Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs

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Abstract

Objective

To investigate the presence of oral bacterial DNAs in serum and synovial fluid (SF) of patients with active rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

Methods

Serum and SF samples from 16 RA patients, 14 PsA patients, and 9 osteoarthritis (controls) patients were extracted for oral bacterial DNA. This was used in a checkerboard DNA-DNA-hybridization set up, to identify 40 different bacteria.

Results

Mean number ± standard deviation (SD) of oral bacterial species in sera were 6.2 (3.2) in the RA group ($p = 0.004$) and 5.4 (2.7) in the PsA group ($p = 0.009$) compared to 2.1 (1.7) in the controls. Periodontitis associated species Porphyromonas gingivalis *and* Prevotella nigrescens *were exclusively detected in RA and PsA. Mean number (± SD) of oral bacterial species in SF were 14.0 (6.8) in the RA ($p = 0.001$) and 19.4 (7.1) in the PsA group ($p < 0.001$) compared to 4.0 (1.7) in controls.* P. gingivalis, Tannerella forsythensis *and* Prevotella intermedia *were exclusively identified in RA and PsA SF. Higher means of DNAs were found in RA SF compared to RA serum ($p < 0.001$), and in PsA SF compared to PsA serum ($p < 0.001$). Higher concentrations of bacterial DNAs were found in RA and PsA compared to controls.*

Conclusion

Higher variety and concentrations of oral bacterial DNAs were found in SF compared to serum of RA and PsA patients. These findings indicate that synovial inflammation in RA and PsA may favor trapping of oral bacterial DNAs, suggesting a perpetuating effect of oral pathogens in joint disease.

Key words

Oral infections, bacteria, synovial fluid, rheumatoid arthritis, psoriatic arthritis.

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Introduction

Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are both rheumatic, systemic disorders characterized by synovial hyperplasia and chronic inflammation affecting joints and connective tissues. PsA occurs in up to 20% of psoriatic patients (1, 2) with skin, nail and spine involvement as the most common clinical features besides an often asymmetric joint affection (3, 4). Despite the fact that RA and PsA are genetically and pathologically distinct, both diseases are thought to be immune driven, with a qualitatively similar immunomodulatory cascade and cytokine profile (4). The prevalence of PsA has varied in different studies, however most data have shown a female/male ratio of approximately 1:1 that clearly contrasts with the ratio of 3:1 in RA. RA is also more common compared to PsA with an incidence of 0.5-1% in western populations (5).

Environmental factors have been shown to play a role in the etiology of RA and PsA and it is assumed that the synovial and adjacent soft tissue inflammation may be initiated by a number of microbial factors, including bacterial DNA, heat shock proteins and lipopolysaccharides (6-11) supported by an underlying immunogenic influence. This theory has been strengthened by reports describing bacterial DNAs in both synovial tissue and synovial fluids (SF) from patients with different forms of arthritis (arthritides) (12-15) indicating that bacteria may act as possible participants in early and late phases of RA and PsA. Although numerous efforts have been made to isolate and cultivate infectious agents from joints, the association between arthritides and microorganisms still remains obscure (16-19). Indeed, experimental models have been established with the intention to demonstrate the arthritogenicity of certain microorganisms, but underlying pathogenic mechanisms still need to be investigated in more detail (16, 20, 21).

Sub-clinical infections, not initially located in the joints, might cause chronic joint inflammation (22). Periodontal diseases is considered chronic, often asymptomatic infectious conditions leading to the loss of supporting tissue around the teeth and is associated with a great number of different bacterial species (23). Interestingly, our group has earlier demonstrated an increased specific antibody response to periodontal bacteria in SF of arthritides patients indicating a possible connection between joint inflammation and oral infections (24). Results have also shown a significant difference in concentration and variation of oral bacterial DNAs in SF between arthritides and controls (25).

In the present study the aim was to identify the frequency of different oral bacterial DNA species in periodontal pocket samples, sera and SF of patients with RA, PsA and controls. Furthermore, we hypothesized that differences in oral bacterial DNAs in serum and SF among the patient groups, were associated with systemic disease status rather than oral status.

