

Adipose-Derived Stromal Cells Are Capable of Restoring Bone Regeneration After Post-Traumatic Osteomyelitis and Modulate B-Cell Response

JOHANNES MAXIMILIAN WAGNER ^a, FELIX REINKEMEIER,^a CHRISTOPH WALLNER,^a MEHRAN DADRAS,^a JULIKA HUBER,^a SONJA VERENA SCHMIDT,^a MARIUS DRYSCH,^a STEPHANIE DITTFELD,^a HENRIETTE JAURICH,^a MUSTAFA BECERIKLI,^a KATHRIN BECKER,^c NICOLE RAUCH,^c VIKAS DUHAN,^b MARCUS LEHNHARDT,^a BJÖRN BEHR^a

Key Words. Post-traumatic osteomyelitis • Bone regeneration • Stem cells • B-cells • Osteoimmunology

^aDepartment of Plastic Surgery, University Hospital BG Bergmannsheil Bochum, Bochum, Germany;

^bInstitute of Immunology, University Hospital Essen, Essen, Germany; ^cPoliklinik für Kieferorthopädie, University Hospital Düsseldorf, Poliklinik für Kieferorthopädie, Düsseldorf, Germany

Correspondence: Björn Behr, M.D., University Hospital Bergmannsheil Bochum, Bürkle-de-la-Camp Platz 1, 44789 Bochum, Germany. Telephone: 0049-234-3023443; e-mail: bjorn.behr@rub.de

Received November 15, 2018; accepted for publication May 10, 2019; first published June 10, 2019.

<http://dx.doi.org/10.1002/sctm.18-0266>

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

ABSTRACT

Bone infections are a frequent cause for large bony defects with a reduced healing capacity. In previous findings, we could already show diminished healing capacity after bone infections, despite the absence of the causing agent, *Staphylococcus aureus*. Moreover, these bony defects showed reduced osteoblastogenesis and increased osteoclastogenesis, meaning elevated bone resorption ongoing with an elevated B-cell activity. To overcome the negative effects of this postinfectious inflammatory state, we tried to use the regenerative capacity of mesenchymal stem cells derived from adipose tissue (adipose-derived stem cells [ASCs]) to improve bone regeneration and moreover were curious about immunomodulation of applied stem cells in this setting. Therefore, we used our established murine animal model and applied ASCs locally after sufficient debridement of infected bones. Bone regeneration and resorption as well as immunological markers were investigated via histology, immunohistochemistry, Western blot, and fluorescence-activated cell scanning (FACS) analysis and μ -computed tomography (CT) analysis. Interestingly, ASCs were able to restore bone healing via elevation of osteoblastogenesis and downregulation of osteoclasts. Surprisingly, stem cells showed an impact on the innate immune system, downregulating B-cell population. In summary, these data provide a fascinating new and innovative approach, supporting bone healing after bacterial infections and moreover gain insights into the complex ceremony of stem cell interaction in terms of bone infection and regeneration. *STEM CELLS TRANSLATIONAL MEDICINE* 2019;8:1084–1091

SIGNIFICANCE STATEMENT

This study focused on reduced bone regeneration after severe bone infections. To restore bone healing in this context, stem cells gained from fat tissue (adipose-derived stem cells) were transferred to bony defects right after surgical treatment of infected bones in mice. The gained study results could help to improve therapy of bone infections and moreover contribute to the understanding of stem cell interaction in bone in an inflammatory state.

INTRODUCTION

Post-traumatic osteomyelitis is a severe complication especially after open fractures, with reported incidences between 5% and 30%, depending on the severity of the fracture and soft tissue injury as well as patient related comorbidities [1]. Besides antibiotic treatment, the gold standard treating bone infections is an adequate surgical debridement, removing all infected purulent bone tissue. The presence of infected bone material reduces the effectiveness of antibiotic

treatment by 10^3 as remaining bacteria can form biofilms [2]. In our previous work, we could show a highly impaired bone regeneration capacity after bone infections in a murine tibia defect model [3]. Besides that, we could underline the importance of surgical debridement versus antibiotic treatment alone for the therapy of post-traumatic osteomyelitis. Despite virtually complete removal of all bacteria, remaining bony defects showed a markedly decreased bone regeneration capacity and moreover elevated bone resorption as a result of increased

osteoclast activity in comparison to the control group (debrided but not infected) in our previously established mouse model. This was mediated by an inflammatory reaction, regulating various cytokines and elevated B-cells [4]. Thus, depending on the extent of the bone infection a large bony defect can remain, showing a markedly reduced bone healing capacity [3].

Bone regeneration is a highly complex regulated process containing bone resorption and bone formation. A distinct feature of bone infections, especially by *Staphylococcus aureus*, cause bone resorption. In detail, proliferation and mineralization of infected osteoblasts is altered and osteoclast activity is increased in a receptor activator of NF- κ B ligand (RANKL)-dependent manner [5–9].

The regenerative capacity of mesenchymal stem cells (MSCs), derived from adipose tissue (adipose-derived stem cells [ASCs]) or bone marrow-derived stem cells (BMSCs) gained a lot of attention in recent years [10]. Besides successful clinical use enhancing regenerative processes [11], ASCs can be easily harvested during a minor invasive liposuction procedure and moreover medical devices processing lipoaspirate into ASCs in real-time are already fully licensed [12].

