ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

Research status of macrophage polarization and osteogenesis and vasculogenesis after polarization

Shangyi Lv[1,2,a], Lisha Ma[1,2,b], Huiyu He[1,2,*]

[1]Department of Prosthodontics and Dental Implantology, The First Affiliated Hospital of Xinjiang Medical University (Affiliated Stomatological Hospital), Urumqi, Xinjiang, 830054, China
[2]Xinjiang Uygur Autonomous Region Institute of Stomatology, Urumqi, Xinjiang, 830054, China
[a]994163038@qq. com, [b]1055128717@qq. com
**Corresponding author : hehuiyu01@163. com*

Abstract: *Macrophages are one of the important innate immune cells in human body. Phenotypic variability and functional diversity are important characteristics of macrophages, which can be polarized under the induction and regulation of different microenvironmental signals. After polarization, macrophages further affect the local immune response, and cooperate with various cytokines in pathogen and microbial infection, tumor and immune regulation, tissue remodeling and regulation. Therefore, the polarization of macrophages and their role after polarization have become a new research hotspot.*

Keywords: *Macrophages, Polarization, Osteogenesi, Angiogenesis*

1. Introduction

Macrophages are an important component of innate innate immunity, which can activate the acquired immune response by releasing a variety of cytokines and participate in the inflammatory response to maintain homeostasis of the environment [1]. Macrophage as a malleable and pluripotent cells group, under the influence of the local microenvironment in vivo and in vitro factors, according to their activation status and function of different type is extremely M1 is classic activated macrophages (classically activated macrophage), and M2 type is a type of macrophages (alternatively activated macrophage) [2]. M1 macrophages secrete pro-inflammatory cytokines and chemokines, and present antigens full-time, participate in the positive immune response, and play the function of immune surveillance; M2 macrophages have weak antigen presentation ability and down-regulate immune response by secreting inhibitory cytokines such as IL-10 and/or TGF-B, which play an important role in immune regulation [3]. Different polarization types of macrophages play different roles in physiological and pathological conditions. This article reviews the effects of polarization of macrophages on osteogenesis and angiogenesis.

2. Polarization phenotype of macrophages and inducing factors

Macrophages are derived from monocytes, which are derived from precursor cells in the human bone marrow. Because macrophages are a diverse cell population, there are great differences in inducing factors and polarization phenotypes of macrophages in different tissues or in the same tissue.

2.1. Polarization phenotype of M1 macrophages and inducing factors

Monocytes can develop into M1 macrophages under the induction of granulocyte-macrophage colony stiMulating factor (GM-CSF) [4]. M1 phagocytes are polarized by Th1 cytokines such as IFN-Y, tumor necrosis factor-A (TNFα), and pathogen-related molecular patterns such as LPS or endogenous danger signals 16. In addition, Janus protein tyrosine kinase, signal transducer and activ ator of transcription (STAT) signaling pathway can change macrophage polarization via NF- κB pathway [5]. STATl, STAT3, STAT4 and STATL-STAT2 can promote the polarization of M1 macrophages [6]. Interferon-regulatory factor (IRF)5 can also control the polarization of M1 macrophages [7]. The virulence factor of Porphyromonas gingivalis (c-di-GMP) can interfere with the transcription of TNF-A, IL-6 and IL-23 genes in macrophages, and the transcription level of c-di-GMP is dose-and time-dependent, so c-di-GMP also suggests the characteristics of polarization of M1 macrophages [8]. M1 macrophages are involved

ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

in acute proinflammatory response, exerting strong antimicrobial activity and anti-proliferation function, and forming reactive oxyg en species (ROS), nitric oxide (NO) and a large number of proinflammatory cytokines such as interleukin1(IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-23 (IL-23), TNF-α and cyclooxygenase 2 [9].

