Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology 31 (2019) 169–174

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Monocyte chemoattractant protein-1 and secreted ectodomain of sialic acid-binding Ig-like lectin-9 enhance bone regeneration by inducing M2 macrophages☆

Jun Ishikawa[a,b], Fumiya Kano[b], Yuji Ando[b,c], Hideharu Hibi[b], Akihito Yamamoto[d,*]

[a] Department of Oral and Maxillofacial Surgery, KARIYA TOYOTA General Hospital, 5-15 Sumiyoshi-cho, Kariya, Aichi, 448-8505, Japan
[b] Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[c] Department of Oral and Maxillofacial Surgery, Chutoen General Medical Center 1-1 Shobugaike, Kakegawa, Shizuoka, 436-8555, Japan
[d] Department of Tissue regeneration, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-5 Kuramoto-cho, Tokushima 770-8504, Japan

ARTICLE INFO

Keywords:
Monocyte Chemoattractant Protein-1
Sialic acid-binding Ig-like lectin-9
M2 macrophage
Bone regeneration
Osteogenesis

ABSTRACT

Objective: The bone-healing process consists of a primary inflammatory phase and a subsequent anti-inflammatory bone-forming phase. It was recently suggested that macrophages play an indispensable role in bone healing. Macrophages are plastic immune cells consisting of several subtypes, including pro-inflammatory M1-type and anti-inflammatory M2-type macrophages. Recently, a set of M2 macrophage inducers was described, monocyte chemoattractant protein-1 (MCP-1) and the secreted ectodomain of sialic acid-binding Ig-like lectin-9 (sSiglec-9), and applied them to a rat spinal cord injury model. Here, we hypothesized that MCP-1/sSiglec-9 enhance bone regeneration by inducing M2 macrophages.

Methods: We investigated the osteogenic activity of MCP-1/sSiglec-9 *in vitro* and in the rat calvarial bone defect model.

Results: We found that MCP-1/sSiglec-9 induced M2 macrophages*in vitro*, which expressed increased levels of multiple mRNAs that encode osteogenic factors, including *Igf-1*, *Tgf-β*, *Hgf*, *Bm*p2, and *Fgf2*. We then demonstrated that MCP-1/sSiglec-9 accelerated bone formation and caused anti-inflammatory M2 macrophages to accumulate in the rat calvarial bone defect *in vivo*.

Conclusions: Collectively, our data suggest that the local administration of MCP-1/sSiglec-9 promotes bone formation by inducing anti-inflammatory M2 macrophages that express a variety of osteogenic factors. MCP-1/sSiglec-9 may be beneficial in bone regenerative therapy.

1. Introduction

Craniofacial bone defects can result from congenital malformation, traumatic injuries, tumor resections, or atrophies, in people of all ages and all over the world [1]. Although successful bone healing is required for aesthetic and functional reasons, it is still a major hurdle for clinicians and researchers [2]. The use of autografts or allografts is considered the gold standard for healing bone [3,4]. However, autografts are restricted due to donor-site damage, morbidity, or insufficient sources, while allografts also have limitations including host rejection, infection, and disease transmission [5,6]. Thus, alternative procedures for bone regeneration are needed to avoid the risks of autogenous and allogeneic transplantations.

Recent studies suggest that macrophages play a pivotal role in bone healing [7]. Macrophages are heterogeneous and plastic immune cells consisting of several subtypes, including pro-inflammatory M1-type and anti-inflammatory M2-type macrophages [8,9]. Although M1 macrophages are important for initiating the inflammatory and regenerative process, a prolonged pro-inflammatory condition is correlated with unsuccessful bone healing [10,11]. A switch from the pro-inflammatory M1 macrophages to the anti-inflammatory M2 phenotype is a key event in successful bone regeneration [12]. Therefore, strategies designed to regulate the M1/M2 polarity in bone healing may provide significant therapeutic benefits.

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

* Corresponding author.
E-mail address: akihito@tokushima-u.ac.jp (A. Yamamoto).

https://doi.org/10.1016/j.ajoms.2018.12.007
Received 1 October 2018; Received in revised form 30 November 2018; Accepted 14 December 2018
Available online 27 December 2018

Recently, a set of M2 macrophage inducers, monocyte chemoattractant protein-1 (MCP-1) and the secreted ectodomain of sialic acid-binding Ig-like lectin-9 (sSiglec-9), were identified from the serum-free conditioned medium of stem cells derived from human exfoliated deciduous teeth (SHED-CM) [13]. MCP-1 is a chemokine that recruits immune cells to inflamed tissues [14], and the Siglecs are a large family of sialic-acid-binding type-I transmembrane immunoglobulin-like lectins that modulate the immune signaling on various types of immune cells [15]. It was further reported that a single intravenous administration of SHED-CM prevents bone destruction in a rheumatoid arthritis model, through the sSiglec-9-mediated induction of M2 macrophages [16]. However, the role of MCP-1/sSiglec-9 in bone regeneration has been unclear.

