Role of skeletal macrophages in fracture repair: A systematic review

ZIHAO WAN1,2, LIH-YING SHIN1, YU-FAN WANG1, ZHIHAO HUANG3, YANJING DONG2, CHIEN-WEI LEE1, SHEKHAR-MADHUCHAR KUMTA1 and OSCAR KUANG-SHENG LEE1,4,5

1Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Sha Tin, Hong Kong, SAR 999077; 2Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou, Guangdong 510005; 3Division of General Surgery, Peking University First Hospital, Peking University, Beijing 100000; 4Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong; 5Li Ka Shing Institute of Health Sciences, Prince of Wales Hospital, The Chinese University of Hong Kong, Sha Tin, Hong Kong, SAR 999077, P.R. China

Received March 19, 2020; Accepted August 13, 2020

DOI: 10.3892/br.2020.1360

Abstract. In the field of bone research, the importance of the function of skeletal macrophages (sMΦ) and their crucial role in immune homeostasis and bone regeneration has been extensively studied. The aim of the present systematic review was to summarize the role of sMΦ in bone fracture healing and to evaluate their potential for immunoregulatory therapy in bone regeneration. A systematic literature search of PubMed and Embase® was performed to retrieve studies on the role of sMΦ in bone injury repair. The Systematic Review Centre for Laboratory animal Experimentation tool was used to assess the risk of bias of the studies included. A total of four articles were included in the present review. A relatively high risk of bias was identified in the included articles as none of the assessors in these studies were blinded. sMΦ were defined by the surface markers F4/80+, Mac-2low, TRAP+, CD169+, Ly6G- and CD115low. All of the studies provided support for the essential role of sMΦ in intramembranous ossification or endochondral ossification during fracture healing. F4/80+Mac-2+CD169+ sMΦ are a promising therapeutic target for immunoregulatory therapy of bone repair due to their essential role in bone formation and homeostasis. Future studies aimed at profiling and modulating sMΦ to promote bone regeneration are required.

Introduction

Trauma is the fifth leading cause of death, resulting in more fatalities than diabetes and infectious diseases in China, and thus places a substantial burden on healthcare systems across the world (1). A recent retrospective study which included >500,000 Chinese subjects reported that the population-weighted incidence rate of traumatic fractures in the general population was ~3.2 per 1,000 individuals (1).

Despite the considerable developments in terms of internal and external fixation systems, bone fractures may still fail to heal under certain circumstances, including bone non-union or pseudarthrosis, causing painful and delayed bone healing (2). Clinical studies focusing on facilitating bone healing and restoration of normal biomechanical properties following bone fracture have shown that such methods may allow patients to recover and return to normal life relatively quicker than conventional methods (1-3).

Healing of fractures is initiated by the inflammatory cascade, followed by the recruitment of various immune and mesenchymal cells, as well as the formation of hematomas that further develop into vascularized and innervated granulation tissue (4). Following this initial stage of repair, callus tissue, characterized by the formation of woven bone, which may bridge the injury sites, is formed, followed by the bone remodeling phase (5). Although the inflammatory response is essential and beneficial to initiate bone repair, dysregulated or chronic inflammation may severely impair bone healing (6). Previous studies have shown that macrophages and other interleukin (IL)-17-producing γδ T cells promote bone healing (7,8), and that cytotoxic T cells may impair bone repair (9). IL-10-producing B cells, which suppress excessive and/or prolonged inflammation, may also contribute to bone healing (4). However, the underlying mechanisms of the effects of immune reaction on bone homeostasis during fracture healing remains to be determined.

In recent years, tissue-resident macrophages have been garnered increasing attention, not only because of their
important roles in innate immunity, but also in homeostasis and regeneration (6,10-12). Multiple subsets of tissue-resident macrophages have been identified in different organs or tissues, including microglial cells in the brain, Kupffer cells in the liver and Langerhans cells in the skin (13). Bone-resident macrophages are divided into erythroblastic island macrophages, haematopoietic stem cell niche macrophages and skeletal macrophages (sMΦ) (4,6,14,15). sMΦ, also called osteal macrophages or osteomacs, have been reported to significantly contribute to bone homeostasis and regeneration (16,17).

