414 ALLOGENIC CHONDROPROGENITORS DISPLAY IMMUNOSUPPRESSIVE PROPERTIES AND ARE NON-IMMUNOGENIC IN VITRO


Purpose: To investigate the interactions between allogeneic articular chondroprogenitors and immune cells in vitro by comparing them to allogeneic MSC.

Methods: Primary articular chondroprogenitors were isolated from the knees of Dark Agouti rats. These cells were used in co-culture systems with allogeneic lymphocytes to determine the immune response elicited by and immunosuppressive ability of the chondroprogenitors. Specific inhibitors of signalling molecules were used to determine the mechanism of chondroprogenitor immunosuppression. Flow cytometric assays were used to assay the allogeneic T cell response to chondroprogenitors. Chondroprogenitor ability to alter the polarisation of allogeneic macrophages was also investigated by flow cytometry, qPCR and zymography.

Results: We found that allogeneic chondroprogenitors elicit allogeneic T cell proliferation similar to Mesenchymal Stem Cells (MSC), indicating that these cells are weakly immunogenic. The chondroprogenitors were not targeted any more than MSC by allo-specific T cell proliferation similar to Mesenchymal Stem Cells (MSC), indicating that these cells are weakly immunogenic. The chondroprogenitors were not targeted any more than MSC by allo-specific T cells and these cytotoxic T cells expressed low levels of the cytotoxic molecule Granzyme-B. The immunogenicity of the chondroprogenitors was not increased by exposure to an intra vitro inflammatory environment. Interestingly we found that allogeneic chondroprogenitors were capable of suppressing allogeneic T cell proliferation. This inhibition was not dependent on cell contact, as is the case with MSC. We found that chondroprogenitors suppress T cell proliferation by IL1β induced nitric oxide production. Chondroprogenitors, like MSC, also produce significant amount of the immunosuppressive molecule PGE2.

Conclusions: These data indicate that allogeneic articular chondroprogenitors are weakly immunogenic, are capable of suppressing allogeneic T cells and altering the polarisation of allogeneic macrophages. This raises the potential for use of allogeneic chondroprogenitors to treat cartilaginous defects in vivo. Such a treatment would greatly reduce the costs of the procedure and indeed, due to the fact that allogeneic chondroprogenitors produce immunosuppressive molecules in an inflammatory environment, may be preferable to autologous treatments as the allogeneic cells may ameliorate the inflammatory component of diseases such as OA, while also repairing the damaged tissue.

Joint Tissue Anabolism & Catabolism

415 EX VIVO TISSUE CULTURES OF BONE, CARTILAGE AND SYNOVIMUM WITH CORRESPONDING BIOMARKERS: A TOOL BOX FOR DRUG DISCOVERY


Purpose: The joint consists of 3 major tissue compartments; articular cartilage, synovium and subchondral bone. Each consists of a specific extracellular matrix (ECM), which is remodelled as part of the pathogenesis of osteoarthritis (OA). The tissues are believed to act as disease drivers and thus may describe three different phenotypes in OA; cartilage-, bone- and synovial-driven OA. Development of potential drugs for treatment is highly dependent on model systems of the different compartments that can accurately describe the activity and mode of action of the candidate drug. We have previously shown that cartilage explant cultures as well as primary osteoclast cultures are viable tools that in combination with biomarkers of ECM turnover can be used to profile potential disease modulating molecules. The aim of the study was to develop and characterize ex vivo culture models of cartilage, bone and synovial turnover with specific biomarkers for each tissue that would allow for testing of pathways and hypotheses. This model includes the development of a novel synovial matrix ex vivo model.

Methods: Synovial membrane explants (SME); Synovial membrane from 4 OA patients undergoing total knee replacement at Gentofte Hospital, Denmark, were cultured as SMEs (30±2mg) for 3 weeks with media alone (w/o), 10 ng/mL TNFα, IL-1β or TGFl-β, or metabolic inactivated. Supernatant was collected 3 times a week and stored at -20°C. In-house neo-epitope biomarkers; C1M, C3M, and active MMP-3 were assessed by ELISA. MMP-2 and -9 were detected by gelatin zymography. Bovine cartilage explants (BEX); Cartilage explants were harvested from the femoral condyle of a cow and cultured as full depth cartilage (FDC) explants for 3 weeks with media alone (w/o) or 20ng/mL TNFα plus 10ng/mL OSM (O-+T). Biomarkers of degradation (AGNx1, C2M) and formation (P2NP) were assessed in the supernatant by ELISA. Human cartilage explants (HEX); Human articular cartilage from 4 OA patients (see SME), were cultured as FDC explants for 3 weeks with media alone (w/o) or O-+T and biomarkers measured as described for BEX.

Human osteoarthritis (hOA): Mature human osteoarthritis were derived from peripheral blood monocytes and differentiated into mature osteoclasts in culture medium containing 25 ng/mL M-CSF and 25 ng/mL RANKL and seeded on bovine bone slices. Bone resorption was assessed by CTX-I and modulated by inhibitors such the cysteine proteinase inhibitor E64. Differences between groups were assessed by ANOVA.

Results: SME: C1M and C3M were increased at day 7 in response to TNFα compared to w/o (10-fold for C1M p<0.05 and 100-fold for C3M: p<0.0001). IL-1β showed similar pattern. TGFl-β did not affect C1M or C3M. Activated MMP-9 and activated MMP-3 (p<0.0001) was increased in SMEs treated with IL-1β or TNFα throughout the study, while activation of MMP-2 was not affected. BEX: The level of AGNx1 and C2M in BEX cultures increased significantly over 3 weeks treatment period with TNFα-alpha (300-fold and 6-fold, respectively, p<0.001) compared to w/o. Furthermore, activated ADAMTS-4, MMP-9 and MMP-13 were increased at the later stages of pro-inflammatory stimuli. All signals could be inhibited by specific inhibitors of ADAMTS and MMP.

HEX: AGNx1 was significantly increased at day 7 (p<0.0001) and 14 (p<0.01) in response to catabolic activation by O-+T, while a trend towards an increase was seen in C2M. No effects were observed on the level II collagen formation marker P2NP.

HOC; The levels of the bone resorption marker CTX-I was significantly increased (<100%) (P<0.001) in response to RANKL as previously reported, and inhibited by bisphosphonates and cathepsin K inhibitors as well acidification inhibitors.

Conclusions: We have characterized 4 primary tissue models with corresponding biomarkers of cartilage, bone and synovium. The toolbox may provide a fast and early signal for the mode of action of novel drug candidates.

416 THE ROLE OF INFLAMMATORY MEDiators IN MEnISCUS AND CARTilAGE CROSSTALK IN OsteoarthRitis

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