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JMJD3 Promotes *Porphyromonas gingivalis* Lipopolysaccharide-Induced Th17-Cell Differentiation by Modulating the STAT3-RORc Signaling Pathway

Doudou Huang,[1,2] Chi Zhang,[1,2] Panpan Wang,[1,2] Xiting Li,[1,2] Li Gao,[1,2] and Chuanjiang Zhao[1,2]

The immune response mediated by Th17 cells is essential in the pathogenesis of periodontitis. Emerging evidence has demonstrated that lipopolysaccharide from *Porphyromonas gingivalis* (Pg-LPS) could promote Th17-cell differentiation directly, while the downstream signaling remains elusive. This study was aimed to explore the role of JMJD3 (a JmjC family histone demethylase) and signal transducers and activators of transcription 3 (STAT3) in Th17-cell differentiation triggered by Pg-LPS and clarify the interaction between them. We found that the expression of JMJD3 and STAT3 was significantly increased under Th17-polarizing conditions. Pg-LPS could promote Th17-cell differentiation from $CD4^+$ T cells, with an increased expression of JMJD3 and STAT3 compared to the culture without Pg-LPS. The coimmunoprecipitation results showed that the interactions of JMJD3 and STAT3, STAT3 and retinoid-related orphan nuclear receptor γt (RORγt) were enhanced following Pg-LPS stimulation during Th17-cell differentiation. Further blocking assays were performed and the results showed that inhibition of STAT3 or JMJD3 both suppressed the Th17-cell differentiation, JMJD3 inhibitor could reduce the expression of STAT3 and p-STAT3, while JMJD3 expression was not affected when STAT3 was inhibited. Taken together, this study found that JMJD3 could promote Pg-LPS induced Th17-cell differentiation by modulating the STAT3-RORc signaling pathway.

Keywords: *P. gingivalis*, lipopolysaccharide, Th17 cell, JMJD3, STAT3

Introduction

It is widely accepted that periodontitis is a microbial-related chronic inflammatory disease that induces the loss of the periodontal ligament and alveolar bone (Könönen *et al.*, 2019). A skewed host immune and inflammatory response initiated by periodontal pathogens is considered to be the main cause for the initiation, establishment and progression of periodontal inflammation and tissue breakdown (Cekici *et al.*, 2014). In this regard, $CD4^+$ T helper (Th) cells, especially Th17 cells, are critically important in the pathogenesis of periodontitis. Exaggerated Th17 responses are proven to promote inflammatory bone loss and tissue damage in periodontitis (Adibrad *et al.*, 2012; Eskan *et al.*, 2012; Moutsopoulos *et al.*, 2014). Notably, Th17 cells, originating from $CD4^+$ T cells, are induced by retinoid-related orphan nuclear receptor γt (RORγt, encoding gene *Rorc*) (Ivanov *et al.*, 2006). However, the underlying mechanisms that regulate Th17-cell differentiation in the periodontal inflammatory context remain undefined.

Porphyromonas gingivalis (P. gingivalis) is strongly correlated with advanced periodontal lesions (Lunar Silva and Cascales, 2021). Lipopolysaccharide (LPS), one of the most important virulence factors of *P. gingivalis*, is believed to induce a strong immune reaction by interacting with toll-like receptors (TLRs), which triggers the expression of proinflammatory cytokines and activates the NF-κB pathway (Yang *et al.*, 2015; Heinbockel *et al.*, 2018). These interactions stimulate the differentiation of $CD4^+$ subtypes (particularly Th1 and Th17) (Candelli *et al.*, 2021). Our

[1]Department of Periodontology, Hospital of Stomatology, Sun Yat-sen University, Guangzhou, China.
[2]Guangdong Provincial Key Laboratory of Stomatology, Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou, China.

previous study demonstrated that LPS from *P. gingivalis* (Pg-LPS) could promote Th17-cell differentiation directly by upregulating TLR2 expression on the T cell membrane (Zhang *et al.*, 2019a). Therefore, the downstream signaling of TLRs in regulating Th17-cell differentiation induced by Pg-LPS needs to be further investigated.

Signal transducers and activators of transcription 3 (STAT3), a component of the IL-6-activated acute phase response factor complex (Heinrich *et al.*, 1998), has been proven to be activated downstream of the TLR signaling pathway and participates in the regulation of inflammatory responses (Park *et al.*, 2013; Lyu *et al.*, 2016). Although STAT3 has been implicated to be closely associated with the differentiation of Th cells (Villarino *et al.*, 2015), we lack insight into whether and how STAT3 regulates Th17-cell differentiation via TLRs. Genome-wide studies have illustrated clear correlations between epigenetic modifications and T cell differentiation (Wei *et al.*, 2009; Zhang *et al.*, 2012; Russ *et al.*, 2014).

