การตอบสนองทางระบบภูมิคุ้มกันโดยกำเนิดต้านต่อไวรัสในเนื้อเยื่อปริทันต์

ANTIVIRAL INNATE IMMUNE RESPONSE IN PERIODONTAL TISSUE

นริศรา วนวิทย์ / นพดล สะอาดเอี่ยม/ พิมพ์ประภา ฤกษ์เย็น / รังสินี มหานนท์
จุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อ

โรคปริทันต์อักเสบเป็นโรคที่พบบ่อยในช่องปาก เกี่ยวข้องกับการอักเสบเรื้อรังของอวัยวะปริทันต์ และอาจนำไปสู่การสูญเสียฟัน สาเหตุหลัก คือ แบคทีเรีย ข้อมูลในเร็วๆนี้บ่งถึงบทบาทของไวรัสในการเกิดและการดำเนินโรคปริทันต์อักเสบ อย่างไรก็ตามความรู้เกี่ยวกับการตอบสนองทางระบบภูมิคุ้มกันโดยกำเนิดต้านต่อไวรัสในเนื้อเยื่อปริทันต์ยังคงมีน้อยมาก ในปัจจุบันยังไม่มีข้อมูลของโปรตีนต้านต่อไวรัสในเนื้อเยื่อปริทันต์ ดังนั้นการศึกษานี้จึงเป็นการศึกษาแรกในการตรวจหาการแสดงออกของเมสเซนเจอร์อาร์เอนเอ (mRNA) ของโปรตีนต้านต่อไวรัส คือ ซีเครโทรี ลิวโคไซท์ โปรทีเอส อินหิบิเทอร์ (secretory leukocyte protease inhibitor,SLPI), โปรตีน ไคเนส อาร์ (protein kinase R, PKR), โอลิโกอะดีไนเลท ซินเทเตส (oligoadenylate synthetase, OAS) และมิกโซไวรัส รีซิสแทนซ์ เอ (myxovirus resistance A, MxA) ในเนื้อเยื่อปริทันต์ของผู้ป่วยที่เป็นโรคปริทันต์อักเสบและเนื้อเยื่อปริทันต์ที่มีสุขภาพดีด้วยวิธีเรียลไทม์ รีเวอร์สทรานส์คริปชัน-โพลิเมอเรส เชน รีแอคชัน (real-time RT-PCR) ผลการทดลองพบว่ามีการแสดงออกของ mRNA ของโปรตีนต้านต่อไวรัสทั้ง 4 ชนิด ทั้งในเนื้อเยื่อที่เป็นโรคปริทันต์อักเสบและเนื้อเยื่อที่มีสุขภาพดี โดยไม่พบความแตกต่างระหว่างกลุ่มอย่างมีนัยสำคัญ เป็นที่น่าสังเกตว่าการแสดงออกของ SLPI อยู่ในระดับสูงกว่าโปรตีนตัวอื่นๆ โดยสรุปเป็นการศึกษาแรกที่รายงานถึงการปรากฏของโปรตีนต้านต่อไวรัสหลายชนิดในเนื้อเยื่อปริทันต์ทั้งที่เป็นและไม่เป็นโรค ซึ่งบทบาทและหน้าที่ของโปรตีนเหล่านี้จำเป็นต้องมีการศึกษาเพิ่มเติมต่อไป

คำสำคัญ : โปรตีนต้านต่อไวรัส/ ระบบภูมิคุ้มกันโดยกำเนิด/ เนื้อเยื่อปริทันต์

ABSTRACT

Periodontitis is a common chronic inflammatory disease in oral cavity. It affects supporting tooth structure-periodontium and may cause tooth loss. Even though the etiologic importance of bacteria in periodontitis has been understood for decades, several recent studies have documented a role of viruses in the development

and progression of periodontitis. Since there is relatively little available information regarding periodontal innate antiviral immunity, we would like to be the first to investigate mRNA expression of different antiviral proteins (secretory leukocyte protease inhibitor (SLPI), protein kinase R (PKR), oligoadenylate synthetase (OAS), and myxovirus resistance A (MxA)) in periodontitis as compared to healthy tissue by real-time reverse transcription-polymerase chain reaction. In this first report of antiviral protein expression, we demonstrated mRNA expression of all studied antiviral proteins (SLPI, PKR, OAS, and MxA), but no significant differences in all parameters were indicated between periodontitis and healthy group. Interestingly, level of SLPI was higher than others. Further research is required to extend understanding of the role of innate antiviral protein in periodontal disease.

Keywords: antiviral protein/ innate immunity/ periodontal tissue

Introduction

Periodontitis is a chronic bacterial infection that affects the gingiva, periodontal ligament, cementum and bone supporting the teeth. Even though the etiologic importance of bacteria in periodontitis has been understood for decades, several recent studies have also documented a role of viruses in the development and progression of periodontitis (Cappuyns, Gugerli, & Mombelli, 2005).

