Periodontitis decreases the antiatherogenic potency of high density lipoprotein

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Abstract Periodontitis, a consequence of persistent bacterial infection and chronic inflammation, has been suggested to predict coronary heart disease (CHD). The aim of this study was to investigate the impact of periodontitis on HDL structure and antiatherogenic function in cholesterol efflux in vitro. HDL was isolated from 30 patients (age 43.6 ± 6.1 years, mean ± SD) with periodontitis before and after (3.2 ± 1.4 months) periodontal treatment. The capacity of HDL for cholesterol efflux from macrophages (RAW 264.7), HDL composition, and key proteins of HDL metabolism were determined. After periodontal treatment, phospholipid transfer protein (PLTP) activity was 6.2% ($P < 0.05$) lower, and serum HDL cholesterol concentration, PLTP mass, and cholesteryl ester transfer protein activity were 10.7% ($P < 0.001$), 7.1% ($P = 0.078$), and 19.4% ($P < 0.001$) higher, respectively. The mean HDL_2/HDL_3 ratio increased from 2.16 ± 0.87 to 3.56 ± 0.48 ($P < 0.05$). HDL total phospholipid mass and sphingomyelin-phosphatidylcholine ratio were 7.4% ($P < 0.05$) and 36.8% ($P < 0.001$) higher, respectively. The HDL-mediated cholesterol efflux tended to be higher after periodontal treatment; interestingly, this increase was significant ($P < 0.05$) among patients whose C-reactive protein decreased (53.7% reduction, $P = 0.015$) and who were positive by PCR for *Actinobacillus actinomycetemcomitans*. These results suggest that periodontitis causes similar, but milder, changes in HDL metabolism than those that occur during the acute-phase response and that periodontitis may diminish the antiatherogenic potency of HDL, thus increasing the risk for CHD.—Pussinen, P. J., M. Jauhiainen, T. Vilkuna-Rautiainen, J. Sundvall, M. Vesanen, K. Mattila, T. Palosuo, G. Alfthan, and S. Asikainen. **Periodontitis decreases the antiatherogenic potency of high density lipoprotein. *J. Lipid Res.* 2004.** 45: **139–147.**

Supplementary key words HDL metabolism • atherosclerosis • reverse cholesterol transport • cholesterol efflux • inflammation

One of the central cells mediating atheroma development in atherosclerosis is a circulating monocyte-derived activated macrophage. Macrophages in arterial intima accumulate excess cholesterol, which is esterified and stored in the cytoplasm. The cholesteryl ester-laden macrophages, or foam cells, that accumulate in the arterial wall are the hallmark of early fatty streak lesions in atherogenesis. Among the bacterial components that trigger macrophage activation, the most widely studied is lipopolysaccharide (LPS), a potent virulence factor of gram-negative bacteria. Increasing evidence suggests that the pathway consisting of LPS-macrophage-inflammatory mediators plays a critical role in infection-associated atherogenesis and thromboembolism by inducing the release of cytokines, by enhancing platelet aggregation and endothelial monocyte adhesion, and by promoting the formation of lipid-laden foam cells (1). LPS also interferes with macrophage cholesterol metabolism by downregulating both scavenger receptor B1 and ATP binding cassette transporter A1 (ABCA1) expression (2). The functions of these two transmembrane proteins have been associated with cholesterol efflux.

HDL is considered an antiatherogenic lipoprotein because of its direct role in neutralizing LPS in circulation (3) and protecting LDL against oxidation (4) as well as its role in reverse cholesterol transport (5). During the first phase of reverse cholesterol transport, HDL functions as an acceptor for cholesterol from cell membranes in a process of cholesterol efflux. This process is facilitated pas-

Abbreviations: apoA-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CRP, C-reactive protein; HL, hepatic lipase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLTP, phospholipid transfer protein; PS, phosphatidylserine; SAA, serum amyloid A; SM, sphingomyelin; TG, triglyceride.

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Manuscript received 11 June 2003 and in revised form 27 August 2003.

Published, JLR Papers in Press, September 16, 2003.
DOI 10.1194/jlr.M300250-JLR200

sively by the diffusion of cholesterol to HDL and actively by the interaction of lipid-poor apolipoprotein A-I (apoA-I) or preβ-HDL and ABCA1 (5, 6). ABCA1-facilitated cholesterol removal is the rate-limiting step in the production of nascent HDL. HDL cholesterol is esterified in circulation and transported directly, or via lipoproteins of lower density, to the liver for excretion. HDL-promoted cholesterol efflux has been studied extensively using different HDL subpopulations as acceptors of cholesterol and has been shown to be indirectly associated with arterial wall thickness (7). Less is known about the action of inflammatory HDL in the cholesterol removal process.

