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C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation

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Abstract

Background and Aim: Periodontitis is associated with elevated C-reactive protein (CRP) in both serum and gingival crevicular fluid (GCF). Although the liver is the primary source of CRP, extra-hepatic production of CRP has been reported. This study aimed to determine whether CRP in GCF is produced locally in the gingivae.

Materials and Methods: Gingivae and GCF were collected from non-periodontitis and periodontitis sites. Presence of CRP in gingivae was assessed by immunohistochemistry. CRP in GCF was measured using ELISA. Gene expression for CRP in gingivae was determined using real-time polymerase chain reaction.

Results: CRP was found in both the gingivae and GCF. No gingivae had detectable amounts of CRP mRNA. Not all patients with periodontitis had detectable levels of CRP in the GCF. Some non-periodontitis patients had detectable levels of CRP in the GCF.

Conclusion: CRP in the GCF appears to be of systemic origin, and therefore may be indicative of systemic inflammation from either a periodontal infection or inflammatory disease elsewhere. The correlation between levels of CRP in GCF and serum requires validation in future studies.

Key words: C-reactive protein; inflammation; gingival crevicular fluid

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Periodontal disease has been associated with a number of other inflammatory diseases, including diabetes mellitus, rheumatoid arthritis and cardiovascular disease (Lagervall et al. 2003). The status of many of these systemic inflammatory diseases can be measured using serum markers of inflammation. One such molecule that has been widely investigated is the acute-phase protein, C-reactive protein (CRP). Serum CRP concentrations are normally in the order of ng/ml, but are capable of rising rapidly 100–1000-fold in response to inflammation or tissue necrosis (Claus et al. 1976). As CRP concentrations closely follow the course of inflammation (Gabay & Kushner 1999), they are used as a measure for many disease processes (Ebersole & Cappelli 2000). Even mildly elevated serum levels of CRP are regarded as evidence of an active tissue-damaging process (Pepys 1981). Aside from being used as a marker of inflammation, CRP is now believed to itself have potent pro-inflammatory properties (Dasu et al. 2007, Ho et al. 2008, Turu et al. 2008).

Not surprisingly, CRP has been detected in the serum of periodontitis patients, and levels are significantly higher than those of non-periodontitis subjects (Cairo et al. 2008, Higashi et al. 2008, Paraskevas et al. 2008). Further, there is mounting evidence that effective periodontal therapy can lower serum CRP levels (Paraskevas et al. 2008, Marcaccini et al. 2009, Offenbacher et al. 2009). CRP has also been detected in the saliva and gingival crevicular fluid (GCF) of periodontitis patients (Sibraa et al. 1991, Pederson et al. 1995, Christodoulides et al. 2005, Hirasaki et al. 2005, Tuter et al. 2007, Fitzsimmons et al. 2009, 2010, Sanders et al. 2009). GCF is a transudate of serum, and as such, contains both serum components and locally produced molecules.

In general, CRP is primarily produced by the liver (Miller et al. 1951, Steel & Whitehead 1994), but several papers have reported extra-hepatic production of CRP by lymphocytes (Kuta & Baum

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1986), neurons of patients with Alzheimer's disease (Yasojima et al. 2000), nasal polyp epithelium (Gould & Weiser 2001), smooth muscle cells and macrophages of atherosclerotic plaques (Yasojima et al. 2001), aortic aneurysmal tissue (Vainas et al. 2003), renal epithelium of acute rejection renal allografts (Jabs et al. 2003a), adipose tissue from patients with coronary artery disease (Ouchi et al. 2003), smooth muscle cells of diseased coronary artery venous bypass grafts (Jabs et al. 2003b) and renal cell carcinoma epithelium (Jabs et al. 2005). As such, the presence of CRP in GCF of periodontitis patients may be due, in part, to the local production of CRP within the periodontal tissues. Local production of CRP would mean that CRP levels detected in the GCF could not be used to make inferences about systemic inflammation.

To date, there have been no published investigations of local production of CRP in the periodontal tissues. Therefore, the aim of this study was to determine if CRP detected in the GCF is the result of local production of CRP within the gingival tissues.

