CHAPTER I

INTRODUCTION

1.1 Background of present study

The immune system consists of innate and adaptive immune response. The innate immune system is a first line of defense that confers non-specific protection against a large number of pathogens. On the contrary, the adaptive immunity is a more slowly developing and highly evolved antigen-specific protective response. Studies of the host defence system in fruit files, Drosophila, provide the first clue as to the mechanism of innate immune recognition. Toll gene products were first discovered in 1985 as critical for embryonic development of dorsal-ventral polarity (Andreson et al.,1985a;1985b). Subsequently, homologues of Drosophila Toll, the so-called Toll-like receptors (TLRs), were identified in mammals (Medzhitov et al., 1997). It is now clear that TLRs function as key pattern recognition-receptors (PRRs) of innate immune system (Janeway and Medzhitov, 2002). The innate immune system utilizes Toll-like receptors (TLRs) to recognize and bind pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), peptidoglycan, lipoproteins, bacteria DNA and double stranded RNA. Bind to of PAMPs leads to TLR activation that transmit this information through intracellular signaling pathway, resulting in activation of innate immune cells. This response involves the secretion of cytokines, chemokines and broad-spectrum antibacterial substances such as defensins.

Mucosal epithelial cells play an integral role in innate immune defense. Gingival epithelium is stratified squamous epithelium surrounding the tooth and forming an attachment to the tooth surface. It is not only considered as a physical barrier, but also able to perform a number of very important protective innate immune responses and maintaining the balance between health and disease. TLR expression on human gingival epithelial cells (HGECs) was investigated in patients' gingival biopsies obtained during periodontal surgery. The cells constitutively express a variety of TLRs: TLR2, TLR3, TLR4, TLR5, TLR6, TLR9, TLR 10 (Kusumoto et al., 2004). Epithelial cells are recognized as metabolically active and capable of reacting to microbes by synthesizing a number of cytokines, adhesion molecules, growth factors and enzymes. More importantly, the epithelial cells generate a family of potent antimicrobial peptides for protection against infection that cause microbial death by lysis through disruption of the integrity of bacterial membranes. These peptides are β-defensins, cathelicidin LL-37, and calprotectin, all of which have been identified in the oral epithelium (Han et al., 2000; Dale et al., 2001; Ross and Herzberg, 2001; Kusumoto et al., 2004). To date, mRNA expression for human β-defensin-1 (HBD-1), human β-defensin-2 (HBD-2), human β-defensin-3 (HBD-3) has been detected in gingival tissues or gingival keratinocytes (Bissell et al.,2004; Dunsche et al., 2001;2002; Krisanaprakornkit et al., 1998; Mathews et al., 1999; Premratanachai et al., 2004). Expression of β- defensins is classically described as constitutive or inducible. Epithelial cells throughout the body express a wide range of TLRs. Recent observations demonstrated the link between the expression of defensins and TLR activation (Birchler et al., 2001; Duits et al., 2003; Jia et al., 2004; Platz et al., 2004; Vora et al.,2004; Ogushi et al., 2001;2004; Sugawara et al., 2006).

Cigarette smoking significantly increases the risk of cardiovascular disease, lung cancer, chronic obstructive pulmonary disease, bacterial meningitis, respiratory infection and periodontitis (Sopori, 2002). Tobacco smoke contains thousands of different compounds such as nicotine, tar, carbon monoxide, ammonia, formaldehyde that are directly noxious/poisonous to living organisms and cells (Palmer et al., 2005). Many of the toxic effects of tobacco have been attributed to nicotine, a major component of the particulate phase of tobacco smoke. Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring that is distilled from burning tobacco and is carried proximally on tar droplets and probably also in the vapor phase which are inhaled (Pool et al., 1985). The adverse effects of smoking may result from the ability of cigarette smoke to weaken the immune system by inhibiting both innate and adaptive immunity. It was found that alveolar macrophage, an important innate immune cell in the lung from smokers have a reduce ability to phagocytose and/or kill microbial pathogens (King et al., 1988; Martin, 1997). As for the adaptive immunity, several studies have demonstrated the marked reduction in serum levels of immunoglobulin in long-term smokers (Ferson et al., 1979).

