J Clin Periodontol 2010; 37: 789–796 doi: 10.1111/j.1600-051X.2010.01600.x

Journal of Clinical Periodontology

Human gingiva is another site of C-reactive protein formation

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Lu Q, Jin L. Human gingiva is another site of C-reactive protein formation. J Clin Periodontol 2010; 37: 789–796. doi: 10.1111/j.1600-051X.2010.01600.x.

Abstract

Objectives: C-reactive protein (CRP) is primarily synthesized in the liver. It is hypothesized that human gingiva per se may produce CRP and its expression could be associated with IL-6. This study elucidated the CRP expression profile in human gingiva and its possible association with IL-6.

Materials and Methods: Ninety-four gingival biopsies were collected from 44 subjects with chronic periodontitis and 18 periodontally healthy subjects. CRP protein was detected by immunohistochemistry and Western blotting, while CRP and IL-6 mRNAs were examined by reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR. CRP protein expression in the reconstituted human gingival epithelia (RHGE) was examined by the particle-enhanced immunoturbidimetric assay and Western blotting.

Results: CRP protein was detected in gingival tissues from patients and healthy subjects by immunohistochemistry and confirmed by Western blotting. Its expression pattern and level at 16 pairs of periodontal pocket tissues and the adjacent clinically healthy tissues from 16 patients were significantly interrelated ($r_s = 0.693$, $p < 0.01$). CRP mRNA expression was strongly correlated with IL-6 ($r = 0.694$, $p < 0.001$). Both CRP protein and mRNA were detected in the RHGE.

Conclusions: The present study shows for the first time that human gingiva is able to produce CRP in situ that may be associated with IL-6 activity.

Key words: C-reactive protein; human gingiva; IL-6; periodontitis

Accepted for publication 3 June 2010

Periodontal diseases are among the most common infections in humans and they are currently recognized as serious infections with profound effects on general health. Emerging evidence indicates that periodontal infections contribute significantly to systemic inflammation, which plays crucial roles in the pathogenesis of atherosclerosis and cardiovascular disease (CVD) (Loos et al. 2000, D'Aiuto et al. 2004, Salzberg et al. 2006). A number of systemic inflammatory biomarkers have been investigated for the association of periodontal infections with systemic inflammation and CVD (Loos 2005, Jin & Wang 2007, Paraskevas et al. 2008). Plasma or serum levels of C-reactive protein (CRP) and IL-6 are significantly higher in periodontitis patients than in healthy controls (Loos et al. 2000, Buhlin et al. 2003, D'Aiuto et al. 2004), and periodontal treatment could reduce their levels (D'Aiuto et al. 2004, Seinost et al. 2005) and improve endothelial function (Seinost et al. 2005, Tonetti et al. 2007). Our recent finding on the association of chronic periodontitis with an increased level of circulating endothelial progenitor cells may be a clue for further exploring the relationship among periodontal infection, systemic inflammation and endothelial function (Li et al. 2009).

CRP is an important acute-phase protein that was discovered in 1930 (Tillet & Francis 1930). The plasma level of CRP may increase rapidly in acute inflammation up to 1000-fold over normal level (Gabay & Kushner 1999, Rattazzi et al. 2003). For instance, patients with primary hypercholesterolaemia are frequently accompanied with an elevated CRP level, and it reduces significantly after treatments (Ridker et al. 2001). CRP is currently recognized as an independent predictor of cardiovascular events (Ablij & Meinders 2002, Rattazzi et al. 2003, Koenig 2005). It may also play an active role in atherosclerosis and its levels could reflect the inflammatory burden within atherosclerotic lesions (Ablij & Meinders 2002).

CRP is closely linked with IL-6, an important pro-inflammatory cytokine, which acts as the principal inducer of

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests. This study was supported by the Hong Kong Research Grants Council (HKU 7518/05M and HKU766909M) and The University of Hong Kong (CRCG 200807176048 and 200907176052).

CRP synthesis by the liver (Mayer 1973, Rattazzi et al. 2003). It has been shown that plasma level of CRP is mainly regulated at the transcriptional level induced by IL-6 (Toniatti et al. 1990, Ganapathi et al. 1991, Volanakis 2001, Ablij & Meinders 2002). Both CRP and IL-6 have been shown to be predictive of CVD outcome and they may even potentially act as active perpetrators of key steps of atherosclerotic diseases (Rattazzi et al. 2003).

