Anti-carbamylated protein antibodies: a specific hallmark for rheumatoid arthritis. Comparison to conditions known for enhanced carbamylation; renal failure, smoking and chronic inflammation

Antibodies that target carbamylated proteins (anti-CarP antibodies) have been described as a biomarker in rheumatoid arthritis (RA).[1] However, little is known about the factors that predispose to the production of anti-CarP antibodies. Carbamylation is a posttranslational modification resulting from the conversion of lysines into homocitrullines 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 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)[6] and in heavy smokers with or without chronic obstructive pulmonary disease (COPD)[7] (see table 1). The presence of anti-CarP antibodies in healthy controls and patients with RA was used as a comparison.[1 8] The collection of these cohorts was approved by the Leiden University Medical Center ethics committee and informed consent was obtained from all patients. Anti-CarP IgG and IgA antibodies were determined by ELISA 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 and 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 carried out as described before.[9]

Table 1 Patient characteristics

		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.

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 matched or gender matched. We did not observe a correlation between anti-carbamylated protein antibodies and age or gender.
Data from patients with RA as in reference (1).
IBD, inflammatory bowel disease; n.d., not determined; RA, rheumatoid arthritis.

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 and without COPD) and patients with IBD, renal failure and RA, respectively (figure 1A). Pearson χ^2 test analyses revealed that a statistically increased frequency of antibody positivity was found in RA ($p=<0.001$) and in patients suffering from renal failure ($p=0.004$), when compared with controls.

As a comparison to the presence of anti-CarP antibodies, a second autoantibody, targeting a structurally similar antigen, namely anti-CCP2, was measured. The percentages of individuals positive for anti-CCP2 antibodies were 1.5%, 1.9%, 0.5%, 2.4% and 50.5% for controls, smokers and patients with IBD, renal failure and RA, respectively (figure 1B). These data indicate that anti-CCP2 antibodies are present in patients with RA but hardly in any of the other conditions. While in patients with RA a certain extent of cross-reactivity between anti-CarP and anti-CCP2 antibodies can be observed, there was no