Methods

Study population

Patients

The University of Adelaide Human Ethics Research Committee and South Australian Dental Service approved the study, in accordance with the guidelines of the National Health and Medical Research Council of Australia. Patients attending the Adelaide Dental Hospital Periodontology Department for periodontal surgery were invited to participate in the study following written and informed consent. Periodontitis-affected tissue was collected during the periodontal flap surgery carried out for the treatment of persistent periodontal pocketing (PD $\geqslant$ 5 mm) following initial non-surgical therapy carried out at least 3 months previously. Tissue samples from non-periodontitis sites included gingival hyperplasia without periodontal attachment loss, gingivae resected during crown lengthening surgery or from soft tissue biopsy before tooth extraction. A thorough medical history and smoking history were taken for each patient, as well as questioning whether anti-inflammatory medication, antibiotics or steroids had been taken within the last 6 months. Patients were not excluded on the basis of their medical history or smoking status. Disease classification was in accordance with the American Academy of Periodontology classification (Armitage 1999), and periodontitis sites exhibited radiographic alveolar bone loss, deepened probing depths and attachment loss to a degree consistent with the rest of the dentition.

Sample collection, processing and analysis

GCF collection and analysis

GCF samples were collected from the deepest periodontal pocket in the area of periodontal surgery before any clinical measurements (Offenbacher et al. 1984). All clinically detectable supragingival plaque was removed without touching the gingiva, to minimize contamination of the paper strips by plaque. GCF samples were taken after air drying of the site, using isolation with cotton rolls and a saliva ejector isolation if necessary, to prevent salivary contamination. One paper strip was used for each collection site. A Periopaper™ strip (OraFlow, Smithtown, NY, USA) was introduced into the gingival crevice 1 mm, with care being taken not to traumatize the tissues. The strip was left in place for 30 s and then transferred for volume determination to a chairside Periotron 8000 unit (OraFlow). The unit was calibrated regularly and standard curves were generated to determine the volume in each strip. The Periopaper™ strip containing the sampled GCF was wrapped in foil and stored at -20°C until further processing.

GCF was subsequently eluted from the paper strips by placing the strips into the wells of a sterile 96-well plate (Flow Laboratories, McLean, VA, USA) and adding 230 µl sterile phosphate-buffered saline (PBS, pH 7.2) into each well. The plate was then sealed and placed on a shaker for 30 min. The amount of CRP in GCF was determined as described previously by Fitzsimmons et al. (2009). Each sample was assayed in duplicate using commercially available ELISA kits (human C-Reactive protein Instant ELISA, Bender MedSystems, Vienna, Austria) in accordance with the manufacturers' instructions. Colour development was monitored using a Bio-Tek Powerwave microplate reader (BioTek Instruments, Winooski, VT, USA) until an optimum optical density was reached, a stop solution was added and the optical density was read at 450 nm. Standard curves were then generated using KC4 software (BioTek Instruments) and used to determine the CRP concentration in each sample. The lower limit of detection for the CRP assay was 3 pg/ml. The concentration of CRP in GCF was determined by dividing the total amount by the volume of GCF collected, as described previously (Tang et al. 2009).

Immunohistochemistry for CRP

Gingival tissue samples for immunohistochemical analysis were fixed in 10% buffered formalin for 24 h before paraffin embedding. The first section cut (5 µm) from each block was stained with haematoxylin and eosin for standard histological assessment. For immunohistochemical detection, sections were dewaxed in two changes of xylene and two changes of alcohol. Endogenous peroxidase activity was inhibited using 0.3% hydrogen peroxide in PBS/0.1% sodium azide. Following rinsing in PBS, the sections were incubated with the primary antibody (monoclonal antibody mouse anti human CRP, clone CRP-8, Sigma, St. Louis, MO, USA) at a concentration 9.1 μg/ml (1 in 1000 dilution) in PBS with 1% (weight/volume) bovine serum albumin (BSA) and incubated overnight in a wet chamber. For control sections, the primary antibodies were omitted or a purified mouse IgG1 isotype control was substituted for the primary antibody (BD Biosciences Pharmingen, Franklin Lakes, NJ, USA). The primary antibody was detected by horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (DAKO, Botany, NSW, Australia), added for 30 min., followed by incubation with HRP-conjugated swine anti-goat IgG antibody (Biosource, Carslbad, CA, USA) for another 30 min. The HRP-conjugated antibodies were diluted in 1% PBS/SA with 10% (volume/volume) normal human serum as blocking serum. All incubations were carried out at room temperature. HRP activity was detected using hydrogen peroxide as a substrate and aminoethylcarbazole (AEC) dye (DAKO). Slides were counterstained with Mayer's haematoxylin, and after washing with distilled water, mounted in Aquatex (Merck, Whitehouse Station, NJ, USA).

