Association between SLE nephritis and polymorphic variants of the CRP and FcγRIIa genes

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Objectives. To study the relationship between clinical manifestations in systemic lupus erythematosus (SLE) with polymorphisms in suggested susceptibility genes encoding FcγRIIa, FcγRIIia, FcγRIIib, CRP and IL-1Ra.

Methods. Genetic polymorphisms were analysed in 323 unrelated SLE patients and 200 healthy blood donors. The genotype frequencies were compared between clinical subsets of SLE patients, as well as with healthy controls. Clinical manifestations included the ACR classification criteria. Nephritis was further classified according to WHO class on renal biopsy.

Results. Presence of a CRP4 A-allele was associated with SLE nephritis (P < 0.01) and inversely correlated with arthritis (P < 0.01), when comparing within the SLE group. The FcγRIIa R/R genotype was associated with nephritis (WHO class III and IV, P = 0.04 for the SLE group) and in combination with the CRP4 A-allele a stronger association was noted (P < 0.001). Furthermore, the FcγRIIib NA2/NA2 genotype was associated with butterfly rash (P < 0.01). An association was found between seizures and the presence of both the FcγRIIa R/R and the FcγRIIa F/F genotypes (P < 0.01) and an inverse correlation between serositis and the CRP4 A-allele when present together with the IL-1Ra 2-allele (P = 0.01). Furthermore, a combination of the FcγRIIa R/R genotype and CRP4 A-allele was associated with lymphopenia (P = 0.02) and a similar result was found for the combination of FcγRIIa F/F and FcγRIIib NA2/NA2 (P = 0.04).

Conclusions. Polymorphic variants of the CRP and Fcγ-receptor genes are associated with the clinical phenotype in SLE. Our findings suggest an immune complex-mediated pathogenesis in nephritis and seizures, while development of arthritis may depend on other pathogenetic pathways.

Key words: Systemic lupus erythematosus, C-reactive protein, Fc receptor, Genetic, Polymorphism, Glomerulonephritis.

Introduction

Systemic lupus erythematosus (SLE) is characterized by inflammation-induced disease manifestations from many organ systems and a heterogeneous autoantibody production. The extent of organ involvement may vary substantially between patients, ranging from a mild disease without organ damage to a severe disease with a high rate of organ damage. The aetiology is not completely understood at present, but the genetic contribution to the development of SLE is considerable [1]. The current view on SLE pathogenesis includes defects in clearance of apoptotic cells and immune complexes, disturbances in peripheral tolerance and dysregulation of inflammatory response. Polymorphic genes coding for proteins involved in these different processes could have synergistic effects on susceptibility and clinical appearance of SLE. There is considerable evidence of the involvement of immune complexes in the pathogenesis of several manifestations in SLE, including nephritis [2]. Fc-receptors (FcR), C-reactive protein (CRP) and complement components are important in facilitating clearance of immune complexes and apoptotic cells from the circulation and tissues [3, 4].

Reduced IgG subclass binding capacity has been reported for variants of the FcγRIIa and FcγRIIia (arginine, R and phenylalanine, F, respectively) [5, 6], which may cause hampered immune complex clearance from the circulation [7]. The association between the FcγRIIa R-allele and SLE nephritis is controversial and suggested to be dependent on the presence of anti-C1q antibodies [8], which may constitute an amplification mechanism for renal inflammation, upon binding to deposited C1q in glomeruli [9]. Furthermore, an association between the FcγRIIia F-allele and nephritis in Caucasian SLE patients has been described [10]. The two allelic variants occurring in the FcγRIIib consist of differences in several amino acids [11]. The NA2/NA2 variant has been shown to confer reduced phagocytic capacity of neutrophils as compared with the NA1/NA1 genotype and is possibly associated with SLE and thrombocytopenia in SLE [12–14].

Two of several polymorphisms in the CRP gene, designated CRP2 (G/C) and CRP4 (G/A) have been demonstrated to have an impact on baseline serum concentration of CRP, with the C- and A-alleles being associated with lower concentrations [4]. Furthermore, the CRP4 A-allele was shown to confer increased susceptibility to SLE [4]. A microsatellite polymorphism in the IL-1Ra gene has been suggested to be involved in several autoimmune diseases [15, 16]. In SLE, an increased frequency of allele 2 (IL-1RN*2) has been found, possibly mediating a pro-inflammatory IL-1/NL-1Ra balance, with a trend towards higher frequency in nephritis and neuropsychiatric involvement [16]. In this study, we analysed the contribution to the clinical phenotype in SLE of polymorphic variants in the following genes, individually or in combination: FcγRIIa, FcγRIIia, FcγRIIib, CRP and IL-1Ra.