The aim of the present review was to systematically summarize the contribution of sMΦ in bone repair, and evaluate their potential as a therapeutic target for promoting bone regeneration and other bone diseases.

Materials and methods

Search strategy. A systematic search of the PubMed and Embase® databases (from inception to December 23rd, 2019) for studies investigating the function of sMΦ in bone injury repair was performed. This review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (18), with search key words including ‘osteal tissue macrophages’, ‘bone resident macrophages’, ‘skeletal macrophages’, ‘bone resident macrophage’ and ‘bone fractures’. The detailed search strategy is presented in Table I including a list of all search items used, names of the database searched and the publication period included.

Inclusion and exclusion criteria. Studies were included if they met the following criteria: i) Relevant to evaluating the effect of sMΦ in bone repair or regeneration; ii) full-length research articles were available; and iii) studies were published in English. Reviews, correspondences, case reports, expert opinions and editorials were all excluded.

Quality assessment and statistical analysis. The Systematic Review Centre for Laboratory animal Experimentation tool (19) was used to assess the risk of bias of included studies, with the types of bias including: Selection bias, performance bias, attrition bias, detection bias, reporting bias and other biases. The response was defined as ‘Low risk of bias’ or ‘High risk of bias’ for each item in the checklist. For ideal methodological quality, the percentage of ‘Low risk of bias’ was required to be ≥80% (20). If it was not possible to make a judgment based on the present information, a rating of ‘Unclear risk of bias’ was assigned. Finally, a sum of the percentage of bias for each study was calculated.

Data extraction. Data extraction was performed by two reviewers independently. Disagreements were resolved by consensus or discussion amongst co-investigators. The extracted data were characteristics of the study samples, general and detailed methodology characteristics, and study results.

Statistical analysis. All statistical analysis was performed using SPSS version 25 (IBM Corp.). A one-way ANOVA and Brown-Forsythe test were used to compare groups. P<0.05 was considered to indicate a statistically significant difference.
Results

Details of the study selection process are presented in Fig. 1. The systematic search resulted in retrieval of 93 articles. After removing duplicates, 87 articles remained for first-stage screening. By reviewing the titles and abstracts, 3 articles were deemed irrelevant. No additional articles were included by checking the references. A total of 9 relevant articles were identified, the full text of which were assessed for eligibility. Finally, 4 articles that met all of the inclusion criteria were identified and included in the present systematic review (10,16,17,21).

The results of the risk of bias assessment are presented in Fig. 2. The mean percentage of low risk bias was 45% [95% confidence interval (CI), 12.6-77.4%], the mean percentage of high risk bias was 7.5%, (95% CI, 0.0-24.5%) and the mean percentage of unclear bias was 45.0%, (95% CI, 12.6-77.4%). P<0.05 in the Brown-Forsythe test indicated there was a significant difference between these 3 bias groups. There was a relatively high risk of bias associated with the blinding of the investigators and animals, since none of the assessors in these studies were blinded, and reports on allocation, random outcome assessment and incomplete outcome data were not well documented. There was a low risk of bias for baseline characteristics, random housing, selective information and other potential biases in the studies evaluated.

Table II presents the major characteristics of the studies included in the present systematic review. In all of the studies, mice were used as the experimental animals, with an age of 11-13 weeks. Of the four studies, three utilized the tibial fracture model and the remaining study used a femoral fracture model. Furthermore, three studies used immunohistochemistry combined with flow cytometry for identification and characterization of sMΦ. Specific surface markers used to define sMΦ were F4/80+, Mac-2^low, TRAP, CD169^, Ly6G^- and CD115low. In addition, all of the studies concluded that sMΦ have an essential role in fracture healing, and the mechanisms are summarized in Fig. 3.