Patients and methods

Study population

Group 1 consisted of 16 in-hospital patients with RA, all fulfilling the revised criteria according to the American College of Rheumatology (formerly the American Rheumatism Association) (26). Fourteen (87.5%) of the RA patients were women and two (12.5%) men. The mean age was 56.0 years (range 28-75). Group 2 consisted of 14 in-hospital PsA patients. Six (42.9 %) were women and eight (57.1%) men. The mean age was 50.3 years (range 26-82). The control group consisted of nine patients with osteoarthritis (OA). Seven (77.8 %) were women and two (22.2%) men, and the mean age was 70.2 years (range 60-85). The OA patients were obtained from a private rheumatology specialist clinic. Two of the RA patients, one of the PsA patients and two of the controls were current smokers, all smoking less than 10 cigarettes per day. The remaining patients in the study were non-smokers, or had not smoked during the last five years. Thirteen of the RA patients, 13 of the PsA patients and all the controls saw their dentist regularly. The remaining four RA and PsA patients went less than once a year. No patient reported prior periodontal treatment except supra-gingival scaling

done by his or her dentist or dental hygienist.

Additional diagnoses and medical treatment

Two (12.5%) of the RA patients and one PsA patient had secondary Sjögren's syndrome (SS) according to the published criteria for SS (27). None of the patients had current gastrointestinal infections or other infections which needed medical attention, however one RA patient received antibiotics for an ongoing urinary tract infection. Other medical data are given in Table I.

Medical examinations

All patients were medically examined at the Rheumatology Clinic, Haukeland University Hospital, Bergen. Disease activity score (DAS28) was calculated for all patients and controls using erythrocyte sedimentation rate (ESR), tender 28 joint score, swollen 28 joint score and patient overall assessments of disease activity (28, 29). The standard Health Assessment Questionnaire (HAQ) was calculated for each patient and control as well (30). SF samples were obtained during therapeutic arthrocentesis of a large joint and transferred to separate 1 ml Eppendorf tubes and stored at -20ºC until used in checkerboard DNA-DNA-hybridization.

Blood analyses

Peripheral blood was collected from all patients after the medical examination. Blood analyses, obtained from routine laboratory analyses included hemoglobin (Hgb), C-reactive protein (CRP), leukocyte count (LC), platelet count (TC), and immunoglobulin G (IgG) concentration (Table II). The cut-off level for a positive rheumatoid factor (RF) was set at a titer of ≥ 64, and was tested either by the Waaler and/or Latex method. CRP, in mg/liter serum, was recorded for each patient. CRP levels were determined by end-point analysis (Tina Quant method) at the hospital biochemical laboratory, Haukeland University Hospital. Sera were also transferred to separate 1 ml Eppendorf tubes and stored at -20ºC until used in checkerboard DNA-DNA-hybridization analyses.

Table I. Medical data of the patients included in the study.

	RA n = 16	PsA n = 14	Control n = 9
Sex (F/M)	12/2	6/8	7/2
Age (mean)	56.0	50.3	70.2
Duration	10.5 (8.3)	12.8 (8.1)	11.3 (8.6)
DMARDs	11	5	1
TNF blocking agents	6	4	0
Oral steroids	8	2	2
NSAIDs	11	10	4

Duration; Mean duration of disease in years (± SD). DMARDs; Disease modifying anti-rheumatic drugs. TNF blocking agents; Anti-TNF treatment (infliximab or etanercept). NSAIDs; Non steroid anti inflammatory drugs.

Table II. Serological data from the patients included in the study.

	RA n = 16	PsA n = 14	Control n = 9
RF positive (64)	7	0	1
CRP	45.0** (42.6)	22.6* (18.4)	12.3 (7.0)
LC[a]	9.9* (3.9)	7.7 (1.8)	6.9 (1.8)
Hgb	12.5* (1.0)	13.3 (1.0)	13.5 (1.0)
TC[a]	398.1* (135.4)	293.3 (60.1)	276.4 (53.2)
IgG	11.3** (3.2)	12.8** (4.5)	10.1 (1.2)

RF: rheumatoid factor; CRP: Mean C-reactive protein (± SD) in mg/liter serum. [a]Cells x 10^9 per liter blood; Hgb: Hemoglobin in g/dl; TC: Trombocyte counts; IgG: total Immunoglobulin G in g/liter. Differences between the patients groups and controls were calculated by the Mann-Whitney U test. *; $p< 0.05$; and **; $p< 0.01$.