Recently, Lamere *et al.* (2017) found that H3K27me3 was demethylated during the early activation of $CD4^+$ T cells. ChIP-seq analysis revealed reduced levels of H3K27me3 in the promoter regions of JAK2 and STAT3. JMJD3, a lysine K27 demethylase, is reported to have an important role in the activation of Th17-related cytokines (Wang *et al.*, 2012). Another study demonstrated that JMJD3 shares direct targets with RORγt and STAT3 (Ciofani *et al.*, 2012), suggesting a potential role of JMJD3 in regulating STAT3 and Th17-cell differentiation. Thus, in this preliminary study, we focused on the potential contribution of JMJD3 and STAT3 to Th17-cell differentiation triggered by Pg-LPS and further explored the interaction between JMJD3 and STAT3.

Materials and Methods

$CD4^+$ T cell purification and in vitro *differentiation*

$CD4^+$ T cells were magnetically separated from the splenocytes of 60 C57BL/6J mice (6- to 8-week-old, female) according to the manufacturer's instructions (130-104-454; Miltenyi Biotech, Germany). This study was approved by the Institutional Animal Care and Use Committee (IACUC), Sun Yat-sen University (SYSU-IACUC-2022-B0003). After sorting, $CD4^+$ T cells were cultured in RPMI medium and stimulated with plate-bound anti-CD3 (5 μg/mL) and soluble anti-CD28 (2 μg/mL) for 5 days under the following conditions (Zhao *et al.*, 2018): 2 ng/mL TGF-β, 30 ng/mL IL-6, 10 μg/mL anti-IFN-γ, 10 μg/mL anti-IL-4, 20 ng/mL IL-23, and 10 ng/mL IL-1β (Biolegend). The medium was replaced on the third day.

Th17 cells stimulated with Pg-LPS

Then, 1 μg/mL Pg-LPS (tlrl-Pglps; InvivoGen) was added to the $CD4^+$ T cells under Th17-polarizing conditions. The cultures were incubated for 5 days for RNA analysis, FACS analysis, western blotting, and enzyme-linked immunosorbent assay (ELISA).

Coimmunoprecipitation

The cultured cells were solubilized in IP buffer. The protein lysate was incubated with 2 μg anti-STAT3 (Cell Signaling Technology) or anti-IgG (Beyotime, China) at 4°C overnight. Then, the pretreated Protein A/G Magnetic Beads (MCE) were incubated with 100 μg of the lysate proteins overnight at 4°C. The precipitates were washed with lysis buffer five times and then used for the immunoblotting assay using anti-JMJD3, anti-STAT3, or anti-RORγt antibody. For the anti-RORγt antibody, the horseradish peroxidase-conjugated anti-rabbit IgG light-chain (Abbkine, China) was used as the secondary antibody to avoid interference from IgG heavy chain.

STAT3 or JMJD3 blocking assay

The STAT3 inhibitor Stattic (MCE) or JMJD3 inhibitor GSK-J4 (Tocris) was added to the Th17-polarizing cultures with or without Pg-LPS. The cultures were further incubated in a humidified atmosphere of 5% CO_2 at 37°C for 5 days for RNA analysis, FACS analysis, western blotting and ELISAs.

Cell viability assay

The effect of Stattic or GSK-J4 on $CD4^+$ T cell viability was assessed by the cell counting kit-8 (CCK-8) assay (Beyotime) according to the manufacturer's instructions.

Flow cytometric analysis

$CD4^+$ T cells were restimulated with 50 ng/mL phorbol-12-myristate-13-acetate and 500 ng/mL ionomycin in the presence of 10 μg/mL Brefeldin A (Sigma-Aldrich, Germany) for 5 h. After permeabilization, cells were stained with FluoresceinIsothiocyanate-conjugated anti-CD4 antibodies and anti-IL-17-antigen-presenting cell (APC) antibodies (BioLegend) for 20 min at room temperature. All flow cytometry analyses were performed on a FACSCalibur (BD Biosciences), and the data were analyzed by FlowJo software.

Enzyme-linked immunosorbent assay

The levels of IL-17A protein in the culture supernatants were measured by an ELISA kit (Wuhan Huamei Biotechnology, China) according to the manufacturer's instructions. The optical density values were measured by GEN5 at 450 nm.

Quantitative real-time PCR

RNA transcripts were quantified by real-time PCR as described (Zhang *et al.*, 2021). The specific primer sequences are described in Table 1 (designed and synthesized by Tianyi Huiyuan Biotechnology, China).

Western blotting

Cells were lysed in RIPA buffer containing 1% protease and phosphatase inhibitor (Beyotime) on ice for 30 min. The lysates were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by immunoblotting with specific antibodies against JMJD3 (1:1000; Novus Biologicals), p-STAT3 (1:1000; Cell Signaling Technology), STAT3 (1:1000; Cell Signaling Technology), IL-17A (1:500; Abcam), and GAPDH (1:1000; Cell Signaling Technology).