During both acute and chronic infections, changes occur in lipoprotein distribution and subclass composition (8–10). Increased catabolism of HDL may be the cause of low HDL cholesterol concentrations during infection (11, 12). The main protein of HDL, apoA-I, is displaced by serum amyloid A (SAA), whose synthesis is largely regulated by inflammation-associated cytokines (13). The displaced lipid-poor apoA-I displays preβ mobility upon electrophoresis (14). On the other hand, HDL triglyceride (TG) concentration increases; the formed TG-rich HDL is a good substrate for hepatic lipase (HL). The function of HL again leads to the formation of lipid-poor apoA-I and its accelerated catabolism via the kidneys (15). In fact, infection and inflammation may cause such dramatic changes in HDL composition that its substrate properties for transforming proteins and receptors change (16, 17). Concentrations and activities of major HDL remodeling factors, which affect reverse cholesterol transport, are also modified during infection. Such factors include HL, cholesteryl ester transfer protein (CETP), lecithin-cholesterol acyltransferase (LCAT), and phospholipid transfer protein (PLTP) (8, 10, 18, 19).

Infection-induced proatherogenic changes in lipoprotein profile may be one of the mechanisms underlying the increased risk of atherosclerosis in patients with chronic infections. Several pathogens that cause chronic infections may induce alterations in lipoprotein metabolism. These pathogens include *Chlamydia pneumoniae* (9), *Helicobacter pylori* (20), and periodontal pathogens (21). Contrary to the single-bacterium infections caused by the two former pathogens, the bacterial flora associated with periodontitis constitute a complex overgrowth of normal oral microbial species. Species with increased periodontopathogenic potential include mainly gram-negative bacteria, particularly *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (22).

Although periodontitis is associated with mild hypercholesterolemia or hypertriglyceridemia (21, 23, 24), its effects on the composition and remodeling factors of HDL are unknown. Therefore, the aim of this study was to investigate in detail the impact of periodontitis on HDL metabolism. Specifically, we investigated the changes in the composition and subclass distribution of HDL, the activities of lipid transfer proteins and enzymes involved in reverse cholesterol transport, and the potential of HDL in the cholesterol efflux in vitro among 30 patients with periodontitis before and after periodontal treatment.

MATERIALS AND METHODS

Study subjects and samples

The study population comprised 30 generally healthy patients [age 49.8 ± 7.4 years (mean ± SD), 16 males and 14 females] with periodontitis. The inclusion criteria of the patients and periodontal examination were described in our previous article (25). Each patient had clinical and radiographic periodontal attachment loss at more than six teeth. In each patient, periodontal probing depths, gingival bleeding on probing, and suppuration were recorded for all teeth at six sites per tooth. Periodontal treatment consisted of conventional mechanical therapy and metronidazole 500 mg twice a day for 7 days when indicated (n = 7). Serum and plasma samples were obtained before periodontal treatment and after approximately 3 months (108 ± 32 days). The patients were not advised to change their diet or smoking habits during the study. The study protocol was approved by the Ethics Committee of the Institute of Dentistry, University of Helsinki, and informed consent was obtained from all participants. The samples were stored at −70°C until use. The results of the PCR detection of *A. actinomycetemcomitans* and *P. gingivalis* from subgingival bacterial samples were available from our earlier study (26).

Determination of serum parameters

Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were determined by fully enzymatic methods. Sensitive C-reactive protein (CRP) and SAA concentrations were determined by UC CRP ELISA (Eucardio Laboratory, San Diego, CA) and the Cytoscreen Immunoassay Kit (BioSource International, Camarillo, CA). PLTP mass and PLTP, CETP, and LCAT activities were assayed as reported (27–30). Specific activity of PLTP was calculated as the ratio of PLTP phospholipid transfer activity and mass. Serum antibodies to *A. actinomycetemcomitans* and *P. gingivalis* were analyzed by multiserotype ELISA using four dilutions of the samples in duplicate (26).

Isolation and characterization of HDL

Total HDL (1.063–1.21 g/ml) was isolated from 1.5 ml of plasma by sequential ultracentrifugation using an Optima TL-100 Tabletop Ultracentrifuge (TL-100.3 rotor, 100,000 rpm, 4°C, 18 h) (Beckman, Fullerton, CA). The density of the samples was adjusted with solid KBr. HDL recovered in the 1 ml top fraction was dialyzed overnight against PBS (10 mM phosphate, pH 7.4, and 150 mM NaCl) and stored at 4°C. HDL particle size and subclass distribution were determined by native gradient gel electrophoresis (31) on 4–30% polyacrylamide gels (PAGE), which, after staining, were photographed with a Kodak Digital Science 120 digital camera (Kodak, Rochester, NY). The densitometric scan was performed using 1D™ analysis software from Kodak. The following HDL subclass division was applied: 1 (13–14 nm), 2b1 (11–12.9 nm), 2b2 (9.7–11 nm), 2a (8.8–9.7 nm), 3a (8.2–8.8 nm), 3b (7.8–8.2 nm), and 3c (7.2–7.8 nm) (31). After densitometric scanning, the HDL_2/HDL_3 ratio was calculated from the percentage distributions individually.

