

# Anti-cyclic citrullinated peptide antibody isotypes in rheumatoid arthritis: association with disease duration, rheumatoid factor production and the presence of shared epitope

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## Abstract Objective

Anti-cyclic citrullinated peptide (anti-CCP) antibodies of IgG isotype are specific diagnostic markers of rheumatoid arthritis (RA). Recent evidence also points to their direct involvement in the pathophysiology. Little information is available, however, regarding the isotype distribution of anti-CCP antibodies and the characteristics of IgA and IgM anti-CCP.

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## Methods

IgG, IgA and IgM anti-CCP2 and rheumatoid factor (RF) levels were measured in the sera of 119 RA patients and 118 controls, including patients with other rheumatic diseases and healthy subjects. We analyzed the diagnostic performance of IgA and IgM anti-CCP2 antibodies and their relationship with IgG anti-CCP2, RFs, disease duration and the presence of HLA-DRB1 shared epitope (SE) alleles.

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## Results

Patients with RA had significantly higher serum IgA and IgM anti-CCP2 antibody levels than healthy subjects and patients with other rheumatic diseases ( $p < 0.0001$ ). IgG, IgA and IgM anti-CCP2 antibodies were present in 74.8%, 52.9% and 44.5% of RA patients, and their diagnostic specificity was 95.8%, 95.8% and 91.6%, respectively. The presence of anti-CCP2 antibodies was significantly associated with SE alleles ( $p = 0.03$ ). The frequency of IgM anti-CCP2 positivity was lower in longstanding disease compared to early RA ( $p = 0.03$ ).

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## Conclusion

IgA and IgM anti-CCP2 antibodies are present in RA patients, and they are similarly specific for RA as IgG anti-CCP2. The higher frequency of IgM anti-CCP2 antibodies in early RA suggests that they are mostly generated during the first phase of immune response; nonetheless, their production seems to be sustained in some patients. Further analysis of IgM and IgA anti-CCP2 antibodies may provide insights into the pathogenesis of RA.

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## Key words

Rheumatoid arthritis, anti-CCP2, IgA, IgM, duration of RA.

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## Introduction

Anti-cyclic citrullinated peptide antibodies (anti-CCP) have recently been identified as highly specific diagnostic markers in rheumatoid arthritis (RA) (1-3). They belong to the family of autoantibodies directed to epitopes containing the non-standard amino acid citrulline (4). Anti-citrullinated protein antibodies (ACPAs) also include anti-perinuclear factor (APF) (5), anti-keratin (AKA) (6) and anti-filaggrin antibodies (7-9). ACPAs have significant predictive value, as they can be found very early, sometimes even during the preclinical phase of RA (10, 11). Their presence is also associated with more destructive joint damage and aggressive course of the disease (12-14). Recent observations point to the direct involvement of ACPAs in the pathophysiology of RA (15, 16).

ACPAs are present in the sera, but also in the synovial fluid of RA patients (17-19), and they are produced by local plasma cells in the inflamed joints (18, 19). These data suggest antigen-driven maturation of citrullinated protein-specific B cells at the site of inflammation. Indeed, synovial fluid CD38<sup>+</sup> B cells from RA patients are characterized by somatic hypermutation and clonal selection (20). These data together with the efficacy of B cell depletion therapy using the anti-CD20 antibody rituximab in RA (21) suggest an important role for B lymphocytes and autoantibodies in the pathogenesis of the disease.

Several citrullinated proteins were identified in the joints of RA patients, which can serve as potential autoantigens. Citrullinated epitopes were observed in extravascular fibrin deposits and extracellular fibrinogen aggregates in the synovium (22, 23). Moreover, citrullinated vimentin, the newly identified target of the anti-Sa antibody (24) is also present in inflammatory macrophages (25, 26). The increasing number of potential autoantigens, the cross-reactivity between antibodies to filaggrin and citrullinated fibrin (27), as well as the demonstration of individual reactivity patterns of sera from RA patients against several citrullinated peptides (2) suggest that citrullinated epitopes, rather than a single citrullinated mole-

cule may be involved in the induction of ACPAs.

The development of autoimmune response against citrullinated epitopes seems to require specific genetic predisposition. The presence of particular HLA-DRB1 alleles, containing a specific amino acid sequence in the third hypervariable region of their  $\beta$ 1 chain (shared epitope) (SE) has been closely associated with anti-CCP positive RA (28-30).