Table III. Clinical data of the patients included in the study.

	RA n = 16	PsA n = 14	Control n = 9
HAQ	2.6** (1.6-3.4)	1.9 (1.0-4.0)	1.5 (1.0-3.8)
DAS28	5.5** (3.1-7.3)	4.8* (1.3-6.1)	3.0 (1.3-4.7)
Di (TMJ)	5.0* (0-12)	6.0* (0-21)	1.0 (0-9)
Teeth	22.4 (9.1)	22.2 (7.6)	20.2 (10.5)
CAL	2.7 (0.7)	2.6 (0.7)	3.3 (1.5)
PPD	2.2 (0.4)	2.3 (0.4)	2.4 (0.5)
Periapical lesions	8 (50.0)	5 (35.7)	4 (44.4)

HAQ: median (range) health assessment questionnaire; DAS28: median (range) disease activity score; Di: median clinical dysfunction index for the masticatory system (range); Teeth: mean number of teeth (± SD); CAL: mean clinical attachment loss in mm (± SD); PPD: mean Periodontal pocket depths in mm (± SD); Periapical lesions: No. of patients (%) with chronic periapical inflammatory conditions (radiology); Differences between the patients groups (RA and PsA) and controls were calculated by the Mann-Whitney U test. *; $p< 0.05$; and **; $p< 0.01$.

Oral examination

Oral examinations of the RA, PsA and OA patients were performed at the Clinic for Oral and Maxillofacial Surgery, Haukeland University Hospital, Bergen. All patients were subjected to a detailed examination of their temporomandibular joints (TMJs). For each patient the Helkimo clinical dysfunction index (Di) for the masticatory system was calculated (31, 32).

The study population underwent a full mouth clinical examination of the oral mucosa (32). The examination included periodontal pocket depth (PPD) and clinical attachment loss (CAL). PPD was defined as the nearest millimeter from the gingival margin to the base of the pocket with a calibrated WHO pocket probe. CAL was calculated for each site by subtracting the distance from the cemento-enamel junction (CEJ) to the free gingival margin (negative values for gingival recession)

from the probing depth (33) and a mean value was established for each group (Table III).

For each patient radiographic full mouth series were recorded. Subsequently, all teeth were examined on the apical, mesial and distal aspects. The patients were then divided into defined categories including mild, moderate or severe periodontitis according to a previous published classification system (34, 35). One of the RA patients and four of the PsA patients had mild periodontitis. The remaining patients and controls were classified as having moderate periodontitis. Chronic inflammation of the periapical region was recorded as present or absent for each tooth of each patient radiograph (Table III).

Sub-gingival plaque sampling procedure

For all patients, the tooth with the deepest pocket was selected for microbial sampling. The sampling was done according to a documented procedure (36) and sent to the microbiological laboratory at the Department of Oral Biology, Dental Faculty, Oslo, for checkerboard DNA-DNA hybridization.

Extraction of bacterial DNA

The whole genomic DNA for checkerboard analysis was extracted from SF, serum and periodontal samples. The DNAs were extracted with the Qiagen DNA extraction kit (Qiagen, Inc., Valencia, CA). One hundred μl of the extracted DNA were used for checkerboard DNA-DNA hybridization. As a control, oral bacterial DNAs were also extracted from 100 μl SF/serum using the Nucleon extraction kit (Amsterdam Life Science, International plc., England).

Checkerboard DNA-DNA-hybridization

A checkerboard method was performed for DNA-DNA-hybridization (25, 36, 37). The sensitivity and the specificity of this test have been shown to be high (37). The same procedure was performed both for gingival samples, sera and SF. Each sample was pipetted in volumes of 0.1 ml and transferred to separate Eppendorf tubes. Hundred microliter of 0.5 M NaOH was added to each sample and the samples were frozen (-20ºC). After storage all samples were placed in a water bath (100ºC) and boiled for 5 min.