After immunohistochemical staining, sections stained with antibodies for CRP

were analysed under a microscope (Nikon Microphot FXA Photomicroscope, Nikon Instruments Inc., Melville, NY, USA).

Real-time polymerase chain reaction (PCR) for CRP gene detection

Gingival tissue samples for real-time PCR analysis of CRP mRNA were placed in RNAlater solution (RNA Stabilization Solution, Applied Biosystems, Austin, TX, USA) at 4°C overnight. The following day, the supernatant was removed before storing at −80°C until isolation of total RNA.

Total RNA was extracted from homogenized tissue specimens using Tri-Reagent Solution according to the manufacturer's protocol (Ambion, Austin, TX, USA). Total RNA was then quantified in triplicate by ultra-violet spectroscopy (Nanodrop 1000, Thermo Scientific, Wilmington, DE, USA) and the quality assessed using the ratio of absorbance at 260 and 280 nm.

One microgram of total RNA was then converted to cDNA by reverse transcription using the Superscript VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting cDNA was stored at −20°C and later assessed for CRP gene expression.

The single copy gene 36B4 (acidic ribosomal phosphoprotein, PO) was used as a housekeeping gene. Previously published primer sequences specific for CRP (Jabs et al. 2003a) and 36B4 mRNA (Cawthon 2002) were used to assess CRP and 36B4 gene expression, respectively. The primer sequences for CRP were 5′GAA CTT TCA GCC GAA TAC ATC TTT T3′ (forward) and 5′CCT TCC TCG ACA TGT CTG TCT3′ (reverse). 36B4 primers were 5′CAG CAA GTG GGA AGG TGT AAT CC3′ and 5′CCCA TTC TAT CAT CAA CGG GTA CAA3′, forward and reverse, respectively, all purchased from Geneworks (Hindmarsh, SA, Australia). The levels of CRP gene expression were analysed using a standard curve of known amounts of reverse transcribed human liver RNA (Stratagene, La Jolla, CA, USA), which served as a positive control.

The final reaction mix consisted of 1x EXPRESS SYBR Green ER qPCR Supermix Universal (Invitrogen), with final concentrations of 200 nM CRP forward and reverse primers, or 250 and 200 nM 36B4 forward and reverse primers, respectively, and 1 μL cDNA in a final volume of 20 μL. Each cDNA sample was measured in triplicate. This mix was then amplified at 95°C for 20 s followed by 40 cycles of 95°C for 3 s, 62°C for 30 s (or 58°C for 36B4) and 72°C for 30 s, followed by a melt curve analysis using a Corbett Research Rotor Gene 3000 real-time thermal cycler (Corbett Life Sciences, Sydney, NSW, Australia).

Amplification efficiency of both CRP and 36B4 PCR runs were assessed using the Corbett Rotor-Gene 6.1 software (Corbett Life Sciences), with efficiency between 90% and 110% and $R^2 \geqslant 0.99$.

Verification of PCR products was conducted using gene sequencing (Molecular Pathology Unit, Institute of Medical and Veterinary Sciences, Adelaide, SA, Australia).

Statistical analysis

Data were entered into a computer database and corrected for implausible data. For the basis of analysis, patients with high blood pressure (including those taking anti-hypertensive medication), cardiovascular disease, high cholesterol, diabetes, arthritis, Crohn's disease or recent antibiotic, anti-inflammatory medication or steroid use were categorized as ''systemically unhealthy'', as these conditions may influence systemic levels of CRP. Patients without these conditions were categorized as ''systemically healthy''. Statistical analysis was performed using GraphPad Prism computer software (GraphPad Software Inc., La Jolla, CA, USA). When comparing the numeric characteristics between sample and patient groups, non-parametric Mann–Whitney test statistics were calculated to test the hypothesis of no difference between the groups. When comparing non-numerical characteristics, non-parametric Fisher's exact test statistics were calculated to test the hypothesis of no difference between the groups. Two-sided tests were performed, with statistical significance set at $p < 0.05$.

