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Research Paper

β_1-Adrenoceptor antibody-induced increase in soluble CD40 ligand release in chronic periodontitis patients: role of prostaglandin E_2

Leonor Sterin-Borda[1,2], Marcela Segovia[1], Silvia Reina[1,2] and Enri Borda[1,2]

[1] *Pharmacology Unit, School of Dentistry, Buenos Aires University, Buenos Aires, Argentina*
[2] *Argentine National Research Council (CONICET), Buenos Aires, Argentina*

In this paper, we demonstrate that circulating antibodies from chronic periodontitis patients reacting with atrial β_1-adrenoceptors (β_1-ARs) act as an inducer of soluble CD40 ligand (sCD40L) release and prostaglandin E_2 (PGE_2) generation. By enzyme-linked immunosorbent assay using β_1 synthetic peptide (with an amino acid sequence identical to the second loop of human myocardial β_1-ARs) as a coating antigen, we demonstrated reactivity against the second extracellular loop on human myocardial β_1-ARs. This autoantibody present in the serum of chronic periodontitis patients was significantly correlated with the release of sCD40L and PGE_2. The release of sCD40L was blunted by atenolol, SP600125 and β_1 synthetic peptide, and PGE_2 generation was inhibited by DuP 697 and slightly by FR122049. The effects of the antibody incubated with isolated rat atria upregulated sCD40L release with an increase of PGE_2 production and c-Jun N-terminal kinase phosphorylation. These results indicate that in chronic periodontitis patients, there is a positive association between sCD40L release and PGE_2 generation via the action of β_1-AR antibodies.

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Corresponding author E. Borda: Pharmacology Unit, School of Dentistry, University of Buenos Aires, Marcelo T. de Alvear 2142–4° 'B', 1122AAH Ciudad Autónoma de Buenos Aires, Argentina. Email: enri@farmaco.odon.uba.ar

Autoantibodies with functional activities against β_1-adrenoceptors (β_1-ARs) were described for the first time in patients with cardiomyopathy (Sterin-Borda *et al.* 1976; Borda *et al.* 1984), followed by the description of such β_1-AR antibodies in patients with dilated cardiomyopathy (Wallukat & Wollenberger, 1987; Limas *et al.* 1989).

A recent study (Sterin-Borda *et al.* 2009) suggests the involvement of serum β_1-AR autoantibodies directed towards extracellular matrix components in the pathogenesis of certain types of periodontal disease. Initial studies of autoimmunity in the pathogenesis of periodontitis focused on the detection of autoantibodies directed towards various self-antigens (Rajapake & Dolby, 2004; Dileep & Pradeep, 2006), such as autoantibodies against the human gingival fibroblast β_1-AR (Furlán *et al.* 2010). Thus, antihuman gingival fibroblast antibodies concomitant with antibacterial antibodies may contribute to the pathogenesis of periodontitis.

Periodontal disease is a multifactorial infection (Beck *et al.* 1992; Oliver *et al.* 1998) caused by the dental plaque biofilm, but the host inflammatory immune response modifies disease outcome (Listgarten & Loomer, 2003). Also, stress and local stimulation of the autonomic adrenergic system are cofactors that could contribute to the prevalence of disease and to disease progression (Silze *et al.* 2004).

We are now able to show that, in certain cardiovascular diseases and in periodontal disease, the second extracellular loop of the β_1-AR is the main antigenic domain recognized by the anti-β_1-AR autoantibodies present in the serum of periodontitis patients (Magnusson *et al.* 1990; Joensen *et al.* 2003), and it could also be a target for autoantibodies with functional activities (Wallukat *et al.* 1995).

In contrast, periodontitis has been linked to systemic illnesses, such as cardiovascular disease and stroke. Increasing evidence indicates that periodontal disease is a risk factor in coronary disease (Armitage, 2000; Karnoutsos *et al.* 2008; Ramseier *et al.* 2009; Nakajima & Yamazaki, 2009) through endothelial cell

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dysfunction induced by periodontopathic bacteria, their products or inflammatory mediators derived from infected periodontal tissue (Nakajima & Yamazaki, 2009). It also raises core questions about the effect of other non-thrombotic factors triggering functional alterations in the myocardium, thus inducing the 'remodelling phenomenon' of the heart. Moreover, although modulation of these inflammatory processes in connection with abnormalities in the cytokine network during this disorder has been suggested, proof is still lacking.

CD40 ligand (CD40L), a transmembrane protein of the tumour necrosis factor superfamily (Schönbeck & Libby, 2001), is subsequently cleaved by a proteolytic process within a period of minutes to hours to form soluble CD40 ligand (sCD40L; Andre *et al.* 2002). This circulating protein has the unique property of promoting both inflammatory and thrombotic processes (Aukrust *et al.* 2004). Soluble CD40L is found to be elevated in coronary artery disease, particularly in patients with acute coronary syndrome (Aukrust *et al.* 1999). The enhanced activity of sCD40L is more prominent in the setting of acute myocardial infarction, in which the upregulation of the sCD40L release mechanism from platelets leads to elevated sCD40L levels (Garlichs *et al.* 2001).

Based on the presence of β_1-AR autoantibodies in patients with chronic periodontitis (CP), the role of CD40 in the inflammatory disease process and the β_1-AR antibody-mediated overexpression of CD40 in human gingival fibroblasts (Sterin-Borda *et al.* 2009), in the present study, we investigated the mechanism of sCD40L release from the serum of human periodontitis patients by β_1-AR autoantibodies and the participation of prostaglandin E_2 (PGE_2) in this phenomenon. It is likely that in CP associated with an upregulation of sCD40L and PGE_2, these two factors would increase the risk of coronary heart disease.

