Unravelling the anti-carbamylated protein antibody response in rheumatoid arthritis

Verheul, M.K.

Chapter 6

Anti-Carbamylated Protein antibodies: a specific hallmark for rheumatoid arthritis - comparison to conditions known for enhanced carbamylation; renal failure, smoking and chronic inflammation

M.K. Verheul1, S.J.H. van Erp2, D. van der Woude1, E.W.N. Levarht1, M.J.K Mallat3, H.W. Verspaget2, J. Stolk4, R.E.M. Toes1, A.E. van der Meulen-de Jong2, P.S. Hiemstra4, C. van Kooten3, L.A. Trouw1

1Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands
2Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, Netherlands
3Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands
4Department of Pulmonology, Leiden University Medical Center, Leiden, Netherlands

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Antibodies that target carbamylated proteins (anti-CarP antibodies) have been described as a disease biomarker in rheumatoid arthritis (RA).[1] However, little is known about the factors that predispose to the production of anti-CarP antibodies, although a likely requirement is the presence of carbamylated antigen(s). Carbamylation is a posttranslational modification resulting from the conversion of a lysine amino acid into a homocitrulline that requires the presence of cyanate. There are several conditions in which the concentration of cyanate (and therefore carbamylation) is increased, such as renal failure, chronic inflammation and heavy smoking.[2-4] We therefore addressed the question whether conditions of enhanced carbamylation could also result in the induction of anti-CarP antibodies.

To investigate this we determined the presence of anti-CarP antibodies in serum samples from patients with renal failure,[5] inflammatory bowel disease (IBD)(expanded from van Erp et. al[6]) and in heavy smokers with or without chronic obstructive pulmonary disease[7] (see table 1). The presence of anti-CarP antibodies in healthy controls and RA patients from the early arthritis clinic (EAC) was used as a comparison.[1,8] The collection of these cohorts was approved by the Leiden University Medical Center ethics committee and informed consents were obtained from all patients. Anti-CarP IgG antibodies were determined by ELISA as described before using carbamylated fetal calf serum as antigen.[1] Positivity for the presence of anti-CarP antibodies was defined as signal higher than the 97th percentile of the healthy controls. Each cohort was tested separately for this study and control samples (between 120-187) were randomly selected from a pool of 209 controls and taken along with each measurement. The anti-CCP2 ELISA (anti-cyclic citrullinated peptide) was also carried out as described before.[9]

The percentage of individuals positive for anti-CarP antibodies was 2.3%, 4.1%, 3.7%, 11.9% and 44.9% for controls, smokers (no differences were observed in anti-CarP antibodies between smokers with or without COPD), patients with IBD, renal failure and RA respectively (Figure 1A). Pearson Chi-square test analyses revealed that next to the highest frequency of anti-CarP antibodies in RA ($p =<0.001$) also a statistically significant increased frequency was found in patients suffering from renal failure ($p=0.004$), when compared to controls.

As a comparison to the presence of anti-CarP antibodies, a second autoantibody, another common and RA-specific autoantibody, namely anti-CCP2, was measured. The percentages of individuals positive for anti-CCP2 antibodies was 1.5%, 1.9%, 0.5%, 2.4% and 50.5% for controls, smokers, patients with IBD, renal failure and RA respectively (Figure 1B). These data indicate that anti-CCP2 antibodies are present in RA patients but hardly in any of the other conditions.

In conclusion, especially RA patients, and to a much lesser extent patients suffering from renal disease, are positive for anti-CarP antibodies, while carbamylation is

reported to be increased in each of these conditions. Therefore, these data indicate that enhanced carbamylation is not sufficient for a break of tolerance against carbamylated proteins. Instead, the presence of antibodies against several post-translational modifications is a rather specific hallmark of the immunological abnormalities present in RA.

		Smokers	IBD	Renal failure	Controls	RA
Patients	*Number*	374	433	85	120-187	557
Age	*Average (SD)*	65 (9)	44 (14)	54 (13)	44 (14)	57 (16)
Gender	*Percentage female*	27	58	29	51	67
Smoking	*Percentage current*	42	21	24	8	23
	Pack years (average)	39	n.d.	n.d.	n.d.	n.d.

Table 1 – Patient characteristics. For the controls, the values are given for the total group of 209 controls. For the assays, controls were randomly selected out of the group of 209 controls. Controls were not age or gender – matched. We did not observe a correlation between anti-CarP antibodies and age or gender. IBD; inflammatory bowel disease, RA; Rheumatoid arthritis, stdev; standard deviation, n.d.; not determined. Data from RA patients as in reference[1].

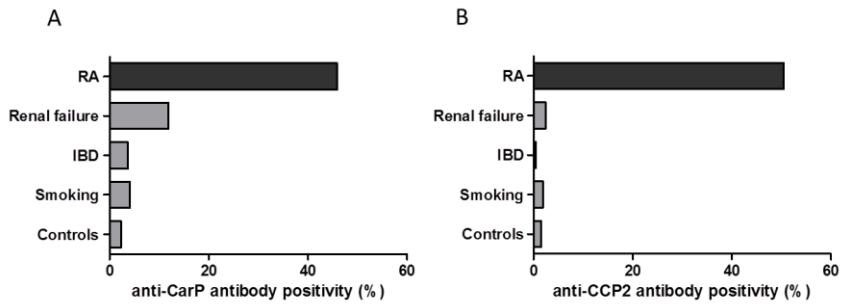

Figure 1 – Anti-CarP and anti-CCP2 antibody positivity. (A) The percentage of anti-CarP antibody positivity in each of the tested cohorts is shown. When compared to controls, significant differences were observed for RA ($p<0.001$) and renal failure ($p=0.004$) (B) The percentage of anti-CCP2 antibody positivity in each of the tested cohorts is shown. When compared to controls, significant differences were observed for RA ($p<0.001$). For both, control percentages are an average of all measurements. IBD; inflammatory bowel disease, RA; rheumatoid arthritis. Percentages of the RA patients are shown as determined in reference[1].

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