Effect of Rheumatoid Arthritis (RA) Susceptibility Genes on the Immune Response to Viral Citrullinated Peptides in RA

FEDERICO PRATESI, ELISABETH PETIT-TEIXEIRA, JOHN SIDNEY, VITOR HUGO TEIXEIRA, ILARIA PUXEDDU, ALESSANDRO SETTE, FRANCOIS CORNELIS and PAOLA MIGLIORINI

J Rheumatol 2012;39;1490-1493
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Effect of Rheumatoid Arthritis (RA) Susceptibility Genes on the Immune Response to Viral Citrullinated Peptides in RA

To the Editor:

Anticitrullinated peptide/protein antibodies (ACPA) are a family of rheumatoid arthritis (RA)-specific antibodies that recognize various protein sequences containing citrulline, the deiminated form of arginine produced posttranslationally by peptidylarginine deiminase (PAD) enzymes. Recently, viral citrullinated peptides (VCP) derived from Epstein-Barr virus nuclear proteins EBNA-1 and EBNA-2 have been described as a target of ACPA in RA sera1,2.

Genetic factors predisposing to RA, namely HLA-DRB1 shared epitope (SE) alleles and PTPN22 gene variants, as well as the TRAF1/C5 locus and PAD4 susceptible haplotype, have been found to be predominately associated with ACPA-positive RA, even if the association with ACPA-negative RA cannot be excluded.

We evaluated the influence of genes conferring susceptibility to RA on the production of anti-VCP antibodies and tested the interaction of VCP with HLA in vitro. We analyzed 172 patients with RA of French white origin3. All provided informed consent and the Ethics Committee of Hôpital Bicêtre (AP-HP, Paris, France) approved the study.

HLA-DRB1 typing and subtyping were performed by a polymerase chain reaction (PCR)-based method, using a panel of sequence-specific oligonucleotide probes. PAD4 haplotypes were obtained by typing 3 single-nucleotide polymorphisms (SNP: rs1886303, PAD4_92, PAD4_96 – CGC haplotype) by PCR-restriction fragment-length polymorphism (RFLP)4. PTPN22 (R620W; rs24766601 – T variant) and C5/TRAF1 region polymorphisms (rs10818488 – A variant) were genotyped by PCR-RFLP5,6.

VCP1 (citrullinated EBNA155-59: GGDNHGCitGcitGcitGcit GcitGGGcitPGAPG) and VCP2 (citrullinated EBNA2338-358: GQSCitGQSCitGcitGcitGcitGKG) were synthesized either as linear peptides (for the peptide-binding assay) or as multiple antigen peptide (MAP) linear peptides (for the peptide-binding assay) or as multiple antigen peptides (for ELISA). Anti-VCP antibodies were detected by ELISA on VCP1 or VCP2 coated plates2, setting the upper limit of normality at the 98.5 percentile of 100 normal controls. Anti-cyclic citrullinated peptide antibodies were detected using an anti-CCP2 kit.

Anti-VCP1 antibodies were detected in 78/172 (45%) patients with RA, anti-VCP2 in 106/172 (62%), and anti-CCP2 in 130/172 (76%). The presence of at least 1 SE allele was not associated with the presence of either anti-CCP2 or anti-VCP antibodies. By contrast, 2 copies of the HLA-SE alleles conferred an increased risk to produce anti-CCP2 (p = 0.01) or anti-VCP2 (p = 0.05) antibodies with respect to 1 or no SE allele.

Analyzing the contribution of individual SE alleles, the *0401 allele conferred an increased risk to produce anti-VCP2 (p = 0.007) and anti-CCP2 (p = 0.02) antibodies; *0404 was associated with the production of anti-VCP1 (p = 0.05) and anti-VCP2 (p = 0.04) antibodies (Table 1).

Patients were subdivided into 3 groups: those carrying no SE alleles (n = 34), 1 SE allele (n = 112), or 2 SE alleles (n = 26). The mean anti-VCP level increased as a function of the SE status; the level of anti-VCP2 antibodies was significantly higher in the double-SE patients compared to the negative patients (p = 0.01; Figure 1B). Anti-VCP1 antibodies were similarly higher in double-HLA-SE patients than in SE-negative patients, but for this there was only a trend to significance (p = 0.06; Figure 1A). Patients carrying *0401 or *0404 alleles had higher levels of anti-VCP1 (p < 0.05; Figure 1C, 1E) and anti-VCP2 antibodies (p < 0.05; Figure 1D, 1F). In contrast, PTPN22, TRAF1/C5, and PAD4 polymorphism did not influence the production or the level of the anti-VCP or anti-CCP2 antibodies (Table 1).

Finally, competitive peptide binding assays were conducted to determine the affinity of VCP1 and VCP2 for purified MHC molecules (DRB1*0101, *0401, *0404, *0301, *0701, *0802, *1101, and *1302) as described7,8. The nanomolar concentration of unlabeled viral peptides necessary for 50% inhibition of the labeled peptide to the purified

Table 1. The association between genetic factors and ACPA was tested in 172 RA patients by comparing (Fisher exact test) the distribution of patients positive and negative for anti-VCP antibodies among carriers and noncarriers for each genetic variant.

