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MEETING REPORT

Meeting report from the 5th International Conference on Osteoimmunology 2014

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Meeting report from the 5th International Conference on Osteoimmunology, Kos, Greece, 15–20 June, 2014

Osteoimmunology is a rapidly developing research field that investigates the interactions between bone, hematopoietic and immune cells in health and disease. To promote recent progress and establishment of novel collaborations in the field, the 5th International Conference on Osteoimmunology: Interactions of the Immune and Skeletal Systems was held in the birthplace of Hippocrates, the island of Kos, Greece from 15–20 June 2014 (http://www.aegeanconferences.org/).

Nineteen senior scientists presented state-of-the-art work of their research, and with the total number of participants being around 100 the meeting enabled formal and informal discussions and interactions between senior and young scientists. The various topics of the meeting included the regulation of osteoclastogenesis at the transcriptional, post-transcriptional, epigenetic and signal transduction level, as well as the interactions of osteoblast and osteoclasts with hematopoietic and immune cells in health and disease. Specific sessions at the meeting were as follows:

Myelopoiesis

Frederic Geissmann from the King's College provided evidence about the origin and fate of tissue-resident macrophages. More specifically, it was presented that tissue-resident macrophages are radioresistant and develop during embryogenesis together with their tissue of residence, independently of hematopoietic stem cells. They therefore represent an independent lineage from bone marrow (BM)-derived monocytes, dendritic cells and monocytes/macrophages that are recruited to tissues during inflammation. In contrast to BM-derived inflammatory cells, tissue-resident macrophages may contribute to tissue homeostasis and tissue response to stress.

Antonios Aliprantis from Harvard Medical School shed light on the role of the nuclear factor of activated T cells (NFAT) transcription factor family in the skeletal system. Even though nuclear factor of activated T cells c1 (NFATc1) was previously identified as a master regulator of osteoclast differentiation, his group investigated the role of NFATs in mature osteoclasts or other cell types. More specifically, conditional deletion of Nfatc1 in mature osteoclasts using Ctsk-Cre resulted in increased bone mass and a marked expansion of both enlarged osteoclasts and CD45- osteoblast precursors in the marrow cavity. In contrast, deletion of Nfatc1 in either limp mesenchymal cells or osteoblasts did not result in a similar phenotype, highlighting the importance of Nfatc1 in the osteoclast cell cycle. Notably, it was also demonstrated that deletion of Nfatc1 in chondrocytes with Col2-Cre in Nfatc2-deficient mice led to early-onset, aggressive osteoarthritis (OA) affecting multiple joints. These results indicate that NFATs are key suppressors of OA.

Joseph Lorenzo of the University of Connecticut examined the ability of injected osteoclast precursor (OCP) cells to differentiate into mature osteoclasts in the bones of mice during normal growth, inflammatory arthritis and fracture repair. Using Ctsk-Cre mice that express a TD-tomato fluorescent reporter protein only in OCPs and mature osteoclasts, it was demonstrated that the engraftment of fluorescent OCPs was successful in arthritic joints and resolving fracture callus but not in wild-type mice. These results demonstrate that in mice during normal growth the majority of osteoclasts in bone derive locally, whereas in inflammatory conditions or in a resolving fracture callus the osteoclasts stem from circulating OCP cells.

Osteoclasts and arthritis

Georg Schett from the University of Erlangen-Nuremberg demonstrated an important role of the adaptive immune system, in particular T-cell CD80/86 costimulation molecules, in the physiological regulation of bone resorption and preservation of bone mass. CD80/86 acts as a negative regulator in osteoclastogenesis. His group showed that engagement of CD80/86 by CTLA-4 promoted the expression of indoleamine 2,3-dioxygenase in OCPs supporting apoptosis. Therefore, future drugs fostering or blocking the effects of CTLA-4 in humans such as in melanoma or rheumatoid arthritis patients need further investigation as regards their effect in bone mass.

Joao Pereira from Yale University showed that the Gai protein-coupled receptor EBI2 and its ligand 7a,25-dihydroxycholesterol guide OCPs toward bone surfaces and promote cell fusion. Interestingly, defective EBI2 signaling led to

increased bone mass and protected mice from age- and estrogen deficiency-induced osteoporosis, suggesting EBI2 as a putative novel therapeutic target.

Transcriptional regulation

Takeshi Miyamoto of Keio University presented data about the transcriptional regulation during osteoclastogenesis. His group identified that B-cell lymphoma 6 (Bcl6), a transcription repressor, is a negative regulator of osteoclastogenesis, as its expression is downregulated during RANKL stimulation, whereas lack of Bcl6 resulted in increased osteoclastogenesis and reduction of bone mass. Moreover, B-lymphocyte-induced maturation protein 1 (Blimp1), another transcription repressor, was identified as a repressor of Bcl6 in osteoclasts, as deletion of Blimp1 in osteoclasts elevated Bcl6 expression, inhibited osteoclastogenesis and resulted in elevated bone mass. Finally, the role of the hypoxia-inducible factor 1 alpha (Hif1a) was addressed in menopausal osteoporosis. Genetic ablation or pharmacological inhibition of Hif1a abrogated ovariectomy-induced bone loss, promoting Hif1a as a target to treat postmenopausal osteoporosis.

