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Porphyromonas gingivalis-Induced Reactive Oxygen Species Activate JAK2 and Regulate Production of Inflammatory Cytokines through c-Jun

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Pathogen-induced reactive oxygen species (ROS) play a crucial role in host innate immune responses through regulating the quality and quantity of inflammatory mediators. However, the underlying molecular mechanisms of this effect have yet to be clarified. In this study, we examined the mechanism of action of ROS stimulated by *Porphyromonas gingivalis* in gingival epithelial cells. *P. gingivalis* induced the rapid production of ROS, which lead to the phosphorylation of JAK2 and increased levels of secreted proinflammatory cytokines interleukin-6 (IL-6) and IL-1β. Neutralization of ROS by *N*-acetyl-L-cysteine (NAC) abrogated the phosphorylation of JAK2 and suppressed the production of IL-6 and IL-1β. ROS-mediated phosphorylation of JAK2 induced the phosphoactivation of c-Jun amino-terminal protein kinase (JNK) and the downstream transcriptional regulator c-Jun. Inhibition of JAK2, either pharmacologically or by small interfering RNA (siRNA), reduced both the phosphorylation of these molecules and the production of proinflammatory cytokines in response to *P. gingivalis*. Furthermore, pharmacological inhibition or siRNA-mediated gene silencing of JNK or c-Jun mimicked the effect of JAK2 inhibition to suppress *P. gingivalis*-induced IL-6 and IL-1β levels. The results show that ROS-mediated activation of JAK2 is required for *P. gingivalis*-induced inflammatory cytokine production and that the JNK/c-Jun signaling axis is involved in the ROS-dependent regulation of IL-1β and IL-6 production.

Reactive oxygen species (ROS) are a group of chemically reactive molecules consisting of radical and nonradical oxygen species formed by the partial reduction of oxygen. Accumulating evidence implicates ROS in the pathogenesis of many diseases, such as rheumatoid arthritis, chronic pulmonary disease, atherosclerosis, and, more recently, periodontitis (1–4). In addition to direct tissue-destructive properties, ROS have been established as secondary signaling molecules with a key role in diverse physiological processes, including cellular proliferation, senescence, cell death and survival, and immune responses (5, 6). Cellular ROS can be generated either through the process of mitochondrial oxidative phosphorylation or upon challenge by exogenous factors, including bacteria and their virulence factors (7, 8). Overwhelming ROS activity, caused by either an increase in ROS levels or a decrease in antioxidant capacity, induces oxidative stress and results in collateral damage to nucleic acids, proteins, and lipids (9, 10). In contrast, restrained ROS production plays an important role in microbial host defense, as ROS can regulate diverse signaling pathways and consequently modify inflammatory responses (11, 12). However, the early signaling events which are impacted by ROS and the molecular mechanisms by which ROS affect inflammatory responses have yet to be fully characterized.

Periodontitis is a chronic immune inflammatory disease which afflicts a large percentage of the adult population and is characterized by the destruction of periodontal tissues, resorption of alveolar bone, and eventual exfoliation of the teeth (13, 14). *Porphyromonas gingivalis*, a Gram-negative black-pigmented anaerobe, is a major pathogen in the initiation and progression of periodontitis (15–18). While *P. gingivalis* can induce robust inflammatory responses, including the generation of a wide variety of cytokines and chemokines, the organism is also capable of subverting and stalling host immunity depending on the context (15, 19–24). Moreover, phagocytosed *P. gingivalis* is resistant to oxidative burst killing by polymorphonuclear neutrophils, in part by producing antioxidant enzymes such as superoxide dismutase, thiol peroxidase, and rubrerythrin (25–28). Additionally, *P. gingivalis* accumulates a hemin layer on the cell surface that provides oxidative stress protection (29). *P. gingivalis* can also invade and survive within epithelial cells (30–33), and recent evidence suggests that within gingival epithelial cells *P. gingivalis* not only upregulates the antioxidant glutathione (GSH) response to create an environment conducive to intracellular growth but also induces the production of ROS in the early stages of infection (34). *P. gingivalis*-induced ROS production requires extracellular ATP, which acts through a complex consisting of $P2X_4$, $P2X_7$, and pannexin-1 (35). However, the functional role of *P. gingivalis*-induced ROS in epithelial cells and the underlying molecular mechanisms that lead to the induction of inflammatory responses are unknown.

JAK2 is one of the four Janus tyrosine kinase members in mammalian cells and is a critical component of signaling pathways involved in cellular survival, proliferation, differentiation, and apoptosis (36). In particular, JAK2 is thought to play a central role in the immune system by regulating the nature and magnitude of inflammatory cytokines. JAK2 both can act as a component of Toll-like receptor (TLR)-initiated signaling pathways and thus di-

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rectly control cytokine production and can be involved in autocrine signaling stimulated by TLR-induced cytokines (37, 38). Recent studies suggest that various ROS components induced by bacteria can phosphoactivate JAK2 signaling and thereby control inflammatory cytokine production in both myeloid and epithelial cells (39, 40). We hypothesized, therefore, that *P. gingivalis*-induced ROS control subsequent inflammatory cytokine production through regulating JAK2 and its downstream signaling cascade. In this study, we show that *P. gingivalis* stimulation of gingival epithelial cells induces the rapid production of ROS, causing phosphoactivation of JAK2. Furthermore, we found that JAK2 activated by ROS in turn positively regulates the production of the inflammatory cytokines interleukin-1β (IL-1β) and IL-6 through downstream c-Jun amino-terminal protein kinase (JNK)-mediated c-Jun activation. These findings characterize the functional role of *P. gingivalis*-induced ROS and elucidate the molecular mechanism by which ROS are involved in the *P. gingivalis*-mediated production of inflammatory cytokines in gingival epithelial cells.

