Heterogeneity of Anticitrullinated Peptide Antibodies and Response to Anti-Tumor Necrosis Factor Agents in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To examine fine specificity of anticitrullinated peptide antibodies (ACPA) in relation to responsiveness to anti-tumor necrosis factor (anti-TNF) agents in rheumatoid arthritis (RA).

Methods. Samples from 450 patients with RA treated with anti-TNF agents were analyzed for antibodies to citrullinated α-enolase, vimentin, and fibrinogen peptides. The Disease Activity Score-28 was measured at baseline and 6 months.

Results. Both anti-cFib antibodies and the number of citrullinated peptides recognized were associated with a poorer response. These findings were not significant following stratification for anti-cyclic citrullinated peptide 2 antibodies.

Conclusion. The presence of any ACPA rather than individual ACPA specificities was associated with a poorer response to anti-TNF agents. We suggest that this reflects distinctive differences in the pathogenesis of ACPA-positive and negative RA. (J Rheumatol First Release April 1 2012; doi:10.3899/jrheum.111315)

Key Indexing Terms:
ANTICITRULLINATED PEPTIDE ANTIBODIES
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The second-generation cyclic citrullinated peptide assay (CCP2) is widely used for the diagnosis of rheumatoid arthritis (RA). However, the peptides it contains were selected from a library in order to maximize diagnostic sensitivity. Consequently it may fail to differentiate subsets of patients with antibodies to particular *in vivo* citrullinated proteins[1,2].

One of the most important subsets in clinical practice consists of nonresponders to anti-tumor necrosis factor (anti-TNF) agents. In the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS) cohort, the presence of anti-CCP antibodies was associated with a poorer response to anti-TNF agents[3]. However, a model comprising both clinical and serological predictors could account for only a small proportion of variance in drug response. As the anti-CCP assay is a generic test for anticitrullinated protein/peptide antibodies (ACPA) this does not account for the possibility that antibodies to specific citrullinated peptides could have greater predictive value. A number of different citrullinated antigens have been reported as reactive with RA sera[4], but only a few have been mapped to immunodominant peptides and their specificity and sensitivity reproducibly examined in several laboratories. These include citrullinated α-enolase (CEP-1, amino acids 4–21)[1,5,6,7,8], vimentin (cVim, amino acids 60–75)[2,8,9,10,11,12], and fibrinogen ß chain (cFib, amino acids 36–52)[8,10,11,12]. The apparent preferential association of anti-CEP-1 and cVim antibodies with important genetic and environmental risk factors for RA[1,2,10] led us to investigate whether these also segregated with clinical response.

MATERIALS AND METHODS

Patient selection, recruitment, and sample collection were as described[3]. Serum samples from 450 patients with RA enrolled in BRAGGSS were available for analysis. Informed consent and ethical approval were obtained.

Serum samples were analyzed for antibodies to CEP-1 (CKIHACitEIFDSCitGNPTVEC), cVim (CVYATCitSSAVCitLCitSSVPC), and cFib (CNEEGFFSACitGHRPLDKKC) peptides by in-house ELISA. Cysteine residues were added at the amino- and carboxy-termini of each peptide to facilitate cyclization, and antibodies to the corresponding arginine-containing control peptides were measured in parallel to ensure positive results were specific for the citrullinated peptide. Anti-CCP2 antibodies were analyzed using a commercial ELISA (Diastat, Axis-Shield, Dundee, UK).

The primary outcome measure was change in 28-joint Disease Activity Score (DAS28) between baseline and 6 months. Associations with the magnitude of change were tested by linear regression. The European League Against Rheumatism (EULAR) response criteria were a secondary outcome measure and associations were tested by ordinal logistic regression.

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RESULTS

Baseline characteristics are detailed in Table 1 and are comparable to those previously reported for the wider British Society for Rheumatology Biologics Register population[3].

Antibodies to CEP-1, cVim, and cFib were positive in 39%, 42%, and 75% of samples, respectively. In comparison, 84% were positive for anti-CCP antibodies and 87% for rheumatoid factor. Only 30 sera (7%) had antibodies to CCP2 but not to 1 or more of cFib, cVim, and CEP-1. Conversely, 19 sera (5%) had antibodies to 1 or more of these 3 peptides, but not to CCP.

