Clinical Significance of Anti-CCP Antibodies in Rheumatoid Arthritis

Tsuneyo Mimori

Abstract

A number of novel autoantibodies have been recently described in rheumatoid arthritis (RA), and their clinical significance and possible pathogenic roles have been discussed. In particular, new autoantibodies to citrullinated proteins such as filaggrin and its circular form (cyclic citrullinated peptide: CCP) are especially noteworthy because of their high sensitivity and high specificity. There are many studies that anti-CCP antibodies may serve as a powerful serologic marker for early diagnosis of RA and prognostic prediction of joint destruction. Anti-citrullinated protein antibodies are locally produced in RA joints, and citrullinated proteins (most are fibrins) are localized in RA synovial tissue. This finding strongly suggests a possibility that local citrullination of intraarticular proteins might be the initial event leading to autoantibody production in RA. Genetic factors such as a gene polymorphism of the citrullinating enzyme, PADI, might be associated with the breakage of self-tolerance and induction of autoimmunity against citrullinated proteins.

(Internal Medicine 44: 1122–1126, 2005)

Key words: citrullinated protein, filaggrin, peptidylarginine deiminase, autoantibody

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic and erosive polyarthritis by abnormal growth of synovial tissue or pannus, and causes irreversible joint disability. Recent studies show that joint injury in RA patients progresses within 2 years from onset, and aggressive treatments from the early stage can prevent the following progression of the disease. Hence, the necessities of early diagnosis and early treatment have been emphasized.

However, RA patients do not always show typical symptoms and signs at their early stage, and are often difficult to be diagnosed since they may not fulfill the classification criteria for RA.

RA is also categorized among systemic autoimmune diseases because of the presence of rheumatoid factor (RF), autoantibodies against the Fc portion of IgG, and other autoantibodies. RF has been clinically utilized as the only serologic marker of RA so far. However, the sensitivity of RF is 60–80% in RA, and the specificity is rather low since RF is also detected widely and frequently in many other conditions including various connective tissue diseases, chronic liver diseases and infectious diseases, and even in a few healthy people. Therefore, despite the fact that RF is adopted into the criteria for classification of RA, its diagnostic value is unsatisfactory especially in the early disease.

In recent years, a number of novel autoantibodies have been described in RA, and their clinical significance and possible pathogenic roles have been discussed. In particular, new autoantibodies to citrullinated proteins such as filaggrin and its circular form (cyclic citrullinated peptide: CCP) are the most remarkable because of the reasonable sensitivity and high specificity in RA patients, which may be able to serve as an early diagnostic marker and a prognostic factor of joint destruction. This article reviews and discusses the nature of target autoantigens as well as clinical and possible etiopathogenic significance of anti-citrullinated antibodies in RA.

Identification of Citrullinated Proteins as RA-specific Autoantigens

In the 1960s, autoantibodies termed as anti-perinuclear factor (APF) were first described as RA-specific autoantibodies, which react with keratohyaline granules scattered around the perinuclear region of human buccal epithelial cells in indirect immunofluorescence (1). In the 1970s, so-called anti-“keratin” antibodies (AKA) were reported as other RA-specific autoantibodies recognized by indirect

From Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto
Reprint requests should be addressed to Dr. Tsuneyo Mimori, Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507
immunofluorescent study using rat esophagus cryostat section (2). Although termed as AKA since the keratin-like structure in the cornified layer of esophageal epithelia was specifically stained, the true target antigen had not been clarified. These two antibodies appeared to be highly specific for RA patients, and had been suspected as the same autoantibodies since they tended to be detected simultaneously. However, these autoantibodies had not been in routine clinical use, since target antigens were not identified and there were some practical difficulties in detecting techniques.

In 1993, Simon et al found that 75% of RA patient sera recognized a 40 kDa protein isolated from human skin tissue (3). They finally demonstrated that this protein was the target antigen of AKA by absorption study, and was a molecule called filaggrin by peptide mapping, which was involved in the aggregation of intracellular cytokeratin filaments. They further recognized that AKA and APF had almost the same specificity, because the target molecule of APF was profilaggrin, the precursor molecule of filaggrin (4).

Filaggrin is produced at first as profilaggrin of ~400 kDa in the late stage of skin differentiation and stored in keratohyaline granules of keratinocytes. Profilaggrin is a phosphorylated protein with 10–12 repeated motifs of 324 amino acid sequences (filaggrin unit), which is dephosphorylated and cleaved during keratinization, and turns to filaggrin molecules. Furthermore, arginine residues of filaggrin molecules are converted to citrullines by an enzyme peptidylarginine deiminase (PADI). These citrulline residues on the filaggrin are important for epitopes recognized by RA autoantibodies (5).