In the setting of bone infections, studies investigating the effects of MSCs are sparse. Subsequently, we sought to improve bone healing after post-traumatic osteomyelitis in mice via stem cell application. Besides the regenerative capacity of MSCs, improving bone formation, this study aimed to investigate the beneficial effects on the highly dysregulated immune system.

For this purpose, ASCs were administered to debrided bony defects after bone infection to increase new bone formation and decrease bone resorption.

MATERIALS AND METHODS

Mouse Osteomyelitis Model

All experiments were performed in adherence to the National Institutes of Health guidelines for the use of experimental animals and after approval by the German legislation. The protocol was approved by the Landesamt für Natur, Umwelt und Verbraucherschutz (NRW, Germany; permit-number: 84-02.04.2014.A044). Animals were housed and caged individually with free access to water and food under specific pathogen free conditions.

C57BL/6J male and female mice, 12 weeks old with an average weight of 25 g, were used for this project. Surgical steps were performed, as previously described in our established mouse model, showing decreased bone formation after debridement following bacterial infection [3]. Briefly, after placement of a skin incision over the proximal medial tibia, a hole (1 mm in diameter) was drilled into the proximal medial tibia. Then *S. aureus* was injected into the medullary cavity of the tibia. Thereafter, the muscle was reapproximated and the wound closed.

Two weeks after bone infection, second surgery was performed. Placing the skin incision once again over the proximal medial tibia the bone defect was exposed. Then, infected bone tissue was debrided, followed by rinsing of debrided defects with isotone sodium chloride solution.

After sufficient debridement, ASCs (10^5 cells in 1 ml) were administered into the bony defect on a collagen sponge. As a control, 1 ml of phosphate-buffered saline (PBS) was administered.

After debridement, the wound was closed and animals were monitored until recovery from anesthesia. Mice were sacrificed 3 and 7 days after debridement.

ASC Isolation

ASCs were isolated and cultivated from inguinal subcutaneous fat pads harvested from C57Bl6 mice. After thoroughly washing the fat pads in sterile PBS, tissue was minced with sterile scissors. Thereafter, 0.075% collagenase type II was administered and incubated at 37°C. Collagenase enzyme reaction was stopped with culture medium. After centrifugation (1,000 rpm, 5 minutes), adipocytes and supernatant were removed and remaining cell pellet was resuspended in culture medium. Cells were then seeded on cells culture flask and passaged later on according to proliferation of cells.

Protein Isolation

Tibiae were quick-frozen after harvest and stored at -80°C . After pestling in liquid nitrogen, bone fragments were collected and homogenized in lysis buffer, containing protease inhibitors until lysis was completed. Cellular debris was removed by centrifugation and isolated protein was stored for further experiments.

Western Blot

Isolated protein was combined and mixed with Laemmli sample buffer. After denaturation at 95°C , samples were kept on ice until loading of the SDS-PAGE. Fifteen percent of polyacrylamide gels were used for electrophoresis of 30 μg total protein per lane. Protein was transferred to a nitrocellulose membrane using wet transfer method before membranes were blocked with 3% bovine serum albumin to prevent unspecific binding. After washing, membranes were incubated with primary antibodies against RANKL, runt-related transcription factor 2 (RUNX2), CD19, interferon- γ (IFN- γ), B-cell activating factor (BAFF), and galectin-9 (GAL9; Abcam, Cambridge, U.K.) overnight at 4°C , followed by washing and incubation with horseradish peroxidase (HRP)-conjugated secondary antibody (Thermo Fisher Scientific, Waltham; Santa Cruz Biotechnologies, Dallas). Proteins were detected for 30–60 seconds by enhanced chemoluminescence.

Flow Cytometry

Tibiae were rinsed with phosphate buffered saline containing 10% fetal calve serum using a fine cannula to wash out bone marrow. A single cell suspension was created and cells of two tibiae per group were combined for flow cytometry analysis. To prevent nonspecific binding, Fc receptors were blocked with anti-CD16/CD32 antibodies (BD Pharmingen, San Diego) before cells were stained with antibodies against CD3, CD19, Gr1 (eBioscience, San Diego), and CD45R (BD Bioscience, San Jose). Flow cytometry was carried out using BD LSRFortessa and corresponding software to compensate fluorescence intensity of antibodies. Further analysis and gating to final cell subpopulations was performed using FlowJo single cell analysis software. Cells were characterized for CD45R+CD19+ B cells, Gr-1+ granulocytes, and CD3+ T cells, respectively.

Microcomputed Tomographic Analysis

Bone specimens were scanned with a μCT device (Viva CT 80; Scanco Medical AG, Brüttisellen, Switzerland) operated at 70 kVp, 114 μA , 8 W, 31.9-mm Field-of-View, an integration time of 1,167 ms and 2x frame averaging. The data sets were