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR for developing anti-VCP antibodies in the presence of susceptible genes (HLA-SE, PTPN22, TRAF1.C5, PAD4)</th>
<th>OR for developing anti-VCP antibodies in the presence of HLA-DRB1 shared epitope alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-SE</td>
<td>Neg 21 12 (0.734–3.687) 1.646 0.23 15 18 1.409 0.42 11 22 1.862 0.17</td>
<td>Neg 66 48 (0.377–3.952) 0.561 0.15 45 84 0.561 0.1 31 98 0.92 0.83</td>
</tr>
<tr>
<td>PTPN22</td>
<td>Yes 73 66 1.473 0.25 44 70 1.028 1 30 84 1.036 0.45</td>
<td>Yes 57 38 1.621 0.126 54 50 2.4 0.007 30 65 2.5 0.02</td>
</tr>
<tr>
<td>TRAF1.C5</td>
<td>Yes 28 30 (0.780–2.780) 1.082 0.87 22 37 0.934 0.87 17 42 1.424 0.35</td>
<td>Yes 37 40 (0.883–2.975) 1.222 0.77 62 98 1.265 0.76 41 119 3.789 0.29</td>
</tr>
<tr>
<td>PAD4</td>
<td>Yes 26 22 1.807 0.2 28 20 1.108 0.83 38 10 1.005 1</td>
<td>Yes 88 72 (0.377–3.952) 1.216 1 63 101 1.039 1 40 124 0.967 1</td>
</tr>
<tr>
<td>HLA-DRB*0101</td>
<td>Yes 28 15 (0.273–2.975) 0.293 0.05 63 90 2.3 0.007 40 113 3.008 0.16</td>
<td>Yes 4 4 (0.294–5.029) 3 5 (0.240–4.501) 2 6 (0.187–4.986) 20</td>
</tr>
<tr>
<td>HLA-DRB*0401</td>
<td>Yes 37 40 (0.883–2.975) 1.222 0.77 62 98 1.265 0.76 41 119 3.789 0.29</td>
<td>Yes 22 12 (0.273–1.396) 0.595 0.24 52 86 0.863 0.7 30 108 0.509 0.12</td>
</tr>
<tr>
<td>HLA-DRB*0404</td>
<td>Yes 28 15 (0.273–2.975) 0.293 0.05 63 90 2.3 0.007 40 113 3.008 0.16</td>
<td>Yes 66 63 0.561 0.15 45 84 0.561 0.1 31 98 0.92 0.83</td>
</tr>
<tr>
<td>HLA-DRB*1100</td>
<td>Yes 28 15 (0.274–1.148) 1.216 1 63 101 1.039 1 40 124 0.967 1</td>
<td>Yes 28 15 (0.274–1.148) 1.216 1 63 101 1.039 1 40 124 0.967 1</td>
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Figure 1. Levels of anti-VCP1 and anti-VCP2 antibodies were analyzed in patients with rheumatoid arthritis subdivided according to HLA-SE (A, B), HLA-DRB1*0401 (C, D), and HLA-DRB1*0404 status (E, F); Kruskal-Wallis test, with Dunn’s multiple comparison test when appropriate and Mann-Whitney U test.
HLA-DRB1 molecules (IC_{50}) was used as an approximation of the affinity of interaction (KD), with expression of results as the inverse of the IC_{50} values measured in nanomolar concentration. Biologically relevant binding (IC_{50} ≤ 5000 nM) was not detected with either peptide for any of the HLA class II molecules tested.

These results indicate that the immune response to citrullinated viral antigens is associated with SE alleles, and in particular with *0401 and *0404. A strong gene-dose effect was observed on both the production and the level of anti-VCP antibodies. Using competitive assays, no binding of the viral sequences to any SE allele was detected.

DRB1*0404 is associated with the production of anti-VCP antibodies and *0401 with anti-VCP and anti-CCP. Similarly to our findings, it has been reported that *0401 and/or *0404 are associated with the production of anti-CCP, anti-citrullinated fibrinogen, and anti-citrullinated enolase peptide 1 antibodies.

We could not detect any influence of PTPN22, TRAF1/C5, and PAD4 on the immune response to VCP and CCP. Lack of statistical power because of the small sample size is likely the cause of these negative findings; associations between these genes and anti-CCP have in fact been found in larger cohorts.

The molecular mechanisms underlying the effect of DRB1 on ACPA production are unknown. It has been proposed that the conversion of arginine into citrulline allows a high affinity interaction with the positively charged pocket of SE alleles. Experimental evidence in support of this hypothesis was obtained in the case of the *0401 allele, which interacted strongly in vitro with a citrullinated vimentin peptide, while the arginine-containing sequence has a low binding affinity. It has been reported that purified DRB1 molecules bind to a similar extent to fibrinogen-derived peptides containing arginine or citrulline. These results, however, were obtained by a qualitative direct binding assay method with sensitivity not comparable to the competitive binding assay. In our study, no binding of citrullinated peptides to HLA alleles was detected by the same stringent competitive assay, with sensitivities in the nanomolar range used for the citrullinated vimentin peptide. It is of interest that despite the association of anti-CCP2 and HLA-DRB1*0401, no binding of the peptide to the purified HLA molecule was detected.

The immune response to VCP is under the genetic control of the *0401 and *0404 alleles, but these molecules do not bind the citrullinated peptides. Thus, the differential binding properties of DRBI* alleles for either arginine- or citrulline-containing sequences do not always explain the HLA control of the immune response to citrullinated epitopes.

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J Rheumatol 2012;39:7; doi:10.3899/jrheum.111548