Here, we evaluated the osteogenic activity of MCP-1 and sSiglec-9 *in vitro*, and investigated their beneficial effects in the rat calvarial bone defect model.

2. Materials and methods

2.1. Animals and surgical procedures

Eight-week-old male Sprague Dawley rats weighing 200–230 g were generally anesthetized with an intraperitoneal injection of pentobarbital (Kyoritsu Seiyaku, Tokyo, Japan). After local anesthesia with lidocaine was applied, the skin on the head was shaved and opened with a parietal incision to expose the surface of the calvarial bones. Two circular bone defects (full-thickness, 5-mm in diameter) were made in the calvarial bones using a trephine bur. Round atelocollagen sponges (KOKEN, Tokyo, Japan) were implanted into the defects. The sponges were soaked in 40 µl of PBS alone, or PBS containing 200 ng recombinant human MCP-1 (Peprotech, London, UK) and 200 ng recombinant human sSiglec-9 (R&D Systems, Minneapolis, MN), respectively. The rats were sacrificed 2 days or 6 weeks after the surgery. All of the animal studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee of Nagoya University.

2.2. Radiographic analysis

Six weeks after surgery, the calvarial bones were harvested, fixed in 4% paraformaldehyde solution, and analyzed by microcomputed tomography (micro-CT) using a laboratory X-ray CT device (SkyScan1176, Toyo Corporation, Tokyo, Japan). Newly generated bone areas were assessed by computerized planimetry using NIH ImageJ software [17].

2.3. Histology

Two days or 6 weeks after surgery, the calvarial bones were harvested, embedded in SCEM gel (8091140; Leica), and frozen in cooled isopentane. Non-decalcified calvarial bone sections were generated using Kawamoto's film method (8091098; Leica) [18]. Cryostat sections (5-µm thick) were subjected to immunohistochemical analysis or stained with hematoxylin and eosin (H-E).

2.4. Immunohistochemistry

Cryostat sections were fixed in 99.5% ethanol for 10 min at room temperature, washed, blocked with 5% bovine serum albumin/PBS for 30 min, and stained with primary antibodies in blocking buffer overnight at 4 °C. The sections were then stained with secondary antibodies for 30 min, mounted with SCMM R3 (Leica, Tokyo, Japan), and examined using a BZ9000 fluorescence microscope (Keyence, Osaka, Japan). The following antibodies were used for immunostaining: mouse anti-CD11b IgG (Abcam, Cambridge, U.K.) and rabbit anti-CD206 IgG (Abcam, Cambridge, U.K.). Secondary antibodies were conjugated with Alexa Fluor 488 or 546 (Invitrogen, Carlsbad, CA). Cell nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA). The average number of CD11b + CD206 + cells was determined by counting 15 random fields at least 3 animals per group.

2.5. Bone marrow macrophage induction assay

Bone marrow cells from the femurs of 8-week-old male Sprague Dawley rats were plated on 6-cm dishes (2.0×10^6 cells per dish) and differentiated into macrophages in DMEM supplemented with 20 ng/ml macrophage colony stimulating factor (M-CSF), at 37 °C in 5% CO_2 for 7 days. The macrophages were then incubated with serum-free DMEM or 100 ng/ml recombinant human MCP-1 (300-04, Peprotech) and recombinant human sSiglec-9 (1139-SL, R&D Systems) for 24- or 48- h. Phase contrast images of the induced macrophages were captured with a microscope digital camera (Leica DFC290 HD, Leica).

Immunostaining was carried out using following antibodies: mouse anti-ED-1 IgG (Merck, Darmstadt, Germany) and rabbit anti-CD206 IgG (Abcam, Cambridge, U.K.). Secondary antibodies were conjugated with Alexa Fluor 488 or 546 (Invitrogen, Carlsbad, CA). Cell nuclei were labeled with DAPI (Invitrogen, Carlsbad, CA).

After a 24- or 48-h incubation at the final concentration, the RNA was extracted using an RNeasy micro kit (Qiagen, Hilden, Germany). The mRNA expressions of a M2-type cell marker and of osteogenic factors were analyzed by qPCR.

