Citation: IBMS BoneKEy **13**, Article number: 812 (2016) | doi:10.1038/ibmsbonekey.2016.39

www.nature.com/bonekey

MEETING REPORT

Osteoimmunology at the 2015 ASBMR Annual Meeting

Archana Sanjay[1] and Joseph A Lorenzo[1,2]

[1]Department of Orthopaedics, UConn Health, Farmington, CT, USA. [2]Department of Medicine, UConn Health, Farmington, CT, USA.

Osteoimmunology was prominently featured in the program of this year's American Society for Bone and Mineral Research Annual Meeting. Although there was no special session devoted to osteoimmunology, several talk and posters featured different aspects of bone–immune cell interaction. Many of the presentations examined cellular modulators of inflammation.

IBMS BoneKEy **13**, Article number: 812 (2016) | doi:10.1038/ibmsbonekey.2016.39; published online 25 May 2016

Meeting Report from the 2015 Annual Meeting of the American Society for Bone and Mineral Research, Seattle, WA, USA, 9–12 October 2015.

Introduction

The immune system is divided into innate and adaptive immune cells. Innate immune cells such as macrophages respond in a generic way to pathogens and do not provide long-term memory of previous infection. In contrast, adaptive immune cells produce memory cells that rapidly respond to pathogens, which have previously invaded an organism to prevent reinvasion. Members of the innate immune system include macrophages, which phagocytize pathogens and produce proinflammatory cytokines, and dendritic cells, which present processed antigen to T lymphocytes to initiate adaptive immune responses. T lymphocytes can be divided into a variety of subcategories. Among these are T-regulatory cells (Tregs), which suppress inflammation, and TH17 cells, which mediate a number of inflammatory responses and produce interleukin-17 (IL-17). After activation, B lymphocytes can differentiate into antibody-producing plasma cells.

Abstracts that examined lymphocyte-mediated regulation of the bone include Liu *et al.* (abstract SU0234) who examined the interactions of Tregs, TH17 cells and immature myeloid precursors, Yang *et al.* (abstract SU0181) who examined interactions of bone marrow mesenchymal cells with Tregs, Mansoori *et al.* (abstract SA0031) who found that neutralization of IL-18 by injection of IL-18 binding protein (IL-18BP) blocked the bone loss that occurs with estrogen withdrawal in mice, an effect that correlated with an increase in Tregs and decreased TH17 cells, Subramaniam *et al.* (abstract 1128) who found femur-specific effects on bone mass with deletion of TGF-β inducible early gene-1 (TIEG) in CD4 T lymphocytes, Sun *et al.* (abstract SA0368) who found that depletion of B lymphocytes in TNF-α transgenic mice, which spontaneously develop an inflammatory arthritis, prevented the decreased bone formation that is seen in peri-articular bone, adjacent to the inflammation, and Wei *et al.* (abstract FR0080) who showed that deletion of ADAMTS-12 enhanced inflammation in a collagen-induced arthritis model and altered the ratio of Tregs to TH17 cells

As a general theme, it appears that cells involved in the adaptive immune response have become a focus of many studies of the interactions of bone and immune cells. However, there were also abstracts at the meeting that examined the interactions of innate immune cells with bone. Nakamura *et al.* (abstract SA0398) found a decreased number of inflammatory macrophages in the fracture callus of aged mice relative to young mice. They speculated that this event might be involved in the delayed fracture healing, which is seen with aging. Rossnagi *et al.* (abstract FR0060) found that conditional deletion of fibronectin in differentiating osteoblasts decreased myelopoiesis, particularly myeloid-derived suppressor cells, and enhanced fibrosis during wound healing.

The following is a more detailed summary of abstracts, which presented new concepts at the meeting about the interactions of bone and the immune system.

Abstract Examining Lymphocyte-Mediated Regulation of the Bone

Signaling interactions of myeloid DC precursors on osteoclastogenesis and bone remodeling: an alternative insight.

Abstract number: SU0234

Yen-Chun Grace Liu, Andy Yen-Tung Teng. Koahsiung Medical University, Taiwan, Center for Osteoimmunology & Biotechnology Research, College of Dental Medicine, Kaohsiung Medical University & KMU-Hospital, Taiwan

Dendritic cells (DCs) are professional antigen-presenting cells, involved in T-cell immunity. In addition, immature

myeloid DC precursors (CD11c+ MHC-II-/lo CD11b – F4/80 – CD31 – Ly-6C – CT-R – Cath-K –) can act as OC precursors. In this work, interactions among myeloid DC precursors (mDCp), T cells (Treg and Th17 cells) and osteotropic cytokines including IL-17 and TGF-β on osteoclastogenesis were investigated in both an established cell culture system and mice. Neutralization of TGF-β activity abolished mDCp development in co-cultures and *in vivo* in adoptive transfer studies. Addition of Foxp3+ CD4+ Treg cells to *in vitro* co-cultures or into NOD/SCID mice, which lack mature immune systems, reduced TRAP expression, osteoclastogenesis and bone resorption both *in vitro* and *in vivo*. In addition, these effects were significantly reversed by IL-17 administration. These data suggest that immature mDCp and CD4+T-cell interactions may serve as a unique model to examine the effects of immunity and inflammation-associated osteoclastogenesis on bone remodeling.