Table 1. Primers for Quantitative Polymerase Chain Reaction Analysis

	Forward primer (5′–3′)	*Reverse primer (3′–5′)*
Jmjd3	CACCGGACCCCAAGAAC	CTGTGGATGTTACCCGCAT
Stat3	GAGAGCAGAAGGGAGCAA	CTCACAGAGTGGGGCAA
Il-17a	TTCACTTTCAGGGTCGAGA	GGGGTTTCTTAGGGGTCA
Rorc	GAACTTGGGGAACCAGAAC	TGGCATGTCTCTCGGAA
Gapdh	GGATGCTGCCCTTACCC	GTTCACACCGACCTTCACC

Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Il-17a, interleukin 17A; Jmjd3, Jumonji domain-containing protein 3; Rorc, RAR-related orphan receptor C; Stat3, signal transducer and activator of transcription 3.

Statistical analysis

Data were analyzed by GraphPad 7.0 software. Student's *t* test or the Mann–Whitney *U* test were used for appropriate comparisons between two groups. One-way analysis of variance was performed to determine the significance among multiple groups. A value of $p < 0.05$ was considered statistically significant. All experiments were repeated more than three times unless otherwise noted.

Results

Pg-LPS promotes the expression of JMJD3 and STAT3 in Th17-cell differentiation

To determine the expression of JMJD3 and STAT3 during Th17-cell differentiation, purified $CD4^+$ T cells were stimulated with specific antibodies for 5 days. Quantitative PCR results showed that the mRNA expression of JMJD3 and STAT3 was significantly increased under Th17-polarizing conditions (Fig. 1a). The Th17-cell group was indicated as the control group in the subsequent experiments.

After stimulation with Pg-LPS, $CD4^+IL\text{-}17A^+$ cells were determined by flow cytometry, and the results showed that the Th17-cell differentiation ratio was upregulated after Pg-LPS treatment compared to that of the control group without LPS (Fig. 1b). Meanwhile, the expression of RORγt and IL-17A was also significantly upregulated with Pg-LPS at both the mRNA and protein levels (Fig. 1d, e), suggesting that Pg-LPS could promote Th17-cell differentiation from $CD4^+$ T cells *in vitro*.

Simultaneously, increased mRNA and protein expression levels of JMJD3 and STAT3 were observed in Th17-cell differentiation after Pg-LPS stimulation compared to the culture without Pg-LPS (Fig. 1c–e), suggesting that Pg-LPS could promote the expression of JMJD3 and STAT3 in Th17-cell differentiation.

STAT3 is correlated with JMJD3 in Pg-LPS-stimulated Th17-cell differentiation

To explore the interaction between JMJD3 and STAT3, coimmunoprecipitation assays were performed. The results showed that JMJD3 directly interacted with STAT3 during Th17-cell differentiation with or without Pg-LPS stimulation (Fig. 2). And RORγt was identified in STAT3 immune complexes suggesting the interaction between STAT3 and RORγt. Furthermore, the interactions between JMJD3 and STAT3, STAT3 and RORγt were enhanced after Pg-LPS stimulation. Thus, we can infer that JMJD3 plays an important role during Pg-LPS-stimulated Th17-cell differentiation through its interaction with STAT3.

STAT3 regulates Th17-cell differentiation induced by Pg-LPS without affecting JMJD3

To determine whether STAT3 is involved in Pg-LPS-stimulated Th17-cell differentiation, a STAT3 blocking assay was performed. A concentration of 200 nM statttic was adopted in the blocking assay according to the CCK-8 assay (Supplementary Fig. S1).

The results showed that statttic could efficiently inhibit STAT3 expression at the mRNA and protein levels ($p < 0.05$) (Fig. 3b, d, e). Furthermore, the effects of STAT3 blockade on the proportion of Th17 cells differentiated from $CD4^+$ T cells were investigated. The frequency of Th17 cells was significantly decreased after STAT3 blockade in both the control and LPS groups ($p < 0.05$) (Fig. 3a). The mRNA and protein expression of RORc and IL-17A were drastically decreased in the stattic-treated groups ($p < 0.05$) (Fig. 3b, d, e). The ELISA results also showed that stattic significantly reduced the concentration of supernatant IL-17A in both the control and LPS groups ($p < 0.01$) (Fig. 3c). However, there was no significant change in JMJD3 expression at either the mRNA or protein level ($p > 0.05$) (Fig. 3b, d, e), indicating that STAT3 can regulate Th17-cell differentiation triggered by Pg-LPS without affecting JMJD3.

JMJD3 regulates Th17-cell differentiation under Pg-LPS through STAT3

To further investigate the interaction between JMJD3 and STAT3 during Th17-cell differentiation by Pg-LPS stimulation, a JMJD3 blocking assay was also conducted. GSK-J4 (80 nM) was used in the blocking assay according to the CCK-8 assay (Supplementary Fig. S2). As shown in Figure 4a, LPS-induced Th17-cell differentiation was completely eliminated with additional GSK-J4 compared with the control group. This was further supported by quantitative real-time PCR and western blot analysis, which indicated that the levels of RORc and IL-17A were also profoundly reduced with GSK-J4 treatment (Fig. 4b, d, e).