Methods

Patients

The study group consisted of 20 (18 male and 2 female) adult patients with CP (group I) who were attending the Periodontology Clinic from the metropolitan area of Buenos Aires. The mean age was 40 years, with a range of 32–50 years. Healthy subjects (group II) were used as controls (16 male and 4 female subjects) with a mean age of 38 years and a range of 30–46 years. The characteristic clinical signs of CP included the following: loss of clinical attachment; horizontal and/or angular alveolar bone loss; periodontal pocket formation; and gingival inflammation. To be included in the study, at least six sites with ongoing periodontal disease were required. Clinical measurements on patients with CP included sites with alveolar bone loss >2 mm and a pocket depth >5 mm, with bleeding and attachment loss >3 mm. In the healthy subjects (control group), the probing depth was <3 mm and the attachment loss <2 mm. None of the subjects (patients in group I and control subjects in group II) had systemic illnesses, and they were not smokers. The patients with CP had not received periodontal treatment or antibiotics within the preceding 5 months or any anti-inflammatory drugs for 3 weeks prior to the study.

Table 1. Characteristics of the study populations

Demographics and risk factors	Periodontitis patients; group I ($n = 20$)	Healthy subjects; group II ($n = 20$)
Sex		
Male	18	16
Female	2	4
Education level		
Elementary school	19	15
High school	1	5
Body mass index (range; kg m^{-2})	22–26	20–24
Blood pressure (mmHg)		
Systolic (mean ± SD)	130 ± 20	116 ± 14
Diastolic (mean ± SD)	85 ± 12	76 ± 10
Laboratory examinations		
Total cholesterol (mean ± SD; mg dl^{-1})	196 ± 19	174 ± 18
Low-density lipoprotein (mean ± SD; mg dl^{-1})	129 ± 14	130 ± 20
High-density lipoprotein (mean ± SD; mg dl^{-1})	34 ± 10	32 ± 12

The clinical characteristics of the study populations are summarized in Table 1. All of the CP patients and control subjects consented to participate in the study, and the investigation was conducted according to the tenets of the Declaration of Helsinki of 1975 as revised in 2000.

Human sera and IgG purification

Sera and the corresponding IgG were obtained from patients with CP (group I) and normal individuals (group II). Six millilitres of blood was obtained by arm venipuncture and allowed to clot at room temperature, and the serum was separated by centrifugation at 2000*g* for 10 min and stored at −20°C until used in assays. The IgG was obtained by precipitation with 50% ammonium sulphate, followed by three washes and reprecipitation with 33% ammonium sulphate. The resulting precipitate was submitted to chromatography on diethylethanolamine-cellolose (DEAE-cellulose), equilibrated with 10 mM phosphate buffer (pH 8). The eluted peaks were concentrated by ultrafiltration to 10 mg protein ml^{-1}. Control immune

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electrophoresis with goat anti-human total serum and goat non-specific anti-human IgG showed only one precipitation line.

Purification of antipeptide antibodies by affinity chromatography

The IgG fraction of group I patients and group II subjects was independently subjected to affinity chromatography on the synthesized peptide covalently linked to Affi-Gel 15 gel (Bio-Rad, Richmond, CA, USA). The IgG fraction was loaded on the affinity column equilibrated with PBS, and the non-peptide fraction was eluted with the same buffer. Specific antipeptide autoantibodies (anti-β_1-AR peptide IgG) were then eluted with 3 M KSCN and 1 M NaCl, followed by immediate extensive dialysis against PBS. The IgG concentrations of both non-antipeptide antibodies and specific anti-β_1-AR peptide antibodies were determined by radial immunodiffusion assay, and the immunological reactivity against the β_1-AR peptide was evaluated by enzyme-linked immunosorbent assay (ELISA).

Enzyme-linked immunosorbant assay

Fifty millilitres of peptide solution (20 μg ml^{-1}) in 0.1 M Na_2CO_3 buffer (pH 9.6) was used to coat microtiter plates at 4°C overnight. After blocking the wells with 2% bovine serum albumin in PBS for 1 h at 37°C, 1:30 dilution of sera from groups I and II were added in duplicate and allowed to react with the peptide for 2 h at 37°C. After thoroughly washing the wells with 0.05% Tween 20 in PBS, 100 μl of 1:6000 goat anti-human IgG alkaline phosphate-conjugated antibodies was added and incubated for 1 h at 37°C. After extensive washing, *p*-nitrophenylphosphate (1 mg ml^{-1}) was added as the substrate, and the reaction was stopped after 30 min. Optical density was measured at 405 nm with an ELISA reader. As a negative control, non-antigen-paired wells with non-specific peptide and wells with no primary antiserum were also processed. The results for each sample were expressed as the mean ± SEM of duplicate values.

Animals and preparation of the atria

Adult male Wistar rats (250–300 g) were used. The animals were housed in standard environmental conditions and fed with a commercial pelleted diet and water *ad libitum*. The experimental protocol followed the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). Rats were anaesthetized with a mixture of ketamine and xylazine (50 and 5 mg kg^{-1}, respectively) and killed by decapitation. The atria were carefully dissected from the ventricles and immersed in a tissue bath containing Krebs–Ringer bicarbonate solution gassed with 5% CO_2 in oxygen and maintained at pH 7.4 and 37°C. The Krebs–Ringer bicarbonate solution was composed as described previously (Sterin-Borda *et al.* 1976).

Prostaglandin E_2 and sCD40L assays

The atria were incubated for 60 min in 0.50 ml Krebs–Ringer bicarbonate solution gassed with 5% CO_2 in oxygen at 37°C. Sera or anti-β_1 IgG were added to the isolated atria 50 min before the end of the incubation period. Blockers were added 10 min before the addition of different concentrations of sera or anti-β_1 IgG. The atria were then homogenized into a 1.5 ml polypropylene microcentrifuge tube. Thereafter, all procedures employed were those indicated in the protocol of Prostaglandin E_2 Biotrak Enzyme Immunoassay (ELISA) System (Amersham Biosciences, Piscataway, NJ, USA). Soluble CD40 ligand levels were measured in serum and homogenized tissues. All samples were stored at −80°C until assayed. Assessment of serum concentrations of sCD40L was performed using a commercially available ELISA kit for quantitative detection of human sCD40L (Human sCD40L Platinum ELISA High Sensitive, Bioscience, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, plasma samples (20 μl per well) or standards (100 μl per well) were incubated for 2 h at room temperature. After washing, 100 μl of HRP conjugate was added for 1 h at room temperature. Following incubation, unbound HRP-conjugated anti-human sCD40L antibody was removed during a wash step, and substrate solution (TMB ELISA substrate) was added to the all wells. A range of 0.08–5.00 ng ml^{-1} of sCD40L was used to establish standard curves and to maximize the sensitivity. The sCD40L levels were determined using a spectrophotometer at a wavelength of 450 nm, and the data are reported in nanograms per millilitre.