MATERIALS AND METHODS

Bacteria, eukaryotic cells, and infection conditions. Wild-type (WT) *P. gingivalis* ATCC 33277 and the isogenic Δ*fimA* mutant (33) were cultured anaerobically in Trypticase soy broth supplemented with yeast extract (1 mg ml^{-1}), hemin (5 μg ml^{-1}), and menadione (1 μg ml^{-1}) at 37°C. Telomerase-immortalized gingival epithelial cells (TIGKs) (41) were maintained in supplemented keratinocyte-SFM (Invitrogen) (19). TIGKs were challenged with *P. gingivalis* at a multiplicity of infection (MOI) of 10. The JAK2 inhibitor AG490 (25 μM) or the antioxidant *N*-acetyl-L-cysteine (NAC) (20 mM), were used for 2 h prior to challenge with *P. gingivalis*. Control cells were pretreated for 2 h with 0.01% dimethyl sulfoxide (DMSO) (solvent control).

Antibodies and reagents. AG490 was from LC Laboratories. NAC and aminophenyl fluorescein (APF) were from Sigma-Aldrich. Lipofectamine and RNAiMax were from Invitrogen. All small interfering RNAs (siRNAs) were from Dharmacon. Cytokine enzyme-linked immunosorbent assay (ELISA) kits were from eBioscience. All antibodies were from Cell Signaling Technology, with the exceptions of phospho-JAK2 (Tyr 221; Assay-Biotech) and anti-rabbit IgG-phycoerythrin (PE) (eBioscience).

Flow cytometry. TIGKs were pretreated with the fluorescent oxidative indicator dye APF (1 μM) for 60 min, washed twice with phosphate-buffered saline (PBS) to remove unincorporated dye, and then stimulated with *P. gingivalis* in the presence or absence of the antioxidant NAC. Cells were harvested, washed twice with 2 ml of fluorescence-activated cell sorting (FACS) buffer (PBS containing 2% fetal bovine serum [FBS] and 0.01% sodium azide), and fixed with formaldehyde at a final concentration of 4% in PBS for 10 min at room temperature. Cells were washed twice in PBS containing 2% FBS and analyzed immediately by flow cytometry.

Transfection. TIGKs were transfected with siRNA (50 nM) using Lipofectamine RNAiMax for 24 h in transfection medium (Invitrogen). The medium was then replaced with regular culture medium, which was left for a further 48 h. The levels of target molecules were assessed by Western blotting.

Western blotting. TIGKs were lysed with radioimmunoprecipitation assay (RIPA) buffer (Sigma), and protein concentrations were determined using the bicinchoninic acid protein assay kit (Thermo). Samples were separated on NuPage Novex 4 to 12% bis-Tris polyacrylamide gels (Invitrogen) and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore). Primary antibody was used at 1 μg/ml at 4°C overnight. Antigen-antibody binding was detected using horseradish peroxidase-conjugated species-specific secondary antibodies followed by ECL Western blotting detection reagents (Thermo). Blots were stripped and probed with β-actin antibodies as a loading control. Images were acquired and analyzed using a GE LAS 4010 Image Station system.

Cytokine analysis. Cytokine levels in cell-free supernatants were determined by ELISA according to the manufacturer's instructions.

Statistical analysis. Statistical significance between groups was evaluated by analysis of variance (ANOVA) and the Tukey multiple-comparison test using the InStat program (GraphPad). Differences between groups were considered significant at a P value of <0.05.

RESULTS

***P. gingivalis* induces rapid production of ROS in TIGKs.** The kinetics of ROS production in TIGKs in response to *P. gingivalis* were investigated by flow cytometry with green-fluorescent APF staining. Stimulation with *P. gingivalis* for 15, 30, and 60 min caused an increase in the levels of ROS (Fig. 1A and B). To confirm the specificity of APF, the antioxidant NAC was used to neutralize ROS and the fluorescence of APF was determined. Addition of NAC abrogated the detection of ROS in *P. gingivalis*-stimulated TIGKs (Fig. 1A and B). To investigate the extent to which the ROS response to *P. gingivalis* is sustained, TIGKs were examined by flow cytometry up to 24 h after challenge with *P. gingivalis*. As shown in Fig. 1C, ROS levels remained elevated through 24 h. *P. gingivalis* rapidly invades gingival epithelial cells in high numbers in a fimbria-dependent manner (31, 33). Hence, we investigated the role of invasion in the generation of ROS by examination of a mutant of *P. gingivalis* lacking the structural subunit protein (FimA) of the major fimbriae. The fimbria-deficient mutant of *P. gingivalis* showed no difference in induction of ROS compared to the parental strain (Fig. 1C), indicating that an intracellular location is not required by *P. gingivalis* to incite ROS generation.