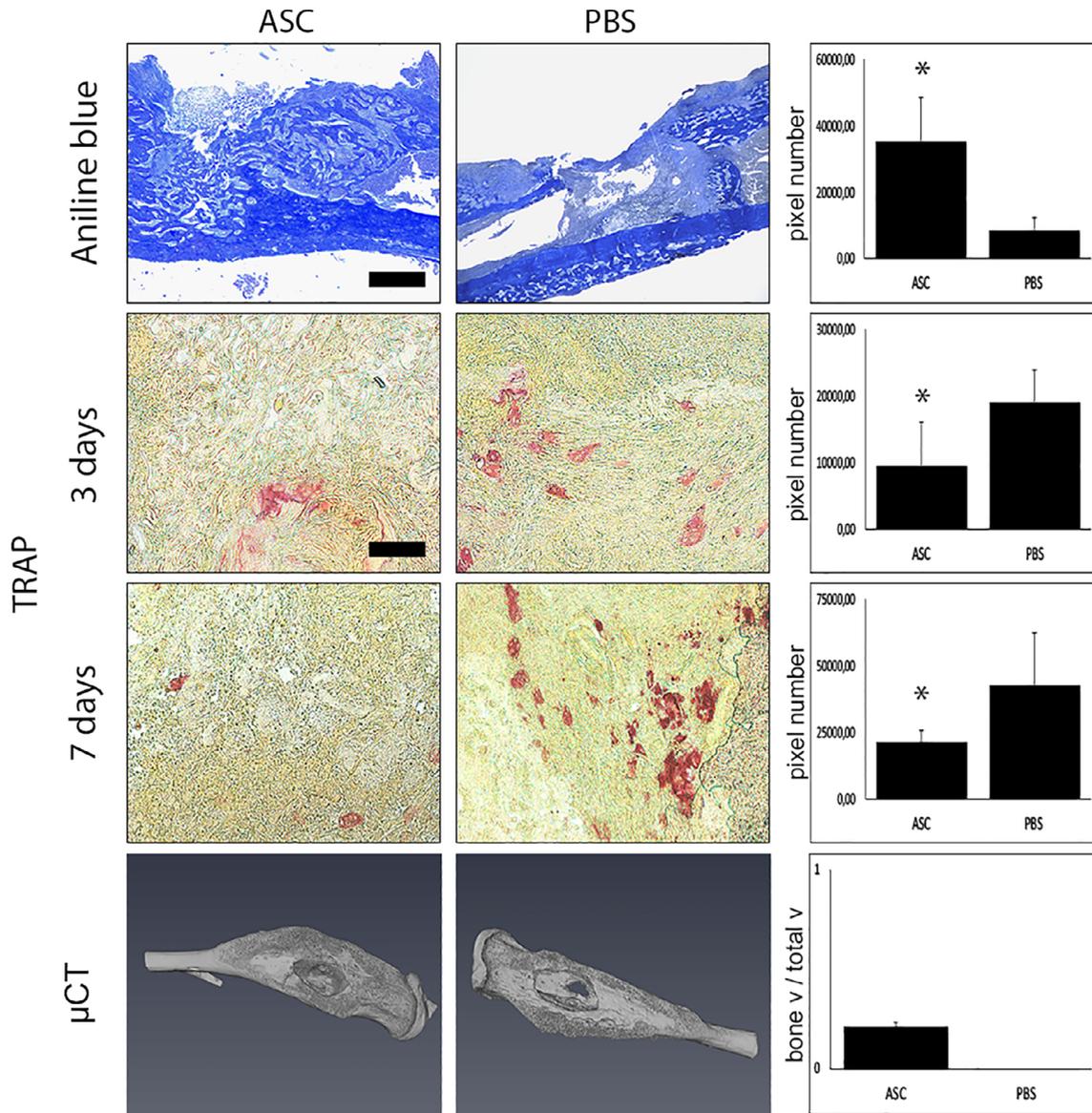


Figure 1. Osteogenesis is elevated and osteoclast activity reduced by adipose-derived stem cell (ASC) treatment. Aniline blue, Tartrate-resistant acid phosphatase (TRAP) stainings, and μ CT-analysis of animals treated with ASCs and phosphate-buffered saline. Aniline blue stainings made after 7 days showed significantly elevated new bone formation after stem cell treatment. In accordance, μ CT scans revealed markedly increased bone volume to total volume of ASC-treated animals in comparison with control animals. Furthermore, TRAP staining indicated a downregulation of osteoclasts 3 and 7 days after surgery due to ASC treatment (seven animals/group). Scale bar: aniline blue represents 200 μ m; scale bar: TRAP represents 100 μ m. *, $p < .05$.

reconstructed into 3D volumes with an isotropic nominal resolution of 15.6- μ m voxel size.

Image Processing

Further processing of the scanned images was performed using μ -CT Evaluation Software Program V6.5 (Scanco Medical AG, Brüttsellen, Switzerland). A standardized cylindrical volume of interest with 16.84 mm in diameter was placed within the defect site. Thereafter, bone volume to total volume was assessed according to the guidelines for assessment of bone microstructures using μ CT (Bouxsein, guidelines for assessment of bone microstructure in rodents using microcomputed tomography 2010).

Histology, Immunohistochemistry, and Immunofluorescence

For all histological procedures, tibiae were taken and fixed in 4% paraformaldehyde solution overnight, decalcified in 19% EDTA solution and finally paraffin embedded. Thereafter, tibiae were longitudinally sectioned at 9 μ m. Following, aniline blue staining was performed as previously described [13]. Moreover, TRAP-staining was performed with TRAP Kit (Sigma-Aldrich, St. Louis) after manufacturer's instruction.