Results

Subject demographics and sample collection

A total of 64 samples were taken from 59 individual patients, as detailed in Table 1. Of the 64 samples, 50 were collected from 46 patients with perio-

Table 1. Subject, sample demographics and clinical parameters in the study population

	Total	Periodontitis	Non-periodontitis
Number of samples	64	50	14
Number of subjects	59	46	13
Age (years)*	53.6 ± 15.2	55.5 ± 12.1	46.9 ± 18.5
Female	36/59 = 61%	29/46 = 63%	7/13 = 54%
Male	23/59 = 39%	17/46 = 37%	6/13 = 46%
PD (mm)*	6.4 ± 2.0	7.2 ± 1.3†	3.6 ± 1.7
Recession (mm)*	1.3 ± 1.6	1.4 ± 1.5‡	0.9 ± 2.0
BOP+	45/64 = 70%	41/50 = 82%§	4/14 = 29%
GCF volume (μL)*	0.88 ± 0.68	0.98 ± 0.69†	0.52 ± 0.55
Current smoker	11/59 = 19%	8/46 = 17%‡	3/13 = 23%
Systemically healthy	24/59 = 41%	18/46 = 39%‡	6/13 = 46%
Systemically unhealthy	35/59 = 59%	28/46 = 61%	7/13 = 54%
Cardiovascular disease	3	1	2
Diabetes	9	8	1
Arthritis	8	7	1
Crohn's disease	1	0	1
Antihypertensive medication	16	13	3
Cholesterol lowering medication	12	10	2
Anti-inflammatory medication (including aspirin)	17	14	3
Antibiotics	2	1	1
Samples with CRP detectable in GCF	26/64 = 41%	19/50 = 38%§	7/14 = 50%

*Numbers represent mean ± SD.

†$p < 0.01$, Mann–Whitney test, compared with non-periodontitis samples.

‡No statistically significant differences between periodontitis and non-periodontitis groups.

§$p < 0.01$, Fisher's exact test, compared with non-periodontitis samples.

Cardiovascular disease included a history of myocardial infarction, angina, or placement of a cardiac stent. PD, periodontal pocketing; BOP, bleeding on probing; GCF, gingival crevicular fluid; CRP, C-reactive protein.

dontitis, and the remaining 14 were collected from 13 patients who were defined as non-periodontitis, but may have exhibited gingival hyperplasia or gingivitis without periodontal bone loss/attachment loss. The pocket depth for the periodontitis sample sites ranged from 5 to 10 mm with a mean of 7.2 (SD ± 1.3 mm). This was significantly deeper than that measured for the non-periodontitis samples with a mean of 3.6 (SD ± 1.7 mm) (Mann–Whitney test, $p < 0.0001$). Similarly, the percentage of sites that bled upon probing (measured following GCF collection) was significantly higher for the periodontitis sites, compared with the non-periodontitis sites (82.0% *versus* 28.6%, Fisher's exact test, $p = 0.0003$). The mean GCF volume for the periodontitis samples was 0.98 (SD ± 0.69) μL, which was also significantly different from the non-periodontitis samples at a mean of 0.52 (SD ± 0.55) μL (Mann–Whitney test, $p = 0.0159$).

Among the periodontitis samples, 39.1% were from systemically healthy patients without any known medical conditions or taking any medications, while among the non-periodontitis samples, 46.2% were systemically healthy, and this difference was not significantly different (Fisher's exact test). The two most common conditions experienced among this sample population were currently taking anti-hypertensive medication or anti-inflammatory medication (including aspirin).

