in secondary spongious zone and diaphyseal cortical bone and slightly in primary spongious area, and iii) bone microarchitecture showed higher values of BV/TV, Tb.th, Tb.N and lower value of Tb.Sp in treated rats. Finally, we demonstrated that the overproduction of RANKL and IL-17 in sera of arthritic animals was prevented by pioglitazone treatment.

Conclusions: In the present study, we demonstrated that pioglitazone had bone protective properties in arthritis-induced inflammation. The concomitant decrease of circulating IL-17 and RANKL levels strongly suggests that pioglitazone anti-resorptive effect is likely supported by the modulation of the IL-17-RANKL osteoclastogenesis pathway.

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 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 reseeded 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 diphyllyn (300nM)). Osteoclastogenesis was assayed 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 precursors 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). Immunocytochemistry 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.

158

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

T. Kasahara1, S. Imai1, H. Kojima2, 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 150 mg·kg−1 STZ (150 mg·kg−1 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.