Phosphoactivation of JAK2 is dependent on ROS in *P. gingivalis*-stimulated cells. Flow cytometry was used to examine the involvement of JAK2 in *P. gingivalis*-induced ROS signaling. Infection with *P. gingivalis* stimulated the phosphorylation of JAK2 in TIGKs over times (15 to 60 min) that ROS levels are increased (Fig. 2A and B). Moreover, neutralization of ROS with NAC attenuated the phosphorylation of JAK2. Western blotting corroborated phosphoactivation of JAK2 by *P. gingivalis* and further showed that neutralization of ROS suppresses the phosphorylation of JAK2 over 15 to 60 min (Fig. 2C and D). Taken together, these results establish that *P. gingivalis*-mediated phosphoactivation of JAK2 in TIGKs requires the production of ROS.

Inhibition of JAK2 or ROS suppresses *P. gingivalis*-induced IL-6 and IL-1β production. To determine if ROS-mediated JAK2 activation plays a functional role in *P. gingivalis*-mediated inflammatory cytokine production in epithelial cells, an antioxidant (NAC) and a pharmacological inhibitor (AG490) were used to suppress activation of ROS and JAK2, respectively. As shown in Fig. 3A and B, neutralization of ROS by NAC or inhibition of JAK2 by AG490 significantly reduced *P. gingivalis*-mediated IL-6 and IL-1β production. Since AG490 has been reported to have nonspecific effects on other kinases, we next used siRNA-mediated gene silencing to confirm the functional role of JAK2 in *P. gingivalis*-mediated production of IL-6 and IL-1β. JAK2 was knocked down with siRNA, which reduced JAK2 levels by >60% compared with those in nontransfected cells or cells transfected with control siRNA (Fig. 3C and D). Reduced JAK2 levels significantly diminished *P. gingivalis*-induced IL-6 (Fig. 3E) and IL-1β (Fig. 3F) secretion, data that support the role of JAK2 in the production of these proinflammatory cytokines.

Influence of JAK2 or ROS inhibition on *P. gingivalis*-mediated phosphorylation of STATs, JNK, and c-Jun. Downstream of

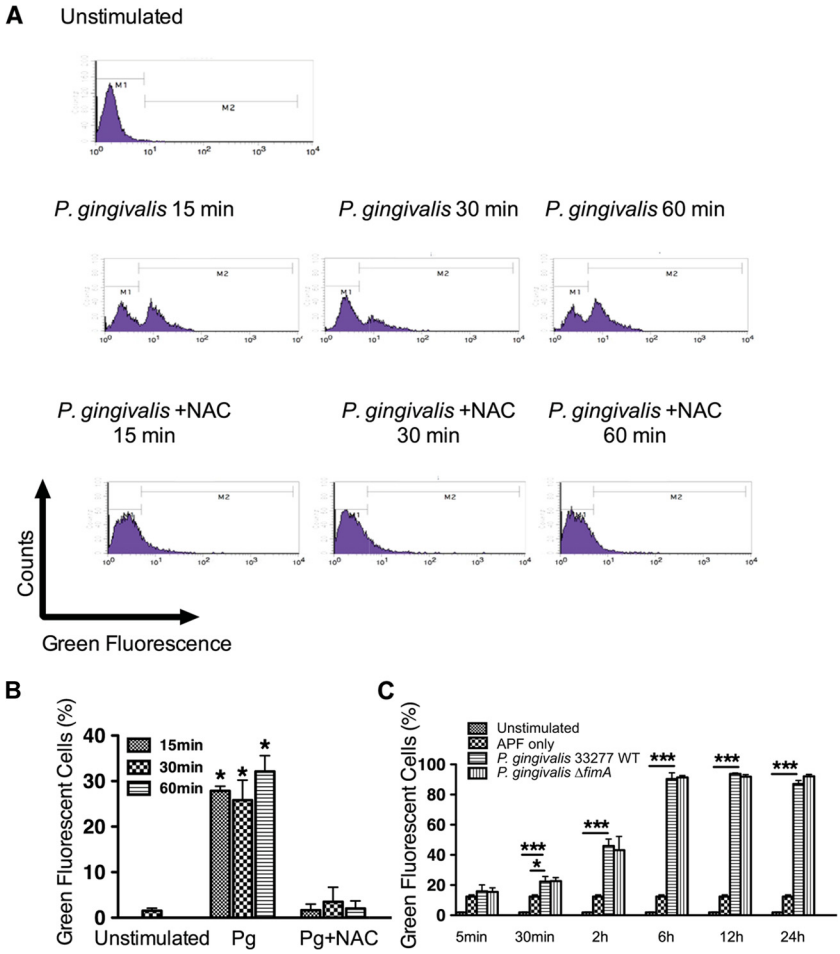

FIG 1 *P. gingivalis* induces production of ROS in TIGKs. TIGKs stimulated with *P. gingivalis* 33277 (WT) or the Δ*fimA* mutant with or without NAC as indicated were stained with APF, and ROS levels were determined by flow cytometry. (A) Typical fluorescence plots of flow cytometry. (B and C) Percentages of cells expressing ROS. Results are means ± standard errors (SE) from 3 independent experiments. *, $P < 0.05$; *** $P < 0.01$.

