Differential roles of *Porphyromonas gingivalis* lipopolysaccharide and *Escherichia coli* lipopolysaccharide in maturation and antigen-presenting functions of dentritic cells

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Abstract. – OBJECTIVE: The aim of this research is to study the roles of *Porphyromonas gingivalis*-lipopolysaccharide (*P. gingivalis*-LPS) and *Escherichia coli*-lipopolysaccharide (*E. coli*-LPS) on maturation and antigen-presenting functions of dendritic cells (DCs), and to provide experimental evidences to explore the possible mechanism of DCs in periodontitis.

MATERIALS AND METHODS: Flow cytometry was used to detect CD11c, MHC-II, CD80, CD86 and CD40 expression on DCs which were stimulated by *P. gingivalis*-LPS or *E. coli*-LPS and ELISA was used to detect IL-12, IFN-γ, IL-10 and IL-13 secreted by DCs. CCK8 was used to assay CD4+T cells proliferation after co-cultured with DCs stimulated by *P. gingivalis*-LPS or *E. coli*-LPS and ELISA was used to detect IL-2, IFN-γ, IL-10 and IL-13 secreted by T cells. TLR4 inhibitor (polymyxin B) or TLR2 and TLR4 inhibitor (OxPA-PC) was added to *P. gingivalis*-LPS group and *E. coli*-LPS group to observe the effects of these two TLR inhibitors on the maturation and antigen-presenting functions of DCs.

RESULTS: The capacity of *P. gingivalis*-LPS to stimulate DCs maturation was similar to that of *E. coli*-LPS. The amount of IL-12 and IFN-γ secreted by DCs in *P. gingivalis*-LPS group was significantly lower than that of *E. coli*-LPS group ($p < 0.05$), meanwhile, IL-10 and IL-13 secreted by DCs in *P. gingivalis*-LPS group was significantly higher than that of *E. coli*-LPS group ($p < 0.05$). DCs stimulated by both *P. gingivalis*-LPS and *E. coli*-LPS could promote the proliferation of CD4+T cells. The amount of IL-2 and IFN-γ secreted by T cells stimulated by DCs in *P. gingivalis*-LPS group was significantly lower than that of *E. coli*-LPS group ($p < 0.05$), meanwhile, IL-10 secreted by T cells stimulated by DCs in *P. gingivalis*-LPS group was significantly higher than that of *E. coli*-LPS group ($p < 0.05$). When TLR4 inhibitor was added to *E. coli*-LPS group, maturation and antigen-presenting functions of DCs were significantly inhibited. When TLR4 inhibitor was added to *P. gingivalis*-LPS group, maturation and antigen-presenting functions of DCs were not significantly inhibited. When TLR2 and TLR4 inhibitor was added to *P. gingivalis*-LPS group, maturation and antigen-presenting functions of DCs were significantly inhibited.

CONCLUSIONS: *P. gingivalis*-LPS could prime DCs maturation and antigen-presenting functions. DCs stimulated by *P. gingivalis*-LPS are prone to induce a stronger Th2 cell responses while DCs stimulated by *E. coli*-LPS are prone to induce a stronger Th1 cell responses. *P. gingivalis*-LPS triggers DCs through TLR2 pathway while *E. coli*-LPS triggers DCs through TLR4 pathway.

Key Words:
   Dendritic cells, *P. gingivalis*-LPS, *E. coli*-LPS, Maturation, Antigen-presenting.

Introduction

Porphyromonas gingivalis (*P. gingivalis*) is a kind of Gram-negative anaerobic rod-shaped bacteria and it is a pathogenic microorganism in the development of chronic periodontitis (CP)[1]. The pathogenic components of *P. gingivalis* include: lipopolysaccharide (LPS), capsular polysaccharide, fimbrial proteins and gingipains[2]. *P. gingivalis*-LPS is one of the main pathogenic factors to periodontitis and *P. gingivalis*-LPS can elicit various types of immune and inflammatory responses in periodontitis[3].

Dendritic cells (DCs) are widely distributed in tissues and organs and they are the body's most efficient antigen-presenting cells[4]. Immature DCs uptake antigens and mature DCs present antigens to naive T-lymphocytes, then stimulate naive T cells to differentiate to effector T cells[5], thus, DCs are important key mediators between innate

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and acquired immune responses[6]. Priming DCs with microbial compounds up-regulates the expression of costimulatory molecules and the production of proinflammatory cytokines, which drives T-helper (Th) cells to differentiate to Th1 or Th2 cells[7]. The first step of the process is that DCs identify various antigenic materials (including pathogenic microorganisms ingredients) by pattern recognition receptors (PRRs), so PRRs and their ligands have important roles in DCs maturation and antigen-presenting function[8,9].

Now, researchers have clearly recognized that the majority of periodontal tissue damage is the host immune responses against pathogen infection, and the host immune system plays a crucial role in the pathogenesis of periodontitis. Previous studies[10] revealed that CD4+T cells infiltrated into gingival tissue and expressed the mRNA of IL-10, IFN-γ, IL-6 and IL-13 which were associated with periodontal diseases. These results suggested that T cells might be actively involved in the immunopathogenesis of periodontitis. How do these CD4+T cells migrate to the gingival tissue? Do DCs play functional roles in the process? What are the interactions between T cells and DCs during periodontitis? These questions remain to be elucidated.

