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Inflammatory response to *Porphyromonas gingivalis* partially requires interferon regulatory factor (IRF) 3

Yazdani B Shaik-Dasthagirisaheb*, Nasi Huang* and Frank C Gibson III

Abstract

Innate immune activation with expression of pro-inflammatory molecules such as TNF-α is a hallmark of the chronic inflammation associated with periodontal disease (PD). *Porphyromonas gingivalis*, a bacterium associated with PD, engages TLRs and activates MyD88-dependent and TIR-domain-containing adapter-inducing IFN-β (TRIF)-dependent signaling pathways. IFN regulatory factor (IRF) 3 is activated in a TRIF-dependent manner and participates in production of cytokines such as TNF-α; however, little is known regarding IRF3 and the host response to PD pathogens. We speculated that IRF3 participates in the host inflammatory response to *P. gingivalis*. Our results show that bone marrow macrophages (MØ) from WT mice respond to *P. gingivalis* with activation and nuclear translocation of IRF3. Compared with WT, MØ from IRF3$^{-/-}$, TRIF$^{-/-}$, and TLR4$^{-/-}$ mice responded with reduced levels of TNF-α on *P. gingivalis* challenge. In addition, full expression of IL-6 and RANTES by MØ to *P. gingivalis* was dependent on IRF3. Lastly, employing MØ from IRF3$^{-/-}$ and IRF7$^{-/-}$ mice we observed a significant role for IRF3 and a modest role for IRF7 in the *P. gingivalis*-elicited TNF-α response. These studies identify a role for IRF3 in the inflammatory response by MØ to the periodontal pathogen *P. gingivalis*.

Keywords

Porphyromonas gingivalis, IRF3, cytokine, chemokine, macrophage

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Introduction

Periodontal disease (PD) is a common chronic oral inflammatory disease.[1] The host response during PD is complex and much of the hard and soft tissue destruction observed is attributed to immune recognition and subsequent inflammatory responses to periodontal pathogens.[2–5] An array of cytokines and chemokines, including TNF-α, IL-1, IL-8, RANTES and others, have been detected at higher levels in samples from individuals with PD than from healthy samples.[4,6,7] Experimental studies identify various cell types, including monocytes, and epithelial and endothelial cells, as sources of cytokines, chemokines and other inflammatory molecules when exposed to oral pathogens, including *Porphyromonas gingivalis*.[8–10]

TLRs are a family of host innate immune receptors that detect the presence of conserved microbial antigens, such as LPS, peptidoglycan and others.[11] These receptors are expressed by a variety of cells, including bone marrow macrophages (MØ). TLR engagement activates signal transduction pathways governed by adaptor molecules MyD88 and TIR-domain-containing adapter-inducing IFN-β (TRIF), culminating in transcription of a variety of genes encoding molecules such as cytokines and chemokines.[12] All TLRs with the exception of TLR3 signal via MyD88.[11] TLR3 utilizes TRIF exclusively, while TLR4 uses both MyD88 and TRIF pathways.[11] TLR signaling pathways are implicated in host responses to *P. gingivalis*, including cytokine and chemokine production,[13,14] osteoclastogenesis[15]

Section of Infectious Diseases, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

*These authors contributed equally.

Corresponding author:
Frank C Gibson III, Section of Infectious Diseases, Department of Medicine, Boston University School of Medicine, Evans Biomedical Research Center Rm. 638, 650 Albany Street, Boston, MA 02118, USA.
Email: fgibson@bu.edu

and oral bone loss.[16,17] Moreover, MyD88-dependent and TRIF-dependent pathways participate in the host response to this organism.[18,19] Little is known about the signaling molecules downstream of TRIF that contribute to host immune response to periodontal pathogens.