Patients and methods

We studied a cohort of 323 SLE patients from southern and mid-Sweden consisting of 39 men and 284 women. Additionally, 200 healthy blood donors were used as controls (100 men, 100 women). Overall, 315 SLE patients displayed four or more ACR classification criteria for SLE [17]. The remaining eight patients had a clinical SLE diagnosis with at least two organ manifestations characteristic of SLE combined with autoimmune phenomena, with no other diagnosis that could better explain the...
Table 1. Distribution of ACR classification criteria for SLE fulfilled in 323 SLE patients

<table>
<thead>
<tr>
<th>ACR criteria</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfly rash</td>
<td>187</td>
<td>58</td>
</tr>
<tr>
<td>Discoid LE</td>
<td>89</td>
<td>28</td>
</tr>
<tr>
<td>Photosensitvity</td>
<td>227</td>
<td>70</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>88</td>
<td>27</td>
</tr>
<tr>
<td>Arthritis</td>
<td>266</td>
<td>82</td>
</tr>
<tr>
<td>Serositis</td>
<td>156</td>
<td>48</td>
</tr>
<tr>
<td>Renal</td>
<td>98</td>
<td>30</td>
</tr>
<tr>
<td>Epilepsy/psychosis</td>
<td>31/9</td>
<td>9.6/28</td>
</tr>
<tr>
<td>Haemolytic anaemia</td>
<td>17</td>
<td>5.3</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>48</td>
<td>14.8</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>96</td>
<td>30</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>69</td>
<td>21</td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>196</td>
<td>61</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>18</td>
<td>5.6</td>
</tr>
<tr>
<td>ANA</td>
<td>320</td>
<td>99</td>
</tr>
</tbody>
</table>

*Wasserman test was not performed on all patients and data were not included in the table.

Fisher's exact test was used in comparisons between groups. Significance was considered if $P < 0.05$. Because of the hypothesis generating nature of the study, correction for multiple comparisons was not applied to the results. For comparison of SLICC/ACR-DI scores, the Mann–Whitney U-test was employed.

Results

The distribution of genetic variants was in Hardy–Weinberg equilibrium (data not shown). The frequency of clinical phenotypes ever presented were analysed in relation to individual genotypes and genotype combinations in the SLE patients. Statistically significant associations are displayed in Table 2, both for comparisons between SLE patients and healthy controls and comparisons between different subgroups of SLE patients. When combining three or more hypothetically unfavourable genetic variants the resulting number of patients was insufficient for meaningful statistical calculations.

The presence of an A-allele in the CRP4 gene variant was associated with SLE nephritis ($P < 0.01$) (Table 2), while the C-allele in CRP2 was not ($P = 0.17$) when comparing SLE subgroups. The CRP4 A-allele was also inversely correlated to arthritis ($P < 0.01$). In addition, we found an increased frequency of the FcyRIIIa F/F genotype among patients with a proliferative nephritis (WHO class III and IV) ($P = 0.04$), although not quite reaching significance when comparing with healthy controls ($P = 0.08$). These associations were even more pronounced when combining the FcyRIIIa F/F genotype with the CRP4 A-allele, the highest significance noted for comparison with other SLE patients ($P < 0.001$). In contrast, the FcyRIIIa R/R genotype was significantly more common in patients with type III and IV SLE nephritis compared with healthy controls ($P = 0.02$), but not when comparing with SLE patients without nephritis ($P = 0.09$).

