M1/M2 macrophage polarity in elderly patients with periodontitis and diabetes mellitus

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Abstract

Background: Periodontitis is a common chronic infectious disease related to diabetes mellitus. The progression of periodontal damage under diabetic condition caused the imbalance of immune defense and inflammation where macrophage plays a role. Age also affects dysregulated macrophage function. Macrophage has the ability to change into an M1 or M2 phenotype.

Aim: This literature aimed to understand M1/M2 polarity in an elderly patient with periodontitis and diabetes mellitus.

Discussion: Periodontitis exacerbated by diabetes mellitus in elderly patients enhanced M1 phenotype macrophage via activation of TLR and overproduction of ROS, and suppressed M2 phenotype by fewer IL-4 receptors available for ligand binding in the elderly.

Conclusion: Enhanced M1 and suppressed M2 polarity in the elderly patient with periodontitis and diabetes mellitus might take responsibility for aggravated periodontal damage.

Keywords: periodontitis, diabetes mellitus, age, M1/M2 macrophage polarity

Introduction

Periodontitis is a common chronic infectious disease caused by plaque biofilms leading to destruction of supporting periodontal tissue and loss of periodontal attachment. Approximately 3.5 billion people in the world with periodontitis and tooth loss, and more than 10% adults affected severe periodontitis. (Sun et al., 2021) [11]. Ministry Health of Indonesia shows 60% adults in Indonesia affected periodontitis. (Depkes RI, 2011). Periodontitis is the most common complication in *diabetes mellitus* (DM) with prevalence up to 75%. Research shows, there were 118 people (93.7%) of 126 DM patients who suffered periodontitis (Berniyanti et al., 2022) [1]. DM is chronic metabolic disease characterized by hyperglycemia and high glycated hemoglobin. Prevalence of DM are higher in elderly than in young people. DM affects 10.9 million adults aged 65 years and older, and this number is projected to reach 26.7 by 2050, which means 55% of all diabetes cases. (Chentli et al., 2015) [2].

In periodontitis, macrophages contribute to inflammation and tissue homeostasis through Toll-like receptor (TLR) associated with immunity to bacteria. Macrophages exhibit two different functional phenotypes in response to different stimuli called polarization. The two outcomes are M1 and M2. M1 macrophage with proinflammatory functions activated by LTA, LPS, and IFN-ɣ in order to eliminate pathogen. M2 macrophage with anti-inflammatory functions activated by IL-4, IL- 13 in order to remodeling tissue and wound healing (Steen et al., 2020) [10]. The balance of M1/M2 ratio can provide useful information concerning the health of periodontal tissue. In this case, increased M1 and decreased M2 can lead to alveolar bone resorption (Sun et al., 2021) [11]. Periodontitis under diabetic condition shows less favorable responses to treatment. Hyperglycemia enhanced M1 polarization upon invasion of periodontal pathogens by excessive oxidative stress. (Zhang et al., 2021) [16].

It has been found that the balance of M1/M2 profile are closely related to periodontitis under diabetic condition. M1 macrophages induced proinflammatory cytokines (TNF-α, IL-6), transforming growth factor (TGF) and represent CD68+ which have function of inhibiting various inflammatory responses and promoting tissue repair, therefore M2 macrophage induced anti-inflammatory cytokines such as IL-10 and represent CD163+ (Yin et al., 2022) [15]. DM is degenerative disease with the high prevalence in elderly, which in the previous study shows that aging affects many aspects of the cellular function of macrophages. Increasing age leads to numerous changes in the immune system and a progressive proinflammatory state (Mahbub et al., 2012) [7]. Based on these backgrounds, we propose a deeper understanding of M1/M1 polarity in elderly patient with periodontitis under a diabetic condition.

Literature review macrophages

Macrophage are released into the peripheral blood as monocytes, the migrate to tissue and differentiate into macrophages or dendritic cells. Macrophage polarization may occur in an inflammatory process. T cell producing IL-4 then polarization toward M2 occur, while T-cell producing IFN-γ

then polarization toward M1 occur. Respectively, polarization depending on the amount of cytokine, exposure time and the competition for cytokine. Polarization is dynamic across time and involves the tissue microenvironment. (Murray, 2017) [9]. Macrophages contribute to the process of infection prevention, tissue repairing, angiogenesis, and immunomodulation (Yao et al., 2019) [14].