IFN regulator factor (IRF) 3 is activated in a TLR/TRIF-dependent manner,[20] following activation of TANK-binding kinase (TBK)-1.[21] IRF3 binding sites have been identified in the promoter region of NF-κB-dependent genes, such as TNF-α,[22] thus identifying a potential mechanism in which IRF3 can influence expression of this and other pro-inflammatory cytokines. IRF3 is one member of a family of nine IRFs identified in humans.[20] IRF3 and IRF7 are among the best-studied IRF family members and participate in immune signaling.[23] In the context of influenza A virus infection, IRF3 is necessary for the expression of IFN-β and a variety of inflammatory mediators, including MCP-1 and, to a lesser extent, TNF-α.[24] Bacterial LPS challenge requires intact IRF3 to induce endotoxic shock response in mice.[25] Use of purified enteric LPS identified TLR-mediated activation of IRF3/IRF7 in a TLR4-mediated, TRIF- and TRIF-related adaptor molecule (TRAM)-dependent manner.[26] This latter finding supports an important role for IRF3 in host response to bacterial pathogens. In the context of host response to periodontal pathogens, there is limited information regarding the involvement of IRF. Dendritic cells cultured with recombinant *P. gingivalis* hemagglutinin B protein increase transcription of the *IRF3* gene,[27] while MØ response to *P. gingivalis* LPS involves IRF7.[19] The aim of the present study is to define the contribution of IRF3 in the host cytokine and chemokine responses to *P. gingivalis*.

Materials and methods

Mice and MØ collection

Male 6–8-wk-old C57BL-6 wild type (WT) mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). IRF3$^{-/-}$[28] and TRIF$^{-/-}$[29] mice were obtained from Dr Egil Lien (University of Massachusetts Medical Center, Worchester, MA, USA), TLR4$^{-/-}$[30] were obtained from Dr Caroline Genco (Boston University Medical Center, Boston, MA, USA), and IRF7$^{-/-}$[31] mice were from Dr Ian Rifkin (Boston University Medical Center, Boston, MA, USA). MyD88$^{-/-}$ mice[32] were from Dr Shizuo Akira (Osaka University, Osaka, Japan). All studies were performed in accordance with Boston University Institutional Animal Care and Use Committee approvals, and animals received chow diet and water *ad libitum*. Bone marrow cells were flushed from femurs of humanely sacrificed mice. The red blood cells were removed by 1 × RBC lysis buffer (eBioscience, San Diego, CA, USA). Remaining bone marrow cells were differentiated into MØ over 7 d in RPMI-1640 + 10% FBS supplemented with 20% conditioned medium from L929 cells (source of M-CSF, a kind gift of Dr Robin Ingalls, Boston University Medical Center, Boston, MA, USA), and penicillin/streptomycin in a CO_2 incubator, under standard cell culture conditions. Following washing and replacement with antibiotic-free and M-CSF-free cell culture medium, adherent MØ were collected and added to wells of tissue culture plates at 5×10^5 cells/ml 2 h prior to challenge.

P. gingivalis *cultivation*

P. gingivalis strain 381 was primarily used in these studies; however, where indicated, *P. gingivalis* strain 33277 was also employed to study host response to different *P. gingivalis* strains. In all cases, bacteria were grown in an anaerobic environment on brain–heart infusion (BHI) yeast extract–blood agar plates for 3–5 d, followed by 24 h cultivation in BHI yeast extract broth, as previously described.[33] Broth-grown bacteria were harvested by centrifugation, washed, adjusted to an OD of 1 at 660 nm (approximately 1×10^9 bacteria/ml) with antibiotic-free RPMI-1640 + 10% FBS, and were added to MØ cultures at the indicated multiplicities of infection (MOI). Gram staining was used to assess purity of all broth cultures.

MØ challenge assays

For IRF3 activation studies MØ from WT mice were placed into wells of tissue culture dishes (5×10^6 total cells), and were incubated with antibiotic-free cell culture medium alone, or medium containing *P. gingivalis* MOI 100. PolyI:C (1 μg/ml; InvivoGen, San Diego, CA, USA) 1 h treatment served as positive control.[34] After 1, 2 and 4 h of incubation, cells were washed and used for isolation of cytoplasmic and nuclear fractions to determine IRF3 activation. For studies investigating cytokine and chemokine production, MØ from WT and mutant mice were placed into wells of tissue culture dishes, and were incubated with fresh antibiotic-free cell culture medium alone, or medium containing *P. gingivalis* at MOIs ranging from 1 to 100 as indicated. In some experiments ultrapure preparations of Pam3CSK4 (100 ng/ml; TLR2/1 agonist; InvivoGen, San Diego, CA, USA) or *Escherichia coli* LPS (10 ng/ml; TLR4 agonist; InvivoGen) were used as specific ligands. Culture supernatant fluids were harvested as indicated, and stored frozen at −80°C until ELISA or multiplex assays were performed.