Table 2. Proportion of samples with detectable C-reactive protein in GCF compared with total sample population

	Total	Periodontitis	Non-periodontitis
Number of samples	26/64 = 41%	19/50 = 38%*	7/14 = 50%
BOP+	16/45 = 36%†	14/41 = 34%*	2/4 = 50%
BOP −	10/19 = 53%	5/9 = 56%	5/10 = 50%
GCF volume (μL)	0.79 ± 0.68	0.90 ± 0.70*	0.51 ± 0.56
Systemically healthy	10/24 = 42%‡	6/18 = 33%*	4/6 = 67%
Systemically unhealthy	16/35 = 46%	13/28 = 46%	3/7 = 43%
Current smoker	4/11 = 36%§	2/8 = 25%	2/3 = 67%
Former or never smoker	22/48 = 46%	17/38 = 45%	5/10 = 50%

*No statistically significant differences between periodontitis and non-periodontitis groups.
†No statistically significant differences in proportion of those with detectable CRP in GCF in BOP+ sites compared with BOP − sites.
‡No statistically significant differences in proportion of those with detectable CRP in GCF who were systemically healthy compared with those who were not.
§No statistically significant differences in proportion of those with detectable CRP in GCF who were current smokers compared with those who were former or never smokers.
BOP, bleeding on probing; GCF, gingival crevicular fluid; CRP, C-reactive protein.

Table 3. Subject and sample demographics and clinical parameters in the samples with detectable C-reactive protein in GCF

	Total	Periodontitis	Non-periodontitis
Number of subjects	26	19	7
Number of samples	26	19/26 = 73%	7/26 = 27%
BOP+	16/26 = 62%	14/19 = 74%*	2/7 = 29%
GCF volume (μL)†	0.79 ± 0.68	0.90 ± 0.70*	0.51 ± 0.56
Systemically healthy	10/26 = 38%	6/19 = 32%*	4/7 = 57%
CRP amount (pg)†	0.12 ± 0.18	0.13 ± 0.21*	0.08 ± 0.09

*No statistically significant differences between periodontitis and non-periodontitis groups.
†Numbers represent mean ± SD.
GCF, gingival crevicular fluid; BOP, bleeding on probing; CRP, C-reactive protein.

Detection of CRP in GCF samples

The subject and sample demographics and clinical parameters for samples with detectable CRP in GCF, in comparison with the total study population are described in Table 2. Approximately, 41% of the 64 GCF samples tested by ELISA had detectable CRP. Numerically, a higher percentage of non-periodontitis GCF samples had detectable CRP compared with the periodontitis GCF samples, but this difference was not statistically significant (50% *versus* 38%, Fisher's exact test). Bleeding on probing (BOP) did not appear to have a positive effect on CRP detection in the GCF with only 35.6% of BOP positive sites subsequently found to be positive for CRP, compared with 52.6% of BOP negative sites, which was not a significant difference (Fisher's exact test). In addition, there were no significant differences in the proportions of BOP positive sites with detectable CRP in the GCF between the periodontitis compared with the non-periodontitis groups (34.1% *versus* 50.0%, Fisher's exact test). Also, there was no significant difference in the volume of GCF for those with detectable CRP compared with the total study population (Mann–Whitney test, data not shown). Systemic health status also did not appear to have a significant influence on CRP detection in the GCF with similar proportions of detection in both systemically healthy and unhealthy groups. Overall, 10 of the 24 (41.6%) systemically healthy patients and 16 of the 35 (45.7%) patients who were not systemically healthy had detectable CRP in GCF, and this was not statistically significant (Fisher's exact test). The 10 systemically healthy patients included two current smokers and two of the systemically unhealthy patients were also current smokers, so that overall four of the 11 (36%) current smokers had detectable CRP in the GCF. Only three of the patients with detectable CRP in the GCF were former smokers, and 19 were never smokers. Owing to the small number of current smokers in the study, statistical significance of smoking on CRP detection was not determined in the current study. However, the proportion of current smokers with detectable CRP was not significantly different from the proportion of former or never smokers with detectable CRP (36.3% *versus* 45.8%, Fisher's exact test).

Table 3 describes the subject and sample demographics and clinical parameters for only the samples with detectable CRP in GCF. Of the 26 samples with CRP detectable in the GCF, 73.1% were periodontitis samples, in keeping with the higher proportion of periodontitis samples in the total sample collection. Seventy-four percent of the CRP positive periodontitis GCF samples and 28.6% of the non-periodontitis GCF

samples were taken from sites that subsequently bled upon probing, this difference approached statistical significance at $p = 0.0687$ (Fisher's exact test), and overall 61.5% of the sites with detectable CRP subsequently bled upon probing. These figures reflect the proportions of BOP+ sites in the periodontitis and non-periodontitis samples seen in the total study population. There were no significant differences in the volume of GCF between periodontitis and non-periodontitis samples (Mann–Whitney test). Interestingly, a numerically higher proportion of non-periodontitis samples with detectable CRP in the GCF were also systemically healthy, compared with the proportion of periodontitis samples that were systemically healthy, but this difference was not statistically significantly (57.1% *versus* 31.6%, Fisher's exact test). Three of the seven patients with both non-periodontitis tissue and CRP detectable in the GCF had other medical conditions that could affect CRP (Type II diabetes, high blood pressure, Crohn's disease), two were current smokers, and the remaining two had no known systemic condition that could affect CRP. The reason for detection of CRP in these otherwise medically and non-periodontitis patients is unknown. There were no significant differences in the amounts of CRP between periodontitis and non-periodontitis samples, or between systemically healthy and systemically unhealthy samples (Mann–Whitney test, data not shown).

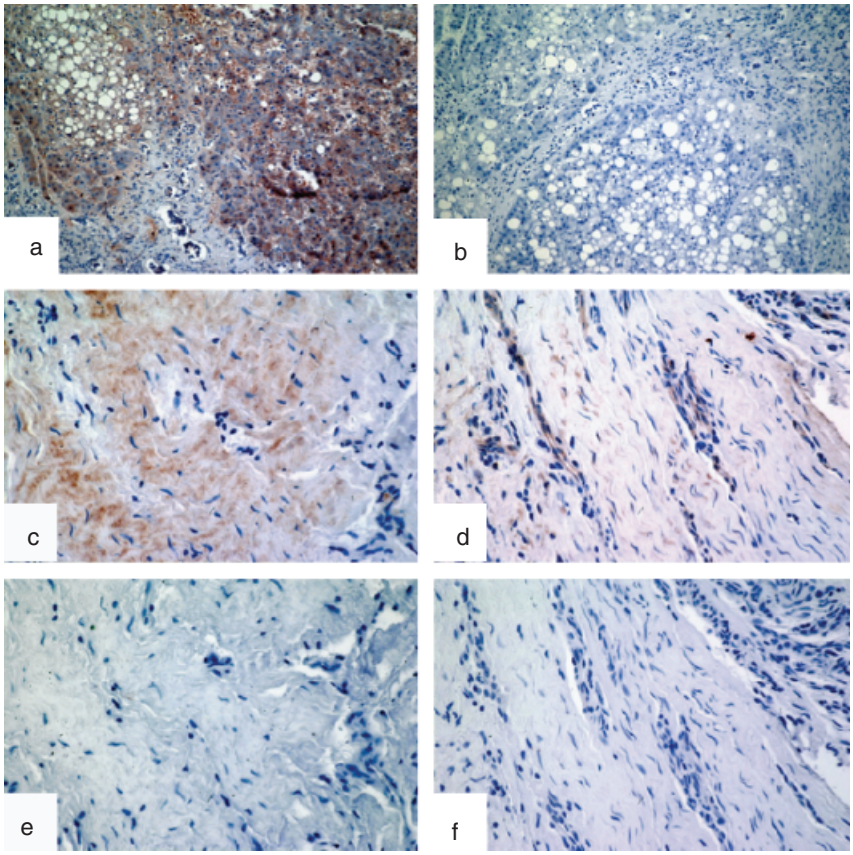

Fig. 1. Immunohistochemical staining of gingival tissue for C-reactive protein. (a) Positive control tissue (liver) immunostaining demonstrates positivity for C-reactive protein antibody in hepatocytes (red), and counterstaining in blue (haematoxylin). Original magnification × 50. (b) Negative control (liver) IgG1 isotype staining in liver tissue. Haematoxylin counterstaining. Original magnification × 50. (c) C-reactive protein immunostaining in gingival tissue demonstrates positivity within connective tissue (red). Haematoxylin counterstaining. Original magnification × 100. (d) C-reactive protein immunostaining in gingival tissue (red) demonstrates positivity around vessels (looks like endothelial cells). Haematoxylin counterstaining. Original magnification × 100. (e) Negative control (omission of antibody) in gingival tissue. Haematoxylin counterstaining. Original magnification × 100. (f) Negative control (IgG1 isotype antibody staining) in gingival tissue. Haematoxylin counterstaining. Magnification × 200.

