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RESEARCH ARTICLE

Porphyromonas gingivalis Lipopolysaccharide-Induced B Cell Differentiation by Toll-like Receptors 2 and 4

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Abstract: ***Background*:** *Porphyromonas gingivalis* (*P. gingivalis*) is a pathogenic bacterium widely present in subgingival plaques of patients with periodontitis. It induces periodontitis with bone loss as its main feature by changing the number and composition of symbiotic microorganisms, as well as inducing the natural immune response of the host. However, the mechanism of the latter remains unclear.

***Objective*:** This study aims to investigate the effect of *P. gingivalis* lipopolysaccharide (LPS) on regulatory B cells (Breg) in the occurrence and development of periodontitis.

***Methods*:** We detected the mRNA levels of IL-10 in B cells under the stimulation of *P. gingivalis* LPS and/or *E. coli* LPS, distinguished IL-10-producing cells from different B cell subgroups using flow cytometry. Through toll-like receptor (TLR) knockout mice, the role of TLR2 and TLR4 in this process was also evaluated.

***Results*:** Results showed that *P. gingivalis* stimulated B cells to produce IL-10 *via* TLR2/4. CD5+B1 subset is the main source of IL-10+Breg cell. Under *P. gingivalis* LPS stimulation, CD5+IgM+CD93-IL-10+B cell subset increased significantly, which was regulated through TLR2/4.

***Conclusion*:** The results of this study provides new insights into the immunopathogenic mechanism of *P. gingivalis*, preliminarily discussed the effect of *P. gingivalis* on the production of Breg, and present a theoretical foundation for subsequent investigations on the occurrence and development of periodontitis.

ARTICLE HISTORY

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1. INTRODUCTION

Porphyromonas gingivalis (*P. gingivalis*) can change the number and composition of symbiotic microorganisms by inducing the natural immune response of the host and lead to periodontitis characterized by bone loss [1, 2]. *P. gingivalis* lipopolysaccharide (LPS) is an important part of its induction of the natural immune response.

B cells play an important role in the innate immune response caused by periodontal pathogens. B cells secrete not only specific antibodies to kill pathogenic bacteria but also inflammatory cytokines and chemokines [3] and receptor activator of nuclear factor-kappa B ligand (RANKL) to destroy tissues when bacteria invade [4]. A small number of B cells called regulatory B cells (Bregs) can secrete IL-10 to protect tissues and inhibit the immune response strongly. Breg plays an immunoregulation role in the occurrence and development of autoimmune diseases [5-9], tumors [10, 11], inflammation [12-14], and other diseases [15-18]. Breg presents different phenotypes in various diseases. However, existing studies have not identified phenotypes of Breg.

Toll-like receptor (TLR) is an important molecule for cells to recognize pattern antigen [19], bacterial LPS can stimulate B cells to produce IL-10 through the TLR on the surface of the B cell membrane; the majority of bacterial LPS is the ligand of TLR4 and purified *P. gingivalis* LPS can activate TLR2 and TLR4 pathways [20-22]. The

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simultaneous activation of TLR2 and TLR4 pathways exerts a variety of effects on Breg. Different ligands of the same TLR activate the pathway to varying degrees, and the interaction between TLR2 and TLR4 may be synergistic or inhibitory.

This study aimed to determine whether a special phenotype exists in Breg under *P. gingivalis* LPS stimulation. TLR2 and TLR4 show synergistic or reciprocal inhibition in this process. Under the effect of *P. gingivalis* LPS, whether the LPS of other periodontal symbiosis bacteria can induce Breg through TLR4 and the production of corresponding cytokines will be changed. Understanding the natural immune response induced by *P. gingivalis* can provide insights into the pathogenesis of periodontitis and help select effective targets for prevention and treatment.

2. MATERIALS AND METHODS

2.1. Animals

C57BL/6 mice were bought from Charles River. TLR2 and TLR4 knockout (KO) mice (background C57BL/6) were purchased from Jackson Laboratory. All mice were placed in pathogen-free laminar flow cabinets. Female rats aged 6-8 weeks were tested. This experiment was approved by the Animal Ethics Committee of Peking University.

2.2. B Cell Isolation and Culture

Mice were euthanized in CO_2 chambers, and their spleens were acquired. Single spleen cells were obtained by grinding spleen samples in a steel mesh and then passing them through a 100 μm cell filter. Erythrocytes were broken down with ammonium-chloride-potassium (ACK) lysis buffer (Life Technologies, USA). Spleen cells were resuspended with PBS and passed through a 40 μm cell filter. Splenocyte suspensions were prepared in MACS buffer (PBS/2 mM EDTA/0.5% BSA). B cells were obtained using negative sorting of immunomagnetic beads according to the instructions of the Miltenyi Biotec Kit (containing >98.5% $CD19^+$ cells). Isolated B cells were adjusted to 1×10^6/ml; added to the complete medium IMDM, which includes 10% FCS, 100 U/ml of penicillin, 100 mg/ml of streptomycin, 2 mM of L-glutamine, 2.5 μg/ml of amphotericin B (Hyclone, Thermo Fisher Scientific, IL), and 50 μM of 2-ME; and incubated in 24-well plates (1 ml/well). Cells were cultured in a humidified incubator at 37°C with 5% CO_2. B cells were cultured in the following groups: control group (without stimulation), 20 μg/ml of *P. gingivalis* LPS (InvivoGen, strain ATCC 33277), 20 μg/ml of *E. coli* LPS (Sigma-Aldrich, strain O55:B5), and 20 μg/ml of *P. gingivalis* LPS+20 μg/ml of *E. coli* LPS. B cells were cultured for 7 hours. PMA (50 ng/ml; Sigma), ionomycin (500 ng/ml; Sigma), and monensin (2 mM; eBioscience) were added to the medium at the last 5 hours.

