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Commentary

Citrullination, a possible functional link between susceptibility genes and rheumatoid arthritis

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Abstract

Antibodies directed to citrullinated proteins (anti-cyclic citrullinated peptide) are highly specific for rheumatoid arthritis (RA). Recent data suggest that the antibodies may be involved in the disease process of RA and that several RA-associated genetic factors might be functionally linked to RA via modulation of the production of anti-cyclic citrullinated peptide antibodies or citrullinated antigens.

Keywords: anti-cyclic citrullinated peptide autoantibodies, citrullination, genetic susceptibility, peptidylarginine deiminase, rheumatoid arthritis

Introduction

The serum of rheumatoid arthritis (RA) patients contains a variety of antibodies directed against self-antigens. The most widely known of these autoantibodies is the rheumatoid factor; antibodies directed against the constant domain of IgG molecules (reviewed in [1]). The rheumatoid factor can only be detected in roughly 75% of RA patients, but also in the serum of patients with other rheumatic or inflammatory diseases, and even in a substantial percentage of the healthy (elderly) population [2]. Its presence is therefore not very specific for RA.

Autoantibodies directed against citrullinated proteins have a much higher specificity for RA (reviewed in [3]). This family of autoantibodies includes the anti-perinuclear factor, the so-called anti-'keratin' antibodies, anti-filaggrin antibodies, anti-cyclic citrullinated peptide (anti-CCP) antibodies and probably also anti-Sa antibodies (for references see [3]). These autoantibodies all recognize epitopes containing citrulline (the naming of the antibody is simply determined by the substrate used to detect them).

Because citrulline is a nonstandard amino acid, it is not incorporated into proteins during translation. It can, however, be generated by post-translational modification (citrullination) of protein-bound arginine by peptidylarginine deiminase (PAD) (EC 3.5.3.15; reviewed in [4]) enzymes (corresponding genes are annotated as PADI).

Anti-citrullinated protein antibodies can be detected (with the CCP2 assay) in up to 80% of RA sera with a specificity of 98%. Besides being very specific for RA, the antibodies can be detected very early in the disease and can predict clinical disease outcome. Furthermore, the antibodies are produced locally in the inflamed synovium, suggesting that they might play a role in the disease process (for references see [3]).

Because citrullinated proteins (e.g. fibrin) have been detected in the synovium of RA patients [5], PAD enzymes must also be present. At least five isotypes of PAD exist in mammals; two of these isotypes (PAD2 and PAD4) are known to be expressed in hemopoietic cells (for references see [4]) and are expressed in the RA synovium [6]. Of special interest is the PAD4 enzyme, which is normally present in the nucleus of granulocytes and CD14+ monocytes, because genetic polymorphisms in the gene encoding this enzyme are associated with RA.
Figure 1

<table>
<thead>
<tr>
<th>SNP ID*</th>
<th>non-susceptible</th>
<th>susceptible</th>
<th>amino acid character</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>89</td>
<td>S AGC</td>
<td>polar → non-polar</td>
<td>0.07</td>
</tr>
<tr>
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<td>A GCG</td>
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<td>0.007</td>
</tr>
<tr>
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<td>92</td>
<td>A GCG</td>
<td>non-polar → non-polar</td>
<td>0.0004</td>
</tr>
<tr>
<td>4</td>
<td>104</td>
<td>L CTG</td>
<td>no substitution</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Summary of the four exonal single nucleotide polymorphisms (SNPs) in PADI4. The actual SNP is indicated in bold. The amino acid that shows most conservation with other known peptidylarginine deiminases [4] is shaded gray. SNP ID* according to Suzuki and colleagues as padi4_x [7].

PAD4 polymorphisms are associated with RA

The existence of numerous single nucleotide polymorphisms (SNPs) in the PADI gene cluster (located on chromosome 1p36 [4]) was recently described by Suzuki and colleagues [7]. Eight of the 17 SNPs in PADI4 were strongly associated ($P < 0.001$) with RA, whereas SNPs in the other PADI genes were not. Because the SNPs within PADI4 are in strong linkage disequilibrium, they segregate together in distinct haplotypes. The two most frequent haplotypes account for more than 85% of all individuals. One of these two haplotypes (referred to as the susceptible haplotype) was more frequent in RA patients than in controls (case: control ratio = 1.28 versus 0.87 for the nonsusceptible haplotype).

Four of the 17 SNPs in PADI4 are located in exons of PADI4. Although three of them result in amino acid substitutions (Fig. 1), possible consequences for the function and activity of the PADI4 enzyme were not analyzed. The three SNPs leading to amino acid changes all appear at nonconserved places, as can be deduced from an alignment of PADI sequences (segment in Fig. 2; for complete alignment see [4]). The susceptible haplotype is more closely conserved to PADI4 sequences of other species (two of the three positions conserved) than the nonsusceptible haplotype (one of the three positions conserved). Interestingly, the fourth SNP, which does not lead to an amino acid substitution, is at a 100% conserved position. Only one of the three amino acid substitutions leads to a change in the electrostatic character of the residue. This SNP (padi4_89) is located directly before the nuclear localization signal of PADI4 [8]. The nuclear localization signal was originally described in the nonsusceptible sequence [8]. The susceptible haplotype is conserved with the mouse sequence at this position and the mouse PAD4 also locates to the nucleus (our unpublished observations). Therefore, consequences for subcellular localization of the enzyme are not very likely. It would still be very interesting, however, to investigate possible effects of the amino acid substitutions on the functional properties of the enzyme (e.g. substrate specificity, calcium dependence, catalytic rate).