Drugs

FR122047 [cyclo-oxygenase (COX)-1 inhibitor] and DuP 697 (COX-2 inhibitor), were purchased from Tocris Cookson Inc. (Menville, MO, USA), while atenolol (β_1-adrenoceptor antagonist), isoprenaline (non-selective β-adrenoceptor agonist), SC19220 (PGE_2 receptor inhibitor), SP600125 [enzymatic c-Jun N-terminal kinase (JNK) inhibitor] and PGE_2 were from Sigma Chemical Co. (St Louis, MO, USA). Stock solutions were freshly prepared in the corresponding buffers. The vehicles used were ethanol for PGE_2 and DMSO in distilled water for DuP 697, FR122047, SC19220 and SP600125. The drugs were diluted in the bath to achieve the final concentration stated in the text. The final concentration of DMSO was

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not more than 1 in 1000 and it lacked pharmacological action.

Statistical analysis

Student's unpaired *t* test was used to determine the levels of significance. When multiple comparisons were necessary, after ANOVA the Student–Newman–Keuls test was applied. Differences between means were considered significant if $P < 0.05$.

Results

To demonstrate the presence of serum IgG directed against β_1-ARs (anti-β_1 IgG), we performed an ELISA using a β_1 synthetic peptide corresponding to the amino acid sequence of the second extracellular loop of the human β_1-AR as a coating antigen. The scatterogram of Fig. 1*A* shows the optical density values for each of the 20 periodontitis patients (group I) and 20 healthy individuals (group II). The optical density values obtained with the reactive autoantibodies were always more than two standard deviations higher than those of healthy individuals. There were significant differences between the two groups ($P < 0.0001$). These results demonstrated that chronic periodontitis patients have circulating autoantibodies in the serum that interact with β_1-ARs.

Figure 1 also shows both PGE_2 (Fig. 1*B*) and sCD40L concentrations (Fig. 1*C*) in the serum of periodontitis patients (group I) and normal subjects (group II) used as controls. The concentrations of PGE_2 and sCD40L in the serum of periodontitis patients were also two standard deviations higher than those of healthy subjects. The values from group I were higher than those from group II, and there was a significant difference between the two groups

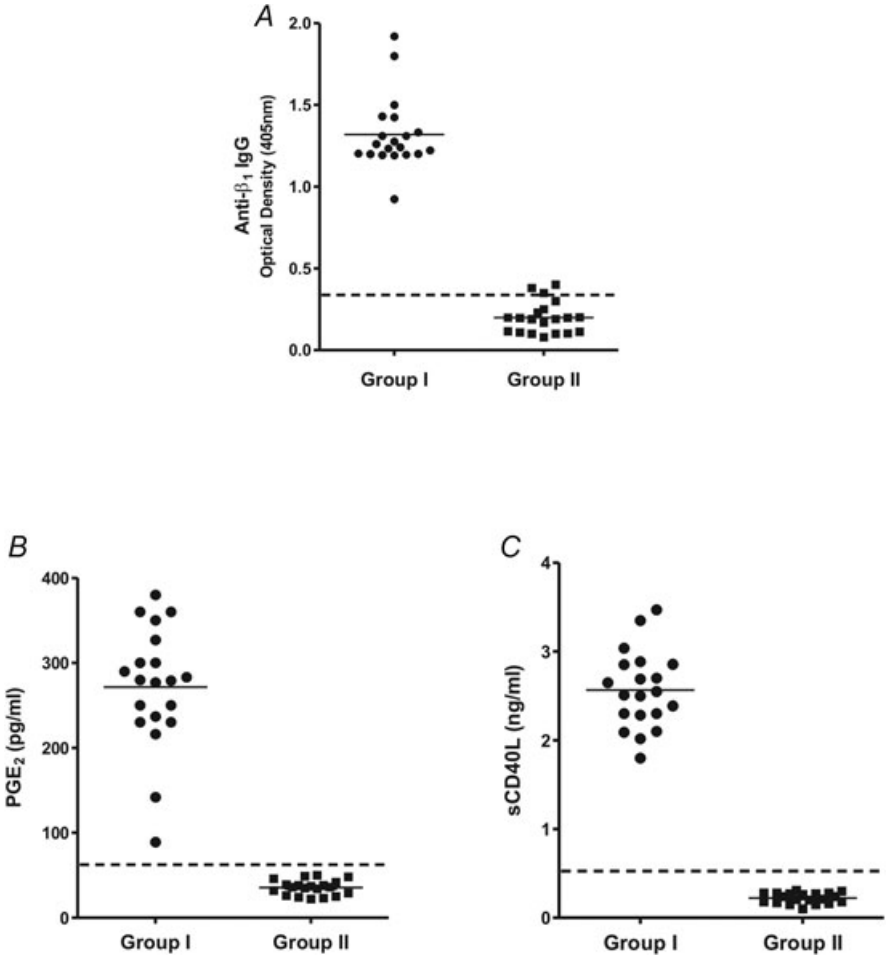

Figure 1. Scattergram showing immunoreactivity of circulating IgG autoantibodies against β_1-adrenoceptor (β_1-AR) synthetic peptide (*A*), values of prostaglandin E_2 (PGE_2) production (*B*) and soluble CD40 ligand (sCD40L) levels (*C*) in serum from periodontitis patients
Individual optical density values for each serum sample (1:30 dilution) from 20 periodontitis patients (group I) compared with 20 healthy individuals (control subjects; group II) are shown. The horizontal lines in *A*, *B* and *C* are mean values. $P < 0.0001$, group I *versus* group II for anti-β_1 IgG, PGE_2 generation and sCD40L levels.

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($P < 0.0001$) in both the serum PGE_2 and the sCD40L levels.

To determine whether the variation in immunoreactivity of the serum against β_1-AR was related to PGE_2 generation and raised serum levels of sCD40L compared with healthy control subjects, a correlation analysis between sCD40L, PGE_2 and anti-β_1 IgG was performed. Figure 2 demonstrates that there was a significant correlation between sCD40L and anti-β_1 IgG and between PGE_2 and anti-β_1 IgG. In addition, PGE_2 serum levels were also positively correlated with serum sCD40L levels (Fig. 3).