JAK are the prototypical STAT (signal transducer and activator of transcription) and mitogen-activated protein kinase (MAPK) pathways (42). To identify the effectors of ROS-JAK2-mediated inflammatory cytokine production, we monitored the effect of *P. gingivalis* on the phosphorylation status of STATs 3, 4, and 5, along with c-Jun amino-terminal kinase (JNK), a member of the MAPK family that is known to be activated by *P. gingivalis* (43). As shown in Fig. 4A and B, STAT 5 was not phosphorylated in response to *P. gingivalis* challenge, and STAT 4 was only weakly phosphorylated after prolonged exposure (12 h). STAT 3 was phosphorylated after 2 h of infection with *P. gingivalis*, a time frame that would indicate autocrine activation rather than direct JAK2-mediated activation. Collectively these results suggest that STATs 3, 4, and 5 do not directly regulate inflammatory cytokine production in gingival epithelial cells. In contrast, stimulation by *P. gingivalis* resulted in the phosphorylation of JNK in TIGKs at multiple time points (Fig. 4C to F). Pretreatment with either antioxidant (NAC) or JAK2 inhibitor (AG490) reduced phosphorylation levels of JNK upon *P. gingivalis* stimulation, confirming that ROS and JAK2 are epistatic to JNK. As JNK-mediated phosphorylation of c-Jun has been defined as an essential regulator in the transcription of IL-6 and IL-1β, we next tested the phosphorylation of c-Jun in *P. gingivalis*-stimulated TIGKs. The Western blot analyses in Fig. 4G to J show that *P. gingivalis* challenge enhanced the phosphorylation of c-Jun at all of the time points tested, and the activation of c-Jun was reversed by inhibition of ROS or JAK2. Collectively, these results demonstrate that induction of ROS by *P. gingivalis* causes phosphoactivation of inflammatory signaling pathways that include c-Jun in a JAK2-dependent manner.

Gene silencing of c-Jun mimics the effect of JAK2 or ROS inhibition on the production of IL-6 and IL-1β in *P. gingivalis*-stimulated TIGKs. Our results established that the *P. gingivalis*-initiated ROS-JAK2 axis phosphoactivates JNK and its downstream c-Jun signaling pathways in TIGKs. Therefore, we next wanted to determine the role of these molecules in *P. gingivalis*-mediated inflammatory cytokine production. To this end, siRNAs were used to silence JNK or c-Jun in TIGKs. siRNA-mediated gene silencing reduced the expression of JNK and c-Jun by more than 60% (Fig. 5A to D) compared to that in nontransfected cells or cells transfected with control siRNA. The reduced amounts of JNK and c-Jun abrogated the production of IL-6 and IL-1β in *P. gingivalis*-stimulated TIGKs (Fig. 5G and H). Furthermore, knockdown of extracellular signal-regulated kinase (ERK) (Fig. 5E and

