Immunobiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Research Paper

Comparative analysis of microbial sensing molecules in mucosal tissues with aging

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ARTICLE INFO

Keywords:
Aging
Oral mucosa
Microbial sensing
Pattern recognition receptors
Nonhuman primates
Periodontitis

ABSTRACT

Host-bacterial interactions at mucosal surfaces require recognition of the bacteria by host cells enabling targeted responses to maintain tissue homeostasis. It is now well recognized that an array of host-derived pattern recognition receptors (PRRs), both cell-bound and soluble, are critical to innate immune engagement of microbes via microbial-associated molecular patterns (MAMP). This report describes the use of a nonhuman primate model to evaluate changes in the expression of these sensing molecules related to aging in healthy gingival tissues. *Macaca mulatta* aged 3–24 years were evaluated clinically and gingival tissues obtained, RNA isolated and microarray analysis conducted for gene expression of the sensing pattern recognition receptors (PRRs). The results demonstrated increased expression of various PRRs in healthy aging gingiva including extracellular (CD14, CD209, CLEC4E, TLR4), intracellular (NAIP, IFIH1, DAI) and soluble (PTX4, SAA1) PRRs. Selected PRRs were also correlated with both bleeding on probing (BOP) and pocket depth (PD) in the animals. These findings suggest that aged animals express altered levels of various PRRs that could affect the ability of the tissues to interact effectively with the juxtaposed microbial ecology, presumably contributing to an enhanced risk of periodontitis even in clinically healthy oral mucosal tissues with aging.

1. Introduction

Mucosal tissues are colonized by an extremely dense and diverse microbiota of commensal bacteria, as well as occasionally having to interact with pathogenic microorganisms. These sites continuously sample foreign material via various cells types, including macrophages (mΦ) and dendritic cells (DCs). These are innate immune cells within the skin and mucosa, including oral and gingival epithelium, that respond rapidly to infection, carrying crucial information about the infection to lymph nodes to trigger an immune response (Kopitar et al., 2006; Nestle et al., 1994; Makino et al., 2001; Jotwani et al., 2001; Cutler and Jotwani, 2006). Historically, both of these APC types were identified to effectively engage microbes using a repertoire of pattern recognition receptors (PRRs) (Hemmi and Akira, 2005; Benko et al., 2008)' which recognize distinct classes of microorganism-associated molecular patterns (MAMPs), including a range of bacterial, viral, and fungal pathogens, through engagement of LPS, LTA, and nucleic acid (*e.g.,* CpG, DNA, dsRNA) ligands (Blach-Olszewska, 2005; Wollenberg et al., 2002; Kawai and Akira, 2006; Kumar et al., 2009). However, it is now recognized that these PRRs exist on virtually all types of host cells with variation in the quality and quantity that are expressed on particular cell-types (Hajishengallis et al., 2012). Moreover, the literature demonstrates that the PRRs exist as cell associated [*e.g.* Toll-like receptors (TLRs), nucleotide-binding oligomerization domains (NODs)] and soluble PRRs (*e.g.* C-reactive protein, serum amyloid A) that are critical to innate immunity (Mogensen, 2009) and help link innate and adaptive immune responses (Werling and Jungi, 2003; Kumar et al., 2011).

While there are numerous reports regarding PRR expression and functions with aging, generally the existing data remains somewhat unclear regarding aging effects on these critical members of the innate immune system (Shaw et al., 2013, 2011). Studies in mice and humans have suggested that the expression and functions of various TLR and other PRRs such as RIG-I and NLRP3 are altered with aging, usually decreasing in level and/or functional capabilities. Beyond adaptive immune senescence that has been uniformly described in aging, these

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http://dx.doi.org/10.1016/j.imbio.2017.10.034
Received 15 November 2016; Accepted 14 October 2017

Table 1
Gene expression targets for microbial sensing molecules.

