OP0175 CHARACTERIZATION OF NOVEL STROMAL-DERIVED AUTOANTIGENS RECOGNIZED BY RA SYNOVIAL MONOCLONAL ANTIBODIES

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Background: We previously showed that up to 40% of RA synovial recombinant monoclonal antibodies (RA-rmAbs) generated from germinal center-like structure (GC-LS+) RA synovium recognize citrullinated antigens contained in neutrophils extracellular traps (NET) (1). The cellular source of other potential autoantigens targeted by the majority of locally differentiated B cells remains undefined. Recently, RA-fibroblast-like synoviocytes (RA-FLS) have been implicated in the release of citrullinated antigens (2, 3). However, whether these cells are targeted by RA-rmAbs is still unknown.
Objectives: Here, we aimed to define the RA-rmAbs immunoreactivity towards i) RA-FLS and ii) identify potential stromal-derived autoantigens.
Methods: 67 RA-rmAbs were generated from single CD19+ B cells FACS-sorted from fresh synovial cell suspensions following IgV_H+V_L genes cloning (1). RA-rmAbs were tested by means of i) cell-based immunofluorescence assays with FLS of RA patients and controls (osteoarthritis (OA)-FLS and RA-dermal fibroblast (RA-DF)), ii) co-localization with stromal specific markers and iii) immunoenzymatic tests with co-localizing antigens. Control rmAbs were also used (Sjögren's syndrome/healthy donor-IgG rmAbs).
Results: Immunofluorescence on RA-FLS demonstrated reactivity of 21% of RA-rmAbs (14/67 rmAbs) towards cytoplasmic components of FLS. Only 4 rmAbs out of 14 were binding both FLS and NET components. For some rmAbs this reactivity was not specific to RA-FLS since it was also observed for OA-FLS and RA-DF. Interestingly, strong co-localization was observed with calreticulin (CRT) which in its citrullinated (cit-CRT) form has been previously shown to recognize the RA "shared epitope" HLA domain sequence (3). When tested in ELISA for native vs cit-CRT, 57% (8/14 rmAbs) of the FLS-reactive RA clones showed binding to CRT with 4 out of 8 rmAbs displaying increased immunoreactivity towards cit-CRT. Controls rmAbs showed no reactivity to either FLS or CRT. Preliminary data suggest that RA patient serum preferentially recognize the lectin-like N-terminal domain of CRT (4).
Conclusions: Here, we provide novel evidence that a subset of locally differentiated B cells within RA synovial GC-LS can react towards RA-FLS derived antigens. Preliminary data suggest that part of this reactivity is directed towards CRT. Identification of immunodominant epitopes within CRT is under investigations.
References:
[1] Corsiero et al, ARD 2015.
[2] Sorice et al, Rheumatology 2016.
[3] Ling et al, AR 2013.
[4] Hong et al, JI 2010.
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OP0176 THE PARACASPASE MALT1 PLAYS A CENTRAL ROLE IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

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Background: One of the hallmarks of many inflammatory arthritides is their strong linkage with MHC-signalling, which is mirrored by the marked role for adaptive immunity. Accordingly, rheumatoid arthritis (RA) is characterized by the activation of auto-reactive T-cells and the development of auto-antibodies. T-cells may additionally respond to non-TCR mediated signals, which are essential in driving their effector functions. Pathways leading to the modulation of both innate and adaptive signals are therefore of marked interest to study in arthritic diseases.
Objectives: The paracaspase MALT1 is a key player in the activation and proliferation of immune and non-immune cells. These cells include the lymphoid, myeloid and mast cells, indicating MALT1's crucial role in both innate and adaptive signaling (1). Therefore, MALT1 is regarded a promising target for the treatment of autoimmune diseases and defining its role in the pathogenesis of inflammatory arthritis is a critical first step.
Methods: To unravel MALT1's role in inflammatory arthritis, we initially assessed MALT1-activation in mice that were challenged with collagen-induced arthritis (CIA), the prototype model for antigen-induced RA. We then addressed the role of MALT1 in the pathogenesis of inflammatory arthritis by challenging MALT1-deficient mice to distinct models of arthritis (CIA and CAIA) or by backcrossing MALT1-deficient mice to TNF^{DARE} mice, representing an SpA-like model. Additionally, CIA was induced in CD4-specific MALT1-deficient mice to determine the importance of MALT1 in T-cells.
Results: We provide evidence that MALT1 plays a crucial role in the pathogenesis of RA as MALT1-deficent mice were completely protected against CIA. This complete protection was additionally observed in CD4-specific MALT1-deficient mice, indicating that the selective ablation of MALT1 in CD4-positive cells is sufficient for the observed resistance against CIA. CAIA on the other hand, which is a T- and B-cell independent model of RA, did not depend on the presence of MALT1, since both $MALT1^{+/+}$ and $MALT1^{-/-}$ mice showed comparable symptoms of RA. Interestingly, TNF^{DARE} mice that were deficient for MALT1 also showed a reduced enthesitis and ileitis phenotype, although TNF-concentration in the serum of these mice was higher compared to $MALT1^{+/+}xTNF^{DARE}$ mice.
Conclusions: Overall, our data highlight that MALT1 plays a crucial role in the pathogenesis of inflammatory arthritis and represents an interesting candidate to target therapeutically.
References:
[1] Thome M. Multifunctional roles for MALT1 in T-cell activation. Nat Rev Immunol 2008; 8 (7): 495–500.
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OP0177 N-GLYCOSYLATION SITES IN THE VARIABLE DOMAIN OF B CELL RECEPTORS SPECIFIC FOR CITRULLINATED ANTIGENS