Additionally, immunohistochemical stainings with primary antibodies against osteocalcin, proliferating-cell-nuclear-antigen (PCNA; Santa Cruz Biotechnologies) and platelet endothelial cell adhesion molecule 1 (PECAM-1; BD Biosciences, Franklin

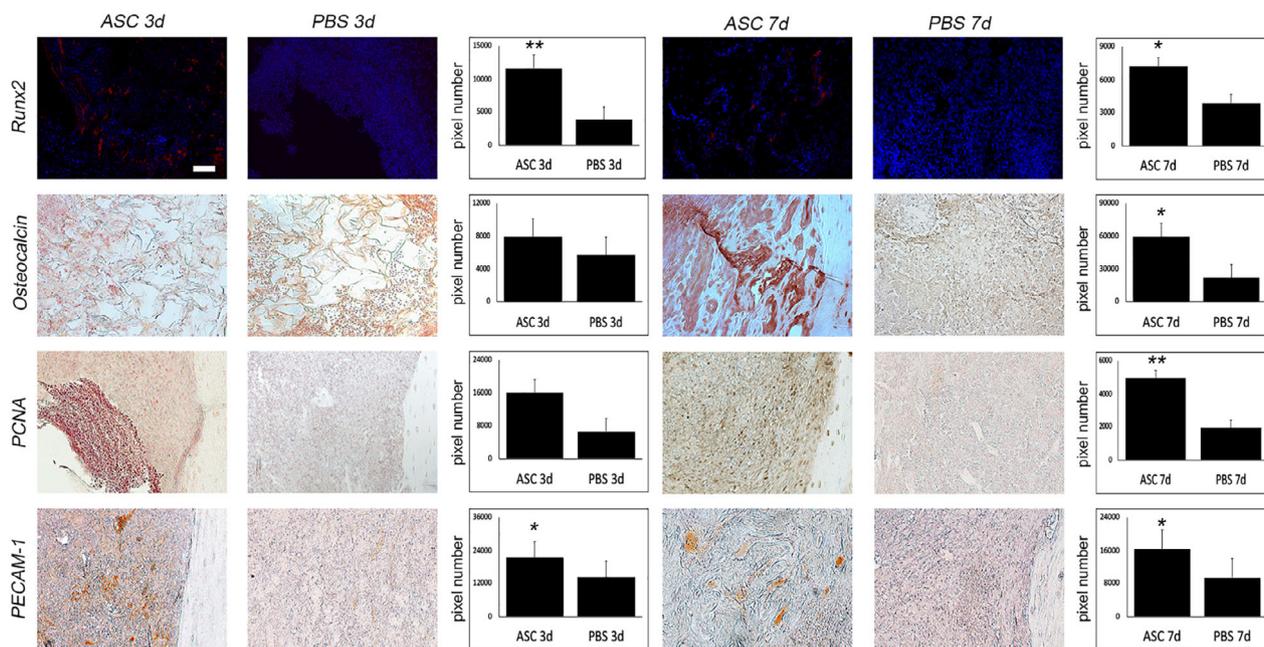


Figure 2. Stem cell application leads to increased osteoblastogenesis, angiogenesis, and proliferation. Immunofluorescent stainings with primary antibody against runt-related transcription factor 2 (Runx2) and immunohistochemical stainings against osteocalcin, proliferating-cell-nuclear-antigen (PCNA), and platelet endothelial cell adhesion molecule 1 (PECAM-1). Subsequently to elevated bone formation after stem cell application, osteoblastogenesis which could be seen in Runx2 stainings was significantly elevated 3 days after surgery. In accordance, osteocalcin, a marker for mature osteoblasts showed significantly enhanced signals after 3 and 7 days. PCNA stainings, indicating cell proliferation was also upregulated after stem cell treatment. Interestingly, angiogenesis seemed to be enhanced by ASC treatment, as signals observed in PECAM-1 stainings were significantly increased (seven animals/group). Scale bar represents 100 μ m. *, $p < .05$; **, $p < .01$.

Lakes) were performed using Vectastain ABC Kit (Vector Laboratories, Burlingame). After deparaffinization and rehydration, specimens were incubated with proteinase K for antigen demasking. Endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide solution. Thereafter, specimens were blocked with normal blocking serum to prevent unspecific binding of primary antibody that was subsequently applied and incubated overnight at 4°C. Following, secondary antibody conjugated to HRP was used and staining reaction was performed by use of NovaRED (HRP) Peroxidase Substrate Kit (Vector Laboratories, Burlingame).

For immunofluorescent stainings, primary antibodies against RUNX2 (Santa Cruz Biotechnologies) and CD19 (Abcam) were used. Initial steps were carried out similar to immunohistochemical staining until application of primary antibody. Thereafter, samples were incubated with secondary antibody conjugated to Alexa Fluor594 (Thermo Fisher Scientific). Three images per specimen were taken with Zeiss Axioplan microscope and histomorphometry of all histological stainings was performed by semiautomatic pixel quantification using Adobe Photoshop as previously been described.

Statistics

Results are presented as mean \pm SEM of at least three independent experiments. Normal distribution was tested using chi square test. p values were calculated by Student's t test comparing two groups. For post hoc comparisons, Tukey's test was used. Statistical significances were set at a p -value $< .05$.

RESULTS

Application of ASCs Leads to Elevated Osteogenesis and Angiogenesis

One of our primary interests after ASC application was the evaluation of new bone formation after treatment. Aniline blue staining revealed significant promotion of osteogenesis by MSCs in comparison to PBS control at day 7 postoperatively. In fact, a 10-fold increase of new bone formation in aniline blue stainings could be made out after stem cell application (Fig. 1). This observation was further validated by μ -CT scans showing elevated bone volume to total volume within the defect site of ASC treated animals (Fig. 1). In this context, we could observe enhanced osteoblastogenesis and osteoblast activity in Runx2 and osteocalcin stainings (Fig. 2). These findings could be further supported by Western blot analysis targeting Runx2 (Fig. 3). Besides osteogenesis, proliferation verified by PCNA staining, could be increased by ASC treatment (Fig. 2). To verify if applied ASCs could likewise increase angiogenesis, stainings with primary antibody against PECAM-1 were performed, elucidating significantly increased number of vessels within the defect (Fig. 2).