Consistently, cytokine levels in the supernatants as determined by ELISA showed that the amount of IL-17A production was much lower in Th17-polarizing conditions with GSK-J4 addition in both the control and LPS groups (Fig. 4c). Moreover, treatment with GSK-J4 dramatically suppressed the phosphorylated and total STAT3 levels, as shown at the protein and RNA levels (Fig. 4b, d, e). Thus, GSK-J4 is likely to inhibit Th17-cell differentiation by blocking the effect of LPS on JMJD3 and further inhibiting STAT3 expression. Taken together, our results demonstrate that JMJD3 regulates Th17-cell differentiation by affecting the expression and phosphorylation of STAT3.

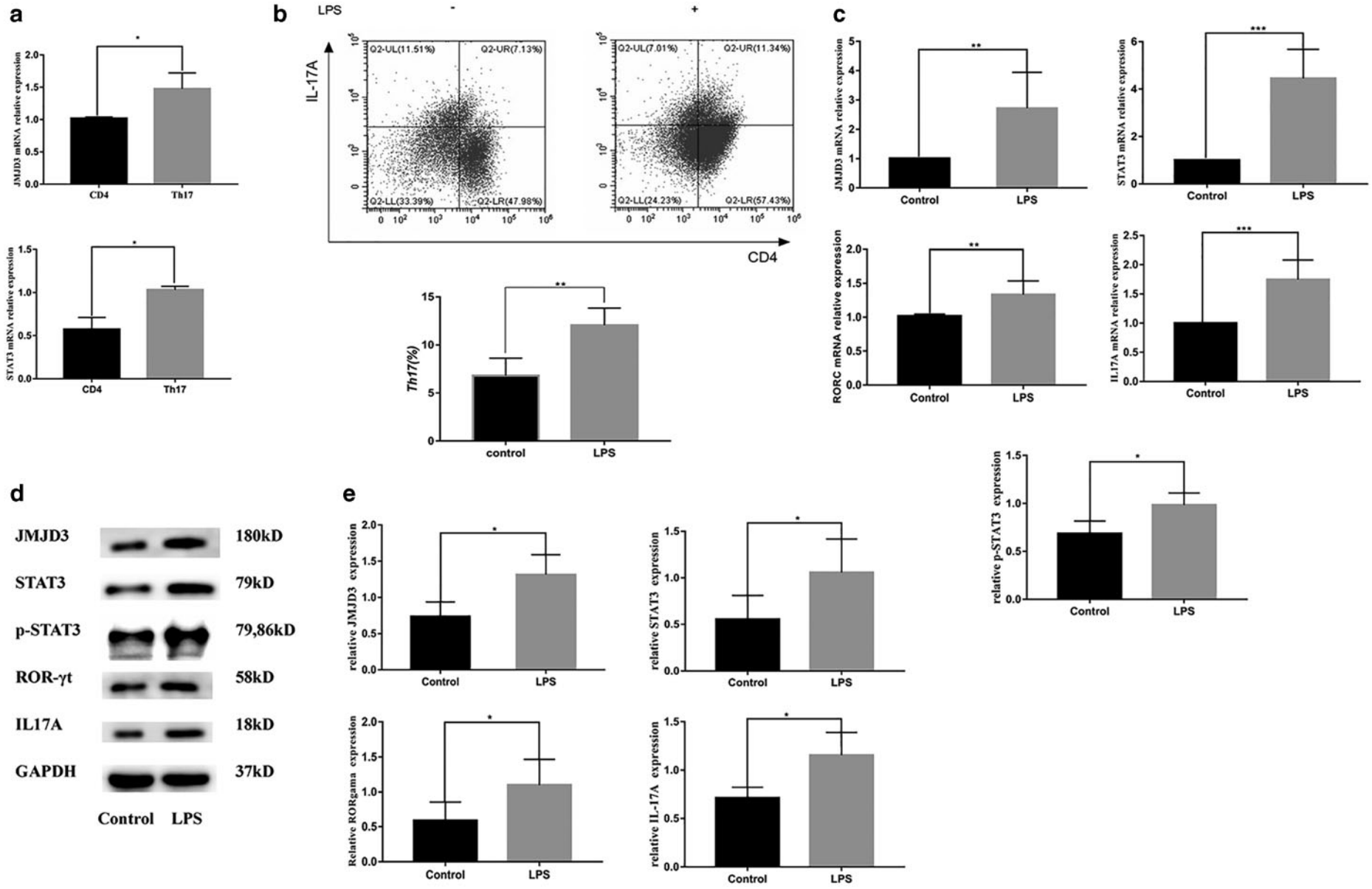

FIG. 1. Pg-LPS promotes the expression of JMJD3 and STAT3 in Th17-cell differentiation. **(a)** The mRNA expression of *Jmjd3* and *Stat3* was significantly increased in freshly isolated $CD4^+$ T cells and activated $CD4^+$ T cells under Th17-cell conditions. **(b)** After stimulation with Pg-LPS, $CD4^+$ $IL\text{-}17A^+$ T cells were identified by flow cytometry. The Th17-cell differentiation ratio was upregulated after Pg-LPS treatment. **(c, d, e)** The expression of RORC, IL-17A, JMJD3, and STAT3 was significantly upregulated with Pg-LPS at both the mRNA **(c)** and protein **(d, e)** levels. Data are presented as the mean ± SEM of at least three independent experiments. Significant changes are marked with $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Jmjd3, Jumonji domain-containing protein 3; Pg-LPS, LPS from *Porphyromonas gingivalis*; Rorc, RAR-related orphan receptor C; Stat3, signal transducer and activator of transcription 3.