Antibodies to cFib, but not CEP-1 or cVim, were associated with a smaller reduction in DAS28 scores at 6 months, indicating a poorer outcome (Table 2). This was statistically significant when analyzed by linear regression (coefficient 0.37; p = 0.02) and controlling for baseline DAS28, concomitant use of disease-modifying antirheumatic drugs (DMARD), sex, Health Assessment Questionnaire results, and smoking status, which were all found to be predictive of response in this cohort.

There was also a trend toward a poorer EULAR response with anti-cFib antibodies and the same adjustments (OR 0.68 for response, 95% CI 0.42, 1.02; p = 0.06), but not with anti-CEP-1 (OR 0.90) or anti-cVim antibodies (OR 1.01).

Patients with antibodies to both CCP2 and cFib (n = 303) were compared to those with antibodies to CCP2 but not cFib (n = 49) in a linear regression model with the same adjustments and with the double antibody-negative patients as referent. Coefficients were 0.55 (95% CI 0.13, 0.97; p = 0.01) and 0.31 (95% CI –0.24, 0.8; p = 0.27), respectively, which did not differ significantly, and the adjusted R^2 for this model did not differ from that with anti-CCP2 alone (both 0.15). The number of positive assays incorporating citrullinated peptides was associated with response, with a coefficient of 0.13 (p = 0.03). Thus the group with 4 positive assays had a mean DAS28 score at 6 months that was 0.57 higher than the group with none (Figure 1). However, there was no association with peptide number when the analysis was restricted to patients with anti-CCP2 antibodies (coefficient 0.02; p = 0.78). These results did not differ according to the anti-TNF inhibitor used (data not shown).

DISCUSSION

Recently, attention has focused on the "fine specificity" of the

Table 1. Baseline demographics of the 450 patients with rheumatoid arthritis included in this study. Values indicate number (%) or mean (SD).

Characteristics	Etanercept	Infliximab	Adalimumab	Combined
RA patients (%)	189 (42.0)	223 (49.6)	38 (8.4)	450 (100)
Female (%)	142 (75.1)	156 (70.0)	33 (86.8)	331 (73.6)
Age (SD)	56.0 (11.5)	57.4 (10.9)	59.8 (11.0)	57.0 (11.2)
Disease duration, yrs (SD)	12.6 (9.3)	15.1 (10.0)	13.9 (11.0)	13.9 (9.9)
HAQ (SD)	2.0 (0.5)	2.2 (0.5)	2.2 (0.5)	2.1 (0.5)
DAS28 (SD)	6.6 (0.95)	6.5 (0.93)	6.5 (0.91)	6.6 (0.94)
Current smokers (%)	37 (19.6)	44 (19.7)	2 (5.3)	83 (18.4)
Ex-smokers (%)	75 (39.7)	83 (37.2)	19 (50)	177 (39.3)
Concurrent DMARD use (%)	113 (59.8)	211 (94.6)	28 (73.7)	352 (78.2)
Concurrent steroid use (%)	67 (35.5)	91 (40.8)	12 (31.6)	170 (37.8)
Previous biologic use (%)	22 (11.6)	12 (5.4)	1 (2.6)	35 (7.8)

HAQ: Health Assessment Questionnaire; DAS28: 28-joint Disease Activity Score; DMARD: disease-modifying antirheumatic drug.

Table 2. Change in DAS28 scores and EULAR response criteria following 6 months of anti-TNF therapy in anti-CEP-1, anti-cVim, and anti-cFib antibody-positive and negative patients with RA. Values indicate number (%) or mean (SD). Linear regression adjusted for baseline DAS28, concomitant DMARD, sex, HAQ, and smoking.

