by significantly increased IL-4 and GM-CSF cytokine production compared to arthralgia subject (P<0.001 and P=0.01) and RA-patient (P<0.001 and P=0.004) synovial tissue. However, not all polyfunctional T-cells are equal in their pathogenic potential. Therefore, in order to identify highly pathogenic synovial T-cells, cluster analysis of flow cytometric data using the unsupervised algorithm Flow-Som was performed and led to the identification of specific T-cell clusters with unique polyfunctionality characteristics. Specifically a cluster of CD4/CD8 double positive (DP) T-cells with high polyfunctionality scores was identified. Hybrid flow cytometry and imaging technique confirmed the co-expression of CD4 and CD8 by a synovial T-cell population. DP-T-cells are enriched in RA-synovial fluid and synovial tissue and arthralgia subject synovial tissue, but are absent from HC synovial tissue. Importantly, DP T-cell synovial accumulation strongly (P=0.0002) correlates with DAS28(CRP) of RA-patients. Initial studies utilising the novel, non-invasive FLIM technique for visualisation of cellular NAD, revealed a novel, non-invasive FLIM technique for visualisation of cellular NAD, revealed that DP T-cells have a metabolic profile indicative of activated memory T-cells.

Conclusion: These data highlight a key early loss of balance between putative and pathogenic synovial T-cell polyfunctionality and the emergence of specific, highly polyfunctional and pathogenic T-cell clusters in RA.

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THE RELATIONSHIP BETWEEN DIFFERENT IGG AND IGA ANTI-MODIFIED PROTEIN AUTOANTIBODIES IN RHEUMATOID ARTHRITIS

C. Grönnwall1, L. Liljefors1, H. Bang1, A. Hensvold1, M. Hansson1, L. Mathsson-Arelli1, E. Svenungsson1, J. Gunnarsson1, G. Serre3, S. Saevardsdott1, A. Kastbom1, L. Alfredsson1, J. Rönnem1, A. Catrina1, K. Lundberg1, L. Klareskog2, Karolinska Institutet, Department of Medicine, Division of Rheumatology, Stockholm, Sweden; OGENTEC Diagnostika GmbH, Mainz, Germany; Rheuma Fischer Scientific, Uppsala, Sweden; Linköping University, Department of Biomedical and Clinical Sciences, Linköping, Sweden; INSERM - Université de Toulouse, Unité d’Interface Union Nationale de Recherche, Toulouse, France; Karolinska Institutet, Department of Medicine Solna, Division of Clinical Epidemiology, Stockholm, Sweden; Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden; Uppsala University, Department of Immunology, Genetics and Pathology, Uppsala, Sweden

Background: Seropositive rheumatoid arthritis (RA) is characterized by the presence of rheumatoid factor (RF) and anti-citrullinated protein autoantibodies (ACPA) with different fine-specificities. Yet, other serum anti-modified protein autoantibodies (AMPA), e.g. anti-carbamylated (Carb), anti-acetylated (KAc), and anti-malondialdehyde acetaldehyde (MAA) modify protein antibodies, have been described. By using RA patient single-cell derived monoclonal antibodies we have previously shown that individual ACPA clones recognize small distinct citrulline-containing epitopes giving them extensive multireactivity when these epitopes are found in many peptides and proteins. Moreover, certain CCP2+ multireactive ACPA clones bind also to carbamylated and acetylated autoantigens [1].

Objectives: To provide a comprehensive evaluation of serum IgG and IgA autoantibodies to different post-translational modifications [2].

Methods: We analyzed 30 different IgG and IgA ACPA reactivities to modified antigens by ELISA and autoantigen arrays, in N=1985 newly diagnosed RA patients and population controls. The study utilized both previously established (i.e. IgG and IgA CCP2; IgG ACPA fine-specificities; IgG anti-Carb fibrinogen and Carb FCS; IgG and IgA Cit/Carb/KAc/Omn(Ac)-vimentin), and novel assays (e.g. anti-MAA and IgG anti-acetylated histones). Association with patient characteristics such as smoking and disease activity were explored. The newly developed assays were also evaluated in SLE disease controls and CCP2+ RA-risk individuals without RA.

Results: Carb and KAc reactivities by different assays were primarily seen in patients also positive for citrulline-reactivity. Modified vimentin (mod-Vim) peptides were used for direct comparison of different ACPA reactivities, revealing that IgA ACPA recognizing mod-Vim was mainly detected in subsets of patients with high IgG anti-Cit-Vim levels and a history of smoking. IgG acetylation reactivity was mainly detected in a subset of patients with Cit and Carb reactivity. Anti-acetylated histone 2B reactivity was RA-specific and associated with high anti-CCP2 IgG levels, multiple ACPA fine-specificities, and smoking. This reactivity was also found to be present in CCP2+ RA-risk individuals without RA. Our data further demonstrate that IgG autoantibody to MAA was increased in RA compared to controls with highest levels in CCP2+ RA, but was not RA-specific, and showed low correlation with other AMPA. Anti-MAA was instead associated with disease activity and was not significantly increased in CCP2+ individuals at risk of RA. Notably, RA patients could be subdivided into four different subsets based on their AMPA IgG and IgA antibodies and disease activity profiles.

Conclusion: We conclude that autoantibodies exhibiting different patterns of ACPA fine-specificities as well as Carb and KAc reactivity are present in RA and may be derived from multireactive B-cell clones. Anti-Carb and anti-KAc could be considered reactivities within the “Cit-umbrella” similar to ACPA fine-specificities, while MAA is distinctly different.

REFERENCES: