J Clin Periodontol 2006; 33: 855–862 doi: 10.1111/j.1600-051X.2006.00996.x

Journal of Clinical Periodontology

Periodontal infection profiles in type 1 diabetes

Evanthia Lalla[1], Selma Kaplan[1], Shu-mei J. Chang[1], Georg A. Roth[1], Romanita Celenti[1], Karen Hinckley[1], Ellen Greenberg[2] and Panos N. Papapanou[1]

[1]Division of Periodontics, Section of Oral and Diagnostic Sciences, College of Dental Medicine, Columbia University, New York, NY, USA; [2]Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA

Lalla E, Kaplan S, Chang SJ, Roth GA, Celenti R, Hinckley K, Greenberg E, Papapanou PN. Periodontal infection profiles in type 1 diabetes. J Clin Periodontal 2006; 33: 855–862. doi: 10.1111/j.1600-051X.2006.00996.x.

Abstract

Objectives: We investigated the levels of subgingival plaque bacteria and serum IgG responses in patients with type 1 diabetes and non-diabetic controls of comparable periodontal status.

Material and Methods: Fifty type 1 diabetes patients (mean duration 20.3 years, range 6–41) were age-and gender-matched with 50 non-diabetic individuals with similar levels of periodontal disease. Full-mouth clinical periodontal status was recorded, and eight plaque samples/person were collected and analysed by checkerboard hybridization with respect to 12 species. Homologous serum IgG titres were assessed by checkerboard immunoblotting. In a sub-sample of pairs, serum cytokines and selected markers of cardiovascular risk were assessed using multiplex technology.

Results: Among the investigated species, only levels of *Eubacterium nodatum* were found to be higher in diabetic patients, while none of the IgG titres differed between the groups, both before and after adjustments for microbial load. Patients with diabetes had significantly higher serum levels of soluble E-selectin ($p = 0.04$), vascular cell adhesion molecule-1 (VCAM-1; $p = 0.0008$), adiponectin ($p = 0.01$) and lower levels of plasminogen activator inhibitor-1 (PAI-1; $p = 0.02$).

Conclusions: After controlling for the severity of periodontal disease, patients with type 1 diabetes and non-diabetic controls showed comparable subgingival infection patterns and serum antibody responses.

Key words: diabetes; microbiology; periodontitis; serum antibody; type 1

Accepted for publication 25 August 2006

Diabetes mellitus has been recognized as an important risk factor for periodontitis (Löe 1993, Papapanou 1996, Borrell & Papapanou 2005). Cross-sectional epidemiologic studies have demonstrated that patients with diabetes have more extensive and severe loss of periodontal tissue support than individuals free of the disease (Thorstensson & Hugoson 1993, Sandberg et al. 2000). Longitudinal studies have shown a higher incidence of progressive periodontitis in patients with diabetes (Seppälä et al. 1993, Taylor et al. 1998, Thomson et al. 2004), and treatment studies have suggested that periodontal treatment outcomes are generally inferior in patients with poor metabolic control when compared with those achieved in non-diabetic subjects (Tervonen & Karjalainen 1997). Several mechanisms conferring an increased susceptibility to periodontitis in the setting of diabetes have been proposed (Lalla et al. 2000a, b). However, it remains unclear whether there are any significant differences in the quality of the periodontal bacterial challenge between subjects with diabetes and non-diabetic subjects (Mealey & Moritz 2003). Although a limited number of studies comparing periodontal microbiota in subjects with and without diabetes do exist, these studies are generally limited with respect to sample size, number of bacterial plaque samples harvested per participant, and diversity of bacterial species assessed (Zambon et al. 1988, Feitosa et al. 1992, Thorstensson et al. 1995, Novaes Junior et al. 1997, Sbordone et al. 1998). Importantly, the control groups involved in these studies typically consist of periodontally healthy individuals (Yuan et al. 2001, Campus et al. 2005). As the clinical periodontal status is by itself a significant determinant of the periodontal bacterial ecology, it is difficult to deduce from these studies whether any reported qualitative and quantitative differences in periodontal microbiota are due to diabetes *per se*, or simply reflect the concurrently prevalent higher extent and severity of periodontitis in diabetic individuals. Arguably, the identification of diabetes-specific subgingival infection profile characteristics should be ideally investigated in pairs of individuals with and without diabetes who display similar periodontal status.

Therefore, the aim of this study was to examine the key features of periodontal infections by microbiological and serological means, in a cohort of

subjects with type 1 diabetes and a control group of age- and gender-matched non-diabetic individuals with similar levels of destructive periodontitis.

Material and Methods

Subject sample

The study protocol was approved by the Columbia University Medical Center Institutional Review Board, and written informed consent was obtained from all study participants. Patients with type 1 diabetes were recruited among those attending the Columbia University Naomi Berrie Diabetes Center. Following a preliminary assessment of eligibility at the Center based on the criteria described below, interested patients were referred for further evaluation and informed consent. Neither periodontal status nor level of metabolic control was considered in the enrolment process. Control subjects were recruited among individuals attending the Columbia University College of Dental Medicine clinics. All control subjects had undergone a physical examination within 1 year before their participation to the study, confirming normal blood glucose levels. Subjects in both groups were at least 18 years old, were on no regular anti-inflammatory medication, and had not received antibiotics for at least 3 months before enrolment. Diabetes duration in the case group was at least 5 years. In order to achieve the target sample size of 50 pairs (50 subjects with type 1 diabetes and 50 age- and gender-matched non-diabetic controls with similar levels of periodontal disease), a total of 111 individuals were examined clinically.

