Receptor (CTR) and TRACP was performed on both precursors release and CTX-I (C-terminal Telopeptide of Type I Collagen) all in the presence or absence of resorption inhibitors. Bone resorption was assayed by calcium release and TRACP staining. Bone resorption was attenuated by more than 70% in the presence of the resorption inhibitors (P<0.01). Imunnochemistry for CTR and TRACP clearly demonstrated the presence of mature osteoclasts. Interestingly human osteoclasts also resorbed the calcified cartilage, the human trabecular and cortical bones, although differences in the level of resorption were observed, suggesting an induction of distinct osteoclast phenotypes that is dependent on the extracellular matrix at which they are activated.

Conclusions: We have demonstrated that CD14+ monocytes isolated from human peripheral blood are a highly useful source of osteoclasts, which are characterized by expression of the CTR, TRACP and bone resorption, all which are hallmarks of osteoclast function. We used this high quality osteoclast preparation to demonstrate that osteoclasts resorb calcified cartilage, and since this process is involved in the pathogenesis of OA this system can be used for characterizing osteoclast related effects on joint turnover.

157
DEVELOPMENT OF A HUMAN OSTEOCLAST SYSTEM FOR THE ASSESSMENT OF OSTEOCLAST ACTIVITIES AND PHENOTYPES IN OSTEOARTHRITIS

Nordic BioSci. A/S, Herlev, Denmark

Purpose: Studies of animal models of osteoarthritis undergoing anti-resorptive therapy have suggested that inactivation of bone resorption leads to improved articular cartilage status. However, the role of osteoclasts, and subchondral bone turnover, still remains to be elucidated. In alignment, an increasing amount of research is devoted to the cellular phenotype associated with disease status. The osteoclast phenotype associated with osteoarthritis has not been investigated. We developed a pure human osteoclast system, and investigated the cellular phenotype on various bone substrates; cortical, trabecular and calcified cartilage.

Methods: CD14+ monocytes were isolated from human peripheral blood using a ficoll gradient and magnetic sorting. For investigation of osteoclastogenesis the CD14+ cells were seeded on either cortical bovine bone slices or plastic with 25ng/mL RANKL and M-CSF in the presence or absence of 17β-estradiol (0.001-10nM) and cultured for 21 days. For mature human osteoclast experiments CD14+ cells were cultured in flasks for 10 days, and the lifted and reseded onto different matrices and cultured for another 10 days in the presence or absence of well-characterized bone resorption inhibitors (calcitonin (1nM-1μM), ibandronate (1μM) and diphillin (300nM)). Osteoclastogenesis was assessed using the osteoclast marker Tartrate Resistant Acid Phosphatase (TRACP), as well as TRACP staining. Bone resorption was assayed by calcium release and CTX-I (C-terminal Telopeptide of Type I Collagen) all in cell culture supernatants. Immunocytochemistry for the calcitonin receptor (CTR) and TRACP was performed on both primers and mature osteoclasts on bone. Finally, mature osteoclasts were seeded on calcified cartilage from bovine knee joints, human trabecular bone from vertebrae and human cortical bone from femurs in the presence or absence of resorption inhibitors.

Results: Osteoclastogenesis was clearly seen when measuring TRACP, CTX-I and calcium release, with TRACP release initiated on day 5 of culture and CTX-I and calcium release on day 7. All markers were increased more than 500% (P<0.01), compared to initial values. TRACP staining confirmed the presence of large multinucleated TRACP positive cells. For the mature human osteoclasts intense bone resorption was observed within 24 hours after seeding on bovine bone slices, and bone resorption was attenuated by more than 70% in the presence of the resorption inhibitors (P<0.01). Imunnochemistry for CTR and TRACP clearly demonstrated the presence of mature osteoclasts. Interestingly human osteoclasts also resorbed the calcified cartilage, the human trabecular and cortical bones, although differences in the level of resorption were observed, suggesting an induction of distinct osteoclast phenotypes that is dependent on the extracellular matrix at which they are activated.

Conclusions: We have demonstrated that CD14+ monocytes isolated from human peripheral blood are a highly useful source of osteoclasts, which are characterized by expression of the CTR, TRACP and bone resorption, all of which are hallmarks of osteoclast function. We used this high quality osteoclast preparation to demonstrate that osteoclasts resorb calcified cartilage, and since this process is involved in the pathogenesis of OA this system can be used for characterizing osteoclast related effects on joint turnover.