CRP is primarily synthesized by hepatocytes (Pepys & Hirschfield 2003), while extrahepatic synthesis of CRP has been reported in peripheral blood lymphocytes (Kuta & Baum 1986), alveolar macrophages (Dong & Wright 1996), brain neurons (Yasojima et al. 2000), respiratory tract (Gould & Weiser 2001), atherosclerotic plaques (Yasojima et al. 2001), coronary artery (Calabro et al. 2003), kidney (Jabs et al. 2003), adipose tissues (Ouchi et al. 2003), lung epithelial cells (Ramage et al. 2004), peripheral blood mononuclear cells (Haider et al. 2006) and conjunctival and corneal epithelial cells (Epstein et al. 2009). In the oral cavity, CRP has been detected in saliva (Aurer et al. 2005) and gingival crevicular fluid (Sibraa et al. 1991), whereas it remains unknown whether gingival tissue per se is capable of producing CRP. It is hypothesized that human gingiva per se is capable of producing CRP and its expression may be associated with IL-6. This study elucidated the CRP expression profile in human gingiva and its possible association with IL-6.

Materials and Methods

Subjects

Forty-four Chinese adults (22 males and 22 females, aged 26–71 years) were recruited for the study, including 12 smokers and 32 non-smokers. Inclusion criteria were: (i) presentation of untreated advanced chronic periodontitis, with probing depth (PD) $\geqslant$5.0 mm, clinical attachment loss (CAL) $\geqslant$3.0 mm and radiographic evidence of alveolar bone loss on at least two teeth per quadrant, excluding the third molars; (ii) healthy systemic condition; (iii) no prior periodontal treatment; and (iv) no use of any immunosuppressive agents, antibiotics or anti-inflammatory drugs within the preceding 6 months. All the subjects were examined at a screening session for checking their suitability for the study. Then, they received baseline examination and a course of non-surgical periodontal therapy including oral hygiene instructions, scaling and root planing, and follow-up monitoring of treatment responses for at least 6 months with routine prophylaxis at an interval of 3 months. At the subsequent re-examination session, all the subjects exhibited unresolved periodontitis with remaining PD $\geqslant$ 5.0 mm and bleeding on probing (BOP) at least in one quadrant of their dentitions in need of periodontal surgery. Eighteen systemically and periodontally healthy subjects (six males and 12 females, aged 13–56 years) were recruited as controls. They were all non-smokers. Written and oral informed consent was obtained from all recruits. The study protocols were approved by the Ethics Committee, Faculty of Dentistry, The University of Hong Kong and subsequently by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Collection of samples

Fifty-seven periodontal pocket tissues were collected during periodontal surgery in unresolved periodontitis sites with PD $\geqslant$ 6 mm, CAL $\geqslant$ 5 mm and loss of alveolar bone $\geqslant$30% of root length on radiographs. Nineteen clinically healthy tissues were collected from the clinically healthy sites adjacent to the pocket sites with PD $\leqslant$ 3 mm, CAL $\leqslant$ 1 mm and absence of BOP (Jin et al. 2004). At least one biopsy of periodontal pocket tissues was obtained from each patient, and when collectable, one biopsy of clinically healthy tissues was also obtained from the same patient. Eighteen gingival biopsies were obtained from the non-inflamed sites of periodontally healthy subjects as healthy controls during tooth extraction for orthodontic reasons.

Reconstituted human gingival epithelia (RHGE)

Areas of 0.5 cm^2 RHGE (Skinethic Laboratories, Nice, France) were reconstituted by culture of normal human gingival keratinocytes (second passage) at the air/liquid interface for 5 days in serum-free and chemically defined medium on inert polycarbonate filters (Lu et al. 2009). This epithelium model features a stratified epithelium with a thin stratum corneum, few granular layer cells and mostly spinous layer cells. Upon arrival, the RHGE were stored in an incubator at 37°C, 5% CO_2 and saturated humidity in the medium containing insulin (5 μg/ml), calcium chloride (1.5 mM), gentamycin (25 μg/ml) and hydrocortisone (0.4 μg/ml). After 24 h of recovery, they were cultured in fresh media for another 24 h.