Although sparse publications mention the occurrence of IgA APF (31) or IgM AKA in RA (32, 33), not much is known about the possible pathogenic and/or prognostic role of isotype distribution of ACPAs in RA. Various antibody isotypes have different effector functions regarding immunocomplex formation and clearance, complement activation and phagocytosis, thus their role in the pathogenesis of RA may be distinct. The only available recent study observed the presence of IgM anti-CCP antibodies early in the course of the disease, but also in follow-up samples several years later (34). The authors concluded that the sustained presence of IgM anti-CCP antibodies indicates ongoing immune response (34).

The aim of the present study was to investigate the occurrence and levels of IgM and IgA anti-CCP2 antibodies in RA, to assess their diagnostic performance and their relationship to IgG anti-CCP2 antibodies, RF isotypes, disease duration and the presence of HLA-DRB1 SE alleles.

## Patients and methods

### Patients and controls

Serum samples were obtained from 119 consecutive patients with RA. All patients met the ACR classification criteria for the disease (35). For comparisons, we tested 118 control subjects, including 74 patients with other well-defined rheumatic diseases, such as 37 patients with primary Sjögren's syndrome (pSS), 30 with polymyositis or dermatomyositis (PM/DM) and 7 with osteoarthritis (OA), as well as 44 healthy subjects. The clinical records of the patients were reviewed for classification. All patients undergo regular follow-ups at the Rheumatology Out-

Competing interests: none declared.

patient Clinic of our institution. Serum samples were stored at  $-80^{\circ}\text{C}$  for less than 1 year until the present analysis. The RA group consisted of 100 women and 19 men. The mean ( $\pm$  SD) age of this group was  $52.7 \pm 12.5$  years (range: 19-77 years), which was not statistically different from that of the control subjects (Table I). The mean duration of RA was  $10.2 \pm 9.1$  years at the time of the study (Table I).

#### Anti-CCP2 IgG ELISA

Anti-CCP2 IgG levels were measured using a second generation ELISA (QUANTA Lite™ CCP ELISA, INOVA Diagnostics Inc., San Diego, CA) utilizing synthetic citrullinated peptides bound to the surface of a microtiter plate as antigen. The test was performed according to the manufacturer's instructions. Instead of categorizing the results by the manufacturer recommended cut-off value (20 U/ml), receiver operating characteristic (ROC) curve analysis was performed, and the optimal cut-off level (12 U/ml) was established by choosing the combination of the highest possible sensitivity and specificity.

#### Anti-CCP2 IgA and IgM ELISA

To measure anti-CCP2 IgA and IgM levels, citrullinated peptides coated plates, sample diluent, wash buffer, TMB substrate and stop solution were used from the QUANTA Lite™ CCP ELISA. Serum samples were diluted to 1:100, and were incubated in the wells of the ELISA plates for 60 minutes. Bound antibodies were detected by HRP-conjugated rabbit anti-human IgA and IgM (DAKO A/S, Glostrup, Denmark). Results were expressed as optical densities (ODs). Optimal cut-off values (0.198 for IgA anti-CCP2 and 0.513 for IgM anti-CCP2 antibodies) were determined by ROC curve analysis.

#### IgM, IgA and IgG RF

IgM, IgA and IgG RFs were assessed by ELISA (ImmuliS™ RF IgM, IgA and IgG, Immco Diagnostics, Buffalo, NY) according to the manufacturer's instructions. Normal upper limits were 9 IU/ml for IgM RF, 25 EU/ml for IgA

**Table I.** Demographic and clinical characteristics of the study population.

	Age (years) (mean $\pm$ SD)	Male/female ratio
RA (n = 119)	52.7 $\pm$ 12.5	19/100
pSS (n = 37)	55.9 $\pm$ 15.2	2/35
PM/DM (n = 30)	47.8 $\pm$ 14.5	6/24
OA (n = 7)	56.6 $\pm$ 16.6	2/5
Healthy subjects (n = 44)	45.8 $\pm$ 10.6	13/29

RA: rheumatoid arthritis; pSS: primary Sjögren's syndrome; PM/DM: polymyositis/dermatomyositis; OA: osteoarthritis.

RF, and 25 EU/ml for IgG RF, respectively.