Innate immunity serves as the first line of defense against invading microorganisms. Innate antibacterial immune response in periodontal disease has been the focus of considerable recent research (Schenkein, 2006; Teng, 2006; Zasloff, 2002). However, to date there is relatively little available information regarding periodontal innate antiviral immunity. Gingival epithelium, the outmost physical barrier of periodontium, plays important role as the local innate non-immune cells. It bears variety of sensing receptors called Toll-like receptors which recognize and activate inflammatory responses not only to bacteria but also to viruses (Mahanonda & Pichyangkul, 2007). In addition to the cellular components, there are the non-cellular components such as secretory innate mediators with antibacterial properties that help protected periodontal mucosa. These include human α-defensins (commonly

known as human neutrophil peptides, HNPs)(Goebel, Mackay, Vickers, & Mather, 2000; Pisano et al., 2005; Puklo, Guentsch, Hiemstra, Eick, & Potempa, 2008), human β-defensins (HBDs)(Diamond, Kimball, Krisanaprakornkit, Ganz, & Dale, 2001; Mathews et al., 1999), cathelicidin (LL37)(Murakami, Ohtake, Dorschner, & Gallo, 2002; Puklo et al., 2008), lactoferrin (Friedman, 2006; McNeely et al., 1995), and secretory leukocyte protease inhibitor (SLPI)(Into et al., 2006; McNeely et al., 1995). Interestingly, these anti-bacterial proteins also have antiviral properties.

It is known that SLPI, protein kinase R (PKR), 2',5'-oligoadenylate synthetase (OAS), and myxovirus resistance A (MxA) are mucosal proteins which implicated in antiviral activity (Franken, Meijer, & Dijkman, 1989; Milush et al., 2007; Santoro et al., 2005; Vijay-Kumar et al., 2005). Secretory leukocyte protease inhibitor (SLPI) is a potent inhibitor of serine proteases (Thompson & Ohlsson, 1986) produced and secreted primarily from epithelial cells lining mucosal surfaces(Franken et al., 1989) and skin (Sorensen et al., 2003), neutrophils (Sallenave, Si Tahar, Cox, Chignard, & Gauldie, 1997), lipopolysaccharide-stimulated macrophages (Jin, Nathan, Radzioch, & Ding, 1997) and cultured human gingival keratinocytes (Jana, Gray, & Shugars, 2005; Westin, Nystrom, Ljungcrantz, Eriksson, & Ohlsson, 2002). It participates in the mucosal defense including by reducing inflammation (Hiemstra, 2002); suppressing matrix metalloproteinase production and activity (Zhang, DeWitt, McNeely, Wahl, & Wahl, 1997); blocking the in vitro growth of selected bacteria (Hiemstra et al., 1996), fungi (Tomee, Hiemstra, Heinzel-Wieland, & Kauffman, 1997), and non-HIV-1 viruses (Beppu et al., 1997). Furthermore, SLPI in GCF has been shown to promote the healing of periodontal tissue after non-surgical treatment in chronic periodontitis patients(Nakamura-Minami, Furuichi, Ishikawa, Mitsuzono-Tofuku, & Izumi, 2003).

At least three major proteins implicated in antiviral activity, such as the protein kinase R (PKR), 2',5'-oligoadenylate synthetase (OAS), and the Mx GTPases, are induced by alpha and beta IFNs, type I IFNs (Frese, Kochs, Feldmann, Hertkorn, & Haller, 1996; Samuel, 2001). When PKR is activated by dsRNA, it leads to halt viral replication (Huang & Schneider, 1991; O'Malley, Mariano, Siekierka, &

Mathews, 1986). PKR may act by shutting down protein synthesis following infection of a cell and limit the transmission of virus to uninfected cells (Clemens, 1997).

OAS constitutively expressed in normal cells in a latent, inactive form, is a marker of immune activation in hepatitis C (Gramenzi et al., 2005; McHutchison et al., 2007; Thimme et al., 2002). Upon binding to dsRNA, this leads to activate RNAseL, then breaks down viral and cellular RNA (Castelli, Wood, & Youle, 1998; Pestka, Langer, Zoon, & Samuel, 1987).

Mx proteins, strictly controlled by type I IFNs, belong to the class of dynamin-like large guanosine triphosphatases (GTPases). The human MxA GTPase accumulates most in the cytoplasm and remains in subcompartment of the endoplasmic reticulum (Accola, Huang, Al Masri, & McNiven, 2002). Low basal levels of human MxA protein may be found in certain cell lines, primary mononuclear cells (Ronni, Melen, Malygin, & Julkunen, 1993), hepatocytes and cholangiocytes (Leifeld et al., 2001). Some Mx GTPases have antiviral activity against diverse viruses, including influenza virus, Thogoto virus, vesicular stomatitis virus, measles virus, bunyavirus, Semliki Forest virus and hepatitis B virus (HBV)(Frese et al., 1996; Frese, Kochs, Meier-Dieter, Siebler, & Haller, 1995; Gordien et al., 2001; Landis et al., 1998; Pavlovic, Zurcher, Haller, & Staeheli, 1990; Schwemmle, Weining, Richter, Schumacher, & Staeheli, 1995; Zhao, De, Das, & Banerjee, 1996). Previous study demonstrated the expression of MxA in plasmacytoid dendritic cells infiltrated in oral mucosa lesions from oral lichen planus (Santoro et al., 2005).