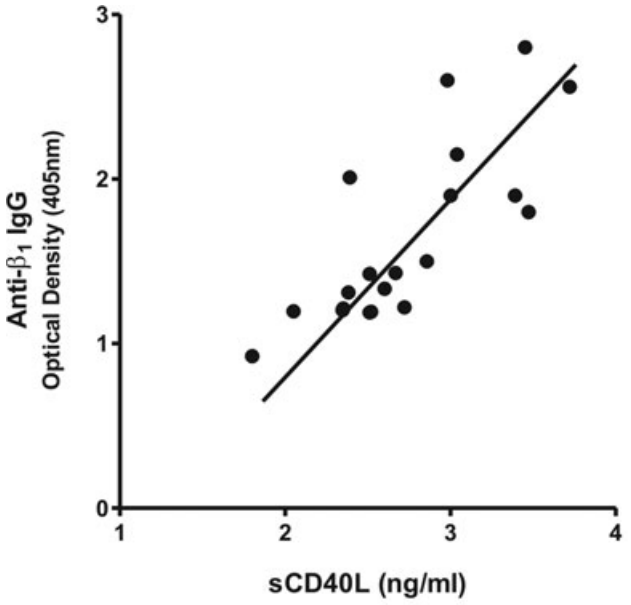
Sera autoantibodies (anti-β_1 IgG) from periodontitis patients significantly induced sCD40L release from atria in a time-dependent manner (Fig. 4*A*). Anti-β_1 IgG affected sCD40L release in as little as 6 min, with the maximal effect occurring at 45 min. Conversely, IgG from healthy subjects failed to change sCD40L release (Fig. 4*A*). When isolated rat atria were pretreated with atenolol (1×10^{-7} M), a specific β_1-AR antagonist, the stimulatory action of anti-β_1 IgG on sCD40L levels was inhibited, suggestomg the activation of cardiac β_1-ARs by the autoantibodies (Fig. 4*B*).

To determine why increased levels of sCD40L triggered by anti-β_1 IgG from periodontitis patients were dependent on the arachidonic acid cascade enzymes, we studied this cascade reaction using several inhibitors. It can be seen in Fig. 5 that inhibition of COX-1 by 5×10^{-8} M FR122047 (Ochi & Goto, 2002) and COX-2 by 5×10^{-8} M DuP 697 (Gans *et al.* 1990) prevented the stimulatory action of anti-β_1 IgG on sCD40L levels. Moreover, when 1×10^{-10} M exogenous PGE_2 was added, the inhibitory effect of FR122047 and DuP 697 was impaired. The addition of 1×10^{-6} M SC19220, a prostaglandin receptor subtype 1 (EP1) antagonist, inhibited the effects of exogenous PGE_2 (Fig. 5). The addition of 1×10^{-5} M SP600125 before anti-β_1 IgG at a concentration known to inhibit JNK phosphorylation (Wylie *et al.* 1999) blunted the anti-β_1 IgG sCD40L-increased release (Fig. 5). None of the inhibitory agents at the concentrations used had any effect on sCD40L release (Table 2).

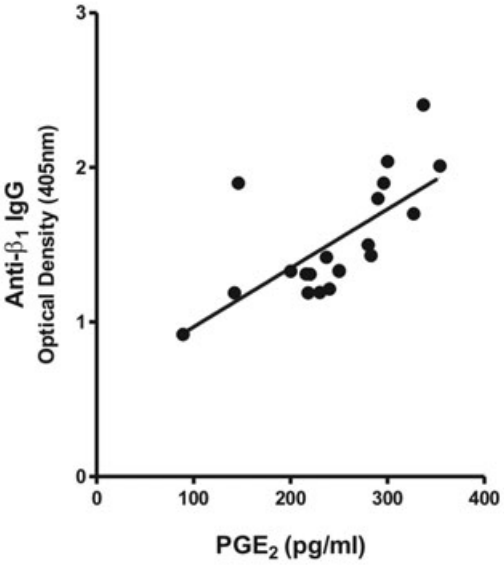
It is important to note that the non-selective β-AR agonist isoprenaline (1×10^{-8} M) can induce the release of sCD40L from rat atria and that the β_1-AR antagonist atenolol (1×10^{-7} M) blunted this action. On the contrary, the sCD40L levels were not changed by the atria alone or in the presence of the β_1-AR antagonist atenolol (Table 3).

Discussion

In this study, we demonstrated the presence of active anti-β_1 IgG in the sera of chronic periodontitis patients, modulating sCD40L levels and PGE_2 generation. Thus, functional studies revealed that serum IgG autoantibodies



Number of XY Pairs:	20
Pearson r:	0,7862
95% confidence interval:	0.5271 to 0.9116
P value (two-tailed):	P<0.0001
Is the correlation significant? (A=0.05):	Yes
R squared:	0,6182



Number of XY Pairs:	20
Pearson r:	0,6842
95% confidence interval:	0.3466 to 0.8649
P value (two-tailed):	0,0009
Is the correlation significant? (α=0.05):	Yes
R squared:	0,4682

Figure 2. Correlation between sCD40L and anti-β_1 IgG serum values of patients from group I (*A*) and between PGE_2 and anti-β_1 IgG serum values of patients from group I (*B*)

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from periodontitis patients enhanced sCD40L levels and PGE_2 generation. Increased levels of sCD40L were also observed with exogenous PGE_2, and a prostaglandin receptor subtype 1 (EP1) blocker impaired this action. Also, the increment of sCD40L levels and PGE_2 generation were almost abolished by treatment of the atria with SP600125, a drug that impairs JNK phosphorylation. Atrial β_1-AR activation by anti-β_1 IgG increased sCD40L; this action was blocked by a specific β_1-AR antagonist, atenolol, and a β_1 synthetic peptide. The arachidonic acid cascade was activated by anti-β_1 IgG, with subsequent activation of COXs (COX-1 and COX-2) inducing PGE_2 generation. The finding that these two features (augmented sCD40L release and PGE_2 generation) involved myocardial β_1-AR activation is consistent with the remodelling process that occurs in human heart failure (Ueland *et al.* 2005). Moreover, modulation of these inflammatory processes has been accompanied by increments of various pro-inflammatory substances, such as PGE_2 and sCD40L, in the sera of patients with chronic periodontitis. The complex interaction between CD40L and CD40 seems to play a pathogenic role in a wide range of inflammatory disorders, such as autoimmune diseases, multiple sclerosis and cardiac allograft rejection, and it has recently been shown to be involved in atherogenesis,