2.3. Flow Cytometry Analysis

Half of the cultured cells were used in the flow cytometry. The following antimouse monoclonal antibodies (mAbs) were used: BV-421-labeled antimouse IgM, PE-labeled antimouse CD1d, BV-510-labeled antimouse CD23, FITC-labeled antimouse CD43, APC-labeled antimouse CD93, PE-Cy7-labeled antimouse CD5 (BD Biosciences), and Alexa Fluor 700-labeled antimouse IL-10. At least 50,000 cells were counted for each sample *via* flow cytometry.

2.4. Real-time PCR

The other half of cultured cells were used for real-time PCR. Total RNA was isolated from cultured B cells using the Purelink RNA Mini Kit (TaKaRa) according to the manufacturer's instructions. The isolated mRNA (0.1 μg each) was reverse-transcribed into cDNA using the SuperScriptII reverse transcription system (TaKaRa) in the presence of random primers. Real-time PCR was carried out in a 20 μl reaction system using SuperScript II Platinum SYBR Green Two-step qRT-PCR Kit (TaKaRa) in an Applied Biosystems 7500/7500 Fast System (Applied Biosystems, USA). The amount of each cDNA sample was 10 ng. Real-time PCR conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 65°C for 10 s, and 72°C for 15 s. Primers are shown in Table **1**.

Table 1. Primers for RT-PCR.

Gene	Forward Sequences	Reverse Sequences
GAPDH	CCCCAGCAAGGACACTGAGCAA	GTGGGTGCAGCGAACTTTATTGATG
IL-4	GGGACGCCATHCACGGAGATG	TGCGAAGCACCTTGGAAGCCC
IL-10	CTCCTAGAGCTGCGGACTGC	GGCCATGCTTCTCTGCCTGGG
IFN-γ	AACCCACAGGTCCAGCGCCA	CACCCCGAATCAGCAGCGACT
TLR2	CCGAATTGCATCACCGGTCAGA	TGGAGCGGCCATCACACACC
TLR4	AGGAGTGCCCCGCTTTCACCT	CCTTCCGGCTCTTGTGGAAGCC
TGF-β	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGGC
p35	TGTCTCCCAAGGTCAGCGT	GTCTTCAGCAGGTTTCGGGA

2.5. Statistics

Data were represented by mean±standard error (SE), and the paired *t*-test was used between the two groups. A *P* value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. *P. gingivalis* LPS Stimulated B Cells to Produce IL-10 *via* TLR2/4

The production of IL-10 in B cells under different stimulation conditions was detected using flow cytometry and RT-PCR. TLR2 and TLR4 KO mice were used to determine the production pathway of IL-10. The flow cytometry analysis showed that the stimulation of B cells *via P. gingivalis* or *E. coli* LPS increases the IL-10 secretion in WT mice compared with that of the control group. *E. coli* LPS+*P. gingivalis* LPS showed no significant difference in IL-10 production in B cells compared with the use of *E. coli* LPS alone (Figure **1**). This phenomenon indicated that the superposition effect is absent when *P. gingivalis* and *E. coli*

LPSs act together (Figure **1A**). The RT-PCR results showed that the production of IL-10 in B cells increases under the stimulation of *P. gingivalis* LPS, *E. coli* LPS, and *E. coli* LPS+*P. gingivalis* LPS (Figure **1B**). The amount of IL-10 stimulated by *P. gingivalis* LPS was not significantly different from that of the control group in TLR2 or TLR4 KO mice. However, TLR2 KO mice produced more IL-10 in B cells than the control group when *E. coli* LPS or *P. gingivalis* LPS+*E. coli* LPS is used as the stimulation. The production of IL-10 in *E. coli* LPS- or *P. gingivalis* LPS+*E. coli* LPS-stimulated B cells were inhibited in TLR4 KO mice (Figure **1A**).

We extracted RNA from B cells to clarify changes in TLR2 and TLR4 pathways during IL-10 production further. The RT-PCR results showed that the expression of TLR2 and TLR4 did not increase in WT mice when *P. gingivalis* or *E. coli* LPS was used alone as the stimulation. However, the increase of TLR2 was significantly different when *P. gingivalis* and *E. coli* LPSs were combined. TLR2 in TLR4 KO mice did not significantly increase when *P. gingivalis* and *E. coli* LPSs were combined (Figure **1B** and **D**, TLR2 KO mice, TLR2 was undetected; TLR4 KO mice, TLR4 was undetected).

3.2. Cytokine Production of B Cells under Different Stimuli

RT-PCR was performed on B cells of WT, TLR2 KO, and TLR4 KO mice under different stimulation conditions to determine whether the B cell production of IL-10 is accompanied by changes in other cytokines as well as that of LPS-related cytokines of other periodontal symbiosis bacteria changes under the action of *P. gingivalis* LPS. The results showed that IL-4, IFN-γ, and TGF-β did not change significantly and p35 increased *via P. gingivalis* and *E. coli* LPS stimulations in WT mice (Figure **2A**). The production of TGF-β was inhibited to different degrees, IFN-γ did not change significantly, and p35 production did not increase under *E. coli* LPS stimulation in TLR2 and TLR4 KO mice (Figure **2B** and **C**).

