Circulating Surfactant Protein D Is Decreased in Systemic Lupus Erythematosus

SILJE VERMEDAL HOEGH, ANNE VOSS, GRITH LYKKE SORENSEN, ANETTE HØJ, CHRISTIAN BENDIXEN, PETER JUNKER, and UFFE HOLMSKOV

ABSTRACT. Objective. Deficiencies of innate immune molecules like mannan binding lectin (MBL) have been implicated in the pathogenesis of systemic lupus erythematosus (SLE). Surfactant protein D (SP-D) and MBL belong to the same family of innate immune molecules — the collectins, which share important structural and functional properties. We aimed to compare concentrations of serum SP-D in patients with SLE and in healthy controls, and to investigate if SP-D is associated with selected disease indicators. We investigated the possible association of the Met11Thr polymorphism with disease, since this polymorphism is an important determinant for serum level, oligomerization pattern, and function of SP-D.

Methods. Serum SP-D was measured using a 5-layer ELISA in 70 SLE patients and 1476 healthy subjects. DNA was genotyped for the Met11Thr variant.

Results. Median SP-D level in serum was 911 ng/ml (95% CI 776–1118) in patients and 1068 ng/ml (95% CI 901–1246) in controls (p = 0.0004). Circulating SP-D did not differ significantly in patients with high, intermediate, or low SLE disease activity. Similarly, SP-D did not correlate with C-reactive protein, erythrocyte sedimentation rate, and anti-dsDNA seropositivity. Genetic analysis did not support an association of the Met11Thr genotype with SLE.

Conclusion. These findings suggest that low SP-D, unrelated to conventional disease indicators, represents an aspect of SLE etiopathogenesis. (First Release Oct 15 2009; J Rheumatol 2009; 36:2449–53; doi:10.3899/jrheum.090069)

Key Indexing Terms: SYSTEMIC LUPUS ERYTHEMATOSUS INNATE IMMUNITY COLLECTINS SURFACTANT PROTEIN D

Systemic lupus erythematosus (SLE) is an autoimmune disorder with a broad range of clinical presentations. Its etiology is unknown, but both genetic and environmental factors are implicated. The collectins, including surfactant protein D (SP-D) and mannan binding lectin (MBL), are pattern-recognition molecules of the innate immune system that recognize a variety of ligands, including oligosaccharide structures expressed by bacteria, viruses, and fungi. Collectins are composed of structurally similar oligomers that contain an N-terminal collagenous tail and a C-terminal globular head group with lectin (carbohydrate-binding) activity. C1q, the first member of the classical complement cascade, is related to the collectins by structure and function, except that its globular head lacks lectin activity. Deficiency of C1q and MBL is associated with an increased risk for SLE or a poor prognosis, and it has been speculated that defects in the clearance of apoptotic material (waste-disposal hypothesis) by early classical complement pathway components as well as other opsonins such as C-reactive protein (CRP) and serum amyloid protein are implicated in the pathophysiology of SLE.

Upon binding oligosaccharide structures on bacteria, viruses, and fungi, SP-D mediates aggregation, phagocytosis, and inflammatory effects. SP-D also plays a role in modulating the immune response by inhibiting T lymphocyte proliferation and interleukin 2 production, and by inhibiting lipopolysaccharide-elicted inflammatory responses in macrophages. SP-D-deficient mice show increased lung inflammation, increased oxidant production, and decreased macrophage phagocytosis after bacterial lung infection. Gardai, et al. provided a model for the appar-
ent paradox that SP-D both enhances and inhibits inflammation. They suggested that SP-D occupied by microbial ligands initiates proinflammatory activity by specific cellular interaction, while free SP-D initiates inhibition of cellular inflammation. In addition to recognizing pathogens, SP-D is also capable of binding to self-derived ligands in the form of apoptotic cells, and thereby facilitating their removal.15

Like MBL, the serum concentration of SP-D is strongly influenced by genetic factors.16 Thus, the Met11Thr polymorphism in the N-terminal part of the protein plays an important role in determining the serum level of SP-D by an unknown mechanism. In addition, this polymorphism is an important determinant for the oligomerization pattern and function of the protein.17

Based on the structural and functional similarities between MBL and SP-D and the association between low MBL and increased risk or a poor prognosis in SLE, our aim was to investigate a possible relationship between SLE and SP-D including disease manifestations and activity. In addition, we studied the distribution of SP-D Met11Thr genotypes in SLE.