HDL protein concentration was determined by the method of Lowry et al. (32). HDL total lipids were extracted (33), and phospholipids were separated on one-dimensional TLC plates (HPTLC; 10 × 20 cm Silica Gel; Merck) with a solvent mixture of chloroform-methanol-acetic acid-formic acid-water (70:30:12:4:2, v/v/v/v/v) using a Camag Automatic TLC sampler 4. After HPTLC runs, the lipids were detected and quantified as described (34). HDL composition was calculated as mass percentages, including total protein, cholesterol (cholesteryl ester and free cholesterol), triglyceride, and phospholipid concentrations. HDL phospholipid subclass distribution was calculated as mass

percentages of the following subspecies: phosphatidylcholine (HDL-PC), sphingomyelin (HDL-SM), phosphatidylethanolamine (HDL-PE), phosphatidylserine (HDL-PS), and phosphatidylinositol (HDL-PI). The HDL-SM/PC ratio was calculated from the mass percentages of HDL-SM and HDL-PC.

HDL-facilitated cholesterol efflux from macrophages

Permanent murine macrophages (RAW 264.7) were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were cultured as monolayers in a humidified atmosphere of 95% air and 5% CO_2 at 37°C and transferred or harvested at 70% confluence. The cells were revived exactly 2 weeks before the efflux experiments and passaged four times. On day 1 before the experiment, the cells were harvested using a rubber policeman, counted under a microscope, and divided at a density of 7×10^4/ml on 12-well plates. The experiment medium was Macrophage-SFM medium (GIBCO, Gaithersburg, MD) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, and 10 ng/ml Leucomax (1.67 million IU Novartis; stock 5 μg/ml in sterile water). On day 2, the medium was changed and the cells were labeled with 50 nCi/ml [1α,2α(n)-^{3}H]cholesterol in ethanol (49.0 Ci/mmol; Pharmacia Amersham, Uppsala, Sweden). The final concentration of ethanol in the medium was 0.1% (v/v). On day 3, the medium with the radioactive label was removed and the cells were allowed to equilibrate overnight in fresh medium. On day 4, the medium was removed, the cells were washed twice with PBS, and 1 ml of fresh medium containing 100 μg (as total protein) of HDL isolated from the patients was added in duplicate wells. The spontaneous cholesterol diffusion (mean 15.6 ± 1.9% from the control HDL, n = 10) was monitored in wells in which no HDL was present. HDL stored at −70°C was used in duplicate wells to control the weekly interexperiment variation (coefficient of variation, 2.9%).

At time points of 1, 3, and 6 h, the medium was removed and centrifuged at 6,000 *g* for 2 min. Of the supernatant, 250 μl was transferred to scintillation vials, stored at 4°C overnight with the scintillation cocktail, and counted for radioactivity. In the preliminary experiments (n = 6), the cells were lysed with 0.3 M NaOH after washing twice with PBS containing 2% BSA and twice with PBS without BSA. The mean recovery, 94.5%, of the total cholesterol-associated ^{3}H radioactivity was counted from the cell lysates and the culture supernatants.

Statistical analyses

The statistical significance of the differences in the continuous variables between the groups was tested with the Mann-Whitney U-test or the Wilcoxon signed ranks test. A two-tailed Pearson correlation was used for correlation analyses. The associations of kinetic parameters of cholesterol efflux as dependent variables with other parameters determined were examined with linear regression analyses. A P value of <0.05 was considered statistically significant. All statistical analyses were performed with SPSS 9.0 for Windows.

RESULTS

The mean periodontal and serum parameters before and after periodontal treatment are summarized in **Table 1**. The proportion of teeth with deepened periodontal pockets and the number of bleeding or suppurating periodontal pockets were significantly ($P < 0.001$) lower after treatment. Additionally, there was a significant ($P < 0.001$) decrease in mean serum IgG-class antibody levels to *P. gingivalis* but not to *A. actinomycetemcomitans*. In the whole study group, the mean CRP and SAA concentrations did not change from the pretreatment value. Serum HDL cholesterol and concomitantly serum total cholesterol concentrations increased 10.7% and 6.7%, respectively, after treatment ($P < 0.001$). PLTP activity decreased (6.2%, $P < 0.05$), whereas PLTP concentration increased (7.1%, not significant), leading to a decrease in PLTP specific activity (9.3%, $P < 0.05$). After periodontal treatment, there was only a nonsignificant increase in LCAT activity but a significant increase in CETP activity (19.4%, $P < 0.001$).

