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Porphyromonas gingivalis-induced inflammatory mediator profile in an *ex vivo* human whole blood model

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Summary

Periodontitis is characterized by an accumulation of inflammatory cells in periodontal tissue and subgingival sites. Leukocytes play a major role in the host response to *Porphyromonas gingivalis*, a major aetiological agent of chronic periodontitis. Secretion of high levels of inflammatory mediators, including cytokines and prostaglandins, by leucocytes is believed to contribute to periodontal tissue destruction. The aim of this study was to investigate the inflammatory response of an *ex vivo* whole blood model to *P. gingivalis* stimulation. The production of interleukin-1 beta (IL-1β), IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), IFN-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), Regulated on Activation Normal T cell Expressed and Secreted (RANTES) and prostaglandin E_2 (PGE_2) were quantified by enzyme-linked immunosorbent assays. *P. gingivalis* induced the secretion of the pro-inflammatory cytokines IL-1β, TNF-α, IL-6 and IFN-γ, the chemokines IL-8, RANTES and MCP-1 and the inflammatory mediator PGE_2 in an *ex vivo* human whole blood model. The secretion levels were dependent on the strain and the infectious dose used. While the mediator profiles were comparable between six healthy subjects, a high interindividual variability in the levels of secreted mediators was observed. This study supports the view that *P. gingivalis*, by inducing high levels of inflammatory mediators from a mixed leucocyte population, can contribute to the progression of periodontitis.

Keywords: cytokine, periodontitis, *Porphyromonas gingivalis*, prostaglandin, whole blood model

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Introduction

Periodontitis is a multifactorial polymicrobial infection characterized by a destructive inflammatory process affecting the tooth supporting tissues and resulting in periodontal pocket formation, alveolar bone resorption, and eventually tooth loss. While human subgingival plaque harbours some 500 bacterial species [1], evidence points to *Porphyromonas gingivalis*, a Gram-negative anaerobic bacterium, as the major aetiological agent of chronic periodontitis [2]. *P. gingivalis* produces a broad range of virulence factors, including cysteine proteinases, lipopolysaccharides (LPS) and fimbriae (FimA), which contribute to the host inflammatory response. Variations of FimA have been associated with pathogenesis of *P. gingivalis*. Indeed, a majority of periodontitis patients harbour type II *fimA* strains, followed by type IV *fimA* strains while type I *fimA* strains are mainly isolated from periodontal healthy subjects [3,4]. The local host response to periodontopathogens and their products includes the recruitment of polymorphonuclear neutrophils and macrophages and the subsequent release of inflammatory mediators, cytokines and matrix metalloproteinases, which are thought to play crucial roles in the pathogenesis of periodontal disease. Indeed, the continuous, high secretion of various cytokines and inflammatory mediators, including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor alpha (TNF-α) and prostaglandin E_2 (PGE_2) by host cells following stimulation by periodontopathogens can modulate periodontal tissue destruction [5,6].

While all types of blood cells are present in normal gingival connective tissue [7], an accumulation of inflammatory cells is observed during periodontitis [8]. Moreover, the number of leucocytes migrating to the gingival sulcus and

periodontal pockets increases during the progression of inflammation [9,10]. While most neutrophils recruited into the gingival tissues migrate to the gingival epithelium and sulcus, the majority of the mononuclear cells, including monocytes/macrophages, T lymphocytes and B cells persist in the connective tissue and form the local cell infiltrate [11]. *P. gingivalis* has been shown to penetrate an engineered human oral mucosa *in vitro* [12] and has been observed within gingival tissues *in vivo* [13,14], indicating that it may reach deeper structures of connective tissues. In addition, *P. gingivalis* can actively invade endothelial cells [15], thus supporting the view that the well-vascularized connective tissues of the periodontium may allow *P. gingivalis* to enter the bloodstream. This indicates that *P. gingivalis* may encounter a heterogeneous leucocyte population depending on where it is in periodontal tissue. In order to take into consideration the interactions between different immune cell types, this study used an *ex vivo* human whole blood model to characterize the inflammatory response to *P. gingivalis* stimulation. This model has the advantage of providing a comprehensive view of the cytokine and PGE_2 responses mediated by this periodontopathogen. In addition, the *ex vivo* whole blood model reduces the confounding factors that may be associated with the isolation procedures, such as activation of isolated cells, and the risk of contamination with biological stimulants such as lipopolysaccharides. Moreover, whole blood represents a more physiological environment for investigating the production of inflammatory mediators in response to challenges by bacterial cells or their components since cellular interactions are preserved in the presence of various plasma proteins (soluble CD14, LPS-binding proteins, hormones, soluble cytokine receptors, etc.). In this study, the *ex vivo* human whole blood model was infected with different strains of *P. gingivalis* at various concentrations and analysed for pro-inflammatory cytokine, chemokine and PGE_2 production.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains used in this study were *P. gingivalis* ATCC 33277, ATCC 53977 and W83. The bacteria were grown in Todd Hewitt broth (THB; BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 0·001% hemin and 0·0001% vitamin K. The cultures were incubated at 37 °C under anaerobic conditions ($N_2 : H_2 : CO_2$/80 : 10 : 10).