Stem Cells Could Decrease Bone Resorption in a RANKL-Dependent Manner

As already shown, osteoclast activity is markedly enhanced during postinfectious, inflammatory state of osteomyelitis. Importantly, applied ASCs were capable to decrease osteoclast

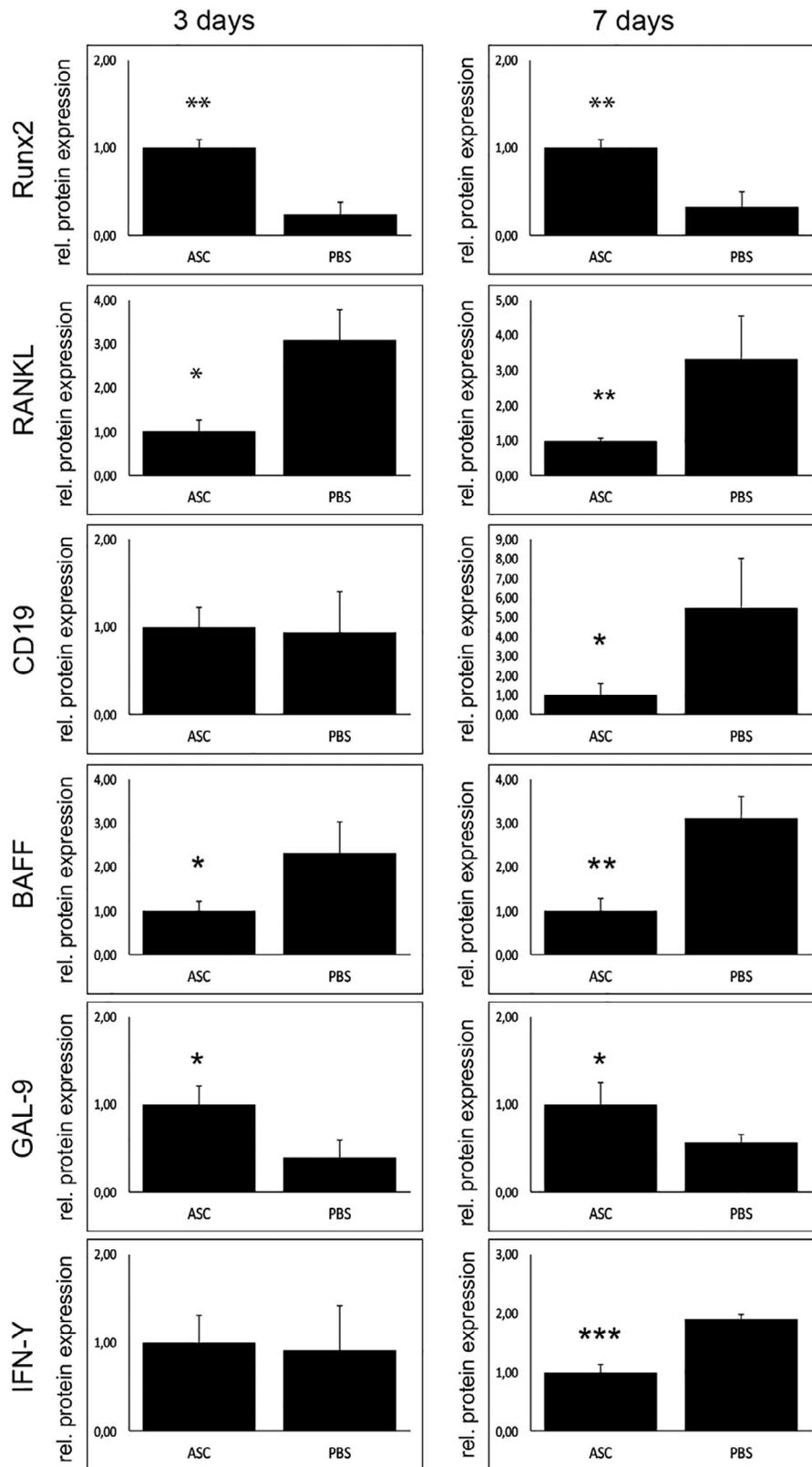


Figure 3. B-cell depletion is mediated via galectin-9 (GAL9) and B-cell activating factor (BAFF). Western blots of markers for osteoblastogenesis, osteoclast, and B-cell activity. In accordance to immunofluorescent stainings, protein levels of runt-related transcription factor 2 could be raised by stem cell application. Decreased osteoclast activity seems to be downregulated in a receptor activator of NF- κ B ligand-dependent manner as levels were significantly reduced in stem cell group. Investigating B-cells, CD19 was downregulated in ASC group 7 days after surgery. Subsequently, GAL9 known to inhibit B-cells is upregulated and moreover, BAFF is downregulated after stem cell application. Interestingly, interferon- γ is decreased 7 days after surgery in stem cell group (nine animals/group). *, $p < .05$; **, $p < .01$; ***, $p < 0.001$.

