Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 823–829 doi: 10.1111/j.1600-051X.2009.01462.x

Differential cytokine expression by human dendritic cells in response to different *Porphyromonas gingivalis* capsular serotypes

Rolando Vernal,[1,2] Rubén León,[1,3] Augusto Silva,[2] Arie J. van Winkelhoff,[4] Jose A. Garcia-Sanz[2]* and Mariano Sanz[3]*

[1]Department of Conservative Dentistry, Dental School, Universidad de Chile, Santiago de Chile, Chile; [2]Department of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas, CIB-CSIC, Madrid, Spain; [3]Graduate Periodontology, Dental School, Universidad Complutense de Madrid, Madrid, Spain; [4]Department of Medical and Oral Microbiology, Center for Dentistry and Oral Hygiene, University Medical Center Groningen, State University of Groningen, Groningen, The Netherlands

Vernal R, León R, Silva A, van Winkelhoff AJ, Garcia-Sanz JA, Sanz M. Differential cytokine expression by human dendritic cells in response to different Porphyromonas gingivalis capsular serotypes. J Clin Periodontol 2009; 36: 823–829. doi:10.1111/j.1600-051X.2009.01462.x.

Abstract

Aim: Capsular polysaccharides play an important role in the virulence of Gram-positive and Gram-negative bacteria. In *Porphyromonas gingivalis*, six serotypes have been described based on capsular antigenicity and its pathogenicity has been correlated both *in vitro* and in animal models. This study aimed to investigate the differential response of human dendritic cells (DCs) when stimulated with different *P. gingivalis* capsular serotypes.

Materials and Methods: Using different multiplicity of infection (MOI) of the encapsulated strains K1–K6 and the non-encapsulated K^- strain of *P. gingivalis*, the mRNA expression levels for interleukin (IL)-1β, IL-2, IL-5, IL-6, IL-10, IL-12, IL-13, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and TNF-β in stimulated DCs were quantified by real-time reverse transcription-polymerase chain reaction.

Results: All *P. gingivalis* capsular serotypes induced a T-helper type 1 (Th1) pattern of cytokine expression. K1- and K2-stimulated DCs expressed higher levels of IL-1β, IL-6, IL-12p35, IL-12p40, and IFN-γ and at lower MOI than DCs stimulated with the other strains.

Conclusions: These results demonstrate a differential potential of *P. gingivalis* capsular serotypes to induce DC responses and a higher capacity of strains K1 W83 and K2 HG184 than other K serotypes to trigger cytokine expression.

Key words: capsular serotypes; cytokines; dendritic cells; *Porphyromonas gingivalis*

Accepted for publication 26 June 2009

Conflict of interest and source of funding statement

The authors declare no competing financial interests.

This investigation has been supported by grants from the Spanish Ministry of Science and Education (SAF2007-63631) and the Spanish Ministry of Health (FIS-060181). R. V. is a recipient of the fellowship CONICYT-26080046 from the Chilean Government.

*Both authors must be considered senior authors.

Periodontitis is a chronic infection elicited by bacteria residing at the subgingival biofilm. Although bacteria may cause direct tissue destruction, their pathogenicity relies mainly on the activation of host immuno-inflammatory mechanisms. These bacterial–host interactions at the biofilm–periodontium interface trigger the synthesis of cytokines and inflammatory mediators that promote the release of enzymes and bone-associated molecules that finally induce the alterations of the connective tissue metabolism and the destruction of the tooth-supporting alveolar bone characteristic of periodontitis (Offenbacher 1996). Only a limited number of bacterial species, from >500 identified in the subgingival biofilm, have been associated with periodontitis (Socransky & Haffajee 1994, van Winkelhoff et al. 2002, Curtis et al. 2005). Among these, *Porphyromonas gingivalis*, a strict anaerobic Gram-negative pathogen, has been strongly associated with periodontitis, playing an important role in its aetiology (van Winkelhoff et al. 1988, Slots & Ting 1999, Herrera et al. 2008).

P. gingivalis expresses a number of virulence factors that may contribute to periodontitis pathogenesis (Curtis et al. 2005). Similar to other Gram-negative bacteria, *P. gingivalis* can synthesize an extracellular capsule, composed of negatively charged polysaccharides, which enables this bacteria to withstand phagocytosis (Schifferle et al. 1989, Farquharson et al. 2000). Six different serotypes based on capsular (K) antigens have been described for *P. gingivalis* and designated K1 (strain W83), K2 (strain HG184), K3 (strain A7A1-28), K4 (strain ATCC-49417), K5 (strain HG1690), and K6 (strain HG1691) (van Winkelhoff et al. 1993, Laine et al. 1996). When subcutaneously inoculated in experimental animals, all six K serotypes were highly virulent, causing a phlegmonous infection, often accompanied by ulceration and/or necrosis, whereas the non-encapsulated (K^-) strain 381 was less virulent, causing localized abscesses (Laine & van Winkelhoff 1998). On murine macrophages, *P. gingivalis* strains K1 W50 and W83 elicited, in a dose- and time-dependent manner, a more potent chemokine expression than the other K serotypes (d'Empaire et al. 2006).

The type of capsule present might have a significant influence on the host–pathogen interactions during infection. Because the serotype K1 of *P. gingivalis* plays a role in inflammatory cell activation and recruitment during the innate immune response (d'Empaire et al. 2006), it can be speculated that it might also play a relevant role in the induction of adaptive immune responses leading to connective tissue and bone destruction during periodontal disease. Dendritic cells (DCs) link the innate and adaptive immune responses and, as specialized antigen-presenting cells, are able to prime naïve T-cells, polarizing them towards T-helper type 1 (Th1), Th2, or Th17 phenotypes, and thus might be able to influence the periodontitis phenotype (Cutler & Jotwani 2006). Hence, we hypothesized that *P. gingivalis* K serotypes trigger differential DCs responses and distinct cytokine expression profiles.

