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Author: Suwannalai, Parawee

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Chapter 4

Low avidity anti-citrullinated protein antibodies (ACPA) are associated with a higher rate of joint destruction in rheumatoid arthritis.

P. Suwannalai^{1,2}, K. Britsemmer³, R. Knevel¹, H.U. Scherer¹, E.W.N. Levarht¹,
A.H. van der Helm-van Mil¹, D. van Schaardenburg³, T.W.J. Huizinga¹,
R.E.M. Toes¹, L.A. Trouw¹

¹Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands. ² Ramathibodi hospital, Mahidol University, Bangkok, Thailand. ³Jan van Breemen Research Institute | Reade, Amsterdam, The Netherlands

submitted

ABSTRACT

Objectives

Anti-citrullinated protein antibodies (ACPA) are specific for Rheumatoid Arthritis (RA) and have been implicated in disease pathogenesis. Previously we have shown that ACPA display a considerably lower avidity as compared to antibodies against recall antigens. Nonetheless, ACPA-avidity did vary between patients. As antibody-mediated effects are influenced by antibody-avidity, we now investigated ACPA-avidity in relation to biological activity and clinical outcome.

Methods

We determined the avidity of ACPA and related this with severity of joint damage in two Dutch early-RA cohorts containing 199 and 132 patients respectively. Differences in effector functions of low and high avidity ACPA were studied.

Results

Extensive variation in ACPA avidity between patients was observed. This allowed the analysis of the relationship between avidity and severity. The presence of low avidity ACPA is associated with a higher rate of joint destruction. This finding was replicated in an independent cohort. Analysis of the properties of low-versus high avidity ACPA revealed that low avidity ACPA are less hampered in their ability to bind 'new' citrullinated antigens. Although no differences could be observed regarding cellular activation via Fc-gamma receptors, low avidity ACPA were more potent in activating the complement system.

Conclusion

Patients with low avidity ACPA display a higher rate of joint destruction. Low avidity ACPA display a higher potency to interact with more citrullinated antigens in time and show that low avidity ACPA are more potent in complement activation. These data indicate that (low) avidity impacts on the biological activity of ACPA and associates with worse radiological outcome.

INTRODUCTION

Rheumatoid Arthritis (RA) is characterized by joint inflammation and destruction (1;2). The presence of anti-citrullinated protein antibodies (ACPA) is associated with both these characteristics and especially with a more destructive phenotype (3-6). Early aggressive treatment of RA improves outcome and limits joint destruction (7-9). The variation in phenotype and outcome observed within the ACPA positive stratum could be influenced by the genetic background that influences immune effector-functions, as well as pathways of bone and cartilage destruction (10). Likewise, other characteristics of the immune response may be involved. *In vitro*, ACPA have been shown to be able to recruit Fc-receptor mediated effector functions (11;12) and mediate complement activation (13). *In vivo*, differences in the levels of ACPA (14), fine specificity or epitope spreading (15;16), isotype usage (17) and glycosylation (18) may be associated with differences in the potential to activate effector-mechanisms, thereby influencing their biological potency.

Another potentially important aspect of ACPA biology is related to avidity. Our previous data (19) show that the ACPA-response differs from antibody responses against recall antigens such as tetanus toxoid, since ACPA display a markedly lower avidity. The relatively low avidity of ACPA was detected using different citrullinated antigens such as the citrullinated CCP-2-peptide as well as citrullinated proteins, such as citrullinated Fibrinogen and citrullinated Vimentin (19). The ACPA response displays overall only limited avidity maturation, which takes place before onset of symptoms (20). However, we also observed considerable variation in ACPA avidity between ACPA-positive patients. In other autoimmune diseases it has been reported that different degrees of antibody avidity are associated with different clinical phenotypes (21-23). We now hypothesize that a similar correlation may be present with respect to ACPA-avidity and outcome of RA-progression and / or the ability to recruit effector-mechanisms.

MATERIALS AND METHODS

Patients and samples

Baseline sera from a total of 199 ACPA positive patients with early RA according to the 1987 criteria were selected based on the availability of serum samples and data on severity at both baseline and follow-up. The patients are part of the Leiden Early Arthritis Clinic (EAC), an inception cohort of patients with recent onset arthritis (symptom duration < 2 years) that was initiated at the Department of Rheumatology of the LUMC in 1993 (24). Characteristics of patients in this

cohort were as follows: 63.3% female, median and IQR of age at inclusion 54.4 (17.1-83.3) years, symptom duration 7.62 (0.17-72.73) months, 80.4% RF positive. The collection and use of patient samples was approved by the local medical ethics committee in compliance with the Helsinki declaration and all participants provided written informed consent.

Diagnosis (at baseline and 1 year), symptom duration at baseline, disease activity at baseline (CRP, 68 swollen / 66 tender joint score and HAQ) and radiographic score (modified Sharp/Van der Heijde score, SHS) at baseline until 6 years follow up were retrieved from the EAC database (25). We evaluated the association of the avidity of ACPA and of the levels of ACPA with these markers by categorizing the patient group into quartiles based on the avidity of ACPA. Similarly, we also categorized the patient group into quartiles based on the levels of ACPA.