The samples were then neutralized using 0.8 ml of 5 M ammonium acetate. The released DNAs from the SF samples in the three groups, together with the pooled DNA standards extracted from 10^5 and 10^6 cells of each of the 40 bacterial test species (Table IV), were placed into the lanes of a Minislot (Immunetics, Cambridge, MA). The samples were then deposited on a nylon membrane (Boehringer Mannheim, Mannheim, Germany). The membranes were fixed by baking at 68ºC for 30 min followed by exposure to ultraviolet light for 30 seconds.

The membrane with the fixed DNA was placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90ºC to the channels of the device. Each channel was used as a hybridization chamber for separate DNA probes. The membranes were prehybridized at 42ºC for 1 hour in 50% formamide, 5 x SSC (1x SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 10% casein (Sigma, St Louis, MO), 5 x Denhardt's reagent, 25 mM sodium phosphate (pH 6.5) and 10 mg/ml yeast RNA

Table IV. Bacterial species used for preparation of standards and probes for checkerboard DNA-DNA hybridization.

Species	Source* and ref. no.
Actinomyces naeslundii I	ATCC 12104^T
Streptococcus constellatus	NCDO 2226^T
Eubacterium nodatum	ATCC 33099^T
Porphyromonas gingivalis	ATCC 33277^T
Actinobacillus actinomycetemcomitans	FDC Y4 (ATCC 43718)
Fusobacterium nucleatum subspecies vincentii	ATCC 49256^T
Campylobacter rectus	FDC 371 (ATCC 33238^T)
Treponema socranskii subspecies socranskii	VPI DR56BR1116^T
Eubacterium saburreum	ATCC 33271^T
Peptostreptococcus micros	ATCC 33270^T
Veillonella parvula	ATCC 10790^T
Actinomyces viscosus	ATCC 27044
Streptococcus anginosus	NCTC 10713^T
Streptococcus sanguis	ATCC 10556^T
Actinomyces gerencseriae	ATCC 23860^T
Streptococcus oralis	ATCC 35037^T
Capnocytophaga ochracea	ATCC 33596
Actinomyces israelii	ATCC 12102^T
Streptococcus intermedius	ATCC 27335^T
Treponema denticola	ATCC 33520
Prevotella nigrescens	ATCC 33563^T
Actinomyces odontolyticus I	ATCC 17929^T
Fusobacterium nucleatum subspecies polymorphum	VPI 5382^T
Campylobacter showae	ATCC 51146^T
Fusobacterium periodonticum	ATCC 33693^T
Neisseria mucosa	ATCC 19696^T
Fusobacterium nucleatum subspecies nucleatum	ATCC 25586^T
Capnocytophaga gingivalis	ATCC 33624^T
Streptococcus gordonii	ATCC 10558^T
Tannerella forsythensis	FDC 338 (ATCC 43037^T)
Selenomonas noxia	ATCC 43541^T
Propionibacterium acnes	ATCC 6919^T
Prevotella melaninogenica	ATCC 25845^T
Porphyromonas endodontalis	ATCC 35406^T
Eikenella corrodens	ATCC 23834^T
Gemella morbillorum	VPI 9802 (ATCC 27824^T)
Capnocytophaga sputigena	ATCC 33612^T
Leptotrichia buccalis	ATCC 14201^T
Campylobacter gracilis	FDC 1084 (ATCC 3323
Prevotella intermedia	ATCC 25611^T

*ATCC: American Type Culture Collection; FDC: Forsyth Dental Institute; NCDO: National Collection of Dairy Organisms; NCTC: National Collection of Type Cultures; VPI: Virginia Polytechnic Institute and State University; ^{T}: type strain.

(Boehringer Mannheim). Digoxygenin-labeled, whole genomic probes (Table 4) and hybridization buffer containing 45% formamide, 5 x SSC, 1x Denhardt's reagent, 20 mM sodium phosphate (pH 6.5), and 10 mg/ml yeast RNA, 20 ng/ml labeled probe, 10% dextran sulfate (Sigma) and 10% casein were placed in individual lanes of the Miniblotter. The whole apparatus was covered with Saran wrap and transferred to a sealed plastic bag. The membranes were hybridized overnight with gentle shaking at 42ºC.

The membranes were then washed in a plastic dish to remove loosely bound probe. To detect hybrids, the membranes were blocked with 10% casein in maleate buffer (100 mM maleic acid, 150 mM NaCl, pH 7.5) and then incubated with antidigi oxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim), diluted 1:20000 in maleic buffer. Signals were detected chromogenically using NTB/BCIP tablets (Boehringer Mannheim) overnight.