**MATERIALS AND METHODS**

*Patients and controls.* A random sample of 70 patients classified as having SLE according to the revised American College of Rheumatology (ACR) criteria18,19 were recruited from a Danish SLE population.20 Median disease damage was scored according to the Systemic Lupus International Collaborating Clinics/ACR Disease Damage Index (SLICC/ACR DI)21,22. Disease activity was scored according to the SLE Disease Activity Index (SLEDAI) score23. CRP and erythrocyte sedimentation rate (ESR) were measured by standard laboratory techniques, and anti-dsDNA was quantified by ELISA (Dako, Glostrup, Denmark).

A group of 1476 healthy Danish twin individuals 18–67 years old matched for age, sex, and smoking status served as controls.24 In total, 425 twins from this group were included for genotyping.

The study was conducted in accord with the Declaration of Helsinki, and signed informed consent was obtained from each patient. The local ethics committee approved the project (J. No. 19940131).

*Measurement of SP-D.* SP-D in serum was assayed using a 5-layer ELISA as described.25 Microtiter plates were coated with Fab’1, anti-human SP-D (K477) at 1 µg/ml in 0.05 M sodium carbonate buffer, pH 9.6. After overnight incubation at 4°C the plates were washed and left in washing buffer (TBS, 0.05% Tween 20, 5 mM CaCl2) for at least 15 min at room temperature. Calibrator, control samples, and samples diluted as appropriate (1:10 to 1:60) in washing buffer were added and incubated overnight at 4°C. This was followed by successive incubations with biotinylated monoclonal anti-human SP-D antibody (Hyb 246-4), horseradish peroxidase-conjugated streptavidin, and o-phenylenediamine in citrate-phosphate buffer, pH 5, containing 0.014% H2O2. Adding H2SO4 stopped the color development. Conjugates of streptavidin and o-phenylenediamine were stored at 4°C (never > 48 h) until they could be read by the fluorescent reader. Fluorescent signals were detected in an Abi-Prism 7900HT sequence detection system (Applied Biosystems). Results were collected and analyzed by Abi-Prism SDS 2.1 software for allelic discrimination.

*Statistical analysis.* Statistical analyses were performed using Intercooled Stata version 8.2 (www.stata.com). The effects of age, sex, current smoking, and Met11Thr genotype on the SP-D serum level were analyzed using multiple regression analysis in the healthy twin population (1476 individuals) with inclusion of the confounders and 2-way interactions. This regression model was used to predict the SP-D values in healthy individuals that were matched to patients according to the parameters given. To approximate a normal distribution, the SP-D was logistically transformed when used as continuous dependent variable in linear regression analysis. Residuals resulting from the linear regression were normally distributed. The equality of matched pairs was tested by the sign test.

Relationships between serum SP-D and disease activity markers were analyzed by multiple linear and logistic regression with inclusion of the confounders age, sex, smoking status, and Met11Thr genotype. Logarithmically transformed SP-D values were used in the analysis. SP-D levels in 2 or 3 groups were compared by Mann-Whitney 2-sample test or Kruskal-Wallis equality of populations rank test, respectively.

Met11Thr genotype and allele frequencies were calculated for patients (n = 70) and controls (n = 425) by means of 2 × 2 and 2 × 3 contingency tables. The twin database was used as the control population, but only one individual in each twin pair was included in the analysis. The observed genotype frequencies were tested for Hardy-Weinberg equilibrium by chi-square analysis. Comparisons of SP-D Met11Thr genotype frequencies in patients and controls were by Fisher’s exact test. P values ≤ 0.05 were considered statistically significant.

**RESULTS**

Patient characteristics are presented in Table 1. SLE patients had significantly lower serum SP-D levels compared with healthy controls matched to each patient for age, sex, smoking status, and Met11Thr genotype (p = 0.0004). Median

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SLE (n = 70)</th>
<th>Control (n = 425)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female/male)</td>
<td>66/4</td>
<td>33/37</td>
</tr>
<tr>
<td>Age, yrs, mean (range)</td>
<td>45 (22–79)</td>
<td>45 (22–79)</td>
</tr>
<tr>
<td>Smoker/nonsmoker</td>
<td>33/37</td>
<td>33/37</td>
</tr>
<tr>
<td>Disease duration, yrs, mean (range)</td>
<td>16 (1–43)</td>
<td>16 (1–43)</td>
</tr>
<tr>
<td>ACR criteria fulfilled, median (range)</td>
<td>6 (4–10)</td>
<td>6 (4–10)</td>
</tr>
<tr>
<td>Drug treatment, no. (%) patients treated</td>
<td>20 (29)</td>
<td>20 (29)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>9 (13)</td>
<td>9 (13)</td>
</tr>
<tr>
<td>Immunosuppressants (azathioprine and cyclophosphamide)</td>
<td>2 (0–8)</td>
<td>2 (0–8)</td>
</tr>
<tr>
<td>SLICC/ACR DI</td>
<td>2 (0–8)</td>
<td>2 (0–8)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>2 (0–9)</td>
<td>2 (0–9)</td>
</tr>
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</table>

| SLICC/ACR DI: Systemic Lupus International Collaborating Clinics/ American College of Rheumatology Disease Damage Index; SLEDAI: SLE Disease Activity Index. |
serum SP-D level was 911 ng/ml (95% CI 776–1118) in patients and 1068 ng/ml (95% CI 901–1246) in controls.

