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In Vitro Stimulation of C1s Proteolytic Activities by C1s-Presenting Autoantibodies from Patients with Systemic Lupus Erythematosus

Shiping He and Ya-Ling Lin

Anti-C1s autoantibodies (IgG forms), which recognize the conjunction of C1s heavy chain and light chain (C1s-presenting autoantibodies) from patients with systemic lupus erythematosus (SLE), have been found to stimulate C1s enzymatic activities. This is due to acceleration of the proteolytic hydrolysis of the synthetic substrate C1-1 by C1s, enhancement of the complex formation of C1s with its natural pseudosubstrate, C1 inhibitor (C1 inh), and promotion of proteolytic activation of its natural substrate, C4. Seven of fifteen samples from patients with SLE were found to contain such autoantibodies. The hydrolysis of the synthetic substrate C1-1 catalyzed by C1s in 25 to 27 min in the presence of anti-C1s autoantibodies was equivalent to the hydrolysis of C1-1 catalyzed by C1s alone or C1s with control IgG from healthy sera in 110 min, approximately fourfold faster than the reaction in the absence of anti-C1s autoantibodies. Densitometry scanning data showed that the formation of the C1s-C1 inh complex in the presence of anti-C1s autoantibodies was three to four times greater than that with control IgG. It was also noticed that the autoantibodies convert almost all of the latent forms of C1s to an active form that binds to C1 inh. Another group of Western blots showed that C1s cleaved C4 α-chain three times faster in the presence of autoantibodies than of control C1 inh. It is likely that the overconsumption of complement components is common in the pathogenesis of tissue damage occurring in SLE.

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; C1s, the first complement component; C1 inh, C1 inhibitor; C4, the fourth complement component; HRP, horseradish peroxidase; ECL, enhanced chemiluminescence; L-H9, a degraded heavy chain linked with light chain by disulfide bonds at 66 kDa; L-H10, a further degraded heavy chain with light chain intact.

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Materials and Methods

Sources of materials

Plasma samples were obtained from 15 SLE patients whose granulocytes ingested large quantities of Ag-Ab complexes and autoantibodies to nucleic acids. These patients had common SLE symptoms such as generalized fever, erythematos lesion, pleurisy, and kidney dysfunction. Seven of them had been found by ELISA testing to have anti-C1s autoantibodies present in their plasma. All 7 patients had early SLE symptoms. The amounts of C1q, C1s, C3, C4, and C1 inh were estimated by ELISA. C1q,
C1s, and C1 inh levels were confirmed to be normal, whereas the amounts of C4 and C3 were only 1/4 to 1/3 of normal (Table I). Isotypes of the autoantibodies were tested according to the previous description (31).

Detection of anti-C1s autoantibodies

ELISA plates (Immunul 4, Dynatech Laboratories, Basingstoke, U.K.) were coated overnight at 4°C with the following complement components (100 μl, 2 μg/ml): C1s (31), C1q, C3, C4, and C6 (Sigma, Poole, U.K.) in coating buffer (0.05 M carbonate/bicarbonate buffer, pH 9.6). Following three washes in isotonic PBS (10 mM Na2 HPO4, 1.8 mM KH2PO4, pH 7.4, containing 2.7 mM KCl and 140 mM NaCl) containing 0.1% (v/v) Tween 20 (PBST), nonspecific sites were blocked by addition of 100 μl of PBS containing 5% (w/v) gelatin (Sigma). After a 2-h incubation at room temperature, the wells were washed an additional three times with PBST.

The following incubation steps were undertaken sequentially at room temperature using 100-μl volumes of reactant and three washes in PBST between each step (31): 1) purified total IgG from healthy plasma or that of SLE patients (3 μg/ml dilution in PBS; for purification step, see below); 2) anti-human IgG labeled with horseradish peroxidase (HRP) (Sigma) for 1 h; 3) the color reaction was developed by addition of 100 μl of ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, 10 mg/25 ml; Sigma) in 0.025 M citric acid, 0.05 M Na2 HPO4, and 0.001% (v/v) H2O2) and read using an automatic ELISA plate reader (Titertek Multiscan, Herts, U.K.) at 405 nm.