Total HDL was isolated from plasma samples obtained before and after treatment and analyzed for size and sub-

TABLE 1. Periodontal status and serum parameters of 30 patients with periodontitis before and after treatment

Parameter	Before	After	P^a
Proportion (%) of teeth with deepened (>5 mm) periodontal pockets	50.9 (25.0)	17.4 (14.8)	<0.001
Number of periodontal pockets with bleeding on probing	60.2 (25.5)	15.4 (14.6)	<0.001
Number of periodontal pockets with suppuration	4.70 (6.04)	1.05 (4.02)	0.001
Antibodies (mm^2) to			
A. actinomycetemcomitans	20.5 (8.9)	20.5 (9.2)	0.490
P. gingivalis	28.4 (11.1)	26.0 (10.5)	<0.001
Cholesterol (mmol/l)	6.28 (1.18)	6.70 (1.18)	<0.001
HDL cholesterol (mmol/l)	1.40 (0.40)	1.55 (0.41)	<0.001
Triglycerides (mmol/l)	1.55 (0.65)	1.74 (0.79)	0.146
CRP (mg/l)	1.55 (1.42)	1.70 (2.71)	0.308
SAA (mg/l)	5.88 (3.68)	6.60 (5.55)	0.234
PLTP activity (μmol/ml/h)	9.67 (2.10)	9.10 (1.57)	0.049
PLTP mass (μg/ml)	15.04 (3.58)	16.11 (4.75)	0.078
PLTP specific activity (μmol/μg/h)	0.680 (0.23)	0.622 (0.24)	0.036
LCAT activity (nmol/ml/h)	98.7 (17.5)	101.8 (17.2)	0.114
CETP activity (nmol/ml/h)	14.22 (5.27)	16.98 (5.25)	<0.001

Values shown are means and (SD). CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein; SAA, serum amyloid A.

[a] Wilcoxon signed ranks test.

class distribution by native gradient gel electrophoresis (**Fig. 1**). The mean proportion of HDL_2 particles increased by 11.3% of the pretreatment values (not significant), whereas the proportion of HDL_3 particles decreased by 33.2% ($P < 0.05$) (Fig. 1, inset). The HDL_2/HDL_3 ratio increased from 2.16 ± 0.87 to 3.56 ± 0.48 ($P < 0.05$). After treatment, the relative amount of HDL particles in subclasses 2b1 and 2a increased by 13.3% (not significant) and 30.6% ($P < 0.05$), whereas the amount of particles in subclasses 2b2, 3a, and 3b decreased by 17.4% (not significant), 33.0% (not significant), and 33.5% ($P < 0.05$), respectively. Subclasses 1 and 3c were not detected in the electrophoresis.

To further characterize HDLs before and after periodontal treatment, their mass composition and phospholipid subclass distributions were analyzed (**Fig. 2**). In the mean mass composition, there were small, nonsignificant decreases in cholesteryl ester and triglyceride concentrations and increases in free cholesterol and protein concentrations. However, there was a significant increase in HDL total phospholipid concentration after periodontal treatment (7.4%, $P < 0.05$). Regarding HDL phospholipid subclass distribution, a statistically nonsignificant decrease in HDL-PC and increases in HDL-PE and HDL-PI concentrations were observed. On the other hand, HDL-PS and HDL-SM concentrations increased significantly ($P < 0.05$) by 15.2% and 16.0%, respectively. These changes caused a highly significant increase in the SM/PC ratio after the treatment (36.8%, $P < 0.001$).