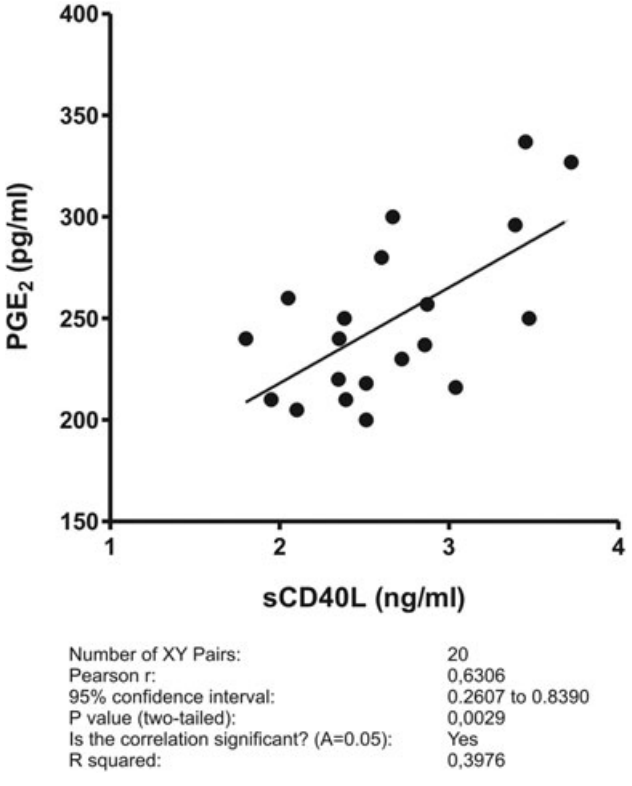

Figure 3. Correlation between sCD40L and levels of PGE_2 in serum of 20 patients with periodontitis
Each symbol represents one patient.

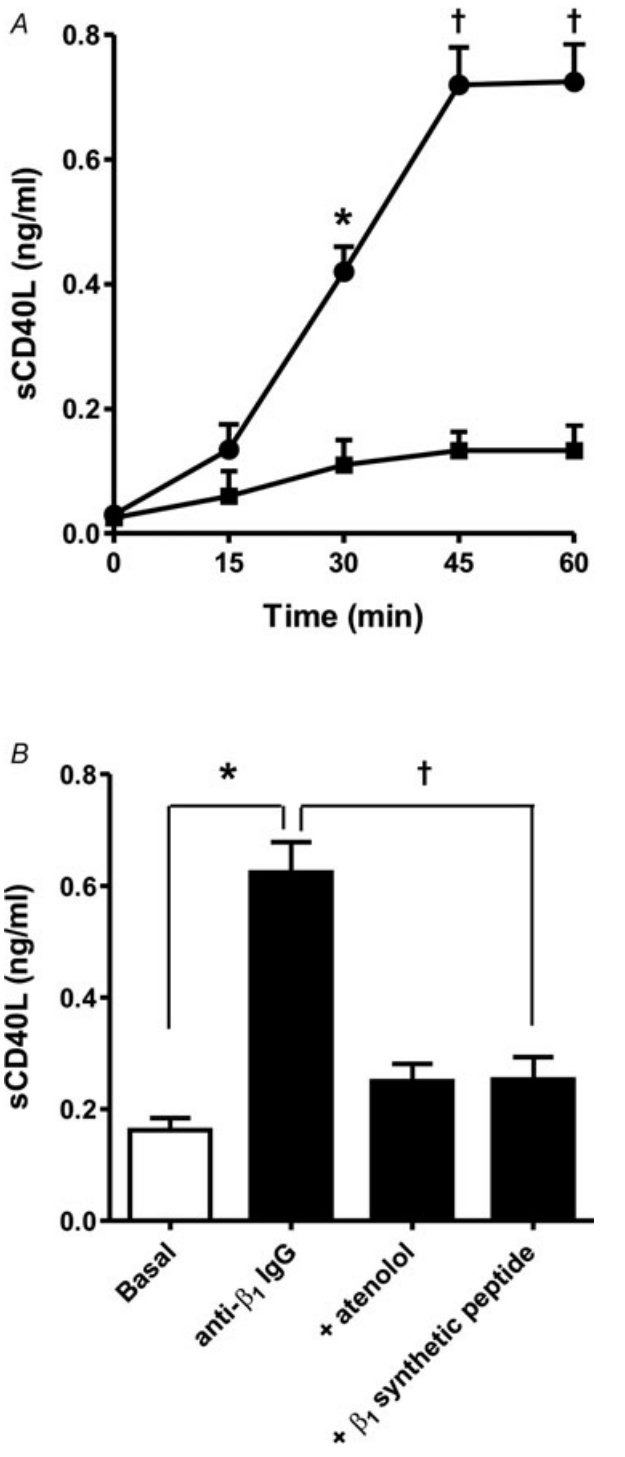

Figure 4. Measurement of sCD40L release.
A, time dependence of the levels of sCD40L from rat atria treated with anti-β_1 IgG. The maximal level of sCD40L release occurred 45 min after addition of anti-β_1 IgG, without modification thereafter. *B*, anti-β_1 IgG (1×10^{-8} M) induced significant compared with basal values ($^*P < 0.0001$) sCD40L release when the antibody was incubated for 1 h with rat atria. Atenolol (1×10^{-7} M) and β_1 synthetic peptide (1×10^{-5} M; $\dagger P < 0.0001$) abrogated the stimulatory action of anti-β_1 IgG. Values are means ± SEM of 20 individual sera from group I.