Periodontitis is a chronic bacterial infection of tooth supporting structures. It causes destruction of periodontal connective tissue and bone and, in severe cases, tooth loss. Key oral plaque bacteria including *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Tanerella forsythia* are recognized as etiologic agents in periodontitis. The disease initiation and progression results from the host response to plaque bacteria. Immunohistochemistry studies reveal dense cellular infiltration including numerous T and B cells in periodontitis lesions. In addition, high

levels of inflammatory mediators such as TNF-α, IL-1β, prostaglandin E_2, IFN-γ, and IL-8 can be detected in inflamed gingival tissues and gingival crevicular fluid (Seymour, 1991; Page et al., 1997). Like other infectious diseases, periodontitis is multi-factorial in nature. Smoking is one of the major risk factors for the disease progression and severity (Sopori, 2002; Tonetti, 1998; Ah et al., 1994; Bergstrom et al., 1989; Haber et al., 1992; Haber et al, 1993; Haber et al., 1994; Preber et al., 1992) . Previous cross-sectional and longitudinal studies provide strong epidemiologic evidence of a positive association between smoking and periodontal disease (papapanou, 1996). Smokers were shown to be at significantly greater risk for further attachment loss when compared to non-smoker, and the odds ratio was 5.4 (Machtei et al., 1997).

Like bronchial epithelial cells in lung, gingival epithelial cells are the first cells that encounter cigarette smokes in the oral cavity. Epithelial cell function can be suppressed by cigarette smoke. Recent data suggests that cigarette smoke inhibit LPS-induced GM-CSF and IL-8 production by bronchial epithelial cells (Laan et al., 2004). The ability of cigarette smoke to suppress the innate immune response of bronchial epithelial cells may contribute to chronic colonization of Gram negative bacteria observed in chronic obstructive pulmonary disease patients (Sethi and Murphy, 2001). To date, the effects of cigarette smoking on HGECs are much less explored. Investigation into how cigarette smoke affects the innate immune response of epithelial cells would provide a better understanding of the association between cigarette smokes and periodontal diseases.

1.2 Objectives

We investigated

1.2.1 full panel of TLR expression (TLR : 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) on HGECs.

1.2.2 the ability to produce HBD-2 in response to TLR ligands, pro-inflammatory cytokine TNF-α, and the combination of *P.gingivalis* LPS and TNF-α.

1.2.3 the effect of nicotine on HBD-2 production from stimulated HGECs.

1.3 Hypothesis

1.3.1 HGECs express a variety of TLRs and produce HBD-2 upon TLR ligation, TNF-α , or the combination of *P.gingivalis* LPS and TNF-α stimulation.

1.3.2 Nicotine suppresses function of stimulated HGECs by decreasing HBD-2 production.

1.4 Field of research

Investigation the TLR expression of HGECs and HBD-2 production in response to TLR ligands, cytokine and nicotine.

1.5 Criteria inclusions

1.5.1 HGECs were grown from gingival biopsies which were obtained from healthy adult subjects.

1.5.2 Subjects who had clinically healthy periodontium with probing depth less than 4 mm were included.

1.5.3 Peripheral blood mononuclear cells (PBMC) were obtained from healthy adult subjects who have no taken any antibiotics or anti-inflammatory drugs within the past 3 months prior to blood donation.

1.5.3 Analysis of TLR expression (TLRs 1-10) were determined by reverse transcriptase polymerase chain reaction (RT-PCR).

1.5.4 The responses of HGECs to different TLR ligands, TNF-α, the combination of *P.gingivalis* LPS and TNF-α, and nicotine (HBD-2 production) were measured by RT-PCR.

1.6 Limitation of research

This study cannot investigate many HGECs samples due to high expenses.

1.7 Application and expectation of research

1.7.1 Better understanding of the role of HGECs in the innate immune defense in the oral cavity.

1.7.2 New scientific information: how nicotine in cigarette smoke affect the innate immune response of human epithelial cells. A

better understanding about the link between nicotine and oral diseases such as periodontitis.

1.7.3 Publication in the international peered-reviewed journal.

1.7.4 The experimental data would be applicable for the anti-cigarette smoking campaign.