Nacksung Kim from the Chonnam National University reported results about the role of the LIM homeobox 2 transcription factor (Lhx2) in osteoclast differentiation. It was shown that the levels of Lhx2 were reduced during osteoclastogenesis, whereas its overexpression in OCPs attenuated osteoclast differentiation by inhibiting the induction of NFATc1. Moreover, genetic ablation of Lhx2 resulted in increased osteoclastogenesis and in an osteoporotic bone phenotype, suggesting a suppressive role for Lhx2 in osteoclast formation.

Hiroshi Takayanagi from the University of Tokyo reviewed the interactions between the various T-cell subtypes and osteoclasts in autoimmune arthritis and focused on the molecules controlling the cell communication in the BM. More specifically, the osteoprotective effect of semaphorin 3A (Sema3A) was demonstrated upon genetic ablation of Sema3A specifically in osteoblasts/osteocytes and neurons, suggesting a novel mechanism that enables interaction between bone, immune cells and neurons. Finally, novel data were presented challenging the role of osteoblasts in hematopoietic stem cell regulation.

Roberto Pacifici of the Emory University demonstrated new mechanisms involved in bone loss induced by continuous PTH (cPTH) treatment. Hyperparathyroidism in humans and cPTH treatment in mice cause bone loss by regulating RANKL production. Data were provided showing that cPTH polarizes the differentiation of CD4+ cells toward the Th17 subset via tumor necrosis factor (TNF) and that cPTH induces bone loss through interleukin (IL)-17. Furthermore, neutralization of IL-17 completely blocked loss of trabecular and cortical bone and decreased TNF and RANKL production induced by cPTH, representing a novel therapeutic strategy for hyperparathyroidism.

Deborah Novack of the Washington University investigated the mechanisms by which the alternative NF-kB pathway controls pathological osteoclast activation. During osteoclastogenesis, RANKL activates the alternative NF-kB pathway through stabilization of the upstream kinase NIK and nuclear translocation of RelB/p52. Data were presented showing that sustained activation of NIK either genetically or pharmacologically enhanced osteoclastogenesis and induced bone loss. Moreover, a possible role of the alternative NF-kB pathway was investigated in mitochondrial biogenesis during osteoclastogenesis.

Innate immunity and epigenetics

Shizuo Akira from Osaka University presented a novel mechanism that controls immune responses involving a novel gene *Regnase-1*. Regnase-1 is an endoribonuclease involved in destabilization of IL-6 and IL-12 mRNA via the stem loop structure within the 3' UTR of these genes, and its genetic ablation caused a spontaneous autoimmune phenotype. Moreover, it was shown that Regnase-1 protein functions as a brake for cytokine production in resting cells, whereas it is rapidly phosphorylated and degraded in response to activation of the IKK complex after LPS stimulation. Thus, Regnase-1 might be a good drug target for controlling immune responses.

Gioacchino Natoli of the European Institute of Oncology examined the transcriptional and epigenetic mechanisms that regulate macrophage identity and responses. The molecular events occurring during lineage commitment were investigated, such as binding of the master transcription factor (TF) Pu.1 to regulatory elements, changes in chromatin structure, recruitment of other TF upon stimulation and subsequent transcriptional activation of proinflammatory genes.

Lionel Ivashkiv from Weill Cornell Medical College investigated the epigenetic mechanisms by which interferon (IFN)-γ, a negative regulator of osteoclastogenesis and bone erosion, silences the capacity of OCPs to differentiate into osteoclasts in response to RANKL and TNF. His group found that IFN-γ alters the epigenetic landscape of human OCPs by inducing decreased histone acetylation (H-Ac), which was associated with gene repression, including repression of osteoclast-related genes. More specifically, IFN-γ silences RANK expression through cooperative mechanisms of decommissioning of enhancers by diminished H-Ac and repression of the promoter by induction of a suppressive chromatin state. These results reveal an important role for epigenetic chromatin-mediated mechanisms in negative regulation of osteoclastogenesis.

Sakae Tanaka of the University of Tokyo examined the molecular mechanisms by which transforming growth factor beta (TGF-β) cooperates with RANKL in promoting osteoclastogenesis. Data were presented showing a cooperation of Smad2/3, direct mediators of TGF-β signaling, with c-Fos that promotes gene expression of Nfatc1, a key regulator of osteoclastogenesis. These findings suggest that TGF-β regulates RANKL-induced osteoclastogenesis through reciprocal cooperation of Smad2/3 with c-Fos.

