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SCREENING FOR ANTIBODY REACTIVITY IN EARLY AXIAL SPONDYLOARTHRITIS IDENTIFIES NOVEL ANTIGENIC TARGETS

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Aim. Diagnosis of axial spondyloarthritis (axSpA) is challenging since clinical manifestations often overlap with other disorders and an appropriate serological test is still lacking. Although autoantibodies are not considered to be a hallmark of axSpA, emerging evidence suggests the involvement of plasma cells and antibodies in the disease. Therefore, we aim to identify novel (auto)antibodies specific for early axSpA patients.
Materials and Methods. An axSpA cDNA phage display library was constructed and screened for antibody reactivity with pooled plasma of early axSpA patients (*n=10*) with validation in additional pooled plasma of early axSpA patients (*n=60*) and healthy controls (HC, *n=30*) and in individual plasma samples of early axSpA patients (*n=79*), patients with non-specific chronic low back pain (NSCLBP, *n=40*), rheumatoid arthritis (RA) patients (*n=60*) and HC (*n=94*) using phage-ELISA.
Results. Antibody reactivity against 9 novel peptide targets was increased in pooled axSpA plasma. Further validation revealed antibody reactivity against at least one of these targets in 53% of early axSpA patients (42/79) compared to 35% of NSCLBP (14/40, p=0.0803), 38% of RA (23/60, p=0.0894) and 26% of HC (24/94, p=0.0003). By combining our 3 best targets, 20% of axSpA patients (16/79) were detected with a specificity of respectively 90.4% (9/94, p=0.0531), 95% (2/40, p=0.0312) and 92% (5/60, p=0.0590) for HC, NSCLBP and RA.
Discussion. Screening our axSpA cDNA phage display library identified antibody responses against 9 novel peptide targets, each one contributing to the detection of a portion of axSpA patients. Antibody reactivity against these targets will be further validated in an independent cohort of early axSpA patients.
Conclusion. The increased antibody reactivity against several novel antigenic targets in early axSpA patients compared to NSCLBP, RA and HC further supports the involvement of the humoral immune response in axSpA.

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A PROBABLE ROLE OF HSP60 IN THE PATHOGENESIS OF SPONDYLOARTHRITIS

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Introduction. The cause of SpA is unknown, although there is an important relationship with gut enterobacteria and the HLA-B27 molecule. This later can misfold during its synthesis causing an altered proteostasis with inflammatory consequences. We identified that patients with ankylosing spondylitis (AS) have serum antibodies against a 60kDa molecule of *Klebsiella pneumoniae*. This molecule was cloned and characterized as the heat shock protein 60 of *K. pneumoniae* (HSP60Kp). Later, we found high titers of plasma and synovial fluid antibodies against the HSP60Kp and the HSP60 of other enterobacteria accompanied by an important lymphoproliferative response against HSP60Kp. Human and enterobacterial HSP60 have a high homology and, given the key role of molecular chaperones in endoplasmic reticulum (ER) stress, we aimed to analyze the relationship of HSP60 with the consequences of protein misfolding.
Materials and Methods. We analyzed the expression and location of HSP60 in THP-1 monocytes suffering tunicamycin-induced endoplasmic reticulum stress. Also, we determined the expression of the ER-stress markers BiP and CHOP.
Results. We found a tunicamycin dose-dependent increase of the expression of HSP60, this expression of HSP60 was associated with the increase of the expression of CHOP, suggesting a role of the PERK/ATF4 pathway. Furthermore, the activation of the ER-stressed monocytes with LPS induced an important induction of HSP60. Interestingly, we found extracellular detection of HSP60 accumulates in the cell cultures of ER-stress suffering cells.
Discussion. Heat shock proteins are potent immunomodulatory molecules, so, these results could help us to understand how the HLA-B27-induced alterations in proteostasis can relate with the inflammatory response.
Acknowledgements.
RLJP receives scholarships from CONACYT and BEIFI-IPN; DLML, JZL and GLE receive grants from EDI and COFAA-IPN and from SNI-CONACYT.
This project was funded by a grant from CONACYT number 000000000240054.