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acute coronary syndrome and acute myocardial infarction (Aukrust *et al.* 1999; Lee *et al.* 1999; Garlichs *et al.* 2001; Peng *et al.* 2002; Yan *et al.* 2002; Heeschen *et al.* 2003). The association between periodontitis and cardiovascular disease makes it likely that periodontitis can be added to the list of factors used to assess a patient's risk for coronary heart disease (Karnoutsos *et al.* 2008). Moreover, anti-β_1-AR autoantibodies present in the serum of patients with periodontitis may be associated with myocardial disease and atherosclerosis, because these autoantibodies are able to release pro-inflammatory substances, such as PGE_2 and sCD40L, that are significant factors in the pathogenesis of coronary heart disease.

There are several biological mechanisms by which CP might be associated with chronic heart disease. First, CP is a chronic infection that results in a chronic inflammatory state (D'Aiuto *et al.* 2004); second, periodontal disease is associated with intermittent bacteraemia, which may have a role either in the chronic inflammatory state or, more directly, on the endothelial tissue surface (Haraszthy *et al.* 2000); and third, some studies conducted using animals have shown an association between atheroma formation and exposure to periodontal pathogens (Genco *et al.* 2002).

Clinical studies of periodontitis have revealed that pathogenic bacteria in the periodontal pocket may cause a cross-reaction with the host atherosclerotic plaque, altering the vascular cell function and providing, together with anti-β_1-AR autoantibodies, the possibility of acute systemic stroke (Chiu, 1999).

All these findings suggest that the chronic inflammation in periodontitis, which is associated with elevated levels of sCD40L and PGE_2, establishes an aetiological relationship between periodontal disease and coronary heart disease (Humphrey *et al.* 2008). Persson & Persson (2008) and Nesse *et al.* (2010) also detected an increased prevalence of cardiovascular and autoimmune diseases among periodontitis patients, and Ueland *et al.* (2005) found that raised serum levels of sCD40L were significantly correlated with the severity of human myocardial failure.

To clarify the link between CP and chronic heart disease will required a consistent body of evidence from longitudinal disease and careful follow-up. The premise

Table 2. Influence of inhibitory agents upon soluble CD40 ligand (sCD40L) release

Agent	sCD40L (ng ml^{-1})	*n*
None (basal conditions)	0.216 ± 0.015	6
FR122047 (5 × 10^{-8} M)	0.218 ± 0.016	5
DuP 697 (5 × 10^{-8} M)	0.198 ± 0.018	5
SP600125 (1 × 10^{-5} M)	0.223 ± 0.019	5
SC19220 (1 × 10^{-6} M)	0.028 ± 0.016	5

Values are means ± SEM of number of experiments (*n*) performed in duplicate.

Table 3. Influence of the non-selective β-AR agonist isoprenaline and the β_1-AR antagonist atenolol upon release of sCD40L from rat atria

Conditions	sCD40L (ng ml^{-1})	*n*
Basal	0.110 ± 0.009	5
Atria alone	0.116 ± 0.010	8
Atria + isoprenaline (1 × 10^{-8} M)	0.567 ± 0.003*	8
Atria + isoprenaline + atenolol (1 × 10^{-7} M)	0.125 ± 0.009†	7
Atria + atenolol (1 × 10^{-7} M)	0.122 ± 0.008	5

Values are means ± SEM of *n* experiments performed in duplicate. *$P < 0.001$ *versus* atria alone; †$P < 0.001$ *versus* atria + isoprenaline.

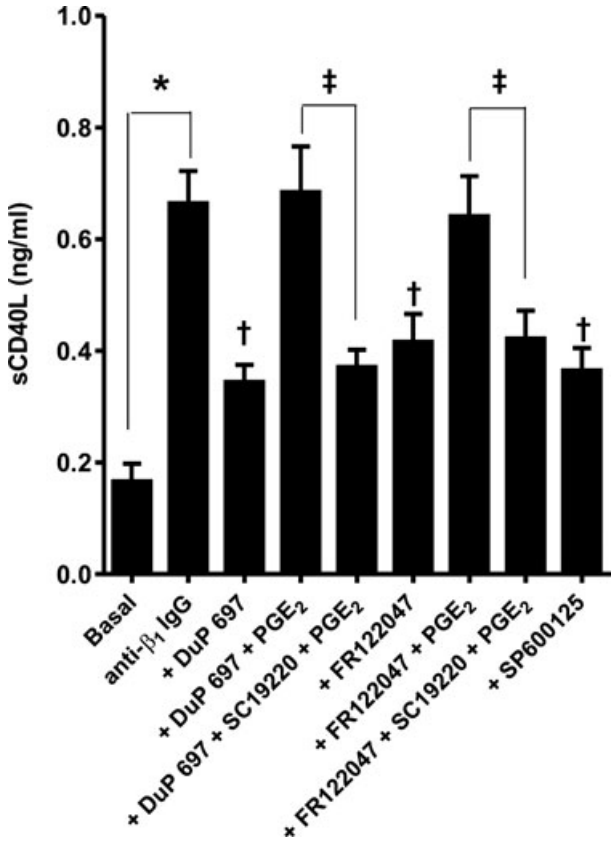

Figure 5. Influence of different inhibitors on sCD40L stimulation by anti-β1 IgG
Rat atria were incubated for 1 h with anti-β_1 IgG (1 × 10^{-8} M) from sera of periodontitis patients with increased sCD40L levels. DuP 697 (5 × 10^{-8} M), FR122047 (5 × 10^{-6} M) and SP600125 (1 × 10^{-5} M) blunted the stimulatory action of anti-β_1 IgG. Exogenous PGE_2 (1 × 10^{-10} M) restored the values of sCD40L in the presence of DuP 697 or FR122047, whereas SC19220 1 × 10^{-6} M inhibited this action. Values are means + SEM of 10 independent periodontitis patients positive for anti-β_1 IgG, with tests performed in duplicate. *$P < 0.0001$ *versus* basal; †$P < 0.0001$ *versus* anti-β_1 IgG; and ‡$P < 0.0001$ *versus* anti-β_1 IgG + DuP 697 + PGE_2 or anti-β_1 IgG + FR122047 + PGE_2.