Previous study[11] found that there were DCs in the gingival epithelium tissues of patients with periodontitis. However, the exact mechanisms of these DCs in the development of periodontitis is not clear. *P. gingivalis* has been recognized to be a causal factor in CP and LPS is the main pathogenic component of *P. gingivalis*. So, in this study we evaluated the effects of *P. gingivalis*-LPS on SD rats' marrow-derived DCs and analyzed the characteristics and the ability to prime T cells of these DCs. It is well accepted that *E. coli*-LPS have the preounced ability to promote DCs maturation and antigen-presenting functions. Being compared with *E. coli*-LPS, how about *P. gingivalis*-LPS? This problem prompted us to compare different roles of *P. gingivalis*-LPS and *E. coli*-LPS in maturation and antigen-presenting functions of DCs to elucidate the possible mechanism of DCs in the process of periodontitis.

Materials and Methods

Rats

6-8 week male SD rats were bred and maintained in a specific pathogen-free (SPF) facility in Medical Animal Experimental Center of Nanjing Command, PLA. All protocols of animal studies were approved by Nanjing University Committee on Use and Care of Animals.

Reagents

Roswell Park Memorial Institute (RPMI) 1640 medium, heat-inactivated fetal bovine serum, streptomycin and penicillin (all from HyClone, South Logan, UT, USA), recombinant rat granulocyte macrophage colony-stimulating factor (rrGM-CSF), recombinant rat interleukin-4 (rrIL-4) (both from R&D, McKinley Place, Minneapolis, MN, USA), standard LPS from *P. gingivalis* (Invitrogen, San Diego, CA, USA), standard LPS from *E. coli 0111:B4* (Sigma-Aldrich, St. Louis, CO, USA), TLR4 signaling inhibitors-Polymyxin B (PmB), LR2 and TLR4 signaling inhibitors – OxPAPC (both from Invivogen, San Diego, CA, USA), phycoerythrin (PE)-conjugated anti-CD11c, allophycocyanin (APC)-conjugated anti-MHCII, fluorescein isothiocyanate (FITC)-conjugated anti-CD86, APC-conjugated anti-CD80 and FITC-conjugated anti-CD40 anti-rat monoclonal antibody (all from eBioscience, San Diego, CA, USA), interferon-γ (IFN-γ), IL-12, IL-2, IL-10 and IL-13 rat ELISA kits (R&D Systems, Minneapolis, MN, USA), CD4 anti-rat monoclonal antibody beads (Miltenyi Biotec, Bergisch Gladbach, Germany), Cell Counting Kit-8 (Dojindo, Mashikimachi, Kumamoto, Japan).

Cell Culture

Total bone marrow cells were freshly isolated from rats' tibias, femurs and humerus. Cells were cultured in complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin, supplemented with 10 ng/ml rrGM-CSF and 1 ng/ml rrIL-4 to generate DCs. Cells were incubated at 37°C in a humidified 5% CO_2 incubator. Culture media were half changed every other day in which all the reagents concentrations were kept and suspension cells were collected. After 6 days culture, the harvested cells were 2×106 per well in 6-well plates and randomly divided into seven groups: control group, E. coli-LPS group, *P. gingivalis*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+OxPAPC group, *P. gingivalis*-LPS+PmB group and *P. gingivalis*-LPS+OxPAPC group. Respectively, nothing, *E. coli*-LPS, *P. gingivalis*-LPS, *E. coli*-LPS+PmB, *E. coli*-LPS+ OxPAPC, *P. gingivalis*-LPS+PmB or *P. gingivalis*-LPS+OxPAPC was

added to the culture media. Each reagent concentration was as following: *E. coli*-LPS: 100 ng/ml; *P. gingivalis*-LPS: 100 ng/ml; PmB: 30 μg/ml; OxPAPC: 30 μg/ml. PmB or OxPAPC was added 30 minutes before *E. coli*-LPS or *P. gingivalis*-LPS was added to the medium. After 48 hours, loosely adhered cells and cells suspension were collected CD11c expressed on cell surface was detected by flow cytometry. The results showed that CD11c+ cells population was > 90%, then these cells were harvested as DCs.

Cells Surface Molecules Expressions Analysis

On the eighth cell culture day, the cells of each group were collected, counted and washed with PBS, then resuspended to 5.0×10^5/ml and transferred to flow tubes. According to the groups, PE-CD11c, APC-MHCII, FITC-CD86, APC-CD80 and FITC-CD40 anti-rat monoclonal antibodies were added to the flow tubes according to the manufacturer instructions to detect double-labeled of CD11c+MHCII+, CD11c+CD80+, CD11c+CD86+, CD11c+ CD40+ cells by flow cytometry. Isotype-matched control antibodies were added to the negative control tubes of each group. Cells were incubated at 4°C in the dark for 30 minutes, then washed twice with PBS and fully resuspended. The expressions of surface molecules on DCs were detected by FACSCalibur. The raw datas obtained in experiments were processed by FlowJo7.6.1 software to analyze the percentage of positive cells in each group. The assays were performed in triplicate for each independent experiment.

Cytokines Detection

On the eighth cell culture day, to measure cytokines secreted by DCs, the supernatants of each group were collected. The levels of the cytokines: IFN-γ, IL-12, IL-10 and IL-13 in the culture supernatants were measured by ELISA according to the manufacturer's protocol and the absorbance was read at 450 nm. The assays were performed in triplicate for each independent experiment.

Mixed Lymphocyte Reaction (MLR) and Allogeneic T-cell Proliferation and Activation assay

To determine the capacity of DCs to prime allogeneic CD4+T cells, SD rats' splenocytes were obtained and CD4+T cells were further purified by using a CD4+T cell magnetic bead sorting kit according to the manufacturer's instructions. In all of the experiments, the isolated cells were 90-95% CD4+ as determined by staining with PE-conjugated anti-CD4 antibody followed by flow cytometry (not shown). DCs of each group, as described above, were cocultured with 2×10^5 CD4+T cells at the ratio of 1:10, in triplicates, in 96-well plate to ensure efficient DC/T cells contact. RPMI-1640 medium supplemented with 10% heat-inactivated FBS and 100 U/ml penicillin and 100 μg/ml streptomycin in a total volume of 200 μl was added to each well. Cells were placed at 37°C, humidified incubator with 5% CO_2 atmosphere for 72 hours. 4 hours before the end, 10 μl Cell Counting Kit-8 (CCK-8) was added to each well according to the manufacturer's instructions, and incubated for 4 hours, then the absorbance was read at 450 nm to measure allogeneic T-cells proliferation. The assays was repeated three times and the mean results were adopted.

