CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Mobile Microbiome: Oral Bacteria in Extra-oral Infections and Inflammation

ABSTRACT

The link between oral infections and adverse systemic conditions has attracted much attention in the research community. Several mechanisms have been proposed, including spread of the oral infection due to transient bacteremia resulting in bacterial colonization in extra-oral sites, systemic injury by free toxins of oral pathogens, and systemic inflammation caused by soluble antigens of oral pathogens. Mounting evidence supports a major role of the systemic spread of oral commensals and pathogens to distant body sites causing extra-oral infections and inflammation. We review here the most recent findings on systemic infections and inflammation complicated by oral bacteria, including cardiovascular disease, adverse pregnancy outcomes, rheumatoid arthritis, inflammatory bowel disease and colorectal cancer, respiratory tract infections, and organ inflammations and abscesses. The recently identified virulence mechanisms of oral species *Fusobacterium nucleatum, Porphyromonas gingivalis, Streptococcus mutans*, and *Campylobacter rectus* are also reviewed. A pattern emerges indicating that only select subtype(s) of a given species, *e.g., F. nucleatum* subspecies *animalis* and *polymorphum* and *S. mutans* non-*c* serotypes, are prone to extra-oral translocation. These findings advocate the importance of identification and quantification of potential pathogens at the subtype levels for accurate prediction of disease potential.

KEY WORDS: virulence mechanism, systemic disease, bacteremia, *Fusobacterium*, pregnancy complications, *Porphyromonas*.

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INTRODUCTION

The theory of focal infection, *i.e.*, the idea that oral conditions can significantly influence diseases elsewhere in the body, first became popular during the late 19th and early 20th centuries, but started to fall out of favor by the 1930s due to lack of scientific support. Interestingly, increasing evidence over the past 3 or so decades suggests that, due to dental bacteremia, the oral cavity can indeed serve as a reservoir for systemic dissemination of pathogenic bacteria and their toxins, leading to infections and inflammation in distant body sites. With the application of culture-independent technologies for microbial detection and identification, a diverse group of oral species has been found to be directly involved in infections at extra-oral sites. Advances in the human microbiome research have also facilitated a shift of focus from oral diseases to oral bacteria in systemic infections. In this review, we will summarize recent advances in the identification and detection of oral bacteria in extra-oral infections, using PubMed searches with key words such as oral bacteria, systemic disease, atherosclerosis, pregnancy, pneumonia, inflammatory bowel disease, colorectal cancer, rheumatoid arthritis, etc. The pathogenic mechanisms of select species in these infections are also reviewed.

SYSTEMIC DISEASES IMPLICATED BY ORAL BACTERIA

Summarized in Table 1 are representative oral species that have been detected in various systemic infections and inflammation. More detailed descriptions are below, with an emphasis on the most recent findings.

Atherosclerotic Disease (AD)

Atherosclerotic disease, including myocardial infarction and stroke, is the leading cause of death worldwide. Although conditions such as smoking, obesity, high blood pressure, and diabetes are well known to contribute to AD, studies show that up to half of individuals with AD may not have any of these traditional risk factors (Seymour *et al.*, 2007), suggesting the presence of additional causes. The implication of oral bacteria in the initiation and progression of atherosclerosis is now widely accepted. Detection of oral bacterial DNA in human atheromatous plaques was first reported in 2000 (Haraszthy *et al.*, 2000). More recently, Ford *et al*. detected oral bacterial DNA in coronary artery biopsies from patients diagnosed with coronary artery disease and in endarterectomy specimens from patients undergoing surgical treatment of atherosclerosis (Ford *et al*., 2005; Ford *et al*., 2006). Collectively, the following species have been detected: *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia*, and *Tannerella forsythia*, with some of the samples containing more than one type of bacteria.

