Published in final edited form as:
J Clin Rheumatol. 2012 April ; 18(3): 117–121. doi:10.1097/RHU.0b013e3182500c95.

Identification of oral bacterial DNA in synovial fluid of arthritis patients with native and failed prosthetic joints

Stéphanie Témoin, PhD[*], **Alia Chakaki, DDS, MSD**[*], **Ali Askari, MD**[†], **Ahmed El-Halaby, DDS, MSD**[*], **Steven Fitzgerald, MD**[‡,¶], **Randall E. Marcus, MD**[‡,¶], **Yiping W. Han, PhD**[*,§,‖], and **Nabil F. Bissada, DDS, MSD**[*]

[*]Department of Periodontics, School of Dental Medicine, Case Western Reserve University, Cleveland, Ohio

[†]Department of Rheumatology, Case Western Reserve University, Cleveland, Ohio

[‡]Department of Orthopaedics, Case Western Reserve University, Cleveland, Ohio

[§]Department of Pathology, Case Western Reserve University, Cleveland, Ohio

[‖]Department of Reproductive Biology, School of Medicine, Case Western Reserve University, Cleveland, Ohio

[¶]Department of Orthopedic Surgery, University Hospitals Case Medical Center

Abstract

Objective—We examined the presence of bacterial DNA in synovial fluids of native or aseptically failed prosthetic joints from patients having periodontal disease and arthritis to determine if there is bacterial spread from the oral cavity to the joints.

Methods—A total of 36 subjects were enrolled in the study. Among these, 11 were diagnosed with rheumatoid arthritis (RA), and 25 with osteoarthritis (OA). Eight patients with OA and are with RA had failed prostheses. Synovial fluid was aspirated from the affected hip or knee joint. Pooled subgingival plaque samples were collected followed by clinical periodontal examination. Bacterial DNA was extracted from the collected synovial fluid and dental plaque samples followed by polymerase chain reactions (PCR) and DNA sequence analysis of the 16S-23S rRNA genes.

Results—Of the 36 subjects, bacterial DNA was detected in the synovial fluid samples from five patients (13.9%), two with rheumatoid arthritis (one native and one failed prosthetic joints) and three with osteoarthritis (one native and two failed prosthetic joints). Of these five patients, two were diagnosed with periodontitis and had identical bacterial clones (*Fusobacterium nucleatum* and *Serratia proteamaculans*, respectively) detected in both the synovial fluid and dental plaque samples.

Conclusions—The present findings of this bacterial DNA in synovial fluid suggest the possibility of infection translocating from the periodontal tissue to the synovium. We suggest that patients with arthritis or failed prosthetic joints be examined for the presence of periodontal diseases and that be treated accordingly.

Keywords

Oral bacteria; Synovial fluid; Rheumatoid arthritis; Osteoarthritis

[*]Corresponding author: Nabil Bissada, DDS, MSD. Mailing address: Department of Periodontics, School of Dental Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4905. Phone: (216) 368-6757. Fax: (216) 368-3204. nabil.bissada@case.edu.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to destruction of the joint architecture and impaired function. DNA from a wide variety of microorganisms (e.g. *Chlamydia*, *Camphylobacter*, *Salmonella*, *Shigella*, and *Yersinia*) has been detected in the synovium associated with various forms of arthritis (1-5).

Periodontal disease, affecting up to 90% of the world population (6), is an inflammatory infection, that destroys the supporting tissues around the teeth. It is classified into two stages, gingivitis and periodontitis. Gingivitis is a mild and reversible form, characterized by inflammatory responses to the bacterial colonization of the tooth surface adjacent to the gingiva. Periodontitis, affecting 15-20% of all adults, is characterized by the irreversible destruction of periodontal ligament fibers and alveolar bone, and is associated with a number of different Gram-negative bacterial species.

In a healthy periodontium, bacteria have no access to the gingival tissue due to the intact epithelial barrier and the high rate of epithelial turnover. However, in the diseased state, bacteria may penetrate through the relatively permeable pocket epithelium to reach the underlying gingival connective tissue (7, 8).