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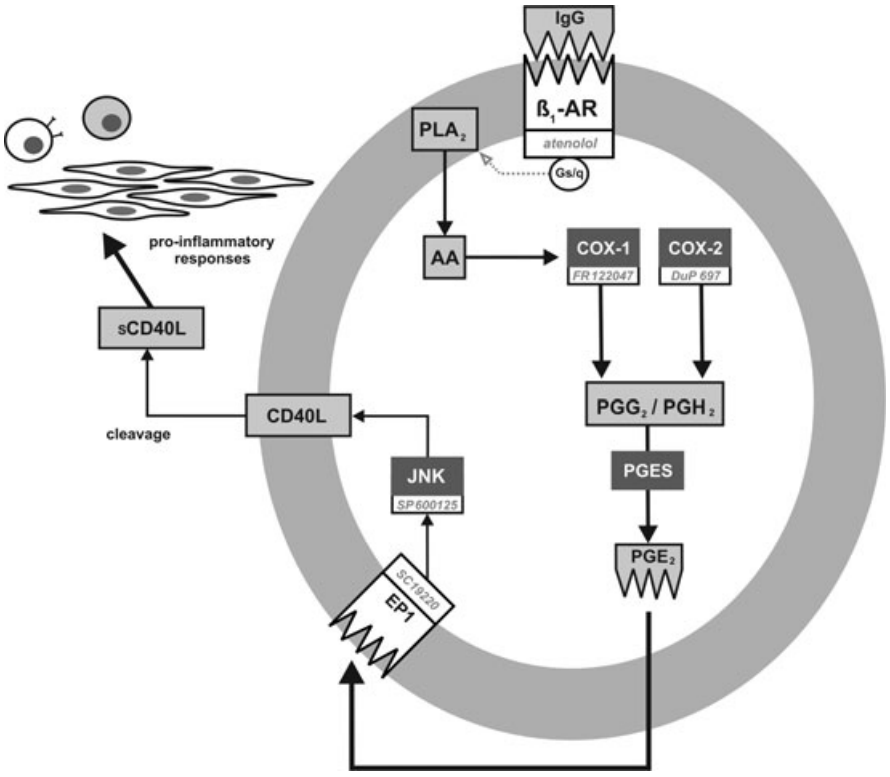

Figure 6. Proposed model to explain the mechanism whereby IgG in chronic periodontitis patients upregulates PGE_2 generation and sCD40L release in isolated rat atria
During chronic periodontitis, anti-β_1 IgG binds and activates atrial β_1-ARs, with subsequent activation of phospholipase A_2 (PLA_2) and cyclo-oxygenase (COX), inducing an increase in PGE_2 generation. Binding of PGE_2 to its own receptor (EP1) leads to JNK phosphorylation and cleavage of CD40, resulting in increased release of sCD40L, which acts as a pro-inflammatory substance together with PGE_2. Inhibitory agents are indicated in italics.

is that CP leads to systemic exposure to oral bacteria and that the resulting production of inflammatory mediators is capable of initiating or supporting different mechanisms associated with the development of atherosclerosis and coronary heart disease.

It has been reported previously that anti-β_1-AR IgG from periodontitis patients can activate the β_1-ARs in gingival human fibroblasts, inducing overexpression of CD40 and PGE_2 generation (Furlán *et al.* 2010). Also, pretreatment of human gingival fibroblasts with a specific β_1-AR antagonist blocked CD40 overexpression.

A potential mechanism for the action of anti-β_1-AR IgG on myocardium that leads to generation of PGE_2, with subsequent sCD40L release, is provided in Fig. 6.

Conclusion

Our findings suggest that β_1-AR IgG autoantibodies from chronic periodontitis patients activate β_1-ARs in rat atria to induce sCD40L release via PGE_2 generation. Indeed, serum levels of sCD40L and PGE_2 were elevated in patients with CP and correlated with serum levels of anti-β_1 IgG in these patients. Moreover, it is probable that inhibition of COX-1 and COX-2 suppressed sCD40L release from the myocardium *in vivo*, with the participation of cardiac JNK phosphorylation. This study provides some new insights into the role of pathways of inflammation in periodontal diseases and cardiovascular diseases.

References

Andre P, Nannizzi-Alaimo L, Prasad SK & Phillips DR (2002). Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. *Circulation* **106**, 896–899.

Armitage GC (2000). Periodontal infections and cardiovascular disease– how strong is the association? *Oral Dis* **6**, 335–350.

Aukrust P, Damas JK & Solum NO (2004). Soluble CD40 ligand and platelets: self-perpetuating pathogenic loop in thrombosis and inflammation? *J Am Coll Cardiol* **43**, 2326–2327.

Aukrust P, Müller F, Ueland T, Berget T, Aaser E, Brunsvig A, Solum NO, Forfang K, Frøland SS & Gullestad L (1999). Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina: possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation* **100**, 614–620.

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Beck JD, Koch GG, Zambon JJ, Genco RJ & Tudor GE (1992). Evaluation of oral bacteria as risk indicators for periodontitis in older adults. *J Periodontol* **63**, 93–99.

Borda E, Pascual J, Cossio P, De la Vega M, Arana R & Sterin-Borda L (1984). A circulating IgG in Chagas' disease which binds to β-adrenoceptor of myocardium and modulates their activity. *Clin Exp Immunol* **57**, 679–686.

Chiu B (1999). Multiple infections in carotid atherosclerotic plaques. *Am Heart J* **138**, s5534–s5536.

D'Aiuto F, Parkar M, Andreou G, Brett PM, Ready D & Tonetti MS (2004). Periodontitis and atherogenesis: causal association or simple coincidence? *J Clin Periodontol* **31**, 402–411.

Dileep CG & Pradeep AR (2006). Anti-neutrophil cytoplasmic autoantibodies: a renewed paradigm in periodontal disease pathogenesis? *J Periodontol* **77**, 1304–1313.

Furlán C, Sterin-Borda L & Borda E (2010). Beta adrenergic-induced CD40 overexpression on gingival fibroblasts: role of PGE_2. *Cell Biol Int* **34**, 365–372.

Gans KR, Galbraith W, Roman RJ, Haber SB, Kerr JS, Schmidt WK, Smith C, Hewes WE & Ackerman NR (1990). Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. *J Pharmacol Exp Ther* **254**, 180–187.