Purification of anti-C1s autoantibodies

Ten milliliters of patient plasma was loaded onto a protein-G Sepharose column (5 ml, Sigma) equilibrated with PBS. Nonspecific binding proteins were removed by washing the column with PBS until the OD 280 nm reached zero. Total IgG was then eluted with 0.58% acetic acid. Fractions containing IgG were combined and dialyzed against PBS (1:2000) over night at 4°C and then subsequently loaded onto a C1s-Sepharose column (5 ml, Sigma) equilibrated with PBS. Nonspecific binding proteins were removed by washing the column with PBS until the OD 280 nm was below 0.02. Fractions containing IgG were combined and dialyzed against 10 mM Tris-HCl buffer (pH 8.0), containing 100 mM NaCl and 1 mM EDTA. The chromogen, p-nitroanilide hydroacetate (C2H5CO-NH-NO2; Immuno Ltd., Heidelberg, Germany) was dissolved in 100 mM sodium phosphate (pH 8.0), 100 mM NaCl, and 1 mM EDTA to a final concentration of 500 μM.

Quantitation of complement component is expressed as μg/ml.

### Table I. Clinical and laboratory features of SLE patients with anti-C1s autoantibodies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>C1q</th>
<th>C1s</th>
<th>C3</th>
<th>C4</th>
<th>C1 inh</th>
<th>Autoantibody Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>F</td>
<td>85</td>
<td>40</td>
<td>212</td>
<td>12</td>
<td>95</td>
<td>IgG-κb</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td>F</td>
<td>92</td>
<td>43</td>
<td>185</td>
<td>23</td>
<td>81</td>
<td>IgG-κ</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>F</td>
<td>93</td>
<td>56</td>
<td>193</td>
<td>10</td>
<td>103</td>
<td>IgG-κb</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>F</td>
<td>87</td>
<td>48</td>
<td>225</td>
<td>&lt;2</td>
<td>94</td>
<td>IgG-κb</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>F</td>
<td>103</td>
<td>45</td>
<td>162</td>
<td>&lt;2</td>
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<td>35</td>
<td>M</td>
<td>116</td>
<td>50</td>
<td>177</td>
<td>&lt;2</td>
<td>108</td>
<td>IgG-κb</td>
</tr>
</tbody>
</table>

Normal range 63–152 45–55 805–1100 70–221 85–158

The quantitation of complement component is expressed as μg/ml.

### Kinetic assays of C1s proteolytic activities in the presence of anti-C1s autoantibodies

Activated C1s was purified using the method of Sim (33), and C1 inh was purified from human plasma using the method described by Pilatte et al. (34). Activated C1s is a two-chain molecule with a molecular mass of 85 kDa (intact C1s). However, the purified C1s contains a degraded heavy chain linked with light chain by disulfide bonds at 66 kDa (referred to as L-Ho) (35, 36), a heavy chain (H) band (a minor band; see below) that was dissociated from a two-chain intact molecule at 55 kDa and a minor light chain (L) band dissociated from an intact two-chain molecule at 30 kDa under nonreducing conditions. The major 55-kDa band may be a "latent form" of C1s that was probably further degraded from the 66-kDa degradation form (referred to as L-Ho); see below.

For each reaction, 3 μg of C1 inh and the same amount of C1s were incubated at 37°C in PBS containing 1% (v/v) Triton X-100 (Sigma) for 2 h in the presence of anti-C1s autoantibodies or control IgG (5.6 μg). The reaction was stopped by adding 1:1 (v/v) nonreducing sample buffer and incubated as described above. Immune blotting and detection of complex formation using an ECL system were conducted according to the description above.

### Activation of C4 by C1s in the presence of anti-C1s autoantibodies

C4, purchased from Sigma, was dialyzed against PBS before use. Under reducing conditions on SDS-PAGE followed by immune blotting with polyclonal goat anti-C4 97 α-chain, C4 was observed to consist of three bands, namely a major band at 96 kDa (α-chain), a minor band at 80 kDa (α-chain), and a medium band at 30 kDa (γ-chain), suggesting that the polyclonal Abs recognize all three subunits with differentiations.