Results from numerous clinical studies point towards a potential association between RA and periodontal disease with several oral bacteria such as *Porphyromonas gingivalis or Prevoltella intermedia* detected in the synovial samples of the patients (4, 9-14). Periodontitis may be a factor in the initiation and maintenance of the autoimmune inflammatory responses that occur in RA (15, 16). Patients with RA may also show an increased risk of developing periodontitis and tooth loss through various mechanisms (16). One hypothesis is that bacteria from a distant site such as the oral cavity can spread to the joints in RA or OA. To test this hypothesis, we investigated the presence of bacterial DNA in the periodontal tissues and synovial fluid from patients with periodontal disease and arthritis.

Material and Methods

This study was conducted in the Department of Periodontics at Case Western Reserve University School of Dental Medicine in conjunction with the Division of Rheumatology and the Department of Orthopedic Surgery at the University Hospitals Case Medical Center/ Case Western Reserve University School of Medicine. The protocol was approved by the University Hospitals Case Medical Center Institutional Review Board (#05-05-17 and #10-08-18).

Patient Selection

Thirty-six subjects diagnosed with either rheumatoid arthritis (n=11) or osteoarthritis (n=25) were enrolled for the study. The diagnoses of RA and OA were made at the Division of Rheumatology at the University Hospitals of Cleveland (UH) based on the criteria established by the American College of Rheumatology or at the Department of Orthopedics at UH based on physical examination and radiographic findings. Among these patients, nine were undergoing revision arthroplasty for aseptic loosening or prosthetic wear. These nine patients were clinically determined to be without infection based on their normal values of complete blood counts, sedimentation rates, and serum C-reactive protein levels. All patients included in the study signed a written informed consent form. Patients being treated with antibiotics for any condition and edentulous subjects were excluded from the study.

Collection of samples and periodontal examination

Synovial fluid was collected from all patients in the examination room or the operating room following skin preparation with providine iodine solution. The synovial fluid was aspirated utilizing sterile technique and transferred to sterile micro-centrifuge tubes. As a control for possible contamination 95 during sample collection, sterile saline solution was aspirated out of a surgical basin and transferred to sterile micro-centrifuge tubes in the same manner following synovial fluid aspiration. Tubes were then either stored at −80°C until PCR analysis or centrifuged for 3 min at 10,000 × g and the pellet was stored. Pooled subgingival plaque samples were collected from all teeth using a sterile 7/8 Gracey curette from each patient. The plaque samples were collected in a sterile 1.5 ml microcentrifuge tube containing 1 ml sterile PBS and stored at −80°C.

Periodontal evaluation was performed by measuring the following parameters: probing pocket depth and clinical attachment level in mm, percentage sites with bleeding on probing, and detection of gingival inflammation using the Loe and Silness index (17).

DNA extraction

Frozen synovial fluid and dental plaque samples were allowed to defrost on ice for 30 minutes. DNA was extracted as previously described (18, 19). DNA extraction was also performed on PBS and on saline solutions taken in the operating room during synovial fluid sampling as negative controls.

PCR amplification

PCR amplification of the 16S-23S rRNA region from synovial fluid samples was performed as previously described (18, 19). The 16S-23S rRNA region (for patients 1-17) or the 16S rRNA gene (for patients 18-36) was amplified using universal primer pairs 785F and 422R or A17F and 1512R, respectively (18, 19). For the amplifications made with primer pair 785F and 422R, a second nested PCR was performed with universal primers 785F and L189R (18). For patients 8 and 32, PCR analyses of the plaque samples were performed using universal primers and *Fusobacteria*- or *Serratia*- specific primers, respectively.

DNA cloning and sequencing

PCR products were cloned into pCR2.1 vector as previously described Han, 2006 #37;Han, 2009 #13}. Plasmid DNA with correct inserts were sent for DNA sequence analysis at the Molecular Biotechnology Core (Lerner Research Institute, Cleveland, OH) or at the Core genomic facility (Case Western Reserve University, Cleveland, OH) using universal primers M13 forward and reverse.