MØ viability assay

For the MØ viability assay, MØ from WT and IRF3$^{-/-}$ mice were placed into wells of tissue culture dishes (six-well) and were incubated with fresh antibiotic-free cell culture medium alone, or medium containing *P. gingivalis* at a MOI of 100 for 24 h. Culture supernatant fluids were aspirated and MØ were washed with antibiotic-free cell culture medium. One milliliter of fresh medium with out antibiotics was added to each well and MØ were gently collected using cell scraper. Viability of recovered MØ was determined by Trypan blue dye exclusion method using hemocytometer.

Detection of IRF3 activation

Cytoplasmic and nuclear fractions were prepared from MØ using a nuclear extraction kit according to the manufacturer's instructions (Active Motif, Carlsbad, CA, USA). Briefly, medium was aspirated from MØ and the adherent cells were washed with ice-cold PBS containing phosphatase inhibitors. Cells were removed from culture dishes by scraping, collected, pelleted by centrifugation at 55 *g* and were suspended in 1 × hypotonic buffer. After 15 min incubation, cells were solubilized by the addition of detergent provided as part of the kit. The cytoplasmic fraction was collected following centrifugation at 14,000 *g* at 4°C, and the cell pellet was suspended in complete lysis buffer and gently mixed for 30 min on ice. The nuclear fraction was obtained from the supernatant by centrifugation at 14,000 *g* at 4°C. Protein concentrations of cytoplasmic and nuclear fractions were calculated using ProStain-Protein Quantification Kit (Active Motif). Levels of IRF3 in cytoplasmic and nuclear fractions were determined on 7 μg of total protein, and presented as arbitrary units at 450 nm using mouse TransAM IRF3 kit, according to the manufacturer's instructions (Active Motif).

Cytokine and chemokine detection

Frozen cell culture supernatant fluids were thawed and the levels of TNF-α, IL-6 and RANTES were determined from unchallenged and challenged MØ using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA), or Luminex-based multiplex assays (Invitrogen, Grand Island, NY, USA) as per manufacturer's instructions.

Statistical analysis

All studies were performed using MØ from individual animals, and all experiments were performed on two separate occasions. Data were imported into Prism statistical analysis software (Graphpad Inc., La Jolla, CA, USA) and are presented as means ± SEM. Comparisons between groups were determined using unpaired *t*-test, or one-way and two-way ANOVA with Tukey's or Bonferroni multiple comparisons respectively. $P < 0.05$ was considered significant.

Results

P. gingivalis *induces IRF3 activation and nuclear localization in MØ*

Here, we investigated IRF3 activation and nuclear translocation in WT mouse MØ to *P. gingivalis* challenge. Medium treatment established baseline IRF3 levels in nuclear and cytoplasmic fractions. A *P. gingivalis* MOI of 100 induced IRF3 levels in nuclear protein fraction compared with unchallenged control (Figure 1A). Moreover, activation of IRF3 was observed at 1, 2 and 4 h of *P. gingivalis* challenge (Figure 1A). As anticipated, 1 h PolyI:C treatment (positive control) induced IRF3 activation and nuclear localization[34] (Figure 1A). Cytoplasmic fractions from medium, *P. gingivalis* and PolyI:C-treated MØ generally contained similar, low-level IRF3 detection (Figure 1B).