For the activation of C4 with C1s, 3 μg of C4 was mixed with 1.5 μg of C1s in the presence of anti-C1s autoantibodies or control IgG (2.5 μg) and incubated at 37°C for 40 min. The reaction was then stopped by adding 1:1 (v/v) reducing sample buffer (32) and heated at 100°C for 4 min before being loading onto a polyacrylamide gel. C4 activation was detected with immune blotting as described above.

### Kinetic assays of C1s proteolytic activities in the presence of anti-C1s autoantibodies

The enhancement of C1s activity by anti-C1s autoantibodies was determined kinetically by the method described by Sim and Reboloul (36) with modifications. Briefly, C1s, C1 inh, and anti-C1s autoantibodies were dialyzed against 10 mM Tris- HCl buffer (pH 8.0), containing 100 mM NaCl and 1 mM EDTA. The chromogen, 3-nitroanilide hydroacetate (C2H5CO-NH-NO2; Immuno Ltd., Heidelberg, Germany) was dissolved in 100 mM sodium phosphate (pH 8.0), 100 mM NaCl, and 1 mM EDTA to a final concentration of 500 μM.

### Determination and quantitation of anti-C1s autoantibody subclasses

Purified anti-C1s autoantibodies (100 μl of 2 μg/ml) were coated on the walls of an ELISA plate in coating buffer overnight at 4°C. Blocking of nonspecific binding sites was conducted with 3% BSA in PBS for 2 h at room temperature. Mouse mAbs (100 μl) to human IgGl (1:3000), IgGl (1:2000), IgGl (1:5000), and IgGl (1:2000) (all FC specific, all from Sigma) were added, and incubation at room temperature was conducted for 2 h before the addition of 100 μl of rabbit anti-mouse Ig conjugated with HRP (1:2000). Three washes in PBS containing 0.1% Tween 20 were conducted after each step. The plates were developed with orthophenylene diamine as described above.

Quantitation of human IgG subclasses was conducted using different concentrations of standard human IgG subclasses (Sigma) to construct standard curves from which the ratios of anti-C1s IgG subclasses were calculated.

### Immune blotting detection for anti-C1s autoantibodies, C1s-C1 inh complex, and C4 activation

Protein samples were incubated in either partial reducing sample buffer (0.125 M Tris-HCl, pH 6.8, 4% (v/v) SDS, and 20% (v/v) glycerol, pH 6.8 (TSG)) containing 0.8% (v/v) 98 β-mercaptoptoethanol (Sigma), complete reducing sample buffer (TSG containing 5% 98 β-mercaptoethanol), or nonreducing sample buffer (TSG) at 37°C for 2 h before loading onto a 7.5% polyacrylamide gel containing 1% SDS. After electrophoresis, transfer of proteins onto nitrocellulose membranes, membrane washing, nonspecific site blocking, binding of primary Abs to Ags and of secondary Abs to the primary Abs, and development using enhanced chemiluminescence (ECL) were conducted as previously described (32). ECL autoradiographs were scanned using a Molecular Dynamics scanner (Molecular Dynamics, Chesham, Buckinghamshire, U.K.). Relative amounts of protein bands were calculated based on the scanning data.

### Purification of anti-C1s autoantibodies

Ten milliliters of patient plasma was loaded onto a protein-G Sepharose column (5 ml, Sigma) equilibrated with PBS. Nonspecific binding proteins were removed by washing the column with PBS until the OD 280 nm reached zero. Total IgG was then eluted with 0.58% acetic acid. Fractions containing IgG were combined and dialyzed against PBS (1:2000) overnight at 4°C and then subsequently loaded onto a C1s-Sepharose column at room temperature (5 mg/ml C1s, 3 ml). Nonspecific binding IgG molecules were washed out with PBS, and anti-C1s autoantibodies were eluted with 0.58% acetic acid followed by immediate dialysis in PBS to remove the acetic acid. Usually, 0.5 to 1.5 mg specific anti-C1s autoantibodies can be purified from 180 mg of total IgG.
The concentrations of purified IgG, C1 inh, and C1s were determined chromatographically (37–39). In all reactions, C1s, anti-C1s autoantibodies, and C1 inh were kept at a fixed concentration of 30 nM. C1s and anti-C1s autoantibodies were incubated at 37°C for 40 min before adding the chromogen to a final concentration of 250 μM, and the absorbency changes at 405 nm were recorded at 1-min intervals for 110 cycles using a Perkin-Elmer spectrophotometer (Norwalk, CT). C1 inh was added to the pre-formed immune complex of C1s-autoantibodies (C1s and autoantibodies were incubated at 37°C for 20 min to form immune complex) followed by incubation at 37°C for a further 20 min before addition of the chromogen. The absorbency changes at 405 nm were recorded as described above.

