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Autoantibodies to β_1-adrenoceptors in human chronic periodontitis induce overexpression of fibroblast CD40 and trigger prostaglandin E_2 generation

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Background and Objective: Autoimmune mechanisms may contribute to the pathogenesis of periodontal disease. Autoantibodies with the potential to bind and activate β_1-adrenoceptors (β_1-AR) of human gingival fibroblasts were studied to provide evidence of altered humoral immune response in chronic periodontal disease.

Material and Methods: Flow cytometry and enzyme-linked immunosorbent assay using cell culture-adherent gingival fibroblasts and/or their purified membranes and/or a synthetic peptide corresponding to the second extracellular loop of human β_1-AR were used to detect serum antibodies. The effects of antibodies from chronic periodontal disease patients on PGE_2 generation and CD40 expression were also tested.

Results: Circulating immunoglobulin G (IgG) from chronic periodontal disease patients (but not from normal individuals) interacted with the fibroblast surface, activating β_1-AR. Atenolol or CGP 20712 (beta 1-AR antagonists) and β_1 synthetic peptide inhibited the interaction of IgG with β_1-AR. Immunoglobulin G from chronic periodontal disease patients also displayed agonist-like activity associated with specific β_1-AR activation, increasing PGE_2 generation and CD40 overexpression. The corresponding affinity-purified anti-β_1-AR peptide IgG mimicked these effects. Both effects were prevented by inhibition of cyclooxygenase.

Conclusion: This article supports the participation of humoral immune alterations in chronic periodontal disease resulting in postsynaptic functional deregulation. Overproduction of proinflammatory mediators (PGE_2 and CD40 expression) is induced as a consequence of antibody–β_1-AR interaction. The PGE_2–CD40–IgG axis may play a part in the pathophysiological mechanisms underlying the inflammatory process in chronic periodontal disease.

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Fibroblasts have been considered important connective tissue cells that construct a supporting framework crucial for the integrity and repair of tissue (1,2). Recently, fibroblasts were found to be important sentinel cells in the immune system. Fibroblasts actively define the structure of tissue microenvironments and regulate the functions of infiltrated hematopoietic cell by production of cytokines and extracellular matrix, in which the CD40 system is a crucial element in the process of fibroblast activation (3).

The receptor CD40, a membrane-bound type I glycoprotein, is a member of the tumor necrosis factor α (TNF-α) receptor superfamily. Initially, it was seen as a B cell-specific receptor (CD40L), but was found to be widely distributed in other lineage cells (4,5), including monocytes, macrophages, epithelial cells, endothelial cells, smooth muscle cells and fibroblasts (5). On fibroblasts, CD40 triggers the production of the proinflammatory cytokines (6–8) interleukin (IL)-1, IL-6 and IL-8 (9), prostaglandin (PG) E_2 (10) and extracellular matrix protein (11). Interaction between CD40 and CD40L is critical for the activation of T lymphocyte proliferation (7). In periodontal disease, CD40 involvement on human gingival fibroblasts with inflammation was demonstrated *in vitro* and *in vivo* (8,12). The CD40–CD40L system is therefore an important pathway for the activation of fibroblast/immune effector functions.

Recent study (13) suggested the involvement of serum autoantibodies directed to extracellular matrix components in the pathogenesis of certain types of periodontal disease. Local production of autoantibodies to autoantigen in granulomatous tissues housed within the periodontal lesion (14) was described. The initial studies of autoimmunity in the pathogenesis of periodontal disease focused on detecting autoantibodies directed toward various self-antigens (15), such as autoantibodies against fibroblasts. Thus, antibody anti-human gingival fibroblast concomitant with antibacterial antibody may contribute to the pathogenesis of periodontal disease. Periodontal disease is multifactorial (1,2); infection is caused by dental plaque biofilm, but the host inflammatory/immune response modifies disease outcome (15). Stress or local stimulation of the autonomic adrenergic system is a cofactor that could contribute to the prevalence of disease and the incidence of disease progression (3).