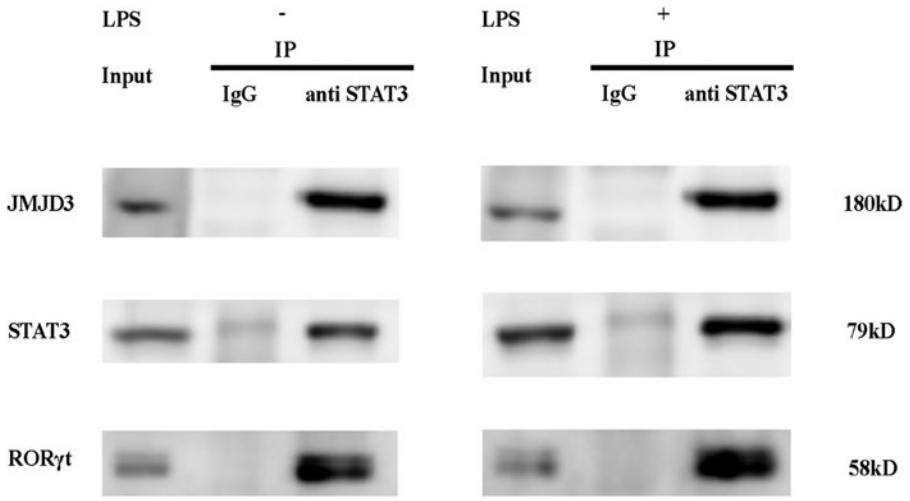

FIG. 2. STAT3 interacts with JMJD3 and RORγt in Th17 cells. The associations of JMJD3 and STAT3, STAT3 and RORγt were enhanced after Pg-LPS stimulation. Total protein extracts were prepared from primary $CD4^+$ T cells cultured for 5 days under Th17-polarizing conditions untreated or stimulated with Pg-LPS and then subjected to IP with anti-STAT3 antibody. The immunocomplexes were separated by SDS-PAGE and analyzed by western blot using indicated antibodies. Five percent of the total protein extracts were loaded as positive control (Input). Meanwhile, total protein extracts were immunoprecipitated with an antibody against rabbit IgG isotype as negative control (IgG). IP, immunoprecipitation; RORγt, retinoid-related orphan nuclear receptor γt; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Discussion

In addition to specific cytokines, the microbiota also plays important roles in inducing Th17-cell differentiation. Increasing evidence indicates that APCs, triggered by microbial infection, can lead to the differentiation of $CD4^+$ T cells by presenting antigens and releasing proinflammatory cytokines (Boccasavia *et al.*, 2021; Yin *et al.*, 2021). In the periodontal microenvironment, *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* have been reported to induce IL-17 production by activating $CD14^+$ monocytes (Cheng *et al.*, 2016). Generally, the recognition of inflammatory signals via pattern recognition receptors by APCs can activate the intracellular pathways involved in $CD4^+$ T cell differentiation, especially TLRs (Mills, 2011; Li *et al.*, 2018; Dias *et al.*, 2019). LPS is widely recognized as an agonist of TLRs (Zeng *et al.*, 2020; Ciesielska *et al.*, 2021), which can induce the differentiation of Th17 cells (Wilson *et al.*, 2009; McAleer *et al.*, 2010; Park *et al.*, 2015).

Interestingly, our previous study showed that Pg-LPS could promote Th17-cell differentiation directly by upregulating TLR2 expression on T cells in the absence of APC *in vitro* (Zhang *et al.*, 2019a). Data from the present study further confirmed that under Th17-polarizing conditions, the expression of JMJD3 in $CD4^+$ T cells was induced by Pg-LPS and further promoted Th17-cell differentiation by modulating the STAT3-RORγt signaling pathway. Taken together, periodontal pathogens can participate in the differentiation of Th17 cells through two different mechanisms. On the one hand, they induce the production of Th17 cells by providing antigens to APCs; on the other hand, they directly induce the production of Th17-related transcription factors, which leads to the differentiation of Th17 cells.

Moreover, it is well known that LPS can induce STAT3 activation (Chen *et al.*, 2009). Upon the binding of LPS ligand to its receptor, intracellular activation of JAK2 is initiated, resulting in downstream STAT3 phosphorylation (Zeinalzadeh *et al.*, 2021). After activation, phosphorylated STAT3 translocates into the nucleus (Pencik *et al.*, 2016; Kitamura *et al.*, 2017; Zheng *et al.*, 2019) and acts as a transcription factor to promote the expression of downstream target genes, including *Rorc* and *Il-17a* (Heim, 1996; Yu *et al.*, 2009; Johnson *et al.*, 2018). ChIP-seq and RNA-seq studies revealed that STAT3 plays a critical role in the Th17 transcriptional program by binding to Th17-related gene loci (Durant *et al.*, 2010). More recently, RORc was also reported to contain direct targets of STAT3 on Th17 cells (Sallusto, 2016; Tripathi *et al.*, 2017; Wu *et al.*, 2017; August, 2018). Consistent with previous studies, our work confirmed that STAT3 could bind to RORγt directly under Th17-polarizing conditions, showing the critical role of STAT3 in Th17-cell differentiation.

