Unresponsiveness of C-reactive protein in the non-infectious inflammation of systemic lupus erythematosus is associated with interleukin 6

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Abstract C-reactive protein (CRP) response is abnormal to a non-infectious inflammation in systemic lupus erythematosus (SLE). We evaluated the role of cytokines in this CRP unresponsiveness. The sera of 138 SLE patients and 71 rheumatoid arthritis patients were collected prospectively. SLE with infection had higher WBC count, ESR, CRP and C4 levels than those without infection. IL-6, IL-10 and IFN-γ levels were higher in SLE with infection than SLE without infection. In SLE with infection, the CRP was correlated with the IL-6 (r = 0.77, P < 0.001) but not correlated with IL-10 and IFN-γ. These data suggest that IL-6 may have a role in the unresponsiveness of CRP to a non-infectious inflammation of SLE.

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nuclear autoantigens such as chromatin and histones for rendering them non-immunogenic [11,12]. It has been demonstrated that CRP inhibits immunization by the epitopes to which it binds [13]. Kinetic analysis has indicated that binding of CRP to cells undergoing apoptosis occurred later than annexin V, which binds to phosphatidylserine. In addition to binding to the membrane of apoptotic cells, CRP also binds to the membranes and nuclear constituents of necrotic cells [14]. There is additional experimental evidence that suggests a participatory role for CRP in the pathogenesis of SLE. CRP treatment significantly prolonged the survival of NZB/NZW mice that were injected with chromatin-coated beads [15]. Furthermore, the endogenously expressed human CRP is protective in a mouse model of SLE [16].

It is reasonable that a relatively low CRP level could cause circulating nuclear autoantigens to become immunogenic, and this is probably due to the decreased cytokine responses. Previous reports have shown that there was no correlation for any cytokine and CRP level in SLE [7,8]. These reports, however, were retrospective, and the number of patients was small. So, we prospectively evaluated CRP levels and cytokine responses in two groups of SLE patients: patients with infection and without infection and also in rheumatoid arthritis (RA) patients.

Patients and methods

Patients

From May 2002 to May 2004, we prospectively recruited consecutive Korean patients who satisfied the American College of Rheumatology (ACR) 1982 revised criteria for the classification of SLE [17] from the rheumatology clinic at Ajou University Hospital. Among a total of 138 SLE patients, 24 SLE patients had a concurrent infection (SLE with infection). The infections were confirmed by both the clinical findings and positive culture, if possible. Herpes zoster was considered when a patient was found to have a vesicular rash in a dermatomal distribution. Three SLE patients were not considered to have infection because the supportive evidence was scant. The remaining 111 patients had no evidence of infection (SLE without infection). As a disease control, we included 71 RA patients who satisfied the ACR 1987 revised criteria for the classification of RA [18]. Serum samples were collected at the time of infection for SLE with infection and at the clinic visits for SLE without infection and RA patients, and the samples were kept at −20°C until they were tested for cytokine and CRP. The disease activity was assessed with SLE disease activity index (SLEDAI) at the time of serum sampling. The study was approved by the ethics committee of Ajou University Hospital, and all the patients gave us an informed consent.

Cytokine assay

Serum concentrations of IL-1β, IL-2, IL-6, IL-10, IL-12, interferon-γ (IFN-γ) and TNF-α were measured in duplicate by sandwich ELISA with using anti-human antibodies (PharMingen, San Diego, CA). Briefly, microplates (Costar, Cambridge, MA) were coated with primary anti-human IL-1β, IL-2, IL-6, IL-10, IL-12, IFN-γ and TNF-α monoclonal antibodies, respectively. After loading the serum, the biotinylated secondary anti-human IL-1β, IL-2, IL-6, IL-10, IL-12, IFN-γ and TNF-α monoclonal antibodies were added, respectively. The wells were incubated with streptavidin horseradish peroxidase conjugate, and the colorimetric reaction was developed with TMB (3,3′,5,5′-tetramethylbenzidine) substrate solution. The reaction was stopped by the addition of 2 N sulfuric acid, and the absorbance was read at 450 nm by an automated microplate reader (Benchmark, Bio-RAD, Hercules, CA). The serum cytokine levels were calculated from the standard curves that were derived from the recombinant human IL-1β, IL-2, IL-6, IL-10, IL-12, IFN-γ and TNF-α in the standard wells, respectively.