Prostanoids are important mediators of inflammation and are involved in various physiological cellular functions (16). The rate-limiting step in prostanoid biosynthesis is mediated through two isoenzymes: cyclo-oxygenase 1 (COX-1) and cyclo-oxygenase 2 (COX-2; 10,11). The former has been regarded as the constitutively expressed isoform involved in homeostatic cellular functions, whereas COX-2 has been regarded as the inducible enzyme that is responsible for prostanoid synthesis at inflammation sites (10). Prostanoids can positively or negatively regulate expression of pro-inflammatory cytokines, depending on the cell type and/or cell activation site. Prostaglandin E_2 augments synthesis of IL-1 (17) and IL-6 (18) in human gingival fibroblasts. Interactions between CD40 and CD40L can trigger PGE_2 synthesis (19).

We considered the possibility that gingival fibroblasts behaving as specific antigen could react with serum antibodies from patients with chronic periodontal disease. We investigated the adrenergic system, screening sera of chronic periodontal disease patients for autoantibodies against β_1-adrenoceptors (β_1-AR). We determined whether autoantibodies against gingival fibroblast β_1-AR could induce PGE_2 generation and CD40 overexpression. We demonstrated that PGE_2 generation and expression of immunoglobulin G (IgG) autoantibodies and/or CD40 in human gingival fibroblasts may be a common pathway that facilitates the initiation or maintenance of the inflammatory process in chronic periodontal disease.

Material and methods

Patients

The study group comprised 25 patients (21 males and four females) with chronic periodontal disease (group I) who were attending periodontology clinics in the metropolitan area of Buenos Aires. The mean age was 40 years (25–58 years). Group I patients exhibited bone loss of > 50% in all teeth, and a probing pocket depth of > 6 mm at > 80% of proximal sites. Age- and sex-matched subjects were used as controls (group II, normal subjects; 15 males and five females). Inclusion criteria for the healthy control group (group II) were no interproximal attachment loss, and no signs of other inflammatory conditions or general diseases. None of the patients or healthy control subjects had a known systemic disorder that could affect the periodontal conditions; no patients had used antibiotics in the last 6 months. Smokers were excluded. All patients and healthy control subjects provided written informed consent to participate in the study. The study was conducted according to the tenets of the Declaration of Helsinki (1964). The Ethical Committee of the School of Dentistry of the University of Buenos Aires approved the study.

Sampling tissue specimens and blood for sera

Reverse-bevel gingivectomy was done at the site of the third molar, and tissues carefully dissected using scalpel blades. Tissues were washed in normal 0.9% saline to remove blood from the surface, and stored in preweighed containers in liquid nitrogen. Six millilitres of blood was obtained by venipuncture immediately before surgery and allowed to clot at room temperature. Serum was separated by centrifugation at $2000 \times g$ and stored at −20°C until use.

Cell culture

Cell cultures of human gingival fibroblasts were established from human normal gingiva and grown in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and penicillin (100 U/mL) and streptomycin (100 μg/mL) in a 5% CO_2 (95%O_2) environment at 37°C as previously

described by Varani *et al.* (20). The medium was replenished every 3–4 days. Confluent cells were subcultured by detaching the monolayer with 0.25% trypsin in phosphate-buffered saline solution (PBS). Assays were done using adherent cultured cells. Cells of passages 6–8 were used throughout.

Purification of human IgG

Immunoglobulin G from groups I and II was obtained by precipitation with ammonium sulphate at 50%, followed by three washes and reprecipitation with 33% ammonium sulphate. The resulting precipitate was submitted to chromatography on diethylaminoethyl–cellulose equilibrated with 10 mM phosphate buffer, pH 8. Eluted peaks were concentrated by ultrafiltration to 10 μg protein/mL. Control immunoelectrophoresis with goat anti-human total serum and goat non-specific anti-human IgG showed only one precipitin line.

Purification of anti-β_1-AR peptide IgG by affinity chromatography

The IgG fractions of chronic periodontal disease patients and normal subjects were independently subjected to affinity chromatography on the synthesized peptide covalently linked to AffiGel 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 first eluted with the same buffer. Specific anti-peptide autoantibodies were eluted with 3 M KSCN and 1 M NaCl after immediate extensive dialysis against PBS. Concentrations of non-anti-peptide IgG and specific anti-β_1-AR peptide IgG were determined by radial immunodiffusion assay and their immunological reactivity against β_1-AR synthetic peptide was evaluated by enzyme-linked immunosorbent assay (ELISA) as previously reported (21).