Whole blood collection

Samples of venous blood were collected from the antecubital vein of six healthy subjects using the Vacutainer™ system and sterile endotoxin-free blood collection tubes containing 150 IU sodium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Informed consent was obtained from all donors prior to the experiments, and the protocol was approved by the ethics committee of Université Laval. Healthy subjects were free of any clinical symptoms of infections, were non-smokers, and had clinically healthy gingiva with no periodontal pockets ≥ 3 mm in depth. Haematological analyses of whole blood samples performed at the Centre Hospitalier de l'Université Laval (Quebec City, Canada) showed that all the subjects had normal leucocyte counts. Whole blood samples were diluted 1 : 3 with RPMI-1640 medium (HyClone Laboratories, Logan, UT, USA) and divided into 6-ml aliquots in 6-well plates.

Whole blood stimulation

Bacteria were grown to the early stationary phase (24 h) at 37°C under anaerobiosis. The cells were harvested by centrifugation at 11 000× *g* for 10 min and suspended in RPMI-1640 medium to a concentration of 1×10^9 bacteria/ml as determined using a Petroff-Hausser counting chamber. The bacterial suspensions were added to the diluted whole blood samples to obtain a final concentration of 10^6 and 10^7 bacteria per ml. The infected whole blood samples were incubated at 37 °C in a 5% CO_2 humidified atmosphere with occasional gentle shaking. After 6 h, the samples were centrifuged at 2000× *g* for 5 min. The supernatants were collected, centrifuged at 11 000 ***g*** for 10 min to eliminate bacteria, and stored at −20 °C until used. Control blood was incubated in the absence of bacteria.

Determination of bacterial viability

Infected whole blood cultures were harvested at times 0 and 6 h. The first time point (0 h) was considered as the 100% viability control. The blood cells were lysed by the addition of sterile distilled water. After 3 min, the bacteria were sedimented at 11 000 ***g*** for 10 min and resuspended in THB. The bacterial suspensions were 10 fold serially diluted (10^{-1}–10^{-8}) in THB culture medium. The bacterial cultures were incubated at 37 °C under anaerobic conditions ($N_2 : H_2 : CO_2$/80 : 10 : 10). The absence of bacterial growth after four days of incubation was an indication that no viable bacteria were present in the suspension.

Determination of cytokine production

Commercial enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN, USA) were used to quantify IL-1β, IL-6, IL-8, TNF-α and Regulated on Activation Normal T cell Expressed and Secreted (RANTES) in cell-free blood supernatants according to the manufacturer's protocols. The absorbance at 450 nm was read using a microplate reader (model 680; Bio-Rad Laboratories, Mississauga, ON, Canada) with the wavelength correction set at 550 nm. Cytokine concentrations were determined in triplicate using a standard curve prepared for each assay. The sensitivities of

the commercial ELISA kits were 31·2 pg/ml for IL-8, 15·6 pg/ml for RANTES and TNF-α, 9·3 pg/ml for IL-6 and 3·9 pg/ml for IL-1β. The concentrations of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-12 (IL-12p70), interferon gamma (IFN-γ), IFN-γ-inducible protein 10 (IP-10) and monocyte chemoattractant protein-1 (MCP-1) in the cell-free blood supernatants were determined by the Upstate testing service using Luminex® multiplex technology (Upstate, Lake Placid, NY, USA).