Immunohistochemistry

Twenty-four patients contributed 24 periodontal pocket tissues and 16 clinically healthy tissues, including 16 pairs of periodontal pocket and clinically healthy tissues (one pair per patient). Among the 24 patients (13 males and 11 females), 10 were smokers and 14 were non-smokers. Sixteen periodontally healthy non-smoking subjects (five males and 11 females) contributed 16 healthy control tissues. Gingival biopsies were embedded in paraffin and routinely sectioned. Serial paraffin sections were deparaffinized, rehydrated and then soaked in deionized water containing 3% hydrogen peroxide for 10 min. to block endogenous peroxides. Sections were incubated at 4°C overnight with mouse monoclonal anti-human CRP IgG (Sigma-Aldrich Corp., St. Louis, MO, USA) at a 1:400 dilution. CRP antibody pre-incubated in an excess of CRP blocking peptide (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was added as a negative control. The reaction was then detected using the ABC technique (Santa Cruz Biotechnology) with 3,3′ diaminobenzidine as the substrate. The expression levels of CRP were recorded as negative (−); very few positive staining (+); or extensive staining (++).

High-sensitivity CRP assay

Total proteins were extracted from homogenized RHGE using CelLytic™-M reagent (Sigma-Aldrich), and their concentrations were measured with Bio-Rad's protein assay (Bio-Rad Laboratories, Hercules, CA, USA). CRP levels in RHGE and culture media were measured by the particle-enhanced immunoturbidimetric assay (Cobas Integra 400/400 plus, Roche Diagnostics GmbH, Mannheim, Germany) with a detection sensitivity of 0.01 μg/ml. In RHGE, the concentrations of total protein and CRP in each sample were

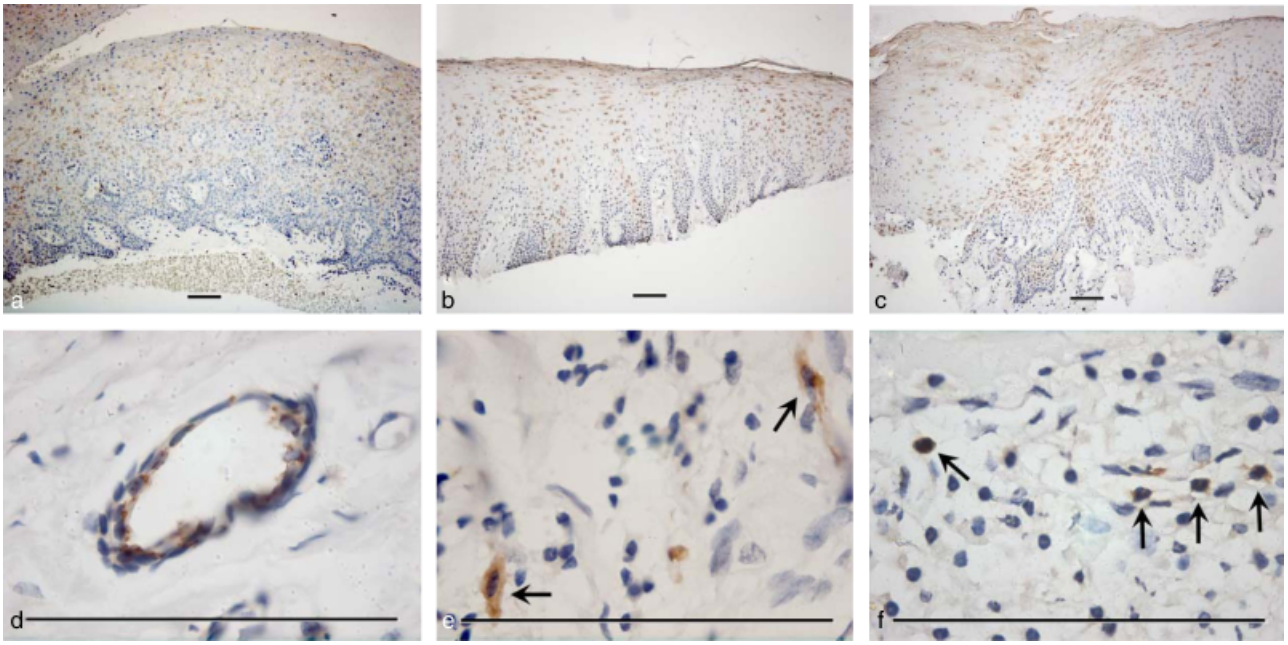

Fig. 1. Immunohistochemical expression of C-reactive protein (CRP) in human gingival tissues. Gingival tissues from healthy subjects (a, d), a clinically healthy site (b) and pocket sites of periodontitis patients (c, e, f) were processed with mouse monoclonal anti-human CRP IgG. The CRP expression was located in gingival epithelia (a, b, c), endothelial cells (d), fibroblast-like cells (e, arrows) and polymorphonuclear neutrophil-like cells (f, arrows). Scale bars: 100 μm.

determined as μg/μl and pg/μl, respectively, and the CRP level was then presented as pg/μg total protein. For the culture media of RHGE, the CRP level was presented as ng/ml. Each assay was performed in duplicate.

