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Extracellular Trap Release and Inflammation Induction in Mast Cells by *Fusobacterium nucleatum*

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Abstract

Mast cells play an important role in the innate immune responses to bacterial infections as the first line of defense such as in the skin and mucosa. Mast cells can produce extracellular traps to kill bacteria by trapping pathogens. Mast cell-extracellular traps (MCETs) are composed of web-like DNA fibers that contain bactericidal substances such as DNA, histones, tryptase, and antimicrobial peptides. At present, it is unknown whether the induction of inflammation in periodontal diseases is due to MCETs induced by periodontal bacteria. We investigated the role of mast cells in the induction of MCET production following infection with *Fusobacterium nucleatum*, a gram-negative anaerobic bacterium associated with periodontal disease. We found that mast cells produced MCETs in response to *F. nucleatum* infection. Furthermore, the MCETs highly expressed macrophage migration inhibitory factor (MIF). Of note, the level of MIF expressed in the MCETs was inhibited by taurolidine, an LPS antagonist. We next investigated whether MCETs can induce inflammatory responses in monocytes. The MCETs induced the production of IL-1β, IL-6, and IL-8 by monocytes. The production of IL-1β, IL-6, and IL-8 was inhibited by an MIF inhibitor. These findings suggest that MCETs produced by mast cells in response to F. nucleatum infection induce proinflammatory cytokine production by monocytes, which may lead to the chronic inflammation observed in periodontal diseases.

Keywords

Chronic periodontitis
Mast cells
Mast cell-extracellular traps
Fusobacterium nucleatum
Lipopolysaccharide

1. Introduction

Chronic periodontitis, which affects approximately 80% of adults over the age of 30, is an infection caused by periodontal disease-associated bacteria. The destruction of periodontal tissue leading to tooth loss is caused by chronic inflammation due to a prolonged immune response to periodontal disease-associated bacteria that chronically infect periodontal pockets. Periodontal pockets contain a biofilm of more than 100 million oral bacteria at 1 mg, known as dental plaque, and the dental plaque flora of patients with chronic periodontitis is predominantly composed of periodontal disease-associated bacteria. The majority of immune cells that accumulate in periodontal pockets in response to periodontal disease-associated bacterial infection are neutrophils, but mast cells have been shown to localize to a large extent in inflamed periodontal tissue in patients with severe chronic periodontitis, suggesting that the immune response by mast cells may be involved in exacerbating inflammation in the pathogenesis of chronic periodontitis.

Upon bacterial infection, neutrophils undergo cell death, known as NETosis, and release cellular contents called neutrophil extracellular traps (NETs), which exert a protective effect against infection. However, persistent or excessive release of NETs from neutrophils has been suggested to exacerbate chronic inflammatory diseases. On the other hand, mast cells also play a role in the defense mechanism against Group A *Streptococcus* infection by releasing mast cell-extracellular traps (MCETs) similar to those of neutrophils.

In this article, we review our findings on the release of MCETs from mast cells upon infection with *Fusobacterium nucleatum*, a periodontal disease-associated bacterium, and the induction of inflammation by MCETs.

2. Release of extracellular traps by mast cells

Upon infection with pathogens, mast cells recognize pathogens with innate immune receptors such as TLRs, Dectin-1, and complement receptors, and exert direct defense mechanisms by phagocytosis, degranulation, and release of reactive oxygen species (ROS). Mast cells indirectly exert defense mechanisms through histamine- and leukotriene C4-induced mucin production from epithelial cells, increase vascular permeability, and migration of eosinophils, neutrophils, and NK cells by IL-8, TNF-α, and eotaxin [1]. Recently, it has been shown that mast cells release MCETs, which consist of DNA and histones similar to NETs released by neutrophils, against Group A *Streptococcus* and *Leishmania* infections [2-4], indicating that MCETs are a defense mechanism by mast cells in bacterial infections.

3. Role of periodontal disease-associated bacteria and mast cells in chronic periodontitis

The periodontitis-associated bacteria comprise three bacterial species, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, which are the three species most strongly involved in the pathogenesis of chronic periodontitis, known as the red complex. *Fusobacterium nucleatum* (*F. nucleatum*) is a Gram-negative anaerobic bacterium frequently detected in periodontal pockets of patients with chronic periodontitis, belonging to the orange complex after the red complex [5] and is endemic in the intestinal tract as well as the human oral cavity, promoting the growth of colon cancer [6]. Dental plaque formation begins with the establishment of early colonizers, Gram-positive commensal bacteria including the caries-causing *Streptococcus mutans*, on the tooth surface. Next, as the dental plaque matures over time, the late colonizers, Gram-negative anaerobes (late-associated bacteria), mainly periodontal disease-associated bacteria, become the main organisms. During this transition (dysbiosis), *F. nucleatum* has a strong co-aggregation ability, which is required for the initial formation of the dental

plaque, and plays a role in binding both early- and late-adherent bacteria [7].