M1 macrophages pathway

M1 macrophage characterized by TLR-2. TLR-4, CD80, CD86, iNOS, and MHC-II surface phenotypes. These cells release various cytokines and chemokines, such as TNF- α, IL-1α, IL-1β, IL-6, IK-12, CXCL9, and CXC10. Key transcription factor such as NF-kβ, STAT1, STAT5, IRF3, and IRF5 shown to regulate M1 genes. It seems that NF-kβ and STAT1 are the two major pathways involved in M1 macrophage polarization and result in microbicidal functions. (Yao et al., 2019) [14].

M1 macrophage activated by *lipoteichoic acid* (LTA), lipopolysaccharide (LPS), and Thelper type 1 such as interferon IFN-ɣ through pattern recognition receptors (PRR) such as Toll-like receptor (TLR). Activation of TLR induce *Myeloid Differentiation Primary-Response Protein 88* (MyD88). MyD88 recruit's *interleukin 1 receptor associated kinase 4* (IRAK4) and phosphorylate IRAK1 then activate TNF *Receptor-Associated Factor 6* (TRAF6). Those two proteins interact with *TGF-β-activated kinase 1* (TAK1) and TAK1-*binding protein* 1(TAB1), TAB2. TAK 1 phosphorylated and activates Ik-β and caused NF-kβ translocate to nucleus then induce proinflammatory cytokines, such as *Tumor necrosis factor αlpha* (TNF- α), and IL-16 (Mukherjee et al., 2016) [8].

Therefore, M1 macrophages are critical for host protection against viruses and bacteria. They also produce microbicidal and tumoricidal reagents, such as nitric oxide (NO) or reactive oxygen intermediates (ROI). However, excessive and persistence M1 macrophages will inhibit cell proliferation and caused tissue damage. (Lee, 2019) [6].

M2 macrophages pathway

M2 changes phenotype happens in response to decrease cytokines such as IL-4, IL-13, IL-10, IL-33 and TGF-β. Activation of M2 macrophage directly induced by IL-4 and IL-13 through producing Th2 cytokines. M2 macrophages identified by their expression of surface markers, such as mannitol receptor, CD206, CD163, and CD209. Up-regulation of cytokines and chemokines, such as IL-10, TGF-β, CCL1, CCL17, CCL18, CCL22, and CCL24. Key transcription factors such as STAT6, IRF4, JMJD3, and PPARɣ have been shown to regulate the expression of M2. STAT6 pathway has been considered to be the pathway to activate M2 macrophages (Yao et al., 2019) [14].

M2 macrophages are stimulated by IL-4 and IL-13, regulated by STAT-6 and they have increased expression of such as Arg-1 and macrophage mannose receptor. High level of Arg-1 impairs Tcell proliferation and IFN-ɣ production. Increased level of Arg-1 competes with iNOS and reduces NO production (Lee, 2019) [6].

M1/M2 polarity in periodontitis with diabetes mellitus

M1 being pro inflammatory and involved in bacterial killing by an increase production of interleukin-6 (IL-6), TNF-α, and inducible nitric oxide synthase (iNOS). In contrast, M2 phenotype plays a role in the resolution of inflammation and tissue repair being characterized by the production of IL-10. Furthermore, M2 has been associated with the presence of chronic infections. Animal models for experimental periodontitis shows high level of CD 80 and TNF-α as a representation of M1 during early inflammation process, while during tissue healing shows high level of CD 206 and TGF-β as a representation of M2 (Viniegra et al., 2018) [12].

Research shows chronic periodontitis group has a higher M1/M2 ratio than gingivitis group. M1 phenotype shows through CD68, MMP-9 and iNOS expression, while M2 shows through CD206 expression. Periodontitis is characterized by accumulation of large number of M1 at the bone destruction which produce large amounts of IL-1β and TNF-α and upregulating expression of RANKL and increasing bone resorption (Wang et al., 2021) [13]. Polarization macrophages from M1 to M2 appears to be reduced in periodontitis than healthy tissue (Garaicoa- Pazmino et al., 2019) [5].