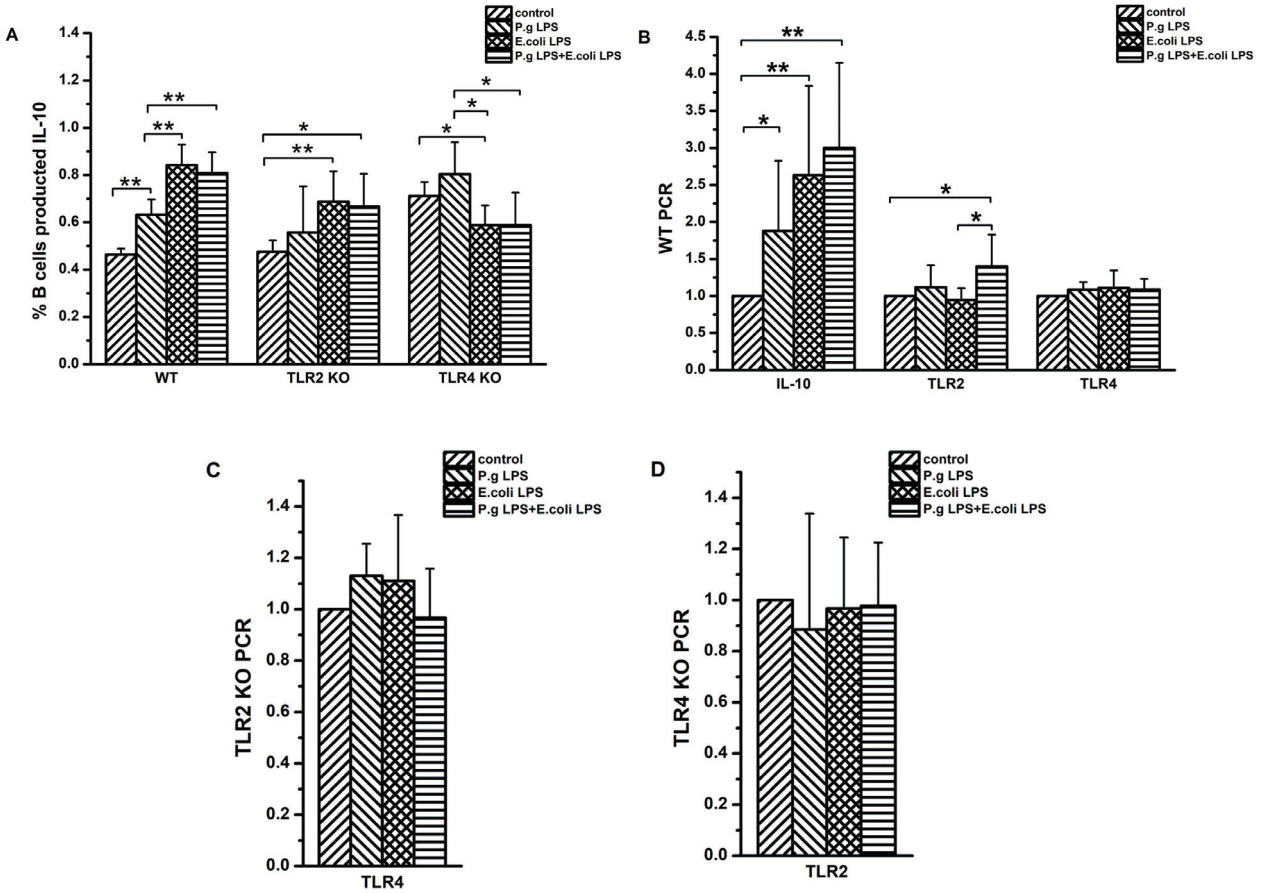

Figure 1. *P. gingivalis* LPS stimulates B cells to produce IL-10 by TLR2/4. (**A**) Flow cytological analysis showed that in WT mice, TLR2 KO mice and TLR4 KO mice, *P. gingivalis* LPS, *E. coli* LPS and *P. gingivalis* LPS+ *E. coli* LPS stimulated B cells and produced IL-10. (**B**) B cells of WT mice were subjected to the PCR test to detect the production of cytokines IL-10, TLR2, TLR4 under the stimulation of *P. gingivalis* LPS, *E. coli* LPS and *P. gingivalis* LPS + *E. coli* LPS, respectively. (**C**) B cells of TLR2 KO mice were subjected to the PCR test to detect the production of cytokines TLR4 under the stimulation of *P. gingivalis* LPS, *E. coli* LPS and *P. gingivalis* LPS+ *E. coli* LPS. (**D**) B cells of TLR4 KO mice were subjected to the PCR test to detect the production of cytokines TLR2 under the stimulation of *P. gingivalis* LPS, *E. coli* LPS and *P. gingivalis* LPS + *E. coli* LPS. n=5. *p<0.05, **p<0.01.

