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Porphorymonas gingivalis induces intracellular adhesion molecule-1 expression in endothelial cells through the nuclear factor-kappaB pathway, but not through the p38 MAPK pathway

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Background and Objective: Porphyromonas gingivalis is a major pathogen in the development and progression of periodontal disease. The aim of this study was to investigate whether endothelial intracellular adhesion molecule-1 (ICAM-1), an inflammation biomarker for periodontitis, could be modified by infection with either of two strains of *P. gingivalis* with different virulence capacities: avirulent ATCC 33277 and virulent W83.

Material and Methods: We examined the expression of ICAM-1, IκBα, phospho-p38 MAPK and nuclear factor-kappaB (NF-κB) p65 in an umbilical vein endothelial cell line (ECV-304) treated with ATCC 33277 and W83, with or without the NF-κB antagonist MG132 and/or a specific p38 inhibitor (SB203580), by real-time PCR, western blotting and immunofluorescence.

Results: Both strains could induce ICAM-1 expression; additionally W83 was able to increase ICAM-1 expression more significantly than ATCC 33277. In *P. gingivalis*-infected endothelial cells, both p38 MAPK and NF-κB signaling pathways were triggered by a rapid increase of p38 MAPK phosphorylation and a more delayed degradation of IκBα, followed by the nuclear translocation of NF-κB. It was found that ICAM-1 production in endothelial cells was abrogated by inhibition of the NF-κB pathway, but not by inhibition of the p38 MAPK pathway, using the inhibitors of the latter two molecules.

Conclusion: The induction of ICAM-1 by infection of umbilical vein endothelial cells with *P. gingivalis* might be mediated through the NF-κB pathway, but not by the p38 MAPK pathway.

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Periodontal disease is a chronic inflammatory disease of the periodontium that is accompanied by loss of alveolar bone and eventual exfoliation of the teeth. It is one of the most common infectious diseases caused by oral bacteria in humans (1). During the inflammatory process of periodontal disease, leukocytes adhere to the endothelial cells and then migrate into the inflammatory site in response to bacterial stimulation (2). The adherence and infiltration of leukocytes to the endothelium requires the expression of cell adhesion molecules on endothelial cells (3). Intercellular adhesion molecule-1 (ICAM-1) is a member of the adhesion molecule family and is constitutively expressed on the cell surface (4). It has been reported that the level of ICAM-1 is increased in response to a variety of inflammatory mediators, including proinflammatory cytokines, cellular stress and infection (5).

The expression of ICAM-1 relies on the activation of cytosolic transcription factors, among which nuclear factor-kappaB (NF-κB) plays a central role in their regulation (6,7). NF-κB can be induced by proinflammatory stimuli (cytokines, bacteria or bacterial toxins), and this induction is regulated by the phosphorylation and degradation of its naturally occurring inhibitor, IκB. Degradation of IκB results in translocation of NF-κB to the nucleus and its activation (8,9). Meanwhile, evidence shows that the MAPK pathways are important for the induction of ICAM-1 expression in endothelial cells (10,11). Specifically, p38 MAPK has been established as crucial in leukocyte proinflammatory signaling, and p38 activity is integral in the up-regulation of ICAM-1 on the pulmonary endothelium, which is stimulated by both lipopolysaccharide and tumor necrosis factor (TNF) (10). The p38 signal-transduction pathway controls many aspects of inflammatory responses and therefore is a priority in research related to many human diseases. However, little is known about signal-transduction pathways that are activated in endothelial cells upon infection with pathogens responsible for the development of periodontal disease.

Porphyromonas gingivalis, a gram-negative anaerobic bacterium, is a major pathogen in the onset and progression of periodontal disease. *P. gingivalis* has been classified into avirulent and virulent strains based on its ability to form necrotic abscesses in animal models (12,13). Virulent strains of *P. gingivalis* are frequently isolated from severe periodontal lesions, whereas avirulent strains are detected more commonly in healthy subjects (14). Several studies have demonstrated different virulence capacities of selected *P. gingivalis* strains (15–17). However, to date, different strains of *P. gingivalis* have not been characterized in regard to their expression of potential virulence factors, although the virulence-associated activities and disease-promoting characteristics *in vitro* and *in vivo* have been compared between avirulent strain ATCC 33277 and virulent strain W83. In this study, we investigated whether the level of ICAM-1 was changed in endothelial cells after infection with two different *P. gingivalis* strains, namely ATCC 33277 and W83. The possible roles of NF-κB and p38 signaling pathways in the regulation of ICAM-1 production were also explored. It was found that both strains of *P. gingivalis* could induce ICAM-1 expression, and that the expression of ICAM-1 was stimulated more strongly by strain W83 than by strain ATCC 33277. In *P. gingivalis*-infected endothelial cells, both p38 MAPK and NF-κB signaling pathways were triggered by a rapid increase of p38 MAPK phosphorylation and a more delayed degradation of IκBα, followed by the nuclear translocation of NF-κB. Further studies indicated that the production of ICAM-1 in endothelial cells was abrogated by inhibition of the NF-κB pathway, but not by inhibition of the p38 MAPK pathway.