DNA sequence analysis

Data from the sequencing was analyzed using Vector NTI software (Invitrogen, Carlsbad, CA). Bacterial sequences were BLASTed against Genbank or HOMD (Human Oral Microbiome Database) to identify the bacteria. The bacterial genus identification was accepted if the sequence identity was ≥ 93% and the species identification was accepted if the sequence identity was ≥ 97%, as previously described (19).

Results

Synovial fluid samples were collected from 36 patients, nine males and 27 females. The patients' ages ranged from 45 to 84 years with a mean of 61.6. Among these, 11 patients were diagnosed with rheumatoid arthritis and 25 with osteoarthritis. One patient with RA and eight with OA were undergoing revision surgery for aseptic prosthetic failure (Table 1).

Using PCR analysis, bacterial DNA was detected in the synovial fluids of two patients with RA (patient 8 and 32) (one with a failed prosthesis), and three patients with OA (two with a failed prosthesis) (patients 19, 20, and 36) (Table 1). Among these five individuals, patients No. 8 and 20 had mild periodontitis; patients No. 32 and 36 had moderate periodontitis; while the periodontal status for patient 19 was unknown.

Bacterial DNA detected in the synovial fluid was further analyzed by cloning and DNA sequencing. A total of 5-15 independent clones were analyzed for each patient with positive PCR results. For patients 8, 19, 20, and 36, multiple species were detected in their synovial fluid samples, including the human pathogen Acinetobacter junii, Serratia proteamaculans, Streptococcus agalactiae, Leptotrichia wadei, Massilia timonae and *Stenotrophomonas maltophilia*. For patient 32, only one species, *Fusobacterium nucleatum*, was detected (Table 2). The presence of synovial bacteria in the dental plaques was selectively analyzed on the two patients with moderate periodontitis. The subgingival plaque sample from patient 32 was analyzed using *Fusobacteria*-specific primers and the one from patient 8 was analyzed using *Serratia*-specific primers.

The presence of synovial bacteria DNA in the dental plaques was selectively analyzed. Since only one bacterial species was identified in patient 32, this patient was selected for further analysis. The subgingival plaque sample from patient 32 was analyzed using *Fusobacteria*-specific primers, followed by clonal analysis. The results were analyzed using the Human Oral Microbiome Database (HOMD). As shown in Table 3, the clone identified in the synovial fluid of patient 32 belonged to *F. nucleatum subspecies (sbsp) polymorphum* Oral Taxon 202. Two clones from the subgingival plaque sample shared 100% identity with that identified from the synovial fluid (Table 3). For patient 8, one clone from the subgingival plaque sample, *Serratia proteamaculans* clone SF8, shared 100% identity with that identified from the synovial fluid (data not shown).

Discussion

The aim of this investigation was to analyze the presence of bacterial DNA in synovial fluid samples in both native and failed prosthetic joints of patients with arthritis. Our hypothesis was that bacterial translocation can occur from the oral cavity to the synovial joint. Synovial fluid samples from 36 patients were analyzed. Bacterial DNA was detected in five out of the 36 patients (13.9%), including two with RA and three with OA. Among these five subjects, three patients had native and two had prosthetic joints.

A diverse group of bacterial species were detected in these samples. Interestingly, *F. nucleatum*, a gram-negative anaerobe ubiquitous to the oral cavity, was detected in four of these five patients (8, 19, 20, and 32). Previous studies in animals and humans have demonstrated that *F. nucleatum* can translocate hematogenously from the mother's oral cavity to her pregnant uterus, causing preterm birth and stillbirth (19-22). Our results suggest that this organism can also translocate from the mouth to the synovial cavity. We examined the source of *F. nucleatum* in the synovial fluid of patient No. 32, who had RA and a prosthetic joint. Identical clones were detected in her plaque sample. Similarly, the *Serratia proteamaculans* clone detected in the synovial fluid of patient 8 with native joint and RA was also detected in her dental plaque.