As the method described above, DCs of each group were cocultured with CD4+T cells at the ratio of 1:10 for 72 hours. Cytokines of IFN-γ, IL-2, IL-10 and IL-13 in the supernatant were measured in triplicate by ELISA, following the manufacturer's instructions. The absorbance was read at 450 nm.

Statistical Analysis

Data were expressed as mean ± SD (standard deviation). Two-sided Student *t*-test was used to determine statistically significant differences between two groups. The one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests was used to analyze differences among multiple groups. Differences between groups were considered statistically significant when the *p* value was < 0.05.

Results

Both E. coli-LPS and P. gingivalis-LPS Could Simulate DCs Maturation

We analyzed the levels of surface molecules expression to detect the effects of DCs maturation simulated by *P. gingivalis*-LPS or *E. coli*-LPS by means of flow cytometry. CD11c+MHCII+, CD11c+CD80+, CD11c+CD86+ and CD11c+CD40+ cells in each group were shown in Figure 1 to Figure 4. As results in histogram shown in Figure 5A, both *P. gingivalis*-LPS (100 ng/ml) and *E. coli*-LPS (100 ng/ml)
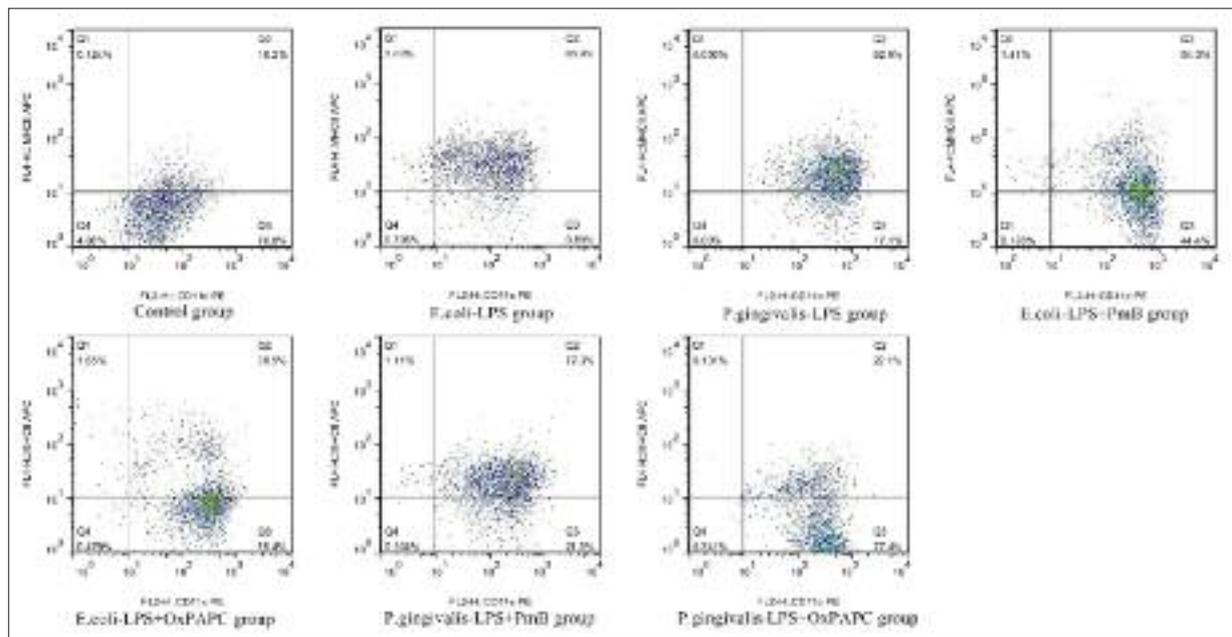
Figure 1. CD11c+MHC‖+ double positive cells ratio on DCs surface of the Control group, *E. coli*-LPS group, *P. gingivalis*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+OxPAPC group, *P. gingivalis*-LPS+PmB group and *P. gingivalis*-LPS+Ox-PAPC group.