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Background: Recent structural analysis of anti citrullinated protein antibodies (ACPA) in serum and synovial fluid of patients with rheumatoid arthritis (RA) revealed that the vast majority (>90%) of secreted ACPA IgG molecules carry N-glycans in the variable (Fab) domain. This remarkable degree of Fab-glycosylation is absent from ACPA-depleted control IgG and from autoantibodies in other diseases. So far, it is unclear why ACPA carry this feature and which biological effects these glycans mediate in the context of RA. Of note, however, N-glycosylation requires a specific amino acid consensus sequence in the protein backbone, which is very rare in germline-encoded variable region genes.
Objectives: To study the B cell receptor (BCR) repertoire of ACPA-expressing B cells to determine the frequency, origin and localisation of N-glycosylation sites in ACPA Fab domains.
Methods: Citrullinated antigen-specific and non citrulline-reactive control B cells were identified in peripheral blood of ACPA-positive RA patients by antigen-specific tetramer staining and isolated by fluorescence activated cell sorting. Cells were either sorted in pools and directly lysed, or sorted as single cells and cultured for two weeks followed by the detection of ACPA-positive culture wells by ELISA. Full-length immunoglobulin (Ig) rearrangements were identified by anchoring reverse transcription of Ig sequences, amplification by nested PCR and either next generation sequencing (NGS, PacBio platform) or, for single cell transcripts, Sanger sequencing (scSeq). Sequence reads were analysed using IMGT V-QUEST tools.
Results: The mean number of nucleotide mutations in heavy chains (HC) of IgG BCRs derived from ACPA-expressing B cells was high (33 in NGS, 48 in scSeq samples; similarity to germline: 88% in NGS, 84% in scSeq). NGS identified 12 unique IgG clones derived from 4 donors, of which 10 (83%) had at least one N-glycosylation site in the HC or light chain (LC). scSeq identified 86 unique IgG clones derived from 6 donors, of which 68 (79%) had N-glycosylation sites. For 57/86 IgG clones, we could determine the combination of HC and LC sequences. In these, only 7 (12%) clones had no sites, while 19 (33%) had one, 23 (41%) had two, 5 (9%) had three and 3 (4%) clones had four sites. 57 sites were found in the HC and 34 in the LC. All N-glycosylation sites were created by somatic mutations and not encoded in the germline sequence. Several sites were located in antigen-engaging regions. No correlation was found between the number of N-glycosylation sites and the number of somatic mutations.
Conclusions: We demonstrate that B cell surface-expressed ACPA-IgG molecules carry a remarkably high frequency of N-glycosylation sites in the Fab domain, all of which are generated by somatic mutation. This could indicate that ACPA-expressing B cells acquire a selective survival advantage by introducing N-glycosylation consensus sequences in the Fab domain, a process that is likely to occur under the influence of T cell help and that could facilitate the break of tolerance to citrullinated antigens.
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OP0178 FIBROBLAST PRIMING IS COMMON TO MANY SITES, AND PSORIATIC SKIN FIBROBLASTS MAY ACQUIRE INFLAMMATORY MEMORY

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Background: Rheumatoid arthritis (RA) is a common chronic inflammatory

disease of the joints. Whilst the autoimmune element continues to be studied intensely, it is evident that the innate inflammatory response propagates the disease. Fibroblast-like synoviocytes (FLS) are the most abundant stromal cell in the synovium. FLS from RA joints are markedly different from their healthy counterparts in their inflammatory characteristics.

