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Antimicrobial peptides and periodontal disease

Sven-Ulrik Gorr and Mahsa Abdolhosseini

Department of Diagnostic and Biological Sciences, University of Minnesota School of Dentistry, Minneapolis, MN, USA

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Abstract

Aims: The goal of this review is to identify the antimicrobial proteins in the oral fluids, saliva and gingival crevicular fluid and identify functional families and candidates for antibacterial treatment.

Results: Periodontal biofilms initiate a cascade of inflammatory and immune processes that lead to the destruction of gingival tissues and ultimately alveolar bone loss and tooth loss. Treatment of periodontal disease with conventional antibiotics does not appear to be effective in the absence of mechanical debridement. An alternative treatment may be found in antimicrobial peptides and proteins, which can be bactericidal and anti-inflammatory and block the inflammatory effects of bacterial toxins. The peptides have co-evolved with oral bacteria, which have not developed significant peptide resistance. Over 45 antibacterial proteins are found in human saliva and gingival crevicular fluid. The proteins and peptides belong to several different functional families and offer broad protection from invading microbes. Several antimicrobial peptides and proteins (AMPs) serve as templates for the development of therapeutic peptides and peptide mimetics, although to date none have demonstrated efficacy in human trials.

Conclusions: Existing and newly identified AMPs may be developed for therapeutic use in periodontal disease or can serve as templates for peptide and peptide mimetics with improved therapeutic indices.

Key words: antibacterial; antibiotics; antimicrobial peptides; cathelicidin; defensin; lipopolysaccharide; peptide mimetics

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Periodontitis is an inflammatory disease that affects approximately half of US adults over 30 years of age. Similarly, 54% of subjects examined in the 1998 UK Adult Dental Health survey exhibited at least moderate pocketing on one or more teeth (Morris et al. 2001). A systematic review of periodontal health in Europe indicates that, on average, 60% of the adult population has clinical attachment loss of >3 mm (König et al. 2010). Periodontal disease is characterized by the formation of mixed biofilms on the teeth and gingival tissues. The oral cavity is an environment exposed to a multitude of bacteria with over 700 possible resident species of which 150–200 are typically found in most individuals. It is thought that this bacterial flora is controlled initially by the innate immune system of oral epithelia, saliva and gingival crevicular fluid, which is rich in antimicrobial proteins and peptides (AMPs) (Table 1). These AMPs constitute a diverse class of host-defense molecules that act early to combat invasion and infection by bacteria and other microorganisms, with over 45 identified to date (Table 2). This group of proteins and peptides has engendered considerable interest in the past decade as a biological paradigm in innate immunity and as a potential source of novel antibiotics (e.g., Brogden 2005, Ganz 2005, Gordon et al. 2005, Wheeler and Hood 2005, Dale et al. 2006, Peschel and Sahl 2006, Schroder and Harder 2006, Talbot et al. 2006, Kinane et al. 2007, Hirsch et al. 2008, Kinane et al. 2008, Sorensen et al. 2008, Gorr 2009). These AMPs presumably protect oral tissues from infection as minor cuts and abrasions or even tooth extractions, which create large lesions in the oral epithelium, typically resolve without major infection or inflammation (Zasloff 2002b). On the other hand, the normal oral flora is in a balance between pathogens and commensals that requires regular cleaning to be maintained. A decrease in oral hygiene is quickly followed by the build-up of oral biofilms on tooth surfaces and, if left untreated, will progress to gingival inflammation and possibly

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periodontitis, alveolar bone loss and loss of teeth. Thus, it appears that the AMPs in the oral cavity do not solely control bacterial growth and prevent biofilm build-up. This critical narrative review catalogs the AMPs found in saliva and gingival crevicular fluid and points to potential roles and uses in control of oral bacteria and periodontal disease. A combination of search strategies was used in an effort to obtain a comprehensive view of the existing literature: PubMed was searched with the MeSH terms ''periodontitis'' and ''anti-bacterial agents''; previous reviews were consulted for relevant proteins and recent updates on specific proteins were identified by a PubMed search for each AMP limited to the publication years 2009–2010. Clinical Trials were initially identified in ''clinicaltrials.gov''. In some cases, these searches were broadened by searching Google using specific protein or drug names in combination with ''periodontal'' or ''periodontitis''.

Oral Bacteria and Infection

The oral cavity and airways are exposed to many of the same bacteria, which are either ingested or inhaled. However, while the lower airways are essentially sterile (Diamond et al. 2008), indicating that airway host-defenses effectively clear invading bacteria, the oral cavity is host to over 700 species of bacteria, with about 400 found in the periodontal pocket. Newer pyrosequencing techniques using short sequence tags for the 16S rDNA V6 region have led to even higher estimates of microbial diversity in saliva and plaque (Keijser et al. 2008). A preliminary estimate identified 5669 and 10,052 phylotypes (species) in saliva and plaque, respectively, using operational taxonomic units (OTUs) at 3% difference. This may represent about 50% of the total species present (Keijser et al. 2008). However, 95% of the sequences were represented by the 1000 most abundant OTUs, which approximates previous estimates. Importantly, in the absence of mechanical or chemical removal of oral bacteria, they quickly form biofilms on tooth surfaces. These biofilms can lead to gingival infections, periodontitis and loss of alveolar bone and teeth. Indeed, oral infections and attendant inflammatory diseases are among the most common human infections.

Of the 400 species of bacteria found in the periodontal pocket not all are found in every individual. As an example, in one study 69 of the 400 periodontal bacteria were found in multiple subjects (Paster et al. 2006). However, only about eight bacterial species have consistently been associated with the development of periodontitis, including *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Eubacterium nodatum*, *Prevotella intermedia and Prevotella nigrescens* (Periodontics 1996, Socransky et al. 1998, Teles et al. 2006). The first three are consensus pathogens (Periodontics 1996) while *P. gingivalis*, *T. forsythia and T. denticola* belong to the red complex described by Socransky et al.(1998).

The periodontal pathogens are typically found in gingival crevices and periodontal pockets of both healthy and diseased sites (Colombo et al. 2006, Teles et al. 2006) and population studies have identified population subgroups with high, moderate or low susceptibility to inflammatory diseases, including periodontitis (Loe et al. 1986). Thus, it is likely that differences in host-defense mechanisms, including antimicrobial protein profiles, determine whether bacterial colonization progresses to overt disease. Similar differences in host-defenses may play a role in the age differences noted for periodontal disease, which is predominantly associated with *A. actinomycetemcomitans* in the young while *P. gingivalis* is the dominant bacterial agent later in life (Slots and Ting 1999).

Biofilms and Periodontitis

Dental plaque is a mixed microbial biofilm that can be composed of hundreds of bacterial species (Kolenbrander et al. 2006). The biofilm bacteria and their toxins perturb gingival epithelial cells as the first stage in a cascade of inflammatory and immune processes that lead to the destruction of gingival tissues and ultimately, in susceptible patients, alveolar bone loss and tooth loss as a result of periodontal disease.