Clinical examination

All subjects underwent a comprehensive, full-mouth clinical periodontal examination at six sites per tooth carried out by a single, calibrated periodontist (author S. K). The periodontal parameters included assessments of:

(i) Probing pocket depth: distance of the free gingival margin to the base of the probeable pocket, recorded to the nearest millimetre.
(ii) Gingival margin: location of the free gingival margin in relation to the cemento-enamel junction (CEJ; positive if located apical to the CEJ, and negative if located coronal to the CEJ).
(iii) Bleeding of probing and dental plaque, assessed dichotomously.

The algebraic sum of probing depth and the gingival margin was used to compute the clinical attachment level.

Blood sample

A 10 ml blood sample was obtained by venipuncture from each participant. Serum was collected by centrifugation, aliquoted, and stored until further analyses at -70°C.

Assessment of periodontal microbiota

Subgingival plaque samples were obtained from the mesiolingual surface of each tooth at the two most posterior teeth in each quadrant (eight samples per subject) using sterile curettes. The samples were analysed by checkerboard DNA–DNA hybridization (Socransky et al. 1994) using whole genomic probes from 12 periodontal bacteria including both putative pathogens and health-related species (*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Campylobacter rectus*, *Micromonas micros*, *Eubacterium nodatum*, *Eikenella corrodens*, *Veillonella parvula*, and *Actinomyces naeslundii*). The sampling technique and the processing of the samples have been described in detail elsewhere (Papapanou et al. 2000).

Assessment of serum IgG responses to periodontal bacteria

The presence and levels of IgG antibodies against all bacteria included in the microbiological panel were assessed by checkerboard immunoblotting (Sakellari et al. 1997) as described earlier (Papapanou et al. 2000).

Assessment of serum inflammatory mediators

In serum samples from a subsample of the examined cohort, we assessed the level of a number of cytokines, chemokines, and mediators relevant to cardiovascular disease risk, using multiplex technology and commercially available kits (Linco Research Inc., St. Charles, MO, USA) according to the manufacturer's instructions. For the assessment of cytokine/chemokine levels, we used the human cytokine multiplex kit and determined serum concentrations of interleukin 1α (IL-1α), IL-1β, IL-2, interleukin-1 receptor antagonist (IL-1ra), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, interferon γ (IFN-γ), granulocyte-colony stimulating factor (G-CSF), granulocyte/macrophage-colony stimulating factor (GM-CSF), tumour necrosis factor α (TNF-α), eotaxin, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), and the chemokine IP-10. The minimum detectable concentration in this assay was 3.2 pg/ml for each analyte.

For the assessment of mediators involved in the pathogenesis of cardiovascular cell disease, we used the CVD Human Biomarker 1 kit, allowing determination of soluble E-selectin, soluble vascular cell adhesion molecule-1 (VCAM-1), soluble intercellular adhesion molecule-1 (ICAM-1), matrix metalloproteinase 9 (MMP-9), myeloperoxidase (MPO), adiponectin, and plasminogen activator inhibitor-1 (PAI-1). The minimum detectable concentrations for this assay were as follows: sE-selectin 79 pg/ml; sVCAM-1 16 pg/ml; sICAM-1 9 pg/ml; MMP-9 1 pg/ml; MPO 7 pg/ml; adiponectin 56 pg/ml; and PAI-1 1 pg/ml.

Data analysis

The SAS statistical analysis package (version 9.1) was used. The analyses included descriptive statistics, two-tailed Student's *t*-test for paired observations, and chi-square analysis to compare frequencies. Adjustments for multiple comparisons were performed using the Bonferroni correction whenever applicable.

Results

Table 1 describes selected demographic and other characteristics of the 50 pairs of patient–control subjects involved in the present report. The gender distribution was well balanced, and the active matching for age within pre-defined 5-year intervals resulted in a very similar mean age in diabetic patients and controls (39.6 and 37.7 years, respectively). With respect to self-reported race and ethnicity, approximately 70% of the study sample consisted of non-Hispanic Whites. Overall, 14% of the participants were current and 18% were former smokers. As expected, a family history of diabetes was more than twice

Table 1. Demographic characteristics in diabetic subjects and controls ($N = 100$, 50/group)

	Diabetic patients	Controls	All
Gender			
Male	26	26	52
Female	24	24	48
Age (years)			
Mean (SD)	39.6 (13.1)	37.7 (12.4)	38.7 (12.2)
Range	18–70	19–70	18–70
18–25	5	5	10
26–35	16	16	32
36–45	16	16	32
46–55	9	9	18
56–65	2	2	4
66–70	2	2	4
Ethnicity			
Hispanic	17	15	32
Non-Hispanic	33	35	68
Race			
White	38	33	71
Black	8	10	18
Other	1	5	6
Unknown	3	2	5
Smoking			
Never	39	29	68
Current	6	8	14
Former	5	13	18
Family history of diabetes			
No	23	40	63
Yes	26	10	36
Unknown	1	0	1
Insurance status			
None	1	12	13
Private	32	25	57
Public (Medicaid)	17	10	27
Unknown	0	3	3

Table 2. Diabetes-related parameters in cases

Duration (years)	20.3 (9.3)
Range	6–41
Treated with	
2 insulin injections/day	9 (18)
3–4 insulin injections/day	13 (26)
Continuous subcutaneous insulin infusion	28 (56)
Mean HbA1c over past 2 years (%)	7.5 (1.3)
Range	5.6–12.6
HDL cholesterol (mg/dl)	62 (14.9)
Range	39–99
LDL cholesterol (mg/dl)	103.2 (26.7)
Range	27–184
Triglycerides (mg/dl)	76.6 (44.4)
Range	23–256

Mean values or frequencies, standard deviations or % in parentheses, $N = 50$.
HbAlc, hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein.

as high in patients with diabetes that in controls (26% *versus* 10%). The vast majority of the participants were covered by private (57%) or public (27%) health insurance.