Cross-talk between BMSCs and regulatory T cells through a GILZ/Del-1-dependent mechanism.

Abstract number: SU0181

Nianlan Yang, Babak Baban, Carlos Isales, Xing-Ming Shi. Georgia Regents University, USA

Bone marrow mesenchymal stem cells (BMSCs) have potent modulatory effects on T cells either through direct cell–cell contact or through the release of soluble factors. However, studies of the interaction or cross-talk between bone cells and immune cells in the bone marrow are sparse. Glucocorticoid-induced leucine zipper (GILZ) inhibits pro-inflammatory cytokine TNF-alpha-induced COX-2 expression and antagonizes TNF-alpha-mediated inhibition of osteogenic differentiation. In the current work, it was found that GILZ can induce the expression in the bone of developmental endothelial locus-1 (Del-1), a secreted protein, implicated in the regulation of neutrophil recruitment and inflammatory bone loss. Further, the induction of Del-1 by GILZ in BMSCs was increased in bone tissues of transgenic mice expressing GILZ under the control of a 3.6-kb type I collagen promoter fragment (Col3.6-GILZ). This response was coupled with a significant increase in the anti-inflammatory cytokine, IL-10, and decreases in the proinflammatory cytokines, IL-6 and IL-12. FACS analysis showed that the percentage of Tregs (FOXP3+ and CD25+) was increased significantly in both the blood and bone marrow of GILZ Tg mice. The authors conclude that GILZ expressed in BMSCs upregulates Tregs number by inducing Del-1 expression and altering cytokines profile. They postulate that GLIZ has a critical role in the cross-talk between BMSCs and Tregs in the bone marrow.

Relation between decreased IL-18BP levels and risk of osteoporosis in post-menopausal osteoporotic patients: role of IL-18BP in preventing bone loss by positively regulating Treg/Th17 balance.

Abstract number: SA0031

Mohd Nizam Mansoori, Priyanka Shukla, Abdul Malik, Kamini Srivastava, Manisha Kakaji, Karam bir Kumar, Manisha Dixi1, Jyoti Kureel, Sushil Kumar Gupta, Divya Singh. CDRI, India

IL-18-binding protein (IL-18BP) is a natural antagonist of IL-18, a proinflammatory cytokine that is increased in several autoimmune disorders. In this abstract, the authors investigated the effect of mIL-18BP administration on T and B cells, Th17/Treg balance and skeletal parameters in estrogen-deficient mice. Sham or ovariectomized (OVX) mice were subcutaneously injected twice weekly for 4 weeks with mIL-18BP (0.5 mg kg^{-1} body weight). Micro-CT analysis showed that mIL-18BP treatment restored trabecular microarchitecture and preserved cortical bone parameters. mIL-18BP-treated OVX mice had decreased proliferation of IL-17-secreting Th17 cells and increased Tregs (CD4+ CD25+ FoxP3+). Importantly, these results were corroborated in human subjects where IL-18BP levels were evaluated in post-menopausal women and serum level of IL-18BP correlated directly with BMD. These results demonstrate that exogenous mIL-18BP administration provides protection against estrogen deficiency-induced bone loss in mice and has the potential to be a therapy for postmenopausal osteoporosis.

T-cell-specific deletion of TIEG alters chemokine expression profiles and results in increased bone mass in mice.

Abstract number: 1128

Malayannan Subramaniam, AKM Khyrul Wara, Fang Fang, Kevin Pitel, Mark Feinberg, John R Hawse. Mayo Clinic, USA, Harvard Medical School, USA

TGF-β inducible early gene-1 (TIEG) has a central role in mediating CD4+ T-cell effector functions and the development of T-reg cells through control of TGF-β1 signaling and FOXP3 expression. This report examined the effects that CD4+ T-cell-specific deletion of TIEG had on the mouse skeleton. pQCT and micro-CT analysis of 2-month-old CD4-Cre control and T-cell-specific TIEG knockout (TKO) mice showed minimal differences within the tibial metaphysis. However, substantial difference was observed in TKO mice in the femoral metaphysis, including increased bone volume/tissue volume (50%), trabecular number (10%) and trabecular thickness (40%), with a concomitant decrease in trabecular spacing (7%). TKO CD4+ T-cells had increased expression of INF and decreased expression of RANKL and TRAF6. These data identify TIEG as an important osteoimmunological molecule with the ability to regulate skeletal homeostasis through CD4+ T cells.