Although it has been suggested that mast cells accumulate in the inflamed gingiva of patients with severe chronic periodontitis [8-12], the functional role of mast cells in the pathogenesis of chronic periodontitis is not fully understood. Recently, we have shown that infection of the mouse oral cavity with *P. gingivalis* results in mast cell-dependent production of the proinflammatory cytokine IL-31, which, when acting on gingival epithelial cells, down-regulates the expression of the tight junction molecule claudin-1 involved in the induction of chronic inflammation [13].

4. Release of extracellular traps from mast cells by *F. nucleatum*

ROS production from neutrophils by *F. nucleatum* has been shown to be higher than that by *P. gingivalis* [14] and ROS is essential for the induction of NETs and MCETs in the cytoplasm, suggesting that *F. nucleatum* is more potent than *P. gingivalis* in releasing MCETs from mast cells. Therefore, *F. nucleatum* is predicted to induce the release of MCETs from mast cells more strongly than *P. gingivalis*. We, therefore, investigated the possibility that mast cells infected with *F. nucleatum* release MCETs. After infecting the cell line HMC-1 with *F. nucleatum*, the DNA was found to be released outside the cells 4 hours after infection. The same extracellular DNA release was also observed when mouse bone marrow-derived mast cells (BMMCs) were infected with *F. nucleatum*. This release of extracellular DNA was also observed in dead *F. nucleatum* bacteria and in mast cells pre-treated with cytochalasin D and nocodazole, indicating that it was independent of bacterial intracellular entry and phagocytosis of bacteria by mast cells. The extracellular DNA released from mast cells by *F. nucleatum* infection proved to be MCETs, as citrullination of histone H3 was clearly detected in the extracellular DNA released from mast cells. The MCETs also expressed a cationic antimicrobial peptide of 18-kDa (CAP-18), an antimicrobial peptide belonging to the cathelicidin family, suggesting that they have infection-defense properties. We analyzed the molecular groups expressed in MCETs released from mast cells due to *F. nucleatum* infection using a cytokine array method with a membrane coated with antibodies to 105 cytokines and other molecules and found that the MCETs released from mast cells due to *F. nucleatum* infection include IL-1 receptor antagonist (IL-1ra) and plasminogen activator inhibitor-1 (PAI-1). In addition, macrophage migration inhibitory factor (MIF) was also found to be expressed at high levels in HMC-1 and BMMCs. On the other hand, we found that human neutrophils release NETs upon *F. nucleatum* infection, which also express high levels of MIF and induce inflammation in vascular endothelial cells.

5. Induction of inflammation by MIF expressing MCETs

MIF was initially discovered as a factor preventing the uncontrolled migration of macrophages from capillaries. Subsequently, the induction of endotoxin shock by LPS was inhibited by anti-MIF antibodies [15], and MIF was found to be an inhibitor of IL-1β, IL-6, IL-8, and TNF-α [16]. MIF is constitutively expressed in the cytoplasm of diverse cell types and is not produced extracellularly by the normal endoplasmic reticulum-mediated protein secretion system due to the lack of an amino terminus, suggesting that MIF is a pro-inflammatory cytokine. MIF is released extracellularly as a result of necrosis-associated cytotoxicity, as in the case of damage-associated molecular patterns (DAMPs) [17]. Interestingly, MCETs released by mast cells infected with *F. nucleatum* showed marked MIF expression, whereas MIF was virtually undetectable in the culture supernatant. Therefore, we investigated the possibility that the LPS of *F. nucleatum* could be responsible for the bacterial components that induce MIF expression of MCETs released by mast cells

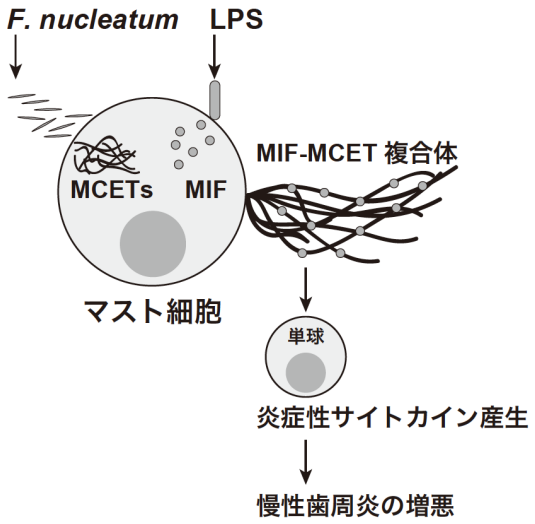

Figure 1. Release of MIF-MCET complexes in mast cells by *F. nucleatum* infection induces inflammatory cytokine production from monocytes. *F. nucleatum* infection induces mast cells to release MCETs, whereas stimulation of *F. nucleatum*-derived LPS induces MIF expression. MIF-bound MCETs are released extracellularly upon cell death and induce the production of pro-inflammatory cytokines from monocytes.