Given the association between periodontal disease and AD, it is not surprising to find periodontal bacterial DNA in AD lesions (Table 1). The finding of

Table 1. Summary of Oral Species Implicated in Extra-oral Infections and Inflammation

Extra-oral Infections Complicated by Oral Bacteria	Oral Species Detected*
Cardiovascular diseases (CVD)	*Aggregatibacter actinomycetemcomitans*
	Campylobacter rectus
	Chlamydia pneumoniae
	Eikenella corrodens
	Fusobacterium necrophorum
	Fusobacterium nucleatum
	Porphyromonas gingivalis
	Prevotella intermedia
	Streptococcus mitis
	Streptococcus mutans
	Streptococcus oralis
	Tannerella forsythia
	Treponema denticola
Adverse pregnancy outcomes (APO)	*Begeyella* spp.
	Campylobacter rectus
	Capnocytophaga spp.
	Eikenella corrodens
	Fusobacterium nucleatum
	Peptostreptococcus micros
	Porphyromonas gingivalis
	Prevotella intermedia
	Prevotella nigrescens
	Rothia dentocariosa
	Streptococcus mutans
	Tannerella forsythia
	Treponema denticola
Rheumatoid arthritis (RA)	*Fusobacterium nucleatum*
	Porphyromonas gingivalis
	Prevotella intermedia
	Prevotella melaninogenica
	Serratia proteamaculans
	Tannerella forsythia
Inflammatory bowel disease (IBD) and colorectal cancer (CRC)	*Campylobacter concisus*
	Fusobacterium nucleatum
	Streptococcus mutans
Respiratory tract infections (RTI)	*Staphylococcus aureus*
	Pseudomonas aeruginosa
	Acinetobacter species
	Candida albicans
	Enteric species
Meningitis or brain abscesses	*Campylobacter rectus*
	Fusobacterium necrophorum
	Fusobacterium nucleatum
	Porphyromonas gingivalis
	Streptococcus intermedius
Lung, liver, or splenic abscesses	*Fusobacterium necrophorum*
	Fusobacterium nucleatum
	Porphyromonas gingivalis
	Prevotella spp.
	Treponema denticola
Appendicitis	*Fusobacterium necrophorum*
	Fusobacterium nucleatum

*References are provided in the Appendix Table.

cariogenic *Streptococcus mutans* in AD, however, was rather intriguing. In a large-scale study of 203 consecutive patients, which included 82 aortic valve specimens, 35 mitral valve specimens, and 86 aortic aneurysmal wall specimens, Nakano *et al.* found that *S. mutans* was the most frequently detected species, with a detection rate of 42.7% in the heart valves and 62.8% in the aneurysm walls, respectively. *S. mutans* DNA was also detected in dental plaque from most of the patients (Nakano *et al*., 2009). Furthermore, while the majority of cariogenic *S. mutans* belongs to serotypes *c, e*, and *f*, the AD-associated *S. mutans* belong to a novel serotype, *k*, characterized by a drastic reduction in the amount of glucose side-chains and reduced cariogenicity (Nakano *et al.*, 2007a; Nakano *et al.*, 2007b; Nakano *et al.*, 2010).

Adverse Pregnancy Outcomes (APO)

The readers are referred to a recent review with regard to epidemiologic and interventional studies on the link between periodontal infections and APO (Han, 2011b). APO is a broad term including preterm labor, preterm premature rupture of membranes, pre-eclampsia, miscarriage, intra-uterine growth retardation, low birthweight, stillbirth, and neonatal sepsis. The oral species implicated in APO are summarized in Table 1.

F. nucleatum is by far the most prevalent oral species in APO and has been detected in a wide variety of placental and fetal tissues, including amniotic fluid, fetal membranes, cord blood, neonatal gastric aspirates, and fetal lung and stomach, associated with preterm birth, stillbirth, and early-onset neonatal sepsis (Gonzales-Marin *et al*., 2011; Han *et al*., 2009, 2010; Wang *et al*., 2013). A case report of term stillbirth caused by oral *F. nucleatum* provided the first human evidence that the bacterium originated from the mother's subgingival plaque and translocated to the placenta and fetus, causing acute inflammation leading to the fetal demise (Han *et al*., 2010). While *F. nucleatum* has been detected as the most prevalent species in amniotic fluid from pregnancies complicated by preterm birth (Han *et al*., 2009), its recent detection in cord blood associated with early-onset neonatal sepsis is rather novel (Wang *et al.*, 2013). The high invasiveness of *F. nucleatum*, and thus its ability to spread to different placental and fetal compartments likely underlies its simultaneous detection in the matching amniotic fluid and cord blood samples (Wang *et al.*, 2013). There are currently 5 subspecies (sbsp) of *F. nucleatum, i.e*., sbsp *nucleatum*, sbsp *polymorphum*, sbsp *fusiform*, sbsp *vincentii*, and sbsp *animalis*. Only 2 of these 5 subspecies have been detected in intra-uterine infections, with the overwhelming majority belonging to sbsp *animalis*, and an occasional few belonging to sbsp *polymorphum* (Table 2).