RA FLS exhibit a form of inflammatory memory named priming. Prolonged exposure to TNFα induces an augmented chemokine response to challenges with interferons [1]. Fibroblast priming leads to significantly augmented IL6, CCL5 and CXCL10 responses to a second challenge by TNFα, with a coincident prolonged NFκB nuclear localization (manuscript in press).

Objectives: We wished to assess whether fibroblasts from sites other than the synovial joint would also exhibit this primed response to second challenge.

Methods: Fibroblasts from the joint, skin, lung, tonsil, bone marrow and gum were stimulated with 10ng/ml TNFα for 24h, before the conditioned medium was removed and the cells were washed free of stimulus. Cells were rested for 24h before once again being washed and stimulated with 10ng/ml TNFα for a further 24h. The conditioned medium was removed and secreted mediators compared between first and second response to stimulus. Transcription and intracellular signalling at time points within each challenge were also determined.

Results: Synovial, lung and tonsil fibroblasts augmented IL6 secretion in response to the second TNFα challenge. RA bone marrow-derived fibroblasts varied in their exhibition of priming. Skin fibroblasts from patients undergoing cosmetic surgery and a gingival sample from non-chronic inflammation displayed no evidence of IL6 priming.

Fibroblasts from the skin of psoriasis (Ps) patients mounted a primed response that was significantly augmented compared to their first response to TNFα, and significantly higher than the second response of healthy skin. This augmented response matches that of FLS, as IL6 but not IL8 was increased. This pattern was also matched by gum fibroblasts from periodontitis.

Mechanistically the Ps skin fibroblasts match FLS, in that NFκB nuclear localization is prolonged in the second challenge compared to the first.

Conclusions: We have shown that inflammatory memory in the form of IL6 priming occurs in fibroblasts from a variety of anatomical sites with diverse functions. Its prevalence implies a shared phenomenon, but the variation between sites suggests a specific role required in some tissues and not others.

The finding that Ps skin fibroblasts acquire this memory response may point towards a pro inflammatory mechanism that contributes to psoriatic diseases, including psoriatic arthritis. Our data may help to explain why an estimated 30% of psoriasis patients go on to develop psoriatic arthritis [2].

References:

[1] Sohn, C., et al., Prolonged TNFαprimes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. Arthritis Rheumatol, 2014.
[2] Mease, P.J., et al., Prevalence of rheumatologist-diagnosed psoriatic arthritis in patients with psoriasis in European/North American dermatology clinics. J Am Acad Dermatol, 2013. 69(5): p. 729–35.

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OP0179 BRAFV600E PROMOTES MYELOID SKEWING IN MULTISYSTEMIC LANGERHANS CELL HISTIOCYTOSIS HUMANIZED MOUSE MODEL

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Background: Multisystemic Langerhans cell histiocytosis (mLCH) is an aggressive disease characterized by the accumulation of mononuclear phagocytes with immunohistochemical features of dendritic cell (histiocytes)[1]. Histiocytes infiltrate mostly the skin, bone marrow (BM), lung and spleen, and produce high levels of proinflammatory cyto/chemokines, leading to organ dysfunction[2,3]. In patients, around 10% of cells in lesions carries an oncogenic mutation in the MAPK pathway, mostly $BRAF^{V600E}$ (70% of cases). $BRAF^{V600E}$ can be detected also in monocytes and BM progenitors (HSPC) from these patients, whereas only a fraction of them carries a mutation in B cells and none in T cells[4].

Objectives: To study the role of $BRAF^{V600E}$ in the pathogenesis of mLCH, we set up a humanized mouse model of mLCH based on the transplantation into immunodeficient mice (NSG) of human HSPC expressing $BRAF^{V600E}$.

Methods: We isolated HSPC from human cord blood and transduced them at two different levels (50% and 20%) with lentiviral vectors that ubiquitously express $BRAF^{V600E}$, $BRAF^{WT}$ or GFP.

Results: All $BRAF^{V600E}$ mice manifested severe weight loss within 7 weeks with a median of 3 and 5 weeks for 50% and 20% transduction groups, respectively. Mice showed dysplastic bone marrow (BM) with infiltration of histiocytes; lesions were present also in lungs, kidneys, CNS, spleen and liver. Immunophenotype of infiltrating histiocytes closely resembles mLCH, staining positive for CD14, CD68, S-100 and langerin. None of the control mice lost weight nor displayed organ alteration. Flow cytometry analyses of BM showed 5- to 7-fold reduction in engraftment of human cells in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). Percentage of myeloid cells increased by 3- to 4-fold in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). On the contrary, percentage of B cells was reduced by 3- to 6,5-fold in $BRAF^{V600E}$ vs GFP groups ($p<0,001$). Moreover, there was no difference in the percentage of GFP-positive cells between myeloid cells, whole BM and *in vitro* sample, suggesting a non-cell autonomous mechanism underlying this myeloid phenotype.