Table 2 shows the partial regression coefficients between disease indicators and serum SP-D. We found no association between serum SP-D and CRP, ESR, anti-dsDNA titer, or SLICC or SLEDAI scores. Stratification of the patients into 3 groups with low (SLEDAI 0–2; n = 50), intermediate (SLEDAI 3–4; n = 11), and high disease activity (SLEDAI > 4; n = 9) revealed median SP-D levels of 949 ng/ml (95% CI 781–1159), 699 ng/ml (95% CI 358–1178), and 901 ng/ml (95% CI 643–1455), respectively (p = 0.35). About half the patients had a history of renal disease (n = 33). SP-D levels in these patients and in the patients without renal disease were 921 ng/ml (95% CI 709–1154) and 901 ng/ml (95% CI 701–1204), respectively (p = 0.86).

In addition, we found no significant differences in serum SP-D stratifying patients according to current/previous and no joint, skin, or lung manifestations (data not shown).

Hardy–Weinberg equilibrium was found in both patients and controls with respect to the Met11Thr polymorphism (data not shown). Thirty-two of the 70 patients (45.7%) carried the Met11/Met11 genotype, 30 (42.9%) the Met11/Thr11 genotype, and 8 (11.4%) the Thr11/Thr11 genotype (data not shown). Thirty-two of the 70 patients (45.7%) carried the Met11/Met11 genotype, 30 (42.9%) the Met11/Thr11 genotype, and 8 (11.4%) the Thr11/Thr11 genotype (data not shown).

When SLE patients were stratified according to Met11Thr genotype, a trend was observed toward decreasing SP-D from Met11/Met11 to the Thr11/Thr11 genotype: 1046 ng/ml (95% CI 810–1206) versus 784 ng/ml (95% CI 589–1124) versus 552 ng/ml (95% CI 303–1541) in the Met11/Met11, Met11/Thr11, and Thr11/Thr11 genotype groups, respectively (p = 0.16).

**DISCUSSION**

In this first study on SP-D in SLE, we found that SP-D in serum was significantly decreased by approximately 15% compared with carefully matched healthy control subjects. Of note, however, no relationship was found between SP-D and clinical phenotype and a selection of disease indicators including CRP, ESR, anti-dsDNA, and SLEDAI and SLICC scores. There was no significant difference in frequencies of Met11Thr genotype between the patient and control groups.

The predicted healthy control levels of SP-D were based on a multiple regression model that included the confounders age, sex, current smoking, and the Met11/Thr11 genotype, implying that the model predicted serum SP-D levels with a same age, sex, smoking status, and Met11Thr genotype as each individual patient with SLE.

Serum SP-D partly reflects pulmonary leakage, and in patients with a range of different lung diseases serum SP-D has been reported to be elevated compared to the healthy control level (as reviewed26,27). By contrast, we previously reported that SP-D is initially decreased on the day of admission to hospital in patients with acute bacterial pneumonia. Following antibiotic treatment SP-D levels increased steeply, reaching peak levels at day 5 after admission25. Similarly, we recently observed that serum SP-D is decreased in patients with newly diagnosed and untreated rheumatoid arthritis. During 1-year followup the patients improved significantly in all measures of disease activity, and in the same period serum SP-D increased significantly and almost reached the level of the healthy matched controls28. In the present study on SLE, we show for the first time that SP-D is also decreased in this chronic autoimmune disease. There was no association with conventional disease activity measures in either disease, indicating that SP-D in serum reflects a distinctive aspect of the disease pathogenesis undetected by currently available disease markers.

**Table 2. Relationship between selected disease indicators and serum SP-D.**

<table>
<thead>
<tr>
<th>Response Variable Y</th>
<th>Partial Regression Coefficient $\beta_{\lnSP-D}$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/ml</td>
<td>–4.42 (–17.35; 8.52)</td>
<td>0.48</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>–7.18 (–21.39; 7.03)</td>
<td>0.31</td>
</tr>
<tr>
<td>Anti-dsDNA (pos/neg)</td>
<td>0.55 (–0.33; 1.44)</td>
<td>0.22</td>
</tr>
<tr>
<td>SLICC</td>
<td>–0.53 (–1.56; 0.49)</td>
<td>0.30</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.34 (–0.55; 1.23)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SLICC: Systemic Lupus International Collaborating Clinics; SLEDAI: SLE Disease Activity Index.