Table 2 describes a number of diabetes-related parameters in the case group. Among the 50 participants with type 1 diabetes, the duration ranged between 6 and 41 years and was on average 20.3 years. Forty-four per cent of the patients used insulin injections at least twice daily, while 56% were on continuous subcutaneous infusion (insulin pump). The average haemoglobin A1c (HbAC) over the 2-year period preceding inclusion into the study was 7.5%, ranging from a low of 5.6% to a high of 12.6%. Overall, the level of metabolic control was good in this patient group. As illustrated in Fig. 1, 59.6% of the patients had good metabolic control, i.e., HbA1c levels below 7.5%, while 8.5% of them had HbA1c levels exceeding 9.5%, indicating poor metabolic control.

The clinical periodontal status of the 50 pairs is described in Table 3. Diabetic subjects and controls had comparable numbers of teeth present, and similar levels of dental plaque, bleeding on probing, probing depths, and attachment loss. However, patients with diabetes had a tendency towards a higher number of deep ($\geqslant 5$ mm) pockets when compared with controls (12.3 *versus* 7.6, $p = 0.062$). On average, patients with diabetes had 12.2 sites with AL $\geqslant 5$ mm (range 0–107), while the control subjects had an average of 10 such sites (range 0–63). Thus, although the average levels of periodontal destruction were rather moderate, there was substantial variation, and cases of severe periodontal disease were included in both groups.

Figure 2 describes the colonization pattern by each species in both cases and controls. Data analysis by means of paired *t*-tests for matched observations after application of the Bonferroni correction to adjust for multiple comparisons revealed that the only statistically significant difference in bacterial colonization between the groups was found for *E. nodatum*, which was significantly elevated in diabetic patients (individual $p = 0.0013$). We subsequently investigated whether there were any differences in bacterial colonization between diabetic patients and controls separately for deep (PD $\geqslant 5$ mm) and shallow sites (data not shown). This analysis revealed no statistically significant differences between the two groups, irrespective of the clinical status (probing depth) of the sampled sites.

We further analysed the levels of serum IgG antibodies against the 12 investigated bacterial species in diabetic patients and controls using checkerboard immunoblotting. Using paired *t*-tests for matched observations and the Bonferroni correction to adjust for multiple comparisons, we detected no statistically significant differences in titres between the two groups (Fig. 3).

Next, we examined whether there were any differences in specific antibody responses to the investigated periodontal bacteria after concomitant consideration of the homologous bacterial load by each species. For this

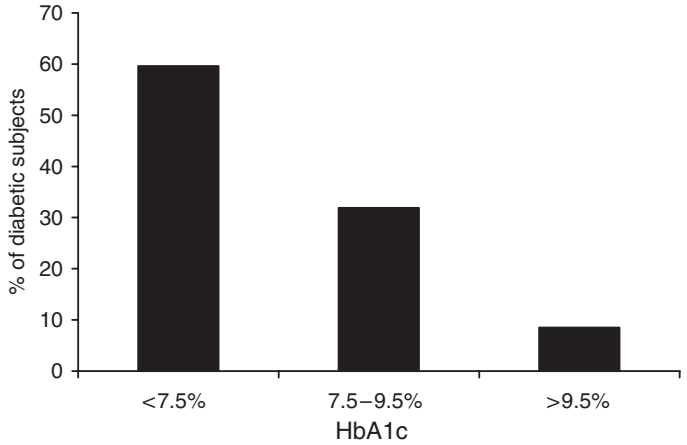

Fig. 1. Level of metabolic control in the diabetic patient group, based on mean HbA1c values over a 2-year period preceding the dental examination.

Table 3. Clinical periodontal status in diabetic subjects and controls ($N = 50$ pairs)

	Diabetic patients	Controls	*p*-value*
# of teeth present	25.9 (3.7)	26.2 (3.0)	0.666
Range	11–28	16–28	
% sites with plaque	32.0 (34.4)	26.0 (37.2)	0.388
Range	0–100	0–100	
% sites with BoP	28.2 (27.5)	24.6 (25.5)	0.467
Range	0.6–100	0–100	
Pocket depth (mm)	2.8 (0.6)	2.7 (0.4)	0.652
range	2.0–4.8	2.0–4.1	
# sites/subject with PD ⩾ 5 mm	12.3 (19.7)	7.6 (12.2)	0.062
Range	0–107	0–55	
% sites/subject with PD ⩾ 5 mm	8.5 (13.1)	5.3 (9.0)	0.078
Range	0–63.9	0–43.7	
Attachment loss (mm)	0.6 (0.8)	0.5 (0.8)	0.415
Range	0–4.1	0–3.5	
# sites/subject with AL ⩾ 5 mm	12.2 (18.9)	10.0 (15.8)	0.457
Range	0–107	0–63	
% sites/subject with AL ⩾ 5 mm	8.3 (12.3)	6.9 (11.2)	0.494
Range	0–64.5	0–45.6	