So far there has been no report of these innate antiviral proteins, except for SLPI, in periodontal tissue. In this study, we investigated mRNA expression of different antiviral proteins (SLPI, PKR, OAS, and MxA) in periodontitis as compared to healthy tissue.

OBJECTIVE

We explored the innate antiviral immunity in periodontal tissue. Our specific aim was to investigate mRNA expression of different antiviral protein such as SLPI, PKR, OAS, and MxA in periodontitis tissues and to compare to those

expressions in healthy tissue by real-time reverse transcription-polymerase chain reaction (real-time RT-PCR).

RESEARCH METHODOLOGY

Periodontal tissue samples

Periodontal tissue samples were collected from subjects who had clinically healthy periodontium and untreated severe chronic periodontitis lesion. The 5 biopsies of healthy gingiva with probing depth less than 4 mm were obtained at the time of crown lengthening procedure for prosthetic reasons or impacted tooth removal. The 5 biopsies of periodontitis lesion with gingival inflammation, evidence of bleeding on probing, radiographic evidence of bone loss, and probing depth not less than 6 mm were obtained at the time of removal the teeth that had hopeless periodontal prognosis and not affected by endodontic problem from Periodontal Clinic or Surgery Clinic, Faculty of Dentistry, Chulalongkorn University in March 2009 to December 2009. The excised tissues were immediately placed in sterile tube containing RNAlater (500µl)(Qiagen, Chatsworth, CA, USA) for analysis mRNA expression by real-time quantitative reverse transcription polymerase chain reaction.

The exclusion criteria for both groups included diabetes, bleeding disorders, gross oral pathology or treatment in the previous six months with antibiotics, anti-inflammatory drugs, or medicinal herbs. Informed consent was obtained prior to inclusion in the study. The protocol was approved by the ethics committee of Faculty of Dentistry, Chulalongkorn University.

mRNA expression of antiviral proteins in gingival tissues

To quantitate the tissue amount of SLPI, PKR, OAS, and MxA mRNA, we used real-time reverse transcription polymerase chain reaction. Periodontal tissue samples kept in RNAlater were washed twice and total RNA were separated by using RNeasy Mini kit (Qiagen, USA). 1 µg of total RNA were reverse transcribed using ImProm-II™ Reverse Transcription System for RT-PCR, according to the manufacturer's instructions (Promega, USA). Real-time PCR was performed on the LightCyclerTM (Roche Molecular Diagnostics) in a total volume of 20 µl containing 0.5 µM of each forward and reverse oligonucleotide primer pairs, 10 µl SYBR Green

PCR Master Mix (FastStart Taq DNA polymerase, reaction buffer, dNTP mix, SYBR Green I dye, and $MgCl_2$), 4 µl water, and 5 µl cDNA template. The primers were specific to a conserved region of SLPI, PKR, OAS, and MxA as shown in Table 1.

The temperature program for SLPI, PKR, OAS, and MxA consisted of an initial denaturation step at 95^0C for 10 min, followed by amplification of the template for 45 cycles of 95^0C for 10 sec, 60^0C for 10 sec, and 72^0C for 20 sec (single acquisition). After amplification was completed, a final melting curve was performed at 95^0C for 5 sec, 65^0C for 1 min, and heating to 97^0C using a ramp rate of 0.11 ^{0}C/sec with continuous monitoring of fluorescence. Determination of product specificity depended upon generation of specific PCR products with well-defined melting temperatures of: SLPI 85 ^{0}C, PKR 79 ^{0}C, OAS-1 86 ^{0}C and MxA 84 ^{0}C. Real-time fluorescence measurement was read and a threshold cycle (C_t) value for each sample was calculated by determining the point at which the fluorescence exceeds a threshold limit. Samples were defined as negative if the C_t values exceeded 40 cycles. The mRNA of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as internal control in each sample to control sample to sample variations in RNA concentration. As a negative control, a PCR reaction would be performed without an RT sample. Peripheral blood mononuclear cell (PBMC) would be used as a positive control. Expression of antiviral proteins mRNA levels were calculated by using the comparative Ct method ($2^{-[delta]Ct}$ formula) after normalization to GADPH.

Statistic analysis

Statistical comparisons between healthy and periodontitis group with respect to mRNA expression of antiviral protein were analyzed using SPSS V14.0 software. The independent-sample t-test was used. A value of $p< 0.05$ was considered statistically significant.