P. gingivalis was first detected in the amniotic fluid in a case of threatened premature labor and periodontitis (Leon *et al*., 2007). *P. gingivalis* antigens were detected by immunocytochemistry in the placental syncytiotrophoblasts, chorionic trophoblasts, decidual cells, amniotic epithelial cells, and vascular cells, with a substantial increase in the intensity of immunostaining of the tissues obtained from women with chorioamnionitis compared with those with normal-term pregnancy (Katz *et al*., 2009).

Uncultivated oral *Bergeyella* spp. is a novel genus only recently associated with APO. It was first detected in the amniotic fluid

Table 2. *F. nucleatum* Subspecies Identified in Intra-uterine Infection

		Fn Subspecies Identified*	Source**	References
Cultivated strains	YWH7052	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2004
	YWH7055	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2004
	YWH7053	*F. nucleatum* ss. *animalis*	PTB placentas	Han *et al.*, 2004
	YWH7056	*F. nucleatum* ss. *animalis*	PTB placentas	Han *et al.*, 2004
	YWH7199	*F. nucleatum* ss. *animalis*	Stillbirth placenta	Han *et al.*, 2010
Accession numbers	EU644455	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644461	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644463	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644464	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644466	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644478	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	EU644480	*F. nucleatum* ss. *animalis*	PTB AF	Han *et al.*, 2009
	JN546083	*F. nucleatum* ss. *animalis*	PTB AF (with EONS)	Wang *et al.*, 2013
	JN546084	*F. nucleatum* ss. *animalis*	PTB AF	Wang *et al.*, 2013
	JN584646	*F. nucleatum* ss. *polymorphum*	PTB AF	Wang *et al.*, 2013
	JN546087	*F. nucleatum* ss. *animalis*	PTB AF	Wang *et al.*, 2013
	JQ901488	*F. nucleatum* ss. *animalis*	PTB CB (with EONS)	Wang *et al.*, 2013
	JN546096	*F. nucleatum* ss. *animalis*	PTB CB	Wang *et al.*, 2013
	JN546097	*F. nucleatum* ss. *animalis*	PTB CB	Wang *et al.*, 2013
	JN546100	*F. nucleatum* ss. *animalis*	PTB CB	Wang *et al.*, 2013
	N/A	*F. nucleatum* ss. *polymorphum*	neonatal gastric aspirates	Gonzales-Marin *et al.*, 2013

*Those with accession numbers were detected by PCR and clone analysis. Species and subspecies identification was performed based on the full-length 16S rRNA gene sequences from the Human Oral Microbiome Database (HOMD). ss, subspecies.
**AF, amniotic fluid; PTB, preterm birth; EONS, early-onset neonatal sepsis; CB, cord blood.

from a case of preterm birth, owing to the use of 16S rRNA gene-based culture-independent technology (Han *et al.*, 2006). The clone identified in the amniotic fluid was detected in the mother's subgingival plaque but not in her vaginal flora. Since then, *Bergeyella* has been repeatedly detected in amniotic fluid associated with preterm birth, and most recently in cord blood associated with neonatal sepsis (Han *et al*., 2009; Wang *et al.*, 2013). Similar to *F. nucleatum, Bergeyella* sp. was detected in paired amniotic fluid and cord blood samples suggesting its ability to invade the fetal compartment (Wang *et al.*, 2013). Oral bacteria are often detected in clinical samples as mixed infections, indicating co-translocation from the oral cavity (Han *et al.*, 2009; Wang *et al.*, 2013). These observations are supported by animal studies in which a variety of cultivated and uncultivated oral species were found to co-translocate to the mouse placenta (Fardini *et al.*, 2010). The majority of oral species translocated to the murine placenta were oral commensals, suggesting that commensals in the oral cavity may become pathogens elsewhere. Identification of species/subspecies involved in APO will make it plausible for early and accurate diagnosis to identify individuals at risk (Han, 2011b).

