Serum Complement Protein C3a Level is Associated With Anti-dsDNA Ab in Systemic Lupus Erythematosus: a Brief Report

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Abstract

Objective.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that is characterized by complement dysfunction and a wide range of autoantibody production. However, data about the relation between C3a and anti-ds DNA Ab in SLE are scarce.

Methods.

Thirteen SLE patients, diagnosed on the basis of SLICC classification criteria (6 patients positive for anti-Sm Ab, 7 patients positive for anti-dsDNA Ab) were enrolled in the present study. Serum levels of C3a, C1q were quantified by Western Blotting. Clinical, biochemical, serological and other markers of disease activity (anti-SM, anti-dsDNA) were measured by standard laboratory procedure.

Results.

Serum C3a levels were significantly higher in anti-dsDNA Ab (+) patients compared to anti-Sm Ab (+) patients (p < 0.01). And serum C3a levels positively correlated with SLE Disease Activity Index (SLEDAI) (p < 0.05, r = 0.6134). Interestingly, C3a was slightly correlated positively with D-Dimer, but no significant difference was found (p = 0.0983, r = 0.4783).

Conclusion.

C3a level is relative to SLE disease activity and may be a promising biomarker for monitoring thrombophilia in SLE.

Introduction

Complement, as an essential part of innate immunity and an evolutionary old system, is highly conserved among a wide variety of species, emphasizing its importance in immune defense throughout evolution [1]. Upon stimulation, complement can be activated within seconds via three different pathways, thereby displaying multiple immune effector functions in controlling infection and maintaining homeostasis[2, 3]. The small activation production C3a and C5a, also called anaphylatoxins, are mainly involved in promoting inflammation, including release of proinflammatory cytokines, degranulation of mast cells, an increase in vascular permeability, smooth muscle cell contraction and chemotaxis of immune cells[4]. However, complement behaves as a “double edged sword”. Dysfunction of complement system often disrupts homeostasis and ultimately leads to severe diseases, such as autoimmune diseases, infection, tissue damage and tumor progression[2].

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that affects all ages, sexes, ethnicities, and backgrounds[5]. It is characterized by complement dysfunction and a wide range of autoantibody production. Furthermore, complement breakdown products are factors that contribute to tissue damage in SLE[6]. The most unique and common autoantibodies are antibodies (Abs) against double-stranded DNA (ds DNA) and Smith (Sm), which are important criteria for the classification of SLE[7]. An important features of anti-ds DNA Ab is its quantitative variation over time, which can essentially disappear with treatment in some cases[8]. In contrast, anti-Sm Ab level is often stable over time and seems resistant to the effects of treatment[7].

Both complement C3a and anti-ds DNA Ab indicate the acute phase responses. However, data about the relation between C3a and anti-ds DNA Ab in SLE are scarce. In this study, we investigated the correlation of serum C3a with anti-ds DNA Ab, Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores and D-Dimer. A significant association between serum C3a and anti-ds DNA Ab, SLEDAI was found, indicating C3a as an important biomarker for disease activity.

Patients And Study Design

Subjects

Blood were obtained from 13 consecutive unselected SLE patients identified according to the Systemic Lupus International Collaborative Clinics (SLICO) classification criteria[9], including 6 patients (46.2%) positive for anti-Sm Ab, 7 patients (53.8%) positive for anti-dsDNA Ab (Table 2), as well as the healthy blood donors. Serum was obtained by allowing whole blood to clot at room temperature for 30 min, then leaving the whole samples stay on ice for another 60 min, followed by centrifugation at 4000 rpm at 4°C for 10 min. The supernatant was collected and stored at – 80°C until use.
Written informed consent was obtained for each participant. This study was approved by the Ethics Committee of Union Hospital at Huazhong University of Science and Technology, and the methods were applied in accordance with the approved guidelines. Disease activity was measured using the Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K).[10]

Routine laboratory investigations

Routine laboratory investigations were performed, including Coagulation Tests (D-Dimer, FDP, ATIII and so on), inflammatory markers (ESR, CRP, PCT), liver and kidney function tests. Immunological examinations such as complement (C3 and C4), cytokines (IL-2, IL-4, IL-6, IL-10) and autoantibody screen were measured by standardized technique.