Table 1: Primer sequences of antiviral proteins and GAPDH

Product	Forward primer	Reverse primer
SLPI *	TTCCCCTGTGAAAGCTTGATTC	GATATCAGTGGTGGAGCCAAGTC
PKR ##	GCCTTTTCATCCAAATGGAATTC	GAAATCTGTTCTGGGCTCATG
OAS-1 **	CATCCGCCTAGTCAAGCACTG	CCACCACCCAAGTTTCCTGTAG
MxA $	GCTACACACCGTGACGGATATGG	CGAGCTGGATTGGAAAGCCC
GAPDH#	GAAGGCTGGGGCTCATTT	CAGGAGGCATTGCTGATGAT

(* primer sequence by Amigo et al., 2006, ## primer sequence by Farrugia & Cann, 1999, ** primer sequence by Kato et al., 2004, $ primer sequence by Antonelli et al., 1999, # primer sequence by Carraro, Albertin, Forneris, & Nussdorfer, 2005)

Results

Antiviral proteins are expressed on many cells and known as non-cellular compartment of the innate immune system. To obtain additional information on the expression of antiviral proteins in gingival tissue, total RNA from gingival tissue was analyzed by real-time RT-PCR using of specific primers. We found that the mRNAs for SLPI, PKR, OAS-1, and MxA were all expressed in gingival tissues irrespective of their disease entities. Moreover, SLPI had the highest expression level of all the antiviral proteins examined. However, the expression levels of SLPI, PKR, OAS-1, and MxA relative to GAPDH were not significantly different between healthy and periodontitis. ($P = 0.865$ for SLPI, $P = 0.623$ for PKR, $P = 0.360$ for OAS-1 and $P = 0.408$ for MxA). (Figure 1A).

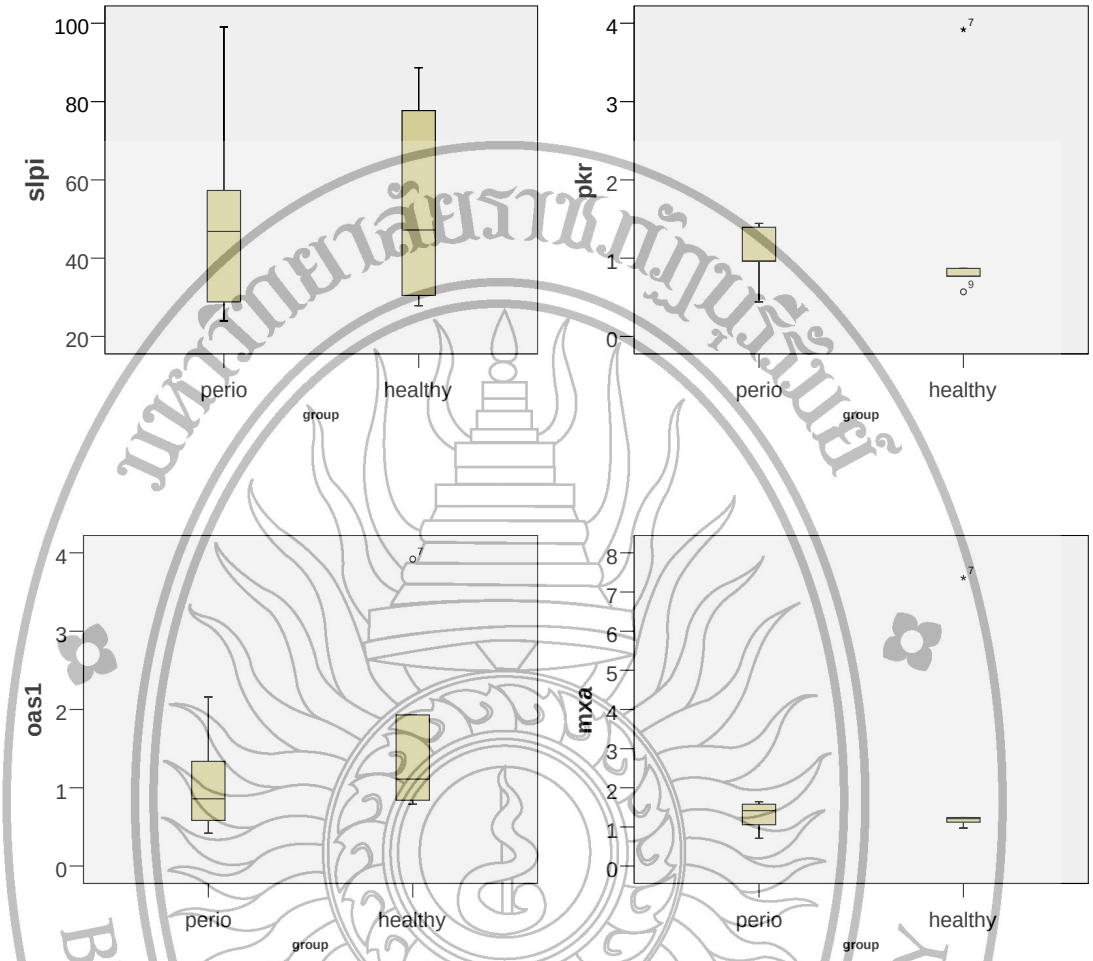

Fig. 1A. Comparison of the relative gene expressions of antiviral proteins between periodontitis (n = 5) and healthy (n = 5) groups. The relative quantity of mRNA was normalized to the relative quantity of GAPDH. The box plots show medians, 25th and 75th percentiles as boxes, and 10th and 90th percentiles as whiskers. Outlying values are shown as open circles. There were no significant differences for all the antiviral proteins examined between periodontitis and healthy.