Mean values (standard deviations).
*Obtained by a two-tailed *t*-test for paired observations.
PD, pocket depth; AL, attachment loss.

analyses, we created a variable termed ''infection ratio'' for each species (Picolos et al. 2005). This variable concomitantly considers host response and bacterial colonization, and provides a more appropriate description of responsiveness to the bacterial challenge than the antibody titre alone. Infection ratios were defined by calculating the log of the ratio of each subject's species-specific antibody titre over the average bacterial load across all sampled sites for each homologous species. This analysis, presented in Fig. 4, revealed similar levels of responsiveness to periodontal bacteria in subjects with type 1 diabetes and control subjects.

Table 4 reports serum concentrations of selected cytokines and chemokines in a sub-sample comprising 13 case–control pairs. No statistically significant differences were detected between the two groups for any of the analytes assessed, although there was a tendency for higher serum levels for IL-1α and IL-6 in diabetic patients ($p = 0.104$ and 0.102, respectively, two-tailed test for paired observations). Lastly, Table 5 presents the levels of selected mediators involved in the pathogenesis of cardiovascular disease in the same sub-sample of 13 diabetic patient-control pairs. Diabetic patients showed higher serum levels of sE-selectin ($p = 0.047$), sVCAM-1 ($p = 0.0008$), and adiponectin ($p = 0.0171$), while controls displayed higher levels of PAI-1 ($p = 0.021$).

Discussion

In this study, we analysed the key features of periodontal infection in pairs of type 1 diabetic patients and age- and gender-matched, non-diabetic controls with similar levels of periodontal disease. In this setting of comparable periodontal status between cases and controls, the periodontal infection profiles (assessed by means of subgingival bacterial levels, serum IgG responses, and infection ratios) were largely similar in the two groups. Our findings add to the substantial but quite conflicting body of literature, part of which has suggested the existence of rather distinct subgingival microbial profiles (Mashimo et al. 1983) or serum antibody response patterns in diabetes (Zambon et al. 1988).

According to recent data from the US National Center for Chronic Disease Prevention and Health (2005), the median duration of diagnosed diabetes in the United States was 6.3 years in 2004. We chose to study a group of type 1 diabetic patients with a long duration of disease (minimum 5 years, mean 20.3 years) rather than both type 1 and type 2 patients for a more focused comparison between a well-established, largely genetically determined diabetic state and a diabetes-free setting. Conceivably, such a strategy maximized our power to detect differences among the infection profiles of case–control pairs with similar periodontal status. After adjusting for multiple comparisons, our data showed that only the subgingival levels of *E. nodatum* were higher in type 1 diabetes patients, although within the same log, the actual difference in mean bacterial counts between the two groups (30×10^4 *versus* 4×10^4) casts some doubt on the biological significance of this finding. In recognition of the possibility that differences in microbial profiles between the two groups would not necessarily be manifested by the use of whole-mouth statistics, we expanded our analysis to include a stratification by pocket depth, i.e., we performed separate comparisons of bacterial levels in deep (PD ⩾ 5 mm) *versus* shallow pockets. This analytical strategy was found to be more discriminatory in earlier studies, notably in the detection of qualitative differences in the subgingival

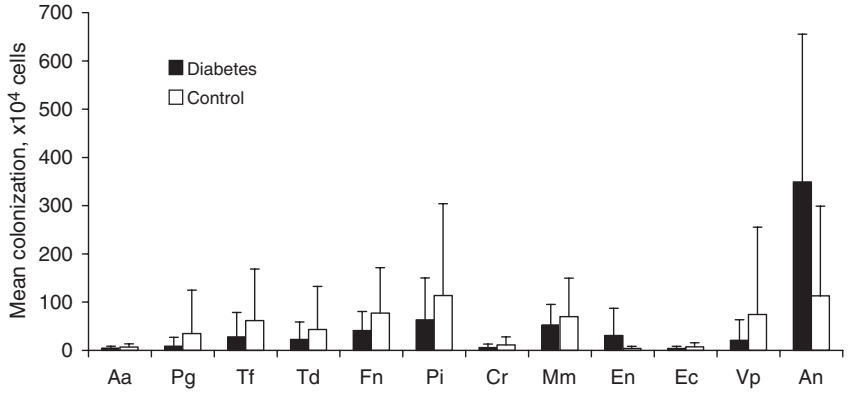

Fig. 2. Mean bacterial colonization by the 12 investigated species in patients with diabetes and non-diabetic controls. Values in the *y*-axis represent bacterial counts. Error bars represent standard deviations.