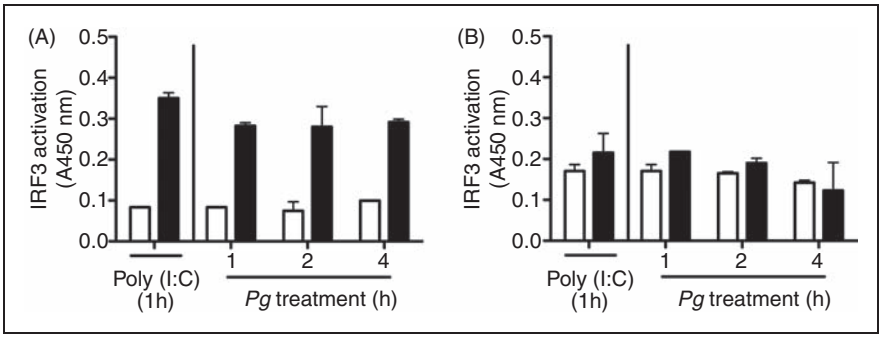

Figure 1. Activation of IRF3 in MØ cultured with *P. gingivalis*. MØ from C57BL-6 WT mice were cultured for 1, 2 and 4 h in medium alone or with *P. gingivalis* strain 381 (*Pg*) at a MOI of 100. One-hour PolyI:C (1 μg/ml) treatment was used as a positive control for IRF3 activation. MØ cultured in medium alone (open bars), and MØ cultured with *P. gingivalis* strain 381 (*Pg*) or PolyI:C (filled bars). MØ IRF3 activation was measured in nuclear fractions (A) or cytoplasmic fractions (B) by spectrophotometric assay per manufacturer's instructions. Data are presented as mean ± SEM of two independent experiments ($n = 1$ mouse per experiment).

Stimulation of MØ TNF-α response by P. gingivalis *is, in part, mediated by IRF3*

As *P. gingivalis* induced IRF3 activation, we speculated that IRF3 may participate in cytokine and chemokine production. To test this we compared TNF-α produced by MØ from WT and IRF3$^{-/-}$ mice cultured with *P. gingivalis*. IRF3$^{-/-}$ MØ cultured with *P. gingivalis* produced significantly less TNF-α than observed with WT ($P < 0.05$; Figure 2A). Based on this finding with *P. gingivalis*, we speculated that an upstream pathway involving TLR4 and TRIF could be involved in host response to this organism. Employing MØ from WT, TRIF$^{-/-}$ and TLR4$^{-/-}$ mice, we observed that TNF-α levels elicited by *P. gingivalis* were significantly reduced in MØ from TRIF$^{-/-}$ and TLR4$^{-/-}$ mice compared with WT respectively (Figure 2B and 2C). In addition we challenged MØ from WT and MyD88$^{-/-}$ mice, and observed a significant reduction in *P. gingivalis*-elicited TNF-α by MØ deficient in MyD88 compared with WT status ($P < 0.05$; Figure 2D).

Role of IRF3 in TNF-α by MØ is conserved between P. gingivalis *strains*

To ensure that the observed reduction in TNF-α levels from IRF3$^{-/-}$ mouse MØ was not specific only to *P. gingivalis* strain 381, we challenged MØ from WT and IRF3$^{-/-}$ with either *P. gingivalis* 381 or *P. gingivalis* 33277 at a MOI of 100, and measured the levels of TNF-α. WT MØ responded less robustly to *P. gingivalis* strain 33277 compared with *P. gingivalis* 381. Although magnitude differences in elicited TNF-α were observed between strains, similar patterns showing significantly reduced levels of TNF-α as a consequence of IRF3$^{-/-}$ were observed (Figure 3).

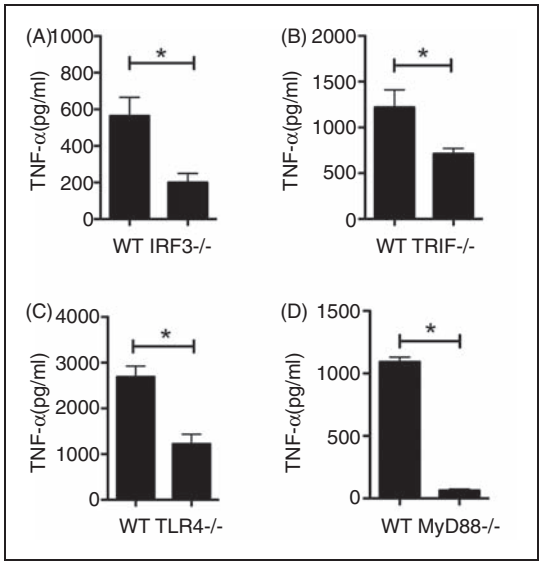

Figure 2. IRF3, TRIF, TLR4 and MyD88 contribute to the production of TNF-α by murine MØ cultured with *P. gingivalis*. MØ from C57BL-6 WT, IRF3$^{-/-}$ (A), TRIF$^{-/-}$ (B), TLR4$^{-/-}$ (C) and MyD88$^{-/-}$ (D) mice were cultured for 24 h in medium alone (open bars) or with *P. gingivalis* strain 381 at a MOI of 100 (filled bars). ELISA was used to measure TNF-α levels in harvested culture supernatant fluids. Data are presented as mean ± SEM from two separate experiments; $n = 3$–6 individual mice per group in total. *$P < 0.05$ as determined by unpaired *t*-test.