#### HLA-DRB1 genotyping

Genomic DNA was isolated from the peripheral blood of 85 RA patients using QIAamp Blood Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. HLA-DRB1 typing and subtyping was performed by polymerase chain reaction (PCR) with sequence specific primers (Olerup SSP™, GenoVision Inc., PA, USA), as described previously (36). We investigated the presence of the following shared epitope alleles: HLA-DRB1\*0101, HLA-DRB1\*0102, HLA-DRB1\*0401, HLA-DRB1\*0404, HLA-DRB1\*0405 and HLA-DRB1\*0408.

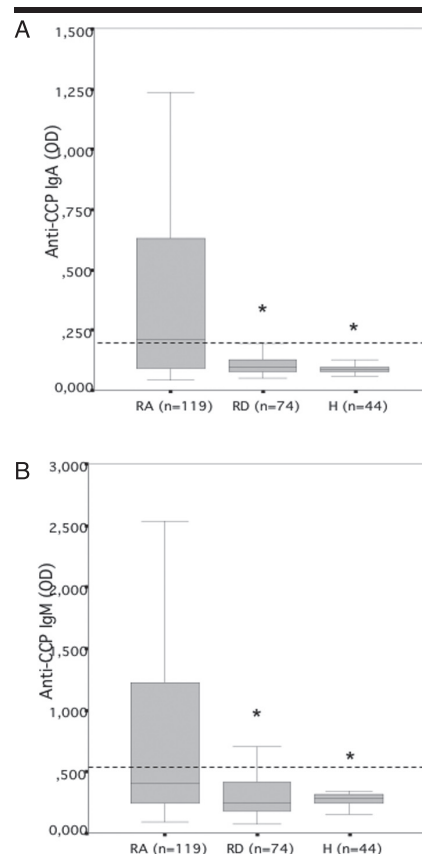
#### Statistical analysis

Antibody levels between different groups were compared by the non-parametric Mann Whitney U test. The diagnostic performance of anti-CCP2 antibody ELISAs was examined by ROC curve analysis, and optimal cut-off levels were determined at the value resulting in the combination of the highest diagnostic sensitivity and specificity. Spearman's rank correlation was used to assess the relationship between IgA, IgM and IgG anti-CCP2 levels. Fisher's exact test was performed to investigate the association between the occurrence of anti-CCP2 antibodies and RFs of different isotypes, as well as between the frequency of IgA, IgM and IgG anti-CCP2 antibodies and disease duration or the presence of HLA-DRB1 SE alleles. *P* values  $<0.05$  were considered significant. All statistical analyses were performed using the statistical package SPSS 11.0 (SPSS Institute Inc., Chicago, IL, USA).

## Results

### IgA and IgM anti-CCP2 levels in the study population

Patients with RA had significantly higher IgA anti-CCP2 antibody levels (median OD: 0.211, interquartile



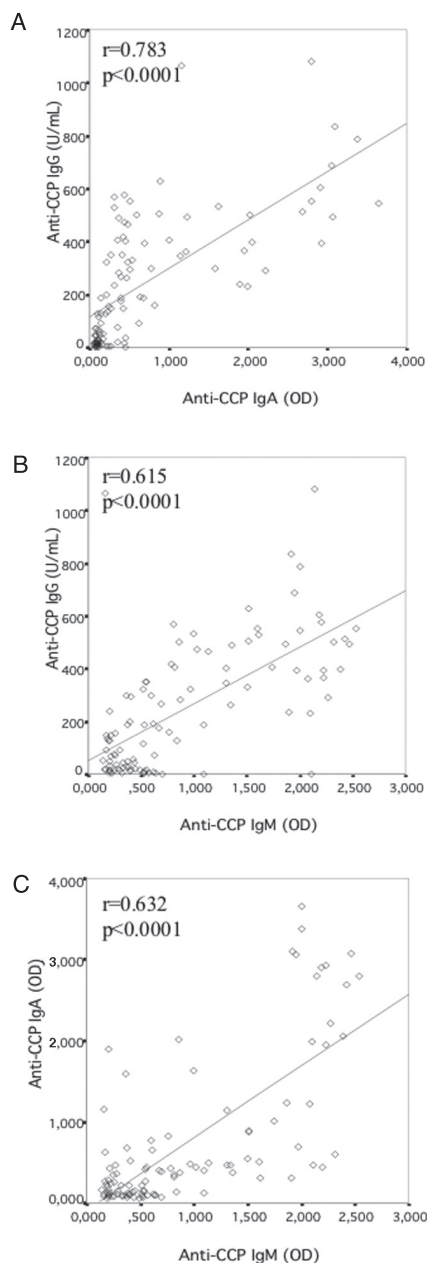
**Fig. 1.** Anti-CCP2 antibody levels in RA patients and in controls. Sera from 119 RA patients (RA), 74 patients with other rheumatic diseases (RD) and 44 healthy subjects (H) were analyzed. Levels of IgA anti-CCP2 antibodies (A) and IgM anti-CCP2 antibodies (B) are shown. Results are expressed as optical densities (OD) and plotted as box plots. Boxes represent the interquartile range of antibodies, lines inside the boxes indicate median values; bars extending from boxes indicate range. Extremes are not shown. Dashed line represents the cut-off OD. \**p* $<0.0001$  between RA and controls