2.2. Polarization phenotype of M2 macrophages and inducing factors

Monocytes can develop into M2 macrophages when cultured with multi-colony stiMulating factor (M-CSF) [4]. M2 macrophages are induced by Th2 cytokines such as IL-4 or IL-13. Studies have confirmed that in the phosphoinositide3-kinases (PI3k)/Akt signaling pathway, Akt1 can express higher levels of iNOS, TNF-α and IL-6 in the deleted macrophages, indicating that Akt1 can promote Polarization of M2 macrophages [10]. In JAK-STAT signaling pathway, STAT6 is an important transcription factor in M2 macrophage polarization induced by IL-4 or IL-13, and STAT6 activates the transcription of genes involved in M2 polarization. [11]M2 macrophages also release angiogenic mediators, the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), which can control inflammation, promote tissue remodeling, angiogenesis and immune regulation, wound healing, and tumor formation and development [9]. Because M2 macrophages can be divided into three subtypes, namely M2a, M2b and M2c[12]. Macrophages induced by IL-4 or IL-13 are called M2a, which can produce a large amount of anti-inflammatory cytokine IL-10 and express mannose reCeptor (MR) and macrophage galactose type c-type lectin (MGL), which is insensitive to inflammatory effects, participates in the removal of killer extracellular pathogen debris, angiogenesis, tissue reconstruction and wound healing steps, and promotes Th2 immunity [13]. IC and TLR or IL-1R ligand induce the generation of M2b, also known as type I activated macrophages, which secrete a large amount of IL-10 and a low secretion of IL-12, and uniquely secrete a large amount of inflammatory cytokines TNF-α, IL-6 and IL-1, which control the acute inflammation caused by bacterial endotoxin, and promote Th2 differentiation and humoral immunity [14]. M2c, also known as inactivated macrophages induced by IL-10 and glucocorticoids to differentiate monocytes, can hypersecrete IL-10 and TGF-B to regulate and control immune inflammation. [13-14]

3. Effect of macrophage polarization on osteogenesis

Macrophages play a bidirectional role in the process of disease and tissue remodeling through their different polarization states. Whether these effects are positive or harmful depends on the transformation and balance between the polarization state of macrophages and the M1/M2 polarization state. At the same time, macrophages secrete a large number of bioactive factors under the action of tissue microenvironment, and the types and secretions of these factors are closely related to the polarization and functional status of macrophages. A large part of these bioactive factors are reported to be related to bone formation.

3.1. Effect of M1 macrophages on osteogenesis

Traditionally, M1 macrophages secreted a large number of inflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-23, TNF-α, iNOS and ROS. These cytokines promote the differentiation and absorption of osteoclasts, resulting in decreased expression of bone mass [15]. Taking IL-1β, IL-6 and TNF-α as an example, in the physiological environment, the concentrations of IL-1β, IL-6 and TNF-α increase rapidly after tissue trauma to initiate inflammatory reaction and bone repair, and then gradually decrease and disappear within 72 hours [16]. Long-term presence or excessive addition of IL-1β and TNF-α in tissues or culture systems can strongly inhibit the expression of bone morphogenetic protein-2 (BMP-2) receptor and collagen synthesis in host tissues, inhibit the chemotactic migration of osteoblast-related cells, and thus hinder bone formation [17-18]. In Gao Shuo etc. [19], however, think that the LPS of inflammatory conditions, between macrophages can enhance the migration of mesenchymal stem cells (mesenchymal stem cells, MSCs) ability. In recent years, more and more attention has been paid to the relationship between M1 macrophages and MSCs. Nicolaidou et al. [20] used the method of in vitro culture to show that monocytes and macrophages can achieve osteogenic differentiation of osteoblast precursor MSCs through direct cell-cell contact, which may be related to tumor suppressor M(OSM) promoting the expression of osteogenic genes through signal transduction and activator of transcription protein 3(STAT3) pathway, and this pathway is dependentIn the presence of cyclooxygenase 2(cox-2) and prostatic cord E2(PGE2). Subsequently, Guihard et al. [21] also proved that M1 macrophages, not M2 macrophages, produced OSM. PGE2 is a known activator of OSM generation by macrophages, and the

ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

paracrine effect between M1 macrophages and MSCs has been gradually proved, that is, after contact with MSCs, M1 macrophages release COX-2 to activate PGE2 and activate OSM in M1 to promote osteogenesis. In addition, mouse osteoblasts co-cultured with LPS-activated macrophages showed more significant calcification in the Transwell system [22]. Omar et al. [23] studied the effect of human Peripheral blood mononuclear cells (PBMCs) on human bone marrow mesenchymal stem cells (hbMSCs). They suggested that PBMCs conditioned culture base stimulated by LPS could induce hbMSCs to enhance bone morphogenetic protein-2 (BMP-2) and Runt-related transcription factor 2(Runx2) and alkaline phosphatase (ALP) expression promote osteogenic differentiation, but no activated or IL-4-treated PBMCs can promote osteogenic differentiation. Lu et al. [24] directly co-cultured bone marrow macrophages from M0, M1 and M2 mice with mouse bone marrow MSCs, and found that all macrophage subtypes promoted bone formation, among which M1 type showed the greatest enhancement effect.

In conclusion, M1 macrophages promote bone growth in the early stage of bone defect repair.

3.2. Effect of M2 macrophages on osteogenesis

M2 macrophages can synthesize large amounts of IL-4, IL-10, Arginase, VEGFA, PDGF-BB and TGF-β. By producing these anti-inflammatory factors, it promotes tissue repair and wound healing. Yang et al. [25] found through co-culture of different subtypes of macrophages and MSCs derived from adipose tissue that although M1 macrophages could significantly promote the osteogenic differentiation of co-cultured MSCs in the early and middle stages of the osteogenic process, significant enhancement of the mineralization effect of M1 macrophages on MSCs could not be observed. In contrast, M2-type macrophages enhanced the mineralization of co-cultured MSCs, and this ability was proportional to the ratio of macrophages to MSCs. Loi et al. [26] have demonstrated that M2a macrophages polarized by M1 and osteoblast precursors after 72h have better osteogenic ability than those polarized after 24h or 48h, which is related to no inhibition of OSM generation after 72h. Although the relationship between OSM and M2a macrophages needs to be further studied, the positive effect of M2a on bone regeneration is well established. The research results of He et al. also confirmed that Bone marrow mesenchynal stem cells (BMMSCs) were cultured by conditioned Medium (CM) generated by cultured macrophages of different subtypes, and the results showed that the cultured BMMSCs were cultured by CM M1 macrophages promoted cell proliferation and matrix formation, but the osteogenic function of MSCs in the co-culture system was greatly reduced. M0 macrophages improved the osteogenic ability of the cells, but the lamellar ability of the cells was significantly impaired compared with M1 and M2 macrophages. In contrast, M2 macrophages not only promote the osteogenic differentiation potential of cultured cells, but also show the ability to form robust cell lamellae [27]. Zhang et al. showed that all types of macrophages affected the osteogenic differentiation of bone marrow mesenchymal stem cells in various ways. M0 and M1 macrophages partially increased the osteogenic effect in the early and middle stages, but failed to improve the extracellular matrix mineralization. However, MSCs co-cultured directly or indirectly with M2 macrophages can obtain more significant matrix mineralization [25].

In conclusion, M2 macrophages can promote the bone formation of MSCs, and M2 macrophages play an important role in the late healing of bone defects.

4. Effect of macrophage polarization on angiogenesis

Macrophages are major vascular effector cells and inflammatory immune cells, which can form a large amount of Nitric Oxide Synthase (NOS) and vascular endothelial growth factor (VEGF) , matrix metalloproteinase (MMP) and other factors interact to promote neovascularization [28]. In recent years, more experiments have shown that it plays a key role in angiogenesis. Experimental results indicate that M2 type promotes angiogenesis, while M1 type has the opposite effect and inhibits angiogenesis [29-30].