To confirm our observations, we replicated the analysis of the association between ACPA avidity and rate of joint destruction in a cohort from the Jan van Breemen Institute (JBI) | Reade in Amsterdam, The Netherlands. Baseline sera from 132 ACPA positive RA patients according to the 1987 criteria enrolled from 1995-2004 with 6 years longitudinal follow-up radiographic score (SHS) were selected from the EAC of the JBI. Characteristic of patients were as follows: 74.2% female, median and IQR of age at inclusion 52.7 (21.8-76.4) years, disease duration 5.04 (2.94-8.02) months, 99.2% RF positive.

ACPA avidity assays

The avidity of ACPA IgG was determined by an elution assay using sodiumthiocyanate (NaSCN) as the chaotropic agent, on a CCP2 ELISA plate (Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands) as described before (19). Antibody avidity is depicted as the “relative avidity index” (AI) (19). The AI is defined as the ratio of the amount of residual antibodies bound to the coated antigen after NaSCN (1M) elution to the amount of binding antibodies in the absence of NaSCN, expressed as percentage.

Number of recognized epitopes and isotype usage of low and high avidity ACPA.

To compare the capacity of ACPA from RA patients with different ACPA avidities to recognize several epitopes of citrullinated peptides/proteins, we determined ACPA reactivity to the following citrullinated antigens; CCP2 (Immunoscan RA Mark 2, Euro-Diagnostica, Arnhem, The Netherlands) according to the manufacturer’s instructions, 2 peptides of vimentin, 2 peptides of fibrinogen (alpha and beta), 2 peptides of enolase (linear and cyclic forms), and reactivity against citrullinated myelin basic protein (MBP) as described before (26). These analyses included non-citrullinated counterparts as controls which were not recognized

by ACPA (Data not shown). Data of immunoglobulin isotypes and subclasses of anti-CCP2 (IgG1, IgG2, IgG3, IgG4, IgM and IgA) have been determined as described (17).

Fluid-phase and solid-phase competition assays

To determine to what extent ACPA binding to solid phase antigens was inhibitable by fluid-phase antigens, we used representative sera with low and high avidity ACPA and performed competition assays. ACPA-positive serum was incubated for 1 hour with citrullinated peptide antigens (CCP), titrated from 0 - 100µg/ml. Following this pre-incubation we determined the avidity of ACPA. We also determined the effect of pre-incubation on solid phase citrullinated antigens by using serial steps incubation on a CCP2 ELISA plate (19). In brief, dilutions of sera with low and high avidity ACPA giving similar absorbance values in the CCP2 ELISA were incubated for 20 minutes in the first well. Then the supernatant was removed and incubated in the next well etc. for 8 steps. Bound ACPA were detected by rabbit anti-human IgG HRP followed by 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) as described before (19).

Complement activation and immune-cell stimulation by ACPA

We analyzed the capacity of low- versus high avidity ACPA to activate complement, as previously described (13). In brief, ACPA containing sera were diluted till the linear phase in the ELISA was reached, thereby allowing equal binding of ACPA in the different samples. Data are expressed as C3c deposition induced by the added exogenous normal human serum (NHS). In a similar approach also the capacity of low- versus high avidity ACPA to trigger cytokine production by immune cells was analyzed. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood obtained from healthy donors using a Ficoll-Paque Plus density gradient (Amersham Biosciences, Uppsala, Sweden). Cells were diluted to 1.5×10^6 cells/ml in RPMI-1640 (Flow Laboratories, Irvine, Scotland, UK) supplemented with 10%FCS, 1% glutamax, 1% penicillin/ streptomycin. PBMC were stimulated by plate bound ACPA, from serial dilutions of 10 high- and 10 low avidity ACPA containing samples. Serum dilutions of 10 ACPA negative RA patients and 10 healthy controls were used as negative controls. After washing, PBMC (3×10^5 cells/well) were incubated in medium on the plates for 20 hr before supernatants were harvested. As controls PBMC were incubated on the same plate, in the absence of ACPA, with LPS (E.coli, Sigma L-2880, 10 µg/ml) or medium only. Supernatants were kept at -20°C before measuring TNF-α concentrations by ELISA (OptEIA Human TNF ELISA; BD Biosciences), as recommended by the manufacturer. Maximal TNF-α secretion from PBMC can be

obtained at different concentrations of ACPA immune complexes, depending on the donor (11). Therefore, we compared the maximal TNF- α levels produced by PBMC upon plate bound ACPA stimulation between samples with low versus high avidity ACPA. The results presented are representative for the three PBMC donors that gave the strongest response.