Determination of PGE_2 production

A competitive enzyme immunoassay (EIA) was performed on the cell-free blood supernatants according the manufacturer's protocol (Cayman Chemical Company, Ann Arbor, MI, USA). The absorbance at 415 nm was read using a microplate reader and PGE_2 concentrations were determined in triplicate. The detection level of the PGE_2 kit was 35 pg/ml.

Degradation of human serum albumin by *P. gingivalis*

Degradation of albumin was monitored to determine whether *P. gingivalis* proteinases are active in the model. Following the 6 h incubation period, uninfected and infected (10^7 bacteria per ml) whole blood supernatants were diluted 1 : 50 in PBS. The degradation of the albumin was monitored by sodium dodecyl sulphate-12% polyacrylamide gel electrophoresis (SDS-PAGE) using the method of Laemmli [16]. The proteins were transferred to a nitrocellulose membrane and reacted with an alkaline phosphatase-conjugated rabbit polyclonal antialbumin antibody (IgG fraction, 1 : 10 000 dilution) for 2 h. Albumin and albumin-related fragments were detected using nitroblue tetrazolium and 5-bromo-chloro-3-indolyl-phosphate in N-N-dimethylformamide diluted in 100 mM Tris-HCl (pH 8·5).

Statistical analysis

Differences between whole blood infections (bacterial strains and infectious doses) were determined using a two-way randomized block design analysis of variance. Statistical analyses of differences between the cytokine levels of uninfected and infected blood were performed using the Student's t paired test. Interactions between variables were studied using Pearson's correlation. Data were considered significant at a P-value < 0·05.

Results

Influence of strains on the *P. gingivalis*-induced cytokine response in human whole blood

Preliminary experiments were first carried out with the blood of only one individual to establish the conditions for determining the capacity of *P. gingivalis* to stimulate cytokine production in the whole blood model (data not shown). Human whole blood was stimulated with *P. gingivalis* ATCC 33277 at infectious doses ranging from 1×10^4 to 5×10^8 bacteria/ml for 6 and 24 h. The whole blood supernatants were then analysed for IL-1β, IL-6, IL-8, TNF-α and RANTES production. When the whole blood was challenged with a *P. gingivalis* concentration of 10^4 or 10^5 bacteria/ml, only a minor cytokine response was detected. The highest IL-1β, TNF-α and IL-6 responses were observed after a 6 h incubation at an infectious dose of 10^7 cells/ml. A higher infectious dose or a 24 h incubation period resulted in increased levels of IL-8 and RANTES whereas the concentrations of the other cytokines were not affected or decreased. Based on the preliminary analysis, an infectious dose of 10^6 and 10^7 bacteria/ml and an incubation period of 6 h were selected for further experiments.

Whole blood from six healthy volunteers was challenged with three strains of *P. gingivalis* (ATCC 33277, ATCC 53977 and W83) at an infectious dose of 10^6 or 10^7 bacteria/ml (Table 1). The viability of *P. gingivalis* in whole blood after a 6 h incubation period was estimated at approximately 10%. To determine whether *P. gingivalis* proteinases were active in the whole blood despite the presence of various plasma proteinase inhibitors, the degradation of albumin, which is the most abundant serum protein, was studied by SDS-PAGE/Western immunoblotting. The albumin was poorly degraded by *P. gingivalis* during the incubation period, suggesting that the proteinases of this bacterium were only weakly active in the whole blood model (data not shown). The amounts of cytokine produced in the model under the various infective conditions were evaluated using a two-way randomized block design analysis of variance. In all cases, the interactions between the two variables (strain and dose) were not significant ($P > 0{\cdot}05$). Furthermore, for all analyses the homogeneity of variance and the normality assumptions were met. All the *P. gingivalis* strains tested induced a significant increase in IL-1β, IL-6, TNF-α, IL-8 and RANTES levels. These cytokine and chemokine responses were bacterial dose-dependent ($P < 0{\cdot}05$). There were significant differences ($P < 0{\cdot}05$) in IL-8 and RANTES responses between *P. gingivalis* strains. *P. gingivalis* ATCC 53977 induced a higher chemokine response than *P. gingivalis* ATCC 33277 and W83. IL-1β and TNF-α responses were also dependent on the *P. gingivalis* strain used ($P < 0{\cdot}05$) with *P. gingivalis* ATCC 33277 inducing lower levels of IL-1β and TNF-α secretion than *P. gingivalis* ATCC 53977 and *P. gingivalis* W83. In addition, *P. gingivalis* ATCC 53977 was the most potent inducer of TNF-α production. While all the combinations of cytokines tested were positively correlated, the strongest correlations were observed for IL-1β/TNF-α ($r = 0{\cdot}91$) and IL-6/IL-8 ($r = 0{\cdot}88$).