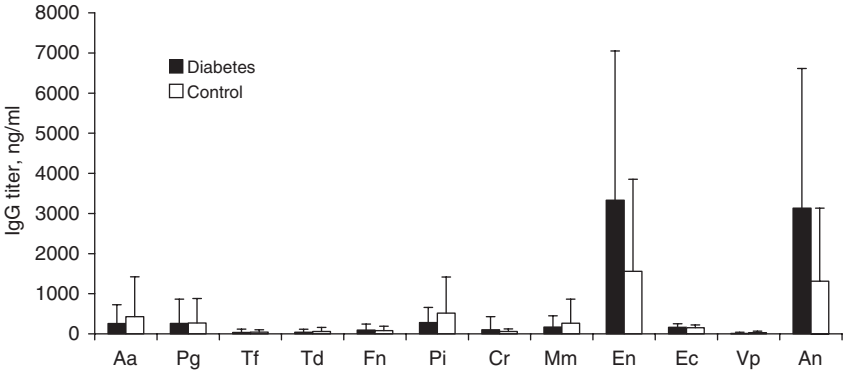

Fig. 3. Serum IgG titres against the 12 investigated species in patients with diabetes and non-diabetic controls. Error bars represent standard deviations.

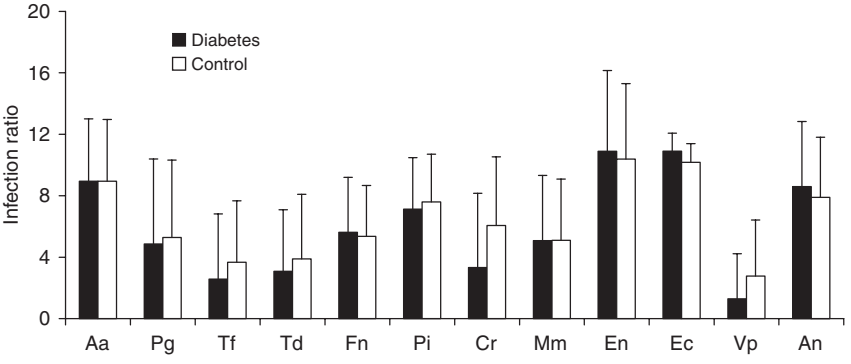

Fig. 4. Infection ratios (log of the ratio of the specific antibody titre over the mean bacterial load across all sampled sites for the homologous species) in diabetic subjects and controls. Error bars represent standard deviations.

microbiota of smokers and non-smokers (Haffajee & Socransky 2001). Nevertheless, the stratified analysis was still not able to reveal differences between diabetes cases and non-diabetic controls.

The present results are in variance with a recent case–control study (Campus et al. 2005), which examined by means of polymerase chain reaction (PCR) the presence of three periodontal species (*P. gingivalis, P. intermedia*, and *T. forsythia*) in subgingival plaque samples of type 2 diabetes patients and non-diabetic controls and reported a higher prevalence of *P. gingivalis* and a lower prevalence of *T. forsythia* in diabetes. Another recent study (Ciantar et al. 2005) compared levels of *Capnocytophaga* spp., assessed through PCR and restriction fragment length polymorphism, in a test group comprising both type 1 and type 2 diabetic patients and a non-diabetic control group. These authors reported that the total mean count of *Capnocytophaga* spp. was overall significantly higher in diabetes-associated periodontitis than in the control group. However, when a direct comparison restricted to only diseased sites in cases and controls was undertaken, the difference in *Capnocytophaga* spp. levels did not reach statistical significance. An earlier study by Thorstensson et al. (1995) compared the subgingival microbiota in a cohort of type 1 (insulin dependent) diabetic patients of long duration with a group of age- and gender- matched controls. These investigators analysed by culture techniques the presence and levels of *A. actinomycetemecomitans, C. rectus, Capnocytophaga* spp., *E. corrodens, F. nucleatum, P. gingivalis*, and *P. intermedia* in both pooled and individual plaque samples and reported a higher prevalence, but no higher proportionate colonization levels, by *P. gingivalis* in the diabetes group. However, when stratified by age, the group of younger diabetic patients also displayed statistically more severe loss of periodontal tissue support than their non-diabetic counterparts.

A few studies have carried out similar comparisons using serum antibody responses to periodontal bacteria. The previously mentioned study by Thorstensson et al. (1995) concomitantly investigated serum IgG responses to a panel of periodontal bacteria by ELISA and identified a limited number of statistically significant differences in titre, notably higher titres for *P. gingivalis* and *F. nucleatum* in older diabetic patients with moderate levels of periodontal disease. Importantly, this study did not adjust for the multiple comparisons performed, and the magnitude of the detected differences was small. In a study involving individuals with and without periodontitis, type 1 (insulin-dependent) diabetes, or impaired glucose tolerance, Zambon et al. (1988) examined serum IgG titres to seven oral bacterial species and reported that diabetic patients with periodontitis had statistically significantly lower serum IgG titres to *P. intermedia* than their non-

Table 4. Serum cytokines (ng/ml) in a sub-sample of the examined cohort ($N = 13$ pairs)