CRP determination

The serum CRP levels were measured by immunoturbidometry using a TBA-200FR chemistry autoanalyzer (Toshiba, Tokyo, Japan). The value limit of CRP detection was 0.2 mg/l. The CRP level was considered as being this value when the CRP levels were lower than 0.2 mg/l. The normal range was less than 8 mg/l with this assay.

Statistical analysis

The serum levels of CRP and cytokines were not normally distributed, and they are expressed as median and interquartile range. Comparisons of CRP levels and cytokine levels between SLE with infection and SLE without infection/RA patients were performed by Mann—Whitney U tests using SPSS version 11.5 (Chicago, IL). To define the relationships between cytokines and CRP levels, Pearson’s correlation coefficients and regression lines were applied after transforming cytokines and CRP levels to log scale. A P value of 0.05 or below was regarded as significant.

Results

Twenty-four infections occurred in 138 consecutive SLE patients; there were 6 pneumonia, 5 sepsis, 4 upper respiratory tract infections, 2 pulmonary tuberculosis, 2 gastrointestinal tract infections, 2 urinary tract infections, 2 herpes zoster skin infections and 1 soft tissue infection. The mean age of SLE with infection was 32.3 ± 13.8 years, and 87.5% of them were female (Table 1). SLE with infection received the daily equivalent of 14.4 ± 16.0 mg prednisolone at the time of infection, which was higher than RA patients (3.7 ± 4.5 mg/day, P < 0.01), but it was not different from SLE without infection (9.6 ± 12.1 mg/day, P = 0.1). There were no demographic and clinical differences between SLE with infection and SLE without infection.

The WBC counts of SLE with infection were in the normal range, but the counts were higher than SLE without infection (Table 1). The platelet counts were lower in SLE with infection than RA patients. Inflammation markers such as ESR and C4 were higher in SLE with infection than RA patients. Inflammation markers such as ESR and C4 were higher in SLE with infection than SLE without infection. The CRP level of SLE with infection (39.1 mg/l (22.2, 70.9)) was higher than that of SLE without infection (1.2 mg/l (0.4, 4.8), P < 0.001) and RA patients (7.1 mg/l (2.0, 31.5), P < 0.001). Like our previous report
CRP levels of SLE without infection were less than 50 mg/l except for 2 patients; one patient that had a large pericardial effusion had a CRP level of 110.9 mg/l, and the other patient, who was immediate postpartum, had a CRP level of 58.8 mg/l (Fig. 1). It is known that serositis can increase the CRP level in SLE. In SLE without infection, six other patients had a serositis at the time of sampling. The CRP levels of them were 22.4 mg/l, 18.7 mg/l, 11 mg/l, 4.1 mg/l, 1.5 mg/l and 0.5 mg/l, respectively, and these are higher than SLE without serositis. The anti-dsDNA positivity was not different according to the presence of infection.

The IL-2 level of SLE with infection (4.5 pg/ml (0, 14.5)) was not different from SLE without infection (3.0 pg/ml (0, 10.1), P = 0.49), but it was lower than RA patients (15.7 pg/ml (8.4, 22.1), P < 0.01) (Fig. 2). In SLE with infection, the IL-6, IL-10 and IFN-γ levels were higher than SLE without infection (41.8 pg/ml (7.6, 86.3) vs. 0 pg/ml (0, 3.5), P < 0.001; 9.0 pg/ml (2.5, 33.7) vs. 3.3 pg/ml (0, 8.4), P < 0.05; 57.0 pg/ml (34.4, 229.2) vs. 31.2 pg/ml (26.4, 39.4), P < 0.001, respectively), and also the IL-10 and IFN-γ levels were higher than RA patients (9.0 pg/ml (2.5, 33.7) vs. 0 pg/ml (0, 0), P < 0.001; 57.0 pg/ml (34.4, 229.2) vs. 27.9 pg/ml (0, 32.8), P < 0.001, respectively). The IL-12 level of SLE with infection (123.0 pg/ml (15.3, 981.1)) was higher than RA patients (39.8 pg/ml (0, 220.7), P = 0.05), but not different from SLE without infection (231.6 pg/ml (18.8, 641.9), P = 0.99). There were no differences for IL-1 and TNF-α in the three groups (data not shown).