Table 1. Secretion of IL-8, RANTES, TNF-α, IL-1β and IL-6 in human whole blood from six healthy individuals following stimulation with *P. gingivalis* ATCC 33277, *P. gingivalis* ATCC 53977 and *P. gingivalis* W83 at final concentration of 10^6- or 10^7 bacteria/ml for 6 h.

Bacterial strain added to the whole blood	Amount of cytokine detected (pg/ml)				
	IL-8	RANTES	TNF-α	IL-1β	IL-6
None	ND	2234 ± 2214	ND	ND	ND
P. gingivalis ATCC 33277					
10^6cells/ml	4 810 ± 5821	4634 ± 3705	3 455 ± 2171	2653 ± 1989	4345 ± 4041
10^7cells/ml	11 675 ± 7886	5720 ± 3767	7 316 ± 2493	6570 ± 3549	8024 ± 2951
P. gingivalis ATCC 53977					
10^6cells/ml	8 281 ± 8293	5276 ± 3544	6 528 ± 3460	5332 ± 3981	5675 ± 3519
10^7cells/ml	11 773 ± 7740	6544 ± 3835	10 091 ± 2389	8943 ± 3180	7801 ± 3210
P. gingivalis W83					
10^6cells/ml	5 445 ± 5885	4218 ± 3982	5 142 ± 2467	4921 ± 3726	5389 ± 3849
10^7cells/ml	10 919 ± 6881	5831 ± 3262	8 412 ± 2886	8024 ± 3229	7531 ± 3361

The cytokine concentrations in the supernatants of whole blood cultures were assessed by ELISA. Control whole blood samples were incubated without bacteria. The data are the means ± standard deviations (SD) of triplicate assays of all six blood samples. ND: Not detected (below the detection level).

Detailed profiles of cytokine and PGE_2 production induced by *P. gingivalis* ATCC 33277 in human whole blood

The inflammatory mediator profile induced by *P. gingivalis* in the *ex vivo* human whole blood model was characterized in more detail. Whole blood from six healthy volunteers was stimulated by *P. gingivalis* ATCC 33277 and analysed for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-12p70, IFN-γ, TNF-α and PGE_2 production. As shown in Table 2, additional mediators induced by *P. gingivalis* were identified. *P. gingivalis* induced a significant increase ($P < 0{\cdot}05$) in IL-1β, TNF-α, IL-6, IFN-γ and PGE_2 secretion. While IL-10 secretion was enhanced in some subjects by *P. gingivalis*, there was no significant induction of this cytokine for the study population. A high interindividual variability in the amounts of inflammatory mediators secreted following the *P. gingivalis* challenge was observed for all the mediators tested. The level of TNF-α secreted was positively correlated with that of IL-1β ($r = 0{\cdot}94$) and IL-6 ($r = 0{\cdot}90$).

Detailed profile of chemokine production induced by *P. gingivalis* ATCC 33277 in human whole blood

The detailed chemokine response induced by *P. gingivalis* ATCC 33277 in human blood from the six healthy volunteers is presented in Table 3. *P. gingivalis* induced a significant ($P < 0{\cdot}05$) increase in IL-8, RANTES and MCP-1 secretion

Table 2. Secretion of IL-1β, TNF-α, IL-6, IFN-γ, IL-10 and PGE_2 in human whole blood from six healthy individuals following stimulation with 10^7 cells/ml of *P. gingivalis* ATCC 33277 for 6 h.