IRF3 ablation limits TNF-α, IL-6 and RANTES production from MØ cultured with P. gingivalis

As IRF3 was involved in TNF-α production by MØ response to *P. gingivalis*, we next investigated the influence of IRF3 on the production of other inflammatory molecules from MØ to *P. gingivalis*. We challenged MØ from WT and IRF3$^{-/-}$ mice with various doses of *P. gingivalis* for 24 h, and measured TNF-α, IL-6 and the IRF3-dependent chemokine RANTES.[35] As anticipated, ablation of IRF3 revealed its role in *P. gingivalis*-elicited TNF-α at MOIs of 100 and 10 (Figure 4A). Little TNF-α was detected with a MOI of 1 in WT, and differences between genotypes at this MOI were not observed. At a MOI of 100, a significant decrease in IL-6 was observed from IRF3$^{-/-}$ MØ compared with WT (Figure 4B). We also observed a reduction in IL-6 from IRF3$^{-/-}$ mouse MØ cultured with *P. gingivalis* at a MOI of 10; however, this did not reach a level of significance ($P > 0.05$; Figure 4B). Focusing on RANTES, a consistent and significant reduction was observed for IRF3$^{-/-}$ at all *P. gingivalis* MOIs studied ($P < 0.05$; Figure 4C). To ensure that MØ from IRF3$^{-/-}$ mice were not intrinsically defective in developing inflammatory responses to bacteria, we challenged WT and IRF3$^{-/-}$ mouse MØ with ultrapure

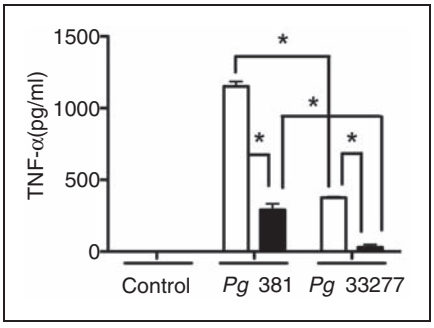

Figure 3. Role of *P. gingivalis* strains on the production of TNF-α in MØ from IRF3$^{-/-}$ mice. MØ from C57BL-6 WT (open bars), and IRF3$^{-/-}$ (filled bars) mice were cultured for 24 h with *P. gingivalis* (*Pg*) strain 381 and *P. gingivalis* strain 33277 at a MOI of 100. ELISA was used to measure TNF-α levels in harvested culture supernatant fluids. Data are presented as mean ± SEM from two separate experiments; $n = 4$ individual mice per group in total. *$P < 0.05$ by two-way ANOVA with Bonferroni post-test.

preparations of Pam3CSK4 (100 ng/ml) or *E. coli* LPS (10 ng/ml), and measured TNF-α production by ELISA. IRF3 ablation resulted in a significant reduction in *E. coli* LPS elicited TNF-α, while levels of TNF-α to Pam3CSK4 were similar between WT and IRF3$^{-/-}$ MØ (Figure 4D).

Next, we were interested in determining whether IRF3 ablation had an effect on MØ viability in response to *P. gingivalis*. MØ were collected after 24 h challenge with medium alone or medium with *P. gingivalis* (MOI 100), and the viability of recovered MØ was determined using Trypan blue dye exclusion assay. No significant difference in the number of viable MØ recovered was observed between WT and IRF3$^{-/-}$ in response to *P. gingivalis* (Table 1). Similarly, we observed no difference in the recovered MØ viability between MØ incubated with medium alone or medium with *P. gingivalis* (Table 1).

IRF3 influences MØ TNF-α response to P. gingivalis *more strongly than IRF7*

Lastly, we were interested to know if MØ cytokine production in response to *P. gingivalis* was specific for IRF3 or was shared by other IRFs. At 6 h challenge, we observed significant reduction in *P. gingivalis*-elicited TNF-α from IRF3$^{-/-}$ MØ, while MØ from IRF7$^{-/-}$ mice produced levels of TNF-α similar to WT (Figure 5A). By 24 h, TNF-α levels from IRF3$^{-/-}$ MØ remained significantly reduced; however, a partial and mild role for IRF7$^{-/-}$ was observed (Figure 5B).

Table 1. Viability of MØ from WT and IRF3$^{-/-}$ mice in response to *P. gingivalis*.