significantly up-regulated the levels of MHC-II, CD80, CD86, CD40 expressed on DCs which were higher than the control group ($p < 0.05$). There was no significant difference between the *E. coli*-LPS group and *P. gingivalis*-LPS group ($p > 0.05$). The above results showed that DCs could undergo maturation by the stimulations of both *E. coli*-LPS and *P. gingivalis*-LPS.
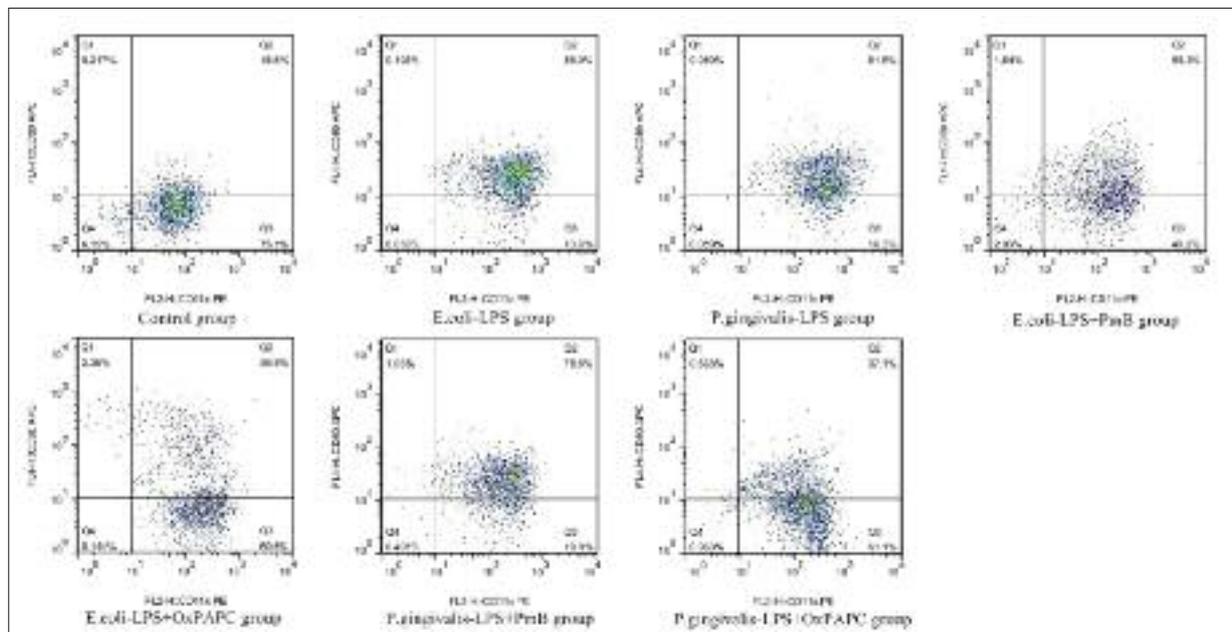

Figure 2. CD11c⁺CD80⁺ double positive cells ratio on DCs surface of the Control group, *E. coli*-LPS group, stimulation on DCs depended mostly on the TLR2 signaling pathway group, *E. coli*-LPS+PmB group, *E. coli*-LPS+OxPAPC group, *P. gingivalis*-LPS+PmB group and *P. gingivalis*-LPS+OxPAPC group.
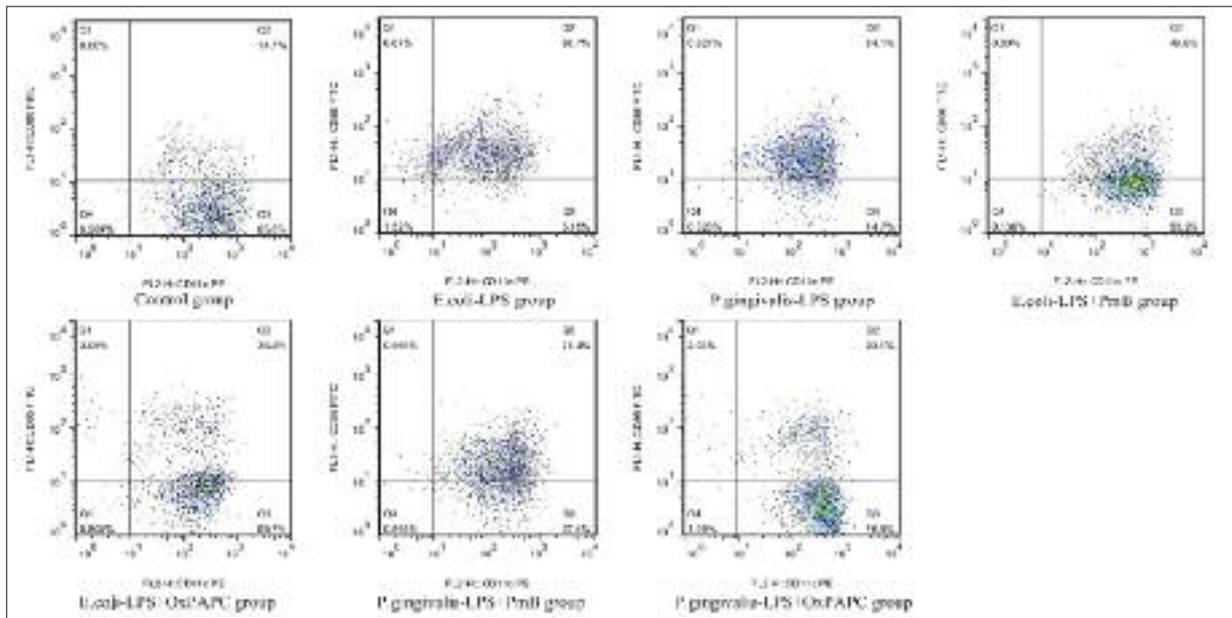
Figure 3. CD11c⁺CD86⁺ double positive cells ratio on DCs surface of the Control group, *E. coli*-LPS group, *P. gingivalis*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+OxPAPC group, *P. gingivalis*-LPS+PmB group and *P. gingivalis*-LPS+Ox-PAPC group.