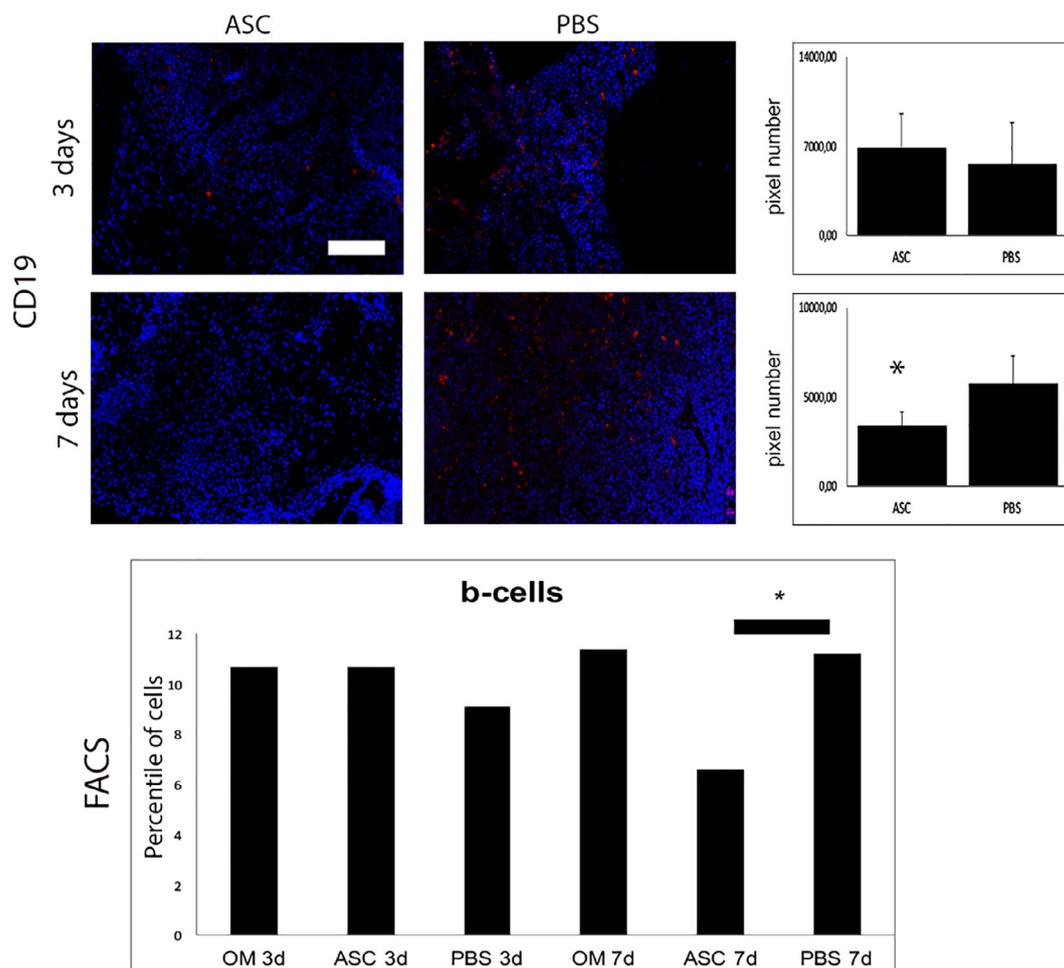


Figure 4. B-cells were decreased 7 days after surgery by stem cell application. Immunofluorescent stainings with primary antibody against CD19 and FACS analysis of B-cells. In accordance to Western blot results, immunofluorescent stainings and FACS analysis revealed B-cell depletion by stem cell application 7 days after surgery (six animals/group). Scale bar represents 100 μ m; *, $p < .05$.

activity, seen in TRAP staining (Fig. 1), and thereby bone resorption. Accordingly, RANKL, essential for osteoclast function, was significantly enhanced in Western blot (Fig. 3).

ASCs Show Immunomodulation of the Adaptive Immune System

Besides studying the osteogenic potential of ASCs in this special setting, a closer look on the immune system was one of the main goals of this work. Detecting an elevated B-cell activity in our previous work, a FACS analysis revealed the depletion of B-cells in stem cell treated group (Fig. 4). Interestingly, this effect could be observed only 7 days after treatment. Accordingly, Western blot levels of BAFF were diminished and GAL9 elevated (Fig. 3). Interestingly, cell numbers of T-cells and granulocytes were not affected by ASC treatment (data not shown).

DISCUSSION

Decreased bone regeneration due to inflammatory processes, especially after bone infections is a common problem of orthopedic surgery [14]. In our previous work, we have demonstrated

decreased bone regeneration after debridement following bacterial infection as a result of a dysregulated inflammatory reaction (3 and 4). In the current study, we sought to investigate the ability of ASCs to restore the regenerative capacity of postinfectious bone defects. In addition, our investigations focused on the immunomodulatory properties of MSCs.

Interestingly, ASC application leads to dramatically enhanced bone formation after debridement of osteomyelitic bone. Accordingly, proliferation and differentiation of osteoblasts as well as angiogenesis was markedly enhanced.

In this context, many studies have already demonstrated the potential of MSCs for osteogenic differentiation [15–17]. Moreover, ASCs have the capacity to differentiate into different types of tissue including cartilage, bone, muscle, vessels, and adipose tissue [10, 15].