158
DIABETIC MICE HAD A DECREASED EXPRESSION OF DENDRITIC CELL-SPECIFIC TRANSMEMBRANE PROTEIN

T. Kasahara1, S. Im1, H. Koijima2, H. Kimura2, Y. Matsusue1
1Orthopedic Surgery, Shiga Univ. of Med. Sci., Otsu Shiga, Japan; 2Molecular Genetics Med., Shiga Univ. of Med. Sci., Otsu Shiga, Japan

Purpose: We have reported that the impairment of osteoclast fusion had important roles for the delayed fracture healing in streptozotocin (STZ)-induced diabetic mice. In this study, we further investigated the mechanism of the malfunction of diabetic osteoclasts using genetic analysis in vivo and cell culture in vitro.

Methods: The C57BL/6 mice were irradiated (9 Gy) and injected with 4 × 10⁶ bone marrow cells isolated from C57BL/6-EGFP mice. The diabetes was induced by intravenous administration of STZ (150 mg/kg in mice). The control was injected with sodium citrate buffer. At 6 weeks after transfer, bone marrow cells were isolated from both the STZ and control groups, and then cultured in the 96well dish with the cortical bone plate. The cortical bone plates were extraction skull bones of wild type C57BL/6, and those were fixed and dehydrated with 100% ethanol. The cell culture was performed in DMEM supplemented with 10% fetal bovine serum under 5% CO₂ and 95% air at 37 °C, and the floating cell was removed after 2 h incubation. On day 3, 5, and 7, the cortical bone plates were examined to analyze the formation of the resorption pits by a scanning electron microscope.

The standardized closed fracture models were created in both the control and STZ group. At 2 weeks after the fracture, the frozen sections of the fracture site were created. The osteoclasts at callus area were captured by laser capture microdissection (LCM). We extracted RNA and synthesized cDNA by using commercial kit. RT-PCR was performed to evaluate the mRNA expressions of MMP9, Catepsin-K, Receptor activator of NF-kappaB (RANK), dendritic cell-specific transmembrane protein (DC-STAMP), and GAPDH.
Results: The resorption pits made by osteoclasts were well demonstrated on the cortical bone plates. The area of the resorption pits became larger sequentially on day 3, 5, and 7 in osteoclast-cell culture. In the STZ group, the resorption-pit area was significantly decreased compared with that in control group. The expression levels of MMP9, Cathepsin-K, and RANK showed no difference in between control and STZ group. On the other hand, expression levels of DC-STAMP were significantly decreased in STZ group.

Conclusions: In the last meeting, we reported that the osteoclast fusion was impaired, the cartilage resorption was decreased, and the endocortical ossification was delayed in the STZ-induced diabetic mice. In this study, we found that the impaired bone resorption in STZ might be produced by a decreased expression of DC-STAMP in osteoclasts. Since DC-STAMP has a central role for the formation of multinuclear osteoclasts, our result indicates that the up-regulation of DC-STAMP may be useful to prevent osteopenia in diabetes mellitus.

159
COMPRESSION INDUCES THE EXPRESSION OF A SCLEROTIC PHENOTYPE IN HUMAN SUBCHONDRAL OSTEOBLASTS

C. Sanchez1, O. Gabay2, L. Pesesse1, P. Msika3, C. Baudouin3, Y.E. Henrotin1

1Univ. of Liège, Liège, Belgium; 2NIH, Bethesda, MD; 3Laboratoires ExpanSci., Epernon, France

Purpose: Recent data showed that subchondral bone plays an important role in osteoarthritis (OA). Metabolic and morphologic modifications in this tissue contribute to the degradation of the overlying cartilage. It was suggested that abnormal mechanical pressure applied to the articulation was responsible to these changes. Here, we evaluated the effects of compression on osteoblasts from subchondral bone.

Methods: Osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) areas of human OA subchondral bone. After 28 days, osteoblasts were surrounded by a newly synthesized matrix and formed a strong membrane. This osteoblasts-containing membrane was then placed onto a Biopress Flexercell plate and submitted to compression (1.67 MPa) for 4 hours at the frequency of 1 Hz. The expression of IL-6, IL-8, COX-2, VEGF, IGF-1, OPG and RANKL was evaluated by RT-PCR. IL-6, IL-8 and PGE2 were quantified by ELISA.