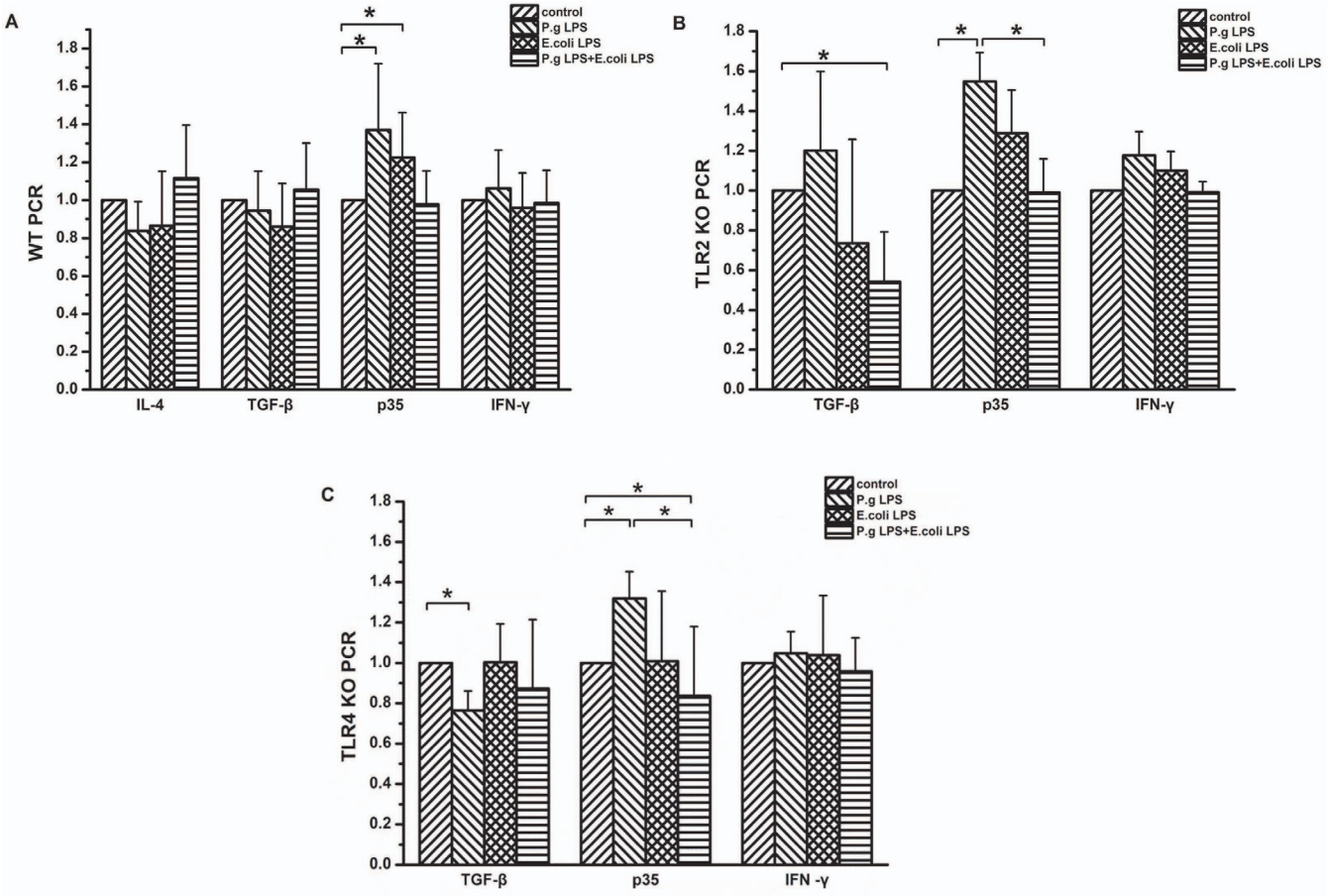

Figure 2. Production of cytokines by B cells under different stimulation. (**A**) RT-PCR was used to detect the production of IL-4, IFN-γ, TGF-β and p35 in B cells of WT mice under different stimulation conditions. (**B**) RT-PCR was used to detect the production of IL-4, IFN-γ, TGF-β and p35 in B cells of TLR2 KO mice under different stimulation conditions. IL-4 was not detected. (**C**) RT-PCR was used to detect the production of IL-4, IFN-γ, TGF-β and p35 in B cells of TLR4 KO mice under different stimulation conditions. IL-4 was not detected. n=5. *p<0.05.

3.3. CD5 was a Surface Marker of IL-10 Breg Subsets and the Phenotype of IL-10-producing B Cells was $CD5^{+}IgM^{+}CD1d^{hi}CD23^{\pm}CD43^{\pm}CD93^{-}$

The flow cytometry analysis (Figure **3**) showed that CD5 can appropriately divide IL-10-producing B cells and isolate subsets with low proportion of B cells but high proportion of IL-10-producing cells (Figure **3A**). By comparison, other markers fail to divide IL-10-producing B cells into whole B cells appropriately, and the area where IL-10-producing B cells are clustered is located in the concentrated area of whole B cells. Hence, CD5 may be a marker for IL-10-producing B cells and the proportion of $CD5^{+}$ in IL-10^{+} B cells was approximately 94% (Figure **3C**). The phenotype of IL-10-producing B cells was analyzed by staining CD1d, IgM, CD23, CD43, and CD93 with antibodies. The results indicated that the phenotype is $IgM^{+}CD1d^{hi}CD23^{\pm}CD43^{\pm}CD93^{-}$ (Figure **3B**). Similar to Tedder [23], this phenotype overlaps with that of B-1a and intersects with MZ. $CD5^{+}IgM^{hi}$ and $CD5^{+}CD1d^{hi}$ accounted for approximately 34% and 20.7% of IL-10-producing B cells, respectively (Figure **3C**).

3.4. $CD5^{+}IgM^{+}$ was the Main B Cell-produced Bregs and $CD93^{-}$ Significantly Increased *via* LPS Stimulation

$CD5^{+}$ was detected as a marker on the B cell surface, and $CD5^{+}CD1d^{hi}$, $CD5^{+}IgM^{+}$, $CD5^{+}CD23^{+}$, $CD5^{+}CD43^{+}$, and $CD5^{+}CD93^{+}$ were detected successively. The proportion of IL-10^{+} B cells produced in each group and the ratio of IL-10^{+} B cells of each subgroup to the total IL-10^{+} B cells were simultaneously analyzed (Figure **4**). The ratio of IL-10^{+} B cells to the total IL-10^{+} B cells in $CD5^{+}IgM^{+}$ and $CD5^{+}CD93^{+}$ subsets was higher than that in other subsets (Figure **4B**). $CD5^{+}IgM^{+}$ subsets were analyzed because the $CD5^{+}IgM^{+}$ subgroup produced a high proportion of IL-10^{+} B cells with a small number of cells (Figure **4A**). The results showed that the proportion of $CD93^{-}$ cells increases, $CD5^{+}IgM^{+}$ produces IL-10 cells, and the proportion of $CD93^{-}$ increases after LPS stimulation (Figure **4C** and **D**).