Discussion and Conclusion

This is the first evidence to show that antiviral protein such as PKR, OAS and MxA are expressed in healthy and periodontitis gingival tissue in addition to SLPI, consistent with the data of Into et al., who showed constitutive expression of

SLPI in healthy gingiva and in inflamed periodontal tissues(Into et al., 2006). Furthermore, the expression of SLPI was much higher compared to other antiviral protein. The reason might be its constitutive expression. It is produced and secreted primarily from epithelial cells lining mucosal surfaces (Franken et al., 1989) and skin(Sorensen et al., 2003), neutrophils (Sallenave et al., 1997), lipopolysaccharide-stimulated macrophages (Jin et al., 1997) and cultured human gingival keratinocytes (Jana et al., 2005; Westin et al., 2002). PKR and OAS are constitutively expressed in normal cells in a latent but inactive form. They have to bind to dsRNA, cytokine, growth factor and stress signals (Tan & Katze, 1999) and be induced by interferon (Stark, Kerr, Williams, Silverman, & Schreiber, 1998), then they can function properly. MxA, in contrast, strictly controlled in a dose-dependent manner by type I IFNs, can be found at low basal levels in certain cell lines, primary mononuclear cells (Ronni et al., 1993), hepatocytes and cholangiocytes (Leifeld et al., 2001).

Due to the etiologic importance of bacteria in periodontitis has been understood for decades, most studies have focused on the anti-bacterial activities, for examples, the expressions of variety of antimicrobial peptides including HNPs, HBDs, LL-37, and lactoferrin in periodontal tissue. Similarly, several recent studies have documented a role of viruses in the development and progression of periodontitis. The present expressions of all of above antiviral proteins in gingival tissue showed that viruses, activate antiviral proteins activity, can be also found in healthy and periodontitis. This is concordant with several studies that reported viral expressions in both healthy and periodontitis lesions. For examples, DNA from herpesviruses such as herpes simplex virus type (HSV-1), herpes simplex virus type 2, human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV) had been detected in subgingival specimens (Contreras & Slots, 1996; Saygun et al., 2002; Saygun, Yapar, Ozdemir, Kubar, & Slots, 2004), gingival crevicular fluid (GCF)(Contreras & Slots, 1996; Parra & Slots, 1996), gingival tissue (Contreras, Nowzari, & Slots, 2000; Ehrlich, Cohen, & Hochman, 1983), and infiltrated immune cells from periodontitis sites (Contreras, Zadeh, Nowzari, & Slots, 1999). Moreover, viral DNA such as herpes simplex virus, Epstein–Barr virus and human cytomegalovirus could also be found at the healthy sites (Contreras et al., 2000; Contreras et al., 1999; Parra & Slots,

1996). Although, periodontal tissue is constantly exposed to multiple assaults by microbes that live harmoniously in the oral niche, but most individuals maintain healthy homeostasis suggesting so effective innate immune response.

Quite unexpectedly, in the present study there were no significant differences for all the antiviral proteins examined between periodontitis and healthy. Although, the periodontitis sites could find viruses, activate antiviral proteins activity, at a higher frequency as compared to healthy sites (Contreras et al., 2000; Contreras et al., 1999; Parra & Slots, 1996). The expression level of all the antiviral proteins examined in periodontitis group was similar to healthy one. This might owing to diminished function of damaged cell in pathological periodontitis tissue.

As mention above, type I IFNs are required for PKR, OAS and MxA expression, therefore the presence of type I IFNs should be explored. Surprisingly, our own observation showed negligible expression level of IFN- α in both periodontitis and healthy gingival tissue agreed with the study of Kajita et al. (Kajita et al., 2007). However, this could be attributable to be nature of its transient expression or inducible by some unknown molecules.

In conclusion, this is the first evidence to show that antiviral protein such as SLPI, PKR, OAS and MxA are expressed in healthy and periodontitis gingival tissue. The roles of innate antiviral protein in periodontal disease remain to be determined.

Suggestions

1. To explore another mechanism that is able to induce MxA, *in vitro* exposure of gingival epithelial cells, lacked of type I interferon, to any molecules remain to be examined.

2. Confirmation of antiviral protein expression at the protein level in periodontal tissues should be done by immunohistochemical staining.