	Total MØ recovered	
Treatment	WT	IRF3$^{-/-}$
Medium alone	$6.54 \pm 1.04 \times 10^5$	$5.16 \pm 0.76 \times 10^5$
P. gingivalis	$5.58 \pm 0.46 \times 10^5$	$4.16 \pm 1.43 \times 10^5$

Viability of WT ($n = 3$) and IRF3$^{-/-}$ mouse MØ ($n = 3$) recovered after 24 h *P. gingivalis* challenge was determined by Trypan blue dye exclusion method using hemocytometer. Comparisons between groups were performed using one-way ANOVA with Bonferroni post-test. No significant differences were observed between any groups.

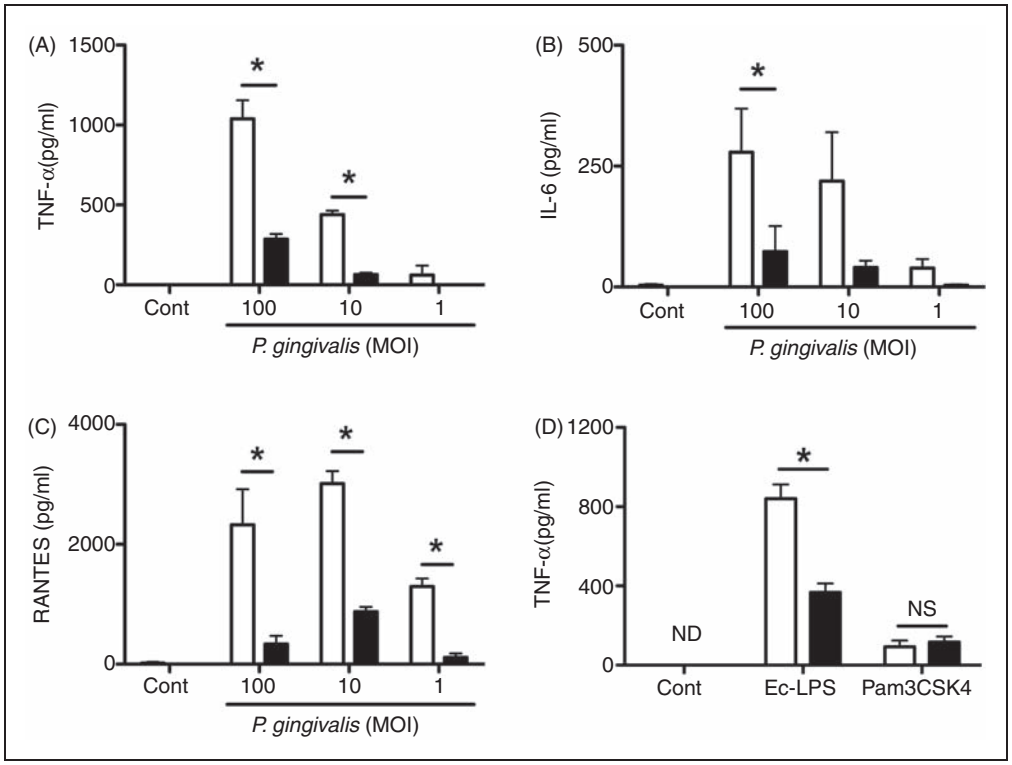

Figure 4. Cytokine and chemokine response of MØ deficient in IRF3 to *P. gingivalis* challenge. MØ from C57BL-6 WT (open bars) and IRF3$^{-/-}$ (filled bars) mice were cultured with medium alone (Cont), medium containing *P. gingivalis* 381 at MOIs of 100, 10, and 1 for 24 h. ELISA was used to measure TNF-α (A), IL-6 (B) and RANTES (C) in culture supernatant fluids. For ligand treatments, MØ from C57BL-6 WT (open bars) and IRF3$^{-/-}$ (filled bars) mice were cultured with medium alone (Cont), medium containing ultra pure *E. coli* 0111:B4 LPS (Ec-LPS, 10 ng/ml) or medium containing ultra pure Pam3CSK4 (100 ng/ml) for 24 h. TNF-α levels in culture supernatant fluids was measured by ELISA (D). Bar represents mean ± SEM from two separate experiments; $n = 3$–5 individual mice per group in total. *$P < 0.05$ by two-way ANOVA with Bonferroni post-test. ND: none detected; NS: not significant.