Ellen Gravallese of the University of Massachusetts presented data about the role of DNA sensors in a novel model of autoimmunity and bone erosion. Lack of the lysosomal endonuclease DNaseII in the DNaseII/IFNaR double-deficient (DKO) mouse resulted in DNA spillover into the cytosol, production of proinflammatory cytokines and inflammatory arthritis. The cytosolic DNA sensor AIM2 results in IL-1β production, whereas other cytosolic DNA sensors signal through the stimulator of interferon genes (STING) resulting in the production of type I interferons. To test the role of DNA sensors in this model, her group generated triple knockout mice (TKO) that address these distinct pathways, including DNaseII/

IFNaR/AIM2 TKO and DNaseII/IFNaR/STING TKO mice. Data were presented showing that the absence of either AIM2 or STING modified the inflammatory phenotype, providing evidence for the role of cytosolic DNA sensors in arthritis.

Up and coming

Charles O'Brien from the University of Arkansas investigated the role of various cellular sources of RANKL in bone remodeling using conditional knockout mice for RANKL. Data were presented demonstrating that osteocytes are an essential source of RANKL for cancellous bone remodeling and bone loss caused by either hindlimb unloading or hyperparathyroidism. It was also shown that deletion of RANKL in B lymphocytes did not affect the skeleton under basal conditions but protected from the loss of the cancellous bone caused by estrogen loss. These results demonstrate that osteocytes are an important source of RANKL during physiological and pathological bone resorption, whereas B cells contribute to pathological bone resorption.

Christina Sobacchi of the CNR-IRGB presented data about the genetic basis of Autosomal Recessive Osteopetrosis (ARO), a rare bone disease characterized by extreme sclerosis of the skeleton caused by failure of osteoclast differentiation or function. Human ARO is genetically heterogeneous involving mutations in various genes such as *TCIRG1*, *CLCN7*, *OSTM1*, *PLEKHM1*, *SNX10*, *RANKL* and *RANK*. Using exome sequencing in osteopetrotic patients lacking a genetic classification, this group identified novel mutations in three genes Cathepsin K (*CTSK*), Carbonic Anhydrase II (*CA2*) and low-density lipoprotein receptor-related protein 5 (*LRP5*), while further investigation is ongoing to verify the causative role of these mutations and unravel the molecular mechanisms leading to ARO. These results confirm the importance of exome sequencing in the molecular classification of genetically heterogeneous diseases.

Mary Nakamura of the University of California presented data about the identification of an OCP population and its regulatory role in rheumatoid arthritis. This group identified a CD11b(-/lo) Ly6C(hi) BM population with OCP potential and T-cell-suppressive activity that is expanded in inflammatory arthritis. Thus, a more complete understanding of the regulation of this OCP population likely has significance for the regulation of bone loss and autoimmunity.

Mark Horowitz from Yale School of Medicine addressed BM adipogenesis, a process that occurs during aging, irradiation or extreme calorie restriction as in anorexia nervosa. BM adipocytes have been implicated as negative regulators of bone mass and hematopoietic microenvironment. To determine the origin of BM adipocytes, his group performed lineage tracing using the fluorescent mT/mG reporter mouse in concert with various cre-recombinase-expressing mouse models. The experimental data demonstrated that BM adipocytes are dynamic and change in response to stress, are derived from a nonhematopoietic adipocyte progenitor cell and are distinct from white or brown adipocytes. Thus, BM contains a distinct fat depot, which needs further characterization.

In addition to the talks from invited speakers, the program contained 14 short (15 min) presentations that were chosen from the submitted abstracts. These included studies of the role of miR-146a in bone loss during inflammatory arthritis, constitutive activation of β-catenin in osteocytes, pathogenic conversion of Foxp3+ T cells into TH17, RANKL and experimental autoimmune encephalomyelitis, signals from commensal microbiota in the expression of proinflammatory cytokine genes, TLR signaling in inflammatory osteolysis, epigenetic regulation by BET, regulation of erythropoiesis, regulation of inflammatory bone loss by MFG-E8, PHD2 and bone homeostasis, regulation of sclerostin levels by glucocorticoids, Wnt5a and osteoblastogenesis, bone regeneration and immune cells and cooperation of PU.1 with NFATc1. Moreover, there were 47 additional posters presented in two poster sessions at the meeting. Our group presented two posters about the analysis of human RANKL transgenic models of osteoporosis and the evaluation of novel small-molecule inhibitors of human RANKL.

Summary

The 5th International Conference on Osteoimmunology fostered fruitful interactions between top scientists and young investigators, allowing exchange of ideas and establishment of novel collaborations that promote and expand the multidisciplinary field of Osteoimmunology. The next meeting has been scheduled for summer 2016 in hospitable Crete. Be prepared!

Conflict of Interest

The author declares no conflict of interest.