E. coli-LPS and *P. gingivalis*-LPS had Differences to Simulate DCs Maturation in the Presence of TLR4 Signaling-Inhibitors or TLR2 and TLR4 Signaling-Inhibitors

CD11c⁺MHCII⁺, CD11c⁺CD80⁺, CD11c⁺CD86⁺ and CD11c⁺CD40⁺ cells in *E. coli*-LPS+TLR4 signaling-inhibitors (polymyxin B, PmB) group and *E. coli*-LPS+TLR2 and TLR4 signaling- inhibitors (OxPAPC) group were shown in Figure 1 to Figure 4. As results in histogram shown in Figure 5-B, in the condition that there were PmB in the *E. coli*-LPS culture milieu, MHCII, CD80, CD86 and CD40 expressed on DCs be-
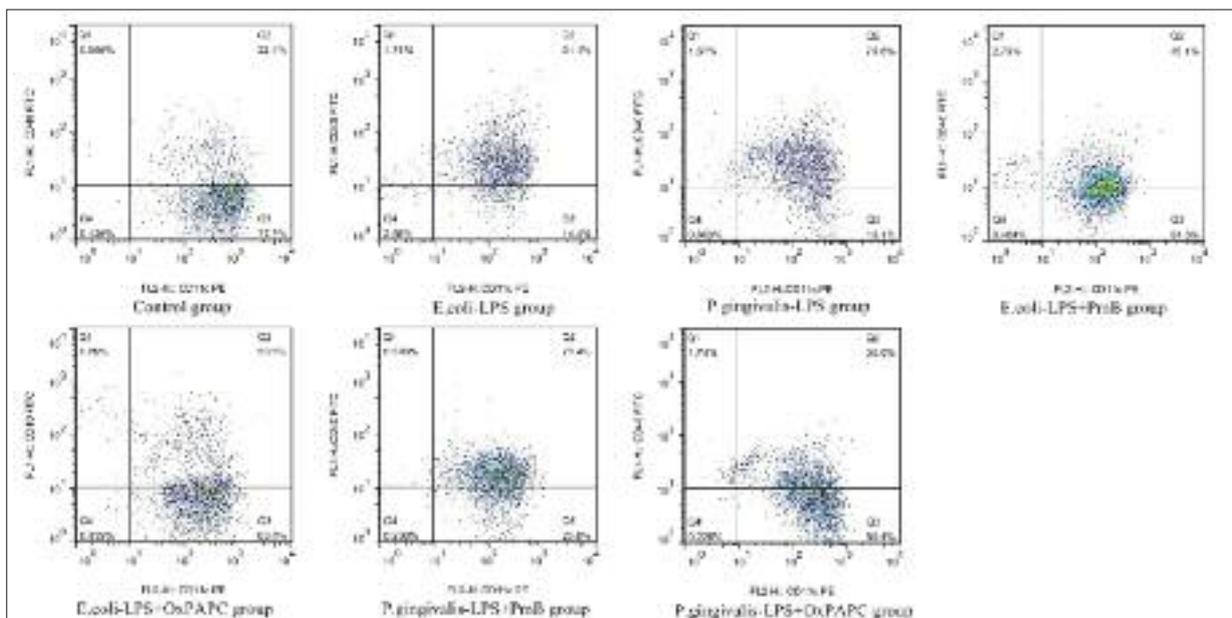

Figure 4. CD11c⁺CD40⁺ double positive cells ratio on DCs surface of the Control group, *E. coli*-LPS group, *P. gingivalis*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+OxPAPC group, P. gingivalis-LPS+PmB group and *P. gingivalis*-LPS+OxPAPC group.

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came much weaker. When OxPAPC were added to the *E. coli*-LPS culture milieu, these molecules expressed on DCs also decreased deeply. MHC-II, CD80, CD86 and CD40 expressed on DCs in both *E. coli*-LPS+PmB group and *E. coli*-LPS + OxPAPC group were significantly lower than *E. coli*-LPS group ($p < 0.05$) and there was no significant difference between *E. coli*-LPS+PmB group and *E. coli*-LPS+OxPAPC group ($p > 0.05$). These results indicated that both TLR4 signaling-inhibitors and TLR2 and TLR4 signaling-inhibitors could inhibit DCs maturation stimulated by *E. coli*-LPS. CD11c^{+}MHCII^{+}, CD11c^{+}CD80^{+}, CD11c^{+}CD86^{+} and CD11c^{+}CD40^{+} cells in *P. gingivalis*-LPS+PmB group and *P. gingivalis*-LPS+OxPAPC group were shown in Figure 1 to Figure 4. As results in histogram shown in Figure 5-C, in the condition that there were PmB in the *P. gingivalis*-LPS culture milieu, there was no apparent influence to MHCII, CD80, CD86 and CD40 expressions on DCs. In the condition that there was OxPAPC in the milieu, MHCII, CD80, CD86 and CD40 expressed on DCs significantly decreased. These molecules expressed in *P. gingivalis*-LPS+OxPAPC group were significantly lower than *P. gingivalis*-LPS group and *P. gingivalis*-LPS+PmB group ($p < 0.05$); and the *P. gingivalis*-LPS+PmB group had no significant difference between *P. gingivalis*-LPS group ($p > 0.05$). The results indicated that TLR4 signaling-inhibitors couldn't suppress DCs maturation stimulated by *P. gingivalis*-LPS, while TLR2 and TLR4 signaling-inhibitors could significantly inhibit DCs maturation stimulated by *P. gingivalis*-LPS. All the above results illustrated that *E. coli*-LPS stimulation on DCs depended mostly on the TLR4 signaling pathway while *P. gingivalis*-LPS stimulation on DCs depended mostly on the TLR2 signaling pathway.

Differential Cytokines Secreted by DCs in Response to *P. gingivalis*-LPS and *E. coli*-LPS

As the cytokines produced by DCs affect subsequent DCs function, we investigated whether *P. gingivalis*-LPS and *E. coli*-LPS are different on the aspect of inducing DCs to secrete cytokines. We analyzed the culture supernatants from DCs of *P. gingivalis*-LPS group and *E. coli*-LPS group. The productions of IFN-γ, IL-12, IL-10 and IL-13 were examined by ELISA. The results were shown in Figure 6A which indicated that IL-12 and IFN-γ secreted by DCs in *P. gingivalis*-LPS group were lower than those in *E. coli*-LPS group ($p < 0.05$), meanwhile, IL-10 and IL-13 secreted by DCs in *P. gingivalis*-LPS group were higher than those in *E. coli*-LPS group ($p < 0.05$). All these findings demonstrated that *E. coli*-LPS were potent to induce a Th1-biased response primed by DCs and *P. gingivalis*-LPS was potent to induce a Th2-biased response primed by DCs.