Materials and Methods

Bacterial growth conditions and curves

The encapsulated strains W83 (K1), HG184 (K2), A7A1-28 (K3), ATCC-49417 (K4), HG1690 (K5), and HG1691 (K6) and the non-encapsulated strain ATCC-33277 (K^-) of *P. gingivalis* were cultured on 5% horse blood agar (Oxoid N°2; Oxoid Ltd., Basingstoke, UK), supplemented with haemin (5 mg/l) and menadione (1 mg/l), under anaerobic conditions (80% N_2, 10% CO_2, and 10% H_2) at 37°C. In order to obtain a reliable number of colony-forming units for the stimulation of DCs, growth curves were obtained in liquid brain–heart infusion medium (Becton Dickinson, Le Pont de Claix, France), supplemented with haemin and menadione, as described previously (Vernal et al. 2008a).

DCs' differentiation and activation

Purified DCs were obtained and stimulated as described previously (Vernal et al. 2008a). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats from 12 healthy donors following a Ficoll gradient (Ficoll-Paque Plus; Amersham Pharmacia Biotech, Uppsala, Sweden). For generating DCs, monocytes were purified from PBMCs by magnetic-cell-sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) and cultured at 10^6 cells/ml in RPMI-1640 containing 10% foetal calf serum (Gibco Invitrogen Corp., Grand Island, NY, USA) and 1000 U/ml of rhGM-CSF and rhIL-4 (Immunotools, Friesoythe, Germany) for 6 days at 37°C. The DCs were then stimulated with increasing multiplicity of infection (MOI) of 0.1, 1, 10, 10^2, or 10^3 (bacteria/DCs ratio) of *P. gingivalis* strains K1-K6 for 2 days. DCs stimulated with increasing MOI of the K^- strain of *P. gingivalis* or 10 ng/ml of the *Escherichia coli* 0111:B4 lipopolysaccharide (LPS) (Fluka, Sigma-Aldrich Chemie, Buchs, Switzerland) were used for comparison. Non-induced DCs served as the control.

Flow cytometry analysis

The efficiency of monocyte purification, their differentiation towards DCs, and their subsequent activation were analysed by flow cytometry as described previously (Vernal et al. 2006). Briefly, cells were stained with 10 μl of phycoerythrin (PE)-, fluorescein isothiocyarate (FITC)- or PE/cyanine 5 (Cy5)-conjugated anti-CD14, CD1a, CD80, CD83, and CD86 monoclonal antibodies (BD Biosciences Pharmingen, San José, CA, USA) for 30 min. at 4°C in the dark. Then, cells were washed and resuspended in 300 μl of PBS to be analysed using flow cytometry (EPICS XL, Beckman Coulter, Fullerton, CA, USA).

RNA isolation and real-time polymerase chain reaction (PCR)

Cytoplasmic RNA was isolated from DCs using the Nonidet-P40 method as described previously (Vernal et al. 2008b). Reverse transcription was performed using a Transcriptor First Strand cDNA synthesis kit following the manufacturer's recommendations (Roche Applied Science, Mannheim, Germany). To examine the mRNA expression for the cytokines interleukin (IL)-1β, IL-2, IL-5, IL-6, IL-10, IL-12, IL-13, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and TNF-β and for the major

Table 1. Forward primers, reverse primers and PCR probes used for cytokine and HLA-DRA mRNA and 18S rRNA amplifications by quantitative real-time PCR

Cytokine	Forward primer	Reverse primer	PCR probe*
IL-1β	ctgtcctgcgtgttgaaaga	ttgggtaatttttgggatctaca	78
IL-2	aagttttacatgcccaagaagg	aagtgaaagtttttgctttgagc	65
IL-5	ctctgaggattcctgttcctgt	cagtacccccttgcacagtt	47
IL-6	gcccagctatgaactccttct	gaaggcagcaggcaacac	45
IL-10	tgggggagaacctgaagac	ccttgctcttgttttcacagg	30
IL-12p35	cactcccaaaacctgctgag	tctcttcagaagtgcaagggta	50
IL-12p40	ccctgacattctgcgttca	aggtcttgtccgtgaagactcta	37
IL-13	agccctcagggagctcat	ctccataccatgctgccatt	17
TNF-α	cagcctcttctccttcctgat	gccagagggctgattagaga	29
TNF-β	ctaccgcccagcagtgtc	gtggtgtcatggggaga	13
IFN-γ	ggcattttgaagaattggaaag	tttggatgctctggtcatctt	21
HLA-DRA	actatactccgatcaccaatgaca	tgcggaaaaggtggtctt	4
18S rRNA	ctcaacacgggaaacctcac	cgctccaccaactaagaacg	77

*Number of the specific FAM dye-labelled probe selected from the quantitative real-time PCR system (Roche).

IL, interleukin; TNF, tumour necrosis factor; IFN, interferon; PCR, polymerase chain reaction; HLA-DRA, major histocompatibility complex class II DRα.

histocompatibility complex class II DRα (HLA-DRA), 50 ng of cDNA were amplified by quantitative real-time PCR in 384-well plates, using the appropriate primers and probes (Table 1) and the FastStart Taqman Probe Master (Roche Applied Science), in an ABI PRISM 7900 Sequence Detector System (Applied Biosystems, Foster City, CA, USA) as described previously (Vernal et al. 2008b). As an endogenous control, 18S rRNA expression levels were determined. A sample of each reaction was run in a 2% agarose gel, stained with 0.5 μg/ml ethidium bromide for 30 min., and de-stained with 1 mM $MgSO_4$ for 20 min. at room temperature.