The possible presence of oral bacterial DNA in the synovial fluid of patients with rheumatoid arthritis has been suggested previously (4, 23). The current investigation differs from the previous studies in at least two aspects. First, the universal primers, rather than species-specific primers, were used in our study allowing the detection of a wider variety of

microorganisms (18, 19). Secondly, the presence of the same bacteria in the oral samples of the same patients was examined by DNA sequence comparison.

Using this approach, we identified the presence of synovial bacteria DNA in the plaque samples to the clonal level, rather than to the species level, thus providing unambiguous evidence of oral-joint spread. To the best of our knowledge, these data are the first genetically proven evidence that oral bacteria can migrate to the synovium in humans. It is also the first study that shows that the same bacterial DNA at the clonal level is detected in both synovial fluid and plaque sample.

There are limitations to our study. There was no control group of synovial fluid samples collected from individuals without arthritis, e.g. those undergoing orthopedic replacement. There were no routine synovial fluid analyses, cultures or synovial biopsies.

Clinical studies of RA and periodontitis have provided evidence for association between the two disorders. Patients with longstanding active RA have a substantially increased frequency of periodontal disease (15, 24). Conversely, patients with periodontal disease have a higher prevalence of RA (13, 25, 26). Furthermore, periodontitis is not only more common but also more severe in patients with RA compared to patients with OA (11). In the present study, oral bacteria DNA was detected in the synovial fluids of patients with RA and OA, as well as in those with failed prostheses. The oral-synovium translocation could be due to the periodontal diseases the patients had. In the presence of periodontal disease, the quantity of oral bacteria increases dramatically. This, in combination with an inflamed gum, increases the chance of oral bacteria entering the circulation, leading to systemic dissemination, as in the case of oral-utero translocation (27, 28).

In summary, this study provides evidence of the presence of bacterial DNA in the synovial fluid and that identical bacterial DNA was detected in the synovial fluid and dental plaque of patients with arthritis and periodontitis. Our study suggests that bacteria from infections at other body sites may lead to subclinical infection manifested in the native or failed prosthesis joint that is not detected otherwise by the current available diagnostic markers for infection. Based on our present and previous studies (10, 14), we suggest that patients with arthritis or failed prosthetic joints be examined for the presence of periodontal diseases and be treated accordingly.

Acknowledgments

We thank Drs. Akihiko Ikegami, Leena Palomo and Valerie Schmedlen for their assistance in the study and the preparation of this manuscript.

This study was supported by the Department of Periodontics at Case Western Reserve University and by NIH grants RO1 DE14924, KO2 DE16102, and R21 DE17165 to Y.W.H.

REFERENCES

1. Beutler AM, Hudson AP, Whittum-Hudson JA, et al. Chlamydia trachomatis Can Persist in Joint Tissue After Antibiotic Treatment in Chronic Reiter's Syndrome / Reactive Arthritis. J Clin Rheumatol. 1997; 3:125–130. [PubMed: 19078167]
2. Gérard HC, Wang Z, Wang GF, et al. Chromosomal DNA from a variety of bacterial species is present in synovial tissue from patients with various forms of arthritis. Arthritis Rheum. 2001; 144:1689–1697. [PubMed: 11465721]
3. Kuipers JG, Sibilia J, Bas S, et al. Reactive and undifferentiated arthritis in North Africa: use of PCR for detection of Chlamydia trachomatis. Clin Rheumatol. 2009; 28(1):11–6. Epub 2008 Aug 8. [PubMed: 18688674]