Immunohistochemical staining for CRP in gingival tissue

Immunohistochemical staining of gingival tissue samples was carried out to determine the distribution of CRP in the periodontal tissue (Fig. 1). Positive staining for CRP was observed around blood vessels within the connective tissue and diffusely within the connective tissues, and no cell-associated staining was seen. Liver tissue was used as a positive control and clearly showed cell associated CRP staining in the hepatocytes. No staining was observed in any of the negative controls included in this study.

Real-time CRP analysis of gingival tissue for CRP mRNA gene expression

Real-time PCR was used to assess whether cells located in gingival tissue were expressing mRNA for CRP. Of the 64 gingival tissue samples that had paired GCF samples, none had detectable levels of CRP mRNA. An additional 22 gingival tissue samples without paired GCF samples were also analysed by PCR for CRP mRNA, but none of these had detectable CRP mRNA. All samples were positive for the expression of the housekeeping gene 36B4 (data not shown).

Discussion

In the current study, as in previous studies, CRP has been detected in the GCF and periodontal tissues of both non-periodontitis and periodontitis sites and subjects. However, the origin of this protein in GCF has not been investigated previously. Based on the absence of CRP mRNA in the periodontal tissues, it can be deduced that the origin of CRP in GCF is not from the local periodontal tissues. Further support for the absence of local production of CRP is that within the periodontal tissues the distribution of CRP was diffuse throughout the connective tissue and was not cell-associated. Therefore, the presence of CRP in the GCF and periodontal tissues appears to be of systemic origin.

The main source of CRP is acknowledged to be the liver (Hurlimann et al. 1966), and although several other tissues have recently been shown to produce CRP (Yasojima et al. 2000, 2001, Gould & Weiser 2001, Jabs et al. 2003a, b); these are regarded as having minimal

contribution to serum levels of CRP (Black et al. 2004). Systemic CRP detected in GCF and periodontal tissue may be a result of systemic inflammation resulting from disease elsewhere in the body (Blake & Ridker 2001, Blake et al. 2003, Aletaha et al. 2007, Bruns et al. 2008, Schutte & Malfertheiner 2008), as well as systemic inflammation induced by periodontitis (Paraskevas et al. 2008). Indeed, in this study the majority of patients with detectable CRP had either periodontitis or systemic inflammatory disease, and it is very possible that the remaining patients had an undiagnosed systemic inflammatory disease or a recent infection, which may explain the detection of CRP, given that it is not of local origin.

In the current study, serum levels of CRP were not analysed. While the collection of serum samples would have enabled a correlation between GCF and serum levels of CRP, the aim of this study was primarily to determine if CRP was locally produced in the periodontal tissues and not to correlate CRP in the GCF with CRP in the serum. Collecting serum samples was not necessary to determine if CRP was produced locally, and GCF samples were primarily included to assess whether CRP was present or absent in the periodontal tissues. As GCF is a transudate of serum, and our results establish that CRP is not produced locally in the periodontal tissues, CRP in the GCF must be derived from serum CRP, and may be indicative of systemic inflammation. Nonetheless, future studies may wish to address the correlation between GCF and serum levels of CRP. If the levels of CRP in GCF mirror those in serum, then the simple collection of GCF could be a non-invasive means of screening for CRP serum levels to determine the severity of systemic inflammation. Indeed, chairside kits for CRP measurement using oral fluids are currently under development (Christodoulides et al. 2005, Floriano et al. 2009) and could provide a novel means to screen patients for systemic inflammation.

We detected CRP in some, but not all, of the GCF and periodontal tissues of both non-periodontitis and periodontitis patients. This is consistent with previous studies (Sibraa et al. 1991, Tuter et al. 2007, Fitzsimmons et al. 2009, 2010, Sanders et al. 2009). Such findings are not surprising if CRP is not produced in the periodontal tissues, as the systemic inflammatory response between individuals is not uniform. Genetic susceptibility to inflammation may modify CRP levels, in response to periodontal destruction or systemic disease, such that patients with a hyperinflammatory phenotype exhibit higher levels of CRP (Beck et al. 2000). As such, probably only a subset of patients produce a systemic CRP response to periodontitis, due to their genetic pre-disposition (Mattila et al. 2002). Indeed, an equivalent hypothesis has been confirmed in genetically hyperinflammatory mice, showing elevated systemic inflammatory markers as a result of experimental periodontitis, compared with hypoinflammatory mice (Trombone et al. 2009). Such patients may be at higher risk of systemic inflammatory diseases due to their hyperinflammatory trait.