Mixed biofilms are communities of bacteria that communicate by quorum sensing to change the bacterial physiology. The biofilm contains channels to aid nutrient transport and is typically encapsulated by an extracellular polysaccharide matrix (Ten Cate 2006). These features combine to make antibiotic treatment difficult. Traditional antibiotics were often selected against metabolically active bacteria in a planktonic state and are therefore less effective against the physiologically dormant bacteria encapsulated in a biofilm (Ten Cate 2006). As an example, the susceptibility of *A. actinomycetemcomitans* to several antibiotics decreases as the biofilm matures (Takahashi et al. 2007). Thus, plaque is typically removed by mechanical debridement, which also remains the main treatment option for periodontitis. Depending on the extent of the gingival infection and attendant inflammation, surgery and tissue regeneration are further treatment option for periodontitis.

The Role of Antimicrobial Proteins in Periodontal Disease

Human saliva and gingival fluid contains at least 45 different AMPs that belong to several different functional classes, ranging from small cationic peptides to enzymes and large agglutinating proteins (Table 1). It is thought that this functional and structural diversity is necessary to protect the oral epithelia from the large number of possible invading microbes and maintain the oral homeostasis of commensal and pathogenic bacteria. Moreover, the expression of anti-microbial proteins is differentially regulated by different periodontal pathogens (Handfield et al. 2005) (Table 2), suggesting that a specific antimicrobial ''cocktail'' constitutes the physiological response to individual pathogens. This mix may also play a role in maintaining an appropriate balance between oral pathogens and commensals.

Proteomic analyses have identified differences in antimicrobial protein expression in periodontal patients compared with healthy or treated controls. A proteomic analysis of salivary proteins from aggressive periodontitis and normal controls revealed differential expression of 11 proteins (Wu et al. 2009b), including the antimicrobial proteins lactotransferrin and PSP/SPLUNC2. A similar study analysed the expression of salivary proteins from periodontitis patients before and after treatment (Haigh et al. 2010). PSP/SPLUNC2, which is up-regulated in periodontitis, was down-regulated after treatment while the calgranulins S100A8 and A9 were up-regulated after

Table 1. Functional classes of antimicrobial proteins

	Cationic peptides	Bacterial agglutination and adhesion	Metal ion chelators	Peroxidases	Protease inhibitors	Activity against bacterial cell walls
1	Adrenomedullin	β-2-microglobulin	Calgranulin A Protein S100-A8	Lactoperoxidase Salivary peroxidase	Cystatin A	Lysozyme C
2	Azurocidin CAP37 Heparin-binding protein	Fibronectin	Calgranulin B Protein S100-A9	Myeloperoxidase	Cystatin B	Peptidoglycan recognition protein 1
3	β defensin-1 hBD-1	Mucin 7	Lactoferrin Lactotransferrin		Cystatin C	Peptidoglycan recognition protein 3
4	β defensin-4A β-defensin-2 hBD-2	Prolatin-inducible protein	Psoriasin Protein S100-A7		Cystatin D	Peptidoglycan recognition protein 4
5	β Defensin 103 β-defensin-3 hBD-3	Proline-rich proteins	Transferrin Serotransferrin		Cystatin S	
6	Calcitonin gene-related peptide 1	Salivary agglutinin GP340 DMBT1			Cystatin SA	
7	Cathelicidin (LL-37)	Surfactant protein A pulmonary surfactant-associated protein A1			Cystatin SN	
8	C-C motif chemokine 28				Secretory leukoprotease inhibitor protein	
9	Hemoglobin β-globin α globin				Skin-derived antileukoproteinase Elafin	
10	Heparin binding growth factor Fibroblast growth factor					
11	Histatin 1					
12	Histatin 3 (Histatin 5)					
13	HNP-1 Neutrophil defensin 1					
14	HNP-2 Neutrophil defensin 2					
15	HNP-3 Neutrophil defensin 3					
16	HNP-4 Neutrophil defensin 4					
17	Neuropeptide Y					
18	Statherin					
19	(Substance P) Protachykinin-1					
20	Vasoactive intestinal peptide					

See Table 2 for additional details for individual proteins.

treatment (Haigh et al. 2010). Direct analysis of the antimicrobial peptide LL-37 in gingival crevicular fluid showed that the peptide is significantly elevated in patients with chronic periodontitis compared with the other groups. Moreover, a positive relationship was found between levels of LL-37 and probing depth, clinical attachment level, plaque index, bleeding on probing and papilla bleeding index at sampled sites (Turkoglu et al. 2009). In addition to understanding the role of specific AMPs in the pathology of periodontal disease, these differences could lead to the development of salivary markers for diagnosis of periodontal disease (Giannobile et al. 2009).

Antimicrobial proteins exhibit striking variation in their ability to kill different species of oral bacteria or different strains of the same species (Diamond et al. 2009). As an example, *Streptococcus gordonii* is not susceptible to hBD-3 or LL-37 while *S. gordonii* 10558 exhibits minimal inhibitory concentrations of 15–31 μg/ml for both peptides (Ji et al. 2007a).

Antimicrobial Protein Deficiency and Periodontitis

Several systemic diseases are associated with an increased risk for periodontitis. In some cases this appears to correlate with reduced expression of antimicrobial proteins.

Diabetes is associated with an increased risk for periodontitis, even in children (Lalla et al. 2007). In a proteomic study of saliva from diabetic children and matched controls, it was noted that the levels of statherin, proline-rich