Furthermore, the FcyRIIIb NA2/NA2 genotype was associated with butterfly rash compared with the subgroup of SLE patients without butterfly rash ($P < 0.01$). There was a correlation between seizures and the combined genotype of FcyRIIIa F/F and FcyRIIIa R/R ($P < 0.01$), which could also be detected when comparing with healthy controls ($P < 0.01$). Additionally, an association was found between reduced frequency of serositis and the combination of the IL-1Ra 2-allele and the CRP4 A-allele ($P = 0.01$), which was also significant in relation to healthy controls ($P = 0.05$). Finally, lymphopenia was more common in patients with the FcyRIIIa R/R genotype compared with healthy controls ($P = 0.03$). When the FcyRIIIa R/R genotype was combined with the CRP4 A-allele significant associations were seen both in comparison to healthy controls ($P = 0.02$) and to the SLE subgroup without lymphopenia ($P = 0.02$). Similar data for lymphopenia was obtained for the combination of genotypes FcyRIIIa F/F and FcyRIIIb NA2/NA2 (healthy controls $P = 0.03$, SLE subgroup without lymphopenia $P = 0.04$).
Anti-C1q antibodies in increased concentrations were found in 25% of the patients and this was associated with nephritis ($P < 0.01, OR 2.8, 95% CI 1.6–4.7$). Anti-dsDNA occurred in an overall frequency of 61% and was also more common in patients with nephritis ($P < 0.01, OR 2.1, 95% CI 1.3–3.5$). In order to clarify the separate roles of autoantibodies and genotypes, stratification for positive anti-C1q or positive anti-dsDNA, was performed. Only the CRP4 A-allele remained significantly associated with nephritis when analysing the anti-dsDNA positive subgroup, while in the anti-C1q positive subgroup no significant associations were found. No synergistic effects could be detected between the different genetic variants and anti-C1q or anti-dsDNA antibodies in determining the nephritis phenotype.

No association was found between any of the genetic variants studied and organ damage measured by SLICC/ACR-DI at the end of follow-up [19]. However, there was a weak association between the FcγRIIa F/F genotype and mortality during follow-up ($P = 0.03, OR = 2.1, 95% CI 1.1–4.1$), which warrants further studies.

### Discussion

The present hypothesis generating study suggests several new associations between genotypes and the clinical phenotype in SLE. Notably, the data suggests an association between SLE nephritis and a polymorphism in the CRP gene (CRP4 A-allele) conferring low CRP-levels [4], which has not been previously reported. Indications of a role of CRP in the pathogenesis of SLE include an association of anti-CRP antibodies with SLE nephritis and disease activity [26]. CRP treatment given to the lupus-prone (NZW × NZB) F1 mice ameliorates renal disease and improves survival [27]. Furthermore, certain mouse strains deficient of another short pentraxin, serum amyloid P component (SAP) develop antinuclear antibodies and occasionally nephritis [28], although the phenotype also seems to be dependent on the overall genetic background [29]. CRP binds to, among other ligands, apoptotic cells and conveys their uptake by phagocytic cells through Fc-receptors [30]. CRP can activate the classical pathway of complement, which constitutes an important scavenging mechanism for apoptotic material [31]. Additionally, monomeric CRP can also bind immune complexes [32]. Altogether, one possible explanation of the association between the CRP4 A-allele and nephritis in our study could be a reduced capacity for clearance of apoptotic cells and immune complexes in patients with the variant allele A. The CRP4 A-allele was also associated with a reduced frequency of arthritis, which perhaps could reflect that different pathogenetic pathways are operating in arthritis and nephritis. In fact, CRP has several putative immunomodulating abilities, such as induction of IL-10 production, inhibition of pro-inflammatory cytokines including TNF-α and inhibition of T-cell proliferation [33].

Studies on the contribution of Fc-receptor polymorphism to susceptibility for SLE nephritis are ample, but have given conflicting results. In a meta-analysis the FcγRIIa R/R genotype was correlated with an increased SLE susceptibility, but not with lupus nephritis [34]. Other studies suggest an increased risk of lupus nephritis in Caucasians with the low affinity IgG binding variant alleles of the FcγRIIa gene (F) [10, 35]. The finding of an increased FcγRIIa F/F frequency in SLE patients with WHO class III and IV nephritis in our study supports the importance of activating FcγR in renal disease in SLE. The observation that FcγRIIa-deficient lupus-prone mice do not develop glomerulonephritis despite immune complex deposition lends further support to the importance of stimulatory Fcγ-receptors in SLE [36], although dependent on the genetic background [37]. Interestingly, the associations with FcγRIIa F/F were more pronounced in patients having the CRP4 A-allele suggesting a combinatory influence on the inflammatory process in the kidney. One pathogenetic mechanism contributing to inflammation in lupus could be an impaired clearance of immune complexes by the FcγR allelic variants with low affinity for IgG, thus increasing the load of immune complexes potentially activating lymphocytes through FcγR in the kidney. As with complement this implies a dual role of FcγR in inflammation and tolerance, the net result upon receptor ligation being dependent on the balance of activating and inhibitory FcγR, as well as the local cytokine environment.