Quantification of oral bacterial DNA

Cell numbers were quantified using a densitometer by comparing signal intensities of unknowns to those of standard suspensions on the same membrane (38). The concentration of each species was recorded as cells per 100 µl serum or SF. The concentration categories included 5: $>10^6$, 4: $=10^6$, 3: $>10^5 < 10^6$, 2: $= 10^5$ and 1: $<10^5$ cells pr 100 µl.

Statistical analysis

Statistical analysis of the differences between the patient groups and the controls was carried out using the Mann-Whitney U test. Associations between CRP, RF and concentration of oral bacterial DNA were analyzed by use of Spearmans rho correlation coefficient. The level of significance in both tests was set at p 0.05. The statistical analysis was performed using SPSS Release 12.0.0 software (SPSS Inc., Chicago, IL).

Results

Medical and blood data in the patient groups

A significantly higher median DAS28 and HAQ score was found among the RA and the PsA patients compared to the controls (Table III). Median TMJ involvement was significantly increased in both patient groups compared to controls according to the index system. The mean serum CRP, LC, HgB TC, IgG and number of RF positive patients is given in Table II. No significant associations were seen between serological markers, DAS28 and concentrations or assortments of oral bacterial DNAs in serum and SF.

Oral bacterial DNA in RA and PsA serum

Oral bacterial DNAs were identified in 14 out of 14 RA serum-samples, 13 out of 13 PsA serum samples and seven out of nine control serum samples (blood samples were missing from one PsA and two RA patients). The mean (± standard deviation (SD)) number of species was 6.2 (3.2) in the RA group and 2.1 (1.7) in the control group (p = 0.004). A higher number of species was found in the PsA group with 5.4 (2.7) compared to the controls (p = 0.009). Differences were most notable for *Actinomyces naeslundii, Eubacterium nodatum, Porphyromonas gingivalis* and *Prevotella nigrescens*. These species were exclusively identified in RA- and PsA sera (Fig. 1).

For nine (64.3%) of the RA and four (33.3%) of the PsA patients, bacterial DNAs were identified in high concentrations (categories 5 and 4, ≥ 10^6 cells pr 100 µl serum). DNAs were found in low concentrations (categories 2 and 1) in the control group. Minor differences were seen both in assortment and concentration of bacterial DNAs sera between RA and PsA patients. The six most frequently detected species in RA, PsA and OA sera were *Treponema denticola, Actinomyces viscosus, P. nigrescens, Capnocytophaga sputigena, Fusobacterium periodonticum* and *Neisseria mucosa* (Fig. 1).

Oral bacterial DNA in RA and PsA synovial fluids

Oral bacterial DNAs were detected in 15 out of 16 RA SF-samples, 14 out of 14 PsA patients and nine out of nine OA patients. The mean (SD) number of species was 14.0 (6.8) in the RA group and 4.0 (1.7) in the control group (p = 0.001). A higher number of species was also found in the PsA group with 19.4 (7.1) as compared to 4.0 (1.7) in the controls (p < 0.001). Differences were most notable for *P. gingivalis, Eubacterium saburreum, Peptostreptococcus micros, A. israelii, Selenomonas noxia, Propionibacterium acnes, Campylobacter showae,*