M1 are the main phenotype that responds to *Poryphormonans gingivalis (Pg)* one of the main periodontitis bacteria. *Pg* inhibits activation of M2 by obstructing the production of α-ketoglutarate in mice. In addition, *Pg* might control the plasticity of macrophage, by preventing M0 transforming into M2 and redirecting M2 cells to be polarized to M1 then leading to over inflammatory environment. In gingival crevicular fluid of periodontitis found oncostatin M (OSM) which induces matrix metalloproteinases (MMPs). (Wang et al., 2021) [13]. *P. gingivalis* activates TLR9, TL2 and TL4 and upregulating M1 and nitric oxide secretion then caused destruction of periodontal health. In addition, NLRP3 and AIM2-like receptors are subset of PRRs that are associated to the occurrence and development of periodontitis. NLRP3 and AIM2 regulates alveolar bone loss in periodontitis then followed by significant upregulation of caspase-1, IL-1β and resulting a large number of inflammatory cell recruitment and promoting osteoclastic differentiation. (Yin et al., 2022) [15].

M1/M2 macrophage phenotype changes in the progression of experimental diabetic periodontitis found induce ROS overproduction, stimulating expression of iNOS, TNF-a, IL-6 and inhibiting mRNA expression of Arg-1, CD206 and CCL18 (M2 cytokine).

M1/M2 polarity in elderly

Aging affects many aspects of the cellular function of macrophages. M1/M2 macrophage phenotype changes in diabetic periodontitis also affected by age. Macrophage activity can be increasingly dysregulated during aging, which possibly the reason of high incidence of diabetic periodontitis in elderly. (Costantini et al., 2018) [3].

Macrophages from aged mice and humans display impairments in their functional activity from defective response in early immune defense to specific immune responses. M1 macrophage induced by LPS bacteria cell wall or a combination of Th1 cytokines, IFN-γ and TNF-α, while M2 macrophage are induced by Th2 cytokines such as IL-4 and IL-13. In aged mice, M1/M2 markers decreased globally. Reduction in M1/M2 cytokine products can affect innate immune effector function, including phagocytic and NO

producrion, as well as the induction of Tcell response. Research shows, TLR4 and others signaling molecules and transcription factor, such as p38, JNK, c-Jun and NFκβ decrease. M2 is mediated by IL-4 in activator of transcription STAT-6. It found that age-related decrease in M2 responses is the level of IL-4 receptor expression significantly lower in aged mice. This suggest that fewer receptor available for ligan binding and downstream signaling. (Mahbub et al., 2012) [7].

Discussion

Bacteria periodontitis, such as *P.gingivalis* might activates TLR9, TLR2, or TLR4 then induces the signaling pathway such as NFκβ and MAPK. Translocation NFκβ into the nucleus, increase proinflammatory cytokines as representation of M1 macrophage to eliminate microbes. Higher M1 macrophage induce production of RANKL and cause alveolar bone resorption in periodontitis (Wang et al., 2021) [13]**.** This periodontitis condition could be exacerbated by hyperglycemia in diabetes. Hyperglycemia condition in diabetic periodontitis can break the balance of ROS production and antioxidant defense. Excessive ROS originated from increased formation of AGEs, overload of the electron transport chain in mitochondria, and enhanced NADPH oxidase. In diabetic condition, increased mitochondrial ROS upregulate the signal of MAPK which has been involved in modulation M1 macrophage signaling (Zhang et al., 2021) [16]. M1 macrophage promote osteoclast genesis caused the direct effects of proinflammatory cytokines and inhibit osteoblast mineralization. ROS and nitric oxide (NO) production by NADPH and iNOS are key to the antimicrobial activity of M1. Indeed NO production also inhibit oxidative metabolism which is important for M2 macrophage (Covarrubias et al., 2013) [4]. Macrophages phenotype changes and functional activity in elderly decrease significantly. M1 and response surpressed compared to the young. Studies show age-associated defects in macrophage polarization. The reduced expression of M2 markers might cause the lower numbers of IL-4R cells which have a function to induce M2 macrophage. These findings suggest that depressed M1/M2 lead to hinder their ability to fight pathogens. (Mahbub et al., 2012) [7].

Conclusion

In conclusion, this literature summarizes that diabetic periodontitis might enhance M1 macrophage phenotype and suppress M2 macrophage phenotype by activation of TLR and overproduction of ROS. M2 phenotype changes also worsen in the elderly due to fewer IL-4 receptors available for ligand binding and downstream signaling. Higher M1 and lower M2 phenotypes could affect the destruction of alveolar bone and inhibit bone remodeling in elderly with diabetic periodontitis.

