Production of interleukin 8 and Monocyte chemoattractant protein–1 on human umbilical vein endothelial cells stimulated by Porphyromonas gingivalis with different fimA genotypes

Shu-Yu Cai1*, Song Ge2

1Department of Dentistry, Dongguan Fifth People’s Hospital, Dongguan 523905
2Affiliated Stomatology Hospital of Zunyi Medical College, Zunyi 563003

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Porphyromonas gingivalis
Interleukin 8
Monocyte chemoattractant protein-1
Atherosclerosis

ABSTRACT

Objective: To study the effects of Porphyromonas gingivalis (Pg) with different fimA genotypes on IL-8 and MCP-1 production by human umbilical vein endothelial cells and to reveal their possible role in the development of atherosclerosis. Methods: Pg with different fimA genotypes were cultured with anaerobic and were used to infect HUVEC cells at a MOI of 100. Supernatant IL-8 and MCP-1 contents of cultured HUVEC cells after Pg stimulation at 2 h, 6 h and 24 h, respectively, were detected by ELISA. Results: Supernatant IL-8 and MCP-1 contents of HUVEC cells after Pg stimulation at 2 h, 6 h and 24 h were significantly higher than those in un-stimulation groups (P<0.05), and supernatant IL-8 and MCP-1 contents of HUVEC cells after II fimA and IV fimA genotypes Pg stimulation were significantly higher than those after I fimA genotypes Pg stimulation (P<0.05). Also, supernatant IL-8 and MCP-1 contents of HUVEC cells after II fimA genotypes Pg stimulation were significantly higher than those after IV fimA genotypes Pg stimulation. Conclusion: Pg with II fimA genotypes show a stronger ability to stimulate HUVEC cells to express IL-8 and MCP-1, which may lead a functional disorder of vascular endothelial.

1. Introduction

Porphyromonas gingivalis (Pg) is a main pathogens involved in the onset of inflammation and tissue destruction during periodontal disease. Recently, many researchers found that Pg infection is a new independent risk factor of atherosclerosis (AS)[1,2]. VEC, key cell of AS pathogenesis, plays an important role in the inflammatory and immune response, especially in endothelial cell injury and dysfunction[3,4]. It has been reported that Pg can adhere or invade into vascular endothelial cells (VEC), resulting in cell damage and irritation[1,2]. Interleukin 8 (IL-8) and Monocyte chemoattractant protein-1 (MCP-1) are two important members of chemokine family, play an important role in chemotaxis and migration of leukocytes into the arterial wall, and closely related to the formation of AS. So far, the effects of Pg with different fimA genotypes on IL-8 or MCP-1 mediated inflammatory had not been reported in the literature. Therefore, inhere we will explore production of IL-8 and MCP-1 on human VEC cells after Pg stimulated and reveal their possible role in the development of AS.

2. Materials and methods

2.1. Strains and cell lines

Pg with I fimA type (ATCC33277), II fimA type (WCSP115) and IV fimA type (W83) were provided by State Key Laboratory of Oral Diseases (Sichuan University). Human umbilical vein endothelial cells (ATCC CRL-2480) were provided by Xiangya School of Medicine, CSU.
2.2. Reagents and instruments

RPMI-1640 medium, fetal bovine serum (FBS) and trypsin were purchased from Gibco Co., Ltd. Epidermal growth factor supplement (ECGS) was purchased from Amresco Co., Ltd. Penicillin, streptomycin were purchased from North China Pharmaceutical Co., Ltd. Bovine heart brain infusion (BHI) were purchased from Britain OXIOD Co., Ltd. Hemin and lipopolysaccharide (LPS) were purchased from Sigma Co., Ltd. Mouse monoclonal anti-human factor VIII was purchased from Wuhan Boster Biological Engineering Co., Ltd. Goat anti-human CD31 monoclonal antibody was purchased from Santa Cruz Co., Ltd. Human IL-8 and MCP-1 ELISA kit were purchased from Haixi Tang biotechnology Co., Ltd. Anaerobic incubator was purchased from American Shellab Co., Ltd. CO2 incubator was purchased from Thermo Co., Ltd. Inverted phase contrast microscope and camera system was purchased from Olympus Corporation of Japan. Microplate reader (ELx800) was purchased from BioTek Co., Ltd. Others were conventional laboratory reagents and instruments.

2.3. Immunocytochemistry

10^5-10^6 HUVEC cell/well were seeded in a 6-well plates containing a cover slips. After cells fully adherent, slips were removed, fixed with acetone for 10 min at 4°C. Then slips were washed with PBS for several times. After blocking, slips were incubated on antibody solution with human factor VIII (1:100) or human CD31 (1:100) antibodies for 60 min at 37°C, respectively. After washed with PBS several times, slips were incubated on secondary antibody solution for 20 min at 37°C. Finally, the slides were observed under a microscope after DAB dyeing.

2.4. ELISA detection of IL-8 and MCP-1 expression

Cell culture supernatant of HUVEC cells after Pg infection with a multiplicity of infection (MOI) of 100:1 for 2 h, 6 h, 24 h, respectively, were collected and IL-8 and MCP-1 expression were detected by ELISA kit according with the operating instructions.

2.5. Statistical analysis

SPSS 13.0 statistical software was used for data analysis. Data were showed as mean±standard deviation. Single-factor analysis of variance (ANOVA) and Wilcoxon test were employed to compare the differences of data. A P value of less than 0.05 was considered as statistical significant.

3. Results

3.1. HUVEC cell identification

HUVEC cells were adherent growth in diamond or polygonal, showing the typical paving stone mosaic-like arrangement. Immunohistochemistry staining showed Factor VIII and CD31 were high expression on cytoplasmic.