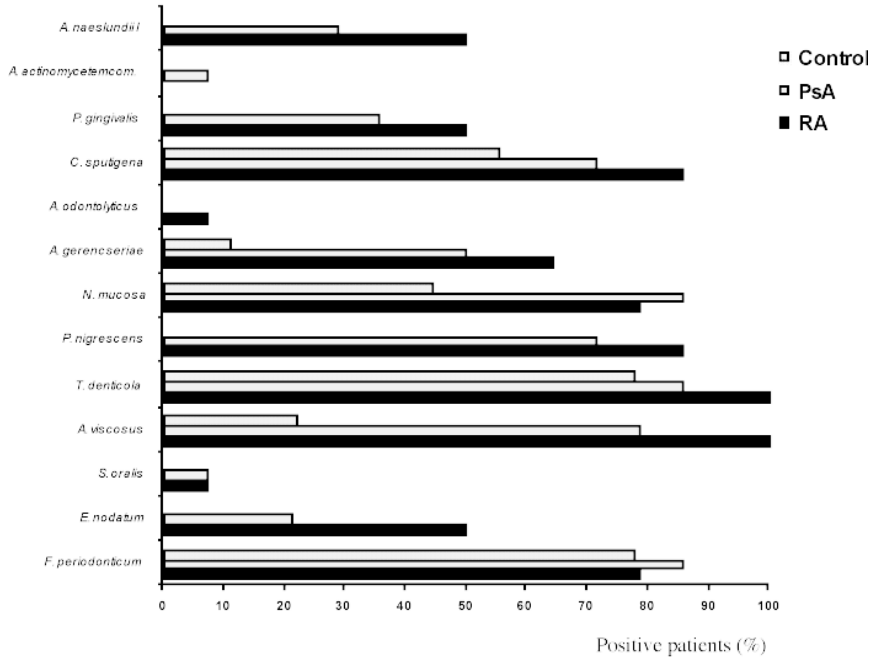

Fig. 1. Oral bacterial DNAs in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and control (osteoarthritis) serum.

Tannerella forsythensis, C. sputigena, Leptotrichia buccalis and *Prevotella intermedia*. These species were exclusively identified in RA SF and PsA SF (Fig 2). For 14 (87.5%) of the RA patients and 13 (92.9%) of the PsA patients, bacterial DNAs were identified in the highest concentration (category 5, > 10^6 cells per 100 µl). DNAs were found in high concentrations for only five (55.6 %) of the controls. Differences were also seen in assortment of bacterial DNAs between RA and PsA patients. Of the 31 bacterial DNAs identified in SF for all groups, the percentage of positive PsA patients was higher for 26 species (Fig 2). Minor differences were seen in concentrations of DNAs between RA and PsA. The six most frequently detected species in general were *T. denticola, A. viscosus, P. nigrescens, Actinomyces gerencseriae, Actinomyces odontolyticus* and *N. mucosa* (Fig. 2).