Gingival fibroblast membrane preparations

Membranes were prepared as previously described (22). Briefly, cells (2×10^6 cells/mL) were homogenized in an Ultraturrax at 4°C in six volumes of potassium phosphate buffer, 1 mM $MgCl_2$, 0.25 M sucrose, pH 7.5, supplemented with 0.1 mM phenylmethylsulphonylfluoride (PMSF), 1 mM EDTA, 5 μg/mL leupeptin, 1 μM bacitracin and 1 μM pepstatin A. The homogenate was centrifuged twice for 10 min at $3000 \times g$, then at $10{,}000 \times g$ and $40{,}000 \times g$ at 4°C for 15 and 90 min, respectively. The resulting pellets were resuspended in 50 mM phosphate buffer with the same protease inhibitors, pH 7.5.

Enzyme-linked immunosorbent assay (ELISA)

Fifty microlitres of peptide solution (20 μg/mL) in 0.1 M Na_2CO_3 buffer, pH 9.6, was used to coat microtitre plates (NUNC, Kastrup, Denmark) at 4°C overnight. After blocking the wells, diluted sera from groups I and II were added in triplicate 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 biotinylated goat anti-human IgG antibodies (Sigma, St Louis, MO, USA) was added and incubated for 1 h at 37°C. Then, a 1:6000 dilution of ExtrAvidin-alkaline phosphatase (Sigma) was allowed to react for an extra 30 min at 37°C. After extensive washings, *p*-nitrophenylphosphate (1 mg/mL) was added as substrate, and the reaction stopped at 30 min. Fifty microlitres of gingival fibroblast membranes (50 μg/mL) in 0.1 M Na_2CO_3 buffer, pH 9.6, was used to coat microtitre plates at 4°C overnight, and the ELISA procedure carried out as described above. The plates were read at 405 nm and the results for each sample expressed as the mean ± SD of triplicate values.

Flow cytometric analysis

To measure the IgG binding to adherent cell fibroablasts after washing cells with PBS, the fibroblasts were resuspended in PBS containing IgG from chronic periodontal disease patients (1×10^{-9} M) or from normal subjects (1×10^{-7} M) as the negative control. After incubation for 1 h at 4°C, cells were washed and further incubated for 30 min with rabbit anti-human IgG fluorescein isothiocyanate (FITC)-conjugated I_gG fragments (1:100). Cells were then fixed with 1% paraformaldehyde. The percentage of positive cells was determined by the threshold set using isotopic controls. Analyses of the expression of cell surface CD40 protein were done using FITC-conjugated monoclonal antibodies (MoAbs) to human CD40 or isotype-matched controls (Becton Dickinson, Franklin Lakes, NJ, USA) in a standard staining procedure. Briefly, fibroblasts (1×10^6 cells/mL), using the same protocol and identical reagents as in the passages of fibroblasts in culture, were incubated or not incubated with IgG from chronic periodontal disease patients (1×10^{-9} M) for 90 min in the presence of isotype-matched serum. When atenolol or CGP 20712 (β_1-AR antagonists), indomethacin (INDO; COX inhibitor), PGE_2 (1×10^{-11} M) and β_1 synthetic peptide were used, they were added 10 min before IgG from chronic periodontal disease patients. Cells with the corresponding antibody were washed with PBS containing 2.5% FBS, fixed in 0.5% paraformaldehyde, and analyzed with a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson), with appropriate forward and side-scatter adjustment for human skin fibroblast cells. Mean fluorescence intensity values obtained by staining with specific MoAbs were corrected by subtraction of background values (isotype-matched control MoAb).

Prostaglandin E_2 assay

Human gingival fibroblasts (2×10^6 cells/mL) were incubated for 60 min in 0.50 mL Krebs–Ringer bicarbonate (KRB) gassed with 5% CO_2 in oxygen at 37°C. Blockers were added to the cell culture and incubated for 30 min, followed by IgG from chronic periodontal disease patients for a further 30 min. Tissues were 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 Immuno Assay (ELISA)

System (Amersham Biosciences, Piscataway, NJ, USA). The results for PGE_2 were expressed as picograms per milligram of tissue wet weight (pg/mg tissue wet wt).

