

## Identification and possible citrullination of antigens in Immune Complexes of Patients with Rheumatoid Arthritis

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### Background/purpose

In autoimmune diseases such as rheumatoid arthritis (RA), antibodies are formed against self-antigens, which may lead to the development of immune complexes (ICs). However, little is known about the identity of the antigens in these ICs. Identifying these antigens may result in a better understanding of the onset and perpetuation of the disease.

### Methods

ICs were isolated from serum of RA patients (n=20) and healthy persons (n=6) by a sucrose gradient (20-65%) and ultracentrifugation. The sucrose gradient was fractionated and the different fractions were submitted to a spectrophotometric analysis at 280nm. The fractions with ICs underwent further purification by immunoprecipitation with immobilized protein G and anti-IgM. Analysis of the ICs was carried out by SDS-PAGE and Western Blotting. Possible citrullination of the proteins was investigated by AMC staining, and mass spectrometry was used to identify the proteins.

### Results

After sucrose gradient ultracentrifugation, the serum samples of RA patients with a high value for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) (n=6) showed a distinct white band. Analysis of the fractions containing this white band showed clearly higher OD<sub>280nm</sub> values than the surrounding fractions. On the contrary, this white band and concomitant higher OD<sub>280nm</sub> values were barely detectable in healthy persons (n=6) and in RA patients with low RF and low CCP (n=4).

AMC (anti-modified citrulline) staining after SDS-PAGE and Western Blot shows that citrullination was present in the fractions of the sucrose gradients that corresponded to the white band containing the ICs.

After further purification of the ICs by immunoprecipitation with protein G sepharose beads followed by 2D-gel electrophoresis and mass spectrometry, different proteins of immune complexes such as IgG, IgM, J-chain and complement factors were identified.

### Conclusions

RA patients with a combination of high RF and high CCP show high amounts of ICs distinct as a clear visual white band after sucrose gradient ultracentrifugation and elevated OD<sub>280nm</sub> measurements. The antigens trapped in these ICs constitute of a variety of different proteins and at least some of them are citrullinated. By means of proteomics it would be possible to identify these antigens.