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Targeting of inflammatory cytokine networks by periodontal pathogens

Kumulative Habilitationsschrift

zur Erlangung der Venia legendi
der Universität Zürich

vorgelegt von

Nagihan Bostanci, DDS, PhD

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The following original publications are submitted as a "Kumulative Habilitationsschrift" thesis, for a post-doctoral Lecturer qualification:

Publication I

Bostanci N, Allaker R P, Belibasakis G N, Rangarajan M, Curtis M A, Hughes F J, McKay I J (2007) *Porphyromonas gingivalis* antagonises *Campylobacter rectus* induced cytokine production by human monocytes. *Cytokine*. 39: 147-156

Publication II

Rangarajan M, Aduse-Opoku, J, Paramonov N, Hashim A, **Bostanci N,** Fraser OP, Tarelli E, Curtis, MA (2008) Identification of a second LPS in *Porphyromonas gingivalis* W50. *J Bacteriol* 190: 2920-2932

Publication III

Hamedi M, Belibasakis G N, Cruchley A T, Rangarajan M, Curtis M A, **Bostanci N** (2009) *Porphyromonas gingivalis* culture supernatants differentially regulate Interleukin-1β and Interleukin-18 in human monocytic cells. *Cytokine.* 45: 99-104

Publication IV

Bostanci N, Emingil G, Saygan, B, Turkoglu O, Atilla G, Curtis M A, Belibasakis G N (2009) Expression and regulation of the NALP3 Inflammasome complex in periodontal diseases. *Clin Exp Immunol*. 157: 415-422

Publication V

Bostanci N, Thurnheer T, Belibasakis G N (2011) Involvement o the TREM-1/DAP12 pathway in the innate immune responses to *Porphyromonas gingivalis*. *Mol Immunol*. 49: 387-394

Targeting of inflammatory cytokine networks by periodontal pathogens

Background and Aim

Periodontal diseases are a widespread group of destructive oral infection-driven inflammatory diseases that affect 50-80% of the general population worldwide (1). These pathologies include the reversible inflammation of the gingiva (gingivitis) and the irreversible chronic inflammatory destruction of the periodontal tissues (periodontitis), with eventual tooth loss. Even though periodontopathogenic bacteria in the form of biofilms are the primary etiologic factor of periodontitis, tissue destruction essentially results from the host immune response to the bacterial challenge (2). Among over 700 bacterial species living in the oral cavity, *Porphyromonas gingivalis* is considered a major component of the microbiota associated with severe forms of periodontitis (3). *P. gingivalis* produces a broad array of potential virulence factors, such as lipopolysaccharide (LPS), gingipains, and fimbriae, involved in tissue colonization and perturbation of the host defences. In this regard, considerable evidence has been accumulated demonstrating its ability to exploit complement receptors, cause chemokine "paralysis" or compete with other pathogens in eliciting inflammatory responses by the host (4, 5). Host defence against *P. gingivalis* requires coordination of multiple signalling pathways, which in turn initiate the production of pro-inflammatory cytokines. Cytokines are secreted signalling molecules that play an important role in the innate immune responses to infection, and they are an integral part of the host inflammatory response in periodontal disease (6). The Interleukin (IL)-1 family (eleven members including IL-1β and IL-18) has a prominent role in these inflammatory responses by activation of other pro-inflammatory cytokines and recruitment of inflammatory cells to sites of infection (6). Their main cellular source is monocytes, cells that function at the front line of inflammatory responses against pathogens. Monocytes express several classes of pattern-recognition receptors, such as nucleotide binding and oligomerization domain-like receptors (NLRs), which sense microbial molecules in the cytosol and thereafter activate IL-1β and IL-18 secretion. NLRP3 is the best characterized member, which forms a multi-protein complex with the adaptor protein ASC and caspase-1 (7). This complex, namely "inflammasome" should be tightly regulated to control the potent proinflammatory activities of IL-1. In addition, monocytes express a recently identified receptor, namely triggering receptor expressed on myeloid cells-1 (TREM-1), whose activation can further amplify inflammatory responses to bacterial challenge, including IL-1 production (8). DAP12 is the intracellular adaptor molecule for TREM-1 that conveys the further downstream signalling that regulates inflammation (9). Further understanding of interactions between *P. gingivalis* and newly identified inflammatory signalling pathways should provide new insights into the pathogenesis of periodontal

diseases. The present thesis includes a series of *in vitro* studies aiming to investigate strategies by which *P. gingivalis* may collectively exploit the host inflammatory responses in monocytes. The complex interactions studied involved: a) *antagonism with other pathogens for pro-inflammatory cytokine production, b) differential effects of* P. gingivalis *virulence factors on pro-inflammatory cytokine production,* c) *interference with the pathogen-sensing NLRP3 inflammasome,* and d) *amplification of pro-inflammatory cytokine production by the TREM-1/DAP12 pathway.*