In the condition that there was either PmB or OxPAPC in *E. coli*-LPS culture milieu, IL-12, IFN-γ, IL-10 and IL-13 secreted by DCs significantly decreased ($p < 0.05$) and there was no significant difference between *E. coli*-LPS+PmB group and *E. coli*-LPS+OxPAPC group ($p > 0.05$) (Figure 6B). When PmB was added to *P. gingivalis*-LPS group, there was no evident impact in IL-12, IFN-γ, IL-10 and IL-13 secretion ($p > 0.05$). When OxPAPC was added to *P. gingivalis*-LPS group, IL-12, IFN-γ, IL-10 and IL-13 secreted by DCs were all significantly decreased.
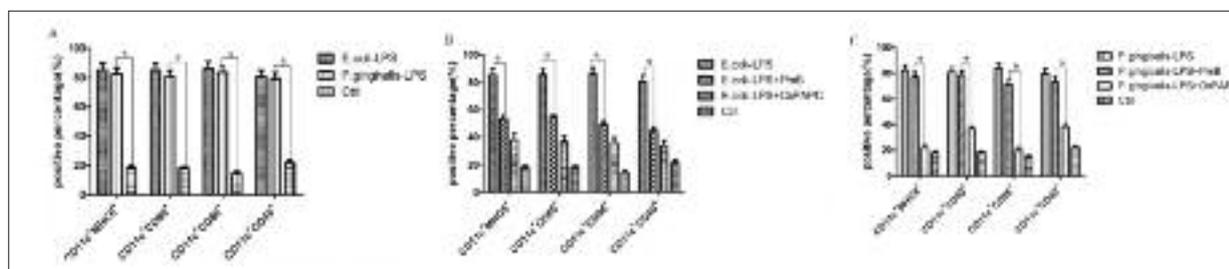

Figure 5. Mean percentage of CD11c, MHCII, CD80, CD86 and CD40 expression on DCs by flow cytometry to detect the effects of *P. gingivalis*-LPS or *E. coli*-LPS, with or without TLR4 inhibitor (PmB) or TLR2/TLR4 inhibitor (OxPAPC), on the maturation of DCs. **A,** The comparison among *P. gingivalis*-LPS group, *E. coli*-LPS group and the Control group. **B,** The comparison among *E. coli*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+ OxPAPC group and the Control group. **C,** The comparison among *P. gingivalis*-LPS group, *P. gingivalis*-LPS+PmB group, *P. gingivalis*-LPS+OxPAPC group and the Control group. *Indicates $p < 0.05$ significant difference between groups.
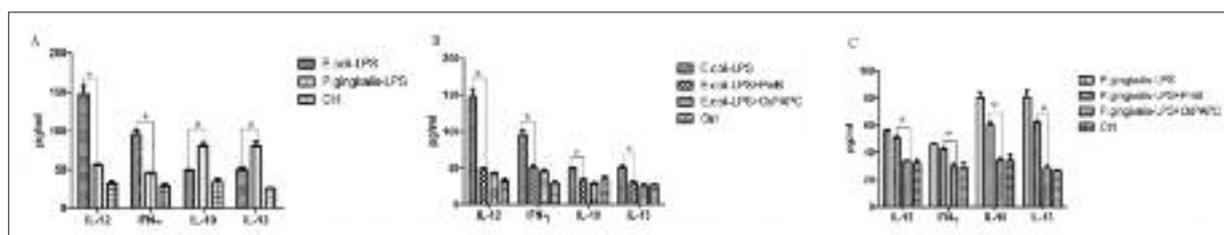
Figure 6. Mean amount of IL-12, IFN-γ, IL-10 and IL-13 secreted by DCs stimulated by *P. gingivalis*-LPS or *E. coli*-LPS, with or without TLR4 inhibitor (PmB) or TLR2/TLR4 inhibitor (OxPAPC). *A,* The comparison among *P. gingivalis*-LPS group, *E. coli*-LPS group and the Control group. *B,* The comparison among *E. coli*-LPS group, E. coli-LPS+PmB group, *E. coli*-LPS+OxPAPC group and the Control group. *C,* The comparison among *P. gingivalis*-LPS group, P. gingivalis-LPS+PmB group, *P. gingivalis*-LPS+OxPAPC group and the Control group. *Indicates $p < 0.05$ significant difference between groups.

The amounts of all these cytokines in the *P. gingivalis*-LPS+OxPAPC group were also statistically lower than those in *P. gingivalis*-LPS+PmB group ($p < 0.05$) (Figure 6C). These results were in accordance with the flow cytometry results that *E. coli*-LPS stimulation on DCs depended on the TLR4 signaling pathway while *P. gingivalis*-LPS stimulation on DCs depended on the TLR2 signaling pathway.

Effects of T-cell Priming by P. gingivalis-LPS Stimulated DCs were Different from Those by E. coli-LPS

The results of CCK8 indicated that in the aspect to prime CD4+T cells proliferation, there was no significant difference between DCs in *P. gingivalis*-LPS group and DCs in *E. coli*-LPS group ($p > 0.05$). Either PmB or OxPAPC added to *E. coli*-LPS group could significantly inhibit the T cell proliferation ($p < 0.05$) and there was no statistical difference between *E. coli*-LPS+PmB group and *E.coli*-LPS+OxPAPC group ($p > 0.05$). When PmB was added to *P. gingivalis*-LPS group, there was no significant decrease in T cell proliferation primed by DCs ($p > 0.05$). When OxPAPC was added to *P. gingivalis*-LPS group, T cell proliferation primed by DCs was significantly lower than that of the *P. gingivalis*-LPS group and *P. gingivalis* LPS+PmB group ($p < 0.05$) (Figure 7).