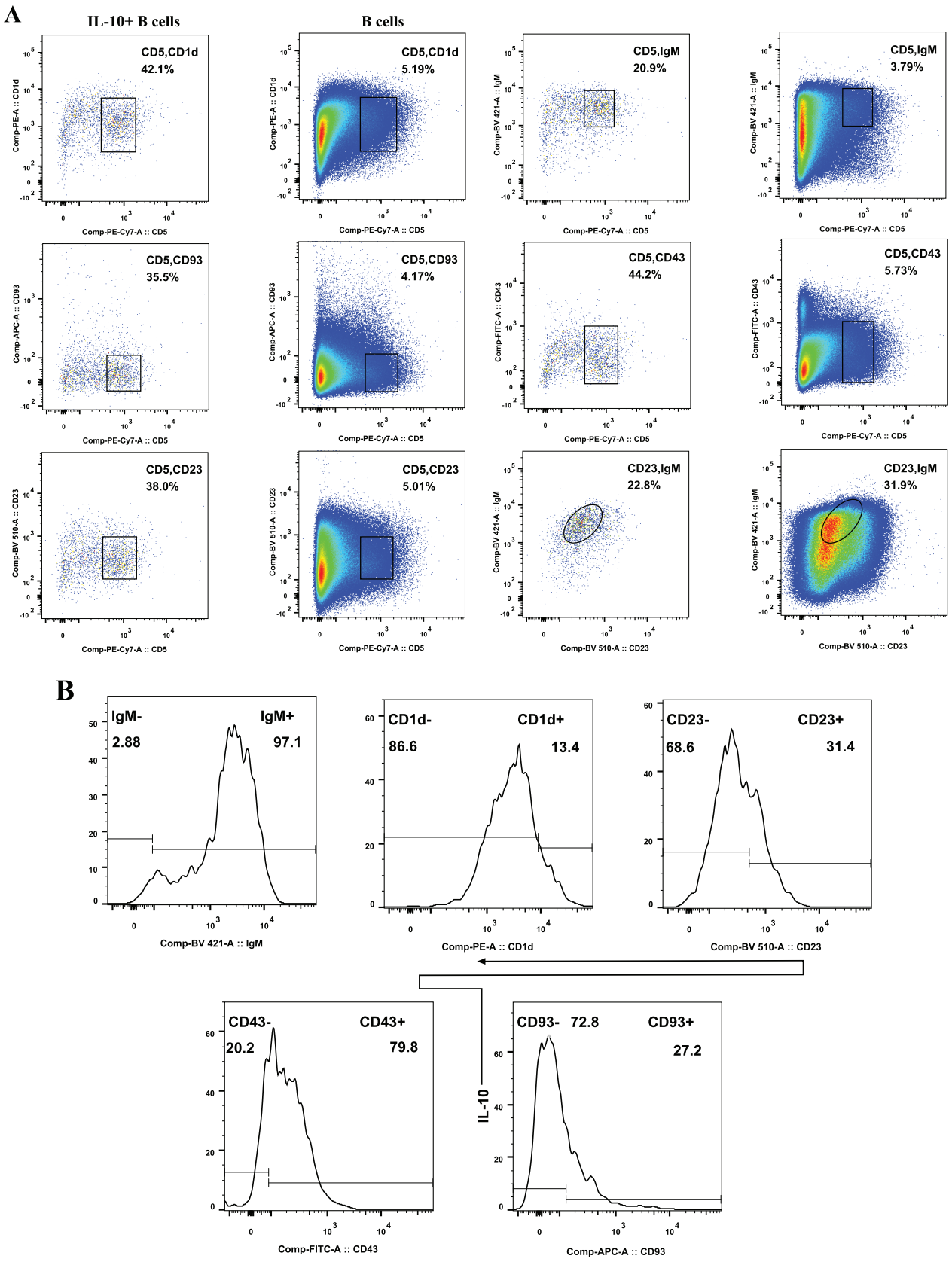

Figure 3. contd....

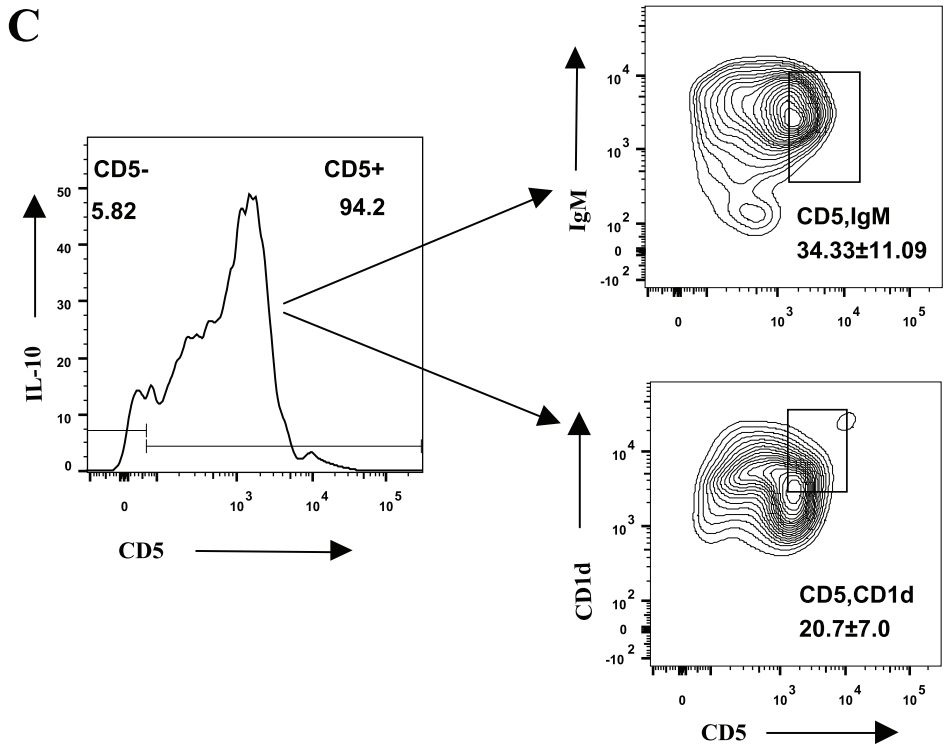

Figure 3. CD5 can differentiate cells that secrete IL-10, and the phenotypes of IL-10-producting B cells. (**A**) CD5 was used to mark cells with different markers, which could significantly distinguish IL-10 cells from B cells. When CD23 was used as a marker to mark cells, it could be seen that IL-10 cells and B cells were coincident in large quantities, and the two could not be distinguished. (**B**) The phenotypes of IL-10-producting B cells. (**C**) The proportion of $CD5^+$, $CD5^+IgM^+$ and $CD5^+CD1d^{hi}$ in IL-10-producing B cells.