Generally, the expression of JMJD3 is low under normal conditions, but a variety of cellular stresses can induce the expression of JMJD3 (Zhang *et al.*, 2019b). De Santa *et al.* (2009) reported that JMJD3 can be induced upon LPS stimulation, mediating inflammation-related genes in peripheral macrophages. Here, we confirmed that Pg-LPS induced JMJD3 expression along with elevated expression of STAT3, p-STAT3, and RORγt, and further enhanced their interactions, suggesting STAT3 and RORγt might be the targets of JMJD3.

A previous study has demonstrated that JMJD3 is involved in H3K27me3 modification at *Rorc* and Th17-related genes (Liu *et al.*, 2015). The study of LaMere *et al.* (2017) confirmed that STAT3 promoter was evidently demethylated after $CD4^+$ T cell activation. However, further ChIP-seq found no JMJD3 peak surrounding the STAT3 locus, and Hi-C did not reveal any distal JMJD3 sites interacting with the promoter. Two JMJD3 peaks surrounding the JAK2 locus were identified, indicating that JMJD3 may activate the phosphorylation of STAT3 by regulating the expression of JAK2 during $CD4^+$ T cell differentiation, which further

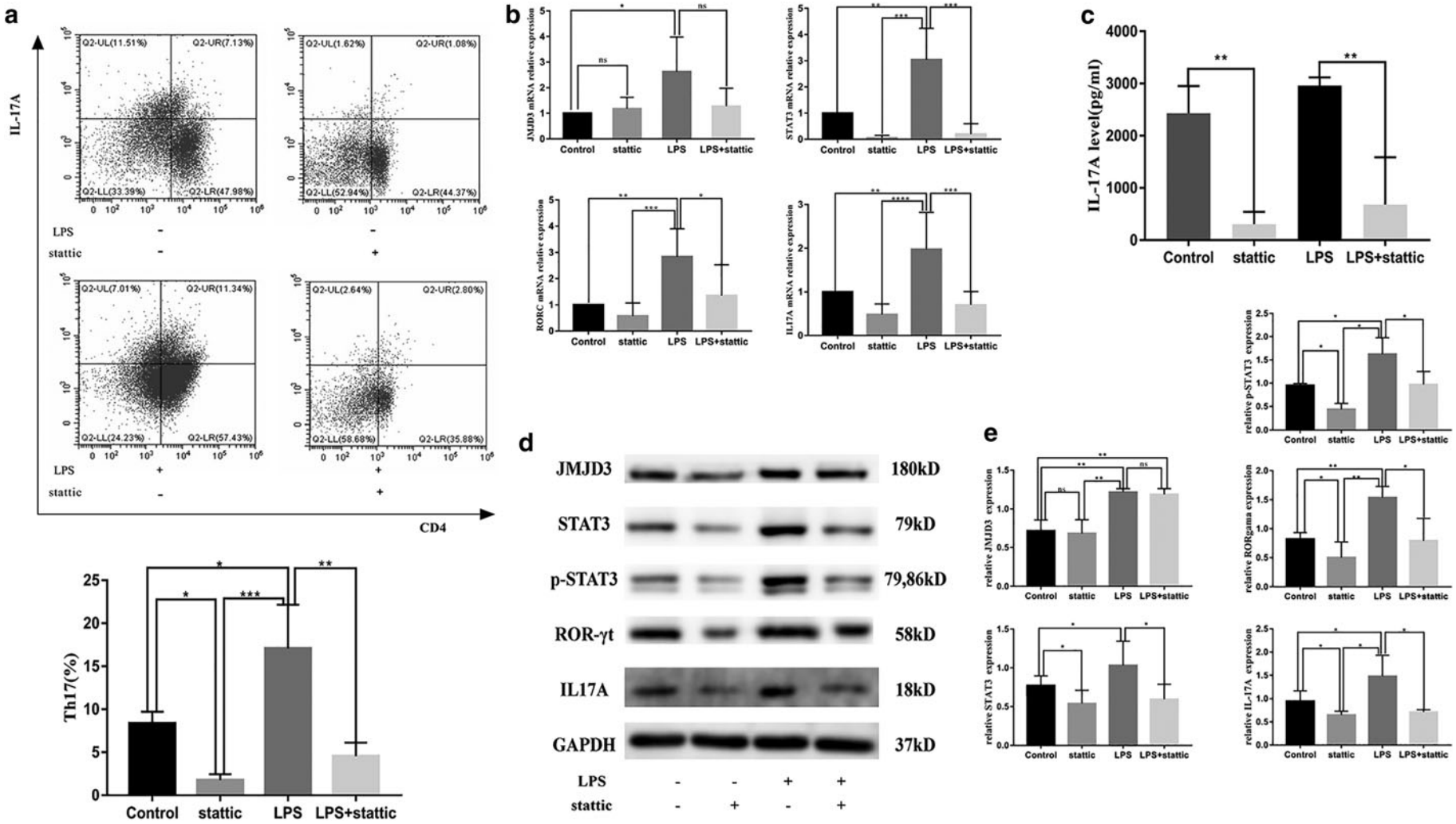

FIG. 3. STAT3 regulates Th17-cell differentiation induced by Pg-LPS without affecting JMJD3. Purified $CD4^+$ T cells were treated with the STAT3 inhibitor stattic (200 nM), Pg-LPS (1 μg/mL), or stattic and Pg-LPS and cultured under the same conditions as the Th17 differentiation system. The cultures were further incubated for 5 days for RNA analysis, FACS analysis, western blotting, and ELISAs. **(a)** The frequency of Th17 cells was significantly decreased after STAT3 blockade in both the control and LPS groups. **(b)** The mRNA expression of *Jmjd3*, *Stat3*, *Rorc*, *and Il-17a* in the control, Stattic, LPS, and LPS+Stattic groups. **(c)** Stattic significantly reduced the concentration of supernatant IL-17A in both the control and LPS groups. **(d, e)** The protein levels of JMJD3, STAT3, RORγt, and IL-17A in the control, Stattic, LPS, and LPS+Stattic groups. Data are presented as the mean ± SEM of four independent experiments. Significant changes are marked with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. ELISA, enzyme-linked immunosorbent assay; ns, no significant difference.