Data analysis

The flow cytometry data were analysed using the WinMDi 2.9 software (The Scripps Research Institute, La Jolla, CA, USA), represented as histograms and expressed as the percentage of positive cells over the total. The quantitative PCR data were analysed using the ABI PRISM software (Applied Biosystems), and the relative quantification was obtained using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen 2001). Data were statistically analysed using the SPSS 15.0 software (Lead Technologies Inc., Charlote, NC, USA). The normality of data distribution was determined using the Shapiro–Wilk test. Differences regarding CD-expression levels were determined using the χ^2 test, and the differences between groups and within each group regarding the cytokine and HLA-DRA mRNA expression were determined using the unpaired Kruskal–Wallis and one-way analysis of variance tests and the Tukey *post hoc* test. Statistical significance was assumed when $p < 0.05$.

Results

A highly purified (>97%) monocyte population (CD14-positive cells) was isolated from buffy coats (Fig. 1). A high percentage (>96%) of DCs differentiated from these monocytes following rhGM-CSF/rhIL-4 stimulation was obtained, as demonstrated by the appearance of CD1a antigen, loss of the monocyte–macrophage marker CD14, and the increase in CD86-positive cells (Fig. 1). DC activation by *P. gingivalis* was confirmed by the increased expression of CD83 and CD80 (Fig. 1).

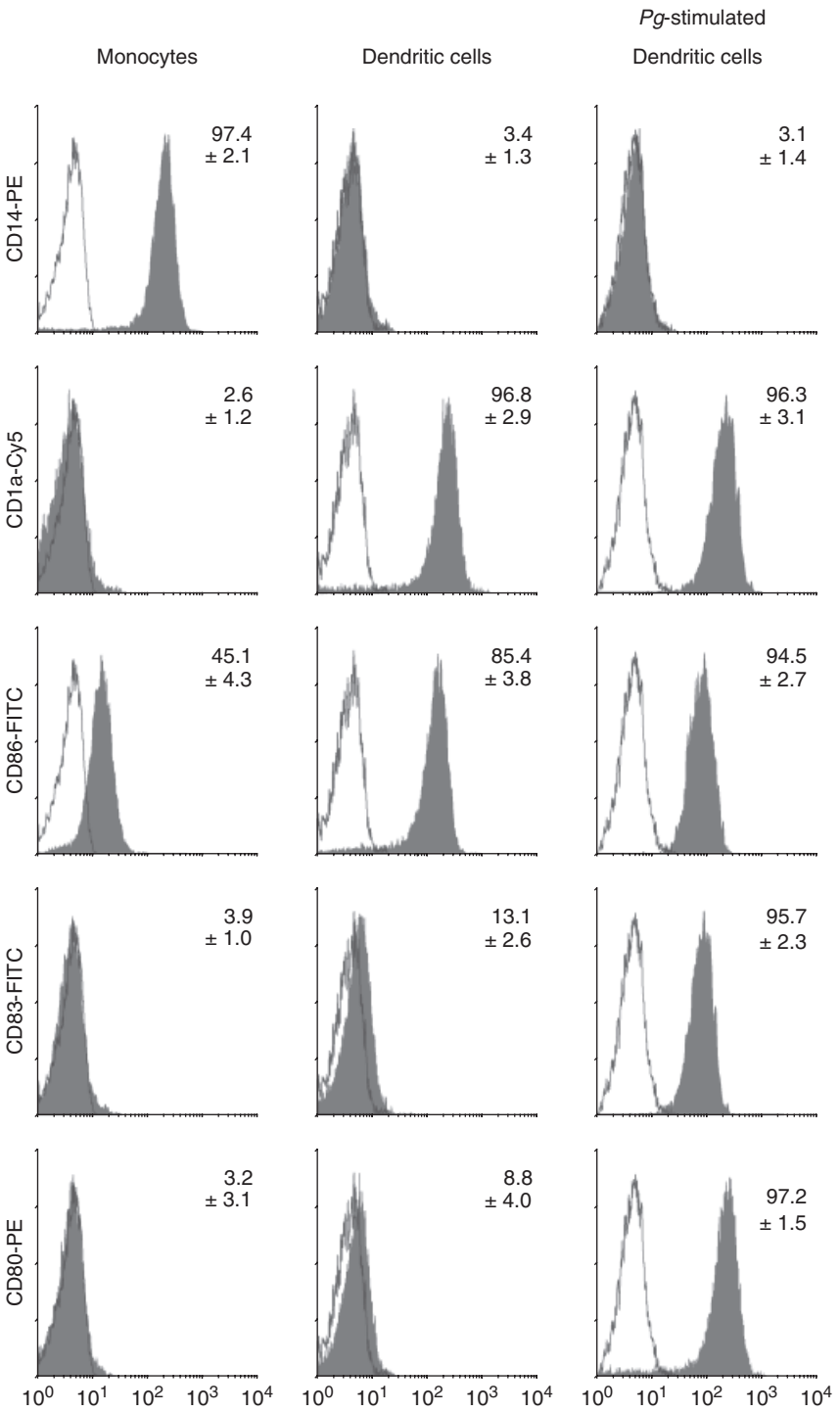

Fig. 1. Monocyte purification, differentiation, and dendritic cell activation. Flow cytometry analyses demonstrating the efficiency of monocyte purification from peripheral blood mononuclear cells previously isolated from buffy coats (CD14-positive cells), their differentiation towards dendritic cells (CD1a- and CD86-positive cells), and their subsequent activation (CD80- and CD83-positive cells) from a representative experiment, after a 48-h stimulation with *Porphyromonas gingivalis* strain K1 W83 (MOI 10^3). The data from each experiment were expressed as percentage of positive cells and represented as mean ± SD from 12 independent experiments. *Pg*, *Porphyromonas gingivalis*; CD, cluster of differentiation; PE, phycoerythrin; Cy5, PE/cyanine 5; FITC, fluorescein isothiocyarate.