4. Martinez-Martinez RE, Abud-Mendoza C, Patino-Marin N, et al. Detection of periodontal bacterial DNA in serum and synovial fluid in refractory rheumatoid arthritis patients. J Clin Periodontol. 2009; 36:1004–1010. [PubMed: 19929953]
5. Schumacher HR Jr, Arayssi T, Crane M, et al. Chlamydia trachomatis nucleic acids can be found in the synovium of some asymptomatic subjects. Arthritis Rheum. 1999; 42:1281–1284. [PubMed: 10366123]
6. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005; 366:1809–1820. [PubMed: 16298220]
7. Lamont RJ, Chan A, Belton CM, et al. Porphyromonas gingivalis invasion of gingival epithelial cells. Infect Immun. 1995; 63:3878–3885. [PubMed: 7558295]
8. Han YW, Shi W, Huang GT, et al. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun. 2000; 68:3140–3146. [PubMed: 10816455]
9. Abou-Raya A, Abou-Raya S, Abu-Elkheir H. Periodontal disease and rheumatoid arthritis: is there a link? Scand J Rheumatol. 2005; 34:408–410. [PubMed: 16234192]
10. Al-Katma MK, Bissada NF, Bordeaux JM, Sue J, Askari AD. Control of periodontal infection reduces the severity of active rheumatoid arthritis. J Clin Rheumatol. 2007; 13:134–137. [PubMed: 17551378]
11. Dissick A, Redman RS, Jones M, et al. Association of periodontitis with rheumatoid arthritis: a pilot study. J Periodontol. 2010; 81:223–230. [PubMed: 20151800]
12. Loyola-Rodriguez JP, Martinez-Martinez RE, Abud-Mendoza C. Rheumatoid arthritis and the role of oral bacteria. J Oral Microbiol. Dec 21.2010 :2. doi: 10.3402/jom.v2i0.5784.
13. Mercado F, Marshall RI, Klestov AC, et al. Is there a relationship between rheumatoid arthritis and periodontal disease? J Clin Periodontol. 2000; 27:267–272. [PubMed: 10783841]
14. Ortiz P, Bissada NF, Palomo L, Han YW, Al-Zahrani MS, Panneerselvam A, Askari A. Periodontal therapy reduces the severity of active rheumatoid arthritis in patients treated with or without tumor necrosis factor inhibitors. J Periodontol. 2009; 80:535–540. [PubMed: 19335072]
15. Bartold PM, Marshall RI, Haynes DR. Periodontitis and rheumatoid arthritis: a review. J Periodontol. 2005; 76:2066–2074. [PubMed: 16277578]
16. de Pablo P, Chapple IL, Buckley CD, et al. Periodontitis in systemic rheumatic diseases. Nat Rev Rheumatol. 2009; 5:218–224. [PubMed: 19337286]
17. Loe H, Silness J. Periodontal Disease in Pregnancy. I. Prevalence and Severity. Acta Odontol Scand. 1963; 21:533–551. [PubMed: 14121956]
18. Han YW, Ikegami A, Bissada NF, et al. Transmission of an uncultivated Bergeyella strain from the oral cavity to amniotic fluid in a case of preterm birth. J Clin Microbiol. 2006; 44:1475–1483. [PubMed: 16597879]
19. Han YW, Shen T, Chung P, et al. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. J Clin Microbiol. 2009; 47:38–47. [PubMed: 18971361]
20. Han YW, Fardini Y, Chen C, et al. Term stillbirth caused by oral Fusobacterium nucleatum. Obstet Gynecol. 2010; 115:442–445. [PubMed: 20093874]
21. Han YW, Redline RW, Li M, et al. Fusobacterium nucleatum induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. Infect Immun. 2004; 72:2272–2279. [PubMed: 15039352]
22. Liu H, Redline RW, Han YW, et al. Fusobacterium nucleatum induces fetal death in mice via stimulation of TLR4-mediated placental inflammatory response. J Immunol. 2007; 179:2501–2508. [PubMed: 17675512]
23. Moen K, Brun JG, Valen M, et al. Synovial inflammation in active rheumatoid arthritis and psoriatic arthritis facilitates trapping of a variety of oral bacterial DNAs. Clin Exp Rheumatol. 2006; 24:656–663. [PubMed: 17207381]
24. Kasser UR, Gleissner C, Dehne F, et al. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. Arthritis Rheum. 1997; 40:2248–2251. [PubMed: 9416864]
25. Greenwald RA, Kirkwood K. Adult periodontitis as a model for rheumatoid arthritis (with emphasis on treatment strategies). J Rheumatol. 1999; 26:1650–1653. [PubMed: 10451056]