upon infection with *F. nucleatum*. The MIF expression of MCETs released by mast cells upon *F. nucleatum* infection was markedly inhibited by pre-treatment of the MCETs with taurolidine, an LPS inhibitor. Therefore, when mast cells were stimulated with LPS from *F. nucleatum*, we found that MIF was not produced in the culture supernatant, but significant MIF expression was induced in the cytoplasm. These findings suggest that when mast cells are infected with *F. nucleatum*, MCETs are formed in the cytoplasm, and at the same time MIF accumulates in the cytoplasm due to LPS stimulation, forming MIF-MCET complexes, which are released from the cells upon cell death (**Figure 1**.) Lastly, the induction of inflammation by MCETs released by *F. nucleatum* infection was examined using monocyte-like cells induced to differentiate from human THP-1 cells by active vitamin D3, and the production of pro-inflammatory cytokines from THP-1 cells by MCETs. Stimulation of THP-1 cells with MCETs markedly induced the production of IL-1β, IL-6, and IL-8. Pre-treatment of THP-1 cells with the MIF antagonist ISO-1 completely inhibited this effect. These findings indicate that *F. nucleatum* infection causes mast cells to release MCETs and that MIF expressed on MCETs induces inflammatory cytokine production from monocytes, as displayed in **Figure 1**.

6. Conclusion

We have shown that infection with the periodontitis-associated bacterium *F. nucleatum* causes mast cells to release MCETs and that the bacterium's LPS induces MIF expression. MIF binding to MCETs also induced pro-inflammatory cytokines production from monocytes. MIFs expressed on mast cell MCETs and neutrophil NETs released by a periodontal disease-associated bacterial infection in chronic periodontitis may be involved in the prolongation of inflammation in chronic periodontitis, and it is a topic for future investigation.

Disclosure statement

The authors declare no conflicts of interest.

References

[1] Urb M, Sheppard DC, 2012, The Role of Mast Cells in the Defense Against Pathogens. PLoS Pathog, 2012(8): e1002619.

[2] Clark M, Kim J, Etesami N, et al., 2018, Group A *Streptococcus* Prevents Mast Cell Degranulation to Promote Extracellular Trap Formation. Front Immunol, 2018(9): 327.

[3] von Köckritz-Blickwede M, Goldmann O, Thulin P, et al., 2008, Phagocytosis-Independent Antimicrobial Activity of Mast Cells by Means of Extracellular Trap Formation. Blood, 2008(111): 3070–3080.

[4] Naqvi N, Ahuja K, Selvapandiyan A, et al., 2017, Role of Mast Cells in Clearance of *Leishmania* Through Extracellular Trap Formation. Sci Rep, 2017(7): 13240.

[5] Socransky SS, Haffajee AD, 2002, Dental Biofilms: Difficult Therapeutic Targets. Periodontol, 2000(28): 12–55.

[6] Kostic AD, Chun E, Robertson L, et al., 2013, *Fusobacterium nucleatum* Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment. Cell Host Microbe, 2013(14): 207–215.

[7] Jiao Y, Hasegawa M, Inohara N, 2014, The Role of Oral Pathobionts in Dysbiosis During Periodontitis Development. J Dent Res, 2014(93): 539–546.

[8] Gupta K, Idahosa C, Roy S, et al., 2017, Differential Regulation of Mas-Related G Protein-Coupled Receptor X2-Mediated Mast Cell Degranulation by Antimicrobial Host Defense Peptides and *Porphyromonas gingivalis* Lipopolysaccharide. Infect Immun, 2017(85): e00246–17.

[9] Huang S, Lu F, Chen Y, et al., 2013, Mast Cell Degranulation in Human Periodontitis. J Periodontol, 2013(84): 248–255.

[10] Huang S, Lu F, Li J, et al., 2014, Quantification of Tryptase-TIM-3 Double-Positive Mast Cells in Human Chronic Periodontitis. Arch Oral Biol, 2014(59): 654–661.

[11] Myint M, Steinsvoll S, Yuan ZN, et al., 2002, Highly Increased Numbers of Leukocytes in Inflamed Gingiva from Patients with HIV Infection. AIDS, 2002(16): 235–243.

[12] Steinvoll S, Halstensen TS, Schenck K, 1999, Extensive Expression of TGF-β1 in Chronically-Inflamed Periodontal Tissue. J Clin Periodontal, 1999(26): 366–373.

[13] Tada H, Nishioka T, Takase A, et al., 2019, *Porphyromonas gingivalis* Induces the Production of Interleukin-31 by Human Mast Cells, Resulting in Dysfunction of the Gingival Epithelial Barrier. Cell Microbiol, 2019(21): e12972.

[14] Wright HJ, Chapple IL, Matthews JB, et al., 2011, *Fusobacterium nucleatum* Regulation of Neutrophil Transcription. J Periodontal Res, 2011(46): 1–12.

[15] Bernhagen J, Calandra T, Mitchell RA, et al., 1993, MIF is a Pituitary-Derived Cytokine that Potentiates Lethal Endotoxemia. Nature, 1993(365): 756–759.

[16] Calandra T, Bernhagen J, Mitchell RA, et al., 1994, The Macrophage is an Important and Previously Unrecognized Source of Macrophage Migration Inhibitory Factor. J Exp Med, 1994(179): 1895-1902.

[17] Roth S, Agthe M, Eickhoff S, et al., 2015, Secondary Necrotic Neutrophils Release Interleukin-16C and Macrophage Migration Inhibitory Factor from Stores in the Cytosol. Cell Death Discov, 2015(1): 15056.