References

1. Berniyanti T, Wening GRS, Palupi R, Setyowati D, Putri CR. Low Levels of Tumor Necrosis Factor-α will Prevent Periodontitis Exacerbation in Type 2 Diabetes Mellitus. European Journal of Dentistry, 2022; 16(2):443-448. https://doi.org/10.1055/s-0041-1739442
2. Chentli F, Azzoug S, Mahgoun S. Diabetes mellitus in elderly. Indian Journal of Endocrinology and Metabolism, 2015; 19(6):744-752. https://doi.org/10.4103/2230-8210.167553
3. Costantini A, Viola N, Berretta A, Galeazzi R, Matacchione G, Sabbatinelli J, et al. Age-related M1/M2 phenotype changes in circulating monocytes from healthy/unhealthy individuals. Aging, 2018; 10(6):1268-1280. https://doi.org/10.18632/aging.101465
4. Covarrubias A, Byles V, Horng T. ROS sets the stage for macrophage differentiation. Cell Research, 2013; 23(8):984-985. https://doi.org/10.1038/cr.2013.88
5. Garaicoa-Pazmino C, Fretwurst T, Squarize CH, Berglundh T, Giannobile WV, et al. Characterization of macrophage polarization in periodontal disease. Journal of Clinical Periodontology, 2019; 46(8):830-839. https://doi.org/10.1111/jcpe.13156
6. Lee KY. M1 and M2 polarization of macrophages: a mini-review. Medical Biological Science and Engineering, 2019; 2(1):1-5. https://doi.org/10.30579/mbse.2019.2.1.1
7. Mahbub S, Deburghgraeve CR, Kovacs EJ. Advanced age impairs macrophage polarization. Journal of Interferon and Cytokine Research, 2012; 32(1):18-26. https://doi.org/10.1089/jir.2011.0058
8. Mukherjee S, Karmakar S, Babu SPS. TLR2 and TLR4 mediated host immune responses in major infectious diseases: A review. Brazilian Journal of Infectious Diseases, 2016; 20(2):193-204. https://doi.org/10.1016/j.bjid.2015.10.011
9. Murray PJ. Macrophage Polarization. Annual Review of Physiology, 2017; 79:541-566. https://doi.org/10.1146/annurev-physiol-022516-034339
10. Steen EH, Wang X, Balaji S, Butte MJ, Bollyky PL, Keswani SG. The Role of the Anti-Inflammatory Cytokine Interleukin-10 in Tissue Fibrosis. Advances in Wound Care, 2020; 9(4):184-198. https://doi.org/10.1089/wound.2019.1032
11. Sun X, Gao J, Meng X, Lu X, Zhang L, Chen R. Polarized Macrophages in Periodontitis: Characteristics, Function, and Molecular Signaling. Frontiers in Immunology, 2021; 12:1-18. https://doi.org/10.3389/fimmu.2021.763334
12. Viniegra A, Goldberg H, Çil Fine N, Sheikh Z, Galli M, Freire M, et al. Resolving Macrophages Counter Osteolysis by Anabolic Actions on Bone Cells. Journal of Dental Research, 2018; 97(10):1160-1169. https://doi.org/10.1177/0022034518777973
13. Wang W, Zheng C, Yang J, Li B. Intersection between macrophages and periodontal pathogens in periodontitis. Journal of Leukocyte Biology, 2021; 110(3):577-583. https://doi.org/10.1002/JLB.4MR0421- 756R
14. Yao Y, Xu XH, Jin L. Macrophage polarization in physiological and pathological pregnancy. Frontiers in Immunology, 2019; 10:1-13. https://doi.org/10.3389/fimmu.2019.00792
15. Yin L, Li X, Hou J. Macrophages in periodontitis: A dynamic shift between tissue destruction and repair. Japanese Dental Science Review, 2022; 58:336-347. https://doi.org/10.1016/j.jdsr.2022.10.002
16. Zhang B, Yang Y, Yi J, Zhao Z, Ye R. Hyperglycemia modulates M1/M2 macrophage polarization via reactive oxygen species overproduction in ligature-induced

periodontitis. Journal of Periodontal Research, 2021; 56(5):991-1005. https://doi.org/10.1111/jre.12912