Statistical analysis

For descriptive statistics, medians and IQR were presented. The differences between groups were analyzed by Mann-Whitney test. To test the effect of ACPA-avidity on rate of joint destruction over time, a multivariate normal regression analysis for longitudinal data was used with radiological score as response variable. This method analyses repeated measurements in one analysis and takes advantage of the correlation between these measurements, which results in a more precise standard error (27). Radiological scores were log-transformed to obtain a normal distribution. The rate of joint destruction over time was tested by an interaction of time with ACPA avidity. The effect of time was assumed to be linear in the interaction term. The effect of time was entered as a factor in the model as well, to allow proper capture of the mean response profile overtime. Age, gender, RF, ACPA-levels and inclusion period as proxy for treatment were included as adjustment variables in all analyses. The results from LUMC and JBI early arthritis cohorts were combined in a fixed effect model meta-analysis with inverse variance weighting in Stata, version 10.1. (Stata Corp. College Station, TX). The other statistical calculations were analyzed by SPSS for Windows (release 16.0, SPSS, Chicago, IL, USA). $P < 0.05$ was considered significant.

RESULTS

The avidity of ACPA is not associated with disease characteristics at baseline.

The avidity of ACPA of 199 ACPA positive early-RA patients was determined. Next, we divided this dataset into quartiles based on the avidity of ACPA IgG. No differences in the disease activity as measured by CRP, swollen/tender joint count and HAQ at baseline was observed comparing patients with low- versus high avidity ACPA (Table 1). Similarly, no differences were observed for these parameters when comparing low versus high levels of ACPA (Table 2). These data indicate that neither avidity nor levels of ACPA are related to disease characteristics at baseline.

Table 1. Disease characteristics of the baseline visit and ACPA avidity

ACPA Avidity	1st quartile	2nd quartile	3rd quartile	4th quartile
Avidity index	1.8 (0.4-3.4)	9.4 (7.8-10.7)	18.1 (14.9-21)	31.1 (26.6-47.9)
Tender joint count*	6 (4-10)	8 (4-15)	7 (5-13)	7 (5-11)
Swollen joint count*	5 (3-7)	5 (3-8)	6 (3-9)	5 (3-8)
C-Reactive Protein	18,5 (7-36.2)	14 (6.5-46)	25,5 (11-51.7)	12,5 (6-32.7)
HAQ	0,8 (0-3.1.2)	1 (0.5-1.3)	1 (0.2-1.5)	0,7 (0.3-1)

Presented are medians and inter-quartile ranges

* Swollen joints were counted in 68 joints and tender joints were counted in 66 joints.

HAQ= health assessment questionnaire

Table 2. Disease activity and ACPA levels

ACPA levels	1st quartile	2nd quartile	3rd quartile	4th quartile
Level †	145 (96-201)	465 (367-620)	1154 (952-1467)	2091 (1970-2692)
Tender joint count*	8 (4-15)	6 (3-12)	7 (4-12)	8 (5-12)
Swollen joint count*	5 (3-8)	4 (2-8)	5 (4-8)	5 (3-8)
C-Reactive Protein	14 (7-39)	15 (6-31)	24 (8-56)	21 (8-41)
HAQ	0,8 (0.3-1.0)	0,9 (0.3-1.3)	0.9 (0.7-1.4)	1 (0.4-1.4)

Presented are medians and inter-quartile ranges

† The lowest limit of ACPA levels in avidity assay = anti-CCP2 50 AU/ml.

* Swollen joints were counted in 68 joints and tender joints were counted in 66 joints.

The avidity of ACPA is associated with more severe joint destruction.

Next we investigated whether the avidity of ACPA IgG was associated with the progression of joint destruction. At baseline we did not observe any differences in joint destruction between patients with low- versus high avidity ACPA (Fig.1A). However, when we compared the progression of joint destruction over time we observed that the patients with the lowest ACPA avidity (1st quartile) displayed a disease course characterized by more joint destruction as compared to patients present in the other three quartiles (Fig. 1A). Radiographic scores, quantified as Sharp-van der Heijde Scores (SHS) of individuals with low and high avidity ACPA are also depicted in a probability plot to provide individual patient data on the progression between baseline and year six (Fig.1B). These data indicate that the association between low avidity ACPA and joint destruction is not driven by a few outliers, but rather found across the entire population of patients harboring low avidity ACPA. In addition, we used repeated measurement analyses to adjust for age, gender and secular trends reflecting e.g. treatment effects as previously described (25). Also these analyses indicate that over 6-years RA patients with the lowest avidity of ACPA display a 1.61 (95%CI 1.17-2.20) fold higher rate of joint destruction compared to patients harboring higher avidity of ACPA. ($P=0.003$)