Material and methods

Bacterial strains and culture

P. gingivalis ATCC 33277 and W83 were cultured in brain–heart infusion (BHI) broth (Nissui Seiyaku, Tokyo, Japan) containing cysteine (0.1%), yeast (0.5%), vitamin K (0.1%) and hemin (0.25%), at 37°C anaerobically. Bacterial cells were cultured overnight until the optical density reached 1.0 at 660 nm, whereupon they were resuspended in RPMI-1640 (Gibco BRL, Carlsbad, CA, USA) at a final concentration of 10^{12} cells/L.

Cell culture and infection with *P. gingivalis*

The human umbilical vein endothelial cell (HUVEC) line, ECV-304, was obtained from the China Center for Type Culture Collection (Wuhan, China). HUVECs were maintained in RPMI-1640, and cell viability was determined, by Trypan Blue exclusion, to be > 90% for all the infection assays. Bacterial suspensions (10^{12} cells/L) were added to confluent HUVEC monolayers at a multiplicity of infection (MOI) of 100 (18,19). After incubation at 37°C in 5% CO_2 for 2 h, the supernatant was replaced with fresh medium containing gentamicin (0.5 g/L) and metronidazole (0.1 g/L) in order to kill the remaining extracellular bacteria. The antibiotic did not affect the morphology of the endothelial cells or alter their ability to exclude Trypan Blue (20,21). Plates were processed for experiments directly or incubated for a further 2, 6, 22 or 46 h. At the time-points indicated, HUVECs were harvested and used in the following experiments.

MG132 (an antagonist of NF-κB) and SB 203580 (a specific inhibitor of p38 MAPK) were obtained from Calbiochem (San Diego, CA) and prepared as stock in dimethylsulfoxide. Cells were pretreated with MG132 (5–20 μM) or SB203580 (5–20 μM) before treatment with *P. gingivalis*.

Antibiotic protection and invasion assays

To compare the invasion efficiencies of *P. gingivalis* strains ATCC 33277 and W83, HUVECs were infected using an antibiotic protection assay, as described previously (21,22). Briefly, HUVEC monolayers in six-well plates were infected at an MOI of 100 (10^8 bacteria to 10^6 HUVECs) and incu-

bated for 2 h. Unattached bacteria were removed by washing with phosphate-buffered saline. Half of the monolayers were lysed with sterile H_2O (20 min). Lysates were diluted, plated on solid BHI medium and incubated for 7 d under anaerobic conditions. Colony-formation units (CFU) of bacteria that had adhered to and/or invaded cells were then enumerated and compared. On the other half of the monolayers, external adherent bacteria were killed by incubating the infected monolayers for 2 h with fresh medium containing gentamicin (0.5 g/L) and metronidazole (0.1 g/L). The monolayers were lysed, diluted and then plated onto solid BHI medium. The CFUs of invasive bacteria were then counted. Invasion was expressed as the percentage of the initial inoculum recovered after antibiotic treatment and cell lysis. The numbers of CFUs of three independent experiments were counted. *Escherichia coli* JM109 was used as a negative control for invasion assays with HUVECs.