Table 2. Antibacterial proteins in saliva and gingival crevicular fluid

Protein	Gene	Saliva	References	GCF	References	Targets	Dose	References	Change in periodontitis	References
Adrenomedullin	ADM	0.06 μg/ml	Kapas et al. (2004)	1.8 μg/ml	Lundy et al. (2006)	*P. gingivalis* *S. mutans*	MIC 7.75×10^4 μg/ml MIC 12.5 μg/ml	Allaker et al. (1999)	Up-regulated twofold *P. gingivalis* up-regulates	Lundy et al. (2006) Kapas et al. (2001)
Azurocidin CAP37 Heparin-binding protein	AZU1	MS				*E. coli*	LD_{50} 1.3 μg/ml	Almeida et al. (1996)		
Bactericidal Permeability-Increasing protein	BPI	0.078 μg/ml	Bartunkovaa et al. (2004)			*Gram-negative bacteria*				
Bactericidal/ permeability-increasing protein-like 1	BPIL1	MS								
β-2-microglobulin	B2M	0.38 μg/ml	Michelis et al. (2007)	9.4 μg/ml	Mogi et al. (1999)	*S. mutans*		Ericson, (1984)	3–10-fold up-regulated	Mogi et al. (1999)
β defensin-1 hBD-1	DEFB1	0.15 μg/ml	Mathews et al. (1999)	+	Diamond et al. (2001)	*P. gingivalis* *A. actinomycetemcomitans* *F. nucleatum*	MIC 50 μg/ml MIC 50 μg/ml MIC 20 μg/ml	Ouhara et al. (2005)	Up-regulated by *P. gingivalis*, *P. intermedia* Down-regulated by T. denticola Not changed by *T forsythia*, and *F. nucleatum*	Vankeerberghen et al. (2005), Ji et al. (2007b) Ji et al. (2007b) Ji et al. (2007b)
β defensin-4 β-defensin-2 hBD-2	DEFB4A	0.15 μg/ml	Mathews et al. (1999)	+	Diamond et al. (2001)	*P. gingivalis* *S. mutans*	MIC 34.6- > 250 μg/ml MIC 4–8 μg/ml	Joly et al. (2004)	Up-regulated by *A. actinomycetemcomitans*, *P. gingivalis*, *F. nucleatum*, and *P. intermedia* Not changed by *T. forsythia* and *T. denticola*	Laube et al. (2008), Ouhara et al. (2006), Chung and Dale (2004), Krisanaprakornkit et al. (2000), Ji et al. (2007b) Brissette et al. (2008) Ji et al. (2007b)
β Defensin 103 β-defensin-3 hBD-3	DEFB103A	0.31 μg/ml	Tao et al. (2005)			*P. gingivalis* *A. Actinomycetemcomitans* *S. mutans* *T. Denticola* *F. nucleatum* *B. cepacia* *S. sanguinis* *P. intermedia*	MIC 42.1 μg/ml MIC 45.6 μg/ml MIC 3–5 μg/ml MIC 15.7 μg/ml MIC 4.5–7.8 μg/ml MIC 6.6 μg/ml MIC 31.3 μg/ml MIC 15.7 μg/ml	Ji et al. (2007a) Joly et al. (2004) Garcia et al. (2001)	Up-regulated by *A. actinomycetemcomitans*, *P. gingivalis*, *F. nucleatum* and *P. intermedia* Down-regulated by *T. forsythia*, and *T. denticola*	Ji et al. (2007b), Ouhara et al. (2006), Vankeerberghen et al. (2005) Ji et al. (2007b)
Calcitonin gene-related peptide 1	CALCA	23.5×10^{-6}	Dawidson et al. (1997)	0.013–0.7 μg/ml	Lundy et al. (1999, [10]Awawdeh et al. (2002)	*P. aeruginosa* *S. mutans*	MIC 5.9 μg/ml MIC > 500 μg/ml	El Karim et al. (2008)	Decreased (Not detected) Decreased 20-fold in gingivitis	Lundy et al. (1999)
Calgranulin A Protein S100-A8	S100A8	1.93 μg/ml	Kleinegger et al. (2001)	240 μg/ml (Calprotectin: 570 μg/ml)	Lundy et al. (2001) Kido et al. (1999)	*P. gingivalis* *S. aureus*	MIC 64 μg/ml	Nisapakultorn et al. (2001) Brandtzaeg et al. (1995)	Increase 2–3-fold Increased after therapy Up-regulated by *P. gingivalis* and *F. nucleatum* (Calgranulin)	Lundy et al. (2001), Kojima et al. (2000), Lundy et al. (2000a) Haigh et al. (2010) Milward et al. (2007)
Calgranulin B Protein S100-A9	S100A9	1.93 μg/ml	Kleinegger et al. (2001)	+ (Calprotectin: 570 μg/ml)	Kido et al. (1999)	*See Calgranulin A (Calprotectin)*			Increased in periodontitis, decreased 2–3-fold after periodontal therapy Increased after therapy	Kojima et al. (2000) Haigh et al. (2010)
	CAMP	1.6 μg/ml		+						

Table 2. (Contd.)

Protein	Gene	Saliva	References	GCF	References	Targets	Dose	References	Change in periodontitis	References
Cathelicidin (LL-37)			Tao et al. (2005), Bachrach et al. (2006)		Puklo et al. (2008)	*P. gingivalis* *A. actinomycetemcomitans* *S. gordonii* *P. intermedia* *F. nucleatum* *S. sanguinis*	MIC > 125 μg/ml MIC 37.8 μg/ml MIC 102.6 μg/ml MIC 15.7 μg/ml MIC 4.9 μg/ml MIC 31.3 μg/ml	Ji et al. (2007a)	Up-regulated (Aggressive and Chronic periodontitis) Up-regulated by *F. nucleatum* and *P. intermedia* Not affected by *P. gingivalis*, *T forsythia* or *T. denticola*	Puklo et al. (2008) Ji et al. (2007b) Ji et al. (2007b)
C–C motif chemokine 28	CCL28	0.9 μg/ml	Hieshima et al. (2003)			*S. mutans*	IC_{50} 1.7 μM		Hieshima et al. (2003)	
Cystatin A	CSTA	93 U/mg protein	Blankenvoorde et al. (1997)	24 U/mg protein	Blankenvoorde et al. (1997)					
Cystatin B	CSTB	MS								
Cystatin C	CST3	0.9 μg/ml	van Gils et al. (2003), Henskens et al. (1996)	1.15 μg/ml (children)	Ulker et al. (2008)	*P. gingivalis*		Blankenvoorde et al. (1998)	Down-regulated by *P. gingivalis*	Elkaim et al. (2008)
Cystatin D	CST5	3.8 μg/ml	Freije et al. (1993)							
Cystatin S	CST4	53 (stim)-116 μg/ml	Baron et al. (1999), Henskens et al. (1996)	ND	Blankenvoorde et al. (1997)	*P. ginigvalis*		Blankenvoorde et al. (1998)	Up-regulated in saliva	
Cystatin SA	CST2	78 μg/ml (stim)	Baron et al. (1999)							
Cystatin SN	CST1	39 μg/ml (stim)	Baron et al. (1999)	ND	Blankenvoorde et al. (1997)					
Fibronectin	FN1	1.2–0.13 (stim) μg/ml	Llena-Puy et al. (2004), Tynelius-Bratthall et al. (1986)	106 μg/ml	Lopatin et al. (1989)	*P. gingivalis* *S. mutans*		Murakami et al. (1998) Llena-Puy et al. (2000)	Decrease 2-fold with less intact fibronectin in periodontitis Decrease 30-fold in gingivitis Down-regulated by *A. actinomycetemcomitans* protease	Lopatin et al. (1989) Lopatin et al. (1989) Wang et al. (2001)
Hemoglobin β-globin α globin	HBB HBA1 HBA2	+		MS		*E. coli*		Parish et al. (2001)	Increased due to bleeding	
Heparin binding growth factor Fibroblast growth factor	FGF1 FGF2	0.87 pg/ml (FGF2)	Ishizaki et al. (2000)	MS (FGF1)		*E. coli* *P. aeruginosa* *B. subtilis*		Malmsten et al. (2007)		
Histatin 1	HTN1	10.1 μg/ml (parotid) 34.7 μg/ml (SM/SL)	Johnson et al. (2000)							
Histatin 3 (Histatin 5)	HTN3	7.3 μg/ml (Parotid) 10.2μg/ml (SM/SL)	Johnson et al. (2000)			*A. actinomycetemcomitans* *Neutralizes leukotoxin*		Murakami et al. (2002b)		
HNP-1 Neutrophil defensin 1	DEFA1	8.6 μg/ml	Goebel et al. (2000)	0.0012 μg/ml*	Puklo et al. (2008)	*S. mutans* *P. aeruginosa* *A. actinomycetemcomitans* *P. gingivalis*	MIC 4.1 μg/ml MIC 10.3 μg/ml No activity (>500 μg/ml) No activity (>200 μM)	Lundy et al. (2008), Miyasaki et al. (1990) Raj et al. (2000)	15-fold up-regulated (aggressive perio.)* 60-fold up-regulated (chronic perio.)*	Puklo et al. (2008)
		5.6 μg/ml		0.0012 μg/ml*						Puklo et al. (2008)