Drugs

Stock solutions of atenolol, CGP 20712 (specific β_1 antagonists) methanesulphonate salt, dobutamine hydrochloride (specific β_1 agonist), indomethacin (inhibitor of COX-1 and COX-2) and PGE_2 were freshly prepared before each experiment (Sigma Chemical Co., St Louis, MO, USA). The β_1 peptide corresponds to the sequence of the second extracellular loop of the human β_1-adrenergic receptor: HWWRA ESDEA RRCYN DPKCC DFVTN RC (Sigma Genosys, St Louis, MO, USA).

Statistical analyses

Student's *t*-test for unpaired values was used to determine significance level. Analysis of variance (ANOVA) and *post hoc* test (Dunnett's method and Student–Newman–Keuls test) were employed when pairwise multiple comparison procedure was necessary. Differences between means were considered significant if $p < 0.05$.

Results

Detection of serum autoantibodies

Flow cytometry and ELISA assays were done to test the ability of autoantibodies from chronic periodontal disease patients to interact with fibroblast membranes.

Figure 1 shows the binding capacity of normal subjects (Fig. 1A) and chronic periodontal disease patients (Fig. 1B) serum antibodies incubated with fibroblast cells (2×10^6 cells/mL). While IgG from normal subjects exhibited low binding ($8 \pm 0.5\%$), IgG from patients with chronic periodontal disease showed binding in almost all fibroblast populations ($91 \pm 8.2\%$).

Figure 2 shows the immune reactivity of sera from chronic periodontal disease patients and normal subjects against human gingival fibroblast membranes that were used as coating antigen. The optical density (OD) for each serum sample from chronic periodontal disease patients was significantly higher than that from normal individuals ($p < 0.001$). The OD of sera from group I was always at least 3 SD that of sera from group II.

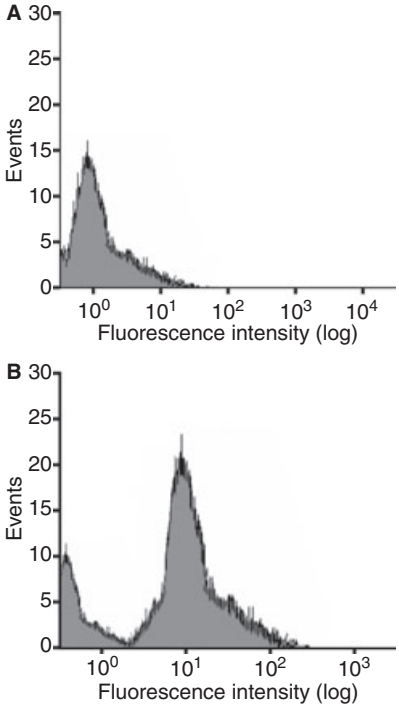

Fig. 1. Representative flow cytometric analysis of binding IgG. Culture fibroblasts were incubated with 1×10^{-7} M IgG from normal subjects (A) or (B) 1×10^{-9} M IgG from patients with chronic periodontal disease. The flow cytometric technique was as described in the text. This figure is representative of separate assays using antibodies from 10 chronic periodontal disease patients and eight normal subjects with similar results.

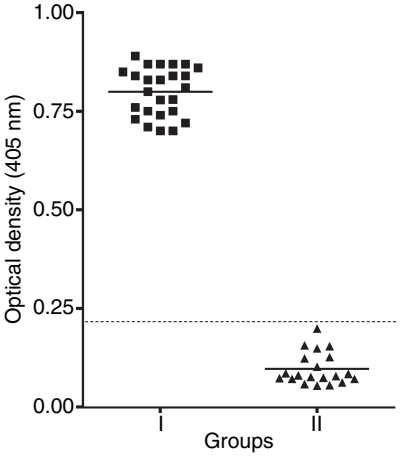

Fig. 2. Immune reactivity between human gingival fibroblast membrane and sera from group I (25 chronic periodontal disease patients and group II (20 normal subjects; control). Serum (1:50 dilution) was assayed on sensitized microplates with 50 μL/mL of fibroblast purified membranes as coating antigen. Dotted line indicates the cut-off value, 0.24 (mean OD ± 3 SD for group - II) and continuous lines indicate the median OD; $p < 0.001$ between group I and group II.