Mediator	Diabetic patients	Controls	*p*-value*
IL-1α	279.9 (318.5)	97.3 (139.7)	0.104
IL-1β	2.1 (6.1)	2.4 (8.7)	0.918
IL-2	21.8 (61.2)	21.7 (66.7)	0.997
IL-1ra	156.8 (128.5)	211.6 (187.2)	0.410
IL-4	353.4 (448.1)	186.9 (349.2)	0.347
IL-5	0.6 (2.1)	1.0 (3.6)	0.731
IL-6	112.7 (116.2)	42.6 (63.6)	0.102
IL-7	13.0 (9.0)	13.9 (17.6)	0.890
IL-8	57.2 (62.8)	93.2 (144.1)	0.421
IL-10	42.3 (93.0)	38.0 (107.7)	0.920
IL-12 p70	18.0 (34.9)	13.0 (38.9)	0.757
IL-13	16.2 (40.2)	17.0 (45.3)	0.963
IL-15	8.8 (19.8)	10.0 (29.1)	0.906
IL-17	26.5 (49.0)	27.8 (55.0)	0.952
IFN-γ	15.5 (38.6)	21.4 (72.9)	0.810
G-CSF	131.6 (219.8)	100.4 (230.5)	0.750
GM-CSF	40.5 (27.9)	27.7 (22.4)	0.236
TNF-α	9.2 (8.6)	10.9 (15.4)	0.756
Eotaxin	131.9 (8.1)	149.9 (82.5)	0.350
MCP-1	263.2 (96.2)	236.8 (75.1)	0.464
MIP-1a	46.5 (52.0)	34.5 (32.2)	0.450
IP-10	103.2 (75.1)	88.4 (58.9)	0.567

Mean values (standard deviations).

*Obtained by a two-tailed *t*-test for paired observations, unadjusted for multiple comparisons; for an overall *p*-value of 0.05, an individual *p*-value of 0.0022 is required after Bonferroni adjustment for 22 tests.

IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte/macrophage-colony stimulating factor; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; IP-10, interferon-inducible protein of 10kDa.

Table 5. Selected serum mediators involved in the pathogenesis of cardiovascular disease in a sub-sample of the examined cohort ($N = 13$ pairs)

Mediator	Diabetic patients	Controls	*p*-value*
E-selectin (ng/ml)	22.2 (13.8)	12.1 (8.7)	0.047
VCAM-1 (μg/ml)	1.2 (0.2)	0.9 (0.1)	0.0008
ICAM-1 (ng/ml)	124.2 (39.9)	111.8 (28.4)	0.320
MMP-9 (μg/ml)	227.1 (97.6)	329.2 (188.9)	0.146
MPO (μg/ml)	123.4 (78.6)	204.2 (213.8)	0.235
Adiponectin (μg/ml)	15.2 (7.5)	9.1 (2.9)	0.0171
PAI-1 (μg/ml)	25.8 (4.5)	31.5 (6.8)	0.021

Mean values (standard deviations).

*Obtained by a two-tailed *t*-test for paired observations, unadjusted for multiple comparisons; for an overall *p*-value of 0.05, an individual *p*-value of 0.0071 is required after Bonferroni adjustment for seven tests.

VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; MMP, matrix metalloproteinase; MPO, myeloperoxidase; PAI, plasminogen activator inhibitor.

diabetic periodontitis counterparts, while subjects with impaired glucose tolerance had significantly elevated titres. Our group has recently analysed ELISA-generated IgG titres to *P. gingivalis* and *A. actinomycetemcomitans* in sera from a cohort of 2,973 subjects, aged 40 years and older, participants of the second phase of the Third National Health and Nutrition Examination Survey (NHANES III, 1991–1994), including 314 diagnosed diabetic subjects (Dye et al. 2005). In this sample, there was no difference in the proportion of subjects with and without diabetes who displayed an elevated titre (i.e., at 90% or higher) to either microorganism (8.35 *versus* 10.1% and 14.4% *versus* 10.5% in diabetic and non-diabetic participants for *A. actinomycetemcomitans* and *P. gingivalis* titre, respectively). In the present study, we additionally used an analysis of infection ratios that simultaneously adjusts for the individual subject's homologous microbial colonization burden, but that analysis similarly failed to establish any difference in responsiveness between type 1 diabetes patients and controls.

As expected, the analysis of serum cytokines and selected markers of cardiovascular disease in a subsample of case–control pairs confirmed that individuals with type 1 diabetes generally display a systemic pro-inflammatory state (Nelson et al. 2005). Although the small sample size did not provide enough power to detect statistical differences in cytokine levels, in agreement with literature findings, a number of mediators suggesting higher risk for cardiovascular disease were found to be elevated in type 1 patients, including soluble E-selectin and VCAM-1 (Clausen et al. 2000, Jude et al. 2002, Fogelstrand et al. 2004) and adiponectin (Imagawa et al. 2002, Frystyk et al. 2005). In contrast, levels of the procoagulant factor PAI-1 were found to be lower in type 1 diabetes patients than controls; however, the literature does not suggest any consistent or independent association of circulating PAI-1 with the presence of vascular disease (McDermott et al. 2003).

Hyperglycaemia and elevated systemic inflammation are thought to play an important role in the pathogenesis of periodontal disease in the diabetic patient (Lalla et al. 2000a). Nevertheless, the present data demonstrate that the extent and severity of periodontal disease is a decisive environmental determinant of the qualitative characteristics of the periodontal infection, and that adjustment for periodontal status results in very similar infection profiles in subjects with and without diabetes. It thus appears that in cross-sectional comparisons between cases with diabetes and disease-free controls, it is the periodontitis phenotype, determined by the extent of bleeding of probing and the depth of the probeable pocket, that largely dictates the composition of the subgingival habitat and the intensity of the serum antibody responses to the periodontal bacterial challenge. Indirectly, our findings are also in agreement with earlier data generated by treatment studies associating periodontal bacterial profiles and metabolic control. For example, in a small-sized longitudinal study of type 1 (insulin-dependent) diabetic patients who underwent intensive insulin therapy that resulted in much improved metabolic control over a 9-month period, Sastrowijoto et al. (1990) reported no improvement in clin-

ical periodontal status and consequently no changes in the subgingival levels of periodontitis-associated bacteria. Similarly, Tervonen et al. (1994) concluded that determinants of diabetic status such as duration, type, and metabolic control had no discernible effect on the prevalence of *A. actinomycetemcomitans, F. nucleatum, E. corrodens, P. gingivalis*, *and P. intermedia*. It must be emphasized, however, that the failure to establish any differences in infection profiles between diabetic patients and disease-free controls in our and other studies is by no means at odds with the concept of diabetes as a major risk factor for periodontitis, but rather suggests that it is the host response that primarily drives the enhanced susceptibility to periodontal disease in diabetic individuals.