HNP-2 Neutrophil defensin 2	DEFA1 DEFA3		Goebel et al. (2000)		Puklo et al. (2008)	*P. gingivalis* *A. actinomycetemcomitans*	No activity (>200 μM) No activity (>500 μg/ml)	Raj et al. (2000) Miyasaki et al. (1990)	15-fold up-regulated (aggressive perio.)* 60-fold up-regulated (chronic perio.)*	
HNP-3 Neutrophil defensin 3	DEFA3	0–2.7 μg/ml	Gardner et al. (2009)	0.0012 μg/ml*	Puklo et al. (2008)	*P. gingivalis* *A. actinomycetemcomitans*	No activity (>200 μM) No activity (>500 μg/ml)	Raj et al. (2000) Miyasaki et al. (1990)	15-fold up-regulated (aggressive perio.)* 60-fold up-regulated (chronic perio.)*	Puklo et al. (2008)
HNP-4 Neutrophil defensin 4	DEFA4			MS		*E. coli*	LD_{50} 0.085 μ μg/ml	Wilde et al. (1989)		
Lactoferrin Lactotransferrin	LTF	20 μg/ml	Shugars et al. (2001)	600 μ/ml but concentration highly variable	Jentsch et al. (2004) Friedman et al. (1983), Adonogianaki et al. (1993)	*P. gingivalis* *A. actinmomycetemcomitans*	35% growth inhibition at 2 mg/ml apoLf 1.9 μM apoLf (iron-free) kills 99.9% in 3 h	Aguilera et al. (1998) Kalmar and Arnold, 1988)	Down-regulated 1.7-fold Highly variable	Wu et al. (2009b) Friedman et al. (1983), Adonogianaki et al. (1993), Jentsch et al. (2004), Adonogianaki et al. (1996)
Lactoperoxidase Salivary peroxidase	LPO	1.9 μg/ml	Thomas et al. (1994a)	+	Ashby, 2008)	*A. actinomycetemcomitans* *Oral Streptococci* *P. gingivalis*		Ihalin et al. (2003) Thomas et al. (1994b) Ihalin et al. (2001)		
Long palate, lung and nasal epithelium carcinoma-associated protein 1 Von Ebner minor salivary gland protein	LPLUNC1	MS								
Lysozyme C	LYZ	40 μg/ml	Allgrove et al. (2008), Klimiuk et al. (2006), Shugars et al. (2001), Rudney and Smith (1985)	+	Friedman et al. (1983), Jentsch et al. (2004)	*Gram-positive bacteria*			Down-regulated in periodontitis Increased in juvenile periodontitis	Ito et al. (2008) Friedman et al. (1983)
Mucin 7 MG2	MUC7	4–10 mg%	Payment et al. (2001)			*Oral Streptococci* *A. actinomycetemcomitans*		Amerongen et al. (1995) Groenink et al. (1996)		
Myeloperoxidase	MPO	3 μg/ml (stim)	Thomas et al. (1994a)	0.3–5.5 μg/ml	Ortiz et al. (1997), Puklo et al. (2008)	*A. actinomycetemcomitans* *Oral Streptococci* *P. gingivalis*		Miyasaki et al. (1986) Ihalin et al. (2001)	Decrease after periodontal therapy	Kaner et al. (2006)
Neuropeptide Y	NPY	41.4×10^{-6} μg/ml	Dawidson et al. (1997)			*P. aeruginosa* *S. mutans*	MIC 134.3 μg/ml MIC 210.9 μg/ml	El Karim et al. (2008)		
Palate lung nasal epithelium clone palate, lung and nasal epithelium carcinoma-associated protein	PLUNC	MS								
Peptidoglycan recognition protein 1	PGLYRP1	MS (SM/SL)		MS		*S. aureus* *E. coli*	LD_{99} 60 μg/ml LD_{99} 30 μg/ml	Wang et al. (2007)		
Peptidoglycan recognition protein 3	PGLYRP3					*S. aureus* *Gram-negative bacteria*	LD_{99} 45 μg/ml LD_{99} 30 μg/ml	Wang et al. (2007)		
Peptidoglycan recognition protein 4	PGLYRP4					*S. aureus* *Gram-negative bacteria*	LD_{99} 45 μg/ml LD_{99} 200 μg/ml	Wang et al. (2007)		

Table 2. (Contd.)

Protein	Gene	Saliva	References	GCF	References	Targets	Dose	References	Change in periodontitis	References
Prolatin-inducible protein	PIP	MS				*Streptococci*		Nistor et al. (2009)		
Proline-rich proteins	PRH1 PRH2 PRB1 PRB3 PRB2 PRB4	MS		MS		*Oral bacteria*		Lamkin and Oppenheim (1993)		
Psoriasin Protein S100-A7	S100A7	MS				*E. coli*	MBC 100 μg/ml	Michalek et al. (2009)		
Salivary agglutinin GP340 DMBT1	DMBT1	MS				*Oral streptococci* *A. actinomycetemcomitans*		Ligtenberg et al. (2007) Groenink et al. (1996)		
Secretory leukoprotease inhibitor protein	SLPI	2.9 μg/ml	Shugars et al. (2001), Lin et al. (2004)			*P. aeruginosa* *P. gingivalis* *S. aureus*	LD_{95} 2.5 μM	Simpson et al. (1999)	79.7 pg/ml in periodontitis Increased 3–4-fold post-treatment	Nakamura-Minami et al. (2003)
Short palate, lung and nasal epithelium carcinoma-associated protein 2 Parotid Secretory Protein	SPLUNC2	MS				*P. aeruginosa*		Geetha et al. (2003)	Up-regulated 3.3-fold Up-regulated by *P. gingivalis*	Wu et al. (2009b) Shiba et al. (2005)
SKALP Skin-derived anti-leukoproteinase Elafin	PI3	0.02 μg/ml	Tjabringa et al. (2005) Lee et al. (2002)			*P. aeruginosa*	LD_{96} 2.5 μM	Simpson et al. (1999)	Up-regulated by *P. gingivalis* Degraded by gingipain	
Statherin	STATH	26.5 μg/ml	Contucci et al. (2005)	MS		*Oral anaerobes*	MIC < 12.5 μg/ml, > 100 μg/ml	Kochanska et al. (2000)		
(Substance P) Protachykinin-1	TAC1	7.5×10^{-6} μg/ml	Dawidson et al. (1997)	0.061–0.11 μg/ml	Awawdeh et al. (2002) Linden et al. (1997)	*P. aeruginosa* *S. mutans*	MIC 15.7 μg/ml MIC 171.6 μg/ml	El Karim et al. (2008)	No change Decreased post-treatment	Linden et al. (1997) Lundy et al. (2000b)
Surfactant Protein A Pulmonary surfactant-associated protein A1	SFTPA1	0.9 μg/ml	Simpson et al. (2005)			*Bacteria*		Korfhagen (2001)		
Transferrin Serotransferrin	TF	6.5 μg/ml (blood contamination?)	Suh et al. (2009)	MS						
Vasoactive Intestinal Peptide	VIP	39.9×10^{-6} μg/ml	Dawidson et al. (1997)			*P. aeruginosa* *S. mutans*	MIC 4.1 μg/ml MIC 150.7 μg/ml	El Karim et al. (2008)		