Rheumatoid Arthritis (RA)

RA is an autoimmune disease characterized by chronic inflammation of the joints and the surrounding tissues, leading eventually to destruction of the joint architecture and impaired function. Numerous clinical studies point toward a potential association between RA and chronic periodontitis (Martinez-Martinez *et al*., 2009; Dissick *et al*., 2010). Two studies reported that treatment of periodontal infections reduced the severity of active RA, suggesting periodontitis as a causal factor in the initiation and maintenance of the autoimmune inflammatory responses (Al-Katma *et al.*, 2007; Ortiz *et al*., 2009). Periodontal bacterial DNA was detected by PCR in the synovial fluid of patients with both RA and periodontal disease, with *Prevotella intermedia* and *P. gingivalis* as the most prevalent (Martinez-Martinez *et al*., 2009). In a recent study, two patients with RA and periodontitis had identical bacterial clones (*Fusobacterium nucleatum* and *Serratia proteamaculans*, respectively) detected in both the synovial fluid and the matching dental plaque samples (Temoin *et al.*, 2012a). The finding of oral bacterial DNA in the synovial fluid suggests the possibility of organisms translocating from the oral cavity to the synovium.

Inflammatory Bowel Disease (IBD) and Colorectal Cancer (CRC)

IBD represents a group of chronic disorders of the gastrointestinal (GI) tract, including ulcerative colitis (UC) and Crohn's disease (CD). Studies have revealed that both genetic and environmental factors are involved in the development of IBD. Dysbiosis of intestinal microbiota has been shown to play a pivotal role in the pathogenesis of IBD.

Oral bacteria, *e.g., Fusobacterium nucleatum* and *Campylobacter concisus*, have recently been associated with IBD (Strauss *et al*., 2011; Ismail *et al*., 2012). *F. nucleatum* strains originating from inflamed biopsy tissues from IBD patients were significantly more invasive than those isolated from healthy tissues from either IBD or control patients (Strauss

et al., 2011). Enteric invasive *C. concisus* oral strains were detected in 50% IBD patients, but none in the normal healthy controls. Multilocus sequence typing (MLST) showed that individuals with Cluster I *C. concisus* had significantly higher incidence of IBD and/or bloody diarrhea compared with those without (Ismail *et al.*, 2012).

IBD has been recognized as a risk factor for CRC. Thus, it is not surprising that the same micro-organisms are implicated in both diseases. Two recent studies reported detection of significantly elevated *F. nucleatum* levels in colorectal carcinomas, compared to the normal colon tissues from the same patients (Castellarin *et al.*, 2012; Kostic *et al*., 2012). The overabundance of *Fusobacterium* in CRC was positively associated with lymph node metastasis. These findings were supported by a subsequent study in which *Fusobacterium, Porphyromonas, Peptostreptococcus*, and *Mogibacterium* were found to be enriched in the mucosa-adherent microbiota in CRC (Chen *et al.*, 2012). *Fusobacterium* is rarely detected in the luminal flora (stools), but it is readily detected in mucosal specimens (Chen *et al.*, 2012). This is likely due to its adherence properties (see below). Whether *F. nucleatum* is a cause or a consequence of CRC is currently under investigation. If a causative link is proven, antibiotics and/or vaccines could be potential therapies to treat and/or prevent CRC.