Real time-PCR analysis of *ICAM-1* expression

Quantitative real-time PCR of *ICAM-1* was performed as previously described (23). The complementary DNA was synthesized using a ThermoScript RT-PCR system (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. Real-time PCR was carried out in an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), with a Carboxyfluorescein (FAM)-labeled probe (5′-FAM-TG ACCTTCTACAGCTTCCCACCTCC A-Eclipse-3′) and a pair of primers specific to the *ICAM-1* transcript (forward 5′-CTGGCAGACGAGAAGGT GGT-3′ and reverse 5′-GCTCGCTCA GGGTCAGGTT-3′) giving a product of 66 bp in length. β-actin, as an internal control, was measured using a FAM-labeled probe (5′-FAM-CCAG GGCGTGATGGTGGGCAT-Eclipse-3′) and a pair of primers specific to the β-actin transcript (forward 5′-CCGTC TTCCCCTCCATCG-3′ and reverse 5′-GTCCCAGTTGGTGACGATGC-3′) giving a product of 155 bp in length. Each sample was amplified in triplicate to obtain average cycle threshold (C_t) values. Reactions without complementary DNA templates were used as negative controls. The results were expressed as relative *ICAM-1* mRNA levels compared with the untreated control, which was considered to be 100%.

Western blot analysis

After treatments, total cell lysates were prepared for western blotting (24). Membranes carrying the blotted proteins were incubated with anti-ICAM-1 (1:300; Santa Cruz, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-IκBα (1:300; Santa Cruz) or anti-phospho-p38 (1:500; Santa Cruz). Then, the membranes were incubated with alkaline phosphatase-conjugated anti-immunoglobulin G (1:2000; Sigma, Sigma-Aldrich, Shanghai, China). To ascertain uniformity of protein loading, the membrane was immunoblotted with anti-β-actin (1:5000; Sigma). Labeled protein bands were visualized, and quantification was performed using a densitometer (Bio imaging systems, Hercules, CA, USA). Band volumes were calculated with the ratio of each normalized to the intensity of β-actin.

Immunofluorescence analysis

Immunofluorescence assays were carried out as previously described (21). Briefly, HUVECs were plated on culture slides. Then, the cells were fixed, permeabilized and incubated with NF-κB p65 antibody (1:300; Santa Cruz) or anti-ICAM-1 (1:400; Santa Cruz). Fluorescein isothiocyanate-conjugated immunoglobulin G (1:500; Sigma) was added as the secondary antibody. For studying ICAM-1, nucleic acids were counterstained with Propidium iodide (0.05 g/L; Sigma). Cells were visualized under a fluorescence microscope equipped with a digital camera (Nikon Eclipse E800, Nikon, Tokyo, Japan).

Statistical analysis

All experiments were performed in triplicate wells for each condition and repeated at least three times. Data are presented as mean ± SD and were statistically analyzed by ANOVA using the SAS 8.12 software package (SAS Institute, Cary, NC, USA). A p-value of < 0.05 was considered statistically significant.

Results

Invasion efficiencies of ATCC 33277 and W83

The ability of the *P. gingivalis* strains ATCC 33277 and W83 to invade HUVECs was quite different. In this set of experiments, about 0.37% (3.70×10^5 CFU/mL) of ATCC 33277 and 0.09% (9.00×10^4 CFU/mL) of W83 were in close contact with HUVECs (adhered or already invaded), as indicated in Table 1. The invasion rate of strain ATCC 33277 was about 0.12% and that of W83 was about 0.02%. Therefore, the invasion rate of strain W83 was approximately sixfold lower than the invasion rate of strain ATCC 33277. The invasion efficiency of *E. coli* JM109 was 0.0003 (data not shown) and was used as a negative control.

Infection with *P. gingivalis* induced ICAM-1 expression in endothelial cells

The over-expression of *ICAM-1* mRNA in ECV-304 was observed to occur in a time-dependent manner after infection with *P. gingivalis* ATCC 33277 and W83 (Fig. 1A). The elevation in

Table 1. Invasion of human umbilical vein endothelial cells (HUVECs) with *Porphyromonas gingivalis*

Strain	Bacteria adhering and invading (%)[a]	Invasion efficiency (%)[b]
33277	0.37 ± 0.15	0.12 ± 0.08
W83	0.09 ± 0.03*	0.02 ± 0.01*

[a]Defined as the percentage of colony-forming units (CFU) that bound to HUVECs; the value represents adhering + invading bacteria. Values are the mean ± SD of triplicate independent determinations of a typical experiment.
[b]Percentage of the inoculum (*P. gingivalis*) protected from killing with antibiotics after the infection period. Values are the mean ± SD of triplicate independent determinations of a typical experiment.
*$p < 0.05$ compared with ATCC 33277.