Individual	*P. gingivalis* ATCC 33277 (cells/ml)	PGE_2 (pg/ml)	Amount of cytokine secreted (pg/ml)				
			IL-1β	TNF-α	IL-6	IFN-γ	IL-10
1	0	ND	ND	ND	ND	77 ± 22	37 ± 18
	10^7	133 ± 11	1 478 ± 39	3 656 ± 233	6 005 ± 137	115 ± 26	110 ± 8
2	0	ND	ND	ND	ND	143 ± 20	36 ± 15
	10^7	67 ± 8	4 334 ± 234	5 616 ± 330	5 779 ± 293	304 ± 21	44 ± 11
3	0	ND	ND	ND	ND	65 ± 3	43 ± 9
	10^7	311 ± 23	7 688 ± 621	9 377 ± 276	10 556 ± 154	132 ± 23	307 ± 16
4	0	ND	ND	ND	ND	60 ± 6	29 ± 1
	10^7	96 ± 13	10 416 ± 316	7 189 ± 413	5 651 ± 179	195 ± 43	77 ± 8
5	0	ND	ND	ND	ND	68 ± 13	26 ± 2
	10^7	84 ± 7	10 325 ± 223	10 557 ± 201	7 435 ± 115	2239 ± 71	49 ± 7
6	0	ND	ND	ND	ND	102 ± 5	41 ± 13
	10^7	263 ± 16	5 180 ± 79	7 502 ± 162	12 721 ± 146	208 ± 35	287 ± 31
Mean	0	ND	ND	ND	ND	85 ± 31	35 ± 6
	10^7	159 ± 102*	6 570 ± 3549*	7 316 ± 2493*	8 024 ± 2951*	532 ± 838*	145 ± 119

The cytokine concentrations in the supernatants of the whole blood cultures were assessed by ELISA. PGE_2 concentration was assessed by EIA. Control whole blood samples were incubated without bacteria. The data are the means ± SD of triplicate assays. *$P < 0{\cdot}05$ compared to an uninfected control. ND: Not detected (below the detection level).

Table 3. Secretion of IL-8, RANTES, MCP-1 and IP-10 in human whole blood from six healthy individuals following stimulation with 10^7 bacteria/ml of *P. gingivalis* ATCC 33277 for 6 h.

Individual	*P. gingivalis* ATCC 33277 (cells/ml)	Amount of chemokine secreted (pg/ml)			
		IL-8	RANTES	MCP-1	IP-10
1	0	ND	565 ± 49	73 ± 5	443 ± 31
	10^7	6 628 ± 408	2 848 ± 180	487 ± 15	1 395 ± 25
2	0	ND	437 ± 57	97 ± 28	425 ± 50
	10^7	2 845 ± 123	3 201 ± 101	1616 ± 206	15 257 ± 316
3	0	ND	6 207 ± 290	60 ± 13	447 ± 38
	10^7	20 981 ± 441	12 544 ± 237	3432 ± 211	1 025 ± 73
4	0	ND	1 278 ± 79	86 ± 6	181 ± 24
	10^7	9 864 ± 248	3 986 ± 194	3002 ± 439	9 150 ± 217
5	0	ND	3 383 ± 201	44 ± 8	187 ± 27
	10^7	7 891 ± 322	7 708 ± 192	2323 ± 135	12 431 ± 227
6	0	ND	1 535 ± 43	103 ± 20	339 ± 31
	10^7	21 842 ± 565	4 036 ± 141	2855 ± 872	950 ± 231
Mean	0	ND	2 234 ± 2214	77 ± 22	337 ± 124
	10^7	11 675 ± 7886*	5 720 ± 3767*	2285 ± 1080*	6 701 ± 6410

The chemokine concentrations in the supernatants of whole blood cultures were assessed by ELISA. Control whole blood samples were incubated without bacteria. The data are the means ± SD of triplicate assays. *$P < 0{\cdot}05$ compared to an uninfected control. ND: Not detected (below the detection level).

by the leucocytes in the whole blood from all six subjects. While *P. gingivalis* induced a large increase in IP-10 levels in some blood samples (2, 4 and 5), there was no statistically significant induction of IP-10 in the study population. There was a high interindividual variability of the amounts of chemokine secreted. The strongest correlations between cytokines were observed for IL-6/IL-8 ($r = 0{\cdot}94$) while the level of MCP-1 was strongly positively correlated with that of IL-1β ($r = 0{\cdot}92$), TNF-α ($r = 0{\cdot}93$) and IL-6 ($r = 0{\cdot}89$).