We characterized the participation of the β-adrenergic system in the reaction with serum antibodies to elucidate the nature of the sympathetic mechanism involved. We determined the molecular interaction between IgG from patients with chronic periodontal disease and human β_1-AR by ELISA, using as coating antigen a synthetic peptide corresponding to the sequence of the second extracellular loop of human β_1-AR. Figure 3 shows the concentration-dependent increase in OD with total IgG, and the corresponding anti-β_1-AR peptide IgG and sera from chronic periodontal disease patients. Figure 3A and B shows the OD values of serum and IgG from chronic periodontal disease patients, which were significantly higher than those from normal individuals ($p < 0.001$). Anti-β_1-AR peptide IgG showed more potency and affinity than the total IgG. Thus, the concentration of affinity-purified anti-β_1-AR peptide IgG (1×10^{-10} M) that maximally increased OD values corresponded to $\sim 1 \times 10^{-9}$ M total IgG concentration in chronic periodontal disease patients. As expected, the IgG fraction from normal subjects purified by affinity chromatography with β_1-AR synthetic peptide gave negative results, as did the non-anti-peptide fraction from chronic periodontal disease patients (data not shown). To determine the specificity of the reaction, IgG samples from patients with chronic periodontal disease were separately incubated with β_1 synthetic peptide for 30 min at 37°C (1 μg/mL) and then added in the microtitre plates. The influence of the

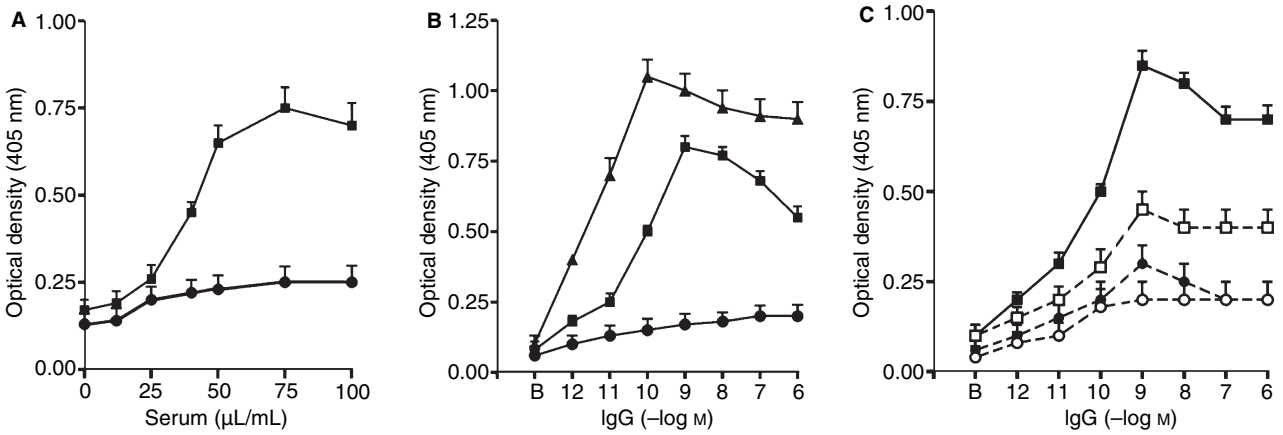

Fig. 3. Immune reactivity of autoantibodies against β_1 synthetic peptide used as coating antigen. (A) Increasing concentrations of sera from chronic periodontal disease patients (–■–) and normal subjects (–●–). (B) Increasing concentrations of IgG from chronic periodontal disease patients (–■–), affinity purified anti-β_1-AR peptide IgG (–▲–) and IgG from normal subjects (–●–). (C) Effect of increasing concentrations of IgG from chronic periodontal disease patients (–■–), alone or when they reacted with 1×10^{-7} M atenolol (-●-) or with 1×10^{-7} M CGP 20712 (-○-) or with 1 μg/mL β_1 synthetic peptide (-□-). Results are means ± SEM of 10 independent patients in each group performed in duplicate. $p < 0.001$ between IgG from chronic periodontal disease and normal subjects.