3. Due to limited numbers of periodontal biopsies in this study, further research should be done more samples.

Acknowledgement

I would like to acknowledge research grant from the The Thailand Research Fund for the partial financial support for this study. My sincere appreciation is also extended to the patients who volunteered to donate their gingival tissue samples. I would like to thank the staff of Periodontal and Surgery Clinic, Faculty of Dentistry, Chulalongkorn University for collecting the samples.

References

Accola, M. A., Huang, B., Al Masri, A., & McNiven, M. A. (2002). The antiviral dynamin family member, MxA, tubulates lipids and localizes to the smooth endoplasmic reticulum. **J Biol Chem,** *277*(24), 21829-21835.

Amigo, M., Schalkwijk, J., Olthuis, D., De Rosa, S., Paya, M., Terencio, M. C., et al. (2006). Identification of avarol derivatives as potential antipsoriatic drugs using an in vitro model for keratinocyte growth and differentiation. **Life Sci,** *79*(25), 2395-2404.

Antonelli, G., Simeoni, E., Turriziani, O., Tesoro, R., Redaelli, A., Roffi, L., et al. (1999). Correlation of interferon-induced expression of MxA mRNA in peripheral blood mononuclear cells with the response of patients with chronic active hepatitis *C* to IFN-alpha therapy. **J Interferon Cytokine Res***, 19*(3), 243-251.

Beppu, Y., Imamura, Y., Tashiro, M., Towatari, T., Ariga, H., & Kido, H. (1997). Human mucus protease inhibitor in airway fluids is a potential defensive compound against infection with influenza A and Sendai viruses. **J Biochem,** *121*(2), 309-316.

Cappuyns, I., Gugerli, P., & Mombelli, A. (2005). Viruses in periodontal disease - a review. **Oral Dis***, 11*(4), 219-229.

Carraro, G., Albertin, G., Forneris, M., & Nussdorfer, G. G. (2005). Similar sequence-free amplification of human glyceraldehyde-3-phosphate dehydrogenase for real time RT-PCR applications. **Mol Cell Probes,** *19*(3), 181-186.

Castelli, J., Wood, K. A., & Youle, R. J. (1998). The 2-5A system in viral infection and apoptosis. **Biomed Pharmacother,** *52*(9), 386-390.

Clemens, M. J. (1997). PKR--a protein kinase regulated by double-stranded RNA. *Int* **J Biochem Cell Biol,** *29*(7), 945-949.

Contreras, A., Nowzari, H., & Slots, J. (2000). Herpesviruses in periodontal pocket and gingival tissue specimens. **Oral Microbiol Immuno***l, 15*(1), 15-18.

Contreras, A., & Slots, J. (1996). Mammalian viruses in human periodontitis. **Oral Microbiol Immunol,** *11*(6), 381-386.

Contreras, A., Zadeh, H. H., Nowzari, H., & Slots, J. (1999). Herpesvirus infection of inflammatory cells in human periodontitis. **Oral Microbiol Immunol,** *14*(4), 206-212.

Diamond, D. L., Kimball, J. R., Krisanaprakornkit, S., Ganz, T., & Dale, B. A. (2001). Detection of beta-defensins secreted by human oral epithelial cells. **J Immunol Methods**, *256*(1-2), 65-76.

Ehrlich, J., Cohen, G. H., & Hochman, N. (1983). Specific herpes simplex virus antigen in human gingiva. **J Periodontol**, *54*(6), 357-360.

Farrugia, J., & Cann, A. J. (1999). Internally controlled quantitative assays for PKR mRNA and protein from liver biopsies and other finite clinical samples. **J Immunol Methods**, *228*(1-2), 59-68.

Franken, C., Meijer, C. J., & Dijkman, J. H. (1989). Tissue distribution of antileukoprotease and lysozyme in humans. **J Histochem Cytochem**, *37*(4), 493-498.

Frese, M., Kochs, G., Feldmann, H., Hertkorn, C., & Haller, O. (1996). Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. **J Virol, 7***0*(2), 915-923.

Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J., & Haller, O. (1995). Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. **J Virol,** *69*(6), 3904-3909.

Friedman, H. M. (2006). Keratin, a dual role in herpes simplex virus pathogenesis. **J Clin Virol,** *35*(1), 103-105.

Goebel, C., Mackay, L. G., Vickers, E. R., & Mather, L. E. (2000). Determination of defensin HNP-1, HNP-2, and HNP-3 in human saliva by using LC/MS. **Peptides**, *21*(6), 757-765.

Gordien, E., Rosmorduc, O., Peltekian, C., Garreau, F., Brechot, C., & Kremsdorf, D. (2001). Inhibition of hepatitis B virus replication by the interferon-inducible MxA protein. **J Virol,** *75*(6), 2684-2691.

Gramenzi, A., Andreone, P., Loggi, E., Foschi, F. G., Cursaro, C., Margotti, M., et al. (2005). Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. **J Viral Hepat**, *12*(5), 525-530.

Hiemstra, P. S. (2002). Novel roles of protease inhibitors in infection and inflammation. **Biochem Soc Trans,** *30*(2), 116-120.

