

of peptidylarginine into peptidylcitrulline, which is catalyzed by peptidylarginine deiminase (PAD) in a calcium-dependent manner. Several citrullinated antigens have been identified in the inflamed joints of RA patients. These include fibrinogen, alpha-enolase, vimentin and collagen type II. Accumulating evidence suggests a role of citrullinated proteins and ACPA in the pathophysiology of RA. The results of many studies indicate that the ACPA response is highly heterogeneous with diverse patterns of reactivity to distinct citrullinated epitopes. This study aimed to identify clinically meaningful ACPA profiles in RA patients using a microarray containing different citrullinated peptides in combination with surface plasmon resonance imaging (iSPR).

Methods: Several pairs of synthetic peptides (citrullinated and the corresponding non-citrullinated control) derived from known ACPA targets (e.g. fibrinogen, alpha-enolase, vimentin) as well as peptides isolated from synthetic citrullinated peptide libraries were used to generate ACPA target arrays. ACPA in RA patient sera were monitored by iSPR, which allows the simultaneous detection of autoantibody-peptide interactions in real-time. The present study was started using a 24-spot microarray and currently peptides are spotted using a continuous flow microspotter resulting in a 48-spot microarray.

Results: Using the 24-spot microarray, a total of 94 RA and 46 control sera were analyzed. The results confirmed the heterogeneous nature of ACPA in RA sera. RA patients displayed different patterns of co-occurrence of autoantibodies directed to distinct citrullinated peptides. The recognition of one peptide was very specific for RA and was observed in 62% of the anti-CCP2 positive RA patients. No reactivity was observed in the anti-CCP2 negative patients and a weak reactivity (2%) was observed in control patients. Another peptide showed reactivity in 68% of the RA patients, both anti-CCP2 positive (74%) as anti-CCP2 negative (54%) patients, whereas patients with other autoimmune diseases showed far less reactivity (13%). The median number of citrullinated peptides recognized by anti-CCP2 positive RA patients (4) was significantly higher than that of anti-CCP2 negative RA patients (1) and disease controls (1). The use of continuous flow microspotting instead of non-contact spotting is being optimized to increase the array size and to improve the quality and reproducibility of the microarrays.

Conclusion: Using microarray-iSPR we have shown that RA sera recognize various citrullinated peptides more frequently than other autoimmune disease sera. Sera from different RA patients frequently recognize different citrullinated peptides. Our data are consistent with the existence of different ACPA profiles that may have diagnostic and/or prognostic value. Microarray-iSPR represents a suitable system for multiplex autoantibody monitoring and allows the identification ACPA profiles.

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Development of a High-Throughput, Multiplex Assay for Profiling the Autoantibody Fine Specificity in Rheumatoid Arthritis. Xiaoyan Zhao¹, P. Scott Eastman¹, Ferhan Qureshi¹, William C. Manning¹, William Robinson² and Lyndal K. Hesterberg¹. ¹Crescendo Bioscience, Inc., South San Francisco, CA, ²Stanford Univ School of Med, Stanford, CA

Background/Purpose: Rheumatoid Arthritis (RA) is an aggressive autoimmune disease that progressively destroys affected joints with frequent systemic complications. Production of autoantibodies, especially those against citrullinated proteins and peptides, is a hallmark of RA. The causal relationship between the development of autoantibodies and radiographic joint damage to this point remains unclear. To better understand this relationship, we developed autoantibody assays in a multiplex format against a panel of common and novel RA epitopes and applied the assays for profiling the fine specificity of these autoantibodies in RA.

Methods: Individual serum samples from 35 RA patients with different disease activities were evaluated for the presence of autoantibodies. Anti-cyclic citrullinated peptide (CCP) reactivity and rheumatoid factor (RF) status were assessed with commercial kits from Euro Diagnostica (CCP 2) and TheraTest Labs (RF). The reactivity of a panel of peptides derived from multiple proteins including both well known and novel antigens was also evaluated. Nine peptides were printed in a 3x3 grid on the bottom of a 96-well plate (Quansys Bioscience) and probed with RA patient samples. HRP-conjugated secondary antibody against human IgG, IgM or IgA was used to measure autoantibodies to specific peptides in a chemiluminescent format.