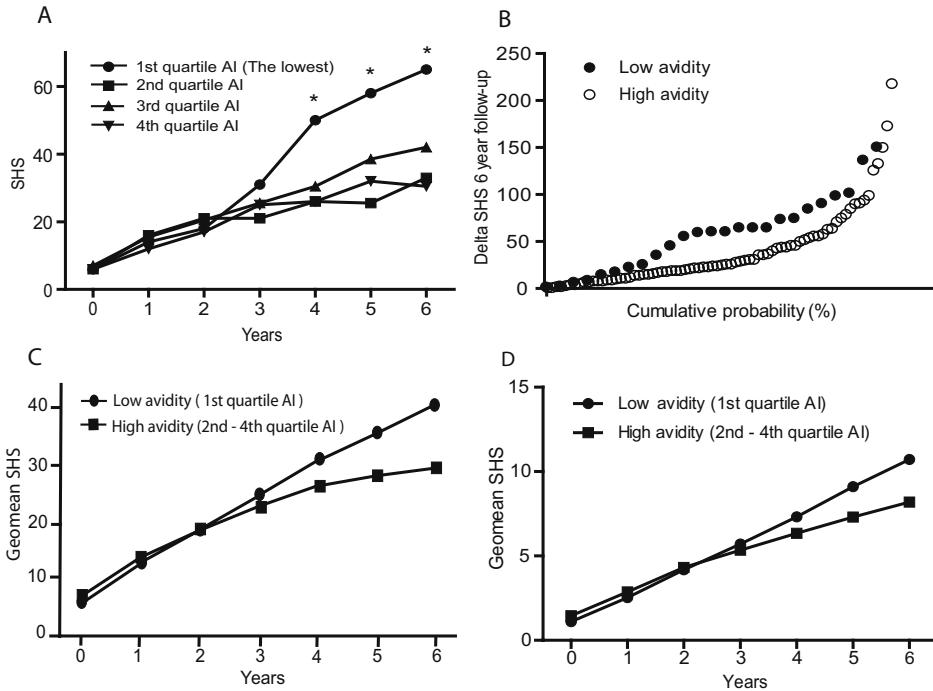


Figure 1. Low avidity ACPA associate with more severe radiological damage

(A) Radiographic damage at 1, 2, 3, 4, 5, 6 years presented as median SHS in 4 quartiles of ACPA avidity in the Leiden Early Arthritis Clinic (EAC) data set of 199 patients. * indicates a significant $p < 0.05$ difference using Mann-Whitney. (B) Cumulative plot of radiographic progression in low avidity ACPA (1st quartile) and high avidity ACPA (2nd – 4th quartiles) over 6 year follow-up (C) Data from repeated measurement analysis, adjusted for age, gender and treatment strategy to compare radiographic progression in low avidity ACPA (1st quartile) and high avidity ACPA (2nd – 4th quartiles). (D) Replication data set from the Amsterdam JBI EAC of 132 patients analyzed in the same way as the Leiden EAC data set.

(Fig.1C). The difference was also observed when we categorized the patient cohort into tertiles based on the ACPA avidity (data not shown). The observed effect remained significant after correcting for RF status and ACPA levels ($p=0.005$). The association between ACPA-avidity and radiological progression is not explained by differences in the ACPA recognition profile or isotype usage as ACPA-avidity did not associate with ACPA fine-specificity (Fig 2A) or isotype usage (Fig 2B). We did observe that the lowest quartile of avidity displayed significantly lower ACPA serum levels than the other quartiles (Fig. 2C). However, no association was observed between the levels of ACPA and the rate of joint damage (data not shown and (26;28;29). Together, these results indicate that the effect of low avidity ACPA on joint destruction was not mediated via enhanced epitope recognition, isotype usage or increased ACPA serum levels.

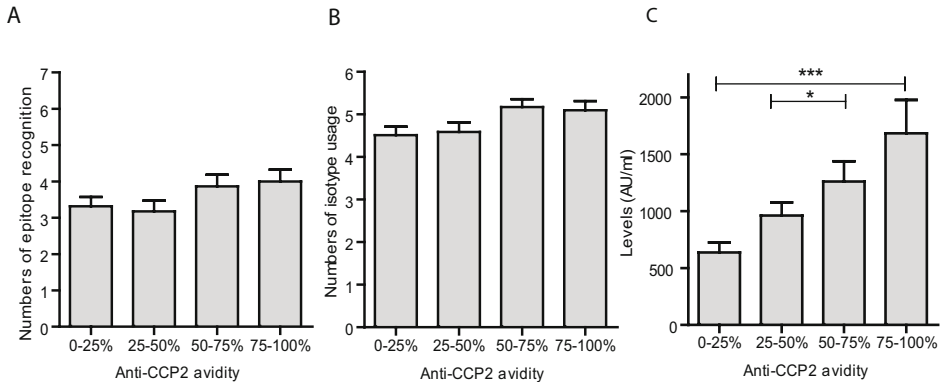


Figure 2. The association of low avidity ACPA with severity is not explained by isotype usage, fine-specificity or levels.

Number of epitopes recognized by ACPA (A), numbers of isotypes used (B) and ACPA titers (C) in 199 patients divided in 4 quartiles of ACPA avidity are shown. Results are the mean and SEM.