Results

Presence of anti-C1s autoantibodies in patient sera

The ELISA results indicated that anti-C1s autoantibodies were found in the plasma of 7 of the 15 patients with SLE but not in plasma from normal controls. The positive results are shown in Figure 1. The ELISA screenings confirmed the presence of IgG1 and IgG3. The results are listed in Table I. The concentration of IgG1 subclass in five patients is ~32 to 41% and that of IgG3 is 29 to 46%, confirming that most of the IgG molecules are IgG1 and IgG3, which are the possible stimulators for the activation of complement components. There are, however, some IgG2 and -4 subclasses that account for 16 to 39% of total IgG, suggesting that the major components of the total IgG are those that are related to complement activation.

Quantitation of IgG subclasses of anti-C1s autoantibodies

Quantitation of IgG subclasses from direct ELISA confirmed the presence of IgG1 and IgG3. The results are listed in Table II. The concentration of IgG1 subclass in five patients is ~32 to 41% and that of IgG3 is 29 to 46%, confirming that most of the IgG molecules are IgG1 and IgG3, which are the possible stimulators for the activation of complement components. There are, however, some IgG2 and -4 subclasses that account for 16 to 39% of total IgG, suggesting that the major components of the total IgG are those that are related to complement activation.

Binding of anti-C1s autoantibodies to the conjunction of C1s heavy and light chains

Western blots confirmed that double affinity-purified anti-C1s autoantibodies bind to the C1s molecule (Fig. 2). Figure 2 shows that all of the purified anti-C1s autoantibodies bound to C1s two-chain intact protein and L-H" form, but not to heavy-chain (H) or light-chain (L) individually. It was also noticed that a small portion of the autoantibodies bound to the L-H" form at 55 kDa. In the control experiment, normal IgG was shown not to bind to C1s as detected by ELISA (Fig. 1) nor to any part of the C1s molecule as detected by Western blot. We also blotted other complement components (C1q, C3, C4, C5, and C6) onto nitrocellulose, using the autoantibodies as primary Abs, but none of these components were shown to bind to the autoantibodies (data not shown).

Acceleration of C1s proteolytic hydrolysis of synthetic substrate in the presence of anti-C1s autoantibodies

Kinetics of the C1s proteolytic hydrolysis of the synthetic substrate C1-1 is shown in Figure 3. In control experiments, the substrate C1-1 showed almost no self-hydrolysis (curve 1 in Fig. 3A) and anti-C1s autoantibodies alone had negligible effect on the hydrolysis of the chromogen (curve II in Fig. 3, A

| Table II. Quantitation of IgG subclasses of anti-C1s autoantibodies* |
|------------------------|--------|--------|--------|--------|--------|
| IgG Subclasses        | 1     | 2     | 3     | 4     | 5     |
| Anti-C1s Autoantibodies (%) Patient No. | 1     | 2     | 3     | 4     | 5     |
| IgG1                   | 35    | 38    | 32    | 41    | 33    |
| IgG2                   | 15    | 8     | 19    | 14    | 15    |
| IgG3                   | 40    | 46    | 29    | 35    | 28    |
| IgG4                   | 10    | 8     | 20    | 10    | 24    |