Results: The levels of anti-CCP antibodies ranged from >5,000 to below the cutoff (<25 arbitrary units) for CCP and from ~600 to 3 units for RF-IgA, suggesting a range of disease. Not surprisingly, levels of autoantibodies, when detected by anti-human IgG, reflected anti-CCP levels, predominantly to citrul-

linated fibrinogen and citrullinated filaggrin peptides. However, a wide range of anti-IgM/A responses were observed regardless of whether anti-CCP levels were high, intermediate or low. Interestingly, when anti-human IgA or IgM was used as detection, CCP negative and low CCP RA subjects frequently demonstrated strong positive reactivity to epitopes derived from citrullinated apolipoprotein, citrullinated biglycan, native histone and/or native fibromodulin. While several of the IgM/A peptide reactivities overlapped with the IgG, in many instances the IgM and/or the IgA profiles demonstrated unique response patterns.

Conclusion: A high-throughput, multiplex assay has been developed to investigate the fine specificity of autoantibody reactivity against a broad variety of RA antigens including both citrullinated and native peptides. While anti-human IgG profiles reflected CCP levels, diverse levels of response were observed with anti-IgM and/or anti-IgA, including at low and intermediate CCP levels. Most studies to date have employed the CCP 2 assay, which uses a mixture of peptides, with mixed results. We observed that profiles of individual peptides may be different with different immunoglobulin isotypes. Thus evaluation of individual peptides in the context of immunoglobulin subtypes may provide insight into disease progression.

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Anti-hnRNP Autoantibodies Detected in Inflammatory Rheumatic Diseases in Use to Close the Sensitivity gap left by Rheumatoid Factor and Anti CCP in Early Rheumatoid Arthritis. Bianka Marklein¹, Zoltan Konthur², Gerd-Rüdiger Burmester³ and Karl Skriner¹. ¹Charité - Universitätsmedizin Berlin, Berlin, Germany, ²Max Planck Institute for Molecular Genetics, Berlin, Germany, ³Charité - University Medicine Berlin, Berlin, Germany

Background/Purpose: Autoantigens are produced in bacteria ex-purified using His-tag and Cm-sepharose under native and denaturated conditions. Bacterially expressed recombinant hnRNPs proteins were used in Elisa for confirming the data obtained by macroarray and immunoblotting. Anti-hnRNP-A/B and hnRNP D proteins were detected in a newly developed Elisa with patient sera and sera from animal model of SLE and RA

Methods: Autoantigens are produced in bacteria ex-purified using His-tag and Cm-sepharose under native and denaturated conditions. Bacterially expressed recombinant hnRNPs proteins were used in Elisa for confirming the data obtained by macroarray and immunoblotting. Anti-hnRNP-A/B and hnRNP D proteins were detected in a newly developed Elisa with patient sera and sera from animal model of SLE and RA.

Results: Using a combination of three hnRNPs A2/D/DL, 76% of RA, 84% of SLE sera and 87% SKG mice which spontaneously develop chronic autoimmune arthritis, can be detected. Moreover hnRNP A2/A3/D/DL identified epitopes as well as identified citrullinated peptides (deduced citrullinated peptides thereof) were used to identify patterns associated with disease severity. No crossreactivity could be detected between the affinity purified anti-hnRNPDL and the highly related hnRNPD. A unique sequenz only found in hnRNP DL between aminoacid 81-120 was identified as indistinguishable for autoantibody binding. With citrullinated peptides out of a mutated form of hnRNPA3 and hnRNPA2/D/DL, 94 % out of 130 early RA sera can be identified but only in <10% of osteoarthritis and healthy control patients. With a combination of citrullinated forms of three hnRNPs (A2, D, DL) 98 % of the sera were tested positive in a cohort of 92 early RA patients (<12month). The hnRNP autoantibody response is dependent on Mod88, Tir8 and both TLR 7 and TLR9 costimulation tested with sera from TLR7, TLR9 deficient and double-deficient mice with an MRL-lpr/lpr background.

Conclusion: The hnRNP antibody response is Myd88, Toll 7 and 9 dependent generated. A combination of hnRNPs (A3/A2/DL/D) can be used to predict disease severity and partially close the sensitivity gap left by rheumatoid factor and anti CCP antibodies in early RA patients.

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Substantial Influence of Rheumatoid Factor Positivity On the Peripheral Memory B Cells and Its Modulation by TNF Inhibition In Rheumatoid Arthritis. Petra Roll¹, Khalid Muhammad¹, Mathias Schumann¹, Stefan Kleinert² and Hans-Peter Tony¹. ¹University of Würzburg, Würzburg, Germany, ²Rheumatology, University of Würzburg, Würzburg, Germany

Background/Purpose: The role of B cells has been appreciated with the advent of B cell targeted therapies in patients with rheumatoid arthritis. However, alterations of peripheral B cell subsets have been described also under TNF inhibition. In this study, we focused on the influence of