Discussion

The aim of the present study was to summarize the results of previous studies assessing the role of sMΦ in bone healing. Previous studies supported the involvement of sMΦ in fracture healing, and identified the underlying cellular and molecular mechanisms and their utility in novel immunoregulatory therapy in bone regeneration.

Alexander et al (17) assessed the effects of sMΦ and inflammatory macrophages in bone healing and showed that F4/80^Mac-2^low sMΦ formed a distinctive canopy-like structure over cuboidal osteoblasts located on the surface of new bone. The number of F4/80^Mac-2^ high inflammatory macrophages was considerably lower than that of sMΦ during the early and late anabolic phases of tibial fracture repair, which heals primarily via intramembranous ossification (16). F4/80^ macrophages were present in all phases of fracture healing and were required for matrix deposition and bone mineralization. Systematic depletion of F4/80^ macrophages notably suppressed bone deposition and mineralization (21). Furthermore, due to the relationship in the lineage of macrophages and osteoclasts, osteoclasts were specifically ablated using osteoporotegerin treatment to study the effect of an absence of osteoclasts on bone healing (21). It was shown that osteoporotegerin treatment resulted in significantly impaired bone resorption, but did not compromise CT1^+ woven bone deposition, which further confirmed the importance of F4/80^ macrophages that were prominently sMΦ in bone healing (22). The systematic depletion approach of macrophages using lysozyme M-driven Cre recombinase, Csf1r promoter, clodronate liposome or antibody is also able to reduce inflammatory macrophages...
and osteoclasts (23). Therefore, specific ablation of sMΦ by targeting a specific surface marker in fracture models is necessary (23).

In addition, macrophages were shown to promote endochondral callus formation following bone fracture (21). In a mouse femoral fracture model, which primarily heals via endochondral ossification, Batoon et al (15) found that F4/80+ Mac-2+ inflammatory macrophages were abundant in the granulation tissue, which was fully established 7 days after fracture surgery. However, the presence of sMΦ, defined as F4/80+Mac-2 cells, were relatively rare at this reparative stage. Furthermore, during soft-to-hard callus transition, both sMΦ and inflammatory macrophages were abundantly present in the maturing callus. F4/80+ macrophage depletion at the start of the early anabolic phase significantly impeded soft callus formation and the progression of anabolism in endochondral ossification (15). Furthermore, Alexander et al (14) suggested that macrophages have a significant influence on both cartilage and bone deposition during endochondral ossification. The presence of F4/80+ macrophages throughout the entire process of fracture repair and macrophage deficiency may result in smaller fracture calluses, but increased fibrotic calluses, which results in delayed bone repair (10).

The crosstalk between sMΦ and osteoblasts/osteoclasts is currently being investigated. Batoon et al (16) demonstrated that...
CD169, a cell surface antigen expressed by mature tissue-resident macrophages, may be used to discriminate osteoclasts and sMΦ. CD169⁺ sMΦ depletion may significantly compromise osteoblastogenesis and bone repair in bone injury, primarily via promoting both endochondral ossification or intramembranous ossification (16). Furthermore, increasing the proliferation of sMΦ in callus tissue by administering colony-stimulating factor-1, which may target sMΦ and promote its proliferation, was reported to promote bone repair (17,21). Although the mechanisms by which sMΦ promotes fracture healing remain elusive, the NF-κB signalling pathway, bone morphogenetic proteins and oncostatin M are thought to be essential in sMΦ-mediated osteogenesis (24,25). Ablation of sMΦ was indicated to significantly impair osteocalcin expression and osteoblast mineralization in vivo and in vitro (11). Furthermore, the interaction between sMΦ and osteoclasts may also be a point of interest. Macrophage-deficient mice exhibited functionally active osteoclast activities, but were characterized by decreased sMΦ at the bone surface and impaired bone formation (10,26). These results emphasize the importance of sMΦ in bone healing, and highlight the potential role of sMΦ as a therapeutic target for bone regeneration. Thus, a more in-depth understanding from a global perspective of molecular profiles and phenotypes adopted by sMΦ in the bone environment is required.