The results of cytokines secreted by activated T cells were showed as Figure 9. The amounts of IL-2 and IFN-γ secreted by T cells in the *P. gingivalis*-LPS group were lower than those in the *E. coli*-LPS group ($p < 0.05$) while IL-10 secreted by T cells in the *P. gingivalis*-LPS group was higher than that in the *E. coli*-LPS group ($p < 0.05$) and IL-13 had no statistical differences between the two groups ($p > 0.05$) (Figure 8A).

These results were in accordance with the differential cytokines secreted by DCs in response to *E. coli*-LPS and *P. gingivalis*-LPS. They revealed that DCs stimulated by *E. coli*-LPS had the tendency to prime Th0 cells to Th1 cells while DCs stimulated by *P. gingivalis*-LPS had the tendency to prime Th0 cells to Th2 cells.

When either PmB or OxPAPC was added to the *E. coli*-LPS group, IL-2, IFN-γ, IL-10 and IL-13 secreted by T cells significantly decreased ($p < 0.05$) and there was no significant difference between *E. coli*-LPS+PmB group and *E. coli*-LPS+OxPAPC group ($p > 0.05$) (Figure 8B). When PmB was added to the *P. gingivalis*-LPS group, there was no significant difference in the amounts of IL-2, IFN-γ, IL-10 and IL-13 secreted by T cells ($p > 0.05$). When OxPAPC was added to the *P. gingivalis*-LPS group, the
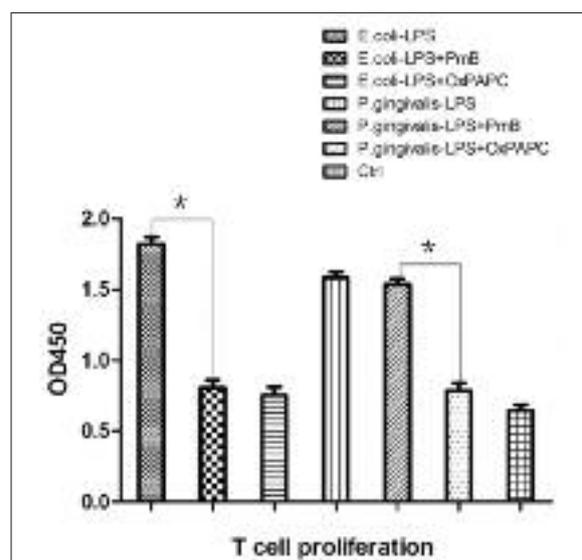

Figure 7. Proliferation of T cells in MLR of each group by assays of CCK-8.
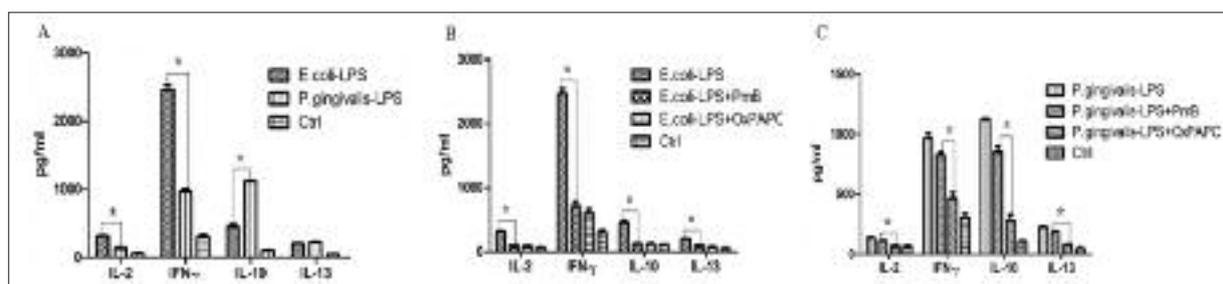
Figure 8. Mean amount of IL-2, IFN-γ, IL-10 and IL-13 secreted by T cells co-cultured with DCs stimulated by *P. gingivalis*-LPS or *E. coli*-LPS, with or without TLR4 inhibitor (PmB) or TLR2/TLR4 inhibitor (OxPAPC). **A,** The comparison among *P. gingivalis*-LPS group, *E. coli*- LPS group and the Control group. **B,** The comparison among *E. coli*-LPS group, *E. coli*-LPS+PmB group, *E. coli*-LPS+ OxPAPC group and the Control group. **C,** The comparison among *P. gingivalis*-LPS group, *P. gingivalis*-LPS+PmB group, *P. gingivalis*-LPS+OxPAPC group and the Control group. *Indicates *p* < 0.05 significant difference between groups.

amounts of IL-2, IFN-γ, IL-10 and IL-13 secreted by T cells were significantly lower than those of the *P. gingivalis*-LPS group and the *P. gingivalis*-LPS+PmB group (*p* < 0.05) (Figure 8C). These results were consistent with the above results that *E. coli*-LPS stimulation on DCs depended on the TLR4 signaling pathway while *P. gingivalis*-LPS stimulation on DCs depended on the TLR2 signaling pathway.