To evaluate the function of HDL in reverse cholesterol transport, we determined the cholesterol efflux capacity of the isolated HDL preparations that were used as acceptors of labeled cholesterol from cultivated macrophages. The mean kinetic parameters describing the efflux capacity are summarized in **Table 2**. K_m, V_{max}, initial velocity (V_{init}), and catalytic efficacy (V_{max}/K_m) were all 1.3–3.4% higher after periodontal treatment, although none of these differences reached statistical significance. To further analyze the efflux capacity, the patients were divided into two groups according to the presence (n = 10) or absence (n = 20) of *A. actinomycetemcomitans* in subgingival bacterial samples by PCR detection and according to the presence (n = 20) or absence (n = 10) of *P. gingivalis* (**Fig. 3**). As determined by V_{max}, the *A. actinomycetemcomitans*-positive patients had a 9.3% lower ($P < 0.01$) efflux

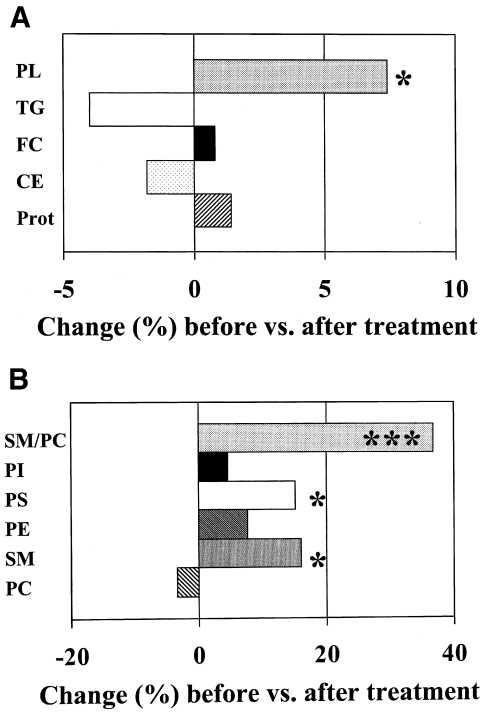

Fig. 2. HDL mass composition and phospholipid subclass distribution. Total HDL was isolated from plasma samples obtained from 30 patients with periodontitis before and after treatment, and the mass composition of HDL was analyzed. A: The percentage of change before versus after periodontal treatment in HDL phospholipid (PL), triglyceride (TG), free cholesterol (FC), cholesteryl ester (CE), and total protein (Prot) mass composition. B: The percentage of change before versus after periodontal treatment in HDL phospholipid subclass composition: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), sphingomyelin (SM), and phosphatidylcholine (PC). The asterisks indicate statistically significant differences (single asterisk, $P < 0.05$; triple asterisks, $P < 0.001$) before versus after treatment.

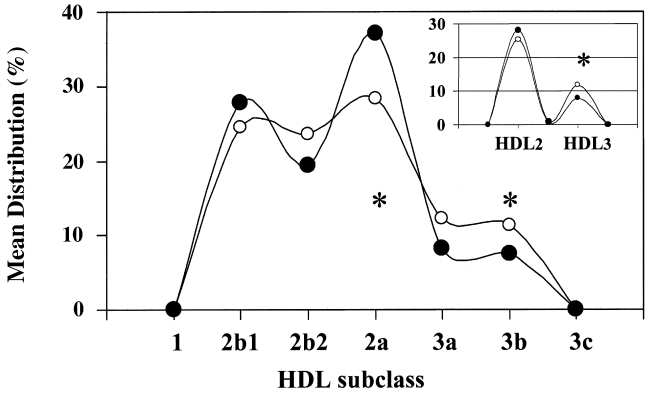

Fig. 1. HDL particle size and subclass distribution. Total HDL was isolated from plasma samples obtained from 30 patients with periodontitis before and after treatment, and HDL particle size was analyzed by 4–30% native gradient PAGE. The mean distribution of HDL_2 and HDL_3 subclasses before (open circles) and after (closed circles) periodontal treatment is shown in detail. The mean distribution of HDL_2 and HDL_3 main subclasses before (open circles) and after (closed circles) periodontal treatment is shown in the inset. The asterisks indicate statistically significant differences ($P < 0.05$) before versus after treatment.

TABLE 2. Kinetic parameters of HDL efflux as determined from samples obtained from 30 patients with periodontitis before and after treatment

Parameter	Before	After	P^a
K_m (h)	1.60 (0.29)	1.62 (0.40)	0.376
V_{max} (pg)	841 (156)	864 (177)	0.154
Initial velocity (V_{init}) (pg/h)	267 (49)	277 (60)	0.105
Catalytic efficacy (V_{max}/K_m) (pg/h)	535 (97)	553 (121)	0.111

Values shown are means and (SD).
[a] Wilcoxon signed ranks test.