*Only five patients’ autoantibodies were examined due to the insufficiency of autoantibodies from patient 6 and 7.
and B) under the experimental conditions used. Furthermore, comparison of reaction from equimolar concentrations of control IgG and C1s to that from C1s alone indicated no significant difference in the absorbency change toward hydrolysis of C1-1 catalyzed by C1s (curve 2 for C1s alone and 10 for C1s with control IgG in Fig. 3A), indicating that the control IgG did not interfere with or enhance C1s enzymatic activities. In the presence of equimolar concentrations of anti-C1s autoantibodies, the amount of C1-1 hydrolysis catalyzed by C1s in 25 to 27 cycles (1-min intervals) was approximately equal to that catalyzed by C1s alone or C1s with control IgG in 110 cycles (curves 4–9 in Fig. 3A), about a 4.4-fold difference. Although we studied only six of the seven autoantibodies in the kinetic assays, due to insufficient protein purified from the seventh patient (Fig. 1), all the rest of the autoantibodies were observed to accelerate the hydrolysis of C1-1 by C1s.

In a further series of kinetic assays for inhibition of C1s in the presence of C1 inh, the C1s proteolytic activities toward chromogen (curve a in Fig. 3C), C1-1, were greatly decreased in the presence of C1 inh (curve b in Fig. 3, C and D) and were further decreased in the presence of C1 inh and autoantibodies (curves c–h in Fig. 3, C and D), probably due to the enhancement of intact C1s-C1 inh complex formation and other complexes formed between the L-H**-C1s and C1 inh (see below).

Promotion of C1s-C1 inh complex formation by anti-C1s autoantibodies

The intact C1s forms an SDS-resistant complex with C1 inh, which is ~195 kDa (lanes 2–9 in Fig. 4), in PBS containing 1% (v/v) Triton X-100. The 55-kDa band seems to bind to C1 inh in the presence of anti-C1s autoantibodies, forming an SDS-resistant complex located at 160 kDa (lanes 3–9 in Fig. 4). However no SDS-resistant complex was formed between this band and C1 inh in the presence of control IgG (lane 2 in Fig. 4). Interestingly, a band located at 66 kDa, which was thought to be a degraded heavy chain linked with a light chain of C1s (L-H**) (35–36), did not form complexes with C1 inh in the presence or the absence of anti-C1s autoantibodies.

The SDS-resistant complexes formed between the intact C1s and C1 inh (195 kDa) were enhanced approximately four to five times in the presence of autoantibodies compared with normal IgG. In addition, most of the 55-kDa band formed the 160-kDa complex with C1 inh in the presence of autoantibodies.

Enhancement of C4 activation by C1s in the presence of anti-C1s autoantibodies

During the activation of C4, C1 complex cleaves C4 α-chain into an 86-kDa and a 9-kDa fragment (C4a) due to the proteolytic activities of C1s and/or C1r (40). C4a is then released from the enzymogen, and the remaining subunits form C4b (activated). Incubation of C4 with C1s in the presence of control IgG in PBS containing 1% Triton X-100 resulted in cleavage of C4 α-chain (lane 2 in Fig. 5) as observed from reducing SDS-PAGE followed by Western blots. Under similar conditions with anti-C1s autoantibodies, the 86-kDa band was increased (lanes 3–8 in Fig. 5) to an average of threefold greater than in the absence of autoantibodies.

Discussion

The present studies demonstrate the biochemical mechanisms by which anti-C1s autoantibodies upgrade the C1s proteolytic/enzymatic activity levels by presenting C1s molecules to their reactive partners, C1 inh, C4, and C1-1. These autoantibodies may convert the L-H** form of C1s to its active form and therefore facilitate the molecular collision between C1s and its reactive counterparts. Alternatively, this process may facilitate C1s reaching a plateau at which the enzyme is in the most reactive state of transition (41) so as to stimulate its proteolytic/enzymatic activities. Finally it remains possible that the autoantibodies may convert the C1s enzymogen to its activated form, C1s. This latter possibility is, however, unlikely, because our activated C1s was purified from C1 complexes rather than from enzymogen (33). These studies provide a mechanism that explains the biochemical and immunologic processes for overconsumption of complement components in the development of SLE.