β_1-specific antagonists atenolol and CGP 20712 (both at 1×10^{-7} M) was also studied. Figure 3C shows that treatment with β_1 synthetic peptide and β_1-specific blockers inhibited the interaction between β_1-AR and IgGfrom chronic periodontal disease patients.

Antibody action on PGE_2 generation and CD40 expression

As shown above, affinity-purified anti-β_1-AR peptide IgG can react with human gingival fibroblasts. We studied the β_1-AR-mediated generation of PGE_2 by autoantibodies from chronic periodontal disease patients in human gingival fibroblasts, behaving as adherent cells in cell culture. Figure 4A shows that IgG from chronic periodontal disease patients and the corresponding affinity-purified anti-β_1-AR peptide IgG triggered a concentration-dependent increase in PGE_2 generation. The action of anti-β_1 antibodies was abolished by 1×10^{-7} M atenolol, 1 μg/mL β_1 synthetic peptide and 5×10^{-6} M INDO (Fig. 4B), indicating the participation of β_1-AR and COX in the action of IgG on PGE_2 generation. Immunoglobulin G from normal subjects had no effect (Fig. 4A).

To elucidate whether CD40 expression resulted in functional postsynaptic β_1-AR activation, we measured CD40 expression in human gingival fibroblasts cultured alone or in 1×10^{-9} M IgG from patients with chronic periodontal disease. As shown in Fig. 5A, fibroblasts cultured in medium alone [cPD IgG(−)] showed a moderate expression of CD40 that was significantly enhanced in anti-β_1 autoantibody-treated cells [cPD IgG(+)]. Results for isotype (control IgG2a) are also shown (Fig. 5A). When β_1 synthetic peptide (1 μg/mL) or atenolol or CGP 20712 (both at 1×10^{-7} M) were added 10 min before anti-β_1 IgG from chronic periodontal disease patients (1×10^{-9} M), the increment in CD40 expression was reduced (Fig. 5B). There were no differences in the level of CD40 expression between fibroblasts cultured alone or in the presence of β_1 peptide or atenolol or CGP 20712 (data not shown). Immunoglobulin G from normal subjects had no effect (Fig. 5B). Affinity-purified anti-β_1-AR peptide IgG was employed to assess whether autoantibodies against β_1-AR induced CD40 overexpression. The affinity-purified anti β_1-AR peptide IgG (1×10^{-10} M) from the same patients also induced CD40 overexpression (Fig. 5B). This effect resembled that of the authentic β_1-AR agonist dobutamine (1×10^{-8} M). These results suggest the participation of β_1-AR in chronic periodontal disease IgG/CD40 overexpression (Fig. 5B).

The effect of INDO (5×10^{-6} M) was studied to assess whether the effect of IgG from chronic periodontal disease patients on CD40 expression partly depended on the increment of PGE_2 generation. Figure 6 shows that the inhibition of COX decreased the number of positive cells [cPD IgG(+) alone, 252 ± 12, $n = 5$; and cPD IgG(+) + INDO, 112 ± 9, $n = 6$]. When exogenous PGE_2 (1×10^{-9} M) was added, the inhibitory effect of INDO was restored (205 ± 15, $n = 6$); inidcating the participation of PGE_2 in CD40 overexpression by β_1-AR autoantibody.

Discussion

The results provide evidence that certain components of the serum IgG fraction from chronic periodontal disease patients can recognize the surface of human gingival fibroblasts and their membrane; this is important new information that autoimmunity may have a role in periodontal disease. The autonomic adrenergic system is an important regulator of the immune response (23). Local production of antibodies to autoantigen in granulomatous tissue housed within the periodontal lesion has been reported (14). Anusaksathien *et al.* (24) have demonstrated that the levels of antibodies to collagen type I in periodontal