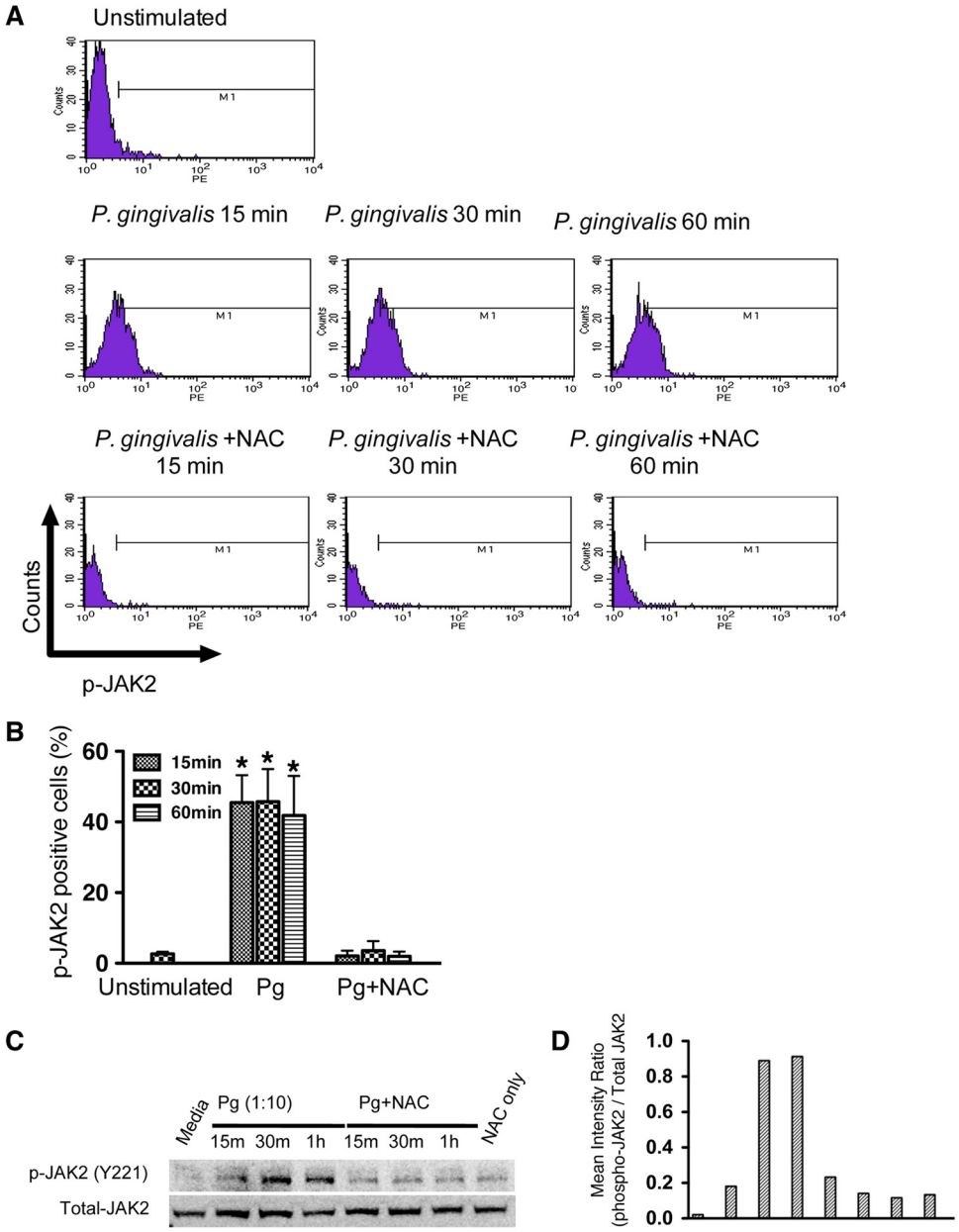

FIG 2 Phosphoactivation of JAK2 is dependent on ROS in *P. gingivalis*-stimulated TIGKs. TIGKs were stimulated with *P. gingivalis* for the times indicated in the presence or absence of NAC, and phospho (p)-JAK2 was measured by flow cytometry or Western blotting. (A) Typical fluorescence plots of flow cytometry. (B) Percentages of cells expressing p-JAK2. Results are means ± SE from 3 independent experiments. *, $P < 0.05$. (C) Western blot of TIGK lysates probed with antibodies to p-JAK2 (Y221). The blot is representative of 3 independent experiments. (D) Densitometric analysis of the image in panel C.

F), which is not activated by *P. gingivalis* in gingival epithelial cells (43), did not exhibit a significant influence on *P. gingivalis*-mediated IL-6 and IL-1β production. These results suggest that the JNK–c-Jun pathway exerts significant transcriptional control over epithelial cell inflammatory responses to *P. gingivalis*-mediated ROS signals.

DISCUSSION

The epithelial cells that line the gingival crevice constitute an interactive interface that senses bacterial colonization and signals the presence of organisms to the underlying cells of the immune system, primarily through the production of cytokines and chemokines. Dysregulation of these inflammatory responses by pathogens such *P. gingivalis* is considered an important component in the development of periodontitis (16, 17). *P. gingivalis* engages in a delicately balanced interaction with gingival epithelial cells and can suppress production of neutrophil and T-cell chemokines while also inciting secretion of proinflammatory cytokines such as IL-1β and IL-6 (19, 20, 44). This "dual personality" with regard to innate immune activation is accomplished through targeted intervention of signaling pathways within epithelial cells. For example, the SerB serine phosphatase of *P. gingi-*