Western blot analysis

Total proteins in the homogenized gingival tissue biopsies and RHGE were isolated with the lysis buffer (Sigma-Aldrich) and protein concentrations were measured using Pierce bicinchoninic acid protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). After denaturation, 50 μg of each protein sample was analysed with 12.5% SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to polyvinylidene fluoride membranes (GE Healthcare, Buckinghamshire, UK). The membranes were blocked with 5% (w/v) non-fat dry milk in Tris Buffered Saline-Tween (TBS-T) (15 mM Tris-HCl, pH7.5, 120 mM NaCl and 0.1% Tween 20) for 1 h at room temperature, and then incubated with primary mouse anti-CRP (1:2000; Sigma) or rabbit anti-α-Tubulin monoclonal antibodies (1:2000; Cell Signaling Technology, Danvers, MA, USA) in blocking solution overnight at 4°C. Membranes were then subjected to incubation with secondary anti-mouse (CRP, 1:10,000, Invitrogen, Carlsbad, CA, USA) or anti-rabbit (α-Tubulin, 1:10,000, Thermo Fisher Scientific Inc.) horseradish peroxidase-conjugated antibodies for 1 h at room temperature. After final washes in TBS-T, membranes were incubated with the Pico chemiluminescent substrate (Thermo Fisher Scientific Inc.) and exposed to CL-X Posture film (Thermo Fisher Scientific Inc.) for detection of protein bands.

RNA isolation and reverse transcription

Twenty patients contributed 33 periodontal pocket tissues and three clinically healthy tissues for the detection of CRP mRNA. Among the 20 patients (nine males and 11 females), two were smokers and 18 were non-smokers. Two periodontally healthy non-smoking subjects (one male and one female) contributed two healthy control tissues for the detection of CRP mRNA. Total cellular RNA was extracted from the homogenized gingival tissues and RHGE using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. One microgram of total RNA from each sample was reverse transcribed into cDNA using the SuperScript™ First-Strand Synthesis System (Invitrogen Corp.) according to the manufacturer's instructions.

Reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR

RT-PCR was performed using the following pairs of primers: human CRP, sense 5′-ATACCCAGGCCACAAGAGTG-3′ and antisense 5′-ACGTCCTCTCAGCT TGGAAA-3′ (Wilson et al. 2007); and human IL-6, sense 5′-ATGAACTCCTT CTCCACAAGCGC-3′ and anti-sense 5′-GAAGAGCCCTCAGGCTGGACTG-3′ (Christodoulides et al. 2002). These primers were tailored to amplify a 204 and 628 bp fragment of the CRP gene and the IL-6 gene, respectively. As an internal mRNA control, another primer pair was used to amplify a 353 bp fragment of human β-actin gene in parallel. The primers for β-actin were forward, 5′-GC TCGTCGTCGACAACGGCTC-3′ and reverse, 5′-CAAACATGATCTGGGTC ATCTTCTC-3′ (Invitrogen). After cycle determination, the samples were subjected to 33 cycles for CRP and IL-6, and 25 cycles for β-actin. An aliquot (13 μl) of each PCR product was electrophoresed through a 1.5% agarose gel for quality control. The product was visualized by ethidium bromide staining, and then scanned using a Uvidoc gel documentation system (Bio-Rad). The band

Table 1. Expression profiles of CRP protein in the gingival tissues of 24 patients with unresolved chronic periodontitis and 16 periodontally healthy subjects

Patients*	CRP expression†	
	periodontal pocket tissues	clinically healthy tissues
1	–	–
2	++ (Epithelium)	++ (Epithelium)
3	–	–
4	–	–
5	+ (Epithelium)	+ (Epithelium)
6	–	–
7	+ (Inflammatory cells)	+ (Fibroblasts)
8	–	–
9	++ (Epithelium)	++ (Epithelium)
10	+ (Fibroblasts, inflammatory cells)	–
11	–	–
12	+ (Endothelial cells, inflammatory cells)	–
13	+ (Epithelium)	+(Epithelium)
14	–	–
15	+ (Epithelium)	+(Epithelium)
16	+ (Fibroblasts)	+(Fibroblasts)
17	–	
18	++ (Epithelium)	
19	+ (Epithelium)	
20	–	
21	+ (Inflammatory cells)	
22	+ (Fibroblasts)	
23	–	
24	++ (Epithelium, fibroblasts)	
Periodontally healthy subjects	Healthy control tissues	
1	++ (Epithelium, endothelial cells, fibroblasts)	
2	+ (Fibroblasts, inflammatory cells)	
3	+ (Epithelium, fibroblasts, inflammatory cells)	
4	–	
5	+ (Fibroblasts)	
6	++ (Epithelium)	
7	–	
8	+ (Epithelium)	
9	–	
10	–	
11	+ (Epithelium)	
12	+ (Endothelial cells)	
13	–	
14	++ (Epithelium, endothelial cells)	
15	–	
16	+ (Epithelium)	