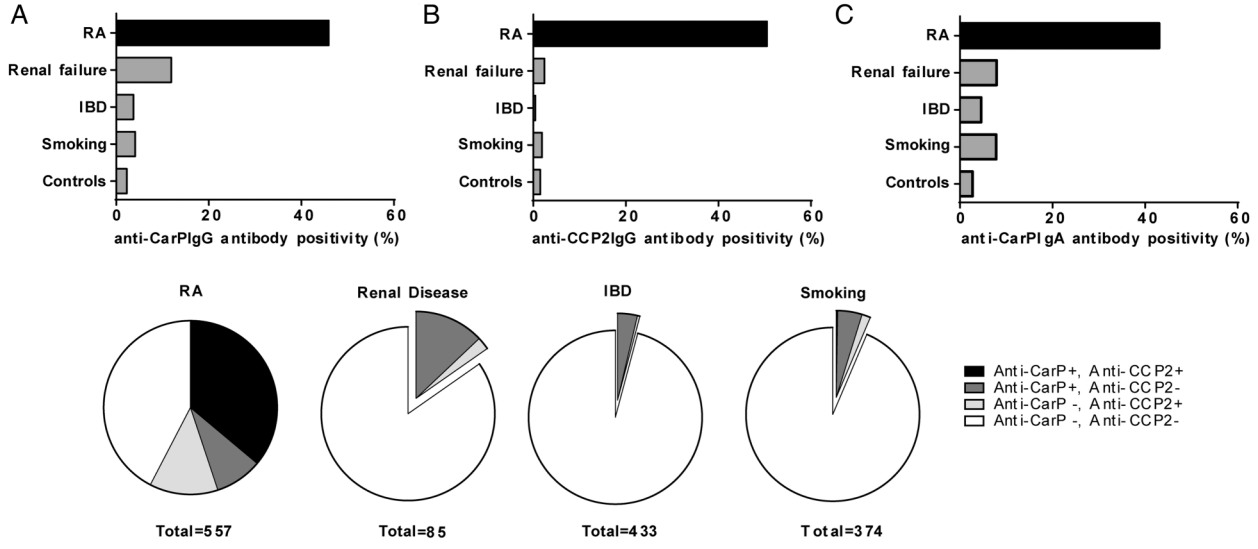

Figure 1 Anti-CarP and anti-CCP2 antibody positivity. (A) The percentage of anti-CarP IgG antibody positivity in each of the tested cohorts is shown. When compared with controls, significant differences were observed for RA ($p<0.001$) and renal failure ($p=0.004$). (B) The percentage of anti-CCP2 antibody positivity in each of the tested cohorts is shown. When compared with controls, significant differences were observed for RA ($p<0.001$). For both, control percentages are an average of all measurements. (C) The percentage of anti-CarP IgA positivity in each of the tested cohorts is shown. When compared with controls, significant differences were observed for RA ($p<0.001$), renal failure ($p=0.048$) and smoking ($p=0.026$). (D) Overlap between anti-CarP IgA and anti-CCP2 IgG antibodies. Groups were divided based on antibody positivity. Percentages of the patients with RA are shown as determined in reference (1). Anti-CarP, Anti-carbamylated protein; IBD, inflammatory bowel disease; RA, rheumatoid arthritis.

overlap between these autoantibodies in renal failure, smoking or IBD (figure 1D). Therefore, the presence of a (low) percentage of anti-CarP IgG antibodies in the individuals analysed is not due to the presence of anti-CCP2 antibodies.

Both IBD and smoking might involve more carbamylation in the lungs or intestine, in which IgA antibodies are thought to be more prevalent than IgG antibodies. Therefore, anti-CarP IgA antibodies were determined as shown in figure 1C (2.7%, 7.8%, 4.6%, 7.9% and 43% for controls, smokers and patients with IBD, renal failure and RA, respectively). Compared with controls, positivity in patients with RA, renal failure and smoking was statistically significant. When correcting for multiple testing, only the difference between controls and patients with RA remained significant.

In summary, we observed an increase in anti-CarP antibodies in patients with RA and to a lesser extent in patients suffering from renal disease. Since an increase in anti-CarP antibodies was not detected in other conditions with enhanced carbamylation, we conclude that increased carbamylation alone is not sufficient for a break of tolerance against carbamylated proteins. Therefore, our data indicate that next to carbamylation, also other (genetic or environmental) factors are required for the induction of anti-CarP antibodies.

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Competing interests REMT and LAT are listed as inventors in a patent application regarding the detection of anti-CarP antibodies for RA.

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REFERENCES

1 Shi J, Knevel R, Suwannalai P, *et al*. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc Natl Acad Sci USA* 2011;108:17372–7.
2 Wang Z, Nicholls SJ, Rodriguez ER, *et al*. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med* 2007;13:1176–84.
3 Shi J, van Veelen PA, Mahler M, *et al*. Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies. *Autoimmunity Rev* 2014;13:225–30.
4 Kalim S, Karumanchi SA, Thadhani RI, *et al*. Protein carbamylation in kidney disease: pathogenesis and clinical implications. *Am J Kidney Dis* 2014;64:793–803.
5 Aydin Z, Mallat MJ, Schaapherder AF, *et al*. Randomized trial of short-course high-dose erythropoietin in donation after cardiac death kidney transplant recipients. *Am J Transplant* 2012;12:1793–800.
6 van Erp SJ, Brakenhoff LK, van Gaalen FA, *et al*. Classifying back pain and peripheral joint complaints in inflammatory bowel disease patients: a prospective longitudinal follow-up study. *J Crohns Colitis* 2016;10:166–75.
7 Chappell S, Daly L, Morgan K, *et al*. Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease. *Hum Mutat* 2006;27:103–9.
8 van Aken J, van Bilsen JH, Allaart CF, *et al*. The Leiden early arthritis clinic. *Clin Exp Rheumatol* 2003;21(Suppl 31):S100–105.
9 Kerkman PF, Fabre E, van der Voort EI, *et al*. Identification and characterisation of citrullinated antigen-specific B cells in peripheral blood of patients with rheumatoid arthritis. *Ann Rheum Dis* 2016;75:1170–6.

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Notes