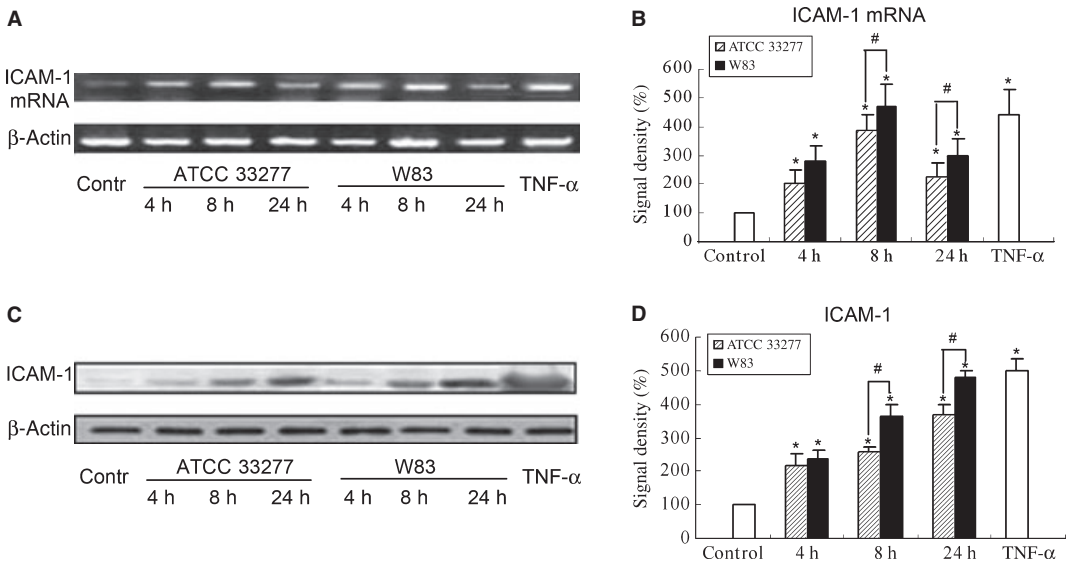

Fig. 1. Intracellular adhesion molecule-1 (ICAM-1) was up-regulated in *Porphyromonas gingivalis*-infected human umbilical vein endothelial cells (HUVECs). HUVECs were cultured in either medium alone (Control) or in medium containing *P. gingivalis* (ATCC 33277 or W83) at a multiplicity of infection (MOI) of 100. (A) Expression of *ICAM1* was determined by RT-PCR. (B) Histogram of real-time PCR shows signal intensities expressed as the ratio of *ICAM1* : β-actin. (C) Western blot analysis of ICAM-1. (D) Histogram of the western blot shows signal intensities expressed as the ratio of ICAM-1 : β-actin. Data are the means ± SD relative to the control (set at 100%) in triplicate experiments from three different cell preparations. $*p < 0.05$ compared with the control; $p < 0.05$ comparing ATCC 33277 with W83. Contr, control; TNF-α, tumor necrosis factor-α.

expression of *ICAM-1* mRNA was found to start at 4 h postinfection, reached a peak at 8 h and remained at this level up to the 24 h time-point (Fig. 1B). The production of ICAM-1 protein was also detected in response to infection with *P. gingivalis*. Western blotting analysis of HUVECs infected with ATCC 33277 showed an increase in ICAM-1 protein expression of 2.6- to 3.7-fold from 8–24 h compared with the control, whereas HUVECs infected with W83 showed an increase in ICAM-1 protein expression of 3.6- to 4.8-fold over the same time-period (Fig. 1C,D). Therefore, the stimulatory effect of *P. gingivalis* W83 was stronger than that of ATCC 33277 at both mRNA and protein levels. In the present study, TNF-α was used, according to a previous report, as a positive control for inducing *ICAM-1* expression (25).

P. gingivalis-induced IκBα degradation and NF-κB p65 translocation in HUVECs

As shown in Fig. 2A, the amount of IκBα in the whole-cell lysate was markedly decreased after infection with *P. gingivalis*, with TNF-α as a positive control. Both strains demonstrated a time-dependent degradation of IκBα, with a maximal effect occurring at 30 min (W83) or 60 min (ATCC 33277) postinfection (Fig. 2B). Immunofluorescence analyses demonstrated that both strains induced NF-κB p65 activation. A time-dependent increase in the fluorescence intensity of the nuclei of HUVECs was observed. It was found that the nuclear translocation of NF-κB p65 started 30 min (W83) or 60 min (ATCC 33277) postinfection. The signal remained elevated for up to 90 min in both strains (Fig. 2C) and had decreased to almost baseline levels by 180 min (data not shown).