3.5. Content of $CD93^-$ Breg Subgroup was Regulated by *P. gingivalis* LPS Through TLR2/4

E. coli LPS was used to simulate the costimulation of other periodontal pathogens with *P. gingivalis* LPS to investigate the pathway through which *P. gingivalis* LPS regulates the content of $CD93^-$ cells and effects of *P. gingivalis* LPS on other periodontal pathogens. The $CD93^-$ regulatory pathway was analyzed in TLR2 and TLR4 KO mice. We detected the content of $CD5^+IgM^+$, $CD5^+IgM^+$ $IL\text{-}10^+$, $CD5^+IgM^+CD93^-$, and $CD5^+IgM^+IL\text{-}10^+CD93^-$ in different animal models using flow cytology (Figure **5**). No significant change was observed in $CD5^+IgM^+$ subsets after *P. gingivalis* LPS stimulation in WT and TLR2 KO groups (Figure **5A**), and $CD5^+IgM^+$ inhibited the IL-10 production after TLR2 knockdown. $CD5^+IgM^+CD93^-$ Bregs did not change significantly when TLR2 and TLR4 were deleted (Figure **5C**). The number of $CD5^+IgM^+IL\text{-}10^+CD93^-$ Bregs in WT mice significantly increased after *P. gingivalis* LPS stimulation, but the changes disappeared when knockout TLR2 and TLR4 (Figure **5D**). These results indicated that *P. gingivalis* LPS regulates the content of $CD5^+IgM^+$ $IL\text{-}10^+CD93^-$ Breg *via* TLR2 and TLR4. $CD5^+IgM^+IL\text{-}10^+$ significantly increased after *E. coli* LPS or *P. gingivalis* LPS+ *E. coli* LPS stimulation (Figure **5B**) while $CD5^+IgM^+CD93^-$ and $CD5^+IgM^+IL\text{-}10^+CD93^-$ remain the same with TLR2 knockout (Figure **5C** and **D**). This finding is different from that when *P. gingivalis* LPS is used as the stimulation. Therefore, $CD5^+IgM^+$ stimulated by *E. coli* LPS can produce IL-10 through other pathways when TLR2 is absent.

4. DISCUSSION

Periodontitis demonstrates high incidence worldwide [2, 24]. Plaque microorganism is the initiator of periodontitis. An excessive host immune response can break the balance between plaque and the body's immunity. *Porphyromonas gingivalis* prevalent in patients with periodontal disease causes periodontitis, which is mainly manifested by bone absorption and induction of the host's natural immune response. Breg can reduce alveolar bone resorption by secreting IL-10 [25, 26]. However, Breg remains unclear, is vaguely defined, and presents neither specific surface markers nor specific functions or transcription factors in the present study. Phenotypes and sources of Breg may vary under different stimulants and pathological states. B cells can secrete IL-10 under the activation of the CD40 signaling pathway in the collagen-induced arthritis mouse model T2-MZ phenotype ($CD21^{hi}CD23^+$) [27]. This subgroup exists in $CD38^+CD24^+$ transitional B cells in systemic lupus erythematosus patients [28]. $CD21^+CD23^-$ MZ B cells are the main subgroup that secretes IL-10 when apoptotic cells induce immune regulation [29]. Tedder laboratory revealed that a subset of cells with the $CD1d^{hi}CD5^+$ phenotype