*Patients 1–16 presented with 16 pairs of periodontal pocket tissues and clinically healthy tissues (one pair per patient).

†The expression levels of CRP protein by immunohistochemistry are presented as negative (–); very few positive staining (+); or extensive staining (++). Components in the brackets indicate the major localization sites of CRP.

intensities of the target genes and the internal control gene were quantified using densitometry (Densitometry Analyzer, Quantity OneTM, Bio-Rad). The relative expression levels of CRP and IL-6 mRNA were determined as the ratios of the band intensities of the target CRP and IL-6 genes to that of β-actin.

Real-time PCR was performed using the StepOne™ Real-time PCR System in a 48-well reaction plate (Applied Biosystems, Foster City, CA, USA). Amplification reactions were undertaken in a total volume of 25 μl of master reaction mix containing 1 μl of cDNA template, appropriate concentrations of primers and QuantiFast™ SYBR® Green (Qiagen). The specific primer sequences (sense/antisense) for each gene were as follows: CRP, 5′-ATACCCAGGCCA CAAGAGTG-3′/5′-ACGTCCTCTCAG CTTGGAAA-3′; IL-6, 5′-AATCATCA CTGGTCTTTTGGAG-3′/5′-GCATTT GTGGTTGGGTCA-3′; and β-actin, 5′-TTGGCAATGAGCGGTT-3′/5′-AGTT GAAGGTAGTTTCGTGGAT-3′. The reaction conditions were set at 95°C for 5 min. followed by 40 cycles at 95°C for 10 s and 60°C for 30 s, and terminated by a cooling step at 4°C. A negative control reaction was performed by replacing the cDNA template with sterile water. CRP and IL-6 mRNA quantification were determined by using the comparative threshold cycle (C_t) method (Lambertini et al. 2002, Lu et al. 2009). The transcription of β-actin was used as an endogenous RNA control for normalization, and the expression level of each target gene (ΔC_t) was then calculated ($\Delta C_t = C_{t_{(\text{target gene})}} - C_{t_{(\beta-\text{actin gene})}}$). The sample with a higher ΔC_t value reflects a relatively lower amount of CRP or IL-6 mRNA content, and vice versa.

Statistical analysis

The correlation between CRP and IL-6 mRNA expression was assessed by the Pearson's correlation coefficient, while the correlation of the expression level (semi-quantitative) of CRP at the paired sites of clinically healthy tissues and periodontal pocket tissues in the same patient was assessed by the non-parametric Spearman's rank correlation analysis. Detection frequency of CRP protein between the groups was analysed by the chi-square test. Statistical significance was defined as a two-tailed p-value < 0.01. Statistical analyses were performed using SPSS version 11 (Chicago, IL, USA).

Results

Localization of CRP in human gingival tissues by immunohistochemistry

CRP was detected in both gingival epithelia and connective tissues in healthy controls (Fig. 1a) and patients (Fig. 1b and c) (Table 1). In epithelia, CRP was expressed in all cell layers (Fig. 1a–c); in connective tissues, it was sporadically expressed in endothelial cells (Fig. 1d), fibroblast-like cells (Fig. 1e), and polymorphonuclear neutrophil-like cells (Fig. 1f). CRP protein was predominantly detected in the cytoplasm. The detection frequency of CRP was 62.5% (10/16) in healthy controls and 58.3% (14/24) in patients, and no significant difference was found