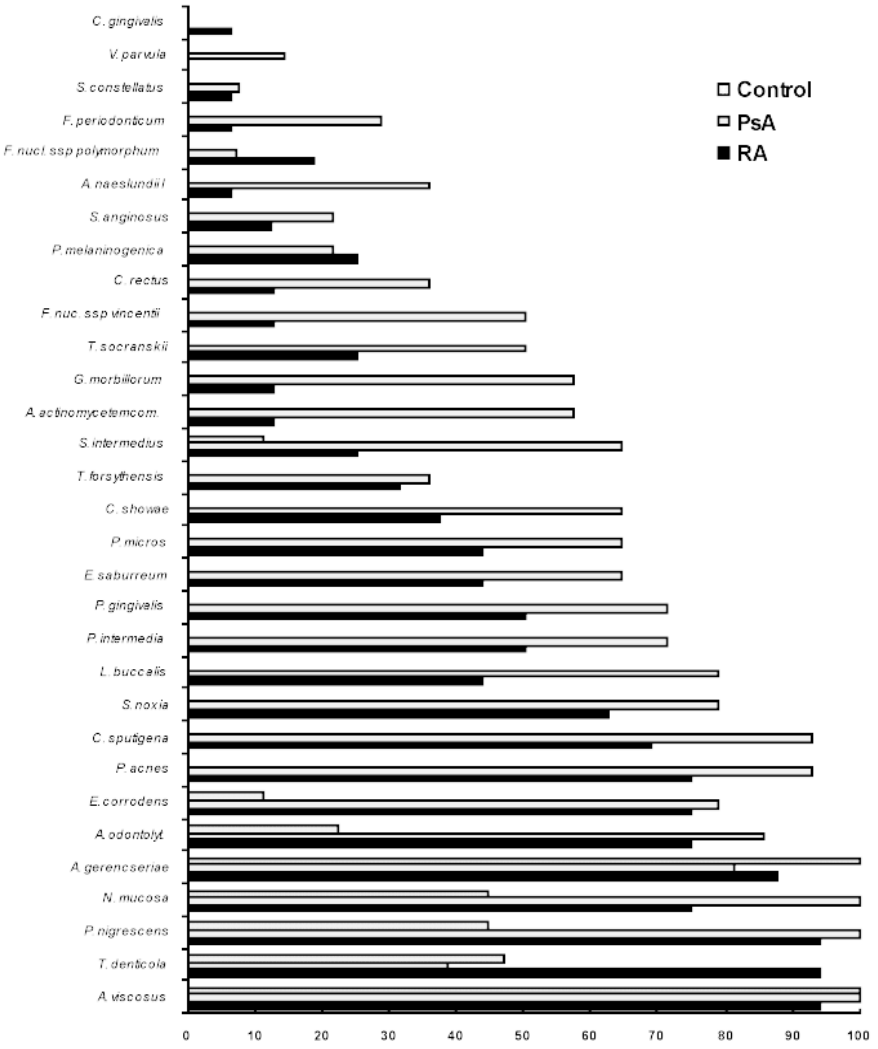

Fig. 2. Oral bacterial DNAs in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and control (osteoarthritis) synovial fluids.

Comparison between oral bacteria in serum and synovial fluids

Significantly higher mean number of DNAs was found in RA SF (14.0) compared to RA serum (6.2), ($p < 0.001$), and in PsA SF (19.4) compared to PsA serum (5.4), ($p < 0.001$). In the OA group no significant difference was seen between the mean numbers of oral bacterial DNAs in SF compared to serum.

Oral bacterial DNA in RA and PsA periodontal pockets

The median depth of the periodontal pockets that was used for sub-gingival sampling was 5.0 mm (range 2.0-8.0) for the RA patients, 6.0 mm (range 2.0-10.0) for the PsA patients and 4.0 (3.0-12.0) for the OA patients. Oral bacterial DNAs were identified in all three patient groups. Five different species were not identified in any patient of the groups including *L. buccalis, Capnocytophaga ochracea, Streptococcus oralis, Veillonella parvula* and *Campylobacter gracilis*. No significant differences in concentrations and assortments of oral bacterial DNAs in PP were seen between the groups. The six most frequently registered species included *Streptococcus intermedius, P. gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Peptostreptococcus micros* and *T. denticola*.

Comparison of the two extraction kits

The two extraction methods (the Qiagen DNA extraction kit and the Nucleon extraction kit) yielded essentially the same concentration of oral bacterial DNAs. (Tables and figures are based on results from only the Qiagen DNA extraction kit).

Other oral infections

Chronic inflammation in the periapical region was detected in eight (50.0%) of the RA patients, five (35.7%) of the PsA patients and in four (44.4%) of the controls (Table III). 1-3 lesions were found in each patient with chronic inflammation in the periapical region. No differences were seen in the frequency of oral mucosal lesions between the RA, PsA and the control groups.

Discussion

The present study demonstrated that chromosomal DNA from a variety of oral bacterial species is present in serum and synovial fluids from patients with various forms of arthritides. Furthermore, both the number of species and concentrations were found to be significantly increased in RA and PsA serum and SF as compared to controls. Although bacterial constituents might be associated with a chronic synovial inflammation (39), proof of a microbial triggering factor is however still not proven (6, 19). Since RA is not gender, age or race specific, it seems possible that an ubiquitous microbial organism,

possibly part of the normal flora, could trigger RA in an appropriate mix of host factors, including genetic predisposition (6, 19). On the other hand, it may be possible that distant infections, such as gastrointestinal or oral infections might cause joint inflammation and a subsequent outbreak of RA or PsA. Periodontitis is a cyclic disease characterized by periods of active tissue breakdown, no breakdown at all and even by tissue repair and seeding of bacterial products from dental plaque to the gingival areas occurs constantly during these conditions (23).

Our results show that translocated oral bacteria/bacterial constituents are not limited to the blood stream, but also occur in joints. The transport of bacterial DNAs from the mouth to joints may occur as free DNA, through whole bacterial cells, or by intracellular capture of immune cells. This study does not however, show whether oral bacterial DNA is found as viable organisms, naked DNA or as cell lysates of SF lymphocytes. Yet, leucocytes in RA synovial fluid contain considerable phagocytated material including IgG, IgM, RF, fibrin, antinuclear factors, immune complexes and DNA particles (40). It is therefore possible that chromosomal DNAs from oral bacteria are found intracellular in SF lymphocytes originating from the oral cavity. Furthermore, studies have also indicated that apparently macrophages containing for instance viral DNA (41) and bacterial DNA may home in a non-specific way to the inflamed compartments, where they subsequently enhance inflammation. Thus, this may indicate that bacterial translocation is not necessary restricted to coral bacteria or bacteria in general.