4.1. Effect of M1 macrophages on angiogenesis

According to the study of Spiller et al. [31], M1 macrophages can secrete factors that trigger angiogenesis (such as vascular endothelial growth factor) in the process of vascular network sprouting, anastomosis and maturation. However, in the study of the effect of macrophage polarization on the formation of choroidal neovascularization (CNV), Apte et al. [32] studied the CNV model established by IL-10-/- gene knockout mice and found that the CNV area in the lesion area decreased with the aggregation of macrophages. Transgenic mice with high expression of IL-10, In the lesion area,

ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

macrophages accumulated and CNV area increased. The results indicated that M2 polarization occurred in macrophages during the formation of CNV, and IL-10 was an important stimulating factor. Once IL-10 was eliminated, M2-type polarization was affected, the ratio of Ml /M2 type was increased, the M1 type was relatively increased, and angiogenesis was inhibited.

4.2. Effect of M2 macrophages on angiogenesis

M2 macrophages secrete TGF-β and VEGF[33], TGF-β is a key factor in collagen synthesis, and VEGF is a central regulator of angiogenesis. Some articles have pointed out that Phd2 factor changes the differentiation of macrophages into M2, which is a necessary condition for blood vessel formation [34]. M2 macrophages have been shown to promote angiogenesis by expressing and secreting high levels of platelet-derived growth factor-BB (PDGF-BB) [35], which is required for the recruitment and differentiation of pericytes, smooth muscle-like cells that are in close contact with the endothelium in capillaries, where they regulate blood vessels during angiogenesisMorphology and function [36]. Migration and angiogenesis of M2-secreted PDGF-BB endothelial progenitor cells (EPCs). It has been found to mobilize mesenchymal derived cells, thereby stabilizing neovascularization and coordinating osteoblast differentiation [36-37]. New study confirmed that severe ROP disease (ROP) fiber hyperplasia in the membrane of the eye have M1, M2, at the same time, most of the M1 cells may from the early inflammatory reaction, promoting other cells infiltration and secretion of chemokines, but not local inflammatory factor levels increased significantly, but the new blood vessels and local inflammatory response to M2Is related to the transformation of tissue repair response [38]. These results suggest that macrophages play an important role in angiogenesis. In addition, other studies have found that if macrophages are eliminated in mice, TGF_B concentration level, collagen production and blood vessel formation all decrease during bone regeneration [33], and in vivo experiments have shown that wound healing and bone regeneration ability are inhibited after macrophage knockout [39].

5. Conclusion

The phenotype and polarization of macrophages have been thoroughly studied, while the cytokines secreted by macrophages polarized into M1 and M2 types and the effects of these cytokines on osteogenesis and vascularization need to be further studied. Dentition defect is one of the most important diseases in clinical dentistry, and the use of implant to repair the missing teeth has become a big trend. Therefore, the osteogenic and vasculogenic reactions of macrophages formed on the surface after implant implantation and their secretions combined with bone tissue need to be further studied, so as to provide immunological theoretical support for better and faster bone integration between implant and alveolar bone in the later stage.