Results: Basal IL-6, VEGF, COX-2, IGF-1 and RANKL mRNA levels were significantly increased in SC osteoblasts as compared to NSC. By contrast, SC osteoblasts expressed less OPG than those from NSC areas. Compressions induced the expression of genes coding for IL-6, IL-8, COX-2, IGF-1, VEGF and RANKL but decreased the expression of OPG in NSC osteoblasts (p<0.01). IL-6, IL-8 and PGE2 productions were also stimulated by compressions. Interestingly, compressed NSC osteoblasts expressed similar levels of these genes than SC osteoblasts, suggesting that mechanical strains could be responsible for SC phenotype.

Conclusions: These results indicate that in response to compression NSC osteoblasts expressed a phenotype similar to that of SC osteoblasts. Moreover, SC osteoblasts are less sensitive to mechanical stimuli than NSC osteoblasts. These results clarify the role of compression in the pathogenesis of subchondral bone sclerosis and allow new perspectives of research in this field.

160
INVOLVEMENT OF CCL20 CHEMOKINE IN BONE TISSUE REMODELING: EVIDENCE IN SUBCHONDRAL BONE OF OSTEARTHRITIS AND RHEUMATOID ARTHRITIS PATIENTS

G. Lisignoli, C. Manferdini, A. Piacentini, E. Gabusi, F. Grassi, L. Cattini, A. Facchini

Istituti Ortopedici Rizzoli, Bologna, Italy

Purpose: Bone tissues are remodeled through cycling of bone resorption and new bone formation that are dependent by the activity of osteoblasts and osteoclasts. Subchondral bone remodelling in osteoarthritis (OA) and rheumatoid arthritis (RA) is mainly characterized by the formation of osteophytes/fibrosis and by the presence of infiltrating cells associated to bone resorption. In this study we analysed on subchondral bone of OA and RA patients both the expression of CCL20 chemokine and its receptor CCR6 on osteoblasts, osteoclasts, osteocytes and infiltrating mononuclear cells. Then we analysed its effects on osteoblasts and osteoclasts.

Methods: CCL20 and CCR6 expression was evaluated in subchondral bone tissue biopsies by immunohistochemical techniques and the percentage of positive cells was manually counted. Functional assays (cell proliferation, matrix proteins expression, apoptotic markers, signalling factor and osteoclast differentiation) on osteoblasts and osteoclasts were performed to assess the functional role of CCL20.

Results: CCL20 was positive on osteoblasts (60%) and osteocytes (20%) from RA patients, while it was expressed only in OA osteoblasts located in area of new bone formation. Both in OA and RA biopsies, osteoblasts were highly positive to CCL20 while mononuclear cells were 10% positive in OA and 60% in RA. The percentage of osteoblasts positive to CCR6 was not different in OA versus RA biopsies, while the percentage of osteocytes and mononuclear cells positive to CCR6 was significantly higher in RA compared to OA. CCL20 did not affect both the expression of different matrix proteins (i.e. bone sialoprotein, collagen type I, osteopontin and osteocalcin) and apoptotic markers (like caspases 3 and 8) in OA and RA osteoblasts. CCL20-stimulated OA osteoblasts showed a significant increase in β-N-acetylhexosaminidase release compared to RA while osteoblasts proliferation was higher only in CCL20-stimulated RA osteoblasts associated to Akt phosphorylation. IL1β and TNFα differently modulated CCL20, RANKL/OPG expression in OA and RA osteoblasts. Moreover, we found that CCL20 was an early inducer (at day3) of cell fusion events, osteoclasts differentiation and MMP-9 release.

Conclusions: This study demonstrates a different expression of CCL20 positive osteoblasts in OA versus RA disease that seems to be associated with the presence of infiltrating mononuclear cells. Moreover, the contemporary action of CCL20 on osteoblasts and osteoclasts that resulted in a greater proliferative response in RA osteoblasts compared to OA and an increased enzymatic activity in OA osteoblasts, clearly suggests a differential role of this chemokine in OA and RA.

161
NUCLEAR RECEPTOR RETINOID-RELATED ORPHAN RECEPTOR ALPHA1 MODULATES OSTEOBLAST METABOLISM WITH ANTI-INFLAMMATORY EFFECTS

M. Benderdour, J.C. Fernandes, P. Lavigne, F. Beaudet, Q. Shi

Univ. of Montreal, Montreal, QC, Canada

Purpose: To elucidate the expression and function of nuclear receptor retinoid-related orphan receptor alpha1 (RORalpha1) in human MG-63 osteoblast-like cells.