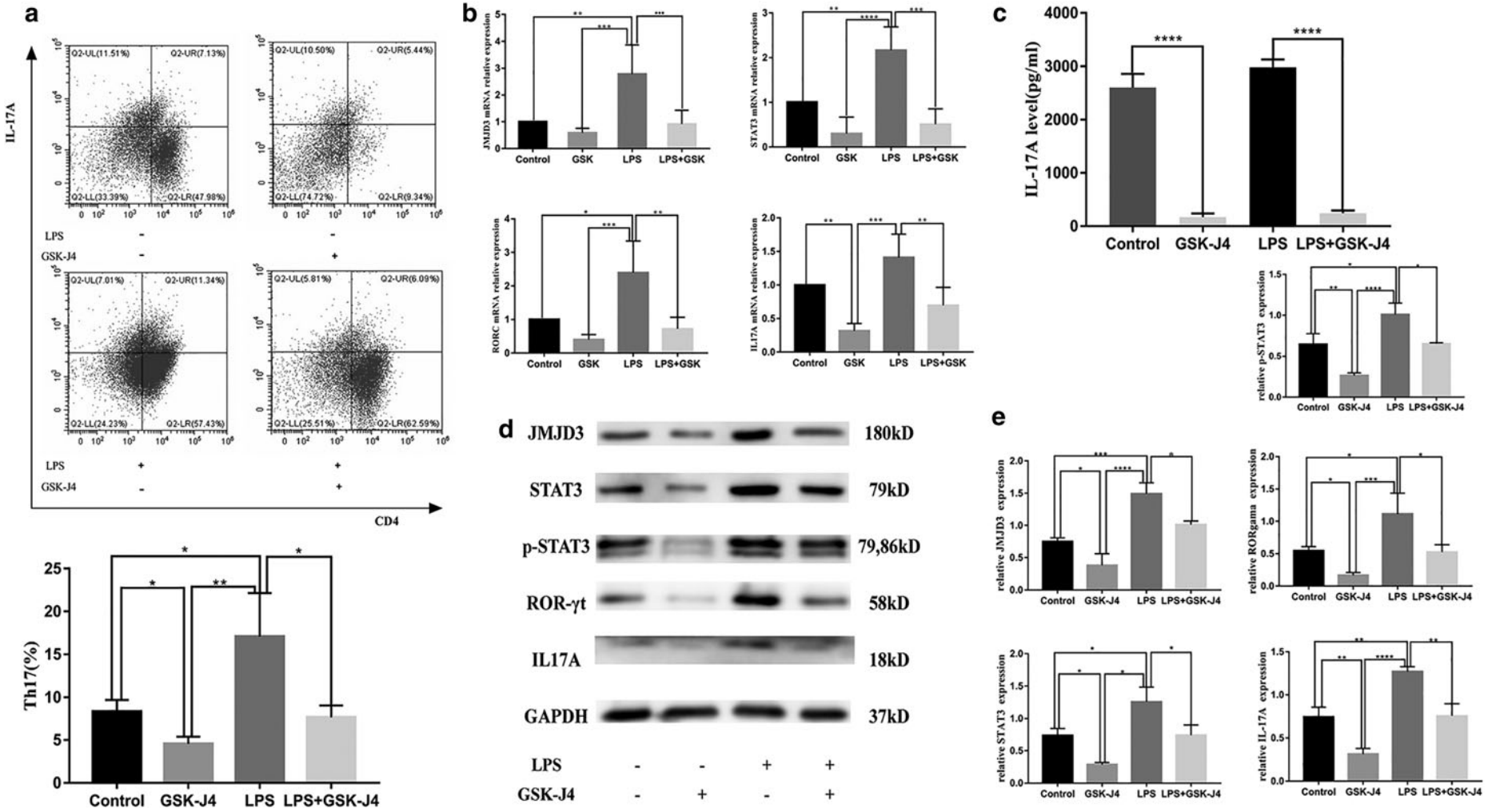

FIG. 4. JMJD3 regulates Th17-cell differentiation induced by Pg-LPS through STAT3. Purified $CD4^+$ T cells were treated with the JMJD3 inhibitor GSK-J4 (80 nM), Pg-LPS (1 μg/mL), or GSK-J4 and Pg-LPS and cultured under the same conditions as the Th17 differentiation system. The cultures were further incubated for 5 days for RNA analysis, FACS analysis, western blotting, and ELISAs. **(a)** The frequency of Th17 cells was significantly decreased after JMJD3 blockade in both the control and LPS groups. **(b)** The mRNA expression of *Jmjd3*, *Stat3*, *Rorc*, *and Il-17a* in the control, GSK-J4, LPS, and LPS+ GSK-J4 groups. **(c)** GSK-J4 significantly reduced the concentration of supernatant IL-17A in both the control and LPS groups. **(d, e)** The protein levels of JMJD3, STAT3, RORγt, and IL-17A in the control, GSK-J4, LPS, and LPS+ GSK-J4 groups. Data are presented as the mean ± SEM of four independent experiments. Significant changes are marked with $*p<0.05$, $**p<0.01$, $***p<0.001$, and $****p<0.0001$.

proved the complicated interaction between JMJD3 and STAT3 (LaMere *et al.*, 2017).

An increasing number of studies have suggested that JMJD3 can interact with coactivators and activate the transcription of target genes as a transcription factor independent of its demethylase activity (Salminen *et al.*, 2014; Zhang *et al.*, 2019b; Ding *et al.*, 2021). Actually, JMJD3 plays an important role in modulating cell-specific pro-inflammatory and anti-inflammatory immune responses, which may also be mediated by H3K27-independent mechanisms (Burchfield *et al.*, 2015). Since there is still a lack of direct evidence to support the role of JMJD3 in regulating the expression of STAT3 by altering the enrichment of H3K27me3 at promoter, the H3K27 demethylation-independent JMJD3-mediated STAT3 gene activation in Th17-cell differentiation process cannot be excluded, therefore further research is required.

It has been reported that the regulation of JMJD3 is highly gene- and context-specific (Burchfield *et al.*, 2015). STAT3 has been proven to bind to the *Jmjd3* promoter in human glioma stem cells (Sherry-Lynes *et al.*, 2017), which is consistent with published genomic data from murine embryonic stem cells (Kidder *et al.*, 2008). The work of Przanowski *et al.