Garlichs CD, Eskafi S, Raaz D, Schmidt A, Ludwig J, Herrmann M, Klinghammer L, Daniel WG & Schmeisser A (2001). Patients with acute coronary syndromes express enhanced CD40 ligand/CD154 on platelets. *Heart* **86**, 649–655.

Genco R, Offenbacher S & Beck J (2002). Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *J Am Dent Assoc* **133**, 14S–22S.

Haraszthy VI, Zambon JJ, Trevisan M, Zeid M & Genco RJ (2000). Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* **71**, 1554–1560.

Heeschen C, Dimmeler S, Hamm CW, van der Brand MJ, Boersma E, Zeiher AM & Simoons MI (2003). Soluble CD40 ligand in acute coronary syndromes. *N Engl J Med* **348**, 1104–1111.

Humphrey LL, Fu R, Buckley DI, Freeman M & Helfand M (2008). Periodontal disease and coronary heart disease incidence: a systematic review and meta-analysis. *J Gen Intern Med* **23**, 2079–2086.

Joensen L, Borda E, Kohout T, Perry S, García G & Sterin-Borda L (2003). *Trypanosoma cruzi* antigen that interacts with the β_1-adrenergic receptor and modifies myocardial contractile activity. *Mol Biochem Parasitol* **127**, 169–177.

Karnoutsos K, Papastergiou P, Stefanidis S & Vakaloudi A (2008). Periodontitis as a risk factor for cardiovascular disease: the role of anti-phosphorylcholine and anti-cardiolipin antibodies. *Hippokratia* **3**, 144–149.

Lee Y, Lee WH & Lee SC (1999). CD40L activation in circulating platelets in patients with acute coronary syndrome. *Cardiology* **92**, 11–16.

Limas CJ, Goldenberg IF & Limas C (1989). Autoantibodies against β-adrenoceptors in human dilated cardiomyopathy. *Circ Res* **64**, 97–103.

Listgarten MA & Loomer PM (2003). Microbial identification in the management of periodontal diseases. A systematic review. *Ann Periodontol* **8**, 182–192.

Magnusson Y, Marullo S, Hoyer S, Waggstein F, Andersson B, Vahine A, Guillet JG, Strosberg AD, Hjalmarson A & Hoebeke J (1990). Mapping of a functional autoimmune epitope on the β1-adrenergic receptor in patients with idiopathic dilated cardiomyopathy. *J Clin Invest* **86**, 1658–1663.

Nakajima T & Yamazaki K (2009). Periodontal disease risk of atherosclerotic coronary heart disease. *Odontology* **97**, 84–91.

Nesse W, Dijkstra PU, Abbas F, Spijkervet FK, Stijger A, Tromp JA, van Dijk JL & Vissink A (2010). Increased prevalence of cardiovascular and autoimmune diseases in periodontitis patients: a cross-sectional study. *J Periodontol* **81**, 1622–1628.

Ochi T & Goto T (2002). Differential effect of FR122047, a selective cyclooxygenase-1 inhibitor, in rat chronic models of arthritis. *Br J Pharmacol* **135**, 782–788.

Oliver RC, Brown LJ & Löe H (1998). Periodontal diseases in the United States population. *J Periodontol* **69**, 269–278.

Peng DQ, Zhao SP, Li YF, Li J & Zhou HN (2002). Elevated soluble CD40 ligand is related to the endothelial adhesion molecules in patients with acute coronary syndrome. *Clin Chim Acta* **319**, 19–26.

Persson GR & Persson RE (2008). Cardiovascular disease and periodontitis: an uptade on the associations and risk. *J Clin Periodontol* **35**, 362–379.

Rajapake PS & Dolby AE (2004). Evidence for local production of antibodies to auto and non-self antigens in periodontal diseases. *Oral Dis* **10**, 99–105.

Ramseier CA, Kinney JS, Herr AE, Braun T, Sugai JV, Shelburne CA, Rayburn LA, Tran HM, Singh AK & Giannobile WV (2009). Identification of pathogen and host-response markers correlated with periodontal disease. *J Periodontol* **8**, 436–446.

Schönbeck U & Libby P (2001). CD40 signaling and plaque instability. *Circ Res* **89**, 1092–1103.

Silze T, Randolph GJ, Kreutz M & Kunz-Schughart LA (2004). The fibroblast: sentinel cell and local immune modulator in tumor tissue. *Int J Cancer* **108**, 173–180.

Sterin-Borda L, Cossio PM, Gimeno MF, Gimeno AL, Diez C, Laguens RP, Meckert PC & Arana RM (1976). Effect of chagasic sera on the rat isolated atrial preparation: immunological, morphological and function aspects. *Cardiovasc Res* **10**, 613–622.

Sterin-Borda L, Furlan C & Borda E (2009). Autoantibodies to β_1-adrenoceptors in human chronic periodontitis induce overexpression of fibroblast CD40 and trigger prostaglandin E_2 generation. *J Periodontal Res* **44**, 330–337.

Ueland T, Aukrust P, Yndestad A, Otterdal K, Froland SS, Dickstein K, Kjekshus J, Gullestad L & Damås JK (2005). Soluble CD40 ligand in acute and chronic heart failure. *Eur Heart J* **26**, 1101–1107.

Wallukat G & Wollenberger A (1987). Effect of gamma globulin fraction of patients with allergic asthma and dilated cardiomyopathy on chronotropic β-adrenoceptor function in cultured neonatal rat heart myocytes. *Biomed Biochim Acta* **46**, 634–639.

Wallukat G, Wollenberger A, Morwinski R & Pitschner F (1995). Anti-β_1-adrenoceptor autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops. *J Mol Cell Cardiol* **27**, 397–406.

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Wylie PC, Challis RA & Blank JL (1999). Regulation of extracellular signal-regulated kinase and c-Jun N-terminal kinase by G-protein-linked muscarinic acetylcholine receptors. *Biochem J* **338**:619–628.

Yan Y, Wu Z, Huang Z, Li L, Zhong R & Kong X (2002). Clinical implications of increased expression of CD40L in patients with acute coronary syndromes. *Chin Med J (Engl)* **115**, 491–493.

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