Next, we wished to replicate this observation using samples from another rheumatology center with an early arthritis cohort, i.e. the Jan van Breemen Institute (JBI) | Reade, Amsterdam, The Netherlands. The avidity of the ACPA response present in the samples from the JBI was in the same range as the avidity from the Leiden samples. Also in the JBI cohort we observed that RA patients with the lowest avidity of ACPA (lowest quartile) were characterized by a higher rate of joint destruction over time than the patients harboring higher avidity ACPA (Fig. 1D). We observed a 1.63 (95%CI 1.06-2.51, $P=0.027$) times higher rate of joint destruction, over the period of 6 years, when comparing patients with low avidity ACPA to patients with a higher avidity using the same calculations as for the Leiden EAC (Fig.1D). The difference was also observed when we categorized group in tertiles (data not shown). Finally, the results were combined in a meta-analysis. Since the heterogeneity of the two cohorts was not significant ($P=0.96$) a fixed effect model was used to combine the results of EAC and JBI. The estimated rate of joint destruction was 1.62 times higher (95%CI 1.26-2.08, $P<0.001$) for patients with the lowest avidity of ACPA as compared to patients harboring a higher avidity ACPA responses.

Together, these data indicate that the avidity of the ACPA-response is associated with the rate of joint destruction.

High avidity ACPA are more strictly retained by either fluid-phase and solid-phase citrullinated antigens.

The results presented above were not expected, as, intuitively, one could speculate that high-avidity antibody responses would mediate stronger biological effects as

compared to low-avidity antibodies. To obtain an indication on the ability of low- versus high avidity ACPA-responses to participate in immune-complex formation, we next performed fluid-phase competition assays to analyze the capacity of low- versus high avidity ACPA to form immune-complexes. Sera containing either low or high avidity ACPA were incubated with increasing concentrations of citrullinated peptides. Subsequently the effect on avidity was analyzed in the standard avidity test. We observed that the avidity of samples with high avidity ACPA was reduced after antigenic competition, whereas the avidity of samples with low-avidity ACPA was not influenced (Fig. 3A,B). These data indicate that during competition with free citrullinated antigen, the high avidity ACPA bind more readily to the fluid-phase antigens, explaining the decrease of the overall avidity of the sample. Next, we also performed a solid-phase competition assay. Low avidity and high avidity samples were diluted in such a way that the samples would give a similar absorbance in ELISA plates coated with the CCP peptide. The samples were incubated at these dilutions in ELISA plates coated with CCP peptide. Following a short incubation in CCP coated plates, the supernatant of these wells was removed and incubated in the next well, etc. Also this solid-phase competition assay showed that in samples harboring high avidity ACPA the amount of free ACPA is decreased more rapidly as compared to samples containing only low avidity ACPA, as reflected by the steeper curve of the high avidity sample (Fig. 3C).

Collectively, these data reveal that high avidity ACPA are more strictly retained in both fluid-phase and solid-phase immune complexes as compared to low

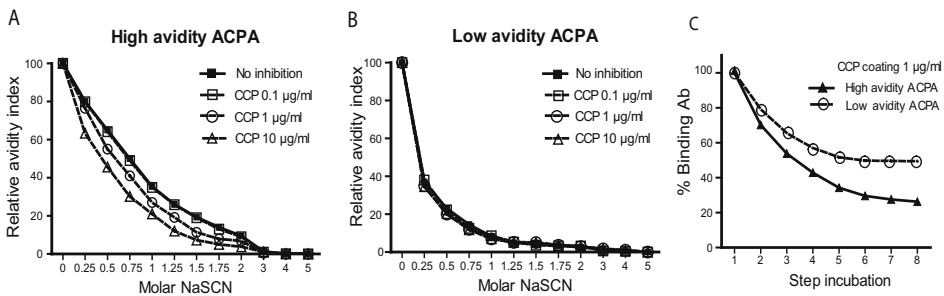


Figure 3. High avidity ACPA are preferentially retained by fluid-phase and solid-phase antigens.

Avidity of ACPA after pre-incubation with fluid-phase antigen of samples containing high avidity (A) and low avidity (B) ACPA. (C) ACPA IgG bound to plates coated with CCP peptide following a step wise incubation of samples containing high- or low avidity ACPA. The difference in slopes of the two lines indicates that High avidity ACPA are retained better as compared to low avidity ACPA. Both sets of data are representative of two other sets of low and high avidity samples.

avidity ACPA. Such differences may have implications for bio-availability, tissue penetration and activation of local effector mechanisms in vivo.

Low avidity ACPA have an enhanced capacity to activate the complement system

Since we observed that RA patients with low avidity ACPA displayed on average the highest radiographic joint destruction, we next determined if there was a difference in the capacity of low- versus high avidity ACPA to recruit effector-functions such as leukocyte activation and complement activation. We first analyzed the capacity of low- versus high-avidity ACPA to activate immune cells. For this purpose we determined immune-cell activation by measuring tumor necrosis factor- α (TNF- α) secretion from PBMC of healthy donors upon stimulation by plate-bound ACPA. We observed dose-dependent stimulation of TNF- α production by ACPA of both low- and high avidity indicating a similar potential of low-vs high avidity ACPA to activate PBMCs to produce TNF upon Fc-Receptor cross-linking (Fig 4C and data not shown).