During the activation of complement components in the classic pathway, an enzymogen, C4, can be activated by C1 complex that is related to C1s and/or C1r proteolytic activities. Complement component activation with lower or higher levels would generate immunity disorders. In the case of higher activation, the inflammatory cells or even the resident tissue cells would be lysed and release inaccessible macromolecules, which could become self-Ags for B cells. The generation of large amounts of immune complexes locally would result in impaired clearance by the circulation, especially in the small capillary blood vessels. The studies reported here demonstrate that anti-C1s autoantibodies increase the speed of activation of C4 by C1s threefold, which may explain the lower level of C4 in SLE patient serum reported previously (27, 29).

The increased C4 activation by C1s proteolytic activity in the presence of autoantibodies is consistent with the increase in C1s enzymatic activity toward the synthetic substrate C1-1, also caused by anti-C1s autoantibodies (see Results). This probably reflects conversion of the L-H** form of C1s to an active form (see below).

C1 inh is a natural inhibitor of C1 complex, which binds to activated C1s and/or C1r to form an SDS-resistant complex. Higher levels of C1 inh-C1s-C1r-C1 inh complex have been observed in patients with SLE (30). In our experiment, C1 inh-C1s

FIGURE 2. A Western blot from partial reducing SDS-PAGE showing the binding of autoantibodies from SLE patients to C1s molecule. Each lane contains 3 μg of C1s and it was blotted onto nitrocellulose sheet after separation by SDS-PAGE. The primary Abs are goat anti-human C1s (lane 1), control human IgG (lane 2) and SLE patients’ autoantibodies (patients 1–7 in lanes 3–9), respectively, and the HRP-conjugated secondary antibodies are rabbit anti-goat IgG (lane 1) and rabbit anti-human IgG (lanes 2–9). Labels on the left are m.w. markers and those on the right are light chain (L), heavy chain (H), latent form C1s (L-Hoo), a degraded two chain C1s (L-H**) and the intact C1s molecule.
complex formation was enhanced in the presence of anti-C1s autoantibodies. The complexes formed between C1 inh and C1s were normally threefold greater in the presence of autoantibodies. The enhancement of inhibition of C1s activities by C1 inh in the presence of autoantibodies not only comes from the enhancement of complex formation between intact C1s and C1 inh but also from complexes formed between C1 inh and the L-Hoo form of C1s. Normally, L-Hoo does not form an SDS-stable complex with C1 inh. However, in the presence of autoantibodies, almost all of the L-Hoo C1s binds to C1 inh to form a 160-kDa SDS-resistant complex, which is consistent with its becoming activated. These experiments were confirmed by kinetic assay with synthetic chromogen, in which the remaining C1s enzymatic activity in the reactions of C1s with C1 inh was further decreased in the presence of autoantibodies. All three sets of experiments showed that the proteolytic/enzymatic activities of C1s were upgraded by anti-C1s autoantibodies, usually by three- to fourfold.

Interestingly, the autoantibodies studied here do not recognize either heavy chain or light chain alone, but both chains are required. Under an electromicroscope, C1s molecule consists of both heavy chain and light chain (42) linked by a fiber-like rod. The

**FIGURE 3.** Plots of kinetic assays of C1s enzymatic activities toward the hydrolysis of synthetic substrate in the presence of anti-C1s autoantibodies. A, Progress curves showing the acceleration of C1s enzymatic activities in the presence of anti-C1s autoantibodies. The reactions in the plots are chromogen only (curve 1), C1s and chromogen (curve 2), C1s/C1 inh and chromogen (curve 3), C1s/autoantibody (patients 1–6) and chromogen (curves 4–9), C1s/control IgG and chromogen (curve 10), autoantibodies and chromogen (curve 11), C1s/autoantibody 1/C1 inh and chromogen (curve 12). B, A more detailed plot for the progress curves of reactions 1, 3, 11, and 12. C, Progress curves showing the enhancement of inhibition of C1s proteolytic activities toward C1 inh. A control reaction (curve a) in the plot is C1s with chromogen. The remaining reactions are: C1s/C1 inh and chromogen (curve b), C1s/autoantibody (patients 1–6)/C1 inh and chromogen (curves c–h). D, A detailed plot of progress curves for reactions b–h.