Measurement of the Complement proteins

The Serum C3a and C1q were detected by western blot. Briefly, serum (0.5ul/sample) was premixed with PBS (7.5ul/sample) and roti-load 1 (2ul/sample), then cooked for 10min at 98°C. The mixed samples were then separated by 12% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. The membranes were blocked in 4% skim milk powder and 1% BSA (supplied with 0.05% Tween-20) at 4°C overnight, followed by incubation at room temperature for 1 h with either Rabbit anti-human C3a (1:2000; Cat No. #A218, Complement Technology, Inc.) or Goat anti-human C1q (1:2000; Cat No. #A200, Complement Technology, Inc.). After washing, horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2000; Cat No. SA00001-2, Proteintech, Inc.) and rabbit anti-goat IgG (1:2000; Cat No. SA00001-4, Proteintech, Inc.) were then added as the secondary antibody. Bands were analysed using a ChemiDoc™ XRS+ Imaging System with Image Lab™ Software version 5.2. (Bio-Rad Laboratories, Inc.).

Statistical analyses

Statistical analyses were performed by using GraphPad Prism (version 5.01). Mean serum level of C3a and C1q in two groups was compared by Student's t test. Correlation of C3a with SLEDAI scores, anti-dsDNA Ab, complement components C3 and C4, IFN-a and IL-6 were analyzed by Pearson correlation test. The results were expressed as median and range. Statistical tests with two-tailed p values less than 0.05 were considered significant.

Results

Clinical characteristics of SLE patients

The comparison of clinical characteristics of SLE patients with anti-Sm Ab (+) and anti-dsDNA Ab (+) is shown in Table 1. A total of 13 SLE patients were enrolled in the present investigation. The mean age (range) of anti-dsDNA Ab and anti-Sm Ab positive patients were 38.5 (23-57) and 48 (16-68) years, respectively. The mean duration of disease (range) were 2.5 (0-13) and 1 (0-18) years, respectively. The distributions of the demographic and laboratory features and scores on the SLEDAI were not statistically different between the two groups.
| Features                              | SLE       | SLE       |
|--------------------------------------|-----------|-----------|
|                                      | anti-Sm Ab(+) | anti-dsDNA Ab(+) |
| Age, years, median (range)           | 38.5 (23-57) | 48 (16-68) |
| Sex, women/men                       | 5:1       | 6:1       |
| Disease duration, years, median (range) | 2.5 (0-13) | 1 (0-18)  |
| SLEDAI score, mean ± SD              | 6±2.10    | 13.14±7.10 |
| D-Dimer (mg/L)                       | 0.47±0.32 | 0.99±0.57 |
| FDP (ug/ml)                          | 3±1.55    | 3.48±1.73 |
| ATIII (%)                            | 98±11.61  | 92.17±6.15 |
| PT (s)                               | 12.9±0.35 | 13.11±0.80 |
| ESR (mm/h)                           | 9.67±4.50 | 25.14±42.08 |
| CRP (mg/dl)                          | 17.55±6.15 | 6.49±1.25 |
| IgE (g/L)                            | 37.08±30.88 | 66.58±56.80 |
| IgG (g/L)                            | 11.93±3.30 | 11.22±3.13 |
| IgA (g/L)                            | 1.72±0.92  | 2.57±1.08 |
| IgM (g/L)                            | 0.66±0.33  | 0.76±0.29 |
| C3 (mg/dl)                           | 0.78±0.19  | 0.47±0.12 |
| C4 (mg/dl)                           | 0.2±0.05   | 0.11±0.04 |
| Albumin (g/dl)                       | 38.61±4.67 | 32.76±5.13 |
| CK (U/L)                             | 31.2±4.67  | 32.76±5.13 |
| LDH (U/L)                            | 226±82.67  | 222.6±50.70 |
| α-HBDH (U/L)                         | 182.25±59.01 | 173.83±34.89 |