An increasing number of studies have shown that tissue-resident macrophages are able to adopt tissue-specific phenotypes and functions and may acquire self-renewal capacity (10,16,17,21). Multiple studies have confirmed the essential roles of macrophages in skeletal homeostasis and bone repair (10,16,17,21); however, direct evidence of the function of sMΦ in bone biology remains insufficient, due to the heterogeneity of macrophage clusters and the lack of sMΦ-specific biomarkers (27). With the development of cutting-edge techniques, including optimized next-generation sequencing technologies (28), for use in life science investigations, a single-cell sequencing approach may be a suitable means of profile the involved macrophages, thus assisting in the identification of the heterogeneity of sMΦ during fracture repair.

The present systematic review provided an overview of the roles of sMΦ in bone healing. Several biomarkers defining sMΦ were identified based on the available literature. The present study is limited by the high risk of bias with regard to blinding and sequence generation in the reviewed studies. Another limitation is that due to the shortage of sufficient studies on this topic, the importance of sMΦ in fracture healing may be under- or overestimated.

In conclusion, a growing body of evidence strongly supports the notion that F4/80⁺Mac-2 CD169⁺ sMΦ may serve as a promising therapeutic target for immunoregulatory therapy in
bone repair, due to their essential role in bone formation and homeostasis. Further investigation aiming to modulate the role of macrophages with the aim of promoting bone regeneration, is required.

Acknowledgements

Not applicable.

Funding

This study was supported by funding from the MWLC Associate Member Programme, Ming Wai Lau Center of Regenerative Medicine of Karolinska Institute (grant no. TK1914020), CUHK Research Committee Funding (grant no. 2018.020) and Hong Kong Government Research Grant Council, General Research Fund (Reference no. 14104620) to CW Lee.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