**FIGURE 4.** Western blot of reaction products of C1s and C1 inh in the presence of anti-C1s autoantibodies. The blot was from a nonreducing SDS-PAGE (7.5%). The reactions are C1s alone (lane 1), C1s/C1 inh/ control IgG (lane 2) and C1s/autoantibodies (patients 1–7)/C1 inh (lanes 3–9). The labels on the right are m.w. markers and those on the left are C1s-C1 inh covalently bound complex (C1s-C1 inh*), latent form C1s and C1 inh covalently bound complex (L-Hoo-C1 inh*), intact C1s molecule, a degraded form C1s (L-H*), latent form C1s (L-Hoo*), C1s heavy chain (H) and light chain (L).
requirement of both heavy chain and light chain for the recognition of autoantibodies indicates that the epitope for the autoantibodies is probably located within or near the conjunction of the light chain and heavy chain.

The presence of anti-C1s autoantibodies in the early stage sera from SLE patients indicates that the autoantibodies may be the direct enhancers responsible for overconsumption of complement components. Although we have no direct evidence to show whether the overconsumption of complement components is responsible for the production of Abs to nuclear macromolecules, the deduction of anti-C1s autoantibodies and the increase of Abs to nuclear macromolecules in the late stage sera from SLE patients suggests a close relationship between the two events.

Anti-C1s autoantibodies were not only found to stimulate C1s-C1 inh complex formation in a purified system, but they were also found to stimulate the formation of C3dg and the 23-kDa subunit of C3c in the whole plasma in which C1 inh was present. In the presence of the autoantibodies, C3dg (38 kDa) and the 23-kDa subunit were found to be generated 3 to 3.5 times as much as in the control reactions (in the presence or absence of normal IgG), as shown by Western blot (data not shown) probed with polyclonal anti-human C3 followed by anti-goat Ig conjugated with HRP. These results suggest that the overall lower levels of complement components (Table I) are related to the stimulation of C1s proteolytic activities. Moreover, the determination and quantitation of IgG subclasses from the purified anti-C1s autoantibodies (Table II) also support the suggestion that overactivation of complement components may have occurred in the SLE patients whose sera contained anti-C1s autoantibodies.

In conclusion, our study reports a new mechanism by which autoantibodies may play a role in the pathogenesis of SLE. This mechanism includes 1) initiation of overactivation of complement components (the overactivation of the complement components may, in turn, convert inaccessible macromolecules to self-Ags by

**FIGURE 5.** Western blot of reaction products of C1s and C4, showing the enhancement of activation of C4 by C1s in the presence of autoantibodies. Protein bands were probed with anti-human C4 \( \alpha \)-chain Abs. The higher band is the uncleaved C4 \( \alpha \)-chain and the lower band is the cleaved form. Purified C4 (lane 1) was incubated with normal IgG (lane 2) or anti-C1s autoantibodies from patients 1–6 (lanes 3–8). Only 6 autoantibodies were examined in the activation of C4 due to the insufficient of patient 7 autoantibodies.

**FIGURE 6.** Schematic diagram showing the enhancement of C1s proteolytic activities toward C4 and C1 inh in the presence of anti-C1s autoantibodies.
and deposition of further immune complexes in capillary blood vessels, such as glomeruli, as a result of activation of C1s by autoantibodies. This stimulation of C1s proteolytic activity by the autoantibodies occurs via two mechanisms: first, by stimulating the two-chain intact C1s molecules; and second, by converting the latent form, C1s, to an active form.

The mechanism studied in this paper is summarized in Figure 6. In route A, activated C1s either activates C4 by cleaving off a 9-KDa fragment (C4a) from C4 α-chain or binds to C1 inh to form a covalently bound complex. The binding of the autoantibodies to C1s molecules may accelerate these processes. In route B, the binding of autoantibodies to L-H11 C1s may activate C1s molecules toward C4 and C1 inh. Therefore, the production and deposition of large amounts of immune complexes (IgG-C1s, IgG-C1s-C1 inh, IgG-C1s (latent form)-C1 inh (C1 inh is referred to as “cleaved C1 inh”)) may be an important event for the development of SLE and the overactivation of complement components leading to the lysis of cells and exposure of inaccessible macromolecules to B cells. This process could lead to the development and deposition of further immune complexes at sites of inflammation in SLE.

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