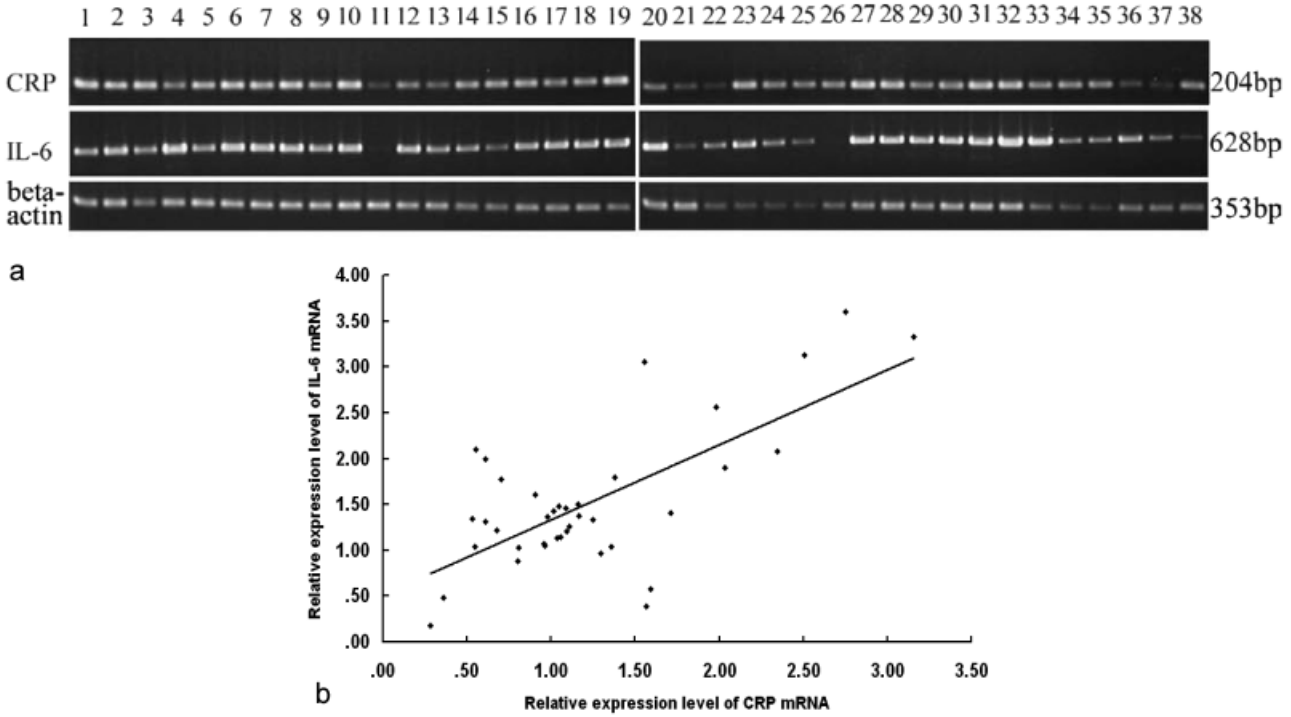

Fig. 2. Expression of C-reactive protein (CRP) and IL-6 mRNAs in human gingival tissues. (a) The mRNA expression of CRP, IL-6 and β-actin in 38 gingival tissues (No. 1–38) from two periodontally healthy subjects and 20 periodontitis patients including both clinically healthy sites and pocket sites was analysed by reverse transcriptase-polymerase chain reaction (RT-PCR). The PCR products of CRP, IL-6 and β-actin are shown as a single band of 204, 628 and 353 bp, respectively. (b) Scatterplot of relative mRNA expression levels of CRP and IL-6 mRNA in the 38 samples shown above. CRP and IL-6 mRNA expression was significantly correlated ($r = 0.694$, $p < 0.001$).

between the groups ($p > 0.05$). Among the subjects with detectable CRP protein, extensive expression was detected in 30% (3/10) of healthy controls and 28.5% (4/14) of patients. The expression pattern and the level of CRP at 16 pairs of periodontal pocket tissues and adjacent clinically healthy tissues within a same subject from 16 patients were significantly interrelated ($r_s = 0.693$, $p < 0.01$), as 87.5% of the patients (14/16) showed a similar CRP profile at the paired sites (Table 1). No significant difference was found in detection frequency and expression pattern of CRP between the smokers and non-smokers.

Expression of CRP and IL-6 mRNAs in human gingival tissues

RT-PCT data showed that CRP and IL-6 mRNAs were expressed in all categories of gingival tissues samples with varied intensities (Fig. 2a). The relative expression levels of CRP and IL-6 mRNAs were 1.22 ± 0.66 and 1.52 ± 0.78, respectively, and a significant correlation was found between them ($r = 0.694$, $p < 0.001$) (Fig. 2b).