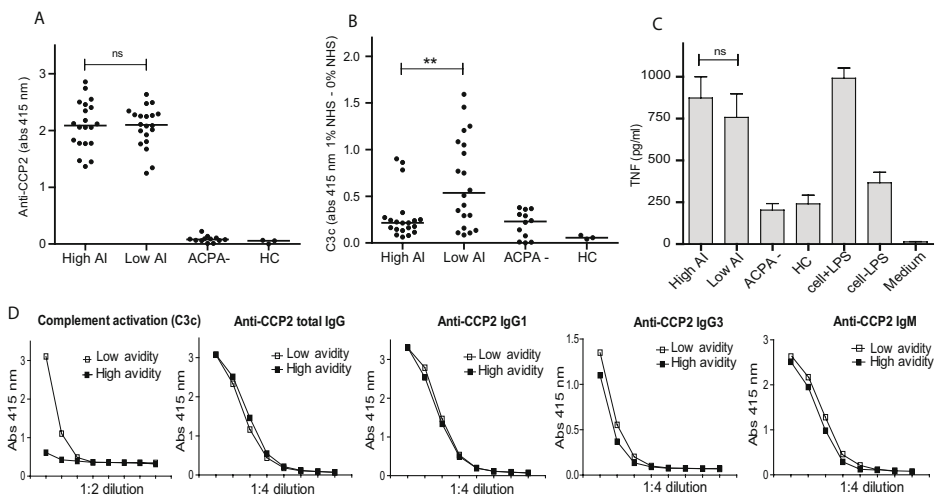


Figure 4. Differential effector mechanism recruitment by low- versus high-avidity ACPA.

Complement activation in low- and high avidity ACPA. (A) ACPA IgG binding to the plate (absorbance at 415 nm), (B) C3c binding to the plate (absorbance at 415 nm). (C) Representative example of one experiment using PBMC from one donor. The maximum concentration of TNF- α obtained upon ACPA-immune complex stimulation from 10 high and 10 low avidity ACPA, 20 ACPA negative sera (10 RA and 10 healthy), including positive control (Peripheral Blood Mononuclear Cells (PBMC) + LPS) and negative controls (PBMC, culture medium alone) is depicted. D) ACPA isotypes in 2 representative samples of low- and high avidity ACPA giving a different degree of complement activation.

For the analyses of complement activation, we titrated sera so that each sample would yield a similar absorbance in ELISA. This indicates that equal amounts of bound antibodies are present in each well for both low- and high avidity ACPA (Fig.4A). This incubation step was performed in a buffer containing EDTA to prevent complement activation during this step, as described before (13). Next the plates were washed and as a source of exogenous complement, active normal human serum was added. C3 activation and binding of C3 to the plate was used as a read-out of complement activation, as described before (13). Using this method we observed significantly more complement activation by plate-bound, low avidity ACPA as compared to high avidity ACPA, despite the presence of equal quantities of low and high avidity antibody bound to the plates (Fig.4B,A). Importantly, the ability to activate the complement system of low avidity ACPA was not the result of the usage of a specific isotype (Fig.4D). Together, these results reveal that low avidity ACPA might be more ‘active’ than higher avidity ACPA as they are more efficient in activating the complement system.

DISCUSSION

Many reports describe the association between the presence of ACPA and a less favorable clinical course of RA (4-6). Although no direct evidence is present showing that ACPA are involved in joint destruction in humans, it has been shown *in vitro* that ACPA-containing immune complexes (IC) can induce TNF- α secretion by macrophages via Fc γ RIIIa engagement (11), can trigger activation of Fc ϵ RI positive cells (12) and can activate the complement system (13). In the current study we hypothesized that the overall avidity of the ACPA response would be involved in the processes leading to joint erosions and that it would be responsible for part of the variation in the joint destruction seen within the ACPA positive patients. Therefore, we analyzed the avidity of ACPA in relation to joint destruction. We observed a higher rate of joint destruction in patients with low avidity ACPA as compared to patients with high avidity ACPA. The reasons for these effects are not known, but our *in vitro* data support the notion that low avidity ACPA mediate more pronounced biological effects.

Citrullinated antigens are enriched in the joint (30). For ACPA to bind to these antigens they should be able to penetrate into the tissue even in case all ACPA would be produced in the inflamed synovium. During this process ACPA may become ‘trapped’ in IC containing citrullinated proteins. We hypothesize that high avidity ACPA might be more avidly trapped in IC as compared to low avidity ACPA. This would predominantly allow the low avidity ACPA to penetrate