range: 0.090-0.630) than healthy subjects (median OD: 0.085, interquartile range: 0.077-0.096) and patients with other rheumatic diseases (median OD: 0.096, interquartile range: 0.076-0.125) ( $p < 0.0001$  for both). They also had significantly higher IgM anti-CCP2 antibody levels (median OD: 0.404, interquartile range: 0.245-1.221) compared to healthy controls (median OD: 0.278, interquartile range: 0.246-0.309) and disease controls (median OD: 0.244, interquartile range: 0.185-0.411) ( $p < 0.0001$  for both) (Fig. 1). IgA and IgM anti-CCP2 levels showed significant correlation with IgG anti-CCP2 levels, and with each other ( $p < 0.0001$  for each) (Fig. 2).

#### Frequency of IgA and IgM and IgG anti-CCP2 antibodies in RA

To examine the overall diagnostic performance of anti-CCP2 assays of different isotypes, ROC analysis was performed. The calculated area under the curve (AUC) value was 0.910 (95% CI: 0.873-0.946) for anti-CCP2 IgG, 0.744 (95% CI: 0.678-0.809) and 0.704 (95% CI: 0.636-0.772) for IgA and IgM anti-CCP2, respectively. The difference between IgA and IgM anti-CCP2 was not significant (Fig. 3.). Categorizing the results according to optimal cut off values, IgG, IgA and IgM anti-CCP2 antibodies were positive in 74.8%, 52.9% and 44.5% of RA patients, respectively. Although most IgA and IgM anti-CCP2 antibodies were present in IgG anti-CCP2 positive RA subjects, two single IgM, and one single IgA positivity was detected in the RA group. No anti-CCP2 antibodies of any isotypes tested positive in healthy controls (Table II). IgG, IgA and IgM anti-CCP2 antibodies occurred in 5, 5 and 9 patients in the disease control group, respectively. In four control subjects two or three antibodies of different isotypes were present.

The overall diagnostic specificity of the IgA and IgM anti-CCP2 tests was 95.8% and 91.6%, respectively. When compared with the diagnostic performance of RFs, the specificity of IgA and IgM anti-CCP2 antibodies proved to be significantly higher than those of any RF isotypes (Table II), and their sensitivity exceeded the diagnostic sensitivity of IgA and IgG RFs (Table II) Within

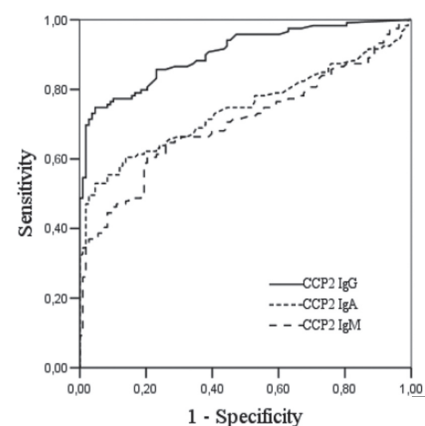


**Fig. 2.** Scatter plot of IgG, IgA and IgM anti-CCP2 antibody titers in RA patients. Only those tested positive for at least one of the antibodies were included ( $n = 92$ ). Anti-CCP2 IgG results are expressed in U/ml, while IgA and IgM anti-CCP2 results as optical densities (OD). Associations between IgG and IgA (A), IgG and IgM (B) and IgA and IgM anti-CCP2 levels (C) are shown. Correlation was examined by Spearman's rank test.

the RA group, being positive for any of the two, or all the three anti-CCP2 isotypes corresponded to a specificity level of 96.6% and 99.2%, respectively.