CRP and IL-6 mRNAs were also detected by real-time PCR in all gingival tissue samples with their mean ΔC_t values of 12.69 ± 1.29 (ranged 9.92–15.62) and 5.47 ± 2.38 (ranged 0.84–11.70), respectively, thus overall showing a relatively higher IL-6 gene expression than CRP. Whereas, CRP gene expression remained to be strongly correlated with IL-6 ($r = 0.659$, $p < 0.001$).

CRP expression in RHGE

CRP mRNA (Fig. 3a) and protein (Fig. 3b) were detected in the RHGE derived from three healthy donors. CRP protein level in the RHGE was 127 ± 4.2, 45.5 ± 2.1 and 79 ± 1.4 pg/μg total protein, respectively (Fig. 3b). CRP protein was also detectable in one culture medium sample at a concentration of 10 ng/ml.

Western blotting analysis of CRP expression in human gingival tissues and RHGE

CRP protein was detected in both gingival tissue and RHGE samples with a molecular weight of approximately 23 kDa (Fig. 4).

Discussion

Our study for the first time demonstrates that human gingiva is another site of CRP production and it constitutively expresses CRP. Most of the cell types in gingival connective tissues are capable of expressing CRP, and CRP mRNA is detected in all gingival biopsies examined. It is interesting to note that gingival epithelia also constitute a source of CRP as detected in both gingival epithelia and RHGE. The latter resembles the outer layers of human gingival epithelia and immunohistochemically expresses various cytokeratins present in the natural gingival epithelia (Peyret-Lacombe et al. 2007). Detection of CRP protein and mRNA in these cultured gingival epithelia demonstrates that gingival epithelia per se are capable of producing CRP. It is worthy of note that CRP protein expression in gingival tissues and RHGE was confirmed by Western blotting analysis with a molecular weight of approxi-

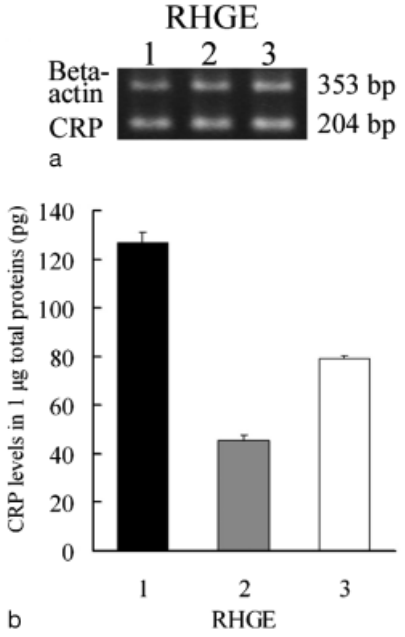

Fig. 3. Expression of C-reactive protein (CRP) mRNA and protein in reconstituted human gingival epithelia (RHGE) from three healthy donors. (a) The mRNA expression of CRP and β-actin was analysed by reverse transcriptase-polymerase chain reaction (RT-PCR). The PCR products were shown as a single band of 204 and 353 bp, respectively. (b) The CRP protein level was measured by a particle-enhanced immunoturbidimetric assay, and its level in the RHGE derived from three healthy donors was 127 ± 4.2, 45.5 ± 2.1 and 79 ± 1.4 pg/μg total protein, respectively. The error bars represent standard deviation.

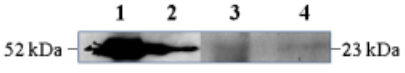

Fig. 4. Western immunoblots. Lane 1: α-Tubulin in reconstituted human gingival epithelia (RHGE) (52 kDa); Lane 2: α-Tubulin in gingival tissue (52 kDa); Lane 3: C-reactive protein (CRP) in RHGE (23 kDa); and Lane 4: CRP in gingival tissue (23 kDa).

mately 23 kDa that is consistent with what has been reported previously (Ablij & Meinders 2002). Therefore, human gingiva constitutes a local source of CRP and might partially contribute to the CRP levels in gingival crevicular fluids, saliva and serum. Recent studies from our group and others show that human gingival epithelia are actively involved in host innate immune responses through production of various pattern recognition receptors, antimicrobial peptides and inflammatory mediators (Dale 2002, Jin et al. 2004, Lu et al. 2004, 2005, Ren et al. 2004, 2005, 2009, Liu et al. 2010). It could be speculated that as other inflammatory mediators commonly produced in gingiva in response to microbial challenge, CRP expression in gingiva could potentially be modulated by various genetic and acquired risk factors. The in vivo activities of CRP, in general, are both anti-inflammatory and pro-inflammatory, and CRP can activate the classical complement cascade to contribute to clearance of bacteria and damaged cells in bloodstream and inflamed tissues (Du Clos & Mold 2004). It has been reported that CRP produced in the epithelial lining of human airway has antibacterial activity (Gould & Weiser 2001) and may play an important role in response to inflammatory stimuli (Ramage et al. 2004). Currently, the exact roles of CRP produced in gingival epithelia remain to be established.