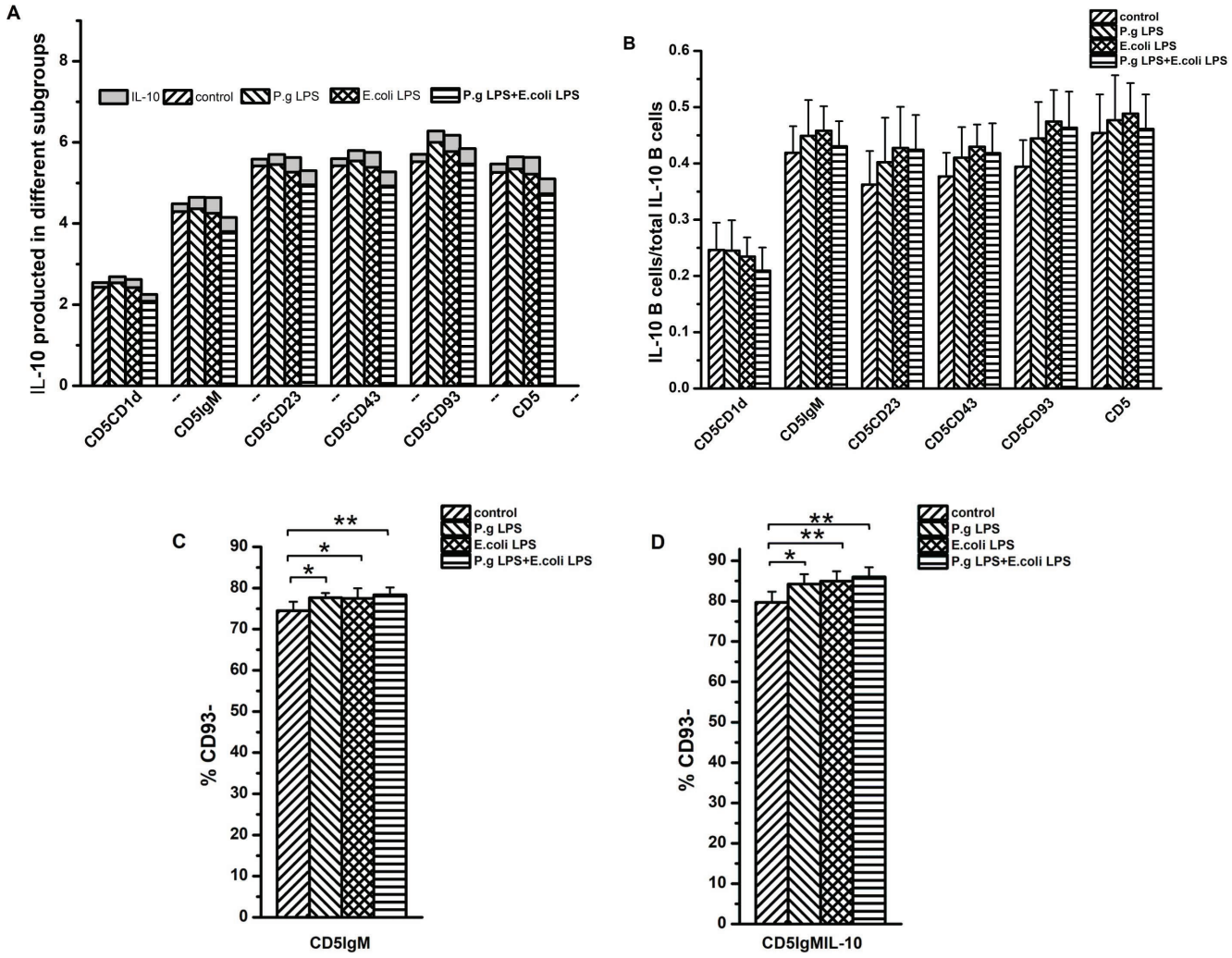

Figure 4. The proportion of each subgroup, IL-10 produced by different subgroups, and the proportion of IL-10 produced in the total IL-10 were different. (**A**) IL -10 producted in different subgroups stimulated by LPS. (**B**) The effects of LPS on IL-10 B cells / total IL-10 B cells in different subgroups were detected. (**C**-**D**) After stimulation by LPS, $CD93^-$ were increased in $CD5^+IgM^+$ subgroups. In $CD5^+IgM^+IL\text{-}10^+$ B cells, $CD93^-$ were increased also. n=5. *p<0.05, **p<0.01.

contains a large number of IL-10-producing B cells named B10 cells [23]. This subtype has been widely used to explore the immunomodulatory function of Breg [30, 31]. However, the Breg phenotype remains unverified and is only limited to a range in existing studies. CD5 is a surface marker of IL-10 Breg subsets, $CD5^+IgM^+$ subsets produce additional Bregs in a small proportion, and the main phenotype of Breg is $CD5^+IgM^+$ under the action of LPS in our study. *E. coli* LPS stimulated B cells to differentiate the production of IL-10 B cells more effectively than the *P. gingivalis* LPS stimulation. We established a combination of *P. gingivalis* and *E. coli* LPS stimulation groups to simulate the coexistence of *P. gingivalis* LPS with other bacteria in the oral environment. The results showed that the combined action of *P. gingivalis* and *E. coli* LPSs exerts no superposition effect on the production of Breg. This finding suggested that LPS of other periodontal bacteria fails to significantly change the induction of Breg under the effect of *P. gingivalis* LPS.

LPS induces the natural immune response mainly through TLRs. LPS of bacteria is typically the ligand of TLR4, and *P. gingivalis* LPS can activate both TLR2 and TLR4 pathways. Both TLR2 and TLR4 ligands can effectively induce the production of Breg in EAE and diabetic animal models [32, 33]. At present, the activation of various TLR pathways can induce Breg generation, and TLR9 and B cell receptor (BCR) is an effective signal combination [34]. The ligand CpG in TLR9 exists in the bacterial nucleus and is released into the intracellular activation TLR9 pathway at a later stage of disease development, given that TLR9 is an intracellular receptor. Therefore, the induction of TLR2 and TLR4 ligands to Breg in the early stage of bacterial infectious diseases is importent in the occurrence of periodontitis. Upregulation of TLR2