2.6. Real-time quantitative polymerase chain reaction analysis

The total RNA from incubated macrophages was quantified by a spectrophotometer, and the RNA integrity was checked on 1% agarose gels. Reverse transcription reactions were performed with Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) using 0.5 µg of total RNA in a 25-µl total reaction volume. Real-time quantitative polymerase chain reaction was carried out using the THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) and the StepOnePlus Real-Time PCR System (Applied Biosystems, Forster City, CA). The primers were designed using DNA Dynamo (Blue Tractor Software Ltd) and Primer3 (Table 1).

2.7. Statistics

An unpaired 2-tailed Student's *t*-test was used for single comparisons. P-values less than 0.05 were considered statistically significant.

Table 1
Rat primers for real time q-PCR.

Origin	Primer	Sequence (forward 5'–3')	Sequence (reverse 5'–3')
rat	*Gapdh*	AACTTTGGCATCGTGGAAGG	CGGATACATTGGGGGTAGGA
rat	*Igf-1*	GCTGTGTAAACGACCCGGGA	ACTGAAGAGCGTCCACCAGC
rat	*Tgf-β*	CCGCAACAACGCAATCTATG	GCACTGCTTCCCGAATGTCT
rat	*Hgf*	GCAAGACATGTCAGCGCTGG	CCAAGGGGTGTCAGGGTCAA
rat	*Bmp2*	GCGTCAAGCCAAACACAAAC	CAGTCATTCCACCCCACATC
rat	*Fgf2*	TGTCCATCAAGGGAGTGTGTG	TCCAGGCGTTCAAAGAAGAA

