

Autoantibodies to C-Reactive Protein in Incomplete Lupus and Systemic Lupus Erythematosus

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Objective: Anti-C-reactive protein (CRP) antibodies have been described in patients with systemic lupus erythematosus (SLE). We investigated the potential of the anti-CRP antibody as a marker for disease activity in SLE patients and as a predictor of progression to SLE in patients with incomplete lupus.

Methods: Immunoglobulin G anti-CRP antibody levels were measured using an enzyme-linked immunosorbent assay.

Results: Patients with incomplete lupus exhibited clinical and immunologic characteristics different from those in SLE patients: no serositis and alopecia, more common oral ulcers and arthritis, lower disease activity index, lower positivity for antinuclear and anti-double-strand DNA antibodies, and higher complement levels. Anti-CRP antibody levels were higher in SLE patients (35.6 [35.1] AU) than in patients with incomplete lupus (23.1 [25.8] AU, $P = 0.016$) and normal controls (21.0 [14.3] AU, $P < 0.001$). Anti-CRP antibody was significantly higher in SLE patients with arthritis and correlated with disease activity markers, including antichromatin antibody. However, no difference in anti-CRP antibody levels was observed between patients with incomplete lupus that progressed to SLE and those whose did not.

Conclusion: These data suggest that anti-CRP antibodies can neither be used as biomarkers in SLE nor predict SLE progression in patients with incomplete lupus.

Key Words: autoantibody, biomarker, C-reactive protein, disease activity, lupus

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Systemic lupus erythematosus (SLE) is an autoimmune disease with multiorgan involvement. Reports have suggested that certain characteristics of SLE can be attributed to specific antibodies, such as lupus nephritis to anti-double-strand DNA antibodies.¹ However, the presence of an autoantibody is not always associated with the symptoms, and up to 5% of healthy people have antinuclear antibodies (ANAs). Although patients with incomplete lupus or lupus syndrome have ANA or other autoantibodies, they meet only 2 or 3 classification criteria of the American College of Rheumatology. Therefore, studies have been conducted to understand how patients with incomplete lupus are protected against organ damage by autoantibodies as observed in SLE.^{2,3} Li et al. investigated the difference in autoantibodies present in patients with SLE and incomplete lupus by using a proteome microarray and suggested that immunoglobulin M autoreactivity was predominant in incomplete lupus.⁴

The C-reactive protein (CRP) is a plasma protein belonging to the pentraxin family and is a useful acute-phase reactant in inflammatory diseases.^{5–7} It also plays a role in phagocytic clearance of circulating opsonized apoptotic materials in innate immunity. In SLE patients, remnant apoptotic debris due to incomplete clearance functions as self-antigens and leads to autoantibody formation. In patients with SLE, only modest elevation in CRP levels is observed despite the presence of active disease, whereas significant elevation is observed in patients with infection.^{5–7} In a study conducted using a more precise assay system, SLE patients with infection were found to have elevated levels of high-sensitivity CRP, whereas patients with active lupus did not.⁸ Such incongruent levels of CRP in inflammatory status may contribute to the accumulation of apoptotic cells during SLE. In addition, CRP treatment in mice models modified the characteristics of autoimmune disease by preventing the exposure of intracellular nuclear antigens to the immune system.⁹ Reports have suggested that CRP gene polymorphism increases the risk for developing SLE by contributing to genetic susceptibility.^{10,11} It is thought that several cytokines, such as type I interferon- α , underlie this mechanism, thereby inhibiting CRP induction in SLE.¹²

Modest elevation in CRP levels in SLE patients may be due to enhanced elimination by autoantibodies. The presence of autoantibodies to CRP in SLE patients was first described by Robey et al.¹³ Several studies have shown different prevalence rates of anti-CRP antibodies in SLE, such as those described by Bell et al.¹⁴ (78%, 39/50), Figueredo et al.¹⁵ (51%, 70/137), Kessel et al.¹⁶ (37%, 34/98), and Shoenfeld et al.¹⁷ (6%, 9/150). Because anti-CRP antibody levels are elevated in patients with lupus nephritis or those with high disease activity, CRP can be potentially used as an assessment tool for diseases.¹⁸ However, these studies did not demonstrate a relationship between CRP levels and presence of anti-CRP antibodies.

