OP0151

MIRNAS CONTRIBUTE TO DYSREGULATED ROS METABOLISM OF IMMUNE CELLS IN THE INFLAMED JOINT

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Background: In the last years miRNAs have emerged as critical regulators of innate and adaptive immune responses and an altered expression or function is associated with several inflammatory and autoimmune diseases. Therefore miRNAs are also believed to promote inflammatory processes within the inflamed joint of juvenile idiopathic arthritis (JIA) patients. It is furthermore known that oxidative stress is associated with JIA. Free radicals are implicated in joint damage and play an important role as secondary messengers in immunological responses. How and if miRNAs contribute to dysregulated reactive oxygen species (ROS) metabolism in JIA remains to be elucidated.
Objectives: We aimed to identify miRNAs and miRNA regulated pathways, which contribute to dysregulated immune cell responses within the inflamed joint.
Methods: miRNA profiling was performed on peripheral blood mononuclear cells (PBMCs) from healthy children, PBMCS from 9 JIA patients and synovial fluid mononuclear cells (SFMCs) from the same JIA patients. Subsequently, GO and pathway enrichment analyses were performed on predicted target genes. Upregulation of miRNAs was confirmed *in vitro* after incubation with synovial fluid by qRT-PCR. Mitochondrial integrity, cellular ROS and Nrf2 protein expression were measured by flow cytometry.
Results: Transcriptome analysis of JIA SFMCs compared to HC PBMCs revealed strongly enhanced expression of *miR23a* and *miR23a*, *miR27a*, *miR146a*, and *miR155*, which are involved in oxidative stress responses. In addition, expression of those could be induced in healthy control PBMCs by synovial fluid *ex vivo*. ROS level in synovial fluid T cells were enhanced, while expression of Nrf2, the main regulator of anti-oxidative responses and a target of *miR27a*, remained low. Furthermore mitochondrial cyclophilin, which regulates ROS escape from mitochondria and is suppressed by *miR23a*, was downregulated in SFMCs as well.
Conclusion: SFMCs within the inflamed joint reveal a distinct miRNA expression profile. Especially miRNAs that are involved in regulation of ROS metabolism are upregulated. In line with this, expression of Nrf2 and mitochondrial cyclophilin which are important regulators of cellular ROS metabolism are reduced while production of ROS is enhanced. We suggest that higher abundance of miRNAs, that are involved in oxidative stress pathways, contribute to redox dysregulations within the inflamed joint and thereby contribute to inflammatory processes.
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OP0152

OLIGOARTICULAR JUVENILE IDIOPATHIC ARTHRITIS DOES NOT SHOW SIGNS OF T-CELL EXHAUSTION, IN SPITE OF INCREASED EXPRESSION OF CO-INHIBITORY RECEPTORS

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Background: Oligoarticular Juvenile idiopathic arthritis (O-JIA) is a common inflammatory joint disease in children, driven by continuous local T-cell activation. [1] T cell activation is counter-balanced via signals generated by co-inhibitory receptors (co-IRs) such CTLA-4, PD-1, LAG-3, and TIM-3.[2]
Objectives: Here we identify the role of co-IRs in the pathogenesis of O-JIA.
Methods: Paired synovial fluid (SF) and plasma, PBMCs and SFMCs, were obtained from O-JIA patients together with clinical data (n=14). Plasma from healthy controls (HC, n=14); paired SF and plasma from 5 non-arthritis juvenile orthopedic patients (OC, n=5) served as controls. Soluble levels of co-IRs were measured by ELISA and their cellular expression by flow cytometry. Spontaneously differentiated fibroblast like synoviocytes (FLS) from SFMCs were co-cultured with autologous PBMCs/SFMCs and used as an *ex-vivo* disease model. Functional effects of co-IRs were evaluated via blocking them with checkpoint inhibitors in these *ex-vivo* disease models.
Results: In O-JIA patients, increased levels of sPD-1, sLAG-3 and sTIM-3, but not sCTLA-4, were present in the SF compared with plasma. (Figure 1A) There was a close correlation between sPD-1 levels in the plasma and SF (r=0.65, p=0.029). In plasma, sTIM-3 levels correlated with sPD-1 levels (r=0.84, p=0.001) and sLAG-3 levels (r=0.68, p=0.021). In the SF, there was a significant correlation between sLAG-3 levels and sPD-1 levels (r=0.54, p=0.047). None of the soluble co-IR levels correlated with disease activity scores. Plasma and SF levels of sLAG-3 and sTIM-3 were higher in O-JIA patients when compared with HC and OC. (Figure 1A) On the CD3+CD4+CD45RO+ T cells, the surface expression of PD-1 (p=0.02), LAG-3 (p=0.001), TIM-3 (p<0.001), but not CTLA-4 (p=0.48), were higher in the SFMC compared with PBMC.
MHC class II expression was induced on FLS when these were co-cultured with autologous PBMCs and SFMCs, together with an increased Monocyte Chemoattractant Protein-1 (MCP-1) production. (Figure 1B) Only addition of neutralizing anti-LAG3 antibodies significantly increased the MCP-1 production in PBMC monocultures (p<0.01) and FLS+PBMC co-cultures (p<0.01). PBMCs and SFMCs produced significantly higher levels of IFN-γ after CD3/CD28 activation both in monocultures and co-cultures (p<0.001), but they were not affected from addition of any of the checkpoint inhibitor.

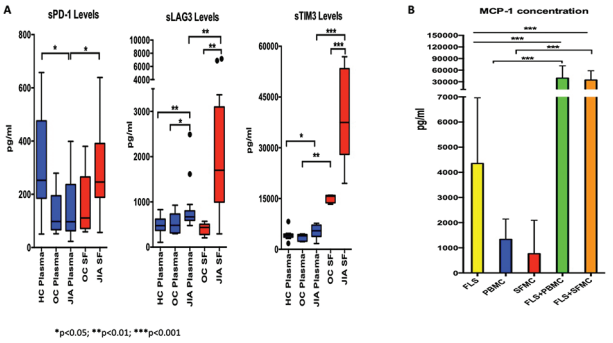

Figure 1

Conclusion: This is the first report studying the effects of different co-IRs in O-JIA. Both the soluble levels and the surface expressions of the co-IRs were higher at the site of inflammation in O-JIA. SFMCs and PBMCs of O-JIA patients are not exhausted, based on their ability to respond to CD3/CD28 activation. This is opposite to what has been shown in adult inflammatory arthritis.[3] Co-cultures of autologous FLSs and PBMCs/SFMCs may serve as an *ex-vivo* arthritis model to perform functional analysis. LAG-3 stands out among the co-IRs and might play a role in O-JIA pathogenesis and a potential therapeutic option for O-JIA.

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OP0153

BROADENING OUR UNDERSTANDING OF THE GENETICS OF JUVENILE IDIOPATHIC ARTHRITIS: INTERROGATION OF THREE DIMENSIONAL CHROMATIN STRUCTURES WITHIN JIA-ASSOCIATED RISK LOCI

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Background: Our group has shown that, like most complex traits, the risk loci for juvenile idiopathic arthritis (JIA) identified on genome-wide association studies (GWAS) and genetic fine mapping studies are highly enriched for enhancers. Enhancers are regulatory elements that fine-tune gene expression to specific