SDS-PAGE. Antigen binding was investigated by CCP3 ELISA. VH-VL structure models were generated using the online tool PIGS and visualised by Jmol, and the GlyProt server was utilised for in silico glycosylation.

**Results** The majority of ACPA exhibited variable region N-linked motifs (83.8%), compared to 14.3% of non-ACPRA RA mAbs and 63.2% of bnAbs, featured in both framework and CDRs. When adjusted for SHM, N-linked motifs were significantly increased in ACPA compared to non-ACPRA RA mAbs (p=0.001) or bnAbs (p=0.002). VH region motif rates were increased in ACPA compared to non-ACPRA mAbs (p=0.002) and bnAbs (p=0.0004), while VL region motifs were only higher compared to non-ACPAs (p=0.002). Deglycosylation revealed that N-linked motifs were indeed glycosylated, although preliminary data suggests glycan removal had no striking effect on antigen-binding. Homology-based structures predicted glycans to be primarily positioned outside of the potential antigen-binding site.

**Conclusions** The results support that variable region glycosylation is a key feature of ACPA. Significant increases in N-linked motifs in ACPA compared to other highly-mutated antibodies signifies that this is not solely linked to hypermutation. Future studies are merited to further investigate the selection mechanisms and functional role of Fab-glycosylated autoantibodies.

**References**

1. Brink M, Hansson M, Mathsson L, et al. Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. *Arthritis Rheumatology*. 2017;69:1850–59.

2. Panza F, Pratesi F, Valori D, et al. Immunoglobulin G subclass profile of anti-citrullinated peptide antibodies specific for Epstein Barr virus-derived and histone-derived citrullinated peptides. *J Rheumatol* 2014;41:407–8.

3. Johansson L, Pratesi F, Brink M, et al. Antibodies directed against endogenous and exogenous citrullinated antigens pre-date the onset of rheumatoid arthritis. *Arthritis Res Ther* 2016;18(1):127.

**08.21 DOMINANT B-CELL RECEPTOR CLONES IN PERIPHERAL BLOOD PREDICT ONSET OF ARTHRITIS IN INDIVIDUALS AT RISK FOR RHEUMATOID ARTHRITIS**

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Background Anti-citrullinated protein/peptide antibodies (ACPA) represent an important tool for the diagnosis of RA and the presence of multiple ACPA specificities is highly correlated with the evolution towards RA.

However, a limited amount of information is available on the predictive value of single specificities on disease manifestations and response to therapy in established RA.

The aim of the present work is to evaluate the diagnostic and prognostic value of the combined use of four well established citrullinated peptides as antigens for ACPA detection: VC1P and VC2P (derived from EBV proteins) and HC1P and HC2P (derived from histone H4).

**Materials and methods**

Four hundred and forty-eight RA patients, followed in the Clinical Immunology Unit, University of Pisa, and in the Rheumatology Unit, University of Perugia, were recruited.

RA patients were evaluated for systemic involvement, disease activity and severity, ongoing and past therapies.

Anti-VC1P, -VC2P, -HC1P, -HC2P were measured by home-made ELISA.

Data were analysed by cluster analysis and principal component analysis.

**Results**

Antibodies to VC1P were detected in 52% of RA patients; anti-VC2P in 59.5%; anti-HC1P in 57%; and anti-HC2P in 57.5%.

Cluster analysis and principal component analysis identify two subpopulations, that differ for ACPA levels, RF positivity, lung involvement and higher use of biological therapy.

Patients were also subdivided in 5 groups according to the number of anti-peptide antibodies (negative for any, positive for one, for two, for three, for four). Mean antibody level and RF positivity progressively increased from the first to the last group, as well as the frequency of lung involvement.

**Conclusions** ACPA are a family of antibodies with overlapping specificities. The results obtained with citrullinated peptides from EBV proteins and histone H4 suggest that fingerprinting of ACPA may be informative in established RA. A higher number of ACPA subtypes is predictive of lung involvement. Moreover, a broader ACPA repertoire also emerged as a feature of patients treated with biological therapy, thus probably affected by a more severe form of the disease.

In conclusion, ACPA typing may be relevant for a better characterisation of some disease features in established RA.

**References**

1. Brink M, Hansson M, Mathsson L, et al. Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. *Arthritis Rheumatology*. 2013;65(4):899–910.

2. Panza F, Pratesi F, Valori D, et al. Immunoglobulin G subclass profile of anti-citrullinated peptide antibodies specific for Epstein Barr virus-derived and histone-derived citrullinated peptides. *J Rheumatol* 2014;41(2):407–8.