The correlations between CRP and cytokines were examined by using Pearson’s correlation coefficients after transforming CRP and cytokine levels to log scale (Fig. 3). In SLE with infection, CRP was positively correlated with IL-6 (r = 0.77, P < 0.001), but not with IL-10 and IFN-γ (data not shown). However, there was no correlation between CRP and any cytokine in SLE without infection and RA. These results reveal that the most important cytokine for the induction of CRP may be IL-6 in SLE patients with infection.

Discussion

The present prospective study confirmed that there were decreased CRP responses to the non-infectious inflammation in SLE. Our previous retrospective study revealed that the CRP level was significantly higher in Korean SLE patients.

Table 1 Clinical characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>SLE with infection (n = 24)</th>
<th>SLE without infection (n = 111)</th>
<th>RA (n = 71)</th>
</tr>
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<tbody>
<tr>
<td>Age, year</td>
<td>32.3 ± 13.8a</td>
<td>33.2 ± 10.9</td>
<td>50.3 ± 12.7a</td>
</tr>
<tr>
<td>Female, %</td>
<td>87.5</td>
<td>86.4</td>
<td>88.7</td>
</tr>
<tr>
<td>Steroid, mg/dayb</td>
<td>14.4 ± 16.0a</td>
<td>9.6 ± 12.8</td>
<td>3.7 ± 4.5a</td>
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<td>Fenretinide, %</td>
<td>41.7</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>SLEDAI</td>
<td>9.6 ± 8.2</td>
<td>6.6 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>CTX percentage</td>
<td>25</td>
<td>14.5</td>
<td></td>
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<tr>
<td>Hematocrit, %</td>
<td>32.7 ± 6.1</td>
<td>35.0 ± 6.1</td>
<td>35.4 ± 4.6</td>
</tr>
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<td>WBC, /mm³</td>
<td>8638 ± 4687c</td>
<td>5860 ± 3046c</td>
<td>7837 ± 3178</td>
</tr>
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<td>Platelet, ×10³/mm³</td>
<td>224 ± 100³</td>
<td>234 ± 93</td>
<td>351 ± 12²</td>
</tr>
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<td>Creatinine, mg/dl</td>
<td>1.07 ± 0.66</td>
<td>1.14 ± 1.86</td>
<td>0.86 ± 0.46</td>
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<tr>
<td>Cholesterol, mg/dl</td>
<td>154.8 ± 65.6</td>
<td>175.0 ± 50.8</td>
<td>175.4 ± 40.0</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>39.1 ± 27.1c</td>
<td>27.2 ± 25.1c</td>
<td>38.1 ± 34.3</td>
</tr>
<tr>
<td>CRP, mg/dld</td>
<td>39.1 (22.2, 70.9)a,c</td>
<td>1.2 (0.4, 4.8)c</td>
<td>7.1 (2.0, 31.5)³</td>
</tr>
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<td>C3, mg/dl</td>
<td>88.8 ± 41.5</td>
<td>71.4 ± 27.8</td>
<td></td>
</tr>
<tr>
<td>C4, mg/dl</td>
<td>21.0 ± 8.6c</td>
<td>14.5 ± 7.3c</td>
<td></td>
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<tr>
<td>Anti-dsDNA, %</td>
<td>62.5</td>
<td>52.3</td>
<td></td>
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</tbody>
</table>

SLEDAI—systemic lupus erythematosus disease activity index.

a SLE with infection vs. RA, P < 0.05.

b mg prednisolone equivalent per day.

c SLE with infection vs. SLE without infection, P < 0.05.

d Values are the median (interquartile range).

Figure 1 C-reactive protein levels in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients. Among the SLE patients without infection, 7 patients with serositis (open circle) showed more highly elevated CRP levels than did the others (closed circle). Also, one patient had a CRP level of 58.8 mg/l during the immediate postpartum period (shaded circle). The horizontal interrupt lines indicate the cutoff level of the normal value. The error bars represent the median and interquartile range.

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with infection than those without infection, all of whom had a CRP level lower than 50 mg/l [10]. However, the CRP levels of two SLE without infection showed higher than 50 mg/dl in this study. One patient had a large pericardial effusion, and the other was in the immediate postpartum period. There were seven SLE without infection who had serositis, and their CRP levels were higher than SLE without serositis. These results suggest that serositis may be a different inflammatory response from other non-infectious inflammations, such as arthritis and nephritis. There were some CRP responses in serositis, but most of non-infectious inflammations could not produce a significant CRP response in SLE.