P. gingivalis infection activated p38 phosphorylation

The phosphorylation of p38 was increased in a time-dependent manner in the presence of *P. gingivalis* (Fig. 3A). The effect peaked 30 min after infection with ATCC 33277 and decreased to almost baseline levels by 90 min. W83 induced a more prolonged effect, whereby phosphorylation remained elevated for up to 90 min (Fig. 3B). TNF-α was used as a positive control to induce p38 MAPK phosphorylation.

Inhibition of the NF-κB-dependent pathway, but not the p38 MAPK pathway, abrogated ICAM-1 production

As shown in Fig. 4, ICAM-1 started to decrease when cells were pretreated with 20 μM MG132 for 15 min and showed a greater decrease at 30 and 60 min. The expression of *ICAM-1* mRNA was decreased at 30 min when the cells were pretreated with 10 μM MG132. By contrast, SB 203580, a specific p38 inhibitor, had no effect on ICAM-1 expression at either protein or mRNA levels (data not shown).

To further confirm the roles of NF-κB and p38 MAPK on *P. gingivalis*-induced ICAM-1 production, immunofluorescent labeling of ICAM-1 was performed in the presence of MG132 or SB 203580. It was clear that infection with *P. gingivalis* could induce

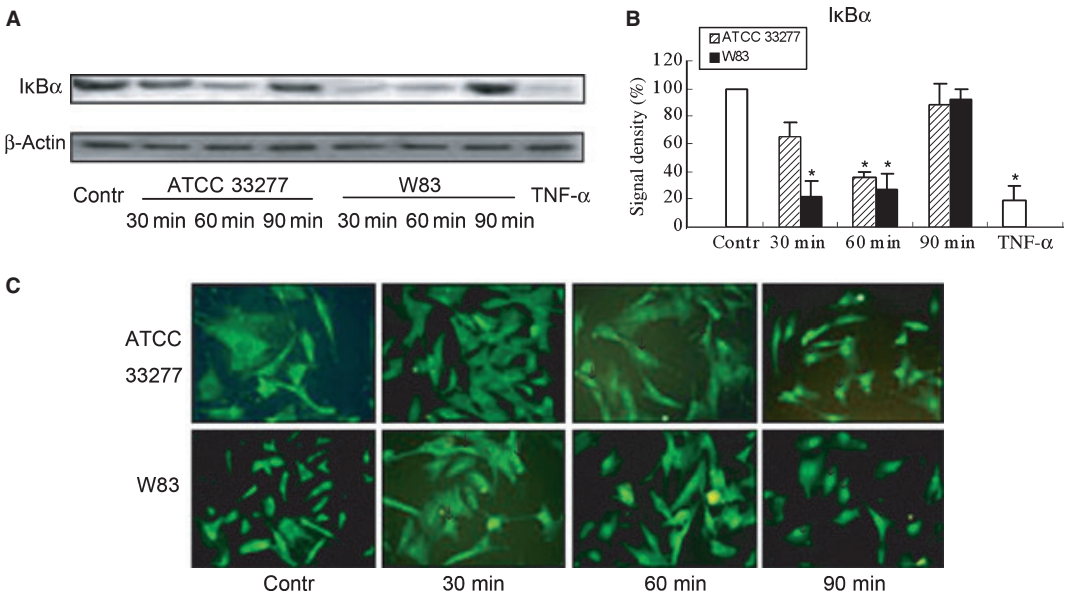

Fig. 2. Infection with *Porphyromonas gingivalis* activates IκBα degradation and nuclear factor-kappaB (NF-κB) translocation. Human umbilical vein endothelial cells (HUVECs) were cultured in either medium alone (Control) or in medium containing *P. gingivalis* (strains ATCC 33277 or W83) at a multiplicity of infection (MOI) of 100. (A) Western blot analysis of IκBα. (B) The histogram shows the densitometric evaluation of signal intensities normalized on β-actin. Data show the means ± SD of three independent experiments from three different cell preparations. $*p < 0.05$ compared with the control. Tumor necrosis factor-α (TNF-α) was used as a positive control. (C) NF-κB p65 activation in *P. gingivalis*-infected HUVECs was analyzed using immunofluorescence. The left line shows mock-treated control cells. Note a time-dependent increasing intensity of fluorescence in the nuclei of HUVECs, demonstrating the nuclear translocation of NF-κB p65 starting 30 min (W83) to 60 min (ATCC 33277) postinfection. Representative pictures (of three independent experiments; magnification ×200) are shown. Contr, control.