26. Rosenstein ED, Greenwald RA, Kushner LJ, et al. Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. Inflammation. 2004; 28:311–318. [PubMed: 16245073]

27. Han YW. Can oral bacteria cause pregnancy complications? Womens Health. 2011; 7:401–404.

28. Han YW. Oral health and adverse pregnancy outcomes - what's next? J Dent Res. 2011; 90:289–293. [PubMed: 21041548]

Table 1
Patients Data and PCR results of the synovial fluid

Patient	Gender	Age	Joints	Diagnosis	PCR results
1	F	56	Natural	RA	−
2	F	45	Natural	RA	−
3	F	57	Natural	OA	−
4	F	70	Natural	RA	−
5	F	60	Natural	RA	−
6	F	47	Natural	RA	−
7	F	80	Natural	OA	−
8	F	48	Natural	RA	+
9	F	65	Natural	OA	−
10	F	69	Natural	OA	−
11	F	66	Natural	OA	−
12	F	55	Natural	RA	−
13	F	61	Natural	OA	−
14	F	56	Natural	RA	−
15	F	50	Natural	RA	−
16	F	63	Natural	OA	−
17	F	51	Natural	RA	−
18	F	56	Natural	OA	−
19	F	51	Natural	OA	+
20	F	65	Natural	OA	+
21	M	55	Natural	OA	−
22	F	62	Natural	OA	−
23	F	66	Natural	OA	−
24	F	73	Natural	OA	−
25	M	57	Natural	OA	−
26	M	84	Natural	OA	−
27	M	55	Natural	OA	−
28	M	50	Prosthesis(LTHA)	OA	−
29	M	76	Prosthesis(RTKA)	OA	−
30	M	70	Prosthesis(RTHA)	OA	−
31	F	68	Prosthesis(RTHA)	OA	−
32	F	68	Prosthesis(LTKA)	RA	+
33	M	70	Prosthesis(RTHA)	OA	−
34	F	67	Prosthesis(LTKA)	OA	−
35	F	74	Prosthesis(LTKA)	OA	−
36	M	52	Prosthesis(LTKA)	OA	+

* OA, Osteoarthritis; RA, Rheumatoid arthritis; R/LTHA, Right /Left total hip arthroplasty; R/LTKA, Right/Left total knee arthoplasty

Table 2
Identified Bacteria in Positive Synovial fluid

Subject	Bacterial species identified
8	*Bacterium JL74*
	Pseudomonas trivialis
	Serratia proteamaculans
	Fusobacterium nucleatum
19	*Fusobacterium nucleatum*
	Nevskia soli/Nevskia ramosa
	Aquabacterium fontiphilum
	Acinetobacter junii
	Streptococcus agalactiae
	Leptrotrichia wadei
20	*Fusobacterium nucleatum*
	Massilia timonae
	Streptococcus agalactiae
32	*Fusobacterium nucleatum*
36	*Pseudomonas synxantha*
	Stenotrophomonas maltophilia
	Agrobacterium tumefaciens
	Flavobacterium-like sp. oral clone/ Pedobacter Kwangyangensis
	Variovorax paradoxus

Table 3

Comparison of *Fusobacterium nucleatum* in synovial fluid and dental plaque ofpatient 32

Samples	Identification	# clones analyzed
synovial fluid	*Fusobacterium nucleatum sbsp. polymorphum* Oral Taxon 202	6*
dental plaque	*Fusobacterium nucleatum sbsp. animalis* Oral Taxon 420	11
	Fusobacterium nucleatum sbsp. polymorphum Oral Taxon 202	2*
	Fusobacterium nucleatum sbsp. vincentii Oral Taxon 200	8
	Fusobacterium nucleatum sbsp. nucleatum Oral Taxon 698	1
	Fusobacterium sp. Oral Taxon 203	1
	Fusobacterium sp. Oral Taxon 205	21

* = 100% sequence match in the 16S rRNA region