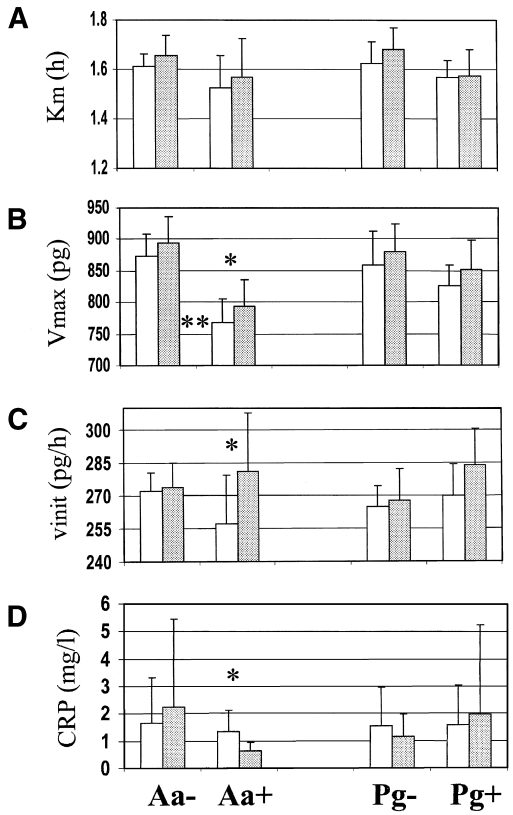

Fig. 3. Kinetic parameters of cholesterol efflux capacity of HDL, and C-reactive protein (CRP) concentrations in serum before and after periodontal treatment. Total HDL was isolated from plasma samples obtained from 30 patients with periodontitis before and after treatment. Cultivated mouse macrophages (RAW 264.7) were labeled with [^{3}H]cholesterol. After removing the label, equilibration, and washing the cells, 100 μg (as total HDL protein) of HDL was added. Cholesterol efflux promoted by HDL was analyzed from the cell medium after 1, 3, and 6 h of incubation. The kinetic parameters K_m (A), V_{max} (B), and initial velocity (vinit; C) were calculated before (white columns) and after (gray columns) treatment of patients positive (+) or negative (−) for *A. actinomycetemcomitans* (Aa) and *P. gingivalis* (Pg). Sensitive CRP was determined from serum samples. One asterisk indicates statistical significance ($P < 0.05$) before versus after treatment, and two asterisks indicate statistical significance ($P < 0.01$) of the difference between Aa-negative and Aa-positive patients before treatment. Error bars indicate SD.

capacity before periodontal treatment compared with the *A. actinomycetemcomitans*-negative patients. As determined by V_{max} and V_{init}, the efflux capacity of the *A. actinomycetemcomitans*-positive patients also increased significantly ($P < 0.05$) after treatment. Interestingly, CRP of *A. actinomycetemcomitans*-positive patients (n = 10) decreased from 1.34 ± 0.81 mg/l to 0.62 ± 0.33 mg/l ($P = 0.015$) (Fig. 3) and SAA concentration decreased from 7.42 ± 2.37 mg/l to 5.92 ± 2.42 mg/l ($P = 0.066$). The HDL efflux capacity of the *P. gingivalis*-positive patients displayed similar trends without statistical significance. There were no differences in HDL efflux capacity between the groups of patients treated with or without systemic administration of metronidazole.

To clarify how the HDL cholesterol efflux parameters related to the other variables determined and to the periodontal variables, correlation analysis was performed on the pretreatment variables (**Table 3**). There was a positive correlation between the efflux parameters and serum HDL cholesterol concentration and CETP activity as well as with HDL free cholesterol, cholesteryl ester, and phospholipid concentrations. The parameters describing the efflux velocity (V_{max} and V_{init}), HDL free cholesterol, and HDL phospholipid concentrations were negatively correlated with the proportion of teeth with periodontal pockets deeper than 5 mm. The proportion of teeth with deep periodontal pockets and the number of periodontal pockets with suppuration correlated negatively with serum HDL cholesterol concentration and PLTP mass. Periodontal pockets with suppuration were also positively correlated with SAA concentration in serum. To ascertain the results from the correlation analysis, two linear regression models with V_{init} as a dependent variable were tested. In a model adjusted for age, V_{init} was significantly associated with PCR positivity for *A. actinomycetemcomitans* ($\beta = 0.240$, $P = 0.049$), CRP ($\beta = -0.262$, $P = 0.044$), and HDL cholesterol concentration in serum ($\beta = 0.474$, $P < 0.001$). Among HDL components, the strongest association with V_{init} was found with free cholesterol ($\beta = 0.298$, $P = 0.098$) and SM/PC ($\beta = 0.558$, $P = 0.104$), but none of the components reached statistical significance.