We determined the levels of anti-CRP antibodies in Korean patients with SLE and incomplete lupus and analyzed their association of CRP levels, disease activity markers, and SLE manifestations. We also compared the anti-CRP levels in patients with incomplete lupus that progressed to SLE with those.

MATERIALS AND METHODS

Patients and Data Collection

All sera from patients were collected at the Department of Rheumatology, Ajou University Hospital, from April 2009 to March 2011. We recruited 99 patients with SLE who fulfilled the revised American College of Rheumatology classification criteria,¹⁹ 60 with incomplete lupus satisfying 2 or 3 of the 11 criteria (38 patients fulfilled 3 criteria; others fulfilled only 2 criteria), and 48 healthy subjects as normal controls (NCs). Fifty-five patients (91.7%) with incomplete lupus had ANA, 7 (11.7%) had anticardiolipin antibodies, and 5 (8.3%) had anti-dsDNA antibodies. During the mean follow-up period of 3.3 years, patients with incomplete lupus were examined every 6 months to monitor changes in their clinical symptoms and laboratory findings. The NCs did not have any autoimmune diseases. Information on their

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medical history and clinical symptoms was registered in a database when serum sampling was performed. The SLE Disease Activity Index (SLEDAI) scores were calculated.²⁰ All subjects who participated provided informed consent. This study was approved by the institutional review of board of our hospital.

Anti-CRP and Antichromatin Antibody Assay

IgG anti-CRP antibodies were measured by performing an enzyme-linked immunosorbent assay. Human CRP (Sigma, St Louis, MO) was diluted in phosphate-buffered saline (PBS) and coated onto 96-well plates overnight. The plates were washed with PBS containing Tween 20 (PBST). The free binding sites were blocked with PBST containing 1% of bovine serum albumin, and the wells were washed. The sera were diluted to 1:50 with PBST, and 100 μ L of sera was incubated and then washed with PBST. Goat antihuman IgG alkaline phosphatase (Sigma, St Louis, MO) was diluted to 1:1000 in a block solution, and 100 μ L of this was added to each well before incubation. After washing, the assay was developed using 1 mg/mL of *p*-nitrophenyl phosphate, and the absorbance was read at 405 nm. The mean optical density for each duplicate set was determined. The optical density of each sample was then calculated with a standard curve generated with stepwise dilution of serum ($r^2 = 0.98$). Antichromatin antibody assays were performed according to the methods described previously.²¹

Statistical Analysis

Differences in the levels of serum anti-CRP antibodies between the groups were identified using the Mann-Whitney *U* test. The optimal cutoff levels were determined by receiver operating characteristic curve analyses. The correlation between anti-CRP antibody levels and clinical manifestations was analyzed using Spearman correlation coefficient. A $P < 0.05$ was considered significant. The statistical analysis was performed using Statistical Package for the Social Sciences version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

The general characteristics of the subjects and their clinical manifestations are shown in Table 1. There was no difference in the gender composition of the groups; however, the mean age was significantly different ($P < 0.05$), and therefore, logistic regression analysis was performed to control for age. None of the patients with incomplete lupus had serositis or alopecia; however, oral ulcers and arthritis were more frequently present than in SLE patients. Patients with incomplete lupus had lower disease activity as assessed on the basis of SLEDAI. The positivity for ANA and anti-dsDNA was lower in patients with incomplete lupus; however, the complement levels were higher.

The serum anti-CRP antibody level in SLE patients was 35.6 (35.1) AU, which was significantly higher than that in patients with incomplete lupus (23.12 [25.82] AU, $P = 0.016$) and NCs (21.01 [14.32] AU, $P < 0.001$) (Fig. 1). However, no difference was observed in the anti-CRP antibody levels between patients with incomplete lupus and NCs.

Serum anti-CRP antibody levels were significantly higher in patients with arthritis than in those without ($P = 0.020$) and in patients with the antichromatin antibody than in those without ($P = 0.006$).

Anti-CRP antibody levels were positively correlated with the levels of CRP ($r = 0.297$, $P = 0.003$), anti-dsDNA antibody ($r = 0.237$, $P = 0.019$), antichromatin antibody ($r = 0.441$, $P < 0.001$), and SLEDAI ($r = 0.241$, $P = 0.017$); however, a negative correlation was observed with leukocyte ($r = -0.243$, $P = 0.016$) and platelet counts ($r = -0.203$, $P = 0.048$).