**Table 3. Distribution of SP-D Met11Thr genotype frequencies and corresponding serum SP-D levels.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SLE Patients, n (%)</th>
<th>Controls, n (%)</th>
<th>p</th>
<th>SP-D, ng/ml (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met11/Met11</td>
<td>32 (45.7)</td>
<td>152 (35.8)</td>
<td>0.20</td>
<td>1046 (810–1206)</td>
</tr>
<tr>
<td>Met11/Thr11</td>
<td>30 (42.9)</td>
<td>196 (46.1)</td>
<td>0.28</td>
<td>784 (589–1124)</td>
</tr>
<tr>
<td>Thr11/Thr11</td>
<td>8 (11.4)</td>
<td>77 (18.1)</td>
<td></td>
<td>522 (303–1541)</td>
</tr>
<tr>
<td>Total</td>
<td>70 (100.0)</td>
<td>425 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Median SP-D values in SLE patients divided into SP-D Met11Thr genotypes. SP-D genotype frequencies were compared by Fisher’s exact test.
These observations in inflammatory conditions with different etiologies indicate that serum SP-D may be affected by an ongoing infection or a chronic inflammatory state within or outside the lung, and is not solely dependent on the permeability of pulmonary lung vessels. The exact mechanism for the low SP-D in SLE is not known.

Virtually all C1q-deficient humans develop SLE\(^2\). Because C1q binds to blebs generated in apoptotic cells\(^3\), it has been speculated that impaired clearance of these autoantigens may be important in the pathogenesis of SLE. Like C1q, SP-D binds to apoptotic cells as well as genomic DNA\(^3\), and SP-D facilitates the clearance of DNA\(^3\) and apoptotic cells\(^3\). Mice deficient in SP-D develop heavy lung infiltrations of inflammatory cells and exhibit increased numbers of dying cells in bronchoalveolar lavage\(^3\). These observations suggest that SP-D plays a critical role in removal of cellular waste. Thus, defective clearance of apoptotic material due to low SP-D could result in prolonged exposure of autoantigens to the immune system, thereby triggering an immune response to nuclear antigens.

A large proportion of the patients with SLE were treated with glucocorticosteroids, mainly in low dose (\(\leq 7.5 \text{ mg/day}\)). However, since glucocorticoids enhance SP-D expression in vitro\(^3\) and in vivo\(^3\), they would tend to decrease the difference between SLE and healthy subjects. Consequently, it would be anticipated that SP-D would be decreased even more in steroid-naive patients. Analysis of sera from steroid-naive patients is needed in order to investigate this issue in detail.

When SP-D levels were compared in SLE subsets with different disease activity, we found no significant differences, indicating that low SP-D in SLE is not primarily due to differences in drug therapy, since patients with high SLEDAI score were generally on more intense immunosuppressive treatment than the SLE patients with low disease activity. Finally, low SP-D did not appear to result from increased renal loss, since SP-D did not differ between patients with and without a history of renal disease.

Serum SP-D in healthy individuals is highly variable and strongly determined by genetic factors, with a heritability coefficient of \(h^2 = 0.83\). The Met11Thr polymorphism, which results in an amino acid substitution from methionine to threonine at position 11 in the mature protein, explains nearly half of the heritability\(^10\). In accord with this, we also observed decreasing serum SP-D levels in SLE patients with Met11/Met11, Met11/Thr11, and Thr11/Thr11 genotype; however, this did not reach statistical significance.

Clinical studies have associated the Met11Thr variation with disease. Thus, the Met11 allele was associated with severe respiratory syncytial virus infection in infants\(^37\), and the Thr11 allele was associated with increased susceptibility to tuberculosis\(^38\). In addition, a haplotype containing the Met11 allelic variant was protective against development of respiratory distress syndrome\(^39\). Our results do not support a significant association of this polymorphism with SLE.

One major limitation of our study is the relatively small sample size, which reduced the statistical power. In addition, only a small proportion of subjects had active disease. However, subgroup comparisons including patients with low, intermediate, and high disease activity and different clinical manifestations did reveal differences with respect to SP-D in the circulation.

We report for the first time that serum SP-D concentration is subnormal in patients with SLE, and that this decrease is unrelated to disease activity, damage, clinical phenotype, and conventional serum markers, indicating that low SP-D represents an aspect of the etiopathogenesis of SLE that is not reflected by conventional disease markers.

**ACKNOWLEDGMENT**

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**REFERENCES**