+, present; *, HNP 1–3 (neutrophil defensins).

MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; MFC, minimal fungicidal concentration; LD_{xx}, concentration that kills XX% of bacteria; HNP, human neutrophil peptide; α-defensin, MS, mass spectrometry detection of proteins in unstimulated whole saliva (Xie et al., 2005, [193]Wilmarth et al., 2004).

Gingival crevicular fluid MS data are from (Ngo et al., 2010).

A. actinomycetemcomitans, Actinobacillus actinomycetemcomitans; *B. cepacia*, *Burkholderia cepacia*; *B. subtilis*, *Bacillus subtilis*; *E. coli, Escherichia coli*; *F. nucleatum, Fusobacterium nucleatum; P. aeruginosa, Pseudomonas aeruginosa; P. gingivalis, Porphyromonas gingivalis*; *P. intermedia, Prevotella intermedia; S. aureus, Staphylococcus aureus; S. gordonii, Streptococcus gordonii; S. mutans, Streptococcus mutans; S. sanguinis, Streptococcus sanguinis; T. denticola, Treponema denticola; T. forsythia, Tannerella forsythia*.

peptides P-B and P-C, Histatin 1 and 3 were significantly reduced in diabetes (Cabras et al. 2010). In contrast, human neutrophil peptide (HNP)-1,2,4 and S100A9 were up-regulated in diabetic patients compared with controls. Thus, the altered complement of salivary antimicrobial proteins may contribute to periodontal disease in young diabetic patients.

Morbus Kostmann disease is a severe congenital neutropenia that is associated with severe periodontitis (Putsep et al. 2002). The saliva, plasma and neutrophils from Kostmann patients are deficient in LL-37 and patients exhibit a 30% decrease in α-defensins. This is not an across-the-board reduction in antimicrobial proteins because plasma lactoferrin content is normal (Putsep et al. 2002). In addition, treatment with granulocyte-colony-stimulating factor restores the number of neutrophils to normal but patients continue to lack LL-37 and exhibit periodontal disease (Putsep et al. 2002, Carlsson et al. 2006). A bone marrow transplant in a single patient restored both neutrophils numbers and the levels of LL-37 and no further dental problems were noted. Similarly, patients with Papillon-Lefèvre syndrome and Haim-Munk syndrome also exhibit low levels of LL-37 and develop periodontitis (de Haar et al. 2006). In these patients, the LL-37 precursor cathelicidin is present at normal levels but little is processed to the active LL-37 peptide due to allelic mutations of the cathepsin C gene CTSC (Hart et al. 2000).

Functional Families of Antimicrobial Proteins in the Oral Cavity, See Tables 1 and 2 for details

Oral tissues express a large variety of AMPs, which may contribute to the host-defense of the oral cavity, although their exact mode of action remains to be determined (Chung et al. 2007, Diamond et al. 2008). At least 45 AMPs are secreted by oral epithelial cells, neutrophils and salivary glands. All are found in saliva and a subset are also found in gingival crevicular fluid (Gorr 2009). Several antimicrobial peptides are highly concentrated in gingival crevicular fluid compared with saliva: Adrenomedulin and β-2-microglobulin are enriched about 30-fold in gingival crevicular fluid while the concentrations of calgranulins, fibronectin, substance P and calcitonin gene-related peptide (CGRP) are 100–10,000-fold higher in gingival crevicular fluid than whole saliva. In contrast, the concentrations of the α-defensins are 1000-fold lower in gingival crevicular fluid than saliva. The high expression of some antimicrobial peptides in gingival crevicular fluid may be due to high local expression rather than saliva contamination of gingival crevicular fluid samples (Griffiths et al. 1992). Alternatively, AMPs may be selectively sequestered by binding to the tissue in the gingival pocket.

The diversity of AMP gene products is further amplified by post-translational modifications (Ramachandran et al. 2006, Messana et al. 2008) or gene polymorphisms (Oppenheim et al. 2007, Whitelegge et al. 2007). This diversity presumably protects the oral tissues from invasion or infection by the large variety of microorganisms that enter the mouth and airways. As noted above, the resident flora is maintained in a balance between pathogenic and commensal bacteria. Interestingly, the minimal inhibitory concentrations of most AMPs to oral bacteria are higher than their concentrations in the gingival crevicular fluid. Thus it is not clear if the AMPs exert direct antibacterial activity, act as a group or if these peptides are acting as sentinels of bacterial status that stimulate other aspects of the immune system (Diamond et al. 2008). The rapid growth of bacterial biofilms in the absence of oral hygiene supports the view that the AMPs do not serve primarily to kill and eliminate oral bacteria but may serve to maintain the balance between resident pathogens and commensals and as sentinels for invading microorganisms.

Individual testing of biological activity of AMPs in vitro has revealed functional families that cover a broad range of biological activities against oral bacteria. However, it is not yet clear why the oral complement of AMPs leads to maintenance of bacterial colonization by commensals and pathogens, which can increase to biofilm formation in the absence of oral hygiene, while the similar complement of AMPs in the airways maintain a near sterile environment (Diamond et al. 2008). The promise of antimicrobial peptide therapy may be realized by over-expressing or supplementing individual antimicrobial peptides for oral therapy or by devising ''cocktails'' of antimicrobial peptides to combat a subset of oral pathogens.