The finding of a correlation between butterfly rash and the FcγRIIIa NA2/NA2 genotype in our study is new. Although the pathogenesis of lupus skin manifestations has not yet been clarified, there is evidence of immune complex induced inflammation. Thus, immunoglobulins are present in the dermal–epidermal junction as evidenced by the lupus band test, correlating with the immune complex solubilizing capacity of the serum and with acquired hypocomplementaemia [38]. The inflammation could be induced by both complement activation and by Fcγ-receptors.

Our finding of a correlation between seizures and a combination of low-affinity variants of FcγRIIa and FcγRIIIa is not previously described. The finding of increased IgG concentration in cerebrospinal fluid from patients with neuropsychiatric SLE (NPSLE) without blood brain barrier alterations suggests that locally produced antibodies may be involved in pathogenesis [39]. It has been proposed that there is a cross-reactivity between anti-dsDNA antibodies and N-methyl-D-aspartate (NMDA) receptors leading to apoptosis of neuronal...
cells [40], although this has not yet been confirmed. Nevertheless, antibodies against a subunit (NR2) of the NMDA receptor has been suggested to be involved in the development of cognitive disorders in SLE-patients [41]. Size and other physical properties of immune complexes could be of vital importance for their ability to induce pro-inflammatory signals through Fcγ-receptors. Additionally, complement activation products may have both pathological and protective effects in models of inflammatory brain disease and are probably of importance also in NPSLE [42].

In a previous study, we have shown that SLE patients with nephritis have a decreased level of circulating IL-1Ra, indicating a possible role for IL-1 in the pathogenesis of lupus nephritis [43]. Blakemore et al. [16] have shown a correlation between SLE and IL-1Ra polymorphism, with a trend towards a higher frequency of the 2-allele in patients with nephritis. In this study, however, no such correlation with nephritis was found ($P = 0.28$, OR 1.3, 95% CI 0.81–2.1). Instead, we found a correlation with reduced frequency of serositis when combining the IL-1Ra 2-allele with the CRP4 A-allele. Previously, several studies have demonstrated ANA in pleural and pericardial fluid of SLE patients [44–46], but the pathogenetic significance of autoantibodies has been unclear. A more recent study indicates a role of both anti-dsDNA antibodies and IL-1β in development of lupus pleuritis [47], making the seemingly protective effect of the above stated genetic variants difficult to interpret. In SLE, an increase in serum CRP concentrations can often be seen in both serositis and arthritis, in contrast to in nephritis. This could possibly be explained by a different pathogenetic mechanism that does not to the same extent involve circulating immune complexes and thereby may not lead to CRP consumption. Another explanation though, could be a correlation of these manifestations with the absence of a low-producing CRP allele.

Furthermore, we found that the combination of the FcγRIIA R/R genotype and CRP4 A-allele was associated with lymphopenia ($P = 0.02$) and a similar result was found for the combination of FcγRIIA F/F and FcγRIIB NA2/NA2 ($P = 0.04$). Suggested mechanisms of lymphopenia in lupus include up-regulation of FAS on lymphocytes and anti-lymphocyte antibodies as well as a decreased expression of complement regulatory proteins on the lymphocytes [48–50]. The role of genetic variants of FcγRI in SLE lymphopenia has not yet been clarified.

In conclusion, this study suggests associations between SLE nephritis and polymorphisms in the CRP and FcγRIIa genes, as well as the possible involvement of genetic variants in other clinical subsets of SLE. However, the results need to be validated in larger SLE cohorts. Clearly, the development of disease manifestations requires additional environmental and/or genetic factors. The autoantibodies studied herein (anti-dsDNA and anti-C1q) did not further explain the associations.

Rheumatology key message

- Genetic variants influence the clinical phenotype in SLE.

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