Eight of the 17 SNPs were significantly associated with RA ($P < 0.001$); only two of these were exonal SNPs ($P$ values presented in Fig. 1). Only one of these two SNPs

Figure 2

Multiple alignment of partial peptidylarginine deiminase (PAD) protein sequences based on a large full alignment described in [4] (available online: http://www.mrw.interscience.wiley.com/suppmat/2003/25/v/25.1106.html). Shown are segments of all five isotypes from the human (Homo sapiens [Hs], PADI1 NM_0037490, PADI2 NM_0031391, PADI3 NM_057317, PADI4 NM_036519 and PADI6 XP_210118) and segments of PAD4 from the mouse (Mus musculus [Mm], NP_058923), the rat (Rattus norvegicus [Rn], NP_058923) and the cow (Bos taurus [Bt], based on BG364986). Conserved residues that are identical in more than 50% of all known PAD sequences are shaded black; fully conserved residues are shaded cyan. Conserved charged residues are also indicated (shaded light gray). Exon boundaries, based on PADI1 sequences, are annotated above the alignment. The monopartite nuclear localization signal (NLS) of PAD4 is shaded green, and conserved NLS residues are bold [8]. The four exonal single nucleotide polymorphisms are shaded pink. The nonsusceptible haplotype (S A A L) is shown in the alignment, and the susceptible (G V G L) haplotype is indicated below it. a.a., amino acid.
Correlation between the PAD4 haplotype and autoantibodies to citrullinated proteins (anti-filaggrin antibodies [AFA]). Homozygous susceptible (homo suscept.) rheumatoid arthritis (RA) patients \((n = 30)\) are significantly more often AFA-positive than homozygous nonsusceptible (homo non-suscept.) RA patients \((n = 33)\) or heterozygous (hetero) RA patients \((n = 66)\) [7].

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death or defects in clearing machinery [13]) this could lead to exposure of the citrullinated proteins to the immune system. Citrullinated proteins may not be recognized as ‘self’ because they have been post-translationally modified, which has consequences for their charge and their structure [4,14]. Many known autoantigens become modified during cell death and, in particular, during apoptosis (for an overview see [15]).

B Correlation between RA and certain human leukocyte antigen haplotypes (e.g. HLA-DR4 [HLA-DRB1*0401 and HLA-DRB1*0404]) has been known for more than 25 years [16]. Recent molecular modeling data indicate that peptides containing citrulline, but not the corresponding arginine variant of the peptide, can efficiently be bound by HLA-DRB1*0401 major histocompatibility complex molecules [17] (Fig. 4b). This citruline-specific interaction might be the basis of a citrulline-specific immune response. T-cell proliferation assays with HLA-DRB1*0401 transgenic mice showed that stimulation with citrullinated peptides, but not with the corresponding arginine peptides, induced proliferation and activation of T cells [17]. Although there is no absolute requirement for HLA-DR4 in order to develop anti-CCP antibodies, there is a strong correlation between HLA-DR4 status and anti-CCP positivity in RA patients [18].

A specific SNP in the IL-10 promoter (−2849[AG/GG]) is associated with high IL-10 production [19]. IL-10 is a pleiotropic cytokine with many anti-inflammatory functions, but it can also stimulate inflammation by enhancing B-cell proliferation, differentiation and antibody production. Anti-CCP-positive RA patients with the ‘high IL-10 haplotype’ have significantly higher anti-CCP titers and more severe erosions than anti-CCP-positive patients with a ‘low IL-10 haplotype’ [19] (Fig. 4c). The anti-CCP antibodies that are locally produced in the inflamed synovium [20] will form immune complexes with locally produced citrullinated proteins [5]. Higher titers of the anti-CCP antibodies allow the formation of more immune complexes, which can be bound by inflammatory cells via their Fcγ receptors. This will activate these cells and cause the release of extra proinflammatory cytokines.

D Various polymorphisms in proinflammatory cytokines and their receptors (for references see [21,22]) are thought to be associated with RA (Fig. 4d). These genetic factors cause the release of larger amounts of cytokines upon stimulation or cause cells to be more sensitive towards these cytokines. The cytokines are the motor of the inflammation, causing influx and activation of more inflammatory cells. These cells will eventually die, allowing their PAD enzymes to become activated by influxing Ca2+. With this the cycle is complete and will continue if not stopped. The cycle will ultimately lead to the chronic inflammatory disease we call RA.

Besides these genetic factors, other susceptibility loci might also be involved. Their precise nature needs to be clarified in order to understand their possible role in the triggering or progression of RA.

Concluding remarks
Recent literature on anti-CCP antibodies (reviewed in [3]) suggests that the antibodies might be involved in the disease process of RA. The antibodies are very specific for the disease, they are present very early in the disease and their presence is correlated with a more severe disease outcome. Anti-CCP antibodies and citrullinated antigens are also both produced at the site of inflammation. Furthermore, drops in anti-CCP titers during rituximab therapy or infliximab therapy are correlated with clinical improvement [23] (G Valesini, personal communication, 2003).

The very interesting study by Suzuki and colleagues [7], showing an association of PADI4 genetic polymorphisms with RA underlines the relationship between citrullination and RA. Their study, however, leaves open some intriguing research questions. What are the effects of the amino acid substitutions on the enzymatic function of PAD? What are the effects on PAD enzyme levels in vivo? How are these PADI4 SNPs distributed in a non-Japanese population? The answers to these and other questions will undoubtedly give a better insight in the etiology of this enigmatic disease.

Competing interests
None declared.

References


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