Respiratory Tract Infections (RTI)

Oral colonization by respiratory pathogens, fostered by poor oral hygiene and periodontal diseases, has been associated with nosocomial pneumonia. Particularly among patients on ventilators in intensive care units, dental plaque has been shown to serve as a reservoir for respiratory pathogens. *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter* species, *Candida albicans*, and enteric species recovered from plaque were indistinguishable from isolates recovered from tracheobronchial sites in the same patient, whereas strains from different patients usually separated into different genotypes (Heo *et al*., 2008, 2011).

Other Organ Inflammation and Abscesses

Oral bacteria have been found to be associated with organ abscesses in the human body (Table 1). For example, *Fusobacterium* is the most common oral genus detected in brain, lung, liver, and splenic abscesses (Han, 2011a). One study reported that 4 periodontopathic bacterial species, including *F. nucleatum, P. intermedia, P. gingivalis*, and *T. denticola*, were detected in a pyogenic liver abscess site of a female patient (Ohyama *et al*., 2009). Two recent studies reported infective appendicitis cases in which oral *Fusobacterium* was shown to play a major role (Swidsinski *et al.*, 2011).

POTENTIAL VIRULENCE MECHANISMS OF ORAL BACTERIA IN EXTRA-ORAL INFECTIONS

Regardless of their pathogenic potentials in the oral cavity, once colonized in the extra-oral sites, oral bacteria often become *bona fide* pathogens, especially in immune-compromised individuals, causing disease manifestation. Here we summarize the recently uncovered virulence mechanisms of *F. nucleatum, P. gingivalis, S. mutans*, and *C. rectus* in extra-oral infections and inflammation.

Fusobacterium nucleatum (Fn)

Fn is ubiquitous in the oral cavity and is the most prevalent species in extra-oral infections (Han, 2011a). It has the ability to adhere to and invade different types of host cells, including human epithelial and endothelial cells (Han, 2011a). These are essential virulence mechanisms because they provide means for colonization, evasion of host defense, and spread to deeper tissues. For example, *Fn* colonizes the murine placentas by binding and crossing the endothelium (Han, 2011a). Once colonized in the placenta, *Fn* proliferates quickly and eventually spreads to amniotic fluid, fetal membranes, and the fetus.

The surface adhesin FadA expressed by *Fn* plays a major role in the cell attachment and invasion processes (Han, 2011a). This adhesin is unique to and highly conserved among *Fn*, but is absent in non-oral fusobacteria (Han, 2011a). *Fn* colonization in the murine placenta requires FadA (Ikegami *et al*., 2009), which exists in 2 forms: the intact pre-FadA, consisting of 129 amino-acid (aa) residues and the secreted mature FadA (mFadA), consisting of 111 aa residues. Both pre-FadA and mFadA are required to form an active complex, FadAc, for binding and invasion (Xu *et al*., 2007). The crystal structure of mFadA reveals a predominantly alpha-helical hairpin structure, with the monomers linked together in a head-to-tail pattern *via* a novel leucine-chain motif (Nithianantham *et al.*, 2009). While the long and thin filaments formed by mFadA alone had no cell-binding activity, the heterogeneous filaments formed by FadAc did (Temoin *et al*., 2012b). We have recently identified vascular endothelial (VE)-cadherin as the endothelial-cell receptor for FadA. FadA binding to VE-cadherin caused the latter to translocate from the cell-cell junctions to intracellular compartments, increasing endothelial cell permeability and allowing bacteria to penetrate (Fardini *et al*., 2011). Thus, FadA allows both direct invasion into the host cells and pericellular invasion *via* loosened cell-cell junctions. We postulate that this is the mechanism used by *Fn* for systemic dissemination. *Fn* is not only invasive by itself, but has also been shown to facilitate both intra- and inter-cellular invasion by other species, such as *Streptococcus cristatus* and *E. coli* (Edwards *et al*., 2006; Fardini *et al*., 2011). This phenomenon explains why *Fn* is often found in mixed infections (Han *et al.*, 2009; Wang *et al*., 2013).