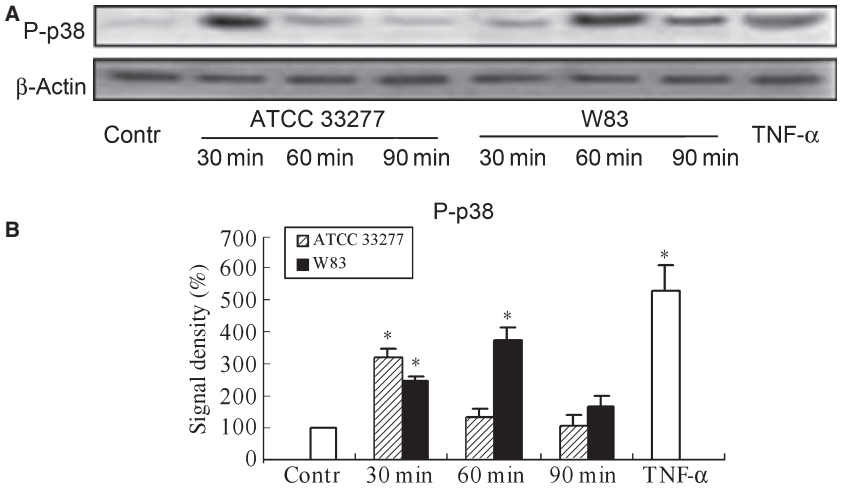

Fig. 3. Infection with *Porphyromonas gingivalis* activates p38 phosphorylation. Human umbilical vein endothelial cells (HUVECs) were cultured in either medium alone (Control) or in medium containing *P. gingivalis* (strains ATCC 33277 or W83) at a multiplicity of infection (MOI) of 100. (A) Western blot analysis of phospho-p38 (P-p38). (B) The histogram shows the densitometric evaluation of signal intensities normalized on β-actin. The data show the means ± SD of three independent experiments from three different cell preparations. $*p < 0.05$ compared with the control. Tumor necrosis factor-α (TNF-α) was used as a positive control. Contr, control.

ICAM-1 production, and pretreatment with 20 μM MG132 inhibited ICAM-1 expression at 15 min. By contrast, SB 203580 had no effect on ICAM-1 expression (Fig. 5). These results indicate that *P. gingivalis* induces ICAM-1 expression in endothelial cells, possibly through the NF-κB pathway, but not through the p38 MAPK pathway.