Interestingly, our findings indicated the potential of ASCs to modulate osteoclast activity and thereby diminish bone resorption. Accordingly, stem cells could modulate RANKL/osteoprotegerin (OPG)-axis, which was dysregulated due to bone infection. Consistent to our findings, MSCs are known to decrease cell number of osteoclasts via modulation of RANKL/OPG-axis [18]. In a murine psoriasis model Th17 cells enhanced osteoclasts in a RANKL-dependent manner, whereas MSC

application could downregulate osteoclasts via OPG production [18]. Moreover, stem cell-based therapy seemed to be promising to reduce bone resorption in rheumatoid arthritis [19].

Besides regenerative abilities, emerging data indicate immunomodulatory properties of MSCs [20]. For instance, BMSCs showed the capacity to reduce the inflammatory response and developing fibrosis in a murine lung injury model, secreting interleukin (IL)-1 receptor antagonist (IL-1ra) [21]. Furthermore, BMSC infusion in a murine myocardial infarct model led to a significant increase of anti-inflammatory protein Tsg-6 [22]. In this context, TSG-6 application as well as human BMSCs have been shown to reduce secretion of tumor necrosis factor α (TNF- α) and IL-1 α during inflammatory state in a peritonitis model. Further beneficial effects of BMSCs were evident in a sepsis model reducing the expression of TNF- α and IL-6 [23]. Accordingly, ASCs could also modulate the immune response of arthritic Dilute Brown Non-Agouti/1 mice. Here, ASC treatment resulted in a decreased incidence and severity of arthritis accompanied by a significant decrease in inflammatory cytokines such as TNF- α , IL-1 β , and IFN- γ . In an experimental colitis and sepsis model, ASCs turned out to have a protective and anti-inflammatory effect via secretion of IL-10 [24]. Investigations concerning cystic fibrosis revealed the ability of MSCs battling bacterial infections via secretion of antimicrobial agents [25]. In this context, the immunomodulatory and antibacterial abilities of MSCs could be used as a new and innovative approach treating bacterial infections and autoimmune disorders like graft versus host disease [26]. More specifically, ASCs showed beneficial effects preventing and treating osteomyelitis [27] and could restore bone regeneration of osteonecrosis in maxillofacial surgery [28].

Accordingly, our data could demonstrate the modulation of the innate immune system via stem cell application, regulating B-cells during late fracture healing. BAFF and GAL9 levels seemed to have a regulatory function in this context.

B-cell function is essential for bone remodeling and osteoclast function. Activated B-cells secrete RANKL and thereby increase osteoclast activity [29]. However, the physiological balance of bone formation and resorption can be shifted toward bone erosion by increased B-cell activity [29]. In inflammatory musculoskeletal diseases like rheumatoid arthritis, B-cells have a major impact on focal bone erosion [30–32]. Supporting this hypothesis, B-cell depleting therapy seems quite effective suppressing bone erosions and synovitis in rheumatoid arthritis [31]. Moreover, B-cells seem to be involved in periodontic bone loss [33].

Recent studies elucidated a downregulation of excessive B-cell proliferation via MSCs. Potential mechanism responsible for this is a cell contact based inhibition mediated by GAL9 [34, 35]. BAFF is indispensable for B-cell maturation and enhances B-cell survival [36]. In terms of B-cell overactivity, MSCs showed the potential to affect B-cells via BAFF regulation [34].

CONCLUSION

Our investigations show how ASCs could overcome the impairment of bone regeneration after osteomyelitis in a murine animal model. Interestingly, bone formation and osteoblastogenesis could be elevated and moreover osteoclastogenesis decreased in a RANKL-dependent manner. Besides that, immunomodulatory effects of applied ASCs on the innate immune system were evident in a B-cell depletion, mediated by GAL9 and BAFF.

ACKNOWLEDGMENT

This work was supported by a grant of Deutsche Forschungsgemeinschaft (DFG; BE 4169/8-1).

AUTHOR CONTRIBUTIONS

J.M.W.: conception and design, collection of data, data analysis and interpretation, manuscript writing, final approval of manuscript; F.R.: collection of data, data analysis and interpretation, manuscript writing, final approval of manuscript; C.W., M.D., J.H.: data analysis and interpretation, final approval of manuscript; S.V.S., M.D., H.J., M.B., V.D., K.B., N.R.: collection of data, data analysis and interpretation, final approval of manuscript; S.D.: collection of data, final approval of manuscript; M.L.: administrative support, final approval of manuscript; B.B.: conception and design, administrative support, data analysis and interpretation, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- 1 Lew DP, Waldvogel FA. Osteomyelitis. *Lancet* 2004;364:369–379.
- 2 Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. *Clin Orthop Relat Res* 2006;437:7–11.
- 3 Wagner JM, Zollner H, Wallner C et al. Surgical debridement is superior to sole antibiotic therapy in a novel murine posttraumatic osteomyelitis model. *PLoS One* 2016;11:e0149389.
- 4 Wagner JM, Jaurich H, Wallner C et al. Diminished bone regeneration after debridement of posttraumatic osteomyelitis is accompanied by altered cytokine levels, elevated B cell activity and increased osteoclast activity. *J Orthop Res* 2017;35:2425–2434.
- 5 Claro T, Widaa A, O'Seaghda M et al. *Staphylococcus aureus* protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. *PLoS One* 2011;6:e18748.
- 6 Dapunt U, Maurer S, Giese T et al. The macrophage inflammatory proteins MIP1alpha (CCL3) and MIP2alpha (CXCL2) in implant-associated osteomyelitis: Linking inflammation to bone degradation. *Mediators Inflamm* 2014;2014:728619.
- 7 Shi S, Zhang X. Interaction of *Staphylococcus aureus* with osteoblasts (review). *Exp Ther Med* 2012;3:367–370.
- 8 Widaa A, Claro T, Foster TJ et al. *Staphylococcus aureus* protein A plays a critical role in mediating bone destruction and bone loss in osteomyelitis. *PLoS One* 2012;7:e40586.
- 9 Josse J, Velard F, Gangloff SC. *Staphylococcus aureus* vs. osteoblast: Relationship and consequences in osteomyelitis. *Front Cell Infect Microbiol* 2015;5:85.
- 10 Behr B, Ko SH, Wong VW et al. Stem cells. *Plast Reconstr Surg* 2010;126:1163–1171.
- 11 Gimble JM. Adipose tissue-derived therapeutics. *Expert Opin Biol Ther* 2003;3:705–713.
- 12 Lin K, Matsubara Y, Masuda Y et al. Characterization of adipose tissue-derived cells

