh, K. J., Rich, H. E., Ramanan, K., Mandalapu, S., et al. (2018). STAT2 Signaling Regulates Macrophage Phenotype During Influenza and Bacterial Super-Infection. Front. Immunol. 9. doi: 10.3389/ fimmu.2018.02151

Gugliando, E., Fusco, R., Ginestra, G., D’Amico, R., Bisignano, C., Mandalari, G., et al. (2019). Involvement of TLR4 and PPARα Receptors in Host Response and NLRP3 Inflammasome Activation Against Pulmonary Infection With Pseudomonas Aeruginosa. Shock 51, 221–227. doi: 10.1097/SHK.0000000000001137

Hageman, J. C., Uyeki, T. M., Francis, J. S., Jernigan, D. B., Wheeler, J. G., Bridges, C. B., et al. (2006). Severe Community-Acquired Pneumonia Due to Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, and the Challenge for Immunology. Clin. Microbiol. Rev. 19, 323–357. doi: 10.1128/CMR.19.2.323-357.2006

Huang, B., Yang, X. D., Lamb, A., and Chen, L. F. (2010). Posttranslational Modifications of NF-KappaB: Another Layer of Regulation for NF-kappaB Signaling Pathway. Cell Signalling 1290. doi: 10.1016/j.cellsig.2010.03.017

Jiao, M., Ren, F., Zhou, L., Zhang, X., Zhang, L., Wen, T., et al. (2014). Peroxisome Promoter-Activated Receptor α Activation Attenuates the Inflammatory Response to Protect the Liver From Acute Failure by Promoting the Autophagy Pathway. Cell Death Dis. 5, e1397. doi: 10.3389/cddis.2014.361

Kim, Y. S., Kim, J. K., Hanh, B. T. B., Kim, S. Y., Kim, H. J., Kim, Y. J., et al. (2020). The Peroxisome Proliferator-Activated Receptor α- Agonist Gemfibrozil Promotes Defense Against Mycobacterium Abscessus Infections. Cells 9 (3), 648. doi: 10.3390/cells9030648

Kim, Y. S., Lee, H. M., Kim, J. K., Yang, C. S., Kim, T. S., Jung, M., et al. (2017). PPAR-Alpha Activation Mediates Innate Host Defense Through Induction of TFEB and Lipid Catabolism. J. Immunol. 198, 3283–3295. doi: 10.4049/jimmunol.1601920

Klyutmys, J., van Belkum, A., and Verbrugh, H. (1997). Nasal Carriage of Staphylococcus Aureus: Epidemiology, Underlying Mechanisms, and Associated Risks. Clin. Microbiol. Rev. 10, 505–520. doi: 10.1128/CMR.10.5.505-520.1997

Kobayashi, S. D., Musser, J. M., and DeLeo, F. R. (2012). Genomic Analysis of the Emergence of Vancomycin-Resistant Staphylococcus Aureus. Mbio 3 (4), e00170–12. doi: 10.1128/mbio.00170-12
Extravascular Migration In Vivo. *Br. J. Pharmacol.* 133, 413–421. doi: 10.1038/sj.bjp.0704087

Zhao, X., Dai, J., Xiao, X., Wu, L., Zeng, J., Sheng, J., et al. (2014). PI3K/Akt Signaling Pathway Modulates Influenza Virus Induced Mouse Alveolar Macrophage Polarization to M1/M2b. *PloS One* 9, e104506. doi: 10.1371/journal.pone.0104506

Zheng, W., and Flavell, R. A. (1997). The Transcription Factor GATA-3 is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. *Cell* 89, 587–596. doi: 10.1016/s0092-8674(00)80240-8

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Lucarelli, Gorrochotegui-Escalante, Taddeo, Buttaro, Beld and Tam. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.