ZW and OKSL conceived and designed the study. ZW acquired the data. ZW, LYS, YFW, ZH, YD, CWL and SMK analyzed and interpreted the data. ZW wrote the manuscript. ZW, LYS, YFW, ZH, YD, CWL and SMK analyzed and interpreted the data. ZW wrote the manuscript. ZW, SMK, and OKSL revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Chen W, Lv H, Liu S, Liu B, Zhu Y, Chen X, Yang G, Liu L, Zhang T, Wang H, et al: National incidence of traumatic fractures in China: A retrospective survey of 512 187 individuals. Lancet Glob Heal 5: e807-e817, 2017.
2. Schneider E, Goldhahn J and Burckhardt P: The challenge: Fracture treatment in osteoporotic bone. Osteoporos Int 16: 1-2, 2005.
3. Holmes D: Non-union bone fracture: A quicker fix. Nature 410: 162-164, 2000.
4. Lui F, Córdova LA, Pajarijien J, Lin TH, Yao Z and Goodman SB: Inflammation, fracture and bone repair. Bone 86: 119-130, 2016.
5. Marsell R and Einhorn TA: The biology of fracture healing. Curr Osteoporos Rep 15: 367‑375, 2017.
6. Mise-Omata S, Alles N, Fukazawa T, Zhang Y, Xu J, Li N, Liu Y, Yang YS, Eisenman M, et al: Discovery of a peristeal stem cell mediating intramembranous bone formation. Nature 562: 133‑139, 2018.
7. Lorenzo J: Interactions between immune and bone cells: New insights with many remaining questions. J Clin Invest 106: 749‑752, 2000.
8. Wijh Bass GS, Whetstone H, Ng A, Wei Q, Poon R, Mylvaganam S, Grynpas M and Alman BA: Macrophages promote osteoblastic differentiation in vivo: Implications in fracture repair and bone homeostasis. J Bone Min Res 30: 1090-1102, 2015.
9. Chang MK, Raggatt LJ, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, Maylin ER, Ripoll VM, Humby A, Pettit AR: Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. J Immunol 181: 1232-1244, 2008.
10. Michalski MN and McCauley LK: Macrophages and skeletal healing. Pharmacol Ther 174: 43-54, 2017.
11. Epstein S, Lavine KJ and Randolph GF: Origin and functions of tissue macrophages. Immunity 41: 21-35, 2014.
12. Alexander KA, Raggatt LJ, Millard S, Batoon L, Chiu-Ku Wu A, Chang MK, Hume DA and Pettit AR: Resting and injury-induced inflamed peristeum contain multiple macrophage subsets that are located at sites of bone growth and regeneration. Immunol Cell Biol 95: 7-16, 2017.
13. Batoon L, Millard SM, Raggatt LJ and Pettit AR: Osteomacs and bone regeneration. Curr Osteoporos Rep 15: 385-395, 2017.
14. Hume DA, Millard SM, Wullschleger ME, Preda C, Wu AC, Kaur S, Tseng HW, Hume DA, Levesque JP, Raggatt LJ and Pettit AR: CD169* macrophages are critical for osteoblast maintenance and promote intramembranous and endochondral ossification during bone repair. Biomaterials 196: 51-66, 2019.
15. Alexander KA, Chang MK, Maylin ER, Kohler T, Millard SM, Wu AC, Van Rooijen N, Sweet MJ, Hume DA, Raggatt LJ and Pettit AR: Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. J Bone Min Res 26: 1517-1532, 2011.
16. Librati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Moher D, Liberati A, Altman DG, Tetzlaff J, et al: The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. J Clin Epidemiol 62: e1-e34, 2009.
17. Hooper MR, CR, Rangwala MM, DE Vries RBM, Leenaars M, Ritskes-Hoitinga M and Langendam MW: SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14: 43, 2014.
18. Stiuso P, Scognamiglio I, Murolo M, Ferranti P, De Simone C, Rizzo MR, Tucillo C, Caraglia M, Loguercio C and Federico A: Serum oxidative stress markers and lipidomic profile to detect NASH patients responsive to an antioxidant treatment: A pilot study. Ovid Med Cell Longev 2014: 169216, 2014.
19. Raggatt LJ, Wullschleger ME, Alexander KA, Wu AC, Millard SM, Kaur S, Maugham ML, Gregory LS, Steck R and Pettit AR: Fracture healing via periosteal callus formation requires macrophages for both initiation and progression of early endochondral ossification. Am J Pathol 184: 3192-3204, 2014.
20. Van Rooijen N, Sanders A and Van Den Berg TK: Apoptosis of macrophages induced by liposome-mediated intracellular delivery of cldonore and paipromidine. J Immunol Methods 193: 93-99, 1996.
21. Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM and Suda T: Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor κB (RANK) receptors. J Exp Med 190: 1741-1754, 1999.
22. Mise-Omata S, Alles N, Fukazawa T, Aoki K, Ohta Y, Jime I, Obata Y and Doi T: NF-κB RELA-deficient bone marrow macrophages fail to support bone formation and to maintain the hematopoietic niche after lethal irradiation and stem cell transplantation. Int Immunol 26: 607-618, 2014.
23. Zhang Y, Xu J, Ruan YC, Yu MK, O'Laughlin M, Wise H, Chen D, Tian L, Shi D, Wang J, et al: Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats. Nat Med 22: 1160-1169, 2016.
24. Wintges K, Beil FT, Álbers J, Jeschke A, Schweizer M, Claass B, Tegs G, Amling M and Schinke T: Impaired bone formation and increased osteoclastogenesis in mice lacking chemeokine (C-C motif) ligand 5 (CCL5). J Bone Miner Res 28: 2070-2080, 2013.
25. Schuldt C, E1 Khusswa T, Serra A, Dienelt A, Wendeler M, Schell H, van Rooijen N, Radbruch A, Lucius R, Hartmann S, et al: Macrophages in bone fracture healing: Their essential role in endochondral ossification. Bone 106: 78-89, 2018.
26. Deb Nath S, Yallowitz AR, McCormick J, Lalani S, Zhang T, Ouyang J, Li N, Liu Y, Yang YS, Eisenman M, et al: Discovery of a peristeal stem cell mediating intramembranous bone formation. Nature 562: 133-139, 2018.