	Anti-CEP-1		Anti-cVim		Anti-cFib	
	Negative, n = 276	Positive, n = 174	Negative, n = 252	Positive, n = 184	Negative, n = 108	Positive, n = 328
Baseline DAS28 (SD)	6.57 (0.94)	6.56 (0.93)	6.58 (0.96)	6.54 (0.91)	6.60 (0.99)	6.55 (0.92)
Change in DAS28 (SD)	–2.50 (1.52)	–2.41 (1.58)	–2.50 (1.53)	–2.43 (1.57)	–2.78 (1.50)	–2.37 (1.55)
Linear regression coefficient (95% CI)	0.04 (–0.24, 0.32), p = 0.77		0.06 (-0.22, 0.33), p = 0.69		0.37 (0.06, 0.69), p = 0.02	
No response (%)	57 (20.7)	39 (22.4)	54 (21.4)	38 (20.7)	20 (18.5)	72 (22.0)
Moderate response (%)	140 (50.7)	89 (51.2)	127 (50.4)	95 (51.6)	51 (47.2)	171 (52.1)
Good response (%)	79 (28.6)	46 (26.44)	71 (28.2)	51 (27.7)	37 (34.3)	85 (25.9)

DAS28: Disease Activity Score-28; DMARD: disease-modifying antirheumatic drug; HAQ: Health Assessment Questionnaire.

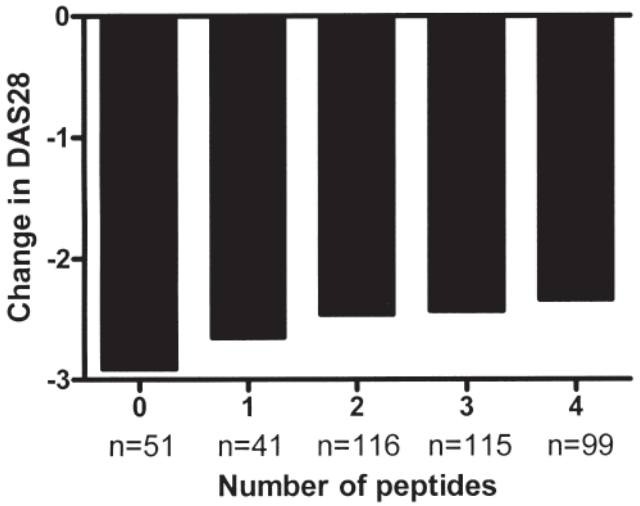

Figure 1. Relationship between change in DAS28 scores after 6 months of anti-tumor necrosis factor treatment and number of positive antibody tests for citrullinated peptides.

ACPA response[1,2,8,10,11]. We have demonstrated that antibodies to cFib are associated with a poorer response to anti-TNF agents at 6 months in patients with RA. In contrast, antibodies to CEP-1 and cVim, which have recently been shown to be strongly associated with the HLA-DRB1 shared epitope (SE)[1,2], were not. These results are consistent with neither the SE nor *PTPN22* predicting response to anti-TNF agents[3], with the same being true for most other RA susceptibility genes[13]. However, the association with anti-cFib antibodies might simply reflect their high frequency in patients with anti-CCP2 antibodies, with the key serological determinant of response being positivity to ACPA, rather than ACPA specificity. Increasing evidence suggests important differences between the pathogenesis of ACPA-positive and ACPA-negative RA. Such evidence includes different HLA associations and other genetic and environmental risk factors that segregate only with ACPA-positive disease. ACPA are also associated with greater disease severity, joint erosions, and possibly responsiveness to methotrexate in early disease (reviewed by Klareskog, *et al*[14]). In accord with this, no statistically significant difference was shown between anti-CCP2-positive patients with and those without anti-cFib antibodies. In addition, we found that the number of peptides recognized by RA sera was associated with response. Antibodies to a higher number of citrullinated peptides are associated with the onset of RA[8,11], and it is possible that this might reflect a more mature immune response that is harder to inhibit with anti-TNF agents. Again, however, this finding was not significant when restricted to patients with anti-CCP2 antibodies, indicating no additional predictive value.