Cyclic citrullinated peptide (CCP) is an artificial molecule in which two serine residues in a major epitope peptide from filaggrin are converted to cysteine and the circular form is made by S-S bond (first generation of CCP). It has been reported that the sensitivity in RA patients was increased and the specificity was unchanged by using CCP as the antigen for ELISA (6). However, the results of anti-filaggrin and anti-CCP antibodies are not always identical, suggesting the diversity of autoantigenic epitopes of citrullinated peptides recognized by the heterogeneous population of autoantibodies (5).

Anti-Sa antibodies were reported as RA-specific autoantibodies that recognized an unknown 50 kDa doublet protein in human spleen and placenta extracts. Anti-Sa antibodies are detected in 31–43% of RA patients with very high specificity (>98%) by immunoblotting (7–9). The target Sa antigen was later identified as a citrullinated vimentin (10). Therefore, anti-Sa antibodies are one of the family members of autoantibodies reactive with citrullinated proteins as well as APF, AKA, anti-filaggrin and anti-CCP antibodies.

It is suspected that the filaggrin molecule distributed in the skin and other keratinated epithelia becomes the target of autoimmune response in the joint-affected disease. However, as discussed later, a possibility has been postulated that citrullination of proteins in the joint, rather than the filaggrin molecule itself, may be involved in the autoantibody production and the etiopathogenesis of RA.

**Clinical Significance of Anti-citrullinated Protein Antibodies in Diagnosis of RA**

To date, a number of reports have demonstrated the clinical significance of autoantibodies to citrullinated filaggrin and CCP in the diagnosis of RA as summarized in Table 1 (3, 5, 6, 11–14). Although the specificity of anti-filaggrin/CCP antibodies in RA is more than 90% in almost all reports, the prevalence (sensitivity) of the same antibodies ranges from 33% to 87.2%. Such a discrepancy in sensitivity might reflect racial and genetic backgrounds as well as the differences of used antigens and detection techniques among reports. In earlier studies, natural filaggrins have been used, and then citrullinized recombinant filaggrins and CCP have been utilized. Generally, anti-CCP appears to be more sensitive than anti-filaggrin. Furthermore, the second generation kit of anti-CCP test has been recently developed, in which highly reactive peptides are selected from random peptide library and are used as antigens for ELISA. The second generation kits maintain the high specificity and have the more improved sensitivity than the first generation kits. Many recent reports of anti-CCP antibodies from Japan have used the second generation kits, and the most of them describe that the sensitivity of anti-CCP in RA is as high as that of RF or even higher.

Anti-citrullinated protein antibodies can be detected in RA patient sera from early stage of the disease. Schelleken et al described that anti-CCP antibodies were detected in 68% of RA patients. Although the sensitivity was decreased to 48% in early RA cases, still the high specificity was maintained at 96% (6). In particular, the combination of anti-CCP and IgM-RF revealed a high positive predictive value for RA. In the report of van Gaalen et al, from 318 patients with undifferentiated arthritis at the first visit, RA had later developed in 93% with positive anti-CCP and in only 25% with negative anti-CCP antibodies (OR=37.8) (15). Rantapää-Da hlqvist et al reported that when preserved sera from 83 cases who had been registered as blood donors and later developed RA were studied, anti-CCP antibodies were detected in 33.7% from the disease-free period (16). A similar result was described in the study of serial measurement in blood donors by Nielen et al, in which 49% of RA patients were positive for IgM-RF and/or anti-CCP before the development of RA symptoms (median of 4.5 years before onset, range 0.1–13.8 years) (17).

Anti-citrullinated protein antibodies may be useful as a new serologic marker for RA, because of their high specificity and high sensitivity in RA, and may also serve as an early diagnostic marker.