Discussion

P. gingivalis interacts with various types of leucocyte that may differ depending on where it is in the periodontal tissues and the phase of the disease process. In order to characterize the overall inflammatory response of mixed leucocyte populations to *P. gingivalis*, whole blood from six subjects was stimulated by *P. gingivalis* and analysed for cytokine and PGE_2 production. The advantage of the *ex vivo* whole blood model is that it contains all the relevant cell populations that are likely to come in contact with periodontopathogens during periodontitis. In addition, it takes into consideration the complex cell/cell interactions that occur *in vivo*. This model is highly relevant for studying periodontitis since the gingival crevicular fluid, which bathes the periodontal pocket, is derived from gingival capillary beds and contains resident and emigrating inflammatory cells.

IL-1β and TNF-α are determinants of the progression of periodontitis [17]. Local inhibition of these two mediators in periodontal tissues significantly reduces the inflammatory response and bone loss in ligature-induced periodontitis in monkeys [18]. *P. gingivalis* showed a strong capacity to induce IL-1β and TNF-α secretion by the mixed leucocyte populations in whole blood, suggesting that it can activate various periodontal tissue destruction pathways mediated by these cytokines. We observed that the levels of IL-1β and TNF-α produced by the whole blood model following *P. gingivalis* challenge were strongly correlated. Stashenko *et al.* also reported a highly significant correlation between the levels of IL-1β and TNF-α in the periodontal tissues of diseased sites, suggesting the coordinated expression of these two mediators [19]. IL-6 plays an important role in regulating the immune response to periodontal pathogens and is notably responsible for the differentiation of osteoclasts and activated B cells into immunoglobulin-secreting plasma cells. IL-6 levels increase in the diseased gingiva of patients with periodontitis compared to periodontally healthy subjects [20]. In the whole blood model used in this study, IL-6 secretion was highly up-regulated by all the *P. gingivalis* challenges, suggesting that this bacterium likely plays a determinant role in IL-6 induction during periodontitis. In addition, IL-6 participates in the recruitment of leucocytes to sites of inflammation by increasing the local production of chemokines such as MCP-1 and IL-8 [21]. This was confirmed by our data, which showed that IL-6 levels were highly positively correlated with MCP-1 and IL-8 levels.

Selective chemokine-mediated recruitment of different cell types to the gingival tissue is potentially involved in the immunopathogenesis of periodontitis. IL-8, RANTES, MCP-1 and IP-10 are chemokines found in diseased periodontal tissues [22,23]. MCP-1 is a potent chemoattractant for monocytes and macrophages [24] whereas IP-10 attracts monocytes and activated T lymphocytes to inflammatory foci [25]. IL-8 and RANTES are involved in the recruitment of neutrophils, eosinophils, monocytes and TH_1 cells to infected sites [26]. RANTES is also a chemotactic factor for

osteoclasts [27]. Our results showed that *P. gingivalis* possesses a strong capacity to induce IL-8, RANTES and MCP-1 production by a mixed leucocyte population. This suggests that *P. gingivalis* can trigger a dense infiltration of various inflammatory cells in periodontal tissue through the induction of these chemokines, which in turn may induce a strong inflammatory reaction at local sites, resulting in the destruction of tissue and alveolar bone. PGE_2 is a potent stimulator of bone resorption [28] and is associated with attachment loss [29]. The secretion of this inflammatory mediator by the whole blood model was enhanced following *P. gingivalis* challenges, suggesting that *P. gingivalis* may cause alveolar bone resorption by stimulating PGE_2 production by leucocytes.