Limitations to our study have been discussed in detail[3]. Of note, serum samples were not taken prior to treatment with anti-TNF agents and antibody status might have changed subsequent to this. However, anti-CCP2 antibodies do not show a large magnitude of change following anti-TNF inhibitor therapy[15] and a change in status from positive to negative is unusual[3]. While we have not been able to demonstrate that antigen-specific ACPA testing shows promise in predicting treatment response, consistent with recent findings for other clinical outcomes[16,17], 2 possibilities have not been excluded. First, it remains possible that there is an unidentified citrullinated autoantigen with a more marked association with poor response, since research into specific ACPA has been relatively recent. Second, our results may have been confounded by differences in dose of concomitant DMARD[16] or the ability of the anti-CCP assay to detect multiple specificities. The latter possibility could be investigated with the multiplex assays for ACPA that are currently in development. However, our findings in this cohort suggest that the presence of any ACPA rather than individual or multiple ACPA specificities is associated with a poorer response to anti-TNF agents. The distinctive difference therefore lies in the differing pathogenesis of ACPA-positive and ACPA-negative RA.

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APPENDIX

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REFERENCES

1. Mahdi H, Fisher BA, Källberg H, Plant D, Malmström V, Rönnelid J, et al. Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis. Nat Genet 2009;41:1319-24.
2. van der Woude D, Alemayehu WG, Verduijn W, de Vries RR, Houwing-Duistermaat JJ, Huizinga TW, et al. Gene-environment interaction influences the reactivity of autoantibodies to citrullinated antigens in rheumatoid arthritis. Nat Genet 2010;42:814-6.
3. Potter C, Hyrich KL, Tracey A, Lunt M, Plant D, Symmons DP, et al. Association of rheumatoid factor and anti-cyclic citrullinated peptide positivity, but not carriage of shared epitope or PTPN22 susceptibility variants, with anti-tumour necrosis factor response in rheumatoid arthritis. Ann Rheum Dis 2009;68:69-74.
4. Wegner N, Lundberg K, Kinloch A, Fisher B, Malmström V, Feldmann M, et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev 2010;233:34-54.
5. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum 2008;58:3009-19.
6. Snir O, Widhe M, von Spee C, Lindberg J, Padyukov L, Lundberg K, et al. Multiple antibody reactivities to citrullinated antigens in sera from patients with rheumatoid arthritis: Association with HLA-DRB1 alleles. Ann Rheum Dis 2009;68:736-43.
7. Montes A, Dieguez-Gonzalez R, Perez-Pampin E, Calaza M, Mera-Varela A, Gomez-Reino JJ, et al. Particular association of clinical and genetic features with autoimmunity to citrullinated a-enolase in rheumatoid arthritis. Arthritis Rheum 2011;63:654-61.
8. van de Stadt LA, van der Horst AR, de Koning MH, Bos WH, Wolbink GJ, van de Stadt RJ, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Ann Rheum Dis 2011;70:128-33.
9. Snir O, Widhe M, Hermansson M, von Spee C, Lindberg J, Hensen S, et al. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. Arthritis Rheum 2010;62:44-52.
10. Verpoort KN, Cheung K, Ioan-Facsinay A, van der Helm-van Mil AH, de Vries-Bouwstra JK, Allaart CF, et al. Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. Arthritis Rheum 2007;56:3949-52.
11. van der Woude D, Rantapää-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;69:1554-61.
12. Pratt AG, Charles PJ, Chowdhury M, Wilson G, Venables PJ, Isaacs JD. Serotyping for an extended anti-citrullinated peptide autoantibody panel does not add value to CCP2 testing for diagnosing RA in an early undifferentiated arthritis cohort. Ann Rheum Dis 2011;70:2056-8.
13. Tan RJ, Gibbons LJ, Potter C, Hyrich KL, Morgan AW, Wilson AG, et al. Investigation of rheumatoid arthritis susceptibility genes identifies association of AFF3 and CD226 variants with response to anti-tumour necrosis factor treatment. Ann Rheum Dis 2010;69:1029-35.
14. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol 2008;26:651-75.
15. Bobbio-Pallavicini F, Caporali R, Alpini C, Moratti R, Montecucco C. Predictive value of antibodies to citrullinated peptides and rheumatoid factors in anti-TNF-alpha treated patients. Ann NY Acad Sci 2007;1109:287-95.
16. Fisher BA, Plant D, Brode M, van Vollenhoven RF, Mathsson L, Symmons D, et al. Antibodies to citrullinated a-enolase peptide 1 and clinical and radiological outcomes in rheumatoid arthritis. Ann Rheum Dis 2011;70:1095-8.
17. 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;70:1461-4.