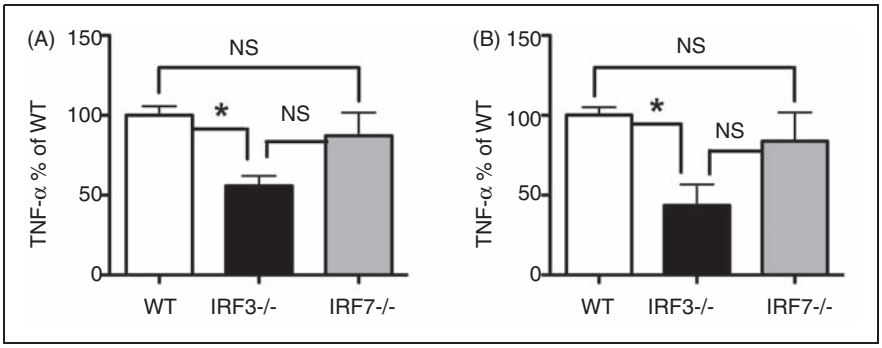

Figure 5. Comparison of IRF3 and IRF7 involvement in TNF-α production by MØ cultured with *P. gingivalis*. MØ from C57BL-6 WT (open bars), IRF3$^{-/-}$ (black filled bars), and IRF7$^{-/-}$ (gray filled bars) mice were cultured for 6 h (A) or 24 h (B) with *P. gingivalis* strain 381 at a MOI of 100. Cell culture supernatant fluids were harvested and ELISA was used to measure TNF-α levels. Data presented as a percentage of WT levels from two separate experiments; $n = 6$ individual mice per group in total. *$P < 0.05$ by one-way ANOVA with Tukey's post test. NS: not significant.

Discussion

Herein, using a murine bone marrow MØ system we report that IRF3 is activated in response to *P. gingivalis* challenge, and IRF3 functionally participates in the development of cytokine and chemokine response to *P. gingivalis*. We observed that the TNF-α response of MØ to *P. gingivalis* is partially dependent on TLR4, TRIF and IRF3. Our data from MyD88$^{-/-}$ mouse MØ is in agreement with others who implicate MyD88 in TNF-α production by the host to *P. gingivalis* challenge.[19,36] The ability of MØ to produce IL-6 and RANTES in response to *P. gingivalis* challenge also involved IRF3. Employing two distinct strains of *P. gingivalis*, we observed that the expression of TNF-α to either *P. gingivalis* strain 381 or *P. gingivalis* strain 33277 by MØ required intact IRF3. Although differences in maximally achieved TNF-α levels were observed between *P. gingivalis* strains, a similar contribution of IRF3 to TNF-α expression was observed. Lastly, the use of MØ from IRF3- and IRF7-deficient mice point to an important role for IRF3 in TNF-α response to *P. gingivalis*.

Our biochemical approach identified activation and nuclear translocation of IRF3 in WT mouse MØ in response to *P. gingivalis* challenge. These data are the first to show activation of IRF3 in primary MØ from mice in response to a pathogen and are in agreement with a previous report using RAW 264.7 cells, where activation of IRF3 was observed.[37] IRF3 binding sites have been identified in the promoter of TNF-α.[22] Our data support that IRF3 is functionally involved in the development of a full TNF-α response by MØ to *P. gingivalis*; however, we did not confirm IRF3 binding to the TNF-α promoter. Similar to that observed with MØ from IRF3 deficient mice, MØ TNF-α response to *P. gingivalis* was also partially dependent on TLR4 and TRIF. TRIF signaling is critical for MØ IRF3 phosphodimerization.[38] Thus, our findings of IRF3 activation and nuclear localization are in agreement with the model that MØ from TRIF$^{-/-}$ and TLR4$^{-/-}$ mice mimic the response seen with IRF3$^{-/-}$ mouse MØ to *P. gingivalis* challenge, and it is in agreement with previous studies that TRIF, IRF3 and TLR4 molecules signal through a common pathway.[39,40] Prior studies using purified ligands including LPS identify a similar trend to what we observed in the context of TNF-α response by MØ when using MyD88$^{-/-}$, TRIF$^{-/-}$ and TRIF^{lps2} mice. Kawai et al.[41] reported a strong reduction in TNF-α response by MyD88$^{-/-}$ MØ to *E. coli* LPS. Yamamoto et al.[29] reported a significant reduction in TNF-α produced from TRIF$^{-/-}$ MØ cultured with LPS. This later finding was substantiated by Hoebe et al.[42] where MØ from TRIF^{lps2} mice also produced little TNF-α to *E. coli* LPS exposure. These findings add complexity to the interpretation of host response in the presence/absence of MyD88 signaling as the three systems report very strong reductions in TNF-α to LPS, with the latter two systems limiting TRIF signaling capacity in the presence of an intact MyD88 pathway. In the context of MyD88 and TRIF, our findings are in broad agreement with those of Burns et al.,[18] who concluded, using an *in vivo* chamber model, that TLR2-dependent host pro-inflammatory cytokine response to *P. gingivalis* challenge was not dependent on the MyD88 pathway but that MyD88 was necessary for clearance of *P. gingivalis*. A recent study by Liang et al.[43] identified TLR2/CR5a interaction and influence of this receptor complex on MØ response to *P. gingivalis*, as well as *P. gingivalis*-induced oral bone loss. In the present study our results indicate that host response to *P. gingivalis* activates IRF3 and the ablation of IRF3 contributes to reduction in secreted levels of TNF-α in response to *P. gingivalis*.