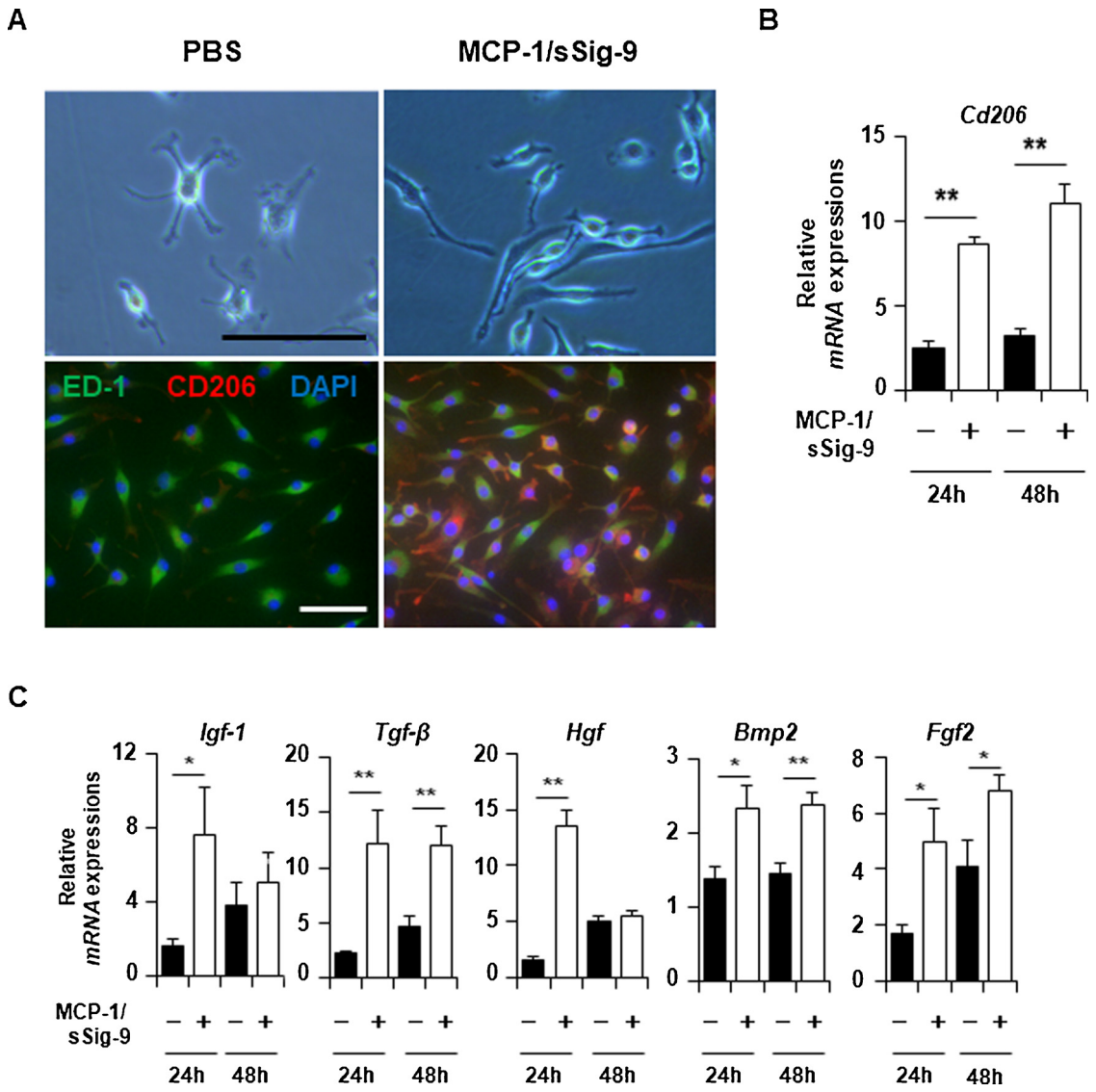

Fig. 1. MCP-1 and sSiglec-9 induce M2 macrophages, which express a variety of osteogenic factors *in vitro*(A)Representative images of BMMs treated with PBS or MCP-1/sSiglec-9. Top, microscopic images; bottom, ED-1/CD206/DAPI immunostaining. Bar = 100 μm. (B) The gene expression level of the M2 macrophage marker, *Cd206*. (C) The gene expression levels of osteogenic markers, *Igf-1, Tgf-β, Hgf, Bmp2, Fgf2*. (B, C) The gene expression levels were evaluated at 24 h or 48 h after treating with PBS or MCP-1/sSiglec-9 (n = 5 per group). Data represent the mean ± SEM; *p < 0.05, **p < 0.01.

3. Results

3.1. MCP-1 and sSiglec-9 induce M2 macrophages, which express a variety of osteogenic factors in vitro

Bone marrow macrophages (BMMs) induced by M-CSF alone (PBS group) had a spherical shape, while BMMs treated with MCP-1/sSiglec-9 (MCP-1/sSiglec-9 group) had an elongated shape and immunohistochemically expressed M2 macrophage markers, ED-1 and CD206. (Fig. 1A) In addition, the MCP-1/sSiglec-9 group was confirmed to increase mRNA expression level of an M2 marker, *Cd206* (Fig. 1B). The MCP-1/sSiglec-9-induced M2 macrophages also expressed increased levels of multiple mRNAs that encode osteogenic factors, including *Igf-1* (Insulin-like growth factor-1), *Tgf-β* (Transforming growth factor *β*), *Hgf* (Hepatocyte growth factor), *Bmp2* (Bone morphogenetic protein 2), and *Fgf2* (Fibroblast growth factor 2) (Fig. 1C). Taken together, these results suggested that the M2 macrophages induced by MCP-1/sSiglec-9 had the potential to function beneficially for bone regeneration.

3.2. MCP-1 and sSiglec-9 accelerate bone regeneration in vivo

To evaluate whether MCP-1/sSiglec-9 could accelerate bone healing *in vivo*, we implanted collagen sponges soaked in MCP-1/sSiglec-9 into rat calvarial bone defects. Six weeks after implantation, radiographic analysis revealed that the new bone area in the MCP-1/sSiglec-9 group (52.0% ± 3.9%) was significantly greater than that in the PBS group (28.3% ± 8.8%) (Fig. 2A and B).

Histological analysis also showed that regenerated bone almost covered the bone defect in the MCP-1/sSiglec-9 group. In contrast, little regenerated bone was observed at the edge of the original bone in the PBS group, which was almost filled with connective tissue, and the collagen sponges remained unabsorbed. Marked inflammatory responses did not appear in either group (Fig. 3). Collectively, these results indicated that MCP-1/sSiglec-9 promoted bone formation *in vivo*.