The different *P. gingivalis* K serotypes induced DC maturation to a similar extent (>94%) as demonstrated by the expression levels of CD83 and CD80 determined by flow cytometry (Fig. 2a) and HLA-DRA mRNA analysed by quantitative real-time PCR (Fig. 2b).

The mRNA expression for the analysed cytokines was determined by quantitative real-time PCR and represented as fold-change for each condition.

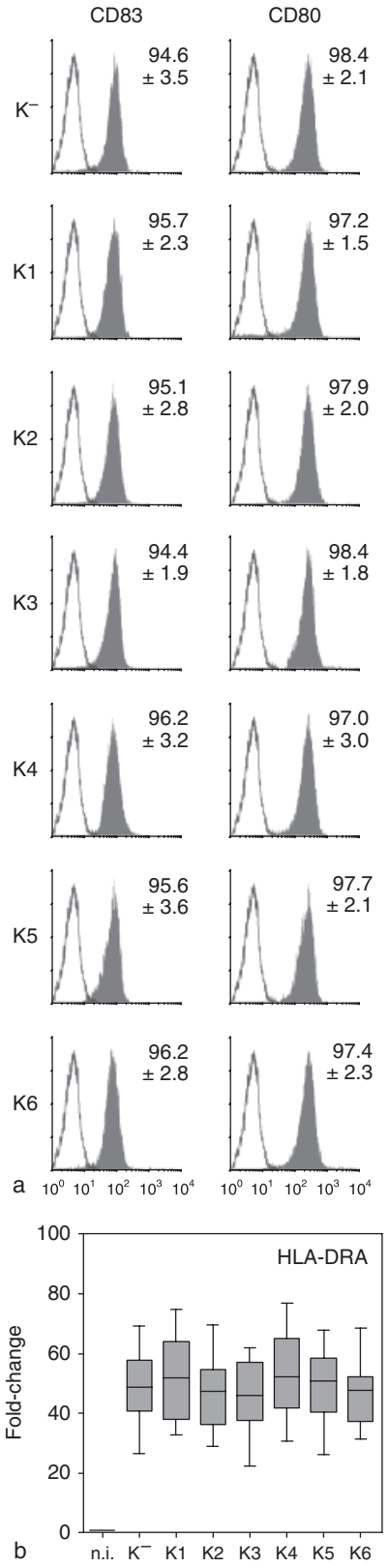

Fig. 2. Dendritic cell activation following stimulation with *Porphyromonas gingivalis* capsular serotypes. (a) Flow cytometry analyses comparing dendritic cell activation (CD80- and CD83-positive cells) from a representative experiment, after a 48-h stimulation with the encapsulated strains K1-K6 or the non-encapsulated (K^-) strain of *Porphyromonas gingivalis* (MOI 10^3). The data from each experiment were expressed as percentage of positive cells and represented as mean ± SD from 12 independent experiments. (b) major Histocompatibility complex class II DRα (HLA-DRA) mRNA expression determined by quantitative real-time polymerase chain reaction in the samples described in (a). For relative expression, the HLA-DRA mRNA expression in non-induced dendritic cells (n.i.) was considered as 1, as a reference for fold-change in expression. Data are represented as fold change for 12 independent experiments. The box plots show the medians, first, and third quartiles as boxes and 10th and 90th percentiles as whiskers. CD, cluster of differentiation.

