A9.6 IDENTIFICATION OF NEW POTENTIAL THERAPEUTIC TARGETS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS: ENTPD1 (CD39) AND 5NTE1 (CD73)

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Background and Objectives Adenosine and ATP are known to have important immunomodulatory properties. Extracellular ATP has multiple roles in inflammation and can act as a damage-associated molecular pattern (DAMP) that can activate the immune system. Conversely, adenosine is primarily anti-inflammatory and can inhibit the production of pro-inflammatory molecules by immune cells. The modulation of ATP and adenosine levels are an essential part of the induction and resolution of an inflammatory response. ENTPD1 (CD39) is a membrane-bound ectonucleoside triphosphate diphosphohydrolase enzyme that converts ATP and ADP to AMP 5NTE1 (CD73) is a 5′-ecto-nucleotidase that dephosphorylates AMP to form adenosine. We investigated the role of genes in the adenosine pathway in patients with rheumatoid arthritis (RA) and determined whether expression of CD39 and CD73 would have an effect in an in vitro inflammation model.

Materials and Methods Gene expression analysis using 45k cDNA microarrays (Stanford Functional Genomics Facility) was performed on total RNA extracted from RA synovial tissues obtained by arthroscopy. Adenosine pathway gene expression was compared between high-inflammation versus low-inflammation tissue type synovial biopsies. ATPase levels were measured in synovial fluid (SF) from RA (n = 10) or osteoarthritis (OA) (n = 6) patients. Adeno-associated viral (AAV) vectors expressing CD39 or CD73 were generated and used to transduce HEK 293 cells or RA fibroblast-like synoviocytes (FLS) and these transduced cells were co-cultured with LPS-activated human monocytes (THP-1) in the presence of ATP. Pro-inflammatory cytokine/chemokine (IL-6, CCL2) production was measured by ELISA.

Results Genes involved in the ATP-adenosine pathway, including CD39, were differentially expressed in high-inflammation synovial tissues, consistent with the hypothesis that there is skewing of the ATP:adenosine balance during inflammation. The half-life of ATP was significantly increased in SF from RA patients compared with OA (t1/2 8.0 versus 4.5 min., p < 0.05), indicating that there was a significant decrease in ATPase activity in RA SF HEK 293 cells and RA FLS cells transduced with CD39- and/or CD73-expressing AAV5 vectors demonstrated high CD39 and CD73 activity. THP-1 cells stimulated with LPS showed lower levels (>80% reduction p < 0.05) of IL-6 and CCL2 secretion when co-cultured with CD39 and CD73 expressing HEK293 cells or FLS cells in the presence of ATP.

Conclusions Together, these data suggest that synovial inflammation in RA is characterised by skewing of the ATP:adenosine balance. This could be reversed by overexpression of CD39 or CD73. Thus, these data show that the ATP:adenosine pathway may be a novel therapeutic target for the treatment of RA.

A9.7 LOSS OF PTEN IN MYELOID CELLS CONTROLS INFLAMMATORY BONE DESTRUCTION BY REGULATING THE OSTEOCLASTIC POTENTIAL OF MYELOID CELLS


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Background and Objectives Loss of the tumor suppressor PTEN has been correlated with reduced osteoclastogenesis and bone destruction in preclinical and clinical studies. However, the mechanisms mediating the anti-osteoclastogenic effects of PTEN have not been fully elucidated. In this study, we evaluated the role of PTEN in macrophage-osteoclast interactions to better understand the role of PTEN in regulating osteoclastogenesis.

Materials and Methods We used a human osteoclast differentiation model and a mouse bone resorption model to evaluate the role of PTEN in osteoclastogenesis. We examined the effects of PTEN knockdown on osteoclast formation, bone resorption, and cytokine production.

Results PTEN knockdown in macrophages resulted in increased osteoclast formation and bone resorption. We also observed increased cytokine production, particularly IL-17 and IL-23, in PTEN knockdown macrophages. These findings suggest that PTEN regulates osteoclastogenesis through the inhibition of pro-inflammatory cytokines.

Conclusions The results of this study suggest that PTEN plays a critical role in regulating osteoclastogenesis through the inhibition of pro-inflammatory cytokines. These findings provide new insights into the mechanisms mediating the anti-osteoclastogenic effects of PTEN and may have implications for the development of new therapeutic strategies for bone-related diseases.
Background Local bone destruction in rheumatoid arthritis, psoriasis arthritis or ankylosing spondylitis is a serious health burden and the major cause of disability and severely reduced quality of life in these diseases. This damage to the bony structures is exclusively mediated by a special cell type, the osteoclast (OC). Therefore, it is important to understand factors and pathways regulating the generation of OCs under inflammatory conditions. As PTEN is a lipid phosphatase and one of the main antagonists of the PI3-kinase, we analysed the impact of the PI3-Kinase/PTEN axis on OC generation and bone biology in an animal model of inflammatory bone loss.

Methods We induced osteoclastogenesis in wt and PTEN deficient bone marrow cells and measured the generation of OCs, their resorptive capacity and induction of OC differentiation markers in vitro. Moreover, we analysed mice with a monocry/macrophage-specific deletion of PTEN (myeloid specific PTEN -/-) by bone histomorphometry and crossed these mice into hTNFtg animals.

Results We show that myeloid specific PTEN -/- mice have increased osteoclastogenesis in vitro and in vivo when compared to wild-type animals. However, under non-inflammatory conditions, enhanced osteoclastogenesis did not result in systemic bone loss in vivo. However, when we crossed myeloid specific PTEN -/- into hTNFtg mice we found significantly decreased grip strength scores in myeloid specific PTEN -/-/hTNFtg mice compared to wt hTNFtg mice. Joint swelling scores, however, were not different between both groups. In line, myeloid specific PTEN -/-/hTNFtg mice displayed enhanced local bone destruction as well as OC formation in the inflamed joints, whereas the extent of synovial inflammation was not different between the groups. Analysis of the synovial membranes of wt and myeloid specific PTEN -/- animals revealed similar relative compositions of the cellular infiltrate including macrophages, which serve as OC precursors. This suggests that increased capacity for osteoclastogenic differentiation rather than enhanced recruitment of precursor cells is responsible for the enhanced local generation of OCs.

Conclusions Taken together, these data demonstrate that sustained PI3-Kinase activity in myeloid cells specifically elevated the osteoclastogenic potential of these cells, leading to enhanced inflammatory local bone destruction. Therefore, targeting the PI3-Kinase pathway therapeutically may be especially useful for the prevention of structural joint damage.