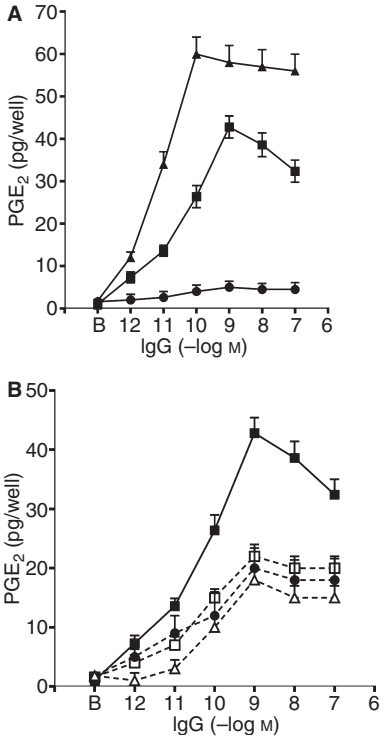

Fig. 4. Generation of PGE_2 by the influence of IgG from chronic periodontal disease patients upon human gingival fibroblasts. (A) Increasing concentrations of IgG from chronic periodontal disease patients (–■–) or anti-β_1-AR peptide IgG (–▲–) or IgG from normal subjects (–●–). (B) Values PGE_2 with increasing concentrations of IgG from chronic periodontal disease patients (–■–) alone or in the presence of 1 μg/mL β_1 synthetic peptide (- - □ - -) or 5×10^{-6} M INDO (- - ▲ - -) or 1×10^{-7} M atenolol (- - ● - -). Results are means ± SEM of 10 independent patients in each group performed in duplicate. $p < 0.001$ between IgG from chronic periodontal disease and normal subjects.

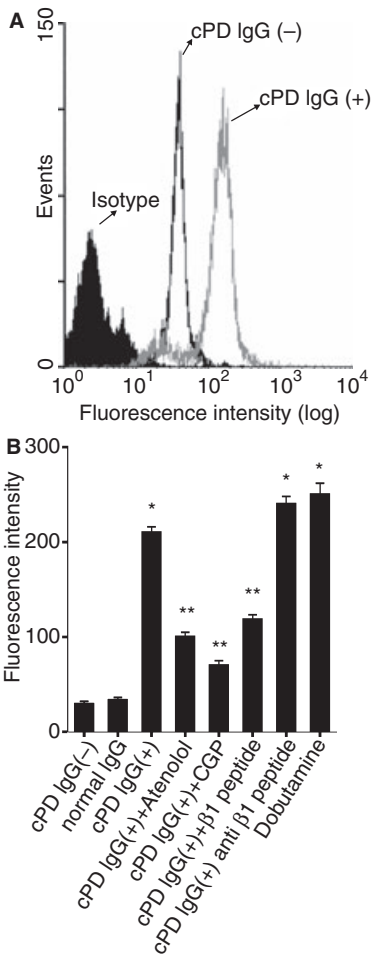

Fig. 5. Representative flow cytometric analysis of CD40 expression. (A) Human gingival fibroblast cells were incubated with IgG2a (isotype, control) or in medium alone [cPD IgG(–)] or in medium with 1×10^{-9} M IgG from chronic periodontal disease patients [cPD IgG(+)], and flow cytometry was performed as described in the Material and Methods section. (B) The total number of fibroblast cells was evaluated in medium alone [cPD IgG(–)] or medium with 1×10^{-9} M IgG from chronic periodontal disease patients [cPD IgG(+)] or medium with cPD IgG(+) plus 1×10^{-7} M atenolol or 1×10^{-7} M CGP 20712 or 1 μg/mL β_1 synthetic peptide. Also shown are the effects of anti-β_1-AR peptide IgG (1×10^{-10} M) [cPD IgG(+) anti-β_1 peptide] from the same patients and 1×10^{-8} M dobutamine. Immunoglobulin G form normal subjects (1×10^{-7} M) is shown as a control. Values are means ± SEM of six different IgGs from 16 chronic periodontal disease patients and 12 normal subjects evaluated in duplicate. *$p < 0.001$ vs. IgG from normal subjects; **$p < 0.001$ vs. cPD IgG(+) alone.