Following colonization, *Fn* stimulates TLR4-mediated inflammatory responses in the fetal-placental unit. Fetal loss was significantly reduced in TLR4 knock-out mice and in wild-type mice treated with a TLR4-antagonist, despite *Fn* colonization in the placenta. These results demonstrate that TLR4-mediated localized inflammation, rather than the bacterium *per se*, is the cause of fetal death (Liu *et al*., 2007). Fetal death occurs after 2 to 3 days of hematogenous infection. The pattern and duration of infection in the pregnant mice were consistent with those observed in humans (Han *et al*., 2010).

Additional virulence factors recently identified from *Fn* include GroEL, which induces factors predisposing to atherosclerosis in human microvascular endothelial cells (HMEC-1) and apolipoprotein E-deficient ($ApoE^{-/-}$) mice (Lee *et al.*, 2012),

and 2 outer-membrane protein adhesins of *Fn*, Fap2 and RadD, which share homology to autotransporter secretion systems (type Va secretion systems) and induce apoptosis of human lymphocytes (Kaplan *et al*., 2010). RadD has been shown to mediate co-aggregation with *Streptococcus sanguinis* (Kaplan *et al*., 2009).

Porphyromonas gingivalis (Pg)

Readers are referred to a recent review on the mechanisms of *Pg* in extra-oral infections, especially those involved in CVD (Hayashi *et al*., 2010). Here we summarize a few recent findings.

Although *Pg* is a long-recognized and well-studied pathogen, novel virulence mechanisms continue to emerge. *Pg* has been postulated as a keystone pathogen in periodontal disease because of its low abundance and high virulence in the oral flora. *Pg* could disrupt host-microbe homeostasis and cause inflammatory responses by modulating the complement system (Hajishengallis *et al*., 2011). A study revealed that immunoreactivity to *Pg* heat-shock protein was predominant in patients with autoimmune disease with ongoing periodontal disease, suggesting a role for *Pg* heat-shock protein in infection-triggered autoimmune diseases (Jeong *et al*., 2012). *Pg* is the only prokaryote described to date to possess peptidylarginine deiminase (PAD), which converts arginines within a peptide (peptidylarginine) into peptidylcitrulline (Liao *et al*., 2009; Mangat *et al*., 2010). Thus, *Pg* can induce anti-citrullinated peptide antibodies and trigger autoimmune inflammation in the host. It has been previously shown that *Pg* PAD plays an important role in the pathogenesis of periodontitis-associated RA (Liao *et al*., 2009; Mangat *et al*., 2010). In susceptible individuals, host protein citrullination by *Pg* PAD in the joint likely induces antibody response, paving the way for the development of chronic arthritis (Mangat *et al*., 2010). Inactivation of arginine gingipains, but not lysine gingipains, resulted in decreased citrullination (Wegner *et al*., 2010), suggesting that *Pg* virulence factors function synergistically in inducing systemic infection and inflammation.

Numerous animal models have been established to investigate the virulence mechanisms of *Pg* in systemic infections. A recent study showed that the subcutaneous infection with *Pg* at different gestation periods of the experimental rat model resulted in lower maternal weight gain, lower placenta/fetus weight, and more fetal-placental resorptions than in controls. The infection at mid-gestation was found to cause more severe consequences with the induction of pro-inflammatory cytokines expression in the fetal compartment (Michelin *et al*., 2012). As observed with *S. mutans, C. concisus*, and *F. nucleatum*, not all *Pg* strains exhibit the same level of virulence. In a mouse periodontitis model, it was shown that different *Pg* strains elicited various systemic responses and various degrees of alveolar bone loss (Marchesan *et al*., 2012). The infectivity of *Pg* in murine placentas was also strain-dependent (Belanger *et al*., 2008).

Streptococcus mutans (Sm)

Oral streptococci, major members of the oral flora, frequently cause bacteremia and systemic diseases, such as infective endocarditis (IE) (Que and Moreillon, 2011). Among them, *Sm* is a primary etiological agent of human dental caries. *Sm* can be divided into 4 different serotypes (*c/e/f/k*), based on its *rhamnose-glucose polymers* (RGPs) (Nakano *et al*., 2007). Serotype *c* is the most common in the oral cavity. Serotypes *e* and *f* have been found to invade endothelial cells (Abranches *et al*., 2011). Serotype *k*, with a defect of the glucose side-chain in RGPs, was found to be more frequently detected in cardiovascular specimens (Nakano *et al*., 2007a; Nakano *et al*., 2007b; Nakano *et al*., 2010).