The incidence of detection of CRP in GCF in this study is consistent with that of recently published studies (Tuter et al. 2007, Fitzsimmons et al. 2009, 2010, Sanders et al. 2009). In our study, CRP was not detected in the GCF of all patients. An explanation for this is that the levels of CRP may have been below the sensitivity of the ELISA assay used, although this was the same as Fitzsimmons et al. (2009, 2010) and Sanders et al. (2009) and greater than that of Tuter et al. (2007). Our study differed from these papers in several aspects. The samples described in Fitzsimmons et al. (2009, 2010) and Sanders et al. (2009) were taken from a population study (Slade et al. 2007), and no history was given of prior periodontal treatment, medical conditions or medication use, all of which are associated with systemic CRP levels (Pradhan et al. 2001, Dehghan et al. 2007, Kinlay 2007, Pearson et al. 2007, Paraskevas et al. 2008). The paper by Tuter et al. (2007) also used untreated periodontitis patients, but excluded those with medical conditions or medications that could increase or decrease CRP levels. In our study, the periodontitis patients had completed a hygienic phase of periodontal therapy while the non-periodontitis patients had not received any such initial therapy. The participants in this study were not excluded on the basis of medical conditions or smoking status. The prior periodontal treatment may have reduced the influence of periodontal inflammation on systemic CRP, as periodontal treatment has been shown to have a moderate effect on reducing systemic CRP levels (Paraskevas et al. 2008). Also, it is acknowledged that many medical conditions do influence systemic CRP, and that many patients in our study had these conditions. However, the exact effect on systemic CRP levels may be difficult to determine. This is because the majority of patients in our study with a medical condition that may affect systemic CRP were also taking medication to counteract that condition and this could further modify systemic CRP. As CRP is not produced locally, the inclusion of patients with medical conditions that may affect CRP does not change the interpretation of this main result.

Because the detection of CRP in both serum and GCF of patients with periodontitis is not uniform, those individuals with detectable CRP may be more susceptible to developing other systemic inflammatory conditions, indeed perhaps due to a hyperinflammatory phenotype (Beck et al. 2000). Those subjects with a hyperinflammatory phenotype may be more susceptible to periodontal bone loss, as demonstrated by recent animal studies (Trombone et al. 2009). It may therefore be desirable to be able to identify these individuals, as they may be at greater risk of periodontal disease progression. Additionally, identifying hyperinflammatory trait individuals may highlight to the clinician the need for a more aggressive management of periodontal and cardiovascular disease risk factors. In our study, a small proportion of non-periodontitis patients had no known medical conditions that could affect CRP, and the reason for detection of CRP in the GCF of these patients is unknown. These patients could have an undiagnosed inflammatory condition.

In conclusion, the findings of this study indicate that CRP detected in the GCF and periodontal tissue is not of local origin. This implies that elevated serum CRP in periodontitis patients is not due to local production but could be indicative of systemic inflammation, either as a result of periodontal infection or systemic disease. As GCF is a serum transudate, we propose that it may be considered as a substitute source with which to assess systemic inflammation as measured by CRP. However, further studies are needed to correlate levels of CRP in the GCF with that of serum before GCF could be considered to be suitable as a source for the non-invasive assessment of the degree of systemic inflammation in both periodontitis and non-periodontitis patients.

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Clinical Relevance

Scientific Rationale for Study: CRP can be detected in GCF. Although the liver is the primary source of CRP, its extra-hepatic production has been reported. We studied whether CRP in the GCF could arise from its local production within the gingivae.

Principal Findings: CRP is not produced within gingivae at sites of periodontitis.

Practical Implications: CRP in GCF is of systemic origin, and therefore indicative of systemic inflammation provoked by periodontal infection or inflammatory disease elsewhere. Testing GCF for CRP may be a non-invasive means of screening for systemic inflammation in periodontitis and non-periodontitis patients.