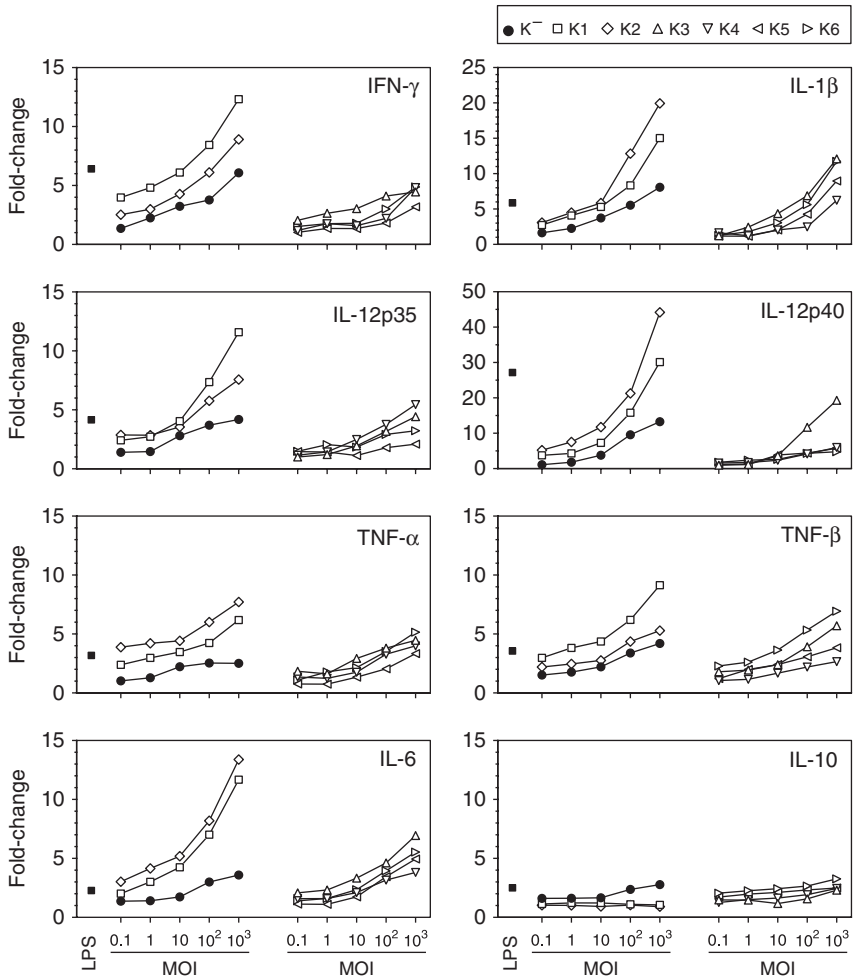

Fig. 3. Cytokine mRNA expression determined by quantitative real-time polymerase chain reaction. The relative expressions for the cytokines IL-1β, IL-6, IL-10, IL-12p35, IL-12p40, IFN-γ, TNF-α, and TNF-β mRNAs in dendritic cells stimulated with increasing MOI (0.1, 1, 10, 10^2, or 10^3) of the encapsulated strains K1–K6 of *Porphyromonas gingivalis* were represented as fold change. Cells stimulated with *Escherichia coli* lipopolysaccharide (LPS, filled squares) or increasing MOI of the non-encapsulated (K^-) strain of *P. gingivalis* (filled circles) were used as controls. Cytokine expression in non-induced dendritic cells (not shown) was considered 1 for relative quantification and used as a reference for the fold change in cytokine expression. Data from 12 independent experiments are expressed as mean. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; MOI, multiplicity of infection (bacteria/dendritic cells ratio).

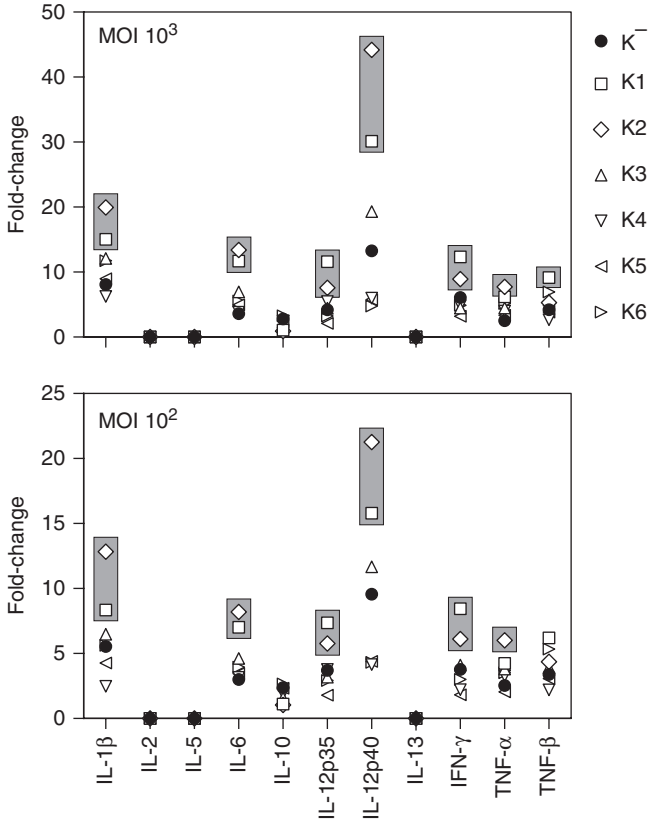

Fig. 4. Polymerase chain reaction analysis of cytokine mRNA expression. The expression for the cytokines IL-1β, IL-2, IL-5, IL-6, IL-10, IL-12p35, IL-12p40, IL-13, IFN-γ, TNF-α, and TNF-β mRNAs was determined in dendritic cells stimulated at MOIs of 10^2 or 10^3 of the *Porphyromonas gingivalis*-encapsulated strains K1–K6 or the non-encapsulated (K^-) strain (filled-circles, used as controls). Data are represented as fold-change mean for 12 independent experiments. Grey area: $p<0.05$. IFN, interferon; IL, interleukin; TNF, tumour necrosis factor; MOI, multiplicity of infection (bacteria/dendritic cells ratio).

A dose-dependent increase in the expression levels for IFN-γ, IL-1β, IL-12p35, IL-12p40, TNF-α, TNF-β, IL-6, and IL-10 was elicited on DC cells following stimulation with each of the K strains of *P. gingivalis* and the observed DC response was characteristic of a Th1 pattern of cytokine expression (Fig. 3). *P. gingivalis* strains K1 and K2 elicited higher expression levels of IFN-γ, IL-1β, IL-12p35, IL-12p40, and IL-6 mRNAs (at MOIs of 10^2 and 10^3 $p<0.05$) on DCs than the strains K3–K6 and the K^- strain of *P. gingivalis* (Fig. 4). The strain K1 elicited a higher increase in the expression of TNF-β mRNA (at an MOI of 10^3 $p<0.05$) as compared with the other *P. gingivalis* strains (Fig. 4). In addition, the strain K2 elicited a higher increase in the expression of TNF-α mRNA (at MOIs of 10^2 and 10^3 $p<0.05$) as compared with the strains K3–K6 and the K^- strain of *P. gingivalis* (Fig. 4). *P. gingivalis* strains K3–K5 induced weak mRNA expression of IFN-γ, IL-1β, IL-12p35, IL-12p40 TNF-β, and IL-10 in stimulated DCs, comparable to the levels obtained with the K^- strain of *P. gingivalis*. Conversely, the strains K1 and K2 down-regulated the IL-10 mRNA expression in DCs as compared with the K^- strain of *P. gingivalis* (Figs 3 and 4). In all experiments, the IL-2, IL-5, and IL-13 mRNA levels remained undetectable for each condition analysed (Fig. 5). Furthermore, our data show that while in DCs stimulated with the strain K1 or K2 the induction of IFN-γ, IL-1β, IL-12p35, IL-12p40, TNF-α, TNF-β, and IL-6 mRNAs was detectable after challenge with an MOI of 0.1, the other K serotypes required a 10^2–10^3-fold higher bacterial challenge for detectable expression of these cytokines (Fig. 3).