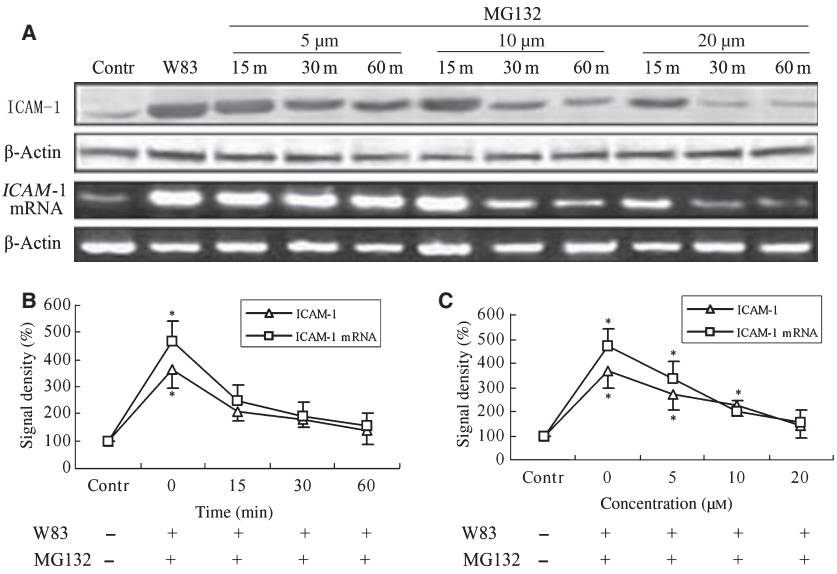

Fig. 4. MG132 inhibits the induction of intracellular adhesion molecule-1 (ICAM-1) in endothelial cells infected with *Porphyromonas gingivalis*. Human umbilical vein endothelial cells (HUVECs) were cultured in either medium alone (Control) or in medium treated with *P. gingivalis* W83 at a multiplicity of infection (MOI) of 100 for 8 h, with or without pretreatment with the nuclear factor-kappaB (NF-κB) inhibitor MG132. (A) Western blot analysis and RT-PCR were performed to detect ICAM-1 protein and *ICAM1* mRNA expression, respectively. (B) The effects of pretreatment with 20 μM MG132, for 15, 30 or 60 min, on ICAM-1 expression. (C) The effects of pretreatment with 5, 10 or 20 μM MG132, for 30 min, on ICAM-1 expression. Data are the means ± SD relative to the control (set at 100%) in triplicate experiments. $*p < 0.05$ compared with the control.

Discussion

Endothelial cell adhesion molecules, such as ICAM-1, play an important role in leukocyte adhesion and transendothelial migration at sites of inflammation (5). Therefore, factors influencing the expression of ICAM-1 are important in regulating vascular inflammatory processes. The present study demonstrated that viable *P. gingivalis*, a major periodontal pathogen, is a potent stimulant for the induction of ICAM-1 in endothelium, which might contribute to the recruitment of leukocytes across the vascular endothelial lining into the extravascular periodontium during the pathogenesis of periodontal disease. An increase in the expression of ICAM-1 protein was found to start at 4 h postinfection and the expression remained elevated for up to 24 h, indicating that *P. gingivalis* might enhance ICAM-1 expression directly, rather than via inflammatory mediators released from HUVECs in an autocrine manner. At the 48 and 72 h time-points, the expression of ICAM-1 showed a dramatic decline at both mRNA and protein levels. We propose that this decrease in ICAM-1 expression might be caused by apoptosis and necrosis occurring after infection with *P. gingivalis*.

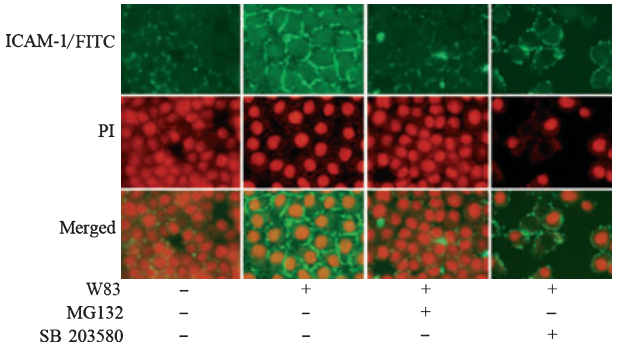

Fig. 5. Immunofluorescence studies show fluorescent labeling of intracellular adhesion molecule-1 (ICAM-1) induced by *Porphyromonas gingivalis* W83 was inhibited by the NF-κB inhibitor, but not by the p38 inhibitor. Human umbilical vein endothelial cells (HUVECs) were pretreated for 30 min with either the nuclear factor-kappaB (NF-κB) inhibitor (MG132) or the p38 inhibitor (SB 203580), and then infected with *P. gingivalis* W83 for 8 h; alternatively, HUVECs were incubated with medium only as a control. Infection with *P. gingivalis* W83 induced ICAM-1 expression compared with the control. Cells stained with fluorescein isothiocyanate-labelled ICAM-1 are shown in green (upper line), nuclei labeled with propidium iodide are artificially colored red (middle line) and merged images are depicted in the lower line. Representative pictures (of three independent experiments; magnification ×400) are shown.

It has been reported that periodontal infection is involved in the initiation and progression of atherosclerosis (26). Oral *P. gingivalis* and its components are transmitted to the serum as a consequence of the infection and may have a direct effect on vascular endothelial cells, which can lead to a systemic

inflammatory process in the blood vessels and heart (27). ICAM-1 is crucial during the binding of monocytes to endothelium and is subsequently recruited into subendothelial areas, which are involved in the early steps of atherogenesis (28). In this study, infection with *P. gingivalis* caused sustained – not acute – expression of ICAM-1, in line with the report of Nakamura (3) that chronic *P. gingivalis* infection may facilitate monocyte recruitment to vascular endothelium through sustained upregulation of ICAM-1. As atherosclerosis is characterized by a chronic, rather than an acute, inflammatory process, we speculate that the *P. gingivalis*-mediated overexpression of ICAM-1 might underlie a mechanistic basis linking *P. gingivalis* to inflammatory atherosclerotic processes. In order to prevent potential atherosclerotic diseases, periodontitis patients have been advised to maintain excellent oral hygiene and suppress further *P. gingivalis* infection as far as possible.