#### Association between anti-CCP2 antibodies and RFs of various isotypes

We compared the proportions of RA



**Fig. 3.** Receiver operating characteristic (ROC) curve analyses of the IgG, IgA and IgM anti-CCP2 assays in the study population. IgA and IgM anti-CCP2 tests have similar, although lower diagnostic efficacy than IgG anti-CCP2 ELISA.

patients in the IgM, IgA and IgG RF positive and negative groups who were positive for the same anti-CCP2 antibody isotypes. Significantly more RA subjects with IgM RF had IgM anti-CCP2 antibodies than those in the IgM RF negative population (56.8% versus 14.7%;  $p < 0.0001$ ). Also, the frequency of IgA anti-CCP2 antibodies was higher in the IgA RF positive group compared to the IgA RF negative patients (70.5% versus 42.6%;  $p = 0.004$ ). Although IgG anti-CCP2 positivity was also more prevalent among IgG RF positive patients (82.2% versus 70.2%), this association was not significant (Table III).

#### Association between anti-CCP2 antibodies and HLA-DRB1 shared epitopes

As the presence of the HLA-DRB1 SE is associated with IgG anti-CCP2 positivity in RA patients, we examined the possibility if the relationship can be extended to anti-CCP2 antibodies of IgA or IgM isotype. HLA-DRB1 typing and subtyping was performed in 85 RA patients, and one or two SE alleles were detected in 54.1% of them. When IgA and IgM anti-CCP2 antibodies were examined separately, only slightly higher IgA and IgM anti-CCP2 antibody frequencies were found in RA patients carrying SEs compared to SE negative ones (54.3% vs. 43.6% for IgA, and 45.7% vs. 33.3% for IgM;  $p = \text{NS}$ ).

**Table II.** Diagnostic sensitivity and specificity of IgG, IgA and IgM anti-CCP2 antibodies and RFs in rheumatoid arthritis.

	IgG anti-CCP2	IgA anti-CCP2	IgM anti-CCP2	IgG RF	IgA RF	IgM RF
Sensitivity (%)	74.8	52.9	44.5	37.8	37.0	71.4
Specificity (%)* (RA/healthy subjects)	100.0	100.0	100.0	100.0	97.7	97.7
Specificity (%)* (RA/all controls)	95.8	95.8	91.6	87.3	89.0	82.2

\*Specificity was calculated for RA versus healthy controls only (RA/healthy subjects), and for RA versus healthy + disease controls (RA/all controls).

**Table III.** Relationship between IgA and IgM anti-CCP2 antibodies and RFs in rheumatoid arthritis (n = 119).

A) Relationship between IgA anti-CCP2 and RF positivity ( $p = 0.004$ )

	Anti-CCP2 IgA pos.	Anti-CCP2 IgA neg.
RF IgA pos.	31	13
RF IgA neg.	32	43

B) Relationship between IgM anti-CCP2 and RF positivity ( $p < 0.0001$ )

	Anti-CCP2 IgM pos.	Anti-CCP2 IgM neg.
RF IgM pos.	48	37
RF IgM neg.	5	29

The association between the occurrence of different antibodies was evaluated by Fisher's exact test. Data are shown as the number of patients in each group.

**Table IV.** Frequency of anti-CCP2 antibodies of IgG, IgA and IgM isotype in early and longstanding rheumatoid arthritis.

	RA duration < 3 years (n = 14)	RA duration > 3 years (n = 105)	RA duration > 10 years (n = 40)
Anti-CCP2 IgG	92.8%	73.8%	70.0%
Anti-CCP2 IgA	71.4%	51.5%	47.5%
Anti-CCP2 IgM	71.4%	41.5%*	37.5%**
Anti-CCP2 IgG+IgA+IgM	64.3%	37.9%	35.0%

\* $p = 0.04$  between RA duration < 3 years and > 3 years by Fisher's exact test.

\*\* $p = 0.03$  between RA duration < 3 years and > 10 years by Fisher's exact test.

However, when anti-CCP2 antibodies of all isotypes were considered, we could demonstrate significant association between anti-CCP2 antibodies and the presence of SE ( $p=0.03$ ). This association was stronger than the association between SE alleles and IgG anti-CCP2 antibodies alone ( $p=0.04$ ). Antibody levels of anti-CCP2 antibodies of any isotype (considering only those patients who tested positive) were not higher in the SE positive group compared to SE negative RA patients (data not shown).