Discussion

In this study, monocytes were purified and differentiated into DCs to compare the cytokine mRNA expression levels following their challenge with the different K serotypes of *P. gingivalis*. DCs' differentiation was associated with an increase in their cell size, loss of the monocyte–macrophage marker CD14, and increased expression of the transmembrane glycoproteins CD1a and CD86 (B7.2), known mediators of the presentation of microbial antigens to T-lymphocytes (Smyth et al. 2005). DCs' activation was associated with the expression of the type-I integral membrane glycoproteins CD80 (B7.1) and CD83, cell surface markers for fully matured DCs (Gemmell et al. 2007, Breloer & Fleischer 2008).

In this investigation, T-cell-depleted DC cultures were used, evidenced by the absence of detectable IL-2 expression levels in each experiment. Stimulation of DCs with K serotypes of *P. gingivalis* induced a predominant Th1 cytokine response, as determined by the high levels of IL-1β, IL-12p35, IL-12p40, and IFN-γ. The DC response induced by the serotypes K1 or K2 showed a significantly higher expression of IL-1β, IL-6, IL-12, IFN-γ, TNF-α, and TNF-β mRNAs in comparison with the strains K3–K6 and the K^- strain of *P. gingivalis*. In addition, distinct thresholds for the cytokine expression were detected, demonstrating that apart from differences in antigenicity among K serotypes, cytokine expression is also a function of the antigenic load.

A virulence increase of the encapsulated *P. gingivalis* strains, as compared with the K^- strain, has been proven when these pathogens were inoculated in a mouse model of experimental infection (Laine & van Winkelhoff 1998). *P. gingivalis* serotypes were unequally distributed among periodontitis patients, the strains K5 and K6 being the predominant serotypes (Laine et al. 1997). In addition, differences in cell adhesion have been reported and it was found that the serotype K4 had higher adhesion capacity to human periodontal pocket epithelial cells than the other serotypes (Dierickx et al. 2003). Also, a higher immunogenic capacity of serotype K1 has been evidenced on murine macrophages (d'Empaire et al. 2006), and a higher resistance of the strains K1 and K2 to phagocytosis and killing by human polymorphonuclear leucocytes has been reported (Sundqvist et al. 1991). Our data are consistent with these results, because significant differences in cytokine expression induced by

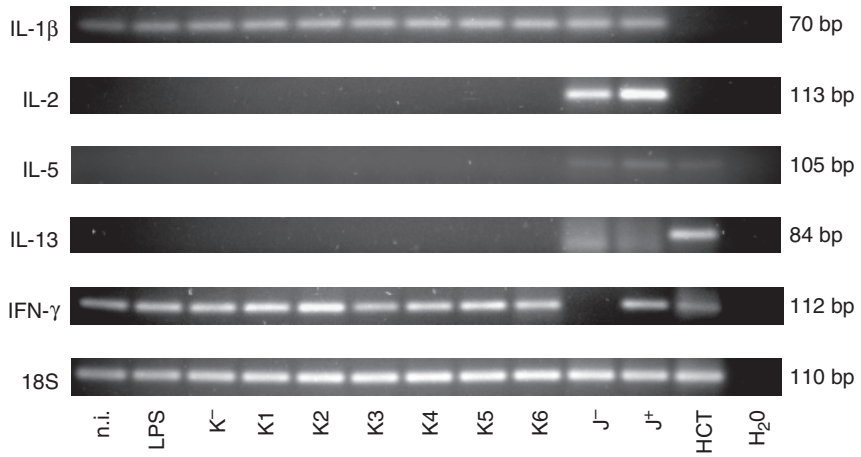

Fig. 5. Expression of IL-1β, IL-2, IL-5, IL-13, and IFN-γ mRNAs and 18S rRNA. The mRNA expression for the cytokines IL-2, IL-5, IL-13, IFN-γ, and IL-1β and for the 18S rRNA was determined in non-induced dendritic cells (n.i.), dendritic cells stimulated with *Escherichia coli* lipopolysaccharide (LPS), and dendritic cells stimulated at an MOI of 10^3 of *Porphyromonas gingivalis* non-encapsulated K^- strain or strains K1–K6. As positive controls, the cytokine mRNA expressions in non-induced Jurkat E61.2 T-lymphocytes (J^-), in Jurkat E61.2 stimulated with a combination of anti-CD3 and anti-CD28 monoclonal antibodies (J^+), and in human colorectal carcinoma HCT116 cells ATCC-CCL247 (HCT) were determined. Water (H_2O) was used as a negative control. IL, interleukin; IFN, interferon; TNF, tumour necrosis factor.

different K serotypes of *P. gingivalis* were demonstrated and these indicate that the strains K1 W83 and K2 HG184 are able to induce a more robust response on DCs, which might lead to the described increased resistance to phagocytosis (Sundqvist et al. 1991).

Our data demonstrate that the different *P. gingivalis* K serotypes analysed induce, rather than a different type of immune response, qualitative differences in this response, suggesting a role of the capsule in the activation of DCs. The relevance of capsular components in virulence has already been described for other Gram-negative bacteria (van Winkelhoff et al. 1986, Taylor & Roberts 2005, Yeh et al. 2007). In particular, it has been suggested that the strains K1 and K5 of *E. coli* are able to evade the immune surveillance by structural similarities of their capsule with host components, a camouflage used by the bacteria as a stratagem for virulence (Jann & Jann 1987, Taylor & Roberts 2005).