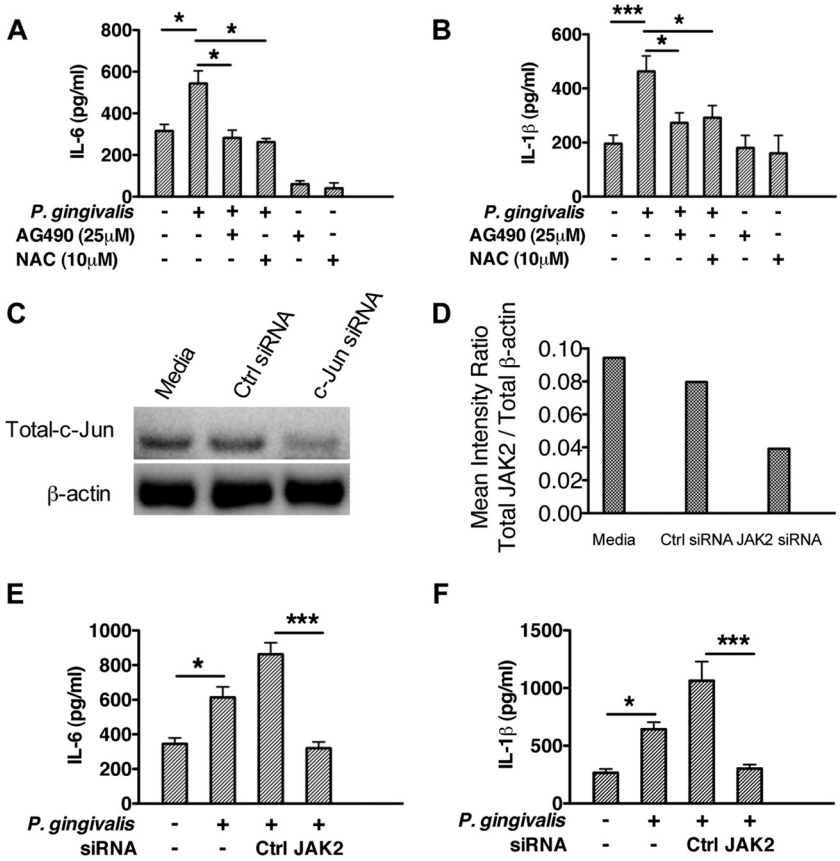

FIG 3 Inhibition of JAK2 or ROS suppresses *P. gingivalis*-induced IL-6 and IL-1β production. (A and B) TIGKs were pretreated with JAK2 inhibitor AG490 or ROS neutralizer NAC for 2 h and then stimulated with *P. gingivalis* for 4 h. Cell-free supernatants were collected, and the levels of IL-6 (A) and IL-1β (B) were determined by ELISA. Results are means ($n = 3$) with standard deviations and are representative of 3 biological replicates. (C to F) TIGKs were pretreated with JAK2-specific siRNA or nontarget siRNA for 72 h and stimulated with *P. gingivalis* for 4 h. Whole-cell lysates and cell-free supernatants were collected to determine the transfection efficiency and cytokine levels, respectively. siRNA-mediated knockdown of JAK2 and total β-actin levels were assessed by Western blotting (C), and the ratio of total JAK2 to total β-actin was determined by densitometry (D). *P. gingivalis*-induced production of IL-6 (E) an IL-1β (F) was suppressed by siRNA-mediated JAK2 inhibition. Results are means ($n = 3$) with standard deviations and are representative of 3 biological replicates. *, $P < 0.05$; *** $P < 0.005$.

valis specifically dephosphorylates the p65 subunit of NF-κB, thus inhibiting IL-8 production (19).

In this study, we investigated the proinflammatory nature of *P. gingivalis* with regard to the stimulation of IL-1β and IL-6 production and secretion from gingival epithelial cells. We found that *P. gingivalis* induces the production of ROS rapidly after infection and that ROS are required for the initiation of subsequent JAK2-mediated signaling pathways, which in turn control the production of IL-1β and IL-6. These findings provide the first documentation of the functional role of *P. gingivalis*-mediated ROS in inflammation and elucidate a molecular mechanism for *P. gingivalis*-induced inflammatory cytokine production by epithelial cells. *P. gingivalis* expresses several microbe-associated molecular patterns (MAMPs), such as lipopolysaccharide (LPS) and fimbriae, which are recognized by TLRs and lead to the production of proinflammatory cytokines (45, 46). The results presented here expand on these fundamental studies and show that the generation of ROS upon *P. gingivalis* infection is necessary for maximal secretion of IL-1β and IL-6. Of relevance to the pathogenesis of periodontitis is that both IL-1β and IL-6 are capable of upregulating osteoclastic activity via RANK/RANKL/OPG pathways and can also induce alveolar bone loss through RANK-independent pathways (47). Furthermore, IL-1β and IL-6 can contribute to tissue degradation through the induction of matrix metalloproteinases and other inflammatory mediators (48–50). A number of studies have demonstrated increased IL-1β and IL-6 levels in the crevicular fluid and periodontal tissues of patients with periodontitis (51, 52), and the application of antagonists to IL-1 reduces the severity of periodontitis in experimental animals (50).

In gingival epithelial cells, ATP stimulation results in both NADPH-induced and mitochondrially derived ROS generation through ligation of $P2X_7$ receptors, indicating that NADPH oxidase and mitochondria produce ROS synergistically (34). Although the underlying mechanism by which *P. gingivalis* stimulates ROS production is yet to be determined, an intracellular location is not required, as an invasion-defective mutant of *P. gingivalis* was equally as effective as the parental strain at increasing ROS amounts. *P. gingivalis* can also induce the production of antioxidants, including glutathione (GSH) and glutathione peroxidase, to suppress the oxidization effect of ROS (34); however, the timing of this process may be dependent on the degree of bacterial challenge. In the current study, using an MOI of 10, ROS