Conclusions: In summary, we generated for the first time a human xenogeneic transplantation mouse model of mLCH, showing that $BRAF^{V600E}$ in human HSPC promotes myeloid skewing rather than proliferation.

References:

[1] Wilejto et al. Curr Opin Rheumatol 2012. DOI: 10.1097/BOR. 0b013e32834db53e.
[2] Donadieu et al. Histiocytic Disorders of Children and Adults 2005.
[3] Kannourakis et al. Br J Cancer 1994, Sep;23:S37–40.
[4] Berres et al. J Exp Med 2014, DOI: 10.1084/jem.20130977.

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Joint health & joint damage: a tale of three tissues –

OP0180 PHARMACOLOGICAL CHARACTERIZATION OF THE ADAMTS-5 INHIBITOR GLPG1972: AN ORAL ANTI-CATABOLIC AGENT FOR THE TREATMENT OF OSTEOARTHRITIS

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Background: Degradation of articular cartilage and alterations of the underlying subchondral bone are hallmarks of osteoarthritis (OA)[1]. A disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5) is a key aggrecan-cleaving enzyme involved in this pathogenic process from the earliest stages of cartilage degradation[2] and as such, is an attractive drug target for the development of a disease-modifying OA drug (DMOAD)[3].

Objectives: In this report we describe the *in vitro* and *in vivo* characterization of the small molecule GLPG1972, an inhibitor of ADAMTS-5. GLPG1972 anti-catabolic activity was evaluated in murine and human cartilage explants and DMOAD activity was investigated in the destabilization of the medial meniscus (DMM) mouse model[4].

Methods: The ADAMTS-5 biochemical assay is based on the cleavage of a fluorescent substrate by recombinant ADAMTS-5. Mouse femoral head cartilage explants were stimulated by interleukin-1a (IL-1a) for 3 days and GAG release quantified[2b]. Human articular cartilage explants were stimulated with IL-1β for 12 or 19 days and the NITEGE epitope quantified using the AGNx1 assay. Unilateral OA was induced in C57BL6 mice by DMM[4]. Mice were treated with vehicle or GLPG1972 at 30, 60 or 120 mg/kg, b.i.d. for 8 weeks. Medial femorotibial joint sections were scored by an evaluator blinded to treatment.

Results: GLPG1972 showed potent inhibition of human ADAMTS-5 (IC_{50}=20 nM). Inhibition of ADAMTS-4 was moderate (IC_{50}=57 nM), and selectivity over 100-fold was observed against a large panel of zinc metalloproteinases. GLPG1972 displayed potent anti-catabolic activity in cartilage explants, with IC_{50} values being 2 μM and <1 μM in mouse and human, respectively. In the DMM mouse model, GLPG1972 demonstrated DMOAD activity, as shown by significant reduction of femorotibial cartilage proteoglycan loss and cartilage damage score, as well as significant impact on subchondral bone sclerosis.

Conclusions: GLPG1972 is an orally bioavailable, potent and selective ADAMTS-5 inhibitor showing significant anti-catabolic activity in cartilage explants. In the DMM model, treatment with GLPG1972 resulted in significant protective effects on both cartilage and subchondral bone pathology. Taken together these results provide support to progress GLPG1972 into the clinic as an oral treatment for OA.

References:

[1] Hunter, D.J., et al. Curr. Opin. Rheumatol. 2009, 21,110–117.
[2] a) Glasson, S.S., et al Nature. 2005, Vol 434: 644–8; b) Stanton, H., et al. Nature. 2005, Vol 434: 648–5; c) Little, C.B., et al. Journal of Clinical Investigation. 2007, Vol 117(6):1627–36.
[3] Larkin, J. et al. Osteoarthritis Cartilage. 2015, 23 (8): 1254–1266.
[4] Little, C.B. and Hunter, D.J. Nat. Rev. Rheumatol. 2013, 9(8):485–497.

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OP0181 DELETION OF THE PROSTAGLANDIN D2 RECEPTOR DP1 EXACERBATES AGING-ASSOCIATED AND INSTABILITY-INDUCED OSTEOARTHRITIS

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Background: *The D prostanoid receptor 1* (D*P1*), a *receptor* for prostaglandin D_2 (PGD_2) plays important roles in inflammation and cartilage metabolism. However, its role in the pathogenesis of osteoarthritis (OA) remains unknown.