B cells contribute to bone erosion in rheumatoid arthritis by directly inhibiting osteoblast differentiation.

Abstract number: SA0368

Wen Sun, Nida Meednu, Hengwei Zhang, Xing Li, Teresa Owen, Alex Rosenberg, Brendan Boyce, Jennifer Anolik, Lianping Xing. University of Rochester Medical Center, USA

B-cell depletion therapy effectively attenuates joint tissue damage in many RA patients. B cells promote osteoclast formation by secreting RANKL and activating T cells. In this report, the authors investigated the effects of B cells on osteoblast differentiation and bone erosion using TNF transgenic (TNF-Tg) mice, a model of RA. Immunofluorescent staining of knee joint sections from TNF-Tg mice showed B220+ B cell aggregates at sites of joint erosion and in the subchondral bone area. Bone marrow CD19+ B cells from TNF-Tg or WT littermate control mice was cocultured with WT bone-derived mesenchymal progenitor cells (MPCs) in OB-inducing medium. TNF-Tg B cell cultures had significantly decreased alkaline phosphatase+ (ALP+) area and Runx2 expression. Furthermore, expression of inflammatory cytokines known to inhibit MPC-OB differentiation was markedly elevated. TNF-neutralizing antibody blocked OB inhibition induced by TNF-Tg B cells. TNF-Tg

mice injected with anti-CD20, which specifically depleted B cells, had decreased joint erosion and an increased number of osteocalcin+ OBs. These results suggest that B cells inhibit OB differentiation in RA by secreting TNF and thus contribute to bone loss.

ADAMTS-12 protects against inflammatory arthritis through interacting with and inactivating proinflammatory CTGF.

Abstract number: FR0080

Jianlu Wei, Wenyu Fu, Qingyun Tian, Chuanju Liu. Hospital for Joint Diseases of NYU, USA

It has been reported that ADAMTS-12 is a susceptibility gene for rheumatoid arthritis and could directly bind and degrade cartilage oligomeric matrix protein. These authors determined whether ADAMTS-12 participates in the pathogenesis of inflammatory arthritis using a collagen-induced arthritis (CIA) model in ADAMTS-12-deficient mice and controls. ADAMTS-12-deficient mice exhibited increased bone and joint destruction. Furthermore, ADAMTS-12-deficient CIA mice expressed higher levels of ROR and lower levels of Foxp3, indicating that the ratio of Treg/Th17 cells was reduced. Isolated CD4+T cells from ADAMTS-12-deficient CIA mice were shown to produce more pro-inflammatory and less anti-inflammatory cytokines. A yeast two-hybrid assay using various ADAMTS-12 deletion mutants demonstrated that the C-terminal mucin and TSP motifs of ADAMTS-12 were required and sufficient for binding CTGF. Intra-articular injection of CTGF blocking antibody attenuated the enhanced inflammation seen in the ADAMTS-12-deficient CIA model. Collectively, this report suggests that ADAMTS-12 is a critical regulator of inflammatory arthritis, at least in part, by inactivating of CTGF.

Abstracts Examining Interactions of Innate Immune Cells with Bone

Fracture repair and effects of aging on macrophages at the fracture callus.

Abstract number: SA0398

Mary Nakamura, Erene Niemi, Yang Frank, Ted Miclau, Ralph Marcucio. University of California, San Francisco/San Francisco VAMedical Center, USA, UCSF/SFVAMC, USA, Orthopaedic Trauma Institute, SFGH, UCSF, USA

Nakamura and colleagues propose that age-related changes in myeloid cells promote dysfunctional bone regeneration during fracture repair. They isolated fracture callus cells from aged (24 months) or young animals and analyzed them by flow cytometry and gene expression. They found that, at day1 post fracture, there was a similar distribution of immune cells in the aged and young fracture callus including T cells, B cells, NK cells, macrophages and granulocytes. On days 3 and 10, in the young fracture callus, there were twofold more F4/80+ (macrophage) cells particularly those expressing an inflammatory phenotype (Ly6C+/MHC class II). These results suggest that decreased recruitment and/or proliferation of inflammatory macrophages and changes in macrophage phenotype contribute to the delayed fracture healing with aging.

Osteoblast fibronectin stimulates myelopoiesis and affects the behavior of myeloid-derived cells *in vivo*.