#### *Association of anti-CCP2 antibodies of various isotypes with the duration of RA*

We examined the frequency and levels of anti-CCP2 antibodies in "early" (disease duration <3 years) and "longstanding" (disease duration >10 years) RA. The tendency of higher rate of positivity was observed for all three antibody isotypes in early RA (Table IV). However, significant difference was detected only in the case of IgM anti-CCP2: the prevalence of this antibody was 71.4% in those with disease

duration of less than 3 years, while it was 37.5% in those having RA for more than 10 years ( $p=0.03$ ) (Table IV). Similar results were obtained when "early" RA was compared to the rest of the RA patients ( $p=0.04$ ) (Table IV). The serum concentrations of anti-CCP2 antibodies were similar, and the frequency of RFs of various isotypes was not significantly different in "early" versus "longstanding" disease (data not shown).

#### **Discussion**

Anti-CCP antibodies are considered as specific diagnostic and prognostic markers in RA (37). Second generation assays detect IgG antibodies against citrullinated epitopes in approximately 70-75% of RA patients (2). However, the prevalence and clinical significance of IgA and IgM anti-CCP antibodies have not been fully revealed. Although autoantibodies of IgG isotype are generally the most relevant ones for the assessment of various autoimmune diseases, IgA or IgM types may represent special significance in certain cases. For example, IgA anti-dsDNA antibodies have been correlated with disease activity in systemic lupus erythematosus (SLE) (38, 39), and higher IgG/IgM anti-dsDNA ratio has been associated with kidney disease (40). The presence of IgM antiphospholipid antibodies (APA) is one of the classification criteria of antiphospholipid syndrome (41), and IgA APAs seem to be associated with specific symptoms of the disease (42, 43). In RA, RFs of all three isotypes can be present in the same patient. The prevalence of IgG and IgA RFs is usually lower than that of IgM RF, but the specificity of IgG and IgA isotypes may be higher (44, 45). The presence of IgA RF may predict the development of RA and is associated with more erosive disease (10, 46).

Our results show that the levels of IgA and IgM anti-CCP2 antibodies are elevated in the sera of RA patients. Although these antibody isotypes are positive only in approximately half of the studied RA population, their diagnostic specificity exceeds that of any RF isotypes. The presence of IgA or IgM anti-CCP2 antibody confirms the diagnosis of RA, and triple positivity increases

the specificity of the anti-CCP2 test to 99.2%. Interestingly, significantly higher frequency of IgM anti-CCP2 antibodies was detected in early RA. This finding seems consistent with the common evolution of antigen-specific immune responses. In a recent study, authors could detect IgA and IgM anti-CCP antibodies in the sera of 62% and 61% of IgG anti-CCP positive RA patients, and after a mean followup of 7 years they observed a decrease in the proportion of patients with IgA, but not in that of subjects with IgM anti-CCP positivity (34). They concluded that the sustained presence of IgM antibodies points to continuous triggering of newly generated B cells (34). However, this relative "stability" in the frequency of IgM anti-CCP antibodies derived from a switch from negative to positive status in 12.5%, and from positive to negative phenotype in 20.3% of their patients (34). The termination of IgM response in one fifth of RA patients, along with the diminished frequency of IgA and IgG anti-CCP positivity, suggests that the anti-CCP response may eventually burn out at least in some of the patients. These data strongly confirm our results, which, besides of the significantly lower prevalence of IgM anti-CCP2 in longstanding RA, show a clear tendency of decreased frequency of IgG and/or IgA anti-CCP2 positivity, and especially that of triple positivity in patients with disease duration of more than 10 years. Whether this is the general course of the disease or an effect of treatment remains to be elucidated.

The development of anti-CCP antibodies later after the onset of the disease in some cases (34), though, may imply the existence of sustained antigenic stimulus. B cells isolated from the synovial fluid of anti-CCP positive RA patients were shown to actively produce IgM anti-CCP antibodies *ex vivo* (19). These data suggest antigen-driven maturation of citrullinated protein-specific B cells at the site of inflammation (20), where citrullinated proteins are present. These findings are supported by our recent observation showing the accumulation of IgD+/CD27+ and IgD-/CD27+ memory B cells in the peripheral blood of RA patients (47). The relative frequency of

these cell types was proportional with the duration of the disease, regardless of age. The relative stability of (IgG) anti-CCP status has been previously demonstrated by a Swedish group (48); although the follow up period in this study was only three years. Another paper, however, in accordance with our findings, reported at least 25% reduction of RF and IgG anti-CCP levels in more than 50% of RA patients during a median follow up of 13 years, regardless of treatment (49). Taken together, these data suggest that the immune response against citrullinated antigens declines or is terminated along the course of the disease in some patients, while it remains sustained in others.