The differences in the molecular composition and structure of *P. gingivalis* capsule among the distinct K serotypes have been poorly defined (Schifferle et al. 1989), although differences in polysaccharide composition between the strains K1 W50 and K3 have been reported, suggesting that structural differences could be involved in the differential immuno-stimulatory potential (Schifferle et al. 1989, Farquharson et al. 2000). Genetic studies have reported on the serotype K1 of *P. gingivalis* at least four potential capsular polysaccharide biosynthesis loci (Nelson et al. 2003), and the lack of the cluster of open reading frames *PG0106–PG0120* in the K^- strain of *P. gingivalis* has been associated with the lack of capsule (Chen et al. 2004). In fact, deletion of *PG0109–PG0118* and *PG0116–PG0120* yielded K1 mutants that neither synthesized a capsule nor showed reactivity with anti-K1 antiserum (Aduse-Opoku et al. 2006). In addition, restriction fragment length polymorphism analysis of the *PG0106–PG0120* locus demonstrated a significant variation among K serotypes, whereas high conservation within the serotypes K1 of *P. gingivalis* was observed (Aduse-Opoku et al. 2006, Brunner et al. 2008). These data suggest that genetic variations in the capsular polysaccharide loci, which would be reflected in differences in either the sugar composition or the capsular structure, might be associated to differential resistance to phagocytosis by neutrophils and a distinct immuno-stimulatory capacity, as we have described on the DCs.

Although the genetic diversity of *P. gingivalis* in the population is high and multiple clones of *P. gingivalis* per individual have been reported (Menard & Mouton 1995, van Winkelhoff et al. 2007), a clonal stability under natural conditions has also been shown (van Winkelhoff et al. 2008); however, an association between a specific clonal type of *P. gingivalis* and disease type or severity has not been established. Gram-negative bacteria express cell surface molecules able to influence the pattern of cytokine expression by host cells, resulting either in down-regulation of the host response as a defensive mechanism or in its over-regulation and tissue destruction (Curtis et al. 2005). The higher immuno-stimulatory potential demonstrated by the encapsulated strains K1 W83 and K2 HG184 of *P. gingivalis* may therefore be an important pathogenic determinant in the initiation, progression, and/or severity of the periodontitis by inducing an over-response of the originally protective host mechanisms.

Acknowledgements

We thank the Unidad de Genómica, Parque Científico de Madrid from Universidad Complutense de Madrid for his help with analysing the quality of the RNAs and performing the PCR quantifications.

References

Aduse-Opoku, J., Slaney, J. M., Hashim, A., Gallagher, A., Gallagher, R. P., Rangarajan, M., Boutaga, K., Laine, M. L., Van Winkelhoff, A. J. & Curtis, M. A. (2006) Identification and characterization of the capsular polysaccharide (K-antigen) locus of *Porphyromonas gingivalis*. *Infection and Immunity* **74**, 449–460.

Breloer, M. & Fleischer, B. (2008) CD83 regulates lymphocyte maturation, activation and homeostasis. *Trends in Immunology* **29**, 186–194.

Brunner, J., Crielaard, W. & van Winkelhoff, A. J. (2008) Analysis of the capsular polysaccharide biosynthesis locus of *Porphyromonas gingivalis* and development of a K1-specific polymerase chain reaction-based serotyping assay. *Journal of Periodontal Research* **43**, 698–705.

Chen, T., Hosogi, Y., Nishikawa, K., Abbey, K., Fleischmann, R. D., Walling, J. & Duncan, M. J. (2004) Comparative whole-genome analysis of virulent and avirulent strains of *Porphyromonas gingivalis*. *Journal of Bacteriology* **186**, 5473–5479.

Curtis, M. A., Slaney, J. M. & Aduse-Opoku, J. (2005) Critical pathways in microbial viru-

lence. *Journal of Clinical Periodontology* **32** (Suppl. 6), 28–38.

Cutler, C. W. & Jotwani, R. (2006) Dendritic cells at the oral mucosal interface. *Journal of Dental Research* **85**, 678–689.

d'Empaire, G., Baer, M. T. & Gibson, F. C. III (2006) The K1 serotype capsular polysaccharide of *Porphyromonas gingivalis* elicits chemokine production from murine macrophages that facilitates cell migration. *Infection and Immunity* **74**, 6236–6243.

Dierickx, K., Pauwels, M., Laine, M. L., Van Eldere, J., Cassiman, J. J., van Winkelhoff, A. J., van Steenberghe, D. & Quirynen, M. (2003) Adhesion of *Porphyromonas gingivalis* serotypes to pocket epithelium. *Journal of Periodontology* **74**, 844–848.

Farquharson, S. I., Germaine, G. R. & Gray, G. R. (2000) Isolation and characterization of the cell-surface polysaccharides of *Porphyromonas gingivalis* ATCC 53978. *Oral Microbiology and Immunology* **15**, 151–157.

Gemmell, E., Yamazaki, K. & Seymour, G. J. (2007) The role of T cells in periodontal disease: homeostasis and autoimmunity. *Periodontology 2000* **43**, 14–40.

Herrera, D., Contreras, A., Gamonal, J., Oteo, A., Jaramillo, A., Silva, N., Sanz, M., Botero, J. E. & Leon, R. (2008) Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. *Journal of Clinical Periodontology* **35**, 106–113.

Jann, K. & Jann, B. (1987) Polysaccharide antigens of *Escherichia coli*. *Reviews of Infectious Diseases* **9** (Suppl. 5), S517–S526.

Laine, M. L., Appelmelk, B. J. & van Winkelhoff, A. J. (1996) Novel polysaccharide capsular serotypes in *Porphyromonas gingivalis*. *Journal of Periodontal Research* **31**, 278–284.

Laine, M. L., Appelmelk, B. J. & van Winkelhoff, A. J. (1997) Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. *Journal of Dental Research* **76**, 1840–1844.