Hiemstra, P. S., Maassen, R. J., Stolk, J., Heinzel-Wieland, R., Steffens, G. J., & Dijkman, J. H. (1996). Antibacterial activity of antileukoprotease. **Infect Immun,** *64*(11), 4520-4524.

Huang, J. T., & Schneider, R. J. (1991). Adenovirus inhibition of cellular protein synthesis involves inactivation of cap-binding protein. **Cell,** *65*(2), 271-280.

Into, T., Inomata, M., Kanno, Y., Matsuyama, T., Machigashira, M., Izumi, Y., et al. (2006). Arginine-specific gingipains from Porphyromonas gingivalis deprive protective functions of secretory leucocyte protease inhibitor in periodontal tissue. **Clin Exp Immunol,** *145*(3), 545-554.

Jana, N. K., Gray, L. R., & Shugars, D. C. (2005). Human immunodeficiency virus type 1 stimulates the expression and production of secretory leukocyte protease inhibitor (SLPI) in oral epithelial cells: a role for SLPI in innate mucosal immunity. **J Virol,** *79*(10), 6432-6440.

Jin, F. Y., Nathan, C., Radzioch, D., & Ding, A. (1997). Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. **Cell**, *88*(3), 417-426.

Kajita, K., Honda, T., Amanuma, R., Domon, H., Okui, T., Ito, H., et al. (2007). Quantitative messenger RNA expression of Toll-like receptors and interferon-alpha1 in gingivitis and periodontitis. **Oral Microbiol Immunol**, *22*(6), 398-402.

Kato, A., Ogasawara, T., Homma, T., Batchelor, J., Imai, S., Wakiguchi, H., et al. (2004). CpG oligodeoxynucleotides directly induce CXCR3 chemokines in human B cells. **Biochem Biophys Res Commun**, *320*(4), 1139-1147.

Landis, H., Simon-Jodicke, A., Kloti, A., Di Paolo, C., Schnorr, J. J., Schneider-Schaulies, S., et al. (1998). Human MxA protein confers resistance to Semliki Forest virus and inhibits the amplification of a Semliki Forest virus-based replicon in the absence of viral structural proteins. **J Virol,** *72*(2), 1516-1522.

Leifeld, L., Ramakers, J., Schneiders, A. M., Dumoulin, F. L., Sterneck, M., Muller, A., et al. (2001). Intrahepatic MxA expression is correlated with interferon-alpha expression in chronic and fulminant hepatitis. **J Pathol**, *194*(4), 478-483.

Mahanonda, R., & Pichyangkul, S. (2007). Toll-like receptors and their role in periodontal health and disease. **Periodontol 2000**, *43*, 41-55.

Mathews, M., Jia, H. P., Guthmiller, J. M., Losh, G., Graham, S., Johnson, G. K., et al. (1999). Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. **Infect Immun**, *67*(6), 2740-2745.

McHutchison, J. G., Bacon, B. R., Gordon, S. C., Lawitz, E., Shiffman, M., Afdhal, N. H., et al. (2007). Phase 1B, randomized, double-blind, dose-escalation trial of CPG 10101 in patients with chronic hepatitis C virus. **Hepatology,** *46*(5), 1341-1349.

McNeely, T. B., Dealy, M., Dripps, D. J., Orenstein, J. M., Eisenberg, S. P., & Wahl, S. M. (1995). Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. **J Clin Invest,** *96*(1), 456-464.

Milush, J. M., Stefano-Cole, K., Schmidt, K., Durudas, A., Pandrea, I., & Sodora, D. L. (2007). Mucosal innate immune response associated with a timely humoral immune response and slower disease progression after oral transmission of simian immunodeficiency virus to rhesus macaques. **J Virol**, *81*(12), 6175-6186.

Murakami, M., Ohtake, T., Dorschner, R. A., & Gallo, R. L. (2002). Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. **J Dent Res,** *81*(12), 845-850.

Nakamura-Minami, M., Furuichi, Y., Ishikawa, K., Mitsuzono-Tofuku, Y., & Izumi, Y. (2003). Changes of alpha1-protease inhibitor and secretory leukocyte protease inhibitor levels in gingival crevicular fluid before and after non-surgical periodontal treatment. **Oral Dis**, *9*(5), 249-254.

O'Malley, R. P., Mariano, T. M., Siekierka, J., & Mathews, M. B. (1986). A mechanism for the control of protein synthesis by adenovirus VA RNAI. **Cell,** *44*(3), 391-400.

Parra, B., & Slots, J. (1996). Detection of human viruses in periodontal pockets using polymerase chain reaction. **Oral Microbiol Immunol**, *11*(5), 289-293.

Pavlovic, J., Zurcher, T., Haller, O., & Staeheli, P. (1990). Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. **J Virol,** *64*(7), 3370-3375.

Pestka, S., Langer, J. A., Zoon, K. C., & Samuel, C. E. (1987). Interferons and their actions. **Annu Rev Biochem**, *56*, 727-777.