TABLE 1. Clinical Features of the Patients With SLE and Incomplete Lupus

Features	Incomplete Lupus,		<i>P</i>
	SLE, n = 99	n = 60	
Age, y	32.8 (11.0)	38.2 (12.4)	0.004
Sex, F:M	85:14	56:4	0.09
Malar rash	13 (13)	8 (13)	0.939
Serositis	8 (8)	0	0.004
Oral ulcer	17 (17)	21 (35)	0.024
Arthritis	26 (26)	32 (53.3)	<0.001
Photosensitivity	11 (11)	5 (8.3)	0.598
Alopecia	12 (12)	0	<0.001
Renal disease	10 (10)	2 (3.2)	0.084
Hematologic disorder	39 (39)	30 (50)	0.128
ANA	99 (100)	55 (91.7)	0.024
Anti-dsDNA antibody	40 (40)	5 (8.3)	<0.001
Hemoglobin, g/dL	11.8 (2.2)	12.3 (1.3)	0.122
Leukocyte, / μ L	6385 (3071)	5884 (2445)	0.233
Lymphocyte, / μ L	1445 (948)	1641 (644)	0.165
Platelet, $\times 10^3$ / μ L	234.4 (83.9)	238.8 (27.4)	0.9
CRP	0.43 (0.95)	0.47 (1.43)	0.84
Complement 3, mg/L	69.9 (26.2)	98.1 (27.3)	<0.001
Complement 4, mg/L	15.46 (7.67)	21.72 (7.38)	<0.001
SLEDAI	5.5 (5.5)	2.7 (2.5)	<0.001

All values are numbers (%) or mean (SD).

On the basis of receiver operating characteristic analyses, an anti-CRP antibody value of 49.63 AU (76% of sensitivity and 50% of specificity) was determined to be the cutoff value. Positive serum anti-CRP antibody levels were observed in 18 patients (18%) with SLE, 5 patients (8%) with incomplete lupus, and 3 (6%) NCs. When the patients with incomplete lupus were divided according to the number of criteria fulfilled (2 or 3), no significant difference in anti-CRP antibody levels was observed between the 2 groups (21.7 [29.6] vs 28.3 [30.7] AU, $P = 0.4$).

Among 60 patients with incomplete lupus, 9 patients (15%) were diagnosed with SLE after a mean of 11 months (5 months to 2 years). The anti-CRP antibody levels in patients with incomplete lupus that progressed to SLE were not significantly different from

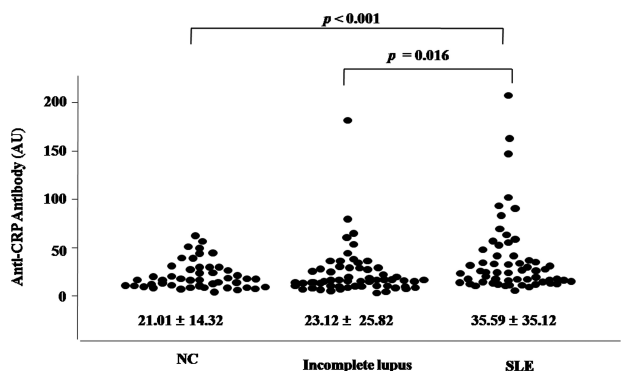


FIGURE 1. Serum anti-CRP antibody levels in patients with SLE, with incomplete lupus erythematosus, and in NCs. The anti-CRP antibody levels (mean [SD]) were determined using enzyme-linked immunosorbent assay in 99 patients with SLE, 60 with incomplete lupus, and 48 NCs. Logistic regression analyses were applied to control for age.

the levels in those who did not (23 [27.6] vs 24.1 [12.3] AU, $P = 0.905$). None of the patients with incomplete lupus that progressed to SLE showed positivity for anti-CRP antibody.

DISCUSSION

Anti-CRP antibodies are expected to be useful markers of SLE because a CRP gene polymorphism has been suggested to increase the risk for developing SLE due to genetic susceptibility. Anti-CRP antibody levels have been shown to increase in SLE patients with high disease activity in some studies but not in others. In this study, the levels of anti-CRP antibodies were higher in patients with SLE than in those with incomplete lupus and the NCs. However, the anti-CRP antibody level showed moderate correlation with several disease activity markers. In addition, anti-CRP antibody levels in patients with incomplete lupus that progressed to SLE were not different from those in patients in whom it did not progress to SLE.