Cationic peptides

Cationic peptides is a large functional family that is represented in oral cavity and airways. Depletion of cationic AMPs from human airway fluid also eliminates the antibacterial activity (Cole et al. 2002). It is not clear if the cationic proteins in saliva play a similar role. However, ion-exchange fractionation of human saliva identified fractions that exhibited antimicrobial activity, which was not apparent in the starting material (S.-U. Gorr, unpublished observation).

The cationic peptide functional family consists of peptides that typically are bactericidal and/or bacteriostatic and includes adrenomedullin, α-defensins (HNP), β-defensins, cathelicidin, histatins 1 and 3, statherin, C–C motif chemokine 28 (CCL28), azurocidin and the neuropeptides CGRP, substance P neuropeptide Y and vasoactive intestinal peptide (Table 1) (Gorr 2009).

As an example of this functional family, LL-37 is a cationic peptide that is derived from the 18 kDa precursor protein cathelicidin by proteolytic cleavage. Cathelicidin is expressed in neutrophils and epithelial cells and LL-37 is found in saliva and gingival crevicular fluid (Murakami et al. 2002a, Puklo et al. 2008). LL-37 exhibits dual function by both killing bacteria and neutralizing the lipopolysaccharide from Gram-negative bacteria. As is the case for several AMPs, the activity of LL-37 is partially inhibited by saliva. On the other hand, saliva protects the peptide from proteolytic inactivation by gingipain proteases secreted by the periodontal pathogen *P. gingivalis* (Gutner et al. 2009).

Bacterial agglutination and adhesion

Several antibacterial proteins are active in bacterial agglutination or adhesion. These include the small salivary mucin-7 (MUC7) (MG2), which promotes bacterial agglutination, surfactant protein-A, proline-rich proteins, prolactin-inducible protein and β-2-microglobulin, which is notably present in most (82%) biopsies from aggressive periodontitis patients but largely absent from normal controls and chronic severe periodontitis specimens (Syrjanen et al. 1985). Saliva from prolactin-inducible protein-knock-out mice exhibit significantly lower agglutination of oral bacteria than saliva from wild-type control

mice, suggesting that prolactin-inducible protein contributes to host-defense of the oral cavity by agglutinating oral bacteria (Nistor et al. 2009). The salivary agglutinin/*GP340*/*Deleted in Malignant Brain Tumors-1* (*DMBT1*) is a large glycoprotein that contains multiple scavenger receptor cysteine-rich repeats. The protein is expressed in mucosal tissues, including salivary glands and is found in saliva (Wilmarth et al. 2004, Xie et al. 2005, Denny et al. 2008) DMBT1 has not been linked directly to periodontitis but *DMBT1* polymorphisms have been associated with a high incidence of caries (Jonasson et al. 2007).

Fibronectin is a 2386 amino acid glycoprotein that is expressed in hepatocytes and epithelial cells and is present in saliva (Llena-Puy et al. 2004). The protein induces bacterial agglutination and plays a role in reducing bacterial adhesion to oral surfaces (Llena-Puy et al. 2000). Fibronectin also binds directly to fimbrillin from *P. gingivalis* and thereby inhibits the fimbrillin-induced expression of inflammatory cytokines in macrophages (Murakami et al. 1998). Low levels of fibronectin are correlated with high levels of *Streptococcus mutans* in children (Llena-Puy et al. 2000) and periodontitis is associated with a relative lack of fibronectin in adults (Murakami et al. 1998).

Metal ion chelators

These proteins inhibit bacterial growth by acting as divalent cation scavengers. The 80 kDa iron-binding glycoprotein lactoferrin/lactotransferrin, which acts as a scavenger of Fe^{3+} ions, exhibits gene polymorphisms that have been associated with aggressive periodontitis (Wu et al. 2009a). The other members of this functional family, Calgranulin A (S100A8) and calgranulin B (S100A9) form a dimer named calprotectin, which is up-regulated in periodontitis and detected in increased levels in gingival crevicular fluid of periodontal patients (Kido et al. 1999). Calprotectin protects cells from bacterial invasion, including the periodontal pathogen *P. gingivalis* (Nisapakultorn et al. 2001).

Protease inhibitors

Proteases are important virulence factors for several bacteria. As an example, *P. gingivalis* secrete gingipains that bind and cleave multiple host-proteins, including activation of coagulation factors, cleavage of fibrinogen (Imamura 2003) and cleavage of IL-8. The IL-8 cleavage products differ by cellular origin of IL-8 and differentially affect chemotaxis and activation of neutrophils in response to IL-8 (Dias et al. 2008). Gingipains also activate protease-activated receptors (e.g., PAR2), which mediates the expression of the AMPs hBD-2 and CCL20 in gingival epithelial cells (Dommisch et al. 2007). Several protease inhibitors are found in saliva and gingival crevicular fluid to inactivate these and other proteases. These include the *cystatins*, a family of 14 human genes and two pseudogenes. Seven of these genes are expressed in saliva and act by blocking the action of bacterial proteases (Dickinson 2002).

Secretory leucocyte protease inhibitor and SKALP (skin-derived anti-leucoprotease)/Elafin, also known as *ESI* (elastase-specific inhibitor). The latter is expressed in human submandibular gland (Lee et al. 2002) and saliva (Tjabringa et al. 2005, Lee et al. 2002). The protein has an N-terminal domain that acts as a transglutaminase substrate and a C-terminal domain that exhibits anti-elastase activity. In addition, the protein kills both Gram-negative and Gram-positive bacteria. This activity depends on the presence of both peptide domains (Simpson et al. 1999). Elafin consists of a single four-disulphide core protein domain, with the reactive site loop expanding to the outside. The rigid, strongly stabilized core renders elafin unusually stable and resistant to proteolysis (Guyot et al. 2005). Elafin expression is induced in inflamed epithelial tissues and *P. gingivalis* up-regulates Elafin expression in gingival epithelial cells. While the protein is highly resistant to most proteases, elafin is degraded by gingipains from *P. gingivalis* (Kantyka et al. 2009). The ability to disturb the balance between proteases and protease inhibitor in infected gingival tissue contributes to the degradation of host proteins. Indeed, the protease inhibitors SLPI and elafin are often inactivated at sites of inflammation. Inactivation may be due to microbial proteases, e.g. gingipains, or host proteases secreted by neutrophils at the site of inflammation (Sallenave 2010). Protease inhibitors based on the sequence of SKALP/Elafin may prevent the tissue destruction caused by inflammatory and bacterial proteases.

Peroxidases

Lactoperoxidase and myeloperoxidase are found in saliva where they form the principal components of the peroxidase system of saliva (Ihalin et al. 2006). Both enzymes catalyse the oxidation of thiocyanate ions (SCN^-) by hydrogen peroxide to form the bactericidal reaction product hypothiocyanite ($OSCN^-$) (Ashby 2008). Further bactericidal products are produced by the oxidation of chloride and iodide (Miyasaki et al. 1986, Ihalin et al. 2001, Ashby 2008). The reaction products produced by both peroxidases are active against *A. actinomycetemcomitans*, *P. gingivalis* and oral streptococci (Miyasaki et al. 1986) (Ihalin et al. 2001). The concentration of myeloperoxidase in gingival crevicular fluid is about 5 μg/ml with no significant differences between chronic periodontitis, aggressive periodontitis and healthy controls, respectively (Puklo et al. 2008). On the other hand, antibiotic treatment of periodontal patients for 3 months resulted in reduced levels of myeloperoxidase in gingival crevicular fluid (Kaner et al. 2006).