Laine, M. L. & van Winkelhoff, A. J. (1998) Virulence of six capsular serotypes of *Porphyromonas gingivalis* in a mouse model. *Oral Microbiology and Immunology* **13**, 322–325.

Livak, K. J. & Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(− Delta Delta C(T)) Method. *Methods* **25**, 402–408.

Menard, C. & Mouton, C. (1995) Clonal diversity of the taxon *Porphyromonas gingivalis* assessed by random amplified polymorphic DNA fingerprinting. *Infection and Immunity* **63**, 2522–2531.

Nelson, K. E., Fleischmann, R. D., DeBoy, R. T., Paulsen, I. T., Fouts, D. E., Eisen, J. A., Daugherty, S. C., Dodson, R. J., Durkin, A. S., Gwinn, M., Haft, D. H., Kolonay, J. F., Nelson, W. C., Mason, T., Tallon, L., Gray, J., Granger, D., Tettelin, H., Dong, H., Galvin, J. L., Duncan, M. J., Dewhirst, F. E. & Fraser, C. M. (2003) Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *Journal of Bacteriology* **185**, 5591–5601.

Offenbacher, S. (1996) Periodontal diseases: pathogenesis. *Annals of Periodontology* **1**, 821–878.

Schifferle, R. E., Reddy, M. S., Zambon, J. J., Genco, R. J. & Levine, M. J. (1989) Characterization of a polysaccharide antigen from *Bacteroides gingivalis*. *Journal of Immunology* **143**, 3035–3042.

Slots, J. & Ting, M. (1999) *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment. *Periodontology 2000* **20**, 82–121.

Smyth, C. M., Logan, G., Boadle, R., Rowe, P. B., Smythe, J. A. & Alexander, I. E. (2005) Differential subcellular localization of CD86 in human PBMC-derived macrophages and DCs, and ultrastructural characterization by immuno-electron microscopy. *International Immunology* **17**, 123–132.

Socransky, S. S. & Haffajee, A. D. (1994) Evidence of bacterial etiology: a historical perspective. *Periodontology 2000* **5**, 7–25.

Sundqvist, G., Figdor, D., Hanstrom, L., Sorlin, S. & Sandstrom, G. (1991) Phagocytosis and virulence of different strains of *Porphyromonas gingivalis*. *Scandinavian Journal of Dental Research* **99**, 117–129.

Taylor, C. M. & Roberts, I. S. (2005) Capsular polysaccharides and their role in virulence. *Contributions to Microbiology* **12**, 55–66.

van Winkelhoff, A. J., Appelmelk, B. J., Kippuw, N. & de Graaff, J. (1993) K-antigens in *Porphyromonas gingivalis* are associated with virulence. *Oral Microbiology and Immunology* **8**, 259–265.

van Winkelhoff, A. J., Kippuw, N. & de Graaff, J. (1986) Serological characterization of black-pigmented *Bacteroides endodontalis*. *Infection and Immunity* **51**, 972–974.

van Winkelhoff, A. J., Loos, B. G., van der Reijden, W. A. & van der Velden, U. (2002) *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* **29**, 1023–1028.

van Winkelhoff, A. J., Rijnsburger, M. C., Abbas, F., Timmerman, M. F., van der Weijden, G. A., Winkel, E. G. & van der Velden, U. (2007) Java project on periodontal diseases: a study on transmission of *Porphyromonas gingivalis* in a remote Indonesian population. *Journal of Clinical Periodontology* **34**, 480–484.

van Winkelhoff, A. J., Rijnsburger, M. C. & van der Velden, U. (2008) Clonal stability of *Porphyromonas gingivalis* in untreated periodontitis. *Journal of Clinical Periodontology* **35**, 674–679.

van Winkelhoff, A. J., van Steenbergen, T. J. & de Graaff, J. (1988) The role of black-pigmented *Bacteroides* in human oral infections. *Journal of Clinical Periodontology* **15**, 145–155.

Vernal, R., Dutzan, N., Hernandez, M., Chandia, S., Puente, J., Leon, R., Garcia, L., Del Valle, I., Silva, A. & Gamonal, J. (2006) High expression levels of receptor activator of nuclear factor-kappa B ligand associated with human chronic periodontitis are mainly secreted by $CD4^+$ T lymphocytes. *Journal of Periodontology* **77**, 1772–1780.

Vernal, R., Leon, R., Herrera, D., Garcia-Sanz, J. A., Silva, A. & Sanz, M. (2008a) Variability in the response of human dendritic cells stimulated with *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans*. *Journal of Periodontal Research* **43**, 689–697.

Vernal, R., Velasquez, E., Gamonal, J., Garcia-Sanz, J. A., Silva, A. & Sanz, M. (2008b) Expression of proinflammatory cytokines in osteoarthritis of the temporomandibular joint. *Archives of Oral Biology* **53**, 910–915.

Address:
Rolando Vernal
Department of Cellular and Molecular Medicine
Centro de Investigaciones Biológicas
CIB-CSIC
Ramiro de Maeztu 9
28040 Madrid
Spain
E-mail: rvernal@uchile.cl

Clinical Relevance

Scientific rationale for the study: In *P. gingivalis*, a strictly anaerobic Gram-negative oral pathogen, a differential virulence based on capsular antigen serology has been hypothesized.

Principal findings: A differential effect was demonstrated when different *P. gingivalis* capsular serotypes stimulated human DCs. Thus, the strains K1 W83 and K2 HG184 induced a more robust cytokine expression than the other K serotypes.

Practical implications: Not all the pathogens belonging to the *P. gingivalis* species have the same virulence and depending on the genetic expression of the different virulence factors, the presence of these bacteria may involve a different pathogenicity. The variability in the host response induced by the different capsular serotypes of *P. gingivalis* in this study should be taken into account when evaluating the role of this bacterial species in the pathogenesis of periodontitis.