DISCUSSION

Periodontitis is a persistent bacterial infection that causes chronic inflammation in periodontal tissues. The disease is characterized by the formation of deep periodontal pockets and the destruction of connective tissue attachment and alveolar bone. Untreated periodontitis may eventually lead to loss of teeth. The systemic response to periodontitis can be measured as increased serum antibody levels against periodontopathogenic bacteria (35) and CRP concentrations (36). Also in the present study, the levels of serum antibodies to *P. gingivalis* and the concentrations of CRP and SAA in *A. actinomycetemcomitans*-positive patients were decreased after periodontal treatment. In addition to data from early cross-sectional clinical studies reporting that dental infections are significantly more prevalent in coronary heart disease (CHD) patients than in their matched controls (37), a number of studies have confirmed and extended these findings (38, 39). Because severe periodontitis is relatively common, affecting ~20% of middle-aged and elderly populations worldwide (40), its relevance to public health is extensive. An increased risk for CHD in individuals with periodontitis may be one of the long-term effects of the daily systemic spread of live subgingival/oral bacteria and their components, which have access to the bloodstream as a result of mechanical injury (e.g., during eating or tooth brushing) to the inflamed gingivae. However, it is still unclear what mechanisms are involved in periodontitis-promoted atherogenesis.

A strong negative association between untreated periodontitis and HDL cholesterol concentration was observed in the present study. This finding is of major importance,

TABLE 3. Correlation coefficients between the variables examined

	Age	S-chol	S-HDL	S-TG	S-SAA	PLTP activity	PLTP mass	CETP activity	LCAT activity	K_m	V_{max}	V_{init}	HDL-CE	HDL-FC	HDL-TG	HDL-PL	HDL-prot	Per. Pockets[a]	Suppuration[b]	CRP
Age	1.00	**0.34**[c]	0.05	0.27	0.11	0.04	−0.31	−0.18	−0.12	−0.03	−0.21	−0.18	−0.22	−0.04	−0.04	0.05	0.12	−0.10	**0.34**	−0.18
S-chol		1.00	0.16	0.19	−0.05	0.26	0.04	0.15	**−0.66**	−0.20	−0.13	0.07	−0.16	−0.06	−0.09	0.08	0.12	**0.35**	**0.39**	0.25
S-HDL			1.00	**−0.38**	−0.02	0.15	**0.59**	0.22	−0.22	−0.19	**0.33**	**0.44**	0.29	**0.58**	**−0.33**	**0.47**	−0.12	**−0.55**	**−0.48**	−0.14
S-TG				1.00	**0.33**	**0.33**	**−0.48**	**−0.41**	−0.05	−0.23	−0.26	−0.15	**−0.48**	**−0.39**	**0.45**	−0.07	−0.09	0.21	0.30	**0.35**
S-SAA					1.00	−0.04	−0.07	**−0.37**	0.11	−0.06	−0.14	−0.04	−0.13	−0.05	−0.03	−0.05	0.11	−0.13	**0.32**	**0.57**
PLTP activity						1.00	0.14	0.06	−0.21	−0.12	0.20	**0.31**	−0.13	0.12	0.02	0.26	−0.09	0.05	0.06	0.20
PLTP mass							1.00	0.15	−0.16	−0.09	0.14	0.24	0.17	**0.40**	−0.29	0.12	0.07	**−0.32**	**−0.42**	−0.15
CETP activity								1.00	−0.19	**0.33**	**0.33**	0.16	0.04	0.25	**−0.42**	0.24	0.21	−0.18	−0.22	−0.21
LCAT activity									1.00	0.11	0.03	0.11	0.21	0.10	0.02	**−0.33**	0.02	**−0.33**	−0.09	−0.11
K_m										1.00	**0.48**	**−0.51**	0.28	**0.34**	−0.12	0.32	−0.20	−0.25	0.00	−0.01
V_{max}											1.00	**0.49**	**0.59**	**0.70**	**−0.32**	**0.68**	**−0.38**	**−0.32**	0.09	−0.22
V_{init}												1.00	0.30	**0.37**	−0.26	**0.34**	−0.10	**−0.34**	0.09	−0.23
HDL-CE													1.00	**0.61**	−0.21	0.26	**−0.45**	−0.06	0.15	−0.02
HDL-FC														1.00	**−0.33**	**0.48**	**−0.32**	**−0.53**	−0.21	−0.01
HDL-TG															1.00	−0.21	**−0.65**	0.26	0.06	0.04
HDL-PL																1.00	**−0.44**	**−0.32**	−0.10	0.14
HDL-prot																	1.00	−0.01	−0.04	−0.08
Per. pockets																		1.00	**0.68**	0.20
Suppuration																			1.00	**0.51**
CRP																				1.00

CE, cholesteryl ester; chol, cholesterol; FC, free cholesterol; Per, periodontal; PL, phospholipid; prot, total protein; TG, triglycerides; V_{init}, initial velocity.

[a] Proportion (%) of teeth with deepened (>5 mm) periodontal pockets of the pockets examined.