References

[1] Xuetao Cao. Medical Immunology [M]. 7th Ed. People's Medical Publishing House. 2018. 07
[2] Murray PJ. Macrophage polarization [J]. Annu Rev Physiol, 2017, 79: 541-566.
[3] Van Ginderachter JA, Movahedi K, Hassanzadeh Ghas- sabeh G, et al, Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion [J]. Immunobiology, 2006, 211: 487-501.
[4] Romo N, Magri G, MuntassellA, et al. Natural killer cellmediated response to human cytomegalovirus-infected macmphages is modulated by their functional polarization [J]. J Leukoc Biol, 2011, 90: 717-726.
[5] Li H, Jiang T, Li MQ, et al. Transcriptional regulation of macrophages polarization by microRNAs [J]. Front Immunol, 2018, 9: 1175.
[6] Jianmei Ma. Polarization phenotype and transcription factor regulation of macrophages [J]. Journal of Dalian Medical University, 2017. 39(1): 1-7. (In Chinese)
[7] LawrenceT, NatoliG. Transcriptional regulation of macrophage polarization: enabling diversity with identity [J]. Nat Rev Immunol. 2011. 11: 750-761.
[8] Jiahui Pan, Gege Li, Qiuling Tang, et al. Effects of cyclodiguanylate on the polarization and proinflammation of M1 macrophages in vitro [J]. Journal of stomatology biomedicine, 2017, 8 (3): 122-126.
[9] Qi Zhou, Donghua Yu, Shumin Liu. The mechanism of M1/M2 polarization of macrophages in different diseases [J]. Chinese Pharmacological Bulletin. 2020. Nov; 36 (11)
[10] Fruman DA, Chiu H, Hopkins BD, et al. The PI3K pathwayin human disease [J]. Cell, 2017, 170(4):

ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

605-635.
[11] Li H, Jiang T, Li MQ, et al. Transcriptional regulation of macrophages polarization by micro RNAs [J]. Front Immunol, 2018, 9: 1175.
[12] Koh Y c, Yang G L, Lai c S, et al. Chemopreventive effects of phytochemicals and medicines on M1/M2 polarized macrophage role in inflammation-related diseases [J]. Int J Mol Sci, 2018, 19(8): 2208.
[13] Kong X, Gao J. Macrophage polarization: a key event in the secondary phase of acute spinal cord injury [J]. J Cell Mol Med, 2017, 21(5): 941-954.
[14] Byrne A J, Mathie SA, Gregory LG, et al. Pulmonary macro- phages:key players in the innate defence of the airways [J]. Thorax, 2015, 70(12) :1189- 1196.
[15] Xiao L, Xiao Y. The autophagy in osteoimmonology: self- eating, maintenance, and beyond [J]. Front Endocrinol (Lausanne), 2019, 10: 490.
[16] Nokhbehsaim M, Deschner B, Winter J, Bourauel C, Rath B, Jager A, et al. Interactions of regenerative, inflammatory and biomechanical signals on bone morphogenetic protein-2 in periodontal ligament cells. J Periodontal Res. 2011;46: 374-81.
[17] Tiessen C, Fehr M, Schwarz C, Baechler S, Domnanich K, Bottler U, et al. Modulation of the cellular redox status by the Alternaria toxins alternariol and alternariol monomethyl ether. Toxicology letters. 2013; 216: 23-30.
[18] Hengartner NE, Fiedler J, Ignatius A, Brenner RE. IL-1beta inhibits human osteoblast migration. Mol Med. 2013;19:36-42.
[19] Shuo Gao, Mengjie Cai, Fei Mao, et al. Effect of macrophages on the migration of bone marrow mesenchymal stem cells in mice under inflammatory environment [J]. Journal of jiangsu university (medical H), 2013, 23(3):201-206.
[20] Nicolaidou V, Wong MM, Redpath AN, et al. Monocytes induce STAT3 activation in human mesenchymal stem cells to promote osteoblast formation [J]. PLoS One, 2012, 7(7): e39871. DOI: 10. 1371/journal, pone. 0039871.
[21] Guihard P, Danger Y. Brounais B, et al. Induction of osteogenesis in mesenchymal stem cells by activated monocytes macrophages depends on oncostatin M signaling [J]. Stem Cells, 2012, 30(4): 762-772. DOI 10. 1002/stem. 1040.