Bacterial chromosomal DNA and CpG motifs have received special attention in the etiology of inflammatory diseases due to their strong immunostimulatory effects (42-45). After stimulation, the innate immune system is activated involving toll like receptors (TLR) (46). Interestingly, this immune activation has been shown to initiate a synovial inflammation in mice (7) with the subsequent secretion of cytokines, illustrating the importance of both TLRs and CpG motifs in the immunopathogenesis of RA and other arthritides.

There are vast numbers of microorganisms colonizing the human body. Notably, oral infectious organisms (Table IV) may cause long-lasting chronic inflammations in the tooth-supporting tissues which sometimes even exhibit ulcers. This implies that oral bacteria have easy access to the heavily vascular connective tissue surrounding the teeth. In this study we have shown that there is a continuous release of oral bacterial constituents to the blood stream from teeth with periodontal disease. Some bacteria, including *T. forsythensis, P. gingivalis* and *Prevotella* species are assumed to be important pathogens in periodontal disease, endodontic infections and jaw osteomyelitis and were exclusively detected in RA and PsA SF indicating a pro-inflammatory potential of these particular species on the synovium. Interestingly, evidence for higher specific antibody levels against *T. forsythensis* and *P. intermedia* in RA SF and other arthritides as compared to controls, has been presented (24). Although antibody analyses were not included in this study, previous publications have found oral and non-oral pathogens such as *T. forsythensis, P. intermedia,* and *enterobacteria* of particular interest in the pathogenesis of RA and other arthritides (24, 25, 47).

The reason for a lower variety of bacterial species in blood as compared to SF may be explained by the advantages of renal filtration and clearance of bacterial products leading to lower concentrations and variations of bacterial DNAs than seen in SF. Higher joint vascularity also increase the continuous seeding of bacterial products to the lumen. Indeed, this may act like a positive feedback maintaining the disease and was particularly strong in PsA (Fig. 2). This is further strengthened by the data showing minor absorption of DNAs from OA serum to OA SF compared to RA and PsA.

It could be asked, however, whether the higher mean age and relatively small number of OA patients compared to the RA and PsA groups, influenced the results in the present study. To our knowledge, no prior studies have findings suggesting a correlation between age and uptake of bacterial DNAs either in blood or synovial fluids. Furthermore, it should be questioned and investigated through experimental studies to which extent age favors trapping of bacterial DNAs in RA and other arthritides. Hypothetically, older subjects are more susceptible to infections due to an impaired immune system than younger subjects. In this study the mean age was higher in the RA and PsA groups compared to the controls. Consequently, this suggestion may strengthen, and not weaken, the results in this study. Furthermore, medical and oral parameters were comparable in all groups suggesting that the groups were comparable despite the age differences. The numbers of patients were relatively small in the present study and could, to some extent be viewed as a pilot study. More studies including a higher number of patients should be initiated in order to investigate the possible role of bacterial DNAs in RA and PsA.

In conclusion, our findings demonstrate a highly variable concentration of oral bacterial DNA species in serum and synovial fluids of patients with RA and PsA. Furthermore, the mean number of oral bacterial species was found to be significantly increased in serum and SF of both RA and PsA as compared to controls. A higher variety and concentration of DNAs were also found in SF of RA and PsA patients compared to serum, indicating that synovial inflammation in RA and PsA may favor a capture of oral bacterial DNAs. In light of this study it might be assumed that oral bacterial DNAs originating from gingival or dental tissue, as well as bacterial products and cell membrane components, might be important factors initiating and perpetuating joint inflammation in RA and PsA.

Ethics approval

Written consent was obtained from all patients. Study approval was given by the Regional Committee for Medical Research Ethics, Faculty of Medicine, University of Bergen (REK III, no 152.1). Study procedures were in accordance with the standards of the responsible local committee or with the Helsinki Declaration of 1975/83.

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