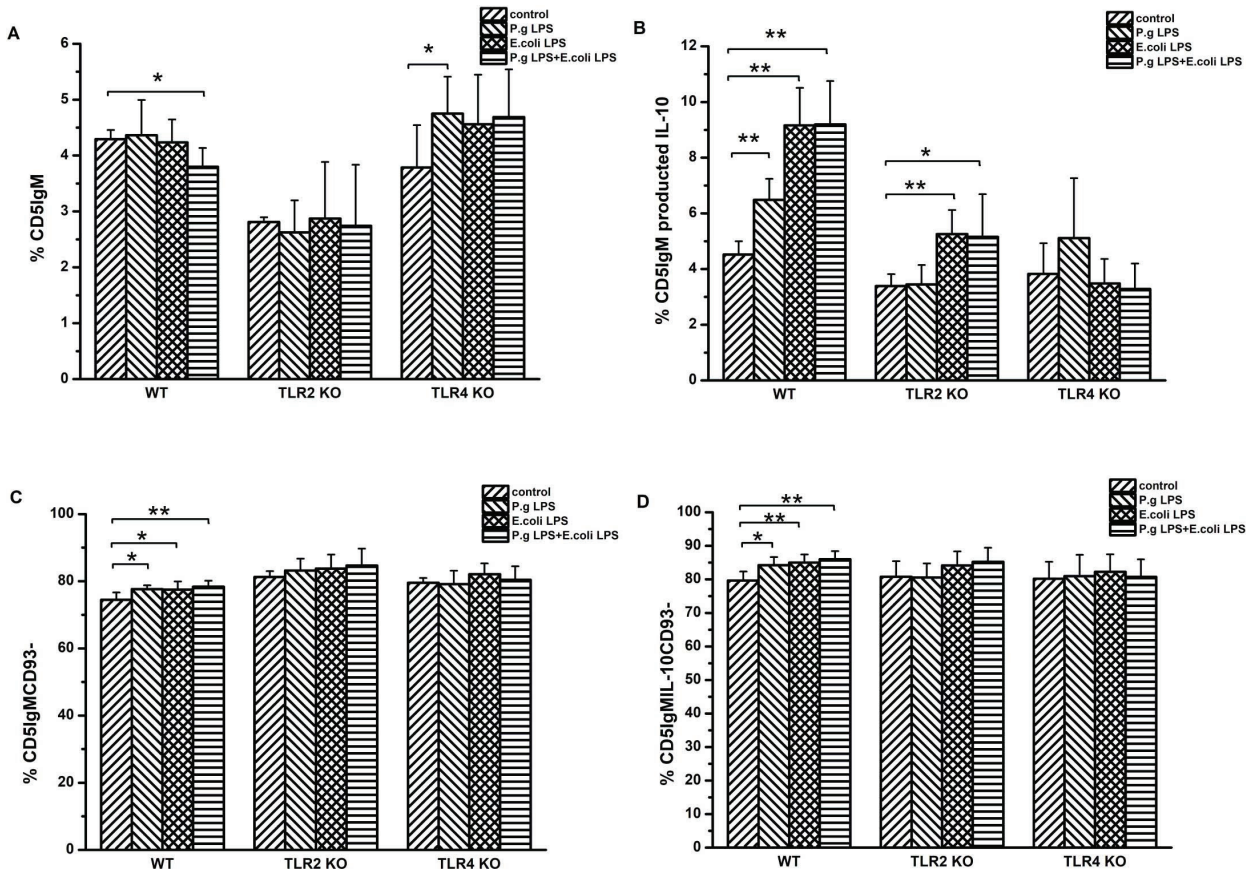

Figure 5. *P. gingivalis* LPS regulated the content of $CD93^-$ Breg subgroup by TLR2/4. (**A**-**D**) $CD5^+IgM^+$ ratio changes, $CD5^+IgM^+$ produces IL-10 ratio, $CD5^+IgM^+CD93^-$ proportion, $CD5^+IgM^+IL\text{-}10^+CD93^-$ proportion were detected in WT, TLR2 KO and TLR4 KO mice, respectively. n=5. *p<0.05, **p<0.01.

and TLR4 can be detected in B cells of infectious diseases, such as periodontitis and enteritis [35, 36]. Similar to the results of previous studies, our findings showed that the differentiation of B cells into Breg induced by *P. gingivalis* LPS requires the simultaneous activation of TLR2 and TLR4 pathways, *E. coli* LPS relies on the TLR4 pathway [22], and upregulation of TLR2 or TLR4 is absent. Mouse primary B cells showed higher TLR2 and lower TLR4 expression [37-39], whereas human primary B cells expressed only low levels of TLR2 [40, 41]. However, TLR4 can be induced to express and activated by LPS with certain stimuli, such as IL-4 [42]. TLR2 was upregulated by *P. gingivalis* LPS+*E. coli* LPS costimulation, when TLR4 knockout, the phenomenon disappeared. Thus, *E. coli* LPS likely increased the activation of TLR2 by *P. gingivalis* LPS in the presence of TLR4. However, failure of the upregulation of TLR2 to increase the amount of Breg may be related to the concentration/duration of the stimulus or the competition of TLR2 and TLR4 for downstream pathway cytokines because TLR2 relies solely on the MyD88 pathway while TLR4 is dependent on MyD88 and TRIF pathways [32].

The mechanism by which B cells produce IL-10 *via* TLR is unclear. An atypical IκB protein (IκBNS) was found to be involved in the induction of IL-10 production by TLR2, TLR4, TLR7, and TLR9 in B cells in 2016. IL-10 secretion of B cells decreased, and the proportion of B10 cells was significantly downregulated in IκBNS knockout mice [43]. $CD93^-$ in $CD5^+IgM^+$ and $CD5^+IgM^+IL\text{-}10^+$ subsets increased under LPS stimulation in our study. *P. gingivalis* LPS-stimulated CD93 was still affected by TLR2 and TLR4 pathways and no significant change occurred in $CD93^-$ cells with TLR2 or TLR4 knockout. By contrast, *E. coli* LPS-stimulated B cell conversion to Breg independent of CD93 changes occurred with TLR2 knockout. These results suggested that the conversion of B cells to Breg by *P. gingivalis* LPS-stimulated TLR2 and TLR4 may be related to the conversion of $CD93^+$ to $CD93^-$ and *E. coli* LPS may present other pathways to stimulate B cell conversion to Breg. Further experiments are needed to confirm whether CD93 is a key factor in the production of Breg.

CONCLUSION

P. gingivalis is a key pathogen of periodontitis that changes the immune balance between plaque microorganisms and hosts through its interaction with the host immune system. The difference in TLR activation pathways shows the significant distinction between *P. gingivalis* LPS and other symbiotic LPSs [20-22]. We

investigated the activation of TLR2 and TLR4 pathways stimulated by *P. gingivalis* LPS in this study. The synergistic effect of the two in the process of Breg generation was clarified. The results showed that the change of CD93 is accompanied by the formation of Breg under the stimulation of *P. gingivalis* LPS and differences between *P. gingivalis* and *E. coli* LPSs in the generation of Breg. Compared with T cell therapy, the use of B cells as therapeutic targets exerts less effect on acquired immunity and is less likely to cause disorders in immune regulation. However, the low proportion distribution of the Breg subgroup and the high efficiency of IL-10 secretion indicate the feasibility of relevant immunotherapy. The results of this study will help us understand the immune pathogenic mechanism of *P. gingivalis* further, clarify characteristics of Breg induction and the influence of this process on the occurrence and development of periodontitis, and provide a theoretical foundation for future investigations on immunotherapy of periodontitis with Breg as the target and similar diseases, such as peri-implant inflammation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study is approved by the Experimental Animal Welfare Ethical Branch of the Biomedical Ethics Committee of Peking University, China (Authorization no. LA201406).

HUMAN AND ANIMAL RIGHTS

No humans were used in the study. All experimental procedures on animals were performed in accordance with institutional guidelines and rules of the National Animal Administration of China.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available on request from the corresponding author [XY].

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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