The anti-CRP antibody is not a specific antibody detected only in SLE patients. These antibodies were also detected in 13% of patients with primary Sjögren syndrome, 22% of those with rheumatoid arthritis, and 54% of those with antiphospholipid syndrome.^{15,22} Because CRP plays a role in the elimination of apoptotic cells that are impaired in SLE patients and its level does not change significantly during active inflammation in SLE, researchers are trying to determine the role of anti-CRP antibodies in SLE.

The possible correlation between anti-CRP antibodies and clinical manifestations or disease activity in SLE has been evaluated previously. The anti-CRP antibody was associated with nephritis,²³ higher SLEDAI,^{24,25} and thrombosis.¹⁵ Formation of an immune complex by anti-CRP antibodies may contribute to lupus nephritis accompanied by activation of the classical pathway through a synergistic effect with other glomerular-targeting autoantibodies. However, in this study, the levels of anti-CRP antibodies were not different in patients with renal involvement and were thought to be caused due to the limited number of renal manifestations (10/99). On the other hand, the levels of anti-CRP antibodies were significantly higher in SLE patients with arthritis than in those without. Although the severity of arthritis varies, higher levels of anti-CRP antibody indicate that it may be involved in activating the inflammation of the joint space in patients with SLE. A previous study reported that anti-CRP antibody levels are more frequently elevated in patients with rheumatoid arthritis than in healthy controls.²²

Anti-CRP antibody levels were positively correlated with CRP and complement 3 levels, the SLEDAI score, and anti-dsDNA and antichromatin antibody levels. However, these correlations were weak, except that with the antichromatin antibody. These findings are inconsistent with other data suggesting that anti-CRP antibody testing could be used to evaluate disease activity.^{15,24,26} We found that the level of anti-CRP antibodies was not a useful predictor of disease activity, as observed by Shoenfeld et al.¹⁷ However, the lupus patients in the present study had low disease activity (mean SLEDAI, 5.5), which may be a reason for the weak correlation observed with disease activity. Although anti-CRP antibodies were positively correlated with CRP in patients with SLE, anti-CRP antibodies were not formed against pentameric CRP, which is the form of CRP measured. An anti-CRP antibody binds to only monomeric CRP dissociated from pentameric CRP, and this autoantibody does not play a role in the downregulation of pentameric CRP.²⁷ In addition, antibodies to serum amyloid P, which is another pentraxin family protein, were raised in patients with SLE, and a correlation was observed with disease activity, although the

level of serum amyloid P is not changed according to disease activity in humans.²⁸

We previously demonstrated that the antichromatin antibody may be a useful SLE diagnostic and assessment marker.²¹ In the present study, the levels of antichromatin antibodies were significantly correlated with the levels of anti-CRP antibody. These 2 autoantibodies may play simultaneous roles in the pathogenesis of lupus; however, the association between the 2 autoantibodies is yet to be determined.

Greer and Panush²⁹ evaluated patients with incomplete lupus and SLE and noted frequent occurrence of cutaneous manifestations and arthritis; however, renal, neurologic, hematologic, or immunologic involvement was rare in patients with incomplete lupus.²⁸ Hallengren et al.³⁰ published 10-year follow-up data indicating that 58% of patients with incomplete lupus developed SLE, and the predictive markers for complete SLE were malar rash and anticardiolipin antibody. Among 60 patients with incomplete lupus in our study, 5 (8%) showed positivity for the anti-CRP antibody, which was not significantly different from the NCs. Nine patients (15%) with incomplete lupus showed progression to SLE during the 3.3-year follow-up, and arthritis and hematologic disorders were frequently observed in patients with recently developed SLE. However, there were no significant similarities between patients with incomplete lupus that progressed to SLE and those whose lupus did not progress. In addition, there were no differences in the levels of anti-CRP antibody between these 2 groups of patients.

As a limitation, most of the patients with SLE were controlled well with low disease activity. In addition, the methodologic differences in detection of anti-CRP antibodies may have led to results that are inconsistent with those of previous studies.

In conclusion, anti-CRP antibodies are detected in some patients with SLE; however, the findings of this study suggest that it may not be a useful disease activity marker. It is also not a reliable biomarker to predict progression to SLE in patients with incomplete lupus.

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