Activity against bacterial cell walls

Two types of proteins show activity against bacterial cell walls. Lysozyme (1,4-β-*N*-acetylmuramidase) is a 14 kDa protein that is expressed widely in mucosal epithelia and found in saliva and gingival crevicular fluid. The enzyme is mainly active against the cell wall of Gram-positive bacteria by hydrolysing peptidoglycans. The other protein type with activity against cell wall peptidoglycans are peptidoglycan recognition proteins 3 and 4, which are expressed in mucosal epithelia, including salivary glands. These large proteins (89–115 kDa disulphide linked homo- or hetero-dimers) bind to cell wall peptidoglycans but do not permeabilize bacterial membranes (Lu et al. 2006). The proteins are bacteriostatic for most Gram-positive and Gram-negative bacteria but not for non-pathogenic bacteria or *C. albicans* (Lu et al. 2006).

Peptides Derived from Host-Defense Proteins

In addition to the already identified AMPs, new peptides are continually discovered or developed from existing proteins. Hundreds of existing AMPs

are accessible in on-line databases, including CAMP: collection of anti--microbial peptides http://www.bicnirrh.res.in/antimicrobial/index.php (Thomas et al. 2010), AMSDb: anti-microbial sequence database (http://www.bbcm.units.it/ ~ tossi/pag1.htm) (A. Tossi, University of Trieste), APD: antimicrobial peptide database (http://aps.unmc.edu/AP/main.php) (Wang and Wang 2004) and PepBank http://pepbank.mgh.harvard.edu/ (Shtatland et al. 2007). These peptides and the numerous possible modifications represent a rich source for the identification and testing of antimicrobials with activity/toxicity profiles that are beneficial against periodontal pathogens.

Peptides of human origin have particular promise as therapeutic agents with low host toxicity. In addition, the co-evolution of these peptides with the oral microflora suggests that they may result in lower rates of bacterial resistance (Peschel and Sahl 2006). It is important to note that bacterial resistance has been observed in vitro and the development of resistance could potentially result in severe consequences for the effectiveness of the endogenous human peptide (Bell and Gouyon 2003). This concern is somewhat mitigated, however, by the alternate host-defense mechanisms that function in the human body such that we do not rely on a single peptide for protection (Hancock 2003).

Human saliva may be a rich source of new AMPs, in addition to the existing proteins described above. The human salivary proteome contains over 1100 proteins (Xie et al. 2005, Denny et al. 2008), many of which have not yet been functionally identified. One approach for the identification of new peptides is the analysis for antimicrobial consensus motifs in peptide sequences (Yount and Yeaman 2004). Structural similarities of new proteins and existing proteins also provide functional clues. Thus, the PLUNC family was recently identified in the oral cavity and airway epithelia (Bingle and Craven 2002). Based on the sequence of the PLUNC proteins and a predicted similarity to the known antibacterial and endotoxin-binding proteins bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP), it was predicted that these proteins contribute to host-defense in the oral cavity and airways (Bingle and Gorr 2004). Comparative analysis of known anti-endotoxin peptides in BPI and LBP (Dankesreiter et al. 2000) with the predicted structure of the PLUNC protein Parotid Secretory Protein, led to the design of a series of antimicrobial peptides that exhibit anti-endotoxin activity (Geetha et al. 2005), bacterial agglutinating activity and act to increase bacterial clearance by macrophages in cell culture (Gorr et al. 2008).

As a further example of antimicrobial peptides derived from human proteins, hemoglobin gives rise to the antibacterial peptides hemocidins. These peptides are active at low pH and potentiate the activity of other AMPs, including LL-37, lysozyme and defensins (Mak et al. 2007). While hemoglobin is found in both saliva and gingival crevicular fluid, the hemocidins have not yet been described in the oral cavity. Their function in conjunction with other AMPs at acidic pH may make them attractive agents for the treatment of dental biofilms.

Anti-microbial peptides constitute a relatively new class of compounds that has shown promise as effective antibiotics to many bacterial species and fungi in vitro. A recent review of the patent literature shows the broad range of peptides in development (Pathan et al. 2010). It is hoped that this class of antibiotics will include clinically useful peptides that could exhibit both high in vivo efficacy and low host toxicity. However, a 2005 review noted the continuing challenges in obtaining approval from the U.S. Food and Drug Administration for these peptides (Gordon et al. 2005). Thus, continued peptide selection and optimization for in vivo conditions is needed to further develop these peptides for therapeutic use.

Targeting of Antimicrobial Peptides

Broad-spectrum antibiotics and AMPs can reduce beneficial commensal bacteria in the oral cavity and broad application of AMPs may be associated with patient toxicity. As an approach to overcome these concerns, systems are being developed to more precisely deliver the AMPs to the target bacteria. Specifically targeted antimicrobial peptides consist of a targeting peptide, linker region and antimicrobial peptide component. The targeted peptides retained antimicrobial activity and selectively killed targeted bacteria in mixed cultures of *Pseudomonas aeruginosa*, *S. mutans*, *Escherichia coli* and *Staphylococcus epidermis* (He et al. 2009). Using this building block approach additional targeting domains were combined with antimicrobial domains to generate peptides that specifically targeted and killed *S. mutans* (He et al. 2010).

Anti-Microbial Peptide Mimetics

As outlined above, the clinical use of AMPs is associated with significant challenges. In some cases the natural peptides have been modified to generate peptides with more favourable efficacy/toxicity profiles (Zasloff 2002a). An alternate approach is the design and synthesis of peptide mimetics that retain the biological activity of AMPs but are more readily produced, exhibit favourable therapeutic index and are stable under physiological conditions (Tew et al. 2006). One such non-peptide compound mPE shows low toxicity, is active against clinical isolates, including antibiotic-resistant bacteria and did not cause resistance in *Staphylococcus aureus* over 17 passages. mPE is active against both Gram-negative and Gram-positive oral pathogens in both the planktonic and biofilm culture (Tew et al. 2006). Similar mimetics based on the structure of defensin have shown a high therapeutic index in pre-clinical studies (Beckloff et al. 2007). The functional domain of BPI protein has been used to design a modified D-enantiomer (XOMA 629, Xoma, Berkeley, CA, USA), which is highly active against a wide variety of bacteria and fungi (Lim et al. 2001). Structure function analysis of naturally occurring peptides will provide additional sources for the design and tuning of peptide mimetics that take advantage of the biological activity of AMPs but avoid some of the challenges associated with their synthesis and therapeutic use. In the oral cavity, it may be of particular importance to develop antibiotics that control harmful pathogens without eliminating beneficial commensals that are needed for microbiological balance.