Acknowledgements

The work was supported by NIH grant DE 14490. We thank Dr. Robin Goland, Co-Director of the Columbia University Naomi Berrie Diabetes Center, for her support of the study.

References

Borrell, L. N. & Papapanou, P. N. (2005) Analytical epidemiology of periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 132–158.

Campus, G., Salem, A., Uzzau, S., Baldoni, E. & Tonolo, G. (2005) Diabetes and periodontal disease: a case–control study. *Journal of Periodontology* **76**, 418–425.

Center for Disease Control (2005) National Center for Chronic Disease Prevention and Health. http://www.cdc.gov/diabetes/statistics/duration/fig2.htm. Accessed 12 April, 2006.

Ciantar, M., Gilthorpe, M. S., Hurel, S. J., Newman, H. N., Wilson, M. & Spratt, D. A. (2005) Capnocytophaga spp. in periodontitis patients manifesting diabetes mellitus. *Journal of Periodontology* **76**, 194–203.

Clausen, P., Jacobsen, P., Rossing, K., Jensen, J. S., Parving, H. H. & Feldt-Rasmussen, B. (2000) Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. *Diabetic Medicine* **17**, 644–649.

Dye, B. A., Choudhary, K., Shea, S. & Papapanou, P. N. (2005) Serum antibodies to periodontal pathogens and markers of systemic inflammation. *Journal of Clinical Periodontology* **32**, 1189–1199.

Feitosa, A. C., de Uzeda, M. & Novaes Junior, A. B. (1992) Actinobacillus actinomycetemcomitans in Brazilian insulin-dependent individuals with diabetes mellitus. *Brazilian Dental Journal* **3**, 25–31.

Fogelstrand, L., Hulthe, J., Hulten, L. M., Wiklund, O. & Fagerberg, B. (2004) Monocytic expression of CD14 and CD18, circulating adhesion molecules and inflammatory markers in women with diabetes mellitus and impaired glucose tolerance. *Diabetologia* **47**, 1948–1952.

Frystyk, J., Tarnow, L., Hansen, T. K., Parving, H. H. & Flyvbjerg, A. (2005) Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. *Diabetologia* **48**, 1911–1918.

Haffajee, A. D. & Socransky, S. S. (2001) Relationship of cigarette smoking to the subgingival microbiota. *Journal of Clinal Periodontology* **28**, 377–388.

Imagawa, A., Funahashi, T., Nakamura, T., Moriwaki, M., Tanaka, S., Nishizawa, H., Sayama, K., Uno, S., Iwahashi, H., Yamagata, K., Miyagawa, J. & Matsuzawa, Y. (2002) Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. *Diabetes Care* **25**, 1665–1666.

Jude, E. B., Douglas, J. T., Anderson, S. G., Young, M. J. & Boulton, A. J. (2002) Circulating cellular adhesion molecules ICAM-1, VCAM-1, P- and E-selectin in the prediction of cardiovascular disease in diabetes mellitus. *European Journal of Internal Medicine* **13**, 185–189.

Lalla, E., Lamster, I. B., Drury, S., Fu, C. & Schmidt, A. M. (2000a) Hyperglycemia, glycoxidation and receptor for advanced glycation endproducts: potential mechanisms underlying diabetic complications, including diabetes-associated periodontitis. *Periodontology 2000* **23**, 50–62.

Lalla, E., Lamster, I. B., Feit, M., Huang, L., Spessot, A., Qu, W., Kislinger, T., Lu, Y., Stern, D. M. & Schmidt, A. M. (2000b) Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. *Journal of Clinical Investigation* **105**, 1117–1124.

Löe, H. (1993) Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care* **16**, 329–334.

Mashimo, P. A., Yamamoto, Y., Slots, J., Park, B. H. & Genco, R. J. (1983) The periodontal microflora of juvenile diabetics. Culture, immunofluorescence, and serum antibody studies. *Journal of Periodontology* **54**, 420–430.

McDermott, M. M., Green, D., Greenland, P., Liu, K., Criqui, M. H., Chan, C., Guralnik, J. M., Pearce, W. H., Ridker, P. M., Taylor, L., Rifai, N. & Schneider, J. R. (2003) Relation of levels of hemostatic factors and inflammatory markers to the ankle brachial index. *American Journal of Cardiology* **92**, 194–199.

Mealey, B. L. & Moritz, A. J. (2003) Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. *Periodontology 2000* **32**, 59–81.

Nelson, C. L., Karschimkus, C. S., Dragicevic, G., Packham, D. K., Wilson, A. M., O'Neal, D., Becker, G. J., Best, J. D. & Jenkins, A. J. (2005) Systemic and vascular inflammation is elevated in early IgA and type 1 diabetic nephropathies and relates to vascular disease risk factors and renal function. *Nephrology Dialysis Transplantation* **20**, 2420–2426.