**Correlation Between Anti-citrullinated Protein Antibodies and Disease Severity**

There have been several reports that anti-citrullinated
protein antibodies might be a predictive marker for the progression of joint destruction as summarized in Table 2. Schellekens et al described that both anti-CCP and IgM-RF at the first visit predicted erosive change at 2 years follow-up in RA patients with 91% of positive predictive value (6). Kroot et al reported that anti-CCP was positive in 70% of 273 RA patients who had had disease symptoms for less than 1 year at study entry, and patients with anti-CCP had developed significantly more severe radiological damage after 6 years follow-up (18). In multiple regression analysis, radiological damage after 6 years follow-up was significantly predicted by IgM-RF, radiological score at entry and anti-CCP status. Forslind et al measured anti-filaggrin antibodies by immunoblotting and AKA in 112 patients with early RA, and showed that positive anti-filaggrin or AKA patients at baseline had significantly higher Larsen scores in 5 years later than the patients without these antibodies (19). Later, they also reported the role of anti-CCP in the radiological outcome in 379 cases with early RA, and concluded that anti-CCP as well as the baseline Larsen score and ESR was an independent predictor of radiological damage and progression in multiple regression analysis (20). In the reports of Meyer et al, in which 191 RA patients within one-year onset were followed up, the likelihood of a total Sharp score increase after 5 years was significantly higher among patients with anti-CCP or APF but not RF and AKA (21). Visser et al showed that anti-CCP had a high discriminating power between persistent and self-limiting arthritis and between erosive and non-erosive arthritis in his clinical prediction model of arthritis outcome (22).

While these reports showed anti-CCP as a good prognostic marker of radiological progression in RA patients, there

Table 1. Clinical Significance of Anti-filaggrin/CCP Antibodies in RA

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Subjects</th>
<th>Antigens (Methods)*</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simon (1993)</td>
<td>RA 48/control 56</td>
<td>human skin FA (IB)</td>
<td>75%</td>
<td>89%</td>
<td>3</td>
</tr>
<tr>
<td>Schellekens (1998)</td>
<td>RA 134/control 154</td>
<td>CCP (ELISA)</td>
<td>76%</td>
<td>96%</td>
<td>5</td>
</tr>
<tr>
<td>Schellekens (2000)</td>
<td>RA 134/control 154</td>
<td>CCP (ELISA)</td>
<td>68%</td>
<td>98%</td>
<td>6</td>
</tr>
<tr>
<td>Goldbach-Mansky (2000)</td>
<td>early arthritis 486</td>
<td>CCP (ELISA)</td>
<td>48%</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td>Schellekens (2000)</td>
<td>arthritis &lt;1 year 238</td>
<td>human skin FA (ELISA)</td>
<td>33%</td>
<td>93%</td>
<td>11</td>
</tr>
<tr>
<td>Kroot (2000)</td>
<td>RA 106/others 122</td>
<td>CCP (ELISA)</td>
<td>41%</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td>Bizzaro (2001)</td>
<td>RA 98/control 232</td>
<td>CCP (ELISA)</td>
<td>41%</td>
<td>97.8%</td>
<td>12</td>
</tr>
<tr>
<td>Vincent (2002)</td>
<td>RA 240/control 471</td>
<td>rat r-cFA (ELISA)</td>
<td>67%</td>
<td>98.5%</td>
<td>13</td>
</tr>
<tr>
<td>Suzuki (2003)</td>
<td>RA 549/control 208</td>
<td>CCP (ELISA)</td>
<td>87.6%</td>
<td>88.9%</td>
<td>14</td>
</tr>
<tr>
<td>Rantapää-Dahlqvist (2003)</td>
<td>RA 83 from blood donors before onset</td>
<td>CCP (ELISA)</td>
<td>68.7%</td>
<td>94.7%</td>
<td></td>
</tr>
</tbody>
</table>


Table 2. Reports of Anti-citrullinated Antibodies as a Predictive Factor for Prognosis of RA

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Subjects</th>
<th>Antibodies (Methods)*</th>
<th>Predictability</th>
<th>Other prognostic factors</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schellekens (2000)</td>
<td>RA 144</td>
<td>αCCP (ELISA)</td>
<td>+</td>
<td>IgM-RF</td>
<td>6</td>
</tr>
<tr>
<td>Kroot (2000)</td>
<td>early RA 273</td>
<td>αCCP (ELISA)</td>
<td>+</td>
<td>baseline X-ray, RF</td>
<td>18</td>
</tr>
<tr>
<td>Bas (2000)</td>
<td>RA 119</td>
<td>AFA (ELISA)</td>
<td>–</td>
<td>RF</td>
<td>23</td>
</tr>
<tr>
<td>Forslind (2001)</td>
<td>early RA 112</td>
<td>AFA (IB)</td>
<td>+</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Paimela (2001)</td>
<td>early RA 78</td>
<td>AFA (ELISA)</td>
<td>–</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Meyer (2003)</td>
<td>early RA 191</td>
<td>αCCP (ELISA)</td>
<td>+</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Rantapää-Dahlqvist (2003)</td>
<td>RA 83 (blood donors)</td>
<td>αCCP (ELISA)</td>
<td>+</td>
<td>IgA-RF</td>
<td>16</td>
</tr>
<tr>
<td>Forslind (2004)</td>
<td>early RA 378</td>
<td>αCCP (ELISA)</td>
<td>+</td>
<td>baseline Larsen score, ESR</td>
<td>20</td>
</tr>
</tbody>
</table>

are also several reports that anti-filaggrin antibodies are not associated with the disease severity. Bas et al measured anti-filaggrin in 199 RA patients and the severity of erosion for a given disease duration was correlated only with RF but not with anti-filaggrin (23). In the report of Paimela et al in which the human skin filaggrin was used as an antigen for ELISA, raised anti-filaggrin levels at entry were associated with an active and treatment-resistant disease, but did not predict radiological progression (24).