anti-Sm Ab: anti-Smith antibodies; anti-dsDNA: anti-double-stranded DNA antibodies; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index;
| Patients Code | AS-1 | AS-2 | AS-3 | AS-4 | AS-5 | AS-6 | AD-1 | AD-2 | AD-3 | AD-4 | AD-5 | AD-6 | AD-7 |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Age           | 57   | 30   | 33   | 50   | 44   | 23   | 64   | 53   | 16   | 32   | 48   | 68   | 44   |
| Disease duration, years | 13   | 2    | 0    | 10   | 3    | 0    | 18   | 1    | 2    | 1    | 0    | 14   | 0    |
| SLEDAl me     | 6    | 7    | 6    | 7    | 8    | 2    | 9    | 8    | 4    | 13   | 22   | 13   | 23   |
| ESR (mm/h)    | 13   | 12   | 14   | 10   | 2    | 7    | 8    | 9    | 19   | 9    | 4    | 7    | 120  |
| Albumin (g/dl) | 35.5 | 42   | 37.6 | 31.8 | 44.8 | 40   | 34.4 | 36.7 | 35.7 | 34.6 | 23.2 | 36.6 | 28.1 |
| CK(U/L)       | -    | 51   | 33   | 27   | 34   | 11   | 66   | 36.7 | 27   | 16   | 9    | 25   | 12   |
| LDH(U/L)      | -    | 369  | 180  | 225  | 187  | 169  | 251  | 273  | 191  | 168  | 201  | 280  |
| α-HBDH(U/L)   | -    | 267  | 130  | 164  | 168  | 205  | 191  | 144  | 131  | 156  | 216  |
| IL-2          | 0.79 | 4.21 | 0.89 | 0.79 | 2.03 | 2.19 | 1.29 | 0.76 | 1.29 | -    | 0.97 | -    | 11.31|
| IL-4          | 1.45 | 2.34 | 1.41 | 1.27 | 2.49 | 2.52 | 1.87 | 1.69 | 0.94 | -    | 1.39 | -    | 3.43 |
| IL-6          | 14.01| 10.73| 3.79 | 1.86 | 4.16 | 5.63 | 25.99| 10.56| 27.17| -    | 196.17| -    | 29.33|
| IL-10         | 2.34 | 2.32 | 2.18 | 1.69 | 3.73 | 4.29 | 2.68 | 3.36 | 6.01 | -    | 5.73 | -    | 9.3  |
| TNF-a         | 17.83| 2.06 | 1.37 | 1.55 | 2.71 | 12.7 | 10.8 | 10   | 2.08 | -    | 49.33| -    | 8.04 |
| IFN-γ         | 1.7  | 1.46 | 1.18 | 1.49 | 2.32 | 2.64 | 1.54 | 1.41 | 2.1  | -    | 1.07 | -    | 39.71|
| CD4/CD8       | 1.17 | 0.3  | 2.05 | 0.29 | 0.96 | 0.65 | 1.24 | 0.87 | 1    | -    | 0.89 | 0.5  | 2.1  |
| anti-Sm Ab(Al)| >8.0 | 1.9  | 5.9  | 1.9  | 2.9  | 1.4  | <0.2 | 0.3  | <0.2 | <0.2 | <0.2 | <0.2 | 0.3  |
| anti-ds DNA Ab(LU/ml) | <1 | 1 | <1 | 2 | 1 | 7 | 84 | 58 | >300 | 29 | 32 | 17 | >300 |
| IgE(g/L)      | 11.7 | 10.62| 62.9 | 10.85| 44.45| 81.98| 67.81| 44.99| 36.92| 189.9| 38.87| 66.07| 21.51|
| IgG(g/L)      | 8.24 | 11.5 | 15.9 | 16   | 10.5 | 9.42 | 8.74 | 11   | 14.8 | 14   | 6.54 | 13.9 | 9.54 |
| IgA(g/L)      | 1.06 | 1.39 | 2.9  | 0.69 | 2.81 | 1.47 | 4.25 | 2.12 | 3.11 | 1.98 | 0.88 | 3.21 | 2.45 |
| IgM(g/L)      | 0.687| 0.649| 1.24 | 0.252| 0.449| 0.718| 1.01 | 0.468| 1.01 | 0.949| 0.285| 0.701| 0.888|
| C3 (g/L)      | 0.635| 0.962| 0.77 | 1.03 | 0.767| 0.524| 0.599| 0.555| 0.54 | 0.26 | 0.426| 0.536| 0.427|
| C4 (g/L)      | 0.16 | 0.256| 0.226| 0.24 | 0.169| 0.147| 0.12 | 0.183| 0.085| 0.1  | 0.13 | 0.125| 0.052|
| CRP(mg/L)     | <2.98| 13.2 | <2.98| 21.9 | <2.98| <2.98| <2.98| <2.98| <2.98| 7.37 | <2.98| <2.98| <2.98| 5.6 |
| PCT(uug/L)    | <0.13| <0.13| <0.13| 0.31 | <0.13| -    | <0.13| 0.24 | -    | -    | <0.13| -    | -    |
| D-D(mg/L)     | 0.49 | 0.22 | 0.57 | 1.04 | 0.27 | 0.22 | 1.74 | 1.50 | 1.43 | 0.31 | 1.06 | 0.33 | 1.09 |
| FDP(uug/ml)   | 4    | 4    | 4    | 4    | 1    | 1    | 5.9  | -    | 4    | 4    | 4    | 1    | 2    |
| ATIII(%)      | 98   | 119  | 92   | 84   | 97   | 98   | 92   | -    | 94   | 98   | 84   | 86   | 99   |
| PT(s)         | 12.7 | 12.7 | 12.9 | 13.6 | 12.7 | 12.8 | 12.7 | -    | 13.3 | 11.9 | 14.3 | 13.1 | 13.4 |
| APTT(s)       | 34   | 34.9 | 34.1 | 39.2 | 33.7 | 40   | 31   | -    | 38.3 | 33.3 | 84   | 38.9 | 28.1 |
| FIB(g/L)      | 3.51 | 3.98 | 2.34 | 5.1  | 3.36 | 2.32 | 2.09 | -    | 3.18 | 3.28 | 1.75 | 2.8  | 2.89 |
### Higher serum C3a levels were identified in anti-dsDNA Ab positive SLE patients