[b] Number of periodontal pockets with suppuration.

[c] Statistically significant ($P < 0.05$) correlation coefficients are shown in boldface.

because low HDL cholesterol concentration is one of the established independent CHD risk factors confirmed in numerous epidemiological studies (41). In earlier clinical studies, patients with periodontitis had higher serum total cholesterol or triglyceride concentrations than did healthy controls (21, 23, 24), but the differences in HDL cholesterol concentrations have not been significant in any of the reports. On the other hand, in a random population sample (n = 1,163) of middle-aged and elderly men, high combined serum antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* were significantly associated with low HDL cholesterol concentration (42). In the present study, the higher HDL cholesterol concentration after periodontal treatment was attributed specifically to the increase of the HDL_{2a} subpopulation, which has been proposed to be more protective than total HDL or HDL_3 (43).

Cell culture as well as genetic family and population studies have revealed that serum HDL cholesterol concentration does not necessarily reflect the efficacy and antiatherogenicity of reverse cholesterol transport (44). Instead, HDL subclass distribution, cholesterol efflux capacity, and the kinetics of HDL metabolism are putatively important parameters that regulate the role of HDL against atherosclerosis. As suggested here, defective cholesterol removal attributable to inflammation-induced changes in HDL may provide one of the mechanisms underlying the relationship between periodontitis and atherosclerosis as well as between other chronic infections and atherosclerosis (45). HDL composition, in addition to HDL cholesterol concentration and subclass distribution, is an important determinant of the cholesterol removal capacity (46). In the present study, the changes in HDL composition before versus after periodontal treatment were substantial, except for HDL phospholipid mass and subclass distribution. It has been shown that the efflux capacity of plasma has the strongest correlation with the HDL-SM concentration (47–49). Moreover, SM is the phospholipid most efficient in solubilizing free cholesterol. The significant increase in the HDL-SM/PC ratio after periodontal treatment thus supports our present observation of the increased cholesterol efflux from macrophages.

Also, the higher LCAT and CETP activities after periodontal treatment support the hypothesis of an enhanced reverse cholesterol transport during periodontitis, because these proteins are among the key factors in this process (5). PLTP provides a supply of primary cholesterol acceptors through HDL conversion (50–52) and is thought to increase the binding of HDL to human skin fibroblasts (53), thus promoting cholesterol efflux. Nevertheless, the exact role of PLTP, especially the balance between recently reported active and inactive forms of PLTP, in the efflux capacity of HDL is not yet resolved (54). In the present study, PLTP activity decreased but PLTP mass increased after periodontal treatment, in agreement with the earlier study of patients with an acute-phase response (10).

Although the statistical power of the present study was limited, the results indicate that there is an association between HDL cholesterol efflux capacity and clinical and microbiological findings of periodontitis. First, we found a significant negative correlation between the proportion of teeth with deep periodontal pockets (indicating the extent of periodontitis in the dentition) and the kinetic parameters of efflux capacity (V_{max} and V_{init}). Second, the differences in the efflux kinetics before and after periodontal treatment were significant only in a subgroup of patients who were PCR-positive for *A. actinomycetemcomitans*. This was not the case, however, for patients who were PCR-positive for *P. gingivalis*. This difference may be attributable to the dissimilar pathogenic characteristics of the two pathogens, for example, LPS structure or activity (55). If substantiated in a larger study population, this difference may also be related to the lower SAA concentration in *A. actinomycetemcomitans*-positive patients after periodontal treatment. Through its association with HDL, SAA results in better binding of SAA-enriched HDL to macrophages, increased cholesteryl ester uptake, impaired activity of LCAT, and reduced cellular cholesterol efflux (16, 17). In addition, SAA effectively displaces apoA-I from HDL and thereby diminishes the formation of lipid-poor apoA-I, the major specific cholesterol acceptor from macrophages.

We found that periodontitis may decrease serum total HDL cholesterol concentration and HDL_2/HDL_3 ratio. Periodontitis-induced changes in HDL composition may impair its efflux capacity; indeed, the efflux capacity of HDL was significantly lower among patients who were PCR-positive for *A. actinomycetemcomitans*. Our results indicate that the changes in HDL metabolism during periodontitis are similar to but milder than those found during an acute-phase response. The present data suggest that periodontitis diminishes the antiatherogenic potency of HDL and may thus increase the risk for CHD.

Tiina Karvonen is acknowledged for skillful and precise technical assistance. This study was sponsored by the Academy of Finland (grants 75953 and 77613 to P.J.P., grants 71413 and 72971 to S.A.), by the Paulo Foundation, and by the Finnish Dental Society.

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