[22] Dong L, Wang CM. Harnessing the power of macrophages/ monocytes for enhanced bone tissue engineering [J]. Trends Biotechnol, 2013, 31(6): 342-346.
[23] Omar OM, Granđi c, Ekström K, et al. The stimulation of an osteogenic response by classical monocyte activation [J]. Bioma-teri- als, 2011, 32(32): 8190-8204.
[24] Lu LY, Loi F, Nathan K, et al. Pro-inflammatory M1 macrophages promote Osteogenesis by mesenchymal stem cells via the COX-2- prostaglandin E2 pathway [J]. J Orthop Res, 2017, 35(11):2378 2385.
[25] Zhang Y, Böse T, Unger RE. Macrophage type modulates osteog- enic differentiation of adipose tissue MSCs [J]. Cell Tissue Res, 2017, 35(11):2378-2385.
[26] Loi F, Cordova LA, Zhang R, et al. The effects of immunomodulation by macrophage subsets on osteogenesis in vitro [J]. Stem Cell Res Ther, 2016, 7:15. DOI:10. 1186/s13287-016-0276-5.
[27] He XT, Li X, Yin Y, et al. The effects of conditioned media gen-erated by polarized macrophages on the cellularbehaviours of bonemarrow mesenchymal stem cells [J]. J Cell Mol Med, 2018, 22(2):1302-1315.
[28] Hao NB, LU MH, Fan YH, et a1. Macrophages in tumormicroenvironments and the progression of tumors [J] OLl. ClinDev Immun01, 2012, 2014: 94809812012-01-19, 1. http://www. hindawi. com/ journals/jir/ 2012/ 948098/
[29] Leitinger N, Schulman IG. Phenotypic polarization of macrophages in atherosclerosis[J]. Arterioscler Thromb Vasc Biol, 2013, 33(6): 1120-1126. DOI:10. 1161/JAHA. 116. 004756.
[30] Jetten N, Verbruggen S, Gijbels MJ, et al. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo [J]. Angiogenesis, 2014, 17(1): 109-118. DOI: 10. 1007/ s10456-013-9381-6.
[31] Spiller KL, Anfang RR, Spiller KJ, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds [J]. Biomaterials, 2014, 35 (15):4477-4488.
[32] Apte RS, Richter J, Herndon J, et al. Macrophages inhibit neovascularization in a murine model of age-related macular degeneration [J/OL]. PLoS Med, 2006, 3(8): 310[2006-08-03]. http://dx. plos. org/ 10. 1371/journal. pmed. 0030310. DOI:10. 1371/ journal. pmed. 0030310.
[33] BRANCATO S K, ALBINAJ E. Wound macrophages as key regulators of repair:origin, phenotype, and function [J]. Am J Pathol, 2011, 178(1): 19-25.
[34] TAKEDA Y, COSTA S, DELAMARRE E, et al. Macrophage skewing by Phd2 haplodeficiency prevents ischaemia by inducing arteriogenesis [J]. Nature, 2011, 479(7371): 122-126.
[35] Kodelja, V. ; Muller, C. ; Tenorio, S. ; Schebesch, C. ; Orfanos, C. E. ; Goerdt, S. Differences in

ISSN 2618-1584 Vol. 4, Issue 11: 47-52, DOI: 10.25236/FMSR.2022.041108

angiogenic potential of classically vs alternatively activated macrophages. Immunobiology 1997, 197, 478-493, doi:10. 1016/s0171-2985(97)80080-0.
[36] Hellberg, C. Ostman, A. ; Heldin, C. H. PDGF and vessel maturation. Recent results in cancer research. Fortschritte der Krebsforschung. Progres dans les recherches sur le cancer 2010, 180, 103-114, doi:10. 1007/978-3-540-78281-0-7.
[37] Stratman, A. N. ; Schwindt, A. E. ; Malotte, K. M. ; Davis, G. E, Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization. Blood 2010, 116, 4720-4730, doi:10. 1182/blood-2010-05-286872.
[38] Ma J, Mehta M, Lam G, et a1. Influence of subretinal fluid in advanced stage retinopathy of prematurity on proangiogenic response and cell proliferation [J]. MoI Vis, 2014. 20: 881—893.
[39] WU A C, RAGGATT L J, ALEXANDER K A, et al. Unraveling macrophage contributions to bone repair [J]. Bonekey Rep, 2013, 2: 373.