Collagen-binding proteins (CBP), have been shown to be directly involved in hemorrhagic stroke (Nakano *et al*., 2011). The detection frequency of CBP-expressing *Sm* in patients with hemorrhagic stroke is significantly higher than in the control individuals. Infection of *Sm* of the *k* serotype, which expresses CBP, aggravates cerebral hemorrhage in mice (Nakano *et al*., 2011).

Two novel CBPs, Cnm and Cbm, have been demonstrated to play a major role in *Sm* attachment and invasion of human endothelial cells (Nomura *et al*., 2013). Cnm is encoded by invasive *Sm* strains and is required for *Sm* adherence and invasion of human coronary artery endothelial cells (Abranches *et al*., 2011). Most of the Cbm-positive strains showed higher levels of binding to type I collagen, a major collagen expressed in human heart valves, and higher rates of adhesion and invasion to human umbilical vein endothelial cells (HUVEC) compared with the Cnm-positive strains (Nomura *et al*., 2013). Cbm was detected significantly more frequently in heart valve specimens from IE patients than from non-IE patients (Nomura *et al*., 2013).

Campylobacter rectus (Cr)

Studies have shown that oral infection with *Cr* induced fetal growth restriction (IUGR), placental inflammation, and activation of TLR4 in a murine pregnancy model (Arce *et al*., 2009). *Cr* infection in TLR4-deficient mice did not lead to IUGR, confirming the role of TLR4 in placental infection, as in the case of *Fn* infection (Arce *et al*., 2012). *Cr* was able to invade and significantly up-regulate IL-6 and TNFα in human trophoblastic cells BeWo (Arce *et al*., 2010). So far, no specific virulence factors from *Cr* have been identified.

CONCLUDING REMARKS AND RECOMMENDATIONS

Oral bacteria have developed aggressive mechanisms to invade and persist in the host cells, to escape host immune surveillance, to adapt to niches at extra-oral sites, and to induce inflammatory responses leading to adverse systemic effects. Based on the knowledge gained thus far, some recommendations are justified.

First, periodontal infection, as a trigger of dental bacteremia, likely plays a facilitating role in enabling the systemic dissemination of oral bacteria. Thus, it must be emphasized that good oral hygiene, especially for immune-compromised patients, is crucial for controlling total bacterial load to prevent bacterial dissemination.

Second, development of accurate bacterial detection techniques is important for targeted patient management. The study of microbes has traditionally focused on bacterial species isolated in culture. Because a great many bacteria are uncultivated,

traditional microbiological cultures provide an incomplete spectrum of the human pathogens. For instance, 16S rRNA gene profiling revealed a far greater microbial diversity in amniotic fluid and cord blood than appreciated based on culture-dependent methods. In pregnancies where there was evidence of an inflammatory host response, approximately two-thirds of the microbes detected by culture-independent methods were not isolated by routine cultures (Han *et al*., 2006, 2009; Wang *et al.*, 2013). Furthermore, it is becoming apparent that not all oral bacteria are created equal. Certain subtypes, such as serotypes *e*, *f* and *k* of *S. mutans*, Cluster I *C. concisus, F. nucleatum* sbsp *animalis*, and certain strains of *P. gingivalis*, are more prevalent in extra-oral infections than others. Thus, bacterial detection methods are needed for accurate identification of individuals with the truly virulent subtypes. Since each detection method has its flaws, it is necessary to develop multiple detection methods for cross-validation.

Third, many of the studies linking oral bacteria to extra-oral infections are still at the stage of association, and detailed mechanistic studies are needed to elucidate the role of these bacteria in systemic health. Novel virulence components and mechanisms continue to emerge, even from well-studied oral pathogens. Only when we have a clear understanding of the mechanisms of oral bacteria in extra-oral infections and inflammation can effective therapies be designed.

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