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AN ENTHESEAL INNATE IMMUNE CELL BIOLOGICAL BASIS FOR DIFFERENTIAL EFFICACY OF PDE4 AND IL-23 PATHWAY BLOCKADE BETWEEN PSORIATIC DISEASE AND RHEUMATOID ARTHRITIS

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Introduction. Both IL-23 and PDE4 inhibition are ineffective in RA but show efficacy in PsA related synovitis despite similar cytokine and molecular profiles between synovitis in both disease settings. We hypothesised that enthesis resident innate immune cells, especially myeloid cells, might be capable of IL-23 production that could be modulated by PDE4 pathway blockade.
Materials and Methods. Human entheses (n=6) were digested and myeloid cells (CD14+) sorted from both the adjacent bone (EB) and soft tissue (ST) fractions. Both CD14+ sorted and CD14- unsorted cells were stimulated with bacterial and fungal adjuvants (TLR and CLR agonists) in the presence and absence of a PDE4 inhibitor and analysed by ELISA and flow cytometry for production of disease relevant mediators (IL-23, TNFα, and CCL20). Corresponding peripheral blood populations were also stimulated with and without a PDE4 inhibitor and other cAMP elevating agents to confirm the role of cAMP in regulating IL-23 associated inflammation.
Results. A CD45+/CD14+ myeloid cell population could be isolated from the normal enthesis in both the ST and EB fractions but with a much higher abundance in EB. This purified population from both ST and EB produced IL-23, TNF-α and CCL20 following TLR/CLR receptor stimulation. IL-23 and TNF-α production was negligible in the CD14- fraction. Moreover, IL-23 and TNF induction was inhibited by the PDE4 inhibitor rolipram. In blood derived myeloid cells, rolipram and other cAMP elevating agents (histamine and 8-br-cAMP), also inhibited IL-23 secretion**.**
Conclusion. These findings demonstrate that the human enthesis harbours an IL-23 producing myeloid cell population which can be modulated by PDE4 pathway manipulation. These findings support the idea of the IL-23/17 pathway genetic architecture of SpA in the context of entheseal biology and offer a "reverse translation" explanation for divergent therapeutic pathways between SpA and RA.

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REGULATION OF CYTOKINE PRODUCTION BY iNKT CELLS REQUIRES IRE1a

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Introduction. iNKT cells represent a prototypic example of innate like T cells which produces large amount of immunomodulatory cytokines. As such they are important in regulation of several forms of inflammatory diseases including SpA and inflammatory bowel diseases. It is currently unclear what determines the unique features of these cells in producing such large quantities of immunoregulatory cytokines. We reasoned that ER stress sensors may be involved such as the inositol-requiring enzyme 1a (IRE-1α), a transmembrane protein which gets activated upon ER stress. Activated IRE1α results in the splicing of X box binding protein 1 (Xbp1) mRNA, which in turn transactivates genes involved in cellular homeostasis. Recent studies have shown that IRE1a also regulates the development and function of B cells, dendritic cells, and eosinophils. We therefore aimed to investigate whether iNKTs require IRE1α to regulate cytokine production following activation both *in vitro* and *in vivo*.
Methods. To assess steady-state IRE1a activity in iNKT cells by flow cytometry, we utilized ERA1$^{FP/WT}$ reporter mice as they express *Venus*^{FP} fused at the sites at which IRE1α splices XBP1. QPCR was performed in *in vitro* expanded iNKT cells to examine the role of TCR-dependent signalling in regulating IRE1α activity within iNKT cells and effector cytokine production. ELISA, flow cytometry and QPCR were performed in CD4^{cre}; IRE1α^{ko} mice were used to study the role of IRE1a in regulating the cytokine production by activated iNKT cells. Intravital