Implication of IRF3 in the development of full immune response to bacterial challenge has been reported for several bacteria. In a lung epithelial cell model, IRF3 knockdown revealed its role in controlling

Legionella pneumophila replication.[44] IRF3-deficient mice are more resistant to *Listeria monocytogenes* infection.[45] MØ cultures and lung infection models support a role for IRF3 in full expression of TNF-α to *Pseudomonas aeruginosa*.[39] The effect of IRF3 deficiency on host response to *P. aeruginosa* was not limited to TNF-α, as defective responses in MØ expressing RANTES and IL-10 were also observed.[39] Our data, that IRF3 is involved in full host cytokine and chemokine response to *P. gingivalis*, parallel findings in studies employing *P. aeruginosa*.[39] Our data support that *P. gingivalis* stimulates RANTES production from MØ cultured with *P. gingivalis* and that this response is partially IRF3-dependent. In the context of IRF3 involvement in TNF-α expression, our results also parallel findings of a recent study employing the influenza A virus, which reported partial involvement of IRF3 in pathogen-elicited TNF-α from MØ.[24]

Recent data identify IRF activation in response to *P. gingivalis* challenge. Gaddis et al.[27] reported activation of IRF3 by dendritic cells cultured with recombinant *P. gingivalis* HagB. Our studies using whole *P. gingivalis* challenge are in broad agreement with the results of this study, which suggest involvement of IRF3 in the host response to *P. gingivalis*. Our data, however, differ from a recent study reporting involvement of IRF7 along with TLR2 and TLR7 in host response by MØ to *P. gingivalis* and its LPS,[19] and no apparent role for IRF3.[19] In our hands we observed activation and nuclear localization of IRF3 in MØ as a consequence of *P. gingivalis* challenge. Moreover, using MØ from IRF3$^{-/-}$ mice, we observed that IRF3 contributed to full TNF-α, IL-6 and RANTES response of these cells to *P. gingivalis* challenge. Lastly, IRF3 played a consistent role in TNF-α response at 6 and 24 h, while only a partial role for IRF7 was detected. The differences observed between our studies and those of Zhou and Amar,[19] may reflect differences in systems used. Both studies utilized murine MØ and employed *P. gingivalis* strain 381; however, we utilized MØ from IRF3 and IRF7 knockout mice, while Zhou and Amar[19] utilized several strains of knockout mice, including TLR2, TLR7 and MyD88 mice, but did not employ IRF3$^{-/-}$ and IRF7$^{-/-}$ mice. Regardless, when taken together, these studies do support a role for IRFs in development of the host response to *P. gingivalis*. More detailed studies are needed to understand the significance of these differences, in the context of other periodontal disease-associated pathogens and to elucidate the bacterial antigens driving IFN pathway activation.

Employing an *in vitro P. gingivalis* mouse MØ challenge model, our data provide new information involving IRF3 in the development of full host inflammatory responses to this organism. Future studies are needed to determine the precise roles of IFN signaling pathways in the host response to *P. gingivalis* and the contribution these pathways have in the pathogenesis of PD.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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