3.3. MCP-1 and sSiglec-9 caused M2 macrophages to accumulate in the bone defect

We immunohistochemically examined the accumulation of M2

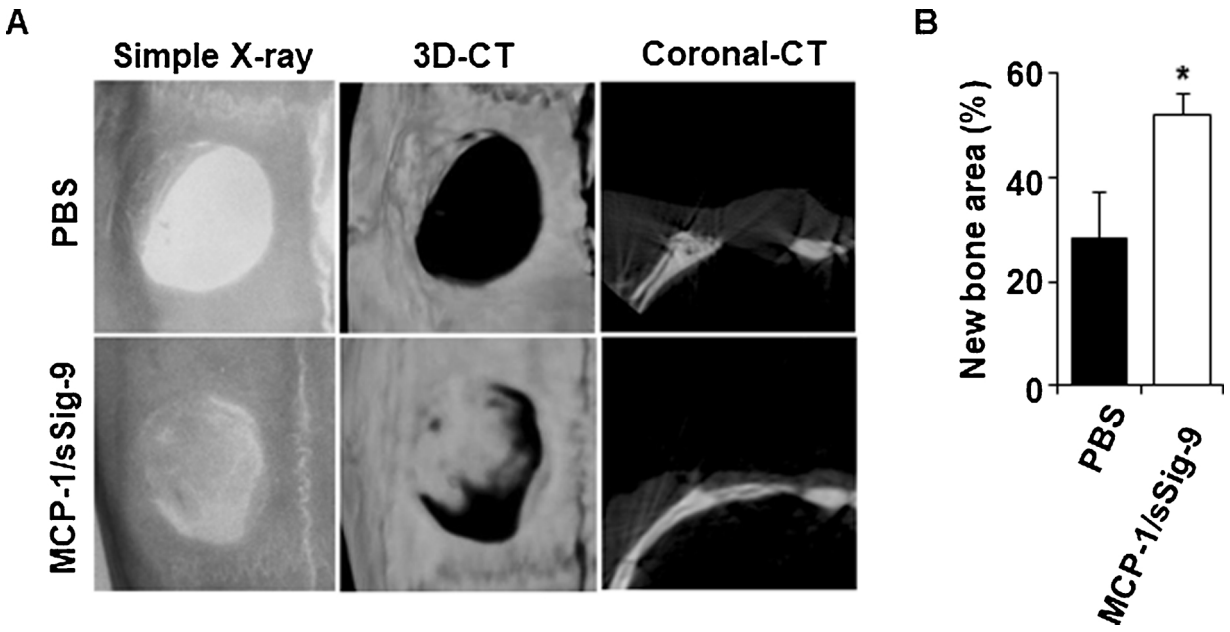

Fig. 2. Radiographic analysis reveals that MCP-1 and sSiglec-9 accelerate bone formation in the rat bone defect.
(A) Representative radiographic images at 6 weeks after surgery. (B) The percentage of the new bone area (n = 6 per group). Data represent the mean ± SEM; *p < 0.05.

macrophages in the bone defect at 2 days after surgery. M2 macrophages express both CD11b and CD206 on their surface [19,20]. The proportion of $CD11b^+$ $CD206^+$ macrophages in the MCP-1/sSiglec-9 group (54.7% ± 2.7%) was significantly greater than that in the PBS group (30.4% ± 3.0%) (Fig. 4A and B). These results demonstrated that MCP-1/sSiglec-9 increased the accumulation of $CD11b^+$ $CD206^+$ M2 macrophages in rat calvarial bone defects

4. Discussion

Various researchers over the years have achieved bone regeneration by applying a variety of osteogenic factors in several animal models. It was previously reported that a single intravenous administration of SHED-CM, a type of stem cell-derived conditioned medium, prevents bone destruction in a mouse experimental arthritis model, and that the induction of M2 macrophages by MCP-1/sSiglec-9 contained in the SHED-CM plays an essential role in this preventive effect [16]. However, the bone regenerative effects of MCP-1/sSiglec-9 have been unclear. This is the first report, to our knowledge, demonstrating a beneficial activity of MCP-1/sSiglec-9 for bone regeneration. Here we showed that MCP-1/sSiglec-9 induced M2 macrophages that expressed various osteogenic genes, *in vitro*. Furthermore, we found that MCP-1/sSiglec-9 caused tissue regenerative, anti-inflammatory M2 macrophages to accumulate in rat calvarial bone defects, resulting in accelerated bone formation. Our findings suggest that MCP-1/sSiglec-9 may provide remarkable benefits for bone regeneration.