3. Johansson L, Pratesi F, Brink M, et al. Antibodies directed against endogenous and exogenous citrullinated antigens pre-date the onset of rheumatoid arthritis. *Arthritis Res Ther* 2016;18(1):127.
significantly associated with arthritis development (validation cohort relative risk (RR) 6.3, 95% confidence interval (CI) 2.7–15, p<1*10^(-4)). Even when adjusted for the recently described clinical prediction rule the association remained intact (relative risk 5.0, 95% CI 1.2–20, p=0.024). When individuals developed arthritis, dominant BCR clones disappeared from peripheral blood and appeared in synovial tissue, suggesting a direct role of these clones in disease pathogenesis.

Conclusion Dominant BCR clones in peripheral blood predict onset of clinical symptoms of RA in at-risk individuals with high accuracy. Our data suggest that during onset of RA these clones shift from peripheral blood to target tissue.

Background TNF plays a key role in immune-mediated inflammatory diseases including rheumatoid arthritis (RA) and spondyloarthritides (SpA). Here we aimed to investigate how TNF can lead to completely different disease phenotypes such as destructive peripheral polyarthritis in RA versus axial and peripheral remodelling arthritis in SpA.

Materials and methods We assessed expression of TNF, TNF-R, and TACE (ADAM17) in synovial fluid, synovial tissue, and synovial fibroblasts from SpA and RA. tmTNFtg mice (TgA86) were clinically scored for development of peripheral and axial disease, and sacrificed at the end of the experiments for radiologic and histologic assessment. Mechanistic studies included bone marrow chimera experiments and crossing with TNF-R1 or TNF-R2 knock-out animals.

Results Arthritis was characterised by lower levels of sTNF and higher levels of tmTNF in SpA versus RA. This misbalance was related to decreased TACE activity in SpA versus RA FLS, as further confirmed by lower levels of other soluble molecules cleaved by TACE (including sTNF-R1, sTNF-RII, and scD163) in the inflamed SpA joint. Assessing whether tmTNF has a functional role in SpA pathology, mice selectively over-expressing the transmembrane form of TNF spontaneously developed a deforming arthritis and spondylitis, starting at 4 weeks of age and reaching a 100% incidence. Histology revealed peripheral and axial synovitis, enthesitis, and osteitis, as well as inflammation of the connective tissue located at the junction of the annulus fibrosus with the vertebral bone. tmTNFtg mice did not develop extra-articular inflammation. Structural phenotyping by histology and radiology revealed mild destructive features in combination with focal of hypertrophic chondrocytes and axial and peripheral new bone formation leading to bridging of tail vertebra over time. Mechanistic experiments revealed that this SpA-like phenotype was mediated by tmTNF expression on stromal cells but not on hematopoietic cells and required the expression of TNF-R1.

Conclusions Collectively, these data suggest that tmTNF expressed by stromal cells is responsible for the key pathological features of SpA.

REFERENCES
1. Alexopoulou L, et al. Eur J Immunol 1997;27(10):2588–92.

08.23 SYNDECAN-4 EXERTS A PROTECTIVE FUNCTION IN EXPERIMENTAL INTESTINAL INFLAMMATION

Background The ubiquitously expressed transmembrane heparan sulfate proteoglycan Syndecan-4 (Sdc4) is crucial in inflammatory diseases, like rheumatoid arthritis. Depending on the tissue, it can either protect or promote an inflammatory process. By its binding of molecules, such as cytokines and growth factors, it can initiate signalling pathways and it has been implicated in cell-matrix adhesion, cell migration, differentiation as well as proliferation. However, the involvement of Sdc4 in intestinal inflammation is unknown so far. Our group revealed a protective function of Sdc4 in experimental intestinal inflammation.

Material and methods We monitored the course of DSS-induced colitis in Sdc4(-/-) and C57Bl/6 WT mice and analysed the changes in body weight, colon length, histology and inflammatory cellular infiltrate. We also evaluated Sdc4 protein- and mRNA-level by immunofluorescence staining (IF) and quantitative real-time PCR. Colon-permeability was examined in vivo by using the Evans Blue method and measuring the clearance for Citrobacter rodentium in vivo. Wound healing effects of Sdc4 were analysed in vitro by scratch assay analysis with human epithelial colon cell line (T-84) and in vivo by mechanically induced wounds in colonoscopies of Sdc4(-/-) compared to WT mice.

Results The expression of Sdc4 is decreased upon the course of colitis and increased during remission. The course of colitis was markedly aggravated in Sdc4(-/-) mice, reflected by dramatically loss of body weight, increased mortality rates and histological damage, emphasised by increased invasion of macrophages and granulocytes into the colon. Also colonic epithelial permeability of DSS-treated Sdc4(-/-) mice was enhanced associated with an altered expression of tight junction proteins. Furthermore, Sdc4 deficiency resulted in a prolonged intestinal wound healing in vivo and in vitro due to reduced proliferation rates in vitro.

Conclusions Our data indicates that Sdc4 is crucial in experimental intestinal inflammation. It exerts protective effects by maintaining epithelial barrier integrity and regeneration. Further studies are needed to explore the mechanisms of Sdc4-signalling in colitis.