Gene ID	Gene Title
Surface Cell Associated PRRs	
CD14	Binds to monomeric lipopolysaccharide and delivers it to the MD-2/TLR4 complex; also soluble molecule
CD209 (DC-SIGN)	Surface of immature dendritic cells (DCs) and involved in initiation of primary immune response
CLEC7A	C-Type Lectin Domain Family 7, Member A, beta-1,3-linked and beta-1,6-linked glucans from fungi
CLEC4E	C-Type Lectin domain family 4, member E, mycobacteria is via trehalose 6,6′-dimycolate; SAP130, a nuclear protein that is released by dead or dying cells
CLEC4M (L-SIGN)	C-Type Lectin Domain Family 4, Member M, parasites to viruses
CLEC6A (Dectin-2)	C-Type Lectin Domain Family 6, Member A, alpha-mannans on *C.albicans* hypheas
COLEC12	Collectin Sub-Family Member 12, Scavenger receptor for phagocytosis of Gram-positive, Gram-negative bacteria and yeast
FCN2	Ficolin (Collagen/Fibrinogen Domain Containing Lectin) 2 (Hucolin), Complement-activating lectin, phagocytosis of *S.typhimurium* by neutrophils
MARCO	Macrophage Receptor With Collagenous Structure, class A scavenger receptor for both Gram-negative and Gram-positive bacteria
MRC1	Mannose Receptor, C Type 1, bind high-mannose structures on the surface of potentially pathogenic viruses, bacteria, and fungi for phagocytosis
TLR1	Toll-like receptor 1, diacylated and triacylated lipopeptides
TLR2	Toll-like receptor 2, bacterial lipoproteins and other microbial cell wall components
TLR4	Toll-like receptor 4, bacterial lipopolysaccharide (LPS)
TLR5	Toll-like receptor 5, bacterial flagellins
TLR6	Toll-like receptor 6, Gram-positive bacteria and fungi
Intracellular Associated PRRs	
AIM2	Cytosolic double-stranded DNA
ARHGEF2	Rho/Rac Guanine Nucleotide Exchange Factor (GEF) 2, intracellular sensing system along with NOD1 for the detection of microbial effectors during cell invasion
IFIH1 (MDA5)	Interferon induced with helicase C domain 1, RIG-1-like receptor family, viral sensor
LGP2 (DEXH58)	DEXH (Asp-Glu-X-His) Box Polypeptide 58, RIG-1-like receptor family, viral sensor
NAIP (BIRC1)	Baculoviral IAP repeat-containing protein 1, effects apoptosis
NLRC4 (IPAF)	NLR family CARD domain-containing protein 4, inflammasome
NOD1	Nucleotide-binding oligomerization domain 1, intracellular bacterial lipopolysaccharides (LPS), senses peptidoglycan (PGN)-derived muropeptides
NOD2	Nucleotide-binding oligomerization domain 2, intracellular bacterial lipopolysaccharides (LPS) by recognizing the muramyl dipeptide
RIG-1 (DDX58)	Retinoic acid-inducible gene 1, RIG-1-like receptor family, viral sensor
TLR3	Toll-like receptor 3, nucleotide-sensing for double-stranded RNA
TLR7	Toll-like receptor 7, nucleotide-sensing for single-stranded RNA
TLR8	Toll-like receptor 8, G-rich oligonucleotides
TLR9	Toll-like receptor 9, nucleotide-sensing for unmethylated cytidine-phosphate-guanosine (CpG) dinucleotides
ZBP1/DAI	Z-DNA Binding Protein 1, cytoplasmic sensor binds to foreign DNA and induces type-I interferon
Soluble PRRs	
CRP (PTX1)	C-reactive protein, promotes agglutination, bacterial capsular swelling, phagocytosis and complement fixation
FCN1	Ficolin (Collagen/Fibrinogen Domain Containing) 1, Complement-activating lectin, 9-O-acetylated 2–6-linked sialic acid derivatives and to various glycans
MBL2	Mannose-Binding Lectin (Protein C) 2, Soluble, soluble mannose-binding lectin or mannose-binding protein found in serum
PTX3	Pentraxin 3, Long, mediating agglutination, complement activation, and opsonization
PTX4	Pentraxin 4, Long, mediating agglutination, complement activation, and opsonization
SAA1	Serum Amyloid A1

findings contribute to the concept of "inflamm-aging" that emphasizes a dysregulation and exacerbated destructive inflammatory responses with aging (Shaw et al., 2013; Franceschi et al., 2000; Fulop et al., 2013). Dunston and Griffiths (Dunston and Griffiths, 2010) suggested that a poor inflammatory response via TLR activation still allowed dysregulated inflammation in aging related to ineffective clearance of pathogens and less well controlled activation of macrophages. Interestingly, Sun et al. (2012) demonstrated a less LPS tolerigenic macrophage in aging with challenge by an oral pathogen, *P. gingivalis*, apparently related to altered TLR2 and TLR expression/function.

The periodontium of the oral cavity is a multi-component tissue comprised of epithelium, connective tissue, a capillary vascular bed, and alveolar bone. Disease of this structure, *ie.* periodontitis, results in chronic inflammation (measured by redness and bleeding), soft tissue destruction (described as clinical attachment loss) and bone resorption, that is generally reflected in increased probing pocket depth. The current paradigm of periodontitis is a chronic destructive inflammatory response with oral bacterial biofilms triggering an influx of host immune cells and release of cytokines/chemokines and inflammatory lipids that have direct effects on the integrity of the tissues that comprise the periodontium. A consistent clinical finding in periodontitis is substantial increases in incidence, extent, and severity of disease with aging (Baelum and Lopez, 2013; Eke et al., 2012). Moreover, a vast literature exists describing the range of molecular inflammatory, innate immune and adaptive immune responses in human health and disease (Ebersole et al., 2013; Bartold and Van Dyke, 2013; Hajishengallis, 2015; Garlet et al., 2014; Souza and Lerner, 2013), as well as a number of animal models that have been used to assess causality (de Molon et al., 2013; Graves et al., 2012; Oz and Puleo, 2011; Madden and Caton, 1994; Holt et al., 1988).

This report uses a nonhuman primate model of naturally-occurring periodontitis to document alterations in expression of these microbial sensing molecules. The hypothesis to be tested was that aging effects on clinically healthy gingival tissues are detected within the pattern of PRR expression and may be related to the aging susceptibility for this mucosal tissue disease.

2. Methods

2.1. Nonhuman primate model and Oral Clinical Evaluation

Rhesus monkeys (*Macaca mulatta*) (n = 41; 21 females and 20 males) housed at the Caribbean Primate Research Center (CPRC) at Sabana Seca, Puerto Rico, were used in these studies. Healthy animals (5–14/group) were distributed by age into four groups: ≤3 years (young), 3–7 years (adolescent), 12–16 years (adult) and 18–23 years (aged). The nonhuman primates are typically fed a 20% protein, 5% fat, and 10% fiber commercial monkey diet (diet 8773, Teklad NIB primate diet modified: Harlan Teklad). The diet is supplemented with fruits and vegetables, and water is provided *ad libitum* in an enclosed corral setting.

A protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Puerto Rico, enabled anesthetized animals to be examined for clinical measures of periodontal