Although our present study shows that human gingiva can directly produce CRP, the mechanisms of expression and regulation remain unclear. Based upon our observations on a similar detection frequency of CRP protein in both healthy subjects and periodontitis patients, great variation of its expression profile among subjects and the highly interrelated expression pattern at the diseased and the adjacent healthy sites within a same patient, these findings imply that CRP expression profile in gingiva may be subject dependent. This is consistent with a previous study that CRP levels in gingival crevicular fluid did not vary significantly between healthy and periodontitis sites (Sibraa et al. 1991). Recent findings that the circulating CRP level is subject to regulation by a common genetic variation at the CRP locus (Brull et al. 2003, D'Aiuto et al. 2005) support this assumption. It is tempting to speculate that the CRP level in gingival tissues may be subject to genetic and epigenetic modulation as well. Further studies are needed to elucidate the correlation between gingival CRP expression and systemic CRP levels, and their potential clinical implications in periodontal medicine.

During periodontitis, bacteria, bacterial products and inflammatory mediators in periodontal lesions may enter the systemic circulation and be disseminated throughout the body, which elicit the production of a series of inflammatory biomarkers like CRP and IL-6. An increased serum level of CRP was associated with the presence of subgingival periodontopathogens (Noack et al. 2001), elevated serum IL-6 (D'Aiuto et al. 2004) and the number of circulating neutrophils (Loos et al. 2000). The plasma level of IL-6 is associated with the extent of periodontitis (Mengel et al. 2002), and IL-6 could significantly induce CRP expression in human hepatocytes (Mayer 1973). In hepatocytes, CRP is induced principally at the transcriptional level by IL-6 (Ganapathi et al. 1991). In the present study, it was interesting to find a significantly positive correlation between CRP and IL-6 mRNA expression in human gingival tissues detected by both RT-PCR and real-time PCR, likely due to complex interactions of different inflammatory mediators in the host immune system.

The present study had a relatively small sample size of human gingival biopsies. The possible contribution of CRP in gingiva to its systemic level was not assessed. Within the limitation of this study, the present data show clearly for the first time that human gingiva is able to produce CRP in situ that may be associated with IL-6 activity. However any further conclusions concerning the role of CRP in periodontal diseases and its possible association with circulating CRP level as well as the clinical implications in periodontal medicine must await confirmation in future studies. Further investigations should therefore be undertaken firstly, to elaborate the possible effects of genetic and acquired environmental factors or major confounders (e.g. CRP gene polymorphism, smoking, diabetes mellitus, obesity and age) on CRP expression profile and level in human gingiva; and secondly to evaluate the association of CRP expression in gingiva with its systemic level and the relevant clinical implications. While, in vitro studies are also needed to determine the mechanisms of CRP expression and regulation in human gingiva as well as its possible roles in host defense response.

Acknowledgements

The authors are grateful to Ms. Bonnie Wong for her technical assistance in the study.

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Clinical Relevance

Scientific rationale for the study: CRP is a strong independent predictor of CVD and it is primarily synthesized in liver. Emerging evidence shows that periodontal diseases is significantly associated with systemic inflammation measured by serum levels of CRP and IL-6, while the mechanisms remain unclear. Currently, it remains unknown whether gingival tissue per se is capable of producing CRP. This study elucidated CRP expression profiles in human gingiva and in the RHGE.

Principal findings: CRP protein was detected in gingival tissues from patients and healthy subjects by immunohistochemistry and confirmed by Western blotting. The expression profile of CRP varied among the patients, while its expression pattern and level at the paired gingival tissues collected from unresolved periodontitis sites and the adjacent healthy sites were interrelated within a same subject. CRP signalling expression was strongly correlated with IL-6. Both protein and mRNA expression of CRP were detected in the RHGE.

Practical implications: The present study shows for the first time that human gingiva is able to produce CRP in situ that may be associated with IL-6 activity. Further studies are warranted to clarify the role of CRP in periodontal diseases and its possible association with the circulating CRP level and to determine the clinical implications in periodontal medicine.