Although the initial inflammatory phase is indispensable for the bone healing process, prolongation of the inflammatory condition is associated with unsuccessful bone healing. Resolving the inflammation and shifting the tissue to an anti-inflammatory condition are important for successful bone healing [11,12]. The classically activated M1 macrophages produce pro-inflammatory cytokines, accelerate tissue destruction, and initiate inflammation, whereas the alternatively activated M2 macrophages release anti-inflammatory cytokines, remove cellular debris, and promote the resolution of inflammation [8]. Thus, control of the M1/M2 macrophage polarization may determine the

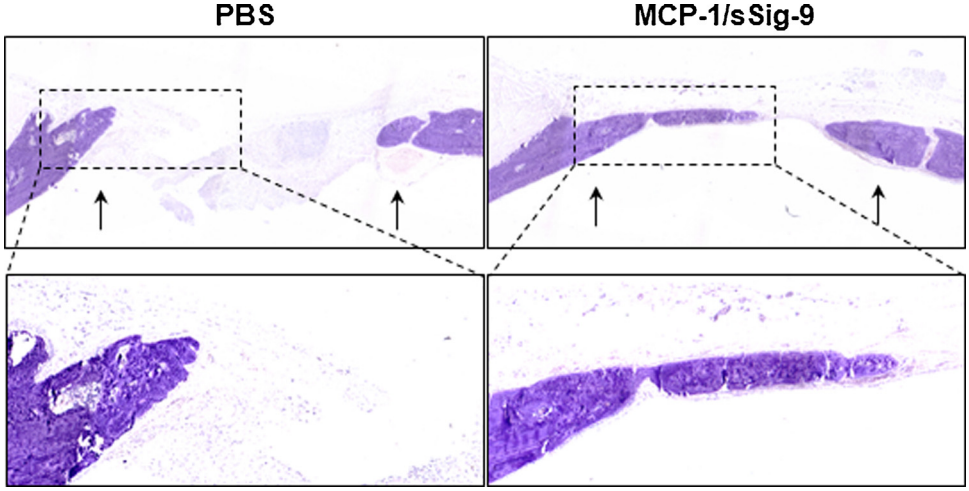

Fig. 3. Histological analysis shows that MCP-1 and sSiglec-9 regenerate almost covering bone in the defect.
Representative histological images at 6 weeks after surgery. Hematoxylin and eosin staining. Arrows indicate the edge of original bones.

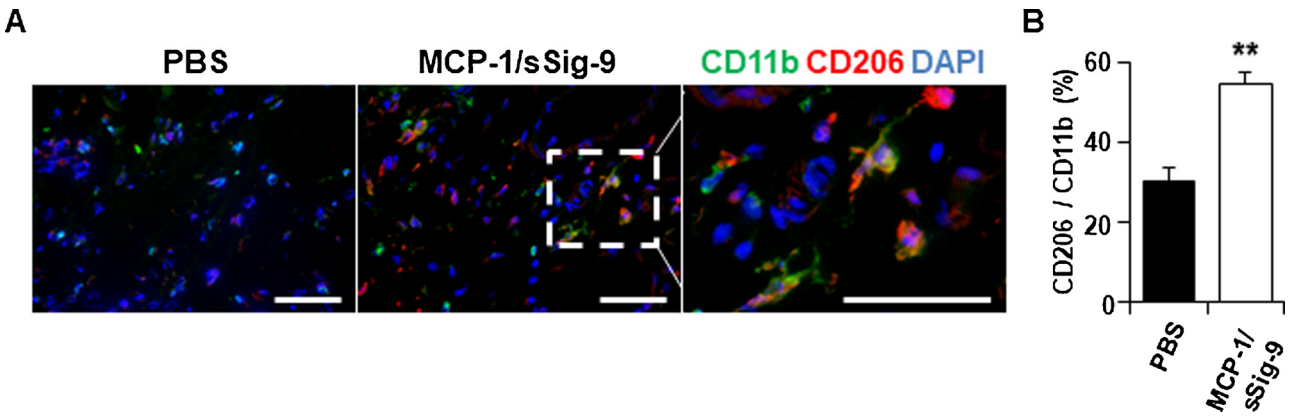

Fig. 4. MCP-1 and sSiglec-9 cause M2 macrophages to accumulate in the defect.
(A) Representative immunohistochemical images at 2 days after surgery. Boxed area highlights $CD11b^+$ $CD206^+$ M2 macrophages of the MCP-1/sSiglec-9 group. Bar = 50 μm. (B) Quantification of the $CD11b^+$ $CD206^+$ M2 macrophage in the calvarial defect (n = 3 per group). Data represent the mean ± SEM; $^{**}p < 0.01$.

initiation and resolution of inflammation, for successful bone regeneration. It was previously reported that MCP-1/sSiglec-9 reduces the pro-inflammatory M1 macrophages in a rat acute liver failure model and a rat peripheral nerve injury model [21,22]. In the present study, it was unclear whether MCP-1/sSiglec-9 reduced the pro-inflammatory M1 macrophages. However, our results suggested that pro-inflammatory circumstances shifted to anti-inflammatory ones, as indicated by the local accumulation of anti-inflammatory M2 macrophages in the defects. Collectively, our data suggested that MCP-1/sSiglec-9 shifts the injured tissue toward the bone regenerative condition by controlling inflammation in the bone defects.