As reviewed here, all reports of anti-CCP indicate a positive correlation with radiological progression, whereas anti-filaggrin and AKA tend to be independent of the disease severity (Table 2). This discrepancy may reflect heterogeneity of autoantigenic epitopes on citrullinated filaggrin molecules, and suggest a possibility that clinical significance may vary among different epitopes and different techniques.

### Protein Citrullination and Etiopathogenesis of RA

Citrullinated proteins are observed in the synovial tissue of RA joints but not in normal joints. Citrulline is expressed mainly in the lining and sublining layers intracellularly or found in interstitial amorphous deposits of RA synovium (25, 26). These citrullinated proteins are not filaggrin but were identified as citrullinated forms of the α- and β-chains of fibrin (26). Recently, the target antigen of anti-Sa antibodies that were reported as RA-specific autoantibodies was identified as citrullinated vimentin. Thus, various citrullinated proteins and peptides have been demonstrated as the specific autoantigens in RA. These results strongly suggest a possibility that citrullinated proteins deposited in the RA synovium are the major targets of autoimmune response in RA patients. In addition, B cells from the synovial fluid, but not peripheral blood B cells, of anti-CCP-positive RA patients spontaneously produce anti-CCP antibodies (27). This fact suggests that an antigen-driven activation of B cells specific for citrullinated proteins occurs at the site of inflammation in RA.

Recently, an interesting report concerning the correlation between the gene polymorphism of the citrullinating enzyme, PADI, and RA susceptibility has been published (28). Japanese researchers conducted a genome-wide screening by SNPs analysis to identify the disease-susceptibility genes for Japanese patients with RA. In this study, the PADI type 4 (PADI4) gene, one of the genes of four types of PADI that are located in chromosome 1p36, was identified as the locus of RA-susceptibility gene. One of the haplotypes (haplotype 2) of PADI4 was found more frequently in RA patients (32%) than in normal controls (25%) (OR=1.97) and was thought to be the RA-susceptible haplotype. The PADI4 was mainly expressed in bone marrow cells and peripheral leucocytes and monocytes as well as RA synovium. Moreover, it was demonstrated that mRNA expressed from the RA-susceptible form of PADI4 had a longer half life than mRNA from the RA-non-susceptible PADI4, and RA patients who had the homozygous RA-susceptible haplotype developed more frequent anti-filaggrin antibodies. These data suggest a possibility that proteins might be easily citrullinated in RA patients, and over-citrullinated proteins such as citrullinated fibrin in the joints might break self-tolerance and promote abnormal immune response.

However, there is also another report against this hypothesis. Shortly after the above study published, Barton et al reported that no correlation was found between RA patients in UK and the PADI4 polymorphism (29). Although the genetic and racial background may be the cause of this discrepancy, further studies will be needed to clarify the role of protein citrullination on the etiopathogenesis of RA.

### Conclusion

In recent studies, it has been demonstrated that RA patients produce not only RF but also a variety of other autoantibodies. Although most of the autoantibodies are not always specific for RA, autoantibodies to citrullinated proteins (APF, AKA, anti-filaggrin, anti-CCP and anti-Sa) appear to be exclusively detected in RA. Anti-CCP antibodies are especially noteworthy because of their high sensitivity as well as high specificity. These antibodies may serve as a powerful serologic marker for early diagnosis and prognostic prediction of RA. New criteria for the early diagnosis or classification of RA should be considered if the routine test of these RA-specific autoantibodies could be utilized. Anti-citrullinated protein antibodies are locally produced in RA joints, and citrullinated proteins identified as citrullinated fibrins are localized in RA synovial tissue. These findings strongly suggest a possibility that local citrullination of intraarticular proteins might be the initial event leading to autoantibody production in RA. Genetic factors such as HLA and a gene polymorphism of the citrullinating enzyme, PADI, (that might express more stable mRNA and cause over-citrullination of proteins) might be associated with the breakage of self-tolerance and induction of autoimmunity against citrullinated proteins. Further research however will be necessary to elucidate the fine mechanism and significance of protein citrullination in etiopathogenesis of RA.

### References

5) Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic peptides.
17) MIMORI