3.2. IL-8 expression detection

HUVEC cells were stimulated with different strains of Pg fimA genotype at different times (2 h, 6 h and 24 h), and the culture supernatant levels of IL-8 were shown in Table 1. Supernatant IL-8 contents of HUVEC cells after Pg stimulation at 2 h, 6 h and 24 h were significantly higher than those in un-stimulation groups (P<0.05), and supernatant IL-8 contents of HUVEC cells after II fimA and IV fimA genotypes Pg stimulation were significantly higher than those after I fimA genotypes Pg stimulation (P<0.05), respectively. Also, supernatant IL-8 contents of HUVEC cells after II fimA genotypes Pg stimulation were significantly higher than those after IV fimA genotypes Pg stimulation. These results suggest that Pg with II fimA genotypes show a stronger ability to stimulate HUVEC cells to express IL-8 than Pg with I fimA genotypes or Pg with IV fimA genotypes.

Table 1. Supernatant IL-8 contents of HUVEC cells after Pg stimulation with different genotypes (pg/mL).

<table>
<thead>
<tr>
<th>Group</th>
<th>2 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I fimA</td>
<td>748.53±44.91</td>
<td>3 410.89±502.94</td>
<td>6 805.30±456.36</td>
</tr>
<tr>
<td>II fimA</td>
<td>2 383.61±388.33</td>
<td>5 421.36±656.37</td>
<td>7 373.05±435.94</td>
</tr>
<tr>
<td>IV fimA</td>
<td>1 151.96±120.98</td>
<td>3 833.02±533.08</td>
<td>6 900.13±285.92</td>
</tr>
<tr>
<td>LPS</td>
<td>2 501.17±581.93</td>
<td>4 809.53±317.75</td>
<td>6 737.17±526.94</td>
</tr>
<tr>
<td>PBS</td>
<td>655.02±126.52</td>
<td>3 104.98±115.24</td>
<td>6 737.17±526.94</td>
</tr>
</tbody>
</table>

*: PBS stimulation group compared with other groups, P<0.05. #: Type II fimA stimulation group compared with other groups, P<0.05. &: Type IV fimA stimulation group compared with other groups, P<0.05. ^: Type I fimA stimulation group compared with other groups, P<0.05.
3.3. MCP–1 expression detection

HUVEC cells were stimulated with different strains of Pg fimA genotype at different times (2 h, 6 h and 24 h), and the culture supernatant levels of MCP–1 were shown in Table 1. Supernatant MCP–1 contents of HUVEC cells after Pg stimulation at 2 h, 6 h and 24 h were significantly higher than those in un-stimulation groups (P<0.05), and supernatant MCP–1 contents of HUVEC cells after II fimA and IV fimA genotypes Pg stimulation were significantly higher than those after I fimA genotypes Pg stimulation (P<0.05), respectively. Also, supernatant MCP–1 contents of HUVEC cells after II fimA genotypes Pg stimulation were significantly higher than those after IV fimA genotypes Pg stimulation. These results suggest that Pg with II fimA genotypes show a stronger ability to stimulate HUVEC cells to express MCP–1 than Pg with I fimA genotypes or Pg with IV fimA genotypes.

4. Discussions

IL–8 and MCP–1 plays an important role in mediating the inflammatory process, especially during the development of the AS[5]. Recently, a large number of epidemiological results showed that periodontal infection, especially Pg infection was closely related to AS pathogenesis[6]. According to the differences nucleotide sequence of fimA gene, Pg was divided into six types, Type I fimA, Type II fimA, Type III fimA, Type IV fimA, Type V fimA and Type Ib fimA genotypes Pg. It has been reported that Type II fimA and Type IV fimA genotypes Pg were highly virulent strains, and closely related to moderate or severe periodontitis, chronic periodontitis and CVD[6–8]. Interestingly, different genotypes Pg infection induced varying degrees of periodontal tissue damage[7–10]. But so far, different genotypes Pg infection on IL–8 or MCP–1 mediated inflammatory, yet few reports.

Here, we detected IL–8 and MCP–1 production on HUVEC cells after different genotypes Pg stimulation. The results suggested that Pg with II fimA genotypes showed a stronger ability to stimulate HUVEC cells to express IL–8 and MCP–1 than Pg with I fimA genotypes or Pg with IV fimA genotypes. Previous studies by other researchers suggested that AS pathogenic factors such as LDL, IL–1 also can induce IL–8 and MCP–1 expression[11,12]. Currently, there are still two different perspectives on IL–8 and MCP–1 expression of endothelial cells after Pg stimulated. Nassna et al. found in P. gingivalis–stimulated HUVEC cells, MCP–1, IL–8 mRNA expression increased, but MCP–1, IL–8 secretion was reduced[13]. In this process, protease of Pg may play an important role in inhibiting the MCP–1, IL–8 secretion[13]. Nassna et al. also found that Pg could temporarily regulate endothelial cell chemokine production to benefit for symbiosis between pathogen and host. Therefore, Pg plays an important role in chronic inflammation of AS. FimA is a gene related to bacterial pili. Earlier, Kang, et al. and Takahashi et al. also found that IL–8 and MCP–1 expression on endothelial cells may be associated with pili, but the exact mechanism is unclear[11,14]. Here, we found supernatant IL–8 and MCP–1 contents of HUVEC cells after II fimA genotypes Pg stimulation were significantly higher than those after I fimA or IV fimA genotypes Pg stimulation. This result suggested that Pg with II fimA genotypes had a stronger ability to stimulate HUVEC cells to express IL–8 and MCP–1, leading a functional disorder of vascular endothelial.

References

[6] Persson GR, Persson RE. Cardiovascular disease and periodontitis: an