Methods

Human peripheral blood mononuclear cells (PBMC) were prepared by Ficoll density gradient sedimentation. For the enrichment of monocytes from PBMC, an antibody-mediated depletion technique was used. The purity of isolated monocytes was analyzed by CD14 staining by flow cytometry. These isolated monocytes, or the monocytic cell line Monomac-6, were challenged with either live *P. gingivalis* W50 or its products (LPS and culture supernatants). To inhibit its cysteine proteinases (gingipains), bacterial supernatants were pretreated with the chemical inhibitor TLCK, or heated to 70 °C to inactivate protein content. The production of cytokines by the cells was measured by cytokine-specific enzyme-linked immunosorbent assay (ELISA). The expression of various genes was assessed by quantitative real-time RT-PCR. Confocal imaging was also used in order to investigate the concomitant presence of bacteria and protein expression at the intracellular level. Microbiological analysis was done by counting of total colony forming units.

Results and discussion of the findings

Two predominant periodontal pathogens, *P. gingivalis* and *Campylobacter rectus* were tested for their capacity to stimulate IL-1β, IL-6 and IL-8 production by human monoyctes (*Publication I*). Both species significantly stimulated the production of all three cytokines, but *P. gingivalis* had a considerably weaker effect. More strikingly, co-stimulation of the cells with *P. gingivalis* and *C. rectus* suppressed the cytokine-stimulatory capacity of the latter, rather than having a synergistic effect. Purified *P. gingivalis* LPS was alone sufficient to antagonise cytokine stimulation by *C. rectus*. This study highlighted the bi-phasic capacity of *P. gingivalis* to manipulate host inflammatory responses, either by inducing cytokine production, or by reducing the cytokine-stimulatory capacity of other pathogens. As the LPS appeared to have a predominant role in these effects, the potential differential effects of the two distinct LPS(s) of *P. gingivalis* were further investigated *(Publication II)*. The newly identified A-LPS showed considerably weaker capacity to elicit IL-1 production by human monocytes, compared to the "classical" LPS. This is in line with previous reports showing that even small alterations in the

LPS structure could alter the capacity of *P. gingivalis* to provoke host immune responses (10). Apart from its LPS, the gingipains of *P. gingivalis* constitute another set of virulence factors that can subvert the host immune responses. Inactivation of its gingipains caused a 50% reduction in the capacity of *P. gingivalis* to stimulate IL-1β expression by monocytes, but it did not affect IL-18 gene expression. On the protein level though, the end-point effect on IL-1β secretion was attributed to the LPS, rather than the gingipains *(Publication III)*. Hence, this study demonstrated that although IL-1β and IL-18 belong to the same cytokine family (11), the stimulation of their individual gene expression by *P. gingivalis* is attributed to different virulence factors. Moreover, the stimulation of gene expression and secretion of IL-1β are regulated by distinct virulence factors of *P. gingivalis*. The regulation and processing of IL-1 cytokines was further considered on the intracellular level, by studying the regulation of the NLRP3 inflammasome complex in monocytes *(Publication IV)*. Interestingly, the expression of the NLRP3 sensor was increased, whereas that of the ASC adaptor was decreased in response to *P. gingivalis*. These opposing actions of *P. gingivalis* may suggest a deregulation of the NLRP3 inflammasome complex, and hence an impairment of the capacity of the monocytes to sense pathogen signals, in order to efficiently process IL-1 production. A further inflammatory pathway considered was the TREM-1/DAP12 pathway, which can amplify inflammatory events (*Publication V*). *P. gingivalis* enhanced TREM-1 gene expression and secretion in monocytes, whereas it had a bi-phasic effect on DAP12 expression, well in line with the bi-phasic effects of *P. gingivalis* on the innate immune responses (12). Activation of TREM-1 in monocytes demonstrated a synergistic effect on the capacity of *P. gingivalis* to induce IL-1β and IL-6 secretion. Collectively, these data suggest that *P. gingivalis* may use the TREM-1/DAP12 pathway to enhance both local and systemic inflammation (13).

Conclusion and significance

Periodontal disease is the outcome of intricate inflammatory networks that are developed following a chronic cross talk between oral bacteria and the host cells in the periodontium. The present studies have revealed a complex interplay between *P.gingivalis* and monocytes. *P. gingivalis* may manipulate the host immune responses, inducing, amplifying or dampening pro-inflammatory cytokine production by the monocytes. This may provide an adaptational superiority to this species, by regulating the local defence mechanisms according to its occasional needs for survival and persistence in the host environment, thus contributing to the disease. A thorough understanding of such host inflammatory responses to *P. gingivalis* also allows for the exploration of new treatment strategies, aiming at their modulation.

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