Discussion

As has been shown in these studies, that although both being LPS, there are differential roles between *P. gingivalis*-LPS and *E. coli*-LPS in different cell lines[12,13]. Kirikae[14] declared that *P. gingivalis*-LPS exhibited activity in C3H/HeJ mice macrophages, which were deficient for TLR4. Jones[15] pointed out that *E. coli*-LPS stimulated gingival fibroblasts to produce more IL-6, iNOS, MCP-1 and *P. gingivalis*-LPS stimulated macrophages to produce more IL-6, IL-1β and MCP-1 *in vitro*. Barksby et al[16] declared that in monocytes, *E. coli*-LPS majorly activates TLR-4 while *P. gingivalis*-LPS activates TLR-2. Jotwani et al[17] indicated that both TLR2 and TLR4 are required for monocyte-derived dendritic cells (MoDC) maturation by *P. gingivalis*-LPS. Diya et al[18] stated that *P. gingivalis*-LPS differs from *E. coli*-LPS in its signaling pathway in THP-1 cells, and that the TLR2-JNK pathway might play a significant role in *P. gingivalis*-LPS induced chronic inflammatory periodontal diseases. Sun et al[19] drew the conclusion that in the aspect of endotoxin tolerance, *P. gingivalis*-LPS triggers TLR2 and *E. coli*-LPS triggers TLR4 in THP-1 cells. All these differences might rely on

that there were unique and heterogenous chemical structures in hydrophilic bisphosphate frames of lipid A between *P. gingivalis*-LPS and *E. coli*-LPS and these structural differences led to differences between *P. gingivalis*-LPS and *E. coli*-LPS in affinity to TLRs[20].

Previous studies[21] demonstrated that Langerhans cells (LCs) and DCs were present in the epithelium and *lamina propria* of healthy gingiva and during gingivitis and periodontitis. DCs played crucial roles to connect innate and adaptive immunity against infective microorganism and both the distribution and the phenotype changes of DCs may regulate immune responses in periodontal tissues and lead to the periodontal destruction[22]. What interested us was that being the main periodontal pathogenic composition, what was the role of *P. gingivalis*-LPS in the interaction with DCs during the occurrence and development of periodontitis?

In the present work, DCs were co-cultured with *P. gingivalis*-LPS or *E. coli*-LPS *in vitro* to evaluate the differential roles of *P. gingivalis*-LPS and *E. coli*-LPS in maturation and function of DCs. In recently researches[23-25], LPS ranging between 10 to 200 ng/ml were used to investigate its effects on various cell types. Hence, an intermediate dose of *P. gingivalis*-LPS (100 ng/ml) and *E. coli*-LPS (100 ng/ml) was used in our study.

Polymyxin B (PmB) is a cyclic cationic peptide antibiotic produced from *Paenibacillus polymixa* and PmB has been proven to be a TLR4 inhibitor to the activation induced by LPS[26,27]. LPS is a main component of the G- bacteria cell wall and Lipid A is an active toxic ingredient of LPS. Lipid A is anionic and cationic PmB binds to Lipid A to inhibit the bi-

ological effects of LPS. OxPAPC is oxidated from 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (PAPC) and OxPAPC is a mixture of oxidated phospholipid with full-length sn-2 and its residue fragments. It has been declared that OxPAPC is TLR2 and TLR4 inhibitor for OxPAPC possesses the capacity to inhibit bacterial lipopeptides and LPS signal[28]. OxPAPC compete with CD14, LPS-binding protein (LBP) and myeloid differential protein 2 (MD2), the accessory proteins that interact with bacterial lipids, to interfere the formation of the LPS receptor complex, thereby, to block the signal transduction of TLR2 and TLR4[29].

Our findings showed that when PmB was added to *E. coli*-LPS group, DCs maturation, inflammatory cytokines secretion and the subsequent CD4+T cells proliferation and inflammatory cytokines secreted by T cells were all significantly inhibited. These results indicated that for DCs, *E. coli*-LPS was their TLR4 ligand. When PmB was added to *P. gingivalis*-LPS group, DCs maturation, inflammatory cytokines secretion and the subsequent CD4+T cells proliferation and inflammatory cytokines secreted by T cells were not significantly inhibited. When OxPAPC was added to *P. gingivalis*-LPS group, DCs maturation, inflammatory cytokines secretion and the subsequent CD4+T cells proliferation and inflammatory cytokines secreted by T cells were all significantly inhibited. These results indicated that for DCs, *P. gingivalis*-LPS were their TLR2 ligand.

Previous authors[30] indicated that incubation with *E. coli*-LPS induced high levels of proinflammatory cytokines from DCs and strong Th1 responses; however, DCs stimulated by *P. gingivalis*-LPS produced low levels of inflammatory cytokines and a skewed Th2 immune response[31,32]. In our studies, we observed that DCs stimulated by *P. gingivalis*-LPS secreted less IFN-γ and IL-12 than DCs stimulated by *E. coli*-LPS while IL-10 and IL-13 secreted by DCs in *P. gingivalis*-LPS group were lower than those in *E. coli*-LPS group. Subsequent MLR showed that DCs in *P. gingivalis*-LPS group to stimulate T cells secreting IFN-γ and IL-2 was weaker than those in *E. coli*-LPS group and in the aspect of IL-10, *P. gingivalis*-LPS group was higher than *E. coli*-LPS group. These results suggested that DCs stimulated by *P. gingivalis*-LPS had the potential to promote Th2 immune responses more prominently than DCs stimulated by *E. coli*-LPS.

Conclusions

The present report explored DCs immune responses induced by *P. gingivalis*-LPS and suggested that one of the potent mechanisms of periodontitis might be the alteration of DC functions stimulated by *P. gingivalis*-LPS. Besides, our study revealed the differential roles of *P. gingivalis*-LPS and *E. coli*-LPS in maturation and antigen-presenting functions of DCs. These results may help to further elucidate the possible mechanism of DCs in the occurrence and development of periodontitis.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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