Among the large array of virulence factors produced by *P. gingivalis*, fimbriae (Fim A), which are filamentous structures on the cell surface, are important stimulators of an inflammatory response [30,31]. There is a close relationship between *P. gingivalis* clones with specific *fimA* fimbriae and periodontitis. Most patients with periodontitis harbour type II *fimA* strains, while type I *fimA* strains are mainly isolated from periodontal healthy subjects [3,4]. Sugano *et al.* [32] recently reported that a *P. gingivalis* strain with type II *fimA* fimbriae induced statistically high levels of IL-1β, IL-8, IL-12 and TNF-α mRNA in the U937 macrophage-like human cell line whereas a *P. gingivalis* strain with type I *fimA* fimbriae caused no significant induction of these cytokines. Interestingly, in a mouse abscess model, type II *fimA P. gingivalis* induced greater inflammatory changes than type I *fimA P. gingivalis* [33]. In the study reported here, the ability of three distinct phenotypes of *P. gingivalis* strains, classified as type I *fimA* (ATCC 33277), type II *fimA* (ATCC 53977) and type IV *fimA* (W83), to induce cytokine production in the whole blood model was investigated. *P. gingivalis* ATCC 53977, which produces type II *fimA* fimbriae, induced significantly higher levels of IL-8, MCP-1 and TNF-α than the other strains tested. Type II *fimA P. gingivalis* ATCC 53977 also induced higher amounts of IL-1β than type I *fimA P. gingivalis* ATCC 33277. These results are in agreement with previous studies [32,33] suggesting that type II *fimA P. gingivalis* strains are more potent inducers of cytokine production by leucocytes than type I *fimA P. gingivalis* strains. However, phenotypic variations other than the type of fimbriae may exist between the *P. gingivalis* strains used and it was recently suggested that FimA may not play a prominent role in the innate immune response to *P. gingivalis* [34]. One should not exclude the possibility that the strains of *P. gingivalis* used in our study may respond in a different way if whole blood is obtained from patients with active periodontitis.

While the cytokine profiles induced by *P. gingivalis* in the whole blood from the six subjects were similar, we noted a high interindividual variability in the levels of cytokines secreted. This could be related, at least in part, to cytokine gene polymorphisms. While reports of genetic polymorphisms associated with periodontitis are increasing, further studies are required to clearly determine the genetic basis of periodontitis [35]. The interindividual variability in cytokine levels observed may also be the consequence of a more or less recent microbial challenge or subinfectious state in certain subjects, resulting in leucocyte preactivation. These quantitative differences in intersubject cytokine responses could explain, at least in part, the various degrees of susceptibility to periodontal infections. Not everyone is equally susceptible to periodontitis [36] and susceptibility also varies greatly between individuals who harbour the same pathogenic microflora [37]. More and more evidence is pointing to the host response to a bacterial challenge as a major determinant of periodontitis susceptibility. In this regard, the whole blood model appears promising for studying host responses to periodontopathogens in different subject categories and may provide interesting data for characterizing variations in the immune response of periodontitis patients.

Transient bacteraemias occur from periodontal infection, surgical dental procedures [38], periodontal probing [39] and mastication [40]. Numerous studies suggested that periodontopathogens, such as *P. gingivalis*, may be involved in the initiation and progression of artherosclerosis and subsequent coronary disease [41]. This was supported by identification of *P. gingivalis* in artherosclerotic plaques [42,43] and in aortic tissue [44]. In addition, *P. gingivalis* infection accelerates artherosclerosis in animal model [45,46]. Both systemic and localized inflammation in the arteries contribute to the initiation and progression of artherosclerosis [47]. As reported here, *P. gingivalis* infection stimulates circulating immune cells to produce inflammatory mediators, such as IL-1β, TNF-α, IL-6, IFN-γ, MCP-1 and PGE_2, which are known to be implicated in artherosclerosis [41,47,48]. This suggest that *P. gingivalis* may contribute to the development of artherosclerosis.

In summary, our study showed that *P. gingivalis* induces the secretion of the pro-inflammatory cytokines IL-1β, TNF-α, IL-6 and IFN-γ, the chemokines IL-8, RANTES and MCP-1 and the inflammatory mediator PGE_2 in the *ex vivo* human whole blood model. The level of secretion of these inflammatory mediators was dependent on the bacterial strain and the infectious dose used. This study supports the view that *P. gingivalis* can contribute to the progression of periodontitis by inducing the production of high levels of inflammatory mediators from a mixed leucocyte population.

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