Regulation of Antimicrobial Peptide Expression

Rather than use AMPs as exogenous therapeutic agents, the stimulation of endogenous peptide expression is a possible approach to antimicrobial therapy. Although many AMPs are regulated by bacteria and bacterial toxins (Diamond et al. 2008, Gorr 2009, Dommisch et al. 2010) this is not an attractive option for

therapy. However, alternative regulatory mechanisms have been described. Thus, LL-37 and hBD-2 are up-regulated by 1,25-dihydroxy vitamin D3 in several human cell types (Wang et al. 2004) and PSP expression is up-regulated by 17-β estradiol in human gingival epithelial cells (Shiba et al. 2005).

An interesting regulatory system for antimicrobial peptides has been described in the intestine (Gudmundsson et al. 2010). Shigellosis is associated with reduced intestinal levels of LL-37 and hBD-1. The rabbit homologue of LL-37, CAP-18 is induced by sodium butyrate in a rabbit model of the disease. This treatment reduced clinical illness and the bacterial load in the stool (Raqib et al. 2006). A clinical trial is underway to determine if butyrate is an effective treatment in human shigellosis patients (ClinicalTrials.gov Identifier: NCT00800930). It is not clear if this approach can be directly applied to periodontal disease since gingival epithelial cells undergo apoptosis and autophagy in the presence of butyrate (Tsuda et al. 2010).

A current clinical trial is examining the expression of chromogranin A in periodontitis. The endocrine protein chromogranin A has been detected in saliva (Kanno et al. 2000) and is the precursor for potential antimicrobial peptides (Shooshtarizadeh et al. 2009). The goal is to determine if chromogranin peptides exhibit antimicrobial activity in gingival crevicular fluid samples from diabetic patients with and without periodontitis (ClinicalTrials.gov Identifier: NCT00399620). This trial is diagnostic and does not include treatment or prevention using the chromogranin peptides.

Clinical Applications

The limits of conventional antibiotic/antimicrobial approaches in the treatment of periodontitis are well recognized (Herrera et al. 2008, Sanz and Teughels 2008, Angaji et al. 2010). Thus, new approaches for non-mechanical periodontal therapy are desirable. An attractive option is to mine the innate host-defense system for potential therapeutic compounds that would be effective against periodontal pathogens with limited side effects and host toxicity. The clinical use of AMPs is associated with several perceived advantages, including their broad-spectrum activity (antibacterial, antiviral, antifungal), rapid onset of killing, cidal activity, potentially low levels of induced resistance, and concomitant broad anti-inflammatory activities. On the other hand a number of disadvantages must be overcome, including the systemic and local toxicity, reduced activity based on salt, serum, and pH sensitivity, susceptibility to proteolysis, pharmacokinetic and pharmacodynamic issues, sensitization and allergy after repeated application, natural resistance, confounding biological functions (e.g., angiogenesis) and high manufacturing costs (Gordon et al. 2005). Despite the discovery of hundreds of AMPs in the past 25 years, only few are in current clinical use. One such peptide is polymyxin B, which is in clinical use for ophthalmic infections, often in formulations that include Neosporin. The peptide shows high antibacterial activity but is also associated with significant toxicity. Thus, polymyxin use was discontinued for many years but has recently resumed in lower doses. Polymyxin E (colistin) is also in clinical use but is associated with similar nephrotoxicity and neurotoxicity at high doses. Despite these drawbacks, the rise in bacterial resistance to other antibiotics has led to a re-evaluation of these ''older'' AMPs (Stein and Raoult 2002).

A recent review noted that no new peptide antibiotics have been approved by the US Food and Drug Administration in recent years (Gordon et al. 2005), although research and clinical trials are ongoing for several promising peptides and peptide mimetics (Zhang and Falla 2009). These include the Histatin 5 derived 12-mer (PAC 113) (PacGen Biopharmaceuticals, Vancouver, British Columbia, Canada), which appeared to prevent the development of experimental gingivitis in healthy subjects (Paquette et al. 2002). PAC-113 has completed phase IIb clinical trials as a mouth rinse for the treatment of oral candidiasis in HIV patients. Other AMPs include the magainin mimetic mPE (Polymedix Inc., Radnor, PA, USA); a synthetic decapeptide KSL-W and a mimetic based on defensins (PMX-30063, Polymedix Inc.) (Zhang and Falla 2009). The latter has passed Phase I safety evaluation in healthy subjects and Phase II trials are planned for 2010. The functional families of AMPs are large and diverse. Thus, while the development of antimicrobial peptides has not yet resulted in new approved therapeutics, the continued development of these drugs is justified by the ongoing struggle with bacterial infections and resistance to existing antibiotics.

Conclusions

While treatment of periodontitis with conventional antibiotics has had mixed success and does not appear to be effective in the absence of mechanical debridement (Herrera et al. 2008), AMPs have unique properties that may make them suitable for the prevention or elimination of oral biofilms and the associated inflammation of gingival tissue. Many AMPs are both bactericidal and anti-inflammatory and can block the inflammatory effects of bacterial toxins. The peptides have co-evolved with oral bacteria, which have not developed significant resistance to these peptides. Although these peptides do not appear to prevent biofilm formation on their own, they are often found in saliva in less than effective concentrations. Thus, they may prove effective when administered in higher doses or as an adjunct to other therapy. Peptides of human origin are unlikely to exhibit toxicity in near physiological concentrations. A key to successful antimicrobial peptide therapy may be the use of multiple AMPs to mimic the in vivo mix of antibacterial activities.

Forty-five antibacterial proteins are found in human saliva and many of these are also found in gingival crevicular fluid. Careful mining of the increasing number of proteins identified in saliva, gingival crevicular fluid and oral epithelial cells by proteomic approaches, promises to reveal additional AMPs. Much work remains to be performed to determine how these peptides interact to achieve the antibacterial properties of healthy oral tissues.

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Address:
Sven-Ulrik Gorr
University of Minnesota School of Dentistry
18-208 Moos Tower
515 Delaware Street SE
Minneapolis
MN 55455
USA
E-mail: sugorr@umn.edu

Clinical Relevance

Scientific rationale for study: Human antibiotic peptides and proteins have promise as novel antibiotic reagents for the treatment of periodontal disease.

Principal Findings: Saliva and gingival crevicular fluid contains at least 45 different AMPs that belong to different functional families. These proteins and peptides may serve as a source of novel antimicrobial agents that are developed to combat periodontal pathogens with low host-toxicity or bacterial resistance.

Practical Implications: Antimicrobial peptide deficiency is linked to the development of periodontitis. Research on antimicrobial peptides and proteins will provide lead compounds that could be developed into new treatments for periodontal disease.