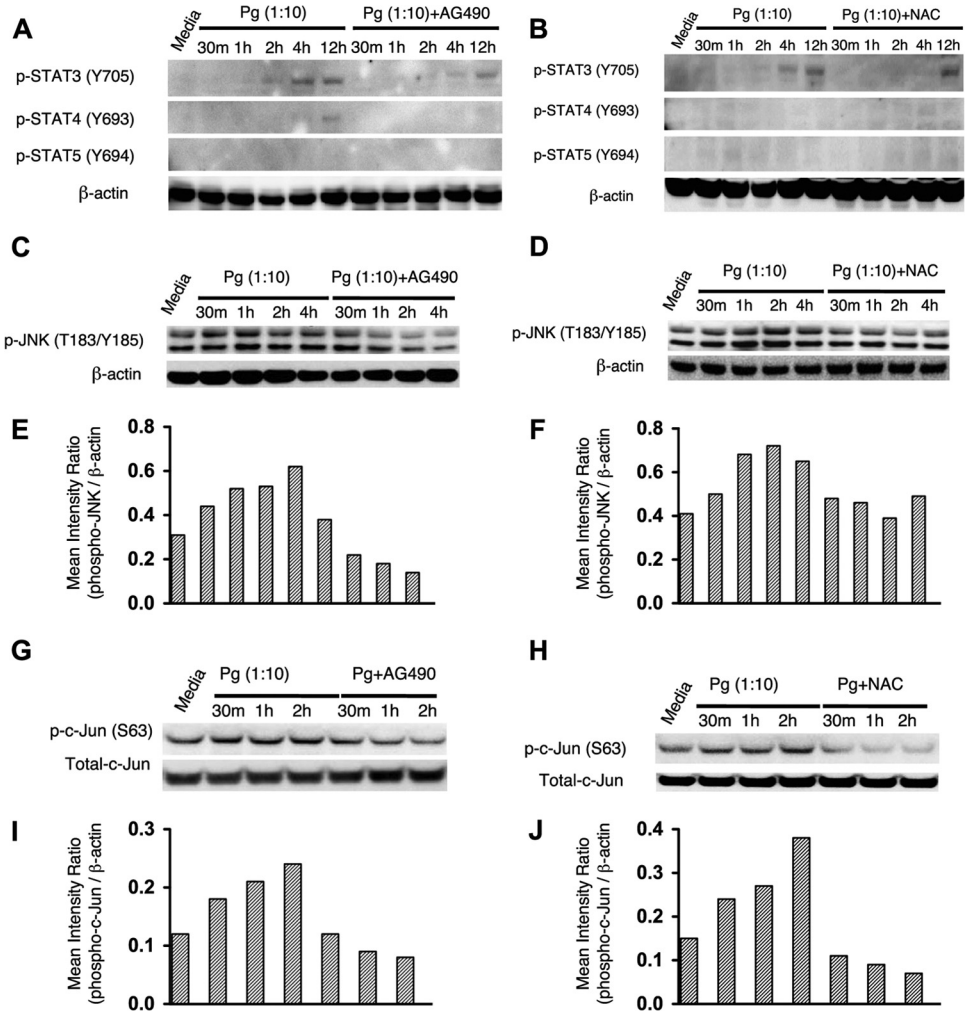

FIG 4 Influence of JAK2 or ROS inhibition on *P. gingivalis*-mediated phosphorylation of STATs (3, 4, and 5), JNK, and c-Jun. Western blots of TIGKs stimulated with *P. gingivalis* with or without SG490 or NAC and probed with antibodies to phospho (p)-STAT 3, 4, or 5 (A and B), antibodies to p-JNK (C and D), or antibodies to p-c-Jun and total c-Jun (G and H) are shown. Antibodies to β-actin were used as a loading control. Panels E and F and panels I and J are densitometric analyses of the blots in panels C and D and panels G and H, respectively. Data are representative of 3 independent experiments.

levels in response to *P. gingivalis* were sustained through 24 h, whereas with an MOI of 100, previous work has shown a reduction in ROS at 6 h postinfection (34). The number of *P. gingivalis* organisms associated with gingival epithelial cells is likely to vary according to stage and severity of infection, and thus *in vivo* ROS production and suppression may be delicately balanced. The function of *P. gingivalis*-induced ROS at early stages of infection has not hitherto been addressed, and we show here that *P. gingivalis*-induced ROS lead to phosphoactivation of JAK2. Activated JAK2 can phosphorylate tyrosine residues within the JAK2-associated receptor, which allows for the recruitment of downstream signaling molecules. These recruited molecules then relay JAK2-mediated signaling to transcription factors. The most well described cell signaling pathway involving JAK2 is phosphorylation and activation of members of the STAT family. *P. gingivalis* activated STAT 3 in TIGKs, consistent with previous studies with primary cultures of gingival epithelial cells (53), and to a lesser extent STAT 4. However, STAT activation was delayed in relation to ROS induction, indicating that the STAT pathway is not the primary mechanism for *P. gingivalis*-induced ROS control of cytokine expression. JAK2 is also involved in the activation of other signaling pathways such as those involving JNK and activating protein 1 (AP-1)/c-Jun (54, 55). JNK has been shown to be phosphorylated in response to *P. gingivalis* infection of primary gingival epithelial cells, and such activation is necessary for efficient internalization by the organism (43). The current results corroborate the activation of JNK by *P. gingivalis* in TIGKs and show that maximal phosphorylation is dependent on ROS and JAK2. A nuclear substrate of JNK is the proto-oncogene product c-Jun, a component of the AP-1 transcription factor family that has been shown to control cytokine production in immune cells (56). In this study, we show that c-Jun was primarily responsible for the upregulation of IL-1β and IL-6 in response to *P. gingivalis*-induced ROS. The literature contains examples of both JAK2/STAT and JAK2/MAPK signal transduction pathways linking microbe-induced ROS-JAK2 with cytokine expression. For example, *Helicobacter pylori* induces an increase in ROS and IL-8 expression through the activation of MAPKs and AP-1/c-Jun in