deeper into the tissues to bind to antigens which are localized at more distant sites of synovial tissue (31). Although the competition assays (fluid-phase IC) and serial steps incubation assays (solid-phase IC) provide a rationale (but no proof) explaining the observations made, they are in line with the idea that low avidity ACPA may have a better bioavailability. This may result in a higher penetrating capacity in tissue as also has been shown for therapeutic monoclonal antibodies that target tumor antigens (32;33). Moreover, the molecular make-up of IC is influenced by the amount of antigen and antibody as well as the avidity of the antibody (34). Therefore, high- and low avidity ACPA can form different types of immune complexes at different concentrations. We observed more pronounced complement activation by low avidity ACPA as compared to high avidity ACPA. Similar observations were also made for low avidity non-protective antibodies (and even pathogenic antibodies) against formalin-inactivated vaccine against measles and respiratory syncytial virus (35;36). The reasons behind the observation that low avidity antibodies could have an enhanced capacity to activate complement is not completely known. However, we excluded the possibility that this was related to a preferential isotype usage of low- versus high avidity ACPA. Since complement activation via the classical pathway requires the presence of at least 2 Fc-tails of antibody to be close together in order to activate C1q (37), one possible explanation may relate to the molecular composition of the IC in low- versus high avidity ACPA. In addition, low avidity ACPA can more easily detach from their antigens than high avidity ACPA. Therefore it is possible that *in situ* ACPA tethering may occur when one arm of immunoglobulin still bound to surface antigen and another arm can swing around for binding to the next antigen (38). As soon as again 2 Fc-tails of antibody are close enough to activate C1q then complement is activated resulting in the covalent binding of complement fragments like C3b and the perpetuation of an inflammatory response (37). Since the low avidity ACPA, in contrast to high avidity ACPA, could bind, detach and rebind at different locations, it could therefore result in an increased activation of complement.

Potentially the presence of RF could be a confounder factor underlying the observation that patients with low-avidity ACPA display more joint damage over time. Approximately 80% of the patients analyzed was positive for RF. However, as also reported before we observed no effect of RF on the avidity of ACPA (19). Likewise, after correcting for RF status the observed effect of low-avidity associating with more joint damage remained intact.

The ACPA response is characterized by limited avidity maturation which takes place in the years before onset of symptoms (20). This suggests that most ACPA positive RA patients started out with low avidity ACPA before disease manifesta-

tion. Nonetheless, most subjects do not develop any bone erosions at this stage and do, by definition, not display arthritis. Although, this seemingly contrasts our observations, it is likely that not only ACPA-avidity influences disease progression, but also several other features such as ACPA-level, isotype-usage or the presence of citrullinated antigens in the joint.

In conclusion, differences in biological properties of ACPA influence the clinical course of RA. Our data show that especially low avidity ACPA are associated with a higher rate of radiographic joint destruction. The bioavailability and the ability to activate complement, are putative mechanisms that drive the differential association of low- and high avidity ACPA with the rate of joint destruction.

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REFERENCE LIST

- (1) Harris ED, Jr. Rheumatoid arthritis. Pathophysiology and implications for therapy. *N Engl J Med* 1990; 322(18):1277-89.
- (2) Smolen JS, Aletaha D, Steiner G. Does damage cause inflammation? Revisiting the link between joint damage and inflammation. *Ann Rheum Dis* 2009; 68(2):159-62.
- (3) Jansen LM, van Schaardenburg D, van der Horst-Bruinsma I, van der Stadt RJ, de Koning MH, Dijkmans BA. The predictive value of anti-cyclic citrullinated peptide antibodies in early arthritis. *J Rheumatol* 2003; 30(8):1691-5.
- (4) Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2000; 43(8):1831-5.
- (5) Lindqvist E, Eberhardt K, Bendtzen K, Heinegard D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2005; 64(2):196-201.
- (6) van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004; 50(3):709-15.
- (7) Korpela M, Laasonen L, Hannonen P, Kautiainen H, Leirisalo-Repo M, Hakala M et al. Retardation of joint damage in patients with early rheumatoid arthritis by initial aggressive treatment with disease-modifying antirheumatic drugs: five-year experience from the FIN-RACo study. *Arthritis Rheum* 2004; 50(7):2072-81.
- (8) van der Kooij SM, le Cessie S, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, van Zeben D, Kerstens PJ et al. Clinical and radiological efficacy of initial vs delayed treatment with infliximab plus methotrexate in patients with early rheumatoid arthritis. *Ann Rheum Dis* 2009; 68(7):1153-8.
- (9) van der Linden MP, le Cessie S, Raza K, van der Woude D, Knevel R, Huizinga TW et al. Long-term impact of delay in assessment of patients with early arthritis. *Arthritis Rheum* 2010; 62(12):3537-46.
- (10) van der Helm-van Mil AH, Wesoly JZ, Huizinga TW. Understanding the genetic contribution to rheumatoid arthritis. *Curr Opin Rheumatol* 2005; 17(3):299-304.
- (11) Clavel C, Nogueira L, Laurent L, Iobagiu C, Vincent C, Sebbag M et al. Induction of macrophage secretion of tumor necrosis factor alpha through Fc gamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. *Arthritis Rheum* 2008; 58(3):678-88.
- (12) Schuerwegh AJ, Ioan-Facsinay A, Dorjee AL, Roos J, Bajema IM, van d, V et al. Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2010; 107(6):2586-91.
- (13) Trouw LA, Haisma EM, Levarht EW, van der WD, Ioan-Facsinay A, Daha MR et al. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. *Arthritis Rheum* 2009; 60(7):1923-31.
- (14) Syversen SW, Gaarder PI, Goll GL, Odegard S, Haavardsholm EA, Mowinckel P et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 2008; 67(2):212-7.