isolated with the Celution system. *Cytotherapy* 2008;10:417–426.

13 Behr B, Leucht P, Longaker MT et al. Fgf-9 is required for angiogenesis and osteogenesis in long bone repair. *PNAS* 2010;107:11853–11858.

14 Schmidt-Rohlfing B, Lemmen SW, Pfeifer R et al. Osteomyelitis in adults. Diagnostic principles and therapeutic strategies. *Unfallchirurg* 2012;115:55–66.

15 Zuk PA, Zhu M, Mizuno H et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* 2001;7:211–228.

16 Behr B, Tang C, Germann G et al. Locally applied vascular endothelial growth factor A increases the osteogenic healing capacity of human adipose-derived stem cells by promoting osteogenic and endothelial differentiation. *STEM CELLS* 2011;29:286–296.

17 Cowan CM, Shi Y-Y, Aalami OO et al. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat Biotechnol* 2004;22:560–567.

18 Cho K-A, Park M, Kim Y-H et al. Mesenchymal stem cells inhibit RANK-RANKL interactions between osteoclasts and Th17 cells via osteoprotegerin activity. *Oncotarget* 2017;8:83419–83431.

19 Tanaka Y. Human mesenchymal stem cells as a tool for joint repair in rheumatoid arthritis. *Clin Exp Rheumatol* 2015;33:S58–S62.

20 Prockop DJ, Oh JY. Mesenchymal stem/stromal cells (MSCs): Role as guardians of inflammation. *Mol Ther* 2012;20:14–20.

21 Ortiz LA, Dutreil M, Fattman C et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci USA* 2007;104:11002–11007.

22 Lee RH, Pulin AA, Seo MJ et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 2009;5:54–63.

23 Nemeth K, Leelahavanichkul A, Yuen PST et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009;15:42–49.

24 Anderson P, Souza-Moreira L, Morell M et al. Adipose-derived mesenchymal stromal cells induce immunomodulatory macrophages which protect from experimental colitis and sepsis. *Gut* 2013;62:1131–1141.

25 Sutton MT, Fletcher D, Ghosh SK et al. Antimicrobial properties of mesenchymal stem cells: Therapeutic potential for cystic fibrosis infection, and treatment. *Stem Cells Int* 2016;2016:1–12.

26 Mezey E, Nemeth K. Mesenchymal stem cells and infectious diseases: Smarter than drugs. *Immunol Lett* 2015;168:208–214.

27 Mohiti-Asli M, Molina C, Diteepeng T et al. Evaluation of silver ion-releasing scaffolds in a 3D coculture system of MRSA and human adipose-derived stem cells for their potential use in treatment or prevention of osteomyelitis. *Tissue Eng Part A* 2016;22:1258–1263.

28 Handschel J, Meyer U. Infection, vascularization, remodelling—Are stem cells the answers for bone diseases of the jaws? *Head Face Med* 2011;7:5.

29 Horowitz M, Fretz J, Lorenzo J. How B cells influence bone biology in health and disease. *Bone* 2010;47:472–479.

30 Meednu N, Zhang H, Owen T et al. Production of RANKL by memory B cells: A link between B cells and bone erosion in rheumatoid arthritis. *Arthritis Rheumatol* 2016;68:805–816.

31 McQueen FM, Issa S. Is rheumatoid arthritis a B-cell haematological disease with a predilection for the joints? Following the B cell thread to its logical conclusion. *Med Hypotheses* 2014;82:266–270.

32 Weitzmann MN. T-cells and B-cells in osteoporosis. *Curr Opin Endocrinol Diabetes Obes* 2014;21:461–467.

33 Hienz SA, Paliwal S, Ivanovski S. Mechanisms of bone resorption in periodontitis. *J Immunol Res* 2015;2015:615486.

34 Fan L, Hu C, Chen J et al. Interaction between mesenchymal stem cells and B-cells. *Int J Mol Sci* 2016;17:650–666.

35 Ungerer C, Quade-Lyssy P, Radeke HH et al. Galectin-9 is a suppressor of T and B cells and predicts the immune modulatory potential of mesenchymal stromal cell preparations. *Stem Cells Dev* 2014;23:755–766.

36 Mackay F, Browning JL. BAFF: A fundamental survival factor for B cells. *Nat Rev Immunol* 2002;2:465–475.