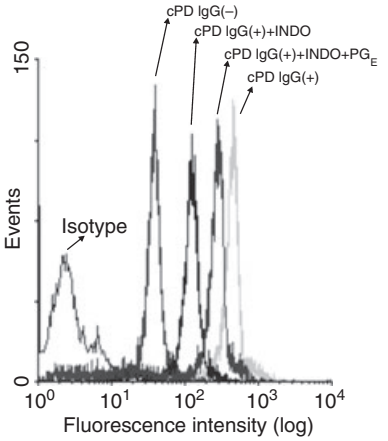

Fig. 6. Representative flow cytometric analysis of CD40 expression on human gingival fibroblast cells. The figure shows isotype (control IgG2a), medium alone [cPD IgG(–)], medium with 1×10^{-9} M IgG from chronic periodontal disease patients [cPD IgG(+)] or medium with IgG from chronic periodontal disease patients plus 5×10^{-6} M INDO [cPD IgG(+) + INDO]. The figure also shows the restitution experiments with 1×10^{-9} M PGE_2 [cPDIgG (+) plus INDO plus PGE]. Values are means ± SEM of 10 chronic periodontal disease patients and 10 normal individuals evaluated in duplicate.

tissue were above the levels detectable in serum from the same patients, suggesting that autoantibody production occurs predominantly at the disease site. Thus, serum antibody could indicate a disturbance in self-recognition of gingival fibroblasts by the immune system after disease-active episodes (25). We cannot exclude the possibility that antibodies detected in individuals with periodontal disease could be due to cross-reactivity with bacteria-elicited, soluble forms of β-ARs.

Sera that reacted positively against the surface of human gingival fibroblasts or their membranes showed positive immune reactivity to human β_1-AR peptide. These results are in agreement with those reported in other autoimmune diseases, in which serum autoantibodies against the second extracellular loops of human neurotransmitter receptors have been described (26–29). These autoantibodies have been shown to be primarily organ specific for each disease, i.e. heart (Chagas' disease), brain (schizophrenia) and salivary glands (Sjögren syndrome).

It is well known that PGE_2 is a proinflammatory agent present in acute and chronic inflammatory processes, as well as in some immunological diseases and cytotoxic tissue damage (16). The inflammatory process described in periodontal disease may be partly attributed to autoantibody fixation on the fibroblast membrane that in turn interacts with β_1-AR, triggering PGE_2 production. This effect was not observed with lymphocyte cells (data not shown) because stimulation of β_1-AR with the authentic agonist, dobutamine, in these

cells did not trigger PGE_2 production (30). Increasing evidence suggests that there is considerable cross-communication between CD40 and PGE_2 in fibroblasts (10). In periodontal tissue, the involvement of CD40 on human gingival fibroblasts with inflammation has been demonstrated (8,12). The potential immune regulatory function of CD40 in the local inflammatory process (31) led us to examine the effect of autoantibodies on CD40 expression.

The results indicate that the increment of PGE_2 generation after β_1 autoantibody stimulation on β_1-AR of human gingival fibroblasts was accompanied by CD40 overexpression. Synthesis of prostanoids depends on arachidonic acid liberation and COX metabolism, rather than activation of phospholipase A_2 (32). The fact that INDO specifically inhibits COX-1 and COX-2 and that low PGE_2 concentrations reversed this inhibition indicates that IgG from patients with chronic periodontal disease led to overexpression CD40, and that this process is upregulated by COX in human gingival fibroblasts. Endothelial cells in chronically inflamed tissues have been shown to express CD40 and employ a CD40-mediated signaling mechanism to acquire a proinflammatory phenotype *in vitro* (33). Recently, Schonbeck *et al*. (34) demonstrated that engagement of CD40 resulted in activation of IL-1β-converting enzyme, with an increase in the production of IL-1β in human endothelial and smooth muscle cells. Zhang *et al*. (10) and Cao *et al*. (11) found that CD40 engagement increased PGE_2 synthesis by upregulation of COX-2 in human fibroblasts.

In conclusion, the β_1 autoantibody IgG–CD40 overexpression axis increased PGE_2 generation. The fact that anti-β_1 autoantibody IgG could overexpress CD40 complex protein, thereby increasing PGE_2 generation, indicates that these interactions are important in initiating or maintaining an inflammatory process in the local periodontal lesion. Our results could be interpreted differently, i.e. PGE_2 generation via autoantibodies is an initial event before CD40 overexpression because the inhibition of COX by INDO attenuated the effect of the autoantibody on CD40 expression. Additional studies are required to clarify this hypothesis.

Chronic periodontal disease could therefore result from one of of two mechanisms: molecular mimicry between the host immune system and the bacterial biofilm, or the deviation of the humoral immune response by generation of non-protective antibodies that recognize host cell neurotransmitter receptors.

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