There has been clinical and experimental evidence that suggests a possible participatory role of CRP in the

Figure 2  Cytokine levels in systemic lupus erythematosus (SLE) and rheumatoid arthritis patients (RA). (A) Cytokine IL-2, (B) cytokine IL-6, (C) cytokine IL-10, (D) cytokine IL-12, (E) IFN-γ. The error bars represent the median and interquartile range.
IL-6 is a multifunctional cytokine and involved in the regulation of immune responses, an acute phase reaction [19,20]. Because polyclonal B cell activation is a principal immunological abnormality in SLE, IL-6 has received a good deal of interest. Since then, several pieces of evidence have suggested the involvement of IL-6 in SLE [21–23]. The study of the expression of IL-6 receptor (R) on the peripheral blood lymphocytes (PBL) showed that IL-6R was constitutively expressed in B cell of SLE patients, but not in normal B cell. By the binding of IL-6R on B cell and so inducing the terminal differentiation of B cell into autoantibody producing plasma cells, IL-6 plays an important role for the B cell’s response in SLE. Experiments looking at the serum IL-6 level in SLE have indicated that there is a correlation between IL-6 production and the disease severity. In contrast to these studies, there was a report that detected no increase in the expression of IL-6R on the PBL of SLE patients [24]. Results from murine systems also support the idea that IL-6 is involved in the pathophysiology of SLE. IL-6 in the sera of MRL/lpr mice increased in an age-dependent manner, and anti-IL-6R antibody treatment of MRL/lpr mice reduced the anti-dsDNA antibody and ameliorated the renal damage [25,26].

Observational studies have shown that elevation of IL-6 level was accompanied with increased CRP level in SLE patients with serositis, with infection and during disease exacerbations, although IL-6 levels did not increase in most SLE patients [27]. However, retrospective studies have shown that IL-6 levels were increased in SLE patients compared to healthy controls and RA patients, but they were not correlated with CRP levels [7,8]. The reason for these contradictory results is not clear. Because SLE has a heterogeneous disease manifestation, discrepant results may be produced in different subgroups of patients. The more important reason probably is that these studies were observational or retrospective in nature, and the patient number was small: from 12 to 37 patients. Our prospective study reveals that cytokine IL-6 and CRP responses were related, and these responses were heterogeneous in SLE; there was a strong response in infection, a mild to moderate response in serositis and tissue injury, and there was nearly no response in other non-infectious lupus disease activities.

In addition to the regulation of immune responses, IL-6 is also directly involved in the modulation of inflammation. IL-6 was initially considered as a proinflammatory cytokine, but it has recently been suggested that IL-6 should be classified as an anti-inflammatory cytokine [28]. The antiinflammatory nature of IL-6 was suggested by studies where recombinant IL-6 was found to induce the release of such antiinflammatory molecules as IL-1 receptor antagonist and soluble TNF-α receptor in normal subjects [29]. Studies on IL-6 knockout mice have demonstrated that these mice possess a compromised ability to control the proinflammatory cytokine response to injected endotoxin [30]. Also, IL-6 can induce corticosteroids and acute phase proteins, and both of these exert antiinflammatory effects. Our results revealed that IL-6 and related CRP responses are intact during infection, but they are variably impaired in the non-infectious inflammation in SLE. These decreased antiinflammatory responses may prolong disease manifestation such as nephritis.

Thus, based on our findings and other studies, a model of IL-6 response to an acute inflammation in SLE can be suggested. When there is an infection, IL-6 is produced normally, and it can induce the terminal differentiation of B cells, which constitute the expression of IL-6R, to the autoantibody producing plasma cells in SLE. In the noninfectious inflammation typically elicited by nephritis, the IL-6 response is not enough to decrease the inflammation and to produce acute phase proteins, which may perpetuate and aggravate the inflammatory responses. These differential IL-6 responses may be the underlying pathophysiology of SLE. There is a need for further study to elucidate the mechanism of these IL-6 responses in SLE.

In conclusion, this study demonstrates that CRP unresponsiveness to non-infectious inflammation is related to IL-6 response. This decreased IL-6 response may be the pathophysiology underlying the unremitting lupus disease activity.

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References