- (15) Ioan-Facsinay A, Willemze A, Robinson DB, Peschken CA, Markland J, van der WD et al. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. *Arthritis Rheum* 2008; 58(10):3000-8.
- (16) van der Woude D, Rantapaa-Dahlqvist S, Ioan-Facsinay A, Onnekink C, Schwarte CM, Verpoort KN et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. *Ann Rheum Dis* 2010; 69(8):1554-61.
- (17) Verpoort KN, Jol-van der Zijde CM, Papendrecht-van der Voort EA, Ioan-Facsinay A, Drijfhout JW, van Tol MJ et al. Isotype distribution of anti-cyclic citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. *Arthritis Rheum* 2006; 54(12):3799-808.
- (18) Scherer HU, van der Woude D, Ioan-Facsinay A, El Bannoudi H, Trouw LA, Wang J et al. Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. *Arthritis Rheum* 2010; 62(6):1620-9.
- (19) Suwannalai P, Scherer HU, van der Woude D, Ioan-Facsinay A, Jol-van der Zijde CM, van Tol MJ et al. Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. *Ann Rheum Dis* 2011; 70(2):373-9.
- (20) Suwannalai P, van de Stadt LA, Radner H, Steiner G, El-Gabalawy HS, Jol-van der Zijde CM et al. Avidity maturation of ACPA in rheumatoid arthritis. *Arthritis Rheum* 2011.
- (21) Cucnik S, Kveder T, Krizaj I, Rozman B, Bozic B. High avidity anti-beta 2-glycoprotein I antibodies in patients with antiphospholipid syndrome. *Ann Rheum Dis* 2004; 63(11):1478-82.
- (22) Villalta D, Romelli PB, Savina C, Bizzaro N, Tozzoli R, Tonutti E et al. Anti-dsDNA antibody avidity determination by a simple reliable ELISA method for SLE diagnosis and monitoring. *Lupus* 2003; 12(1):31-6.
- (23) Zhang Y, Gao Y, Li M, Xie L, Huang Y, Gao Y et al. Avidity of thyroglobulin antibody in sera from patients with Hashimoto's thyroiditis with different thyroid functional status. *Clin Exp Immunol* 2010; 161(1):65-70.
- (24) de Rooy DP, van der Linden MP, Knevel R, Huizinga TW, van der Helm-van Mil AH. Predicting arthritis outcomes--what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011; 50(1):93-100.
- (25) van der Linden MP, Feitsma AL, le CS, Kern M, Olsson LM, Raychaudhuri S et al. Association of a single-nucleotide polymorphism in CD40 with the rate of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2009; 60(8):2242-7.
- (26) Willemze A, Bohringer S, Knevel R, Levarht EN, Stoeken-Rijsbergen G, Houwing-Duistermaat JJ et al. The ACPA recognition profile and subgrouping of ACPA-positive RA patients. *Ann Rheum Dis* 2012; 71(2):268-74.
- (27) van der Helm-van Mil AH, Knevel R, van der HD, Huizinga TW. How to avoid phenotypic misclassification in using joint destruction as an outcome measure for rheumatoid arthritis? *Rheumatology (Oxford)* 2010; 49(8):1429-35.
- (28) Scherer HU, van der Woude D, Willemze A, Trouw LA, Knevel R, Syversen SW et al. Distinct ACPA fine specificities, formed under the influence of HLA shared epitope alleles, have no effect on radiographic joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2011; 70(8):1461-4.

- (29) Ursum J, Bos WH, van Dillen N, Dijkmans BA, van Schaardenburg D. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010; 12(1):R8.
- (30) Bang H, Egerer K, Gauliard A, Luthke K, Rudolph PE, Fredenhagen G et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum* 2007; 56(8):2503-11.
- (31) Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis Rheum* 2004; 50(11):3485-94.
- (32) Adams GP, Schier R, McCall AM, Simmons HH, Horak EM, Alpaugh RK et al. High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. *Cancer Res* 2001; 61(12):4750-5.
- (33) Rudnick SI, Lou J, Shaller CC, Tang Y, Klein-Szanto AJ, Weiner LM et al. Influence of Affinity and Antigen Internalization on the Uptake and Penetration of Anti-HER2 Antibodies in Solid Tumors. *Cancer Res* 2011; 71(6):2250-9.
- (34) GERMUTH FG, Jr. A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J Exp Med* 1953; 97(2):257-82.
- (35) Delgado MF, Coviello S, Monsalvo AC, Melendi GA, Hernandez JZ, Batalle JP et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009; 15(1):34-41.
- (36) Polack FP, Hoffman SJ, Crujeiras G, Griffin DE. A role for nonprotective complement-fixing antibodies with low avidity for measles virus in atypical measles. *Nat Med* 2003; 9(9):1209-13.
- (37) Daha NA, Banda NK, Roos A, Beurskens FJ, Bakker JM, Daha MR et al. Complement activation by (auto-) antibodies. *Mol Immunol* 2011; 48(14):1656-65.
- (38) Kaufman EN, Jain RK. Effect of bivalent interaction upon apparent antibody affinity: experimental confirmation of theory using fluorescence photobleaching and implications for antibody binding assays. *Cancer Res* 1992; 52(15):4157-67.