* (2014) also indicates that JMJD3 cooperates with STAT1 and STAT3 and acts as their novel target to drive the expression of inflammatory genes in microglia. However, in the present study, JMJD3 and STAT3 blocking assays indicated that JMJD3 had a regulatory effect on STAT3 in the Th17-cell differentiation process with Pg-LPS stimulation, but STAT3 had no regulatory effect on JMJD3.

Consistent with our results, LaMere *et al.* (2017) reported that the expression of STAT3 and its phosphorylation in GSK-J4-treated cells or in JMJD3 knockdown cells were both impaired during $CD4^+$ T cell activation. Therefore, different cell sources and disease states may be important reasons for the discrete function of STAT3 on JMJD3 in different experimental settings. Thus, further studies are required to elucidate the exact mechanism by which JMJD3 regulates STAT3 in the context of Th17 cells.

Conclusion

In this study, we demonstrated that JMJD3 was induced in $CD4^+$ T cells stimulated with Pg-LPS under Th17-cell-polarizing conditions *in vitro* and further mediated STAT3 to enhance the expression of the key transcription factor RORγt, resulting in promoted Th17-cell differentiation. Nevertheless, the detailed mechanism still needs to be further investigated, as the effect of JMJD3 on STAT3 is complicated and not completely understood.

Authors' Contributions

L.G. and C.Z. conceptualized and designed the original idea. D.H. performed the experiments, acquired the figures, and revised the first draft. D.H., C.Z., and P.W. performed the literature search and analysis and finished the draft. X.L., L.G., and C.Z. provided valuable feedback and approved the final draft.

Authorship Confirmation Statement

All authors have read and approved the final article, and agree to submit it for consideration for publication in your journal.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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Address correspondence to:
Chuanjiang Zhao, MD, PhD
Department of Periodontology
Hospital of Stomatology
Sun Yat-sen University
Guangzhou City 510055
P.R. China

E-mail: zhaochj@mail.sysu.edu.cn

Li Gao, MD, PhD
Department of Periodontology
Hospital of Stomatology
Sun Yat-sen University
Guangzhou City 510055
P.R. China

E-mail: gaol7@mail.sysu.edu.cn

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