Abstract number: FR0060

Stephanie Rossnagl, Sabrina Kraft, Eva Altrock1, Carla Sens, Katrin Rau, Verena Klemis, Inaam Nakchbandi. University of Heidelberg & Max-Planck Institute of Biochemistry, Germany, Max-Planck Institute of Biochemistry & University of Heidelberg, Germany

It is known that osteoblasts affect hematopoietic stem cell differentiation and produce fibronectin isoforms with autocrine effects. In this report, conditional deletion of fibronectin in differentiating osteoblasts (cKO) caused a 30% decrease in myeloid cells in bone marrow due to defective differentiation of progenitors. Fibronectin isoform containing the extra domain A (EDA-FN), which is produced by osteoblasts, enhanced myelopoiesis *in vitro*, whereas other isoforms did not. $\alpha5\beta1$ integrin was identified as the mediator of CD11b+ cell differentiation in response to EDA-FN. The myeloid cells of the bone marrow can differentiate to myeloid-derived suppressor cells (MDSCs). Fibrosis represents a wound healing process where MDSCs prevent an excessive immune response. Induction of liver fibrosis in mice resulted in enhanced fibrosis in cKO animals with less MDSCs. In addition, mRNA expression of cytokines involved in MDSCs responses in CD11b+ cells showed that IL-6, iNOS and IFN-γ expression was higher and the anti-inflammatory Arg-1 was lower in CD11b+ cells isolated from cKO mice, or *in vitro* differentiated cells not exposed to EDA-FN. In summary, EDA-containing fibronectin originating from osteoblasts acts via $\alpha5\beta1$ to enhance differentiation of anti-inflammatory myeloid cells.

Abstracts on Other Topics in Osteoimmunology

Inflammatory cytokines alter gene expression of osteocyte signaling molecules by human osteocytes cultured in their native matrix.

Abstract number: SU0238

Janak L Pathak, Astrid D Bakker1, Frank P Luyten, Patrick Verschueren, Willem F Lems, Jenneke Klein-Nulend, Nathalie Bravenboer

Bone remodeling is compromised in rheumatoid arthritis (RA), leading to bone loss, possibly as a result of elevated levels of circulating inflammatory cytokines. This report examined the effect of RA serum or exogenous inflammatory cytokines and chemokines on human osteocyte signaling molecules. Human trabecular bone chips were denuded by collagenase-2 treatment. The resultant bone chips, containing osteocytes embedded in native matrix, were cultured with RA serum or recombinant IL-1β, IL-6, IL-17 or TNFα. Osteocytes in the bone chips expressed sclerostin, FGF23, DMP1 and MEPE, and the cytokines IL-1β, IL-6 and TNFα at day 0 and 7. Active RA serum, individual exogenous recombinant cytokines, chemokines and a combination of cytokines modulated gene expression of cytokines, and Wnt inhibitors in human osteocytes cultured. Hence, osteocytes could be a new target for developing therapies to prevent bone loss in inflammatory diseases.

Identification of senescent cells in the bone microenvironment: a key role for osteocytes in skeletal aging.

Abstract number: FR0399

Joshua Farr, David Monroe, Matthew Drake, Daniel Fraser, Tamara Tchkonia, Nathan LeBrasseur, James Kirkland, Sundeep Khosla. Mayo Clinic, USA

Cellular senescence is a fundamental mechanism by which cells cease dividing and undergo distinct phenotypic alterations, characterized by upregulation of p16Ink4a, a robust biomarker

and principle mediator of senescence. Senescent cells accumulate in multiple tissues with aging where they secrete pro-inflammatory factors and matrix remodeling proteins. They have been proposed to promote degenerative pathologies, including osteoporosis. In this study, the senescence marker, senescence-associated β-galactosidase, and pro-inflammatory markers were examined in enriched preparations of B cells, T cells, myeloid cells, osteoprogenitors, osteoblasts and osteocytes in young (6 months) and old (24 months) mice. In both young and old mice, p16Ink4a was not expressed in B cells, T cells, osteoblasts or osteoprogenitors. With aging, however, expression of p16Ink4a and senescence-associated b-galactosidase increased in the osteocyte-enriched fraction. In addition, there were significant increases in the expression of pro-inflammatory markers in the old osteocyte fractions. The authors speculated that, with aging, a subset of osteocytes become senescent and produce a SASP signal that is communicated to neighboring myeloid lineage cells, which, in turn, stimulates the production and secretion of pro-inflammatory cytokines and chemokines, creating a toxic local microenvironment that may contribute to age-related bone loss.

Conflict of Interest

The authors declare no conflict of interest.