B cell depletion therapy results in significant decrease in the titers of RFs and IgG anti-CCP antibodies (50), associated with marked clinical improvement. Relapse, however, was found to closely correlate with rises in the level of at least one autoantibody (50). It remains to be investigated whether the production of IgM and/or IgA anti-CCP2 antibodies may precede the appearance of IgG anti-CCP2 in this setting.

The diagnostic sensitivity of anti-CCP assays was reported to be lower in very early arthritis (2, 51). This seems to contradict with the higher frequency of anti-CCP2 antibodies in early RA in our cohort. However, our patients were not in the phase of undifferentiated arthritis, but all had the diagnosis of definitive RA based on the ACR classification criteria. The duration of RA is calculated from the time of the diagnosis, which, according to several studies, sometimes is established with substantial delay after the first symptoms (52, 53).

Anti-CCP antibodies of IgG isotype can be present in as much as half of RA patients before the development of symptoms (10, 11). The predictive value of IgA and IgM anti-CCP antibodies has not been studied so far. The high prevalence of IgM anti-CCP2 antibodies in early RA strongly suggests, though, that these antibodies can be present along with, or even earlier than IgG anti-CCP2. Differentiating RA from other rheumatic diseases, especially Sjögren's syndrome accompanied with RA-like polyarthritides, is a difficult diagnostic problem.

Whether the high diagnostic specificity of double and triple anti-CCP2 positivity facilitates this discrimination, remains to be investigated. Anti-CCP antibodies (similarly to our results) have been demonstrated in as much as 7.5% of patients with Sjögren's syndrome not fulfilling the ACR criteria for RA (54). The possibility of developing RA cannot be ruled out, and the anti-CCP2 positive pSS and PM patients in our study group (especially those with two or three anti-CCP2 antibody isotypes) require close clinical, laboratory and radiographic follow-up.

The evolution of the autoimmune response against citrullinated epitopes is facilitated by the carriership of HLA-DRB1 SE alleles (28-30). HLA-DR genes are known to exert a major influence on the CD4<sup>+</sup> αβ T cell repertoire, and HLA molecules coded by SE alleles are thought to efficiently present self peptides to CD4<sup>+</sup> T cells in the thymus (55). The conversion of arginine to citrulline significantly increases peptide-MHC affinity and leads to the activation of CD4<sup>+</sup> T cells (56). The frequency and distribution of SE alleles in Hungarian RA patients has been reported to be similar, although somewhat lower than in other European populations (57). We were able to confirm the association between the presence of SE and anti-CCP2 antibodies in this Hungarian cohort, and demonstrated a stronger association when antibodies of all three isotypes were considered instead of the IgG isotype alone.

RF and anti-CCP antibodies are present in the majority of RA patients. The nature of these autoantibody systems is different, as it was elegantly demonstrated by cluster analysis in a recent study (58). Nevertheless, the agreement rate between IgM RF and IgG anti-CCP antibodies is usually high. We examined whether an association also exists between RFs and anti-CCP2 antibodies of the same isotype. Interestingly, the presence of IgA and IgM anti-CCP2 antibodies was strongly associated with IgA and IgM RF positivity, but there was no significant relationship between IgG anti-CCP2 antibodies and IgG RF. This finding provides additional evidence on the differences between the two antibody systems.

In summary, IgA and IgM anti-CCP2 antibodies are present in patients with RA, and they are similarly specific for the disease as IgG anti-CCP2. The production of IgA and IgM anti-CCP2 antibodies has been closely associated with that of IgG anti-CCP2 and with the presence of HLA-DRB1 SE alleles. IgM and IgA anti-CCP2 positivity strongly confirms the diagnosis of RA, as triple positivity represents 99.2% diagnostic specificity. IgM anti-CCP2 antibodies are more prevalent in early than in longstanding RA, which suggests that they are mostly produced during the first phase of immune response against citrullinated antigens. Our data suggest that the antibody response declines along the course of the disease in some RA patients, while it remains sustained in others. Further studies on the possible association of these antibody isotypes with the activity, prognosis and responsiveness of the disease to different therapeutic approaches may facilitate our understanding of the pathophysiology of RA.

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