Pisano, E., Cabras, T., Montaldo, C., Piras, V., Inzitari, R., Olmi, C., et al. (2005). Peptides of human gingival crevicular fluid determined by HPLC-ESI-MS. **Eur J Oral Sci**, *113*(6), 462-468.

Puklo, M., Guentsch, A., Hiemstra, P. S., Eick, S., & Potempa, J. (2008). Analysis of neutrophil-derived antimicrobial peptides in gingival crevicular fluid suggests importance of cathelicidin LL-37 in the innate immune response against periodontogenic bacteria. **Oral Microbiol Immunol**, *23*(4), 328-335.

Ronni, T., Melen, K., Malygin, A., & Julkunen, I. (1993). Control of IFN-inducible MxA gene expression in human cells. **J Immunol,** *150*(5), 1715-1726.

Sallenave, J. M., Si Tahar, M., Cox, G., Chignard, M., & Gauldie, J. (1997). Secretory leukocyte proteinase inhibitor is a major leukocyte elastase inhibitor in human neutrophils. **J Leukoc Biol**, *61*(6), 695-702.

Samuel, C. E. (2001). Antiviral actions of interferons. **Clin Microbiol Rev**, *14*(4), 778-809, table of contents.

Santoro, A., Majorana, A., Roversi, L., Gentili, F., Marrelli, S., Vermi, W., et al. (2005). Recruitment of dendritic cells in oral lichen planus. **J Pathol,** *205*(4), 426-434.

Saygun, I., Sahin, S., Ozdemir, A., Kurtis, B., Yapar, M., Kubar, A., et al. (2002). Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters. **J Periodontol***, 73*(12), 1437-1443.

Saygun, I., Yapar, M., Ozdemir, A., Kubar, A., & Slots, J. (2004). Human cytomegalovirus and Epstein-Barr virus type 1 in periodontal abscesses. **Oral Microbiol Immunol***, 19*(2), 83-87.

Schenkein, H. A. (2006). Host responses in maintaining periodontal health and determining periodontal disease. **Periodontol 2000***, 40*, 77-93.

Schwemmle, M., Weining, K. C., Richter, M. F., Schumacher, B., & Staeheli, P. (1995). Vesicular stomatitis virus transcription inhibited by purified MxA protein. **Virology**, *206*(1), 545-554.

Sorensen, O. E., Cowland, J. B., Theilgaard-Monch, K., Liu, L., Ganz, T., & Borregaard, N. (2003). Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. **J Immunol***, 170*(11), 5583-5589.

Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., & Schreiber, R. D. (1998). How cells respond to interferons. **Annu Rev Biochem,** *67*, 227-264.

Tan, S. L., & Katze, M. G. (1999). The emerging role of the interferon-induced PKR protein kinase as an apoptotic effector: a new face of death? **J Interferon Cytokine Res,** *19*(6), 543-554.

Teng, Y. T. (2006). Protective and destructive immunity in the periodontium: Part 1--innate and humoral immunity and the periodontium. **J Dent Res***, 85*(3), 198-208.

Thimme, R., Bukh, J., Spangenberg, H. C., Wieland, S., Pemberton, J., Steiger, C., et al. (2002). Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. **Proc Natl Acad Sci U S A,** *99*(24), 15661-15668.

Thompson, R. C., & Ohlsson, K. (1986). Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. **Proc Natl Acad Sci U S A,** *83*(18), 6692-6696.

Tomee, J. F., Hiemstra, P. S., Heinzel-Wieland, R., & Kauffman, H. F. (1997). Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. **J Infect Dis,** *176*(3), 740-747.

Vijay-Kumar, M., Gentsch, J. R., Kaiser, W. J., Borregaard, N., Offermann, M. K., Neish, A. S., et al. (2005). Protein kinase R mediates intestinal epithelial gene remodeling in response to double-stranded RNA and live rotavirus. **J Immunol**, *174*(10), 6322-6331.

Westin, U., Nystrom, M., Ljungcrantz, I., Eriksson, B., & Ohlsson, K. (2002). The presence of elafin, SLPI, IL1-RA and STNFalpha RI in head and neck squamous cell carcinomas and their relation to the degree of tumour differentiation. **Mediators Inflamm,** *11*(1), 7-12.

Zasloff, M. (2002). Innate immunity, antimicrobial peptides, and protection of the oral cavity. *Lancet, 360*(9340), 1116-1117.

Zhang, Y., DeWitt, D. L., McNeely, T. B., Wahl, S. M., & Wahl, L. M. (1997). Secretory leukocyte protease inhibitor suppresses the production of monocyte prostaglandin H synthase-2, prostaglandin E2, and matrix metalloproteinases. **J Clin Invest,** *99*(5), 894-900.

Zhao, H., De, B. P., Das, T., & Banerjee, A. K. (1996). Inhibition of human parainfluenza virus-3 replication by interferon and human MxA. **Virology,** *220*(2), 330-338.