Serum levels of C3a and C1q in SLE patients (anti-Sm Ab (+) (n=6); anti-dsDNA Ab (+) (n=7)) were quantified by western blot (Figure 1(a) and 1(b)). As shown in Figure 1(c), anti-dsDNA Ab (+) patients displayed a significantly higher level of serum C3a integrated density (mean=1.24, SD=0.561) compared to anti-Sm Ab (+) patients (mean=2.59, SD=0.79) (P < 0.01; n = 13). While the level of serum C1q don’t show differences between two groups (P = 0.1812; n = 13) (Figure 1 (d)), indicating the appearance of anti-dsDNA closely correlates with complement overactivation.

**Serum C3a correlates positively with SLEDAI score**

Correlation between serum C3a and SLEDAI scores was analyzed by Spearman rank correlation coefficient and the result is shown in Figure 2(a). We observed a significant positive correlation of serum C3a levels with SLEDAI score (p < 0.05, r = 0.6134), while C3a was slightly correlated positively with D-Dimer, but no significant difference was found (Figure 2(b)) (p = 0.0983, r = 0.4783). These data indicate C3a level, to some extent might be used for predicting the SLE disease severity.

### Discussion

SLE is an autoimmune disease characterized mainly by inflammation. After onset, the clinical manifestations of patients are relatively complex, the condition is unstable, and the recurrence rate is high[11]. Complement system is a central immune surveillance system, which can be activated through classical, lectin and alternative pathways. Dysfunction of complement leads to many diseases’ progression, such as SLE, a-HUS, PNH, DIC and so on[12]. Currently, C3 and C4 have become mainly biomarkers for monitoring SLE disease activity. But several limitations exist as low levels of C3, C4 are found, even more infrequently in very early and milder disease, instead of acute phase. [13].

The present study observed elevated C3a levels in anti-dsDNA Ab positive SLE patients, but not in anti-Sm Ab positive patient. Further C3a level positively correlates with disease activity scores. C3a and C5a are fragments, immediately generated upon activation of the complement cascades, which reflects complement activation more accurately than the levels of the individual intact proteins[14]. Previously, it was shown that increased levels of complement split products are associated with disease activity, however, they were not used to study the relationship between serum C3a and Disease Activity considering extremely short half-lives of C3a[15].

In addition, the current investigation also identified a slightly association between serum C3a levels and D-Dimer. It was shown that complement anaphylatoxins C3a and C5a can activate macrophages and neutrophils, release more vasoactive substances, leading to local vascular inflammation. Therefore, for normal people, the levels of complement C3a and C5a are relatively low, but when the complement is overactivated, its expression level will increase rapidly. Those indicates that injury of the vascular endothelium caused by complement overactivation and immune complex formation might contribute to the vasculopathy in SLE. Observations in the current study indicates C3a shows promise as a biomarker for monitoring SLE disease activity and thrombophilia.

However, a possible limitation to the use of C3a for evaluation of SLE disease activity is that not all increase of C3a is relative to SLE disease activity, many other stimuli, like pathogenic microbes, tumors and other autoimmune diseases can also cause complement overactivation. In addition, how anti-dsDNA positively correlates with complement overaction and what is the mechanism behind are still unclear. These aspects can be an interesting senarios for future research.

### Declarations

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Conflict of interest

There is no conflict of interest.

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Figures
Figure 1 Serum C3a levels in anti-Sm Ab (+) and anti-dsDNA Ab (+) SLE patients. (a) and (b): Serum samples from anti-Sm Ab (+) (n=6), anti-dsDNA Ab (+) (n=7) SLE patients and healthy blood donors (3) were quantified by WB according to the manufacturers’ instructions. (c): Anti-dsDNA Ab (+) SLE patients displayed significantly higher concentrations of C3a compared to anti-Sm Ab (+) SLE patients (p < 0.01). (d): Levels of serum C1q don’t show significant differences between two groups. Dots represent individual samples. The Student’s t test was used.

Figure 1

See image above for figure legend.
**Figure 2** Correlation of serum C3a levels with SLEDAI score and D-Dimers. Serum C3a levels correlated positively with SLEDAI score(a) and slightly correlated positively with D-Dimers(b). Dots represent individual samples. Correlation analysis was performed using the Spearman correlation coefficient. A p value less than 0.05 was considered significant. SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

See image above for figure legend.

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