In vitro, the M2 macrophages induced by MCP-1/sSiglec-9 expressed increased mRNA levels of multiple osteogenic factors, including *Igf-1*, *Tgf-β*, *Hgf*, *Bmp2*, and *Fgf2*. IGF plays important roles in bone growth and development by promoting the proliferation and differentiation of osteoblasts [23,24] and enhances neovascularization [25]. TGF-β promotes the migration of osteoprogenitor cells [26] and regulates extracellular matrix synthesis [27,28]. HGF is believed to have a direct angiogenic effect [29,30] and improves bone regeneration by modulating the NF-kB signaling pathway [31]. BMP2 promotes bone growth and formation, and enhances the migration, proliferation, and differentiation of mesenchymal cells [32,33]. FGF2 also plays key roles in the proliferation and differentiation of osteoblasts, and in angiogenesis [34]. Importantly, our *in vivo* study revealed that MCP-1/sSiglec-9 enhanced the accumulation of M2 macrophages in the defects. Taken together, our findings suggested that various osteogenic factors produced by the MCP-1/sSiglec-9-induced M2 macrophages pleiotropically accelerated bone regeneration in the rat calvarial defects.

The Siglecs are a large family of immune regulatory receptors predominantly found on various types of immune cells, including macrophages, and modulate immune-cell signaling through recognition of their glycan ligands [15]. Siglec-15 is constitutively expressed in osteoclasts, and positively regulates osteoclastogenesis [35]. Siglec-9 is reported to contribute the prevention of bone destruction in an arthritis model [16]. MCP-1 is generally known as a member of the C-C chemokine family that recruits monocytes into inflamed tissues [14,36]. Macrophages, a kind of monocyte, play an indispensable role in the bone healing process [7]. The administered MCP-1 may effectively recruit macrophages into the bone defects, resulting in the enhancement of bone regeneration. Our present study demonstrated that MCP-1/sSiglec-9 functioned to induce M2 macrophages, which accelerated bone regeneration. However, the precise roles of MCP-1 and sSiglec-9 in bone regeneration still need to be revealed in future studies.

In vivo, we investigated bone regenerative effects of MCP-1/sSiglec-9 using the rat calvarial bone defect model. In this model, it was considered that macrophages derived from many kinds of tissue, including bone marrow, periosteum, and peripheral blood, were involved in bone regeneration. In the present study, it has not become clear what kind of tissue macrophage plays a key role in bone regeneration. To clarify the detailed mechanisms of bone regeneration by MCP-1/sSiglec-9-induced M2 macrophages, the future studies using another animal model or experimental system are desired.

5. Conclusion

We found that the M2 macrophages induced by MCP-1/sSiglec-9 expressed various osteogenic genes. Furthermore, our results suggested that MCP-1/sSiglec-9 caused M2 macrophages to accumulate in the rat calvarial bone defect, consequently accelerating bone formation. Therefore, MCP-1/sSiglec-9 may be a promising candidate for providing beneficial effects in bone regenerative therapy.

Conflict of interest

None.

Acknowledgment

We thank the Division of Experimental Animals and Medical Research Engineering, Nagoya University Graduate School of Medicine, for housing the animals and for microscope maintenance. This work was supported by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (15K15736).

References

[1] Miura M, Miura Y, Sonoyama W, Yamaza T, Gronthos S, Shi S. Bone marrow-derived mesenchymal stem cells for regenerative medicine in craniofacial region. Oral Dis 2006;12:514–22.
[2] Futran ND, Mendez E. Developments in reconstruction of midface and maxilla. Lancet Oncol 2006;7:249–58.
[3] Misch CM. Autogenous bone: is it still the gold standard? Implant Dent 2010;19:361.
[4] Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Glogauer M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: a review. Biomater Res 2017;21:9.
[5] Block MS, Degen M. Horizontal ridge augmentation using human mineralized particulate bone: preliminary results. J Oral Maxillofac Surg 2004;62:67–72.
[6] Kaigler D, Mooney D. Tissue engineering's impact on dentistry. J Dent Educ 2001;65:456–62.
[7] Sinder BP, Pettit AR, McCauley LK. Macrophages: their emerging roles in bone. J Bone Miner Res 2015;30:2140–9.
[8] Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 2011;11:723–37.
[9] Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity

2014;41:14–20.
[10] Lienau J, Schmidt-Bleek K, Peters A, Haschke F, Duda GN, Perka C, et al. Differential regulation of blood vessel formation between standard and delayed bone healing. J Orthop Res 2009;27:1133–40.
[11] Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol 2012;8:133–43.
[12] Schlundt C, El Khassawna T, Serra A, Dienelt A, Wendler S, Schell H, et al. Macrophages in bone fracture healing: their essential role in endochondral ossification. Bone 2018;106:78–89.
[13] Matsubara K, Matsushita Y, Sakai K, Kano F, Kondo M, Noda M, et al. Secreted ectodomain of sialic acid-binding Ig-like lectin-9 and monocyte chemoattractant protein-1 promote recovery after rat spinal cord injury by altering macrophage polarity. J Neurosci 2015;35:2452–64.
[14] Bachmann MF, Kopf M, Marsland BJ. Chemokines: more than just road signs. Nat Rev Immunol 2006;6:159–64.
[15] Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol 2007;7:255–66.
[16] Ishikawa J, Takahashi N, Matsumoto T, Yoshioka Y, Yamamoto N, Nishikawa M, et al. Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis. Bone 2016;83:210–9.
[17] Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. Tissue Eng Part A 2012;18:1479–89.
[18] Kawamoto T. Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. Arch Histol Cytol 2003;66:123–43.
[19] Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010;11:889–96.
[20] Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 2012;122:787–95.
[21] Ito T, Ishigami M, Matsushita Y, Hirata M, Matsubara K, Ishikawa T, et al. Secreted ectodomain of SIGLEC-9 and MCP-1 synergistically improve acute liver failure in rats by altering macrophage polarity. Sci Rep 2017;7:44043.
[22] Kano F, Matsubara K, Ueda M, Hibi H, Yamamoto A. Secreted ectodomain of sialic acid-binding Ig-like lectin-9 and monocyte chemoattractant protein-1 synergistically regenerate transected rat peripheral nerves by altering macrophage polarity. Stem Cells 2017;35:641–53.
[23] Schmidmaier G, Wildemann B, Heeger J, Gabelein T, Flyvbjerg A, Bail HJ, et al. Improvement of fracture healing by systemic administration of growth hormone and local application of insulin-like growth factor-1 and transforming growth factor-beta1. Bone 2002;31:165–72.
[24] Guntur AR, Rosen CJ. IGF-1 regulation of key signaling pathways in bone. Bonekey Rep 2013;2:437.
[25] Bach LA. Endothelial cells and the IGF system. J Mol Endocrinol 2015;54:R1–13.
[26] Bostrom MP, Asnis P. Transforming growth factor beta in fracture repair. Clin Orthop Relat Res 1998:S124–31.
[27] Balooch G, Balooch M, Nalla RK, Schilling S, Filvaroff EH, Marshall GW, et al. TGF-beta regulates the mechanical properties and composition of bone matrix. Proc Natl Acad Sci U S A 2005;102:18813–8.
[28] Tang SY, Alliston T. Regulation of postnatal bone homeostasis by TGFbeta. Bonekey Rep 2013;2:255.
[29] Morishita R, Nakamura S, Hayashi S, Taniyama Y, Moriguchi A, Nagano T, et al. Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. Hypertension 1999;33:1379–84.
[30] Frisch RN, Curtis KM, Aenlle KK, Howard GA. Hepatocyte growth factor and alternative splice variants – expression, regulation and implications in osteogenesis and bone health and repair. Expert Opin Ther Targets 2016;20:1087–98.
[31] Zhen R, Yang J, Wang Y, Li Y, Chen B, Song Y, et al. Hepatocyte growth factor improves bone regeneration via the bone morphogenetic protein2mediated NFkappaB signaling pathway. Mol Med Rep 2018;17:6045–53.
[32] Boden SD. The ABCs of BMPs. Orthop Nurs 2005;24:49–52. quiz 53-4.
[33] Gomes-Ferreira PH, Okamoto R, Ferreira S, De Oliveira D, Momesso GA, Faverani LP. Scientific evidence on the use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in oral and maxillofacial surgery. Oral Maxillofac Surg 2016;20:223–32.
[34] Komatsu DE, Warden SJ. The control of fracture healing and its therapeutic targeting: improving upon nature. J Cell Biochem 2010;109:302–11.
[35] Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol 2014;14:653–66.
[36] Sica A, Invernizzi P, Mantovani A. Macrophage plasticity and polarization in liver homeostasis and pathology. Hepatology 2014;59:2034–42.