P. gingivalis produces a broad array of potential virulence factors involved in tissue destruction (25). It was documented that invasive *P. gingivalis* strains and fimbria induced endothelial cells to produce ICAM-1 (29), while Walter *et al*. (21) showed that the induction was independent of the expression of bacterial fimbriae. Mao's (30) study indicated that some heat-stable proteins might be the stimulatory factors responsible for inducing ICAM-1 expression in endothelial cells. It has been reported that the ability of *P. gingivalis* to form abscesses plays an important role in the initiation and progression of periodontal disease. Based on the ability to form abscesses, *P. gingivalis* are divided into avirulent (e.g. ATCC 33277) and virulent (e.g. W83) strains (12,13). In the present study, W83 was found to increase ICAM-1 expression more strongly than ATCC 33277. Interestingly, W83 was less invasive than ATCC 33277, as shown in our results. However, definitive factors specific to virulent strains, or factors involved in abscess formation, have not yet been identified. The biochemical profiles of potential virulence factors, such as lipopolysaccharide (LPS) and proteinases, are almost the same in avirulent and virulent strains of *P. gingivalis* (13). Further investigation of the differences between the two stains may offer insight into the mechanisms of *P. gingivalis* pathogenesis because approximately 93% homology is present between ATCC 33277 and W83. In addition, it is important to note that *P. gingivalis* produces a variety of different virulence factors. Whether whole bacteria, specific virulence factors, or a combination of both, primarily interacts with endothelial cells remains to be determined.

To clarify the signaling pathway that regulates ICAM-1 expression in endothelial cells infected with *P. gingivalis*, we investigated the role of specific inhibitors of p38 and NF-κB. NF-κB has been implicated to play a vital role in the initiation and development of inflammatory diseases (6,7,31). Here, it was demonstrated that infection of endothelial cells with *P. gingivalis* induced the degradation of IkBα, the nuclear translocation of NF-κB p65 and the overexpression of ICAM-1 in endothelial cells, which was attenuated by the proteasome inhibitor MG132. The mechanism by which *P. gingivalis* activates the NF-κB pathway is unknown, but kinases upstream of IκBα may be activated, because IκBα degradation is one of the earliest end points that is affected by *P. gingivalis*. We observed that there is an increase in IκBα at 90 min. The rapid degradation of IκB results in the migration of the NF-κB complex to the nucleus and binding to the promoter region of target genes, while subsequent regeneration of IκB could terminate the activation of NF-κB (32). Voraberger *et al*. (7) first described the presence of NF-κB-binding sites upstream of the ICAM-1 translational start site, strongly indicating that NF-κB p65 is part of the intracellular signal-transduction pathway leading to *P. gingivalis*-induced production of ICAM-1 and that NF-κB inhibitors may be a candidate for preventing infection with *P. gingivalis*.

p38 is one of the three best-characterized MAPK cascade signaling pathways. Walter's (21) study demonstrated that infection with *P. gingivalis* may induce a rapid increase of p38 phosphorylation in endothelial cells. Wu *et al*. (33) reported that the activity of p38 might be initiated by *P. gingivalis*-conditioned medium. In the present study, we demonstrated that viable ATCC 33277 and W83 could stimulate the phosphorylation of p38. However, the responses of virulence factors in different cell types appear to be quite diverse. For example, LPS, one of the virulence factors in *P. gingivalis*, up-regulated TNF-α production in macrophages via various pathways, including p38 (34). Another example showed that *P. gingivalis* LPS did not activate p38 in endothelial cells but did so in human monocytes (35). In the present study, inhibition of the p38 pathway had no effect on ICAM-1 production. It was concluded that p38 signaling pathways may be initiated by *P. gingivalis*, but further investigations are required to elucidate which virulence factors of *P. gingivalis* are also required.

In summary, the present study indicated that *P. gingivalis* could up-regulate ICAM-1 expression via two signal-transduction pathways, including p38 phosphorylation, degradation of IκBα and NF-κB translocation. Further studies should be performed to delineate the detailed roles of other signaling pathways involved in *P. gingivalis*-induced inflammatory activation.

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