Novaes Junior, A. B., Gonzalez Gutierrez, F., Grisi, M. F. & Novaes, A. B. (1997) Periodontal disease progression in type II non-insulin-dependent diabetes mellitus patients (NIDDM). Part II–Microbiological analysis using the BANA test. *Brazilian Dental Journal* **8**, 27–33.

Papapanou, P. N. (1996) Periodontal diseases: epidemiology. *Annals of Periodontology* **1**, 1–36.

Papapanou, P. N., Neiderud, A.-M., Papadimitriou, A., Sandros, J. & Dahlén, G. (2000) ''Checkerboard'' assessments of periodontal microbiota and serum antibody responses: a case–control study. *Journal of Periodontology* **71**, 885–897.

Picolos, D. K., Lerche-Sehm, J., Abron, A., Fine, J. B. & Papapanou, P. N. (2005) Infection patterns in chronic and aggressive periodontitis. *Journal of Clinical Periodontology* **32**, 1055–1061.

Sakellari, D., Socransky, S. S., Dibart, S., Eftimiadi, C. & Taubman, M. A. (1997) Estimation of serum antibody to subgingival species using checkerboard immunoblotting. *Oral Microbiology and Immunology* **12**, 303–310.

Sandberg, G. E., Sundberg, H. E., Fjellstrom, C. A. & Wikblad, K. F. (2000) Type 2 diabetes and oral health: a comparison between diabetic and non-diabetic subjects. *Diabetes Research and Clinical Practice* **50**, 27–34.

Sastrowijoto, S. H., van der Velden, U., van Steenbergen, T. J., Hillemans, P., Hart, A. A., de Graaff, J. & Abraham-Inpijn, L. (1990) Improved metabolic control, clinical periodontal status and subgingival microbiology in insulin-dependent diabetes mellitus. A prospective study. *Journal of Clinical Periodontology* **17**, 233–242.

Sbordone, L., Ramaglia, L., Barone, A., Ciaglia, R. N. & Iacono, V. J. (1998) Periodontal status and subgingival microbiota of insulin-dependent juvenile diabetics: a 3-year longitudinal study. *Journal of Periodontology* **69**, 120–128.

Seppälä, B., Seppälä, M. & Ainamo, J. (1993) A longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. *Journal of Clinical Periodontology* **20**, 161–165.

Socransky, S. S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) ''Checkerboard'' DNA–DNA hybridization. *Biotechniques* **17**, 788–792.

Taylor, G. W., Burt, B. A., Becker, M. P., Genco, R. J. & Shlossman, M. (1998) Glycemic control and alveolar bone loss progression in type 2 diabetes. *Annals of Periodontology* **3**, 30–39.

Tervonen, T. & Karjalainen, K. (1997) Periodontal disease related to diabetic status. A

pilot study of the response to periodontal therapy in type 1 diabetes. *Journal of Clinical Periodontology* **24**, 505–510.

Tervonen, T., Oliver, R. C., Wolff, L. F., Bereuter, J., Anderson, L. & Aeppli, D. M. (1994) Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. *Journal of Clinical Periodontology* **21**, 375–379.

Thomson, W. M., Slade, G. D., Beck, J. D., Elter, J. R., Spencer, A. J. & Chalmers, J. M. (2004) Incidence of periodontal attachment loss over 5 years among older South Australians. *Journal of Clinical Periodontology* **31**, 119–125.

Thorstensson, H., Dahlén, G. & Hugoson, A. (1995) Some suspected periodontopathogens and serum antibody response in adult long-duration insulin-dependent diabetics. *Journal of Clinical Periodontology* **22**, 449–458.

Thorstensson, H. & Hugoson, A. (1993) Periodontal disease experience in adult long-duration insulin-dependent diabetics. *Journal of Clinical Periodontology* **20**, 352–358.

Yuan, K., Chang, C. J., Hsu, P. C., Sun, H. S., Tseng, C. C. & Wang, J. R. (2001) Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. *Journal of Periodontal Research* **36**, 18–24.

Zambon, J. J., Reynolds, H., Fisher, J. G., Shlossman, M., Dunford, R. & Genco, R. J. (1988) Microbiological and immunological studies of adult periodontitis in patients with noninsulin-dependent diabetes mellitus. *Journal of Periodontology* **59**, 23–31.

Address:
Evanthia Lalla
Division of Periodontics
Section of Oral and Diagnostic Sciences
College of Dental Medicine
Columbia University
630 W 168th Street
PH7E-110
New York
NY 10032
USA
E-mail: EL94@columbia.edu

Clinical Relevance

Scientific rationale: Diabetes mellitus is an established risk factor for periodontitis. However, it is unclear whether the subgingival microbial ecology of the established periodontitis lesion in patients with diabetes is different from that prevalent in non-diabetic patients of corresponding extent and severity. Similarly, it is not clear whether diabetes aggravates or suppresses the systemic antibody responses to the periodontal bacterial challenge.

Principal findings: After adjustment for clinical periodontal status, diabetic and non-diabetic patients showed similar subgingival plaque patterns, and comparable serum antibody responses.

Practical implications: The findings of the present study do not support the notion of a diabetes-specific periodontal infection profile.