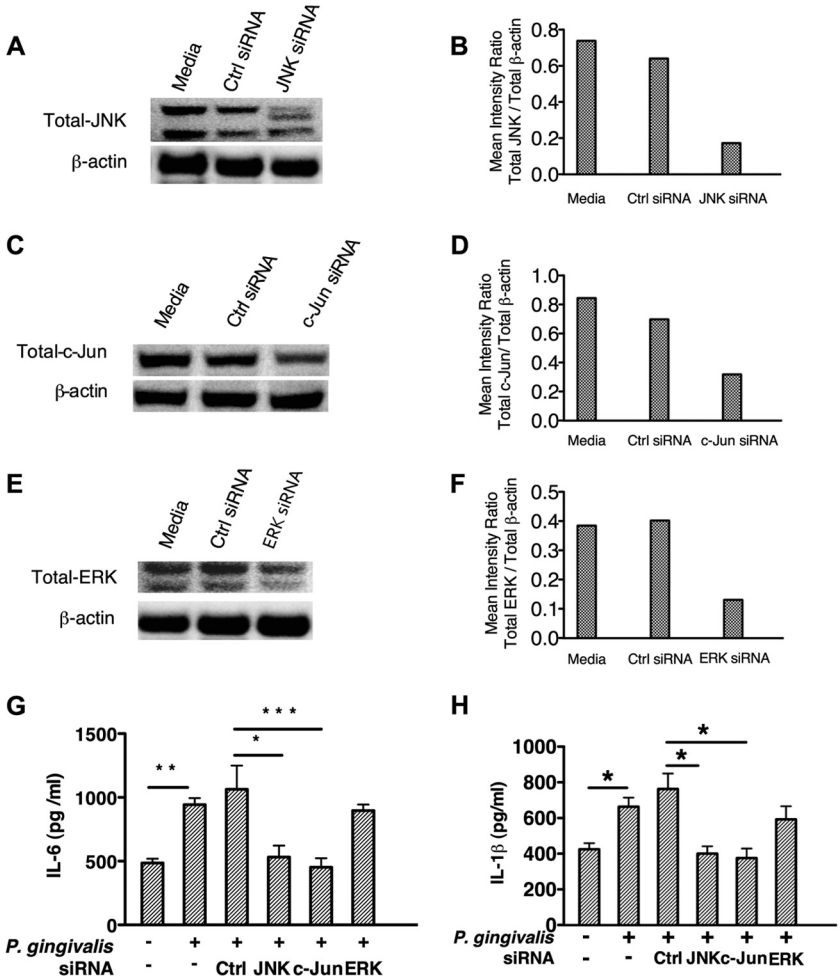

FIG 5 Gene silencing of JNK or c-Jun inhibits the production of IL-6 and IL-1β in *P. gingivalis*-stimulated TIGKs. TIGKs were pretreated with ERK, JNK, c-Jun, or nontarget (Ctrl) siRNA for 72 h and stimulated with *P. gingivalis* for 4 h. Whole-cell lysates and cell-free supernatant were collected to determine the transfection efficiency and cytokine levels, respectively. siRNA-mediated knockdown of c-JNK (A and B), c-Jun (C and D), or ERK (E and F) was assessed by Western blotting and densitometric analysis. *P. gingivalis*-induced production of IL-6 (G) an IL-1β (H) in TIGKs with JNK, c-Jun, or ERK knockdown was determined by ELISA. Results are means ($n = 3$) with standard deviations and are representative of 3 independent experiments. *, $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

gastric epithelial cells (57). However, in pulmonary epithelial cells stimulated with lipid-associated membrane proteins from *Mycoplasma pneumoniae*, ROS mediate the phosphorylation of JAK2/STAT3 and the consequent expression of IL-8 (39). Hence the nature of the signaling induced by ROS is likely to depend on a number of factors, such as the nature and duration of the stimulus along with the cell type.

The role of JAK2 in multiple downstream pathways indicates that *P. gingivalis*-mediated activation of JAK2 through ROS could impact a number of epithelial cell processes, a topic that requires further investigation. In the case of IL-1β, secretion of the mature cytokine requires cleavage of immature pro-IL-1β by capsase-1, an enzyme that is activated in the inflammasome. Assembly of the inflammasome occurs in response to MAMPs and also endogenous danger-associated molecular patterns (DAMPs) such as ATP. ROS can act as secondary signaling molecules for the formation of the NALP3 inflammasome (58), and *P. gingivalis*-induced ROS production in gingival epithelial cells can activate the NLRP3 inflammasome and caspase-1, leading to IL-1β secretion (35). Hence, ROS may play a role in both transcriptional and posttranslational regulation of IL-1β by *P. gingivalis*.

In summary, we have demonstrated that *P. gingivalis*-induced ROS is required for the phosphoactivation of JAK2, which in turn activates downstream signaling pathways including JNK and c-Jun in gingival epithelial cells. Moreover, activation of the transcription factor c-Jun regulates production of the inflammatory cytokines IL-6 and IL-1β. These findings suggest that ROS-JAK2 signaling represents a potential therapeutic target in the control of inflammatory diseases such as periodontitis.

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