Generation of hydrolyzed complement component C3 is substantially elevated in SLE

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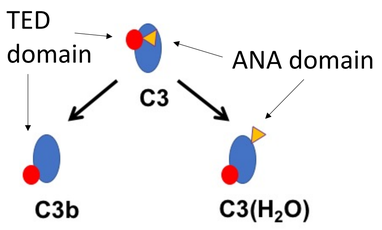
Introduction

Complement activation is a central pathophysiologic event in several autoimmune diseases. A key activation event is the conversion of native C3 to C3(H_2O), where a highly reactive thioester bond in C3 is hydrolyzed. C3(H_2O) can be utilized to generate C3 convertase, which further drives complement activation via the alternative pathway. C3(H_2O) has been elusive to measure, but we have recently developed a novel ELISA-based assay allowing for its accurate measurement. We hypothesized that in autoimmune diseases where complement activation is a central feature, C3(H_2O) levels will be elevated reflecting a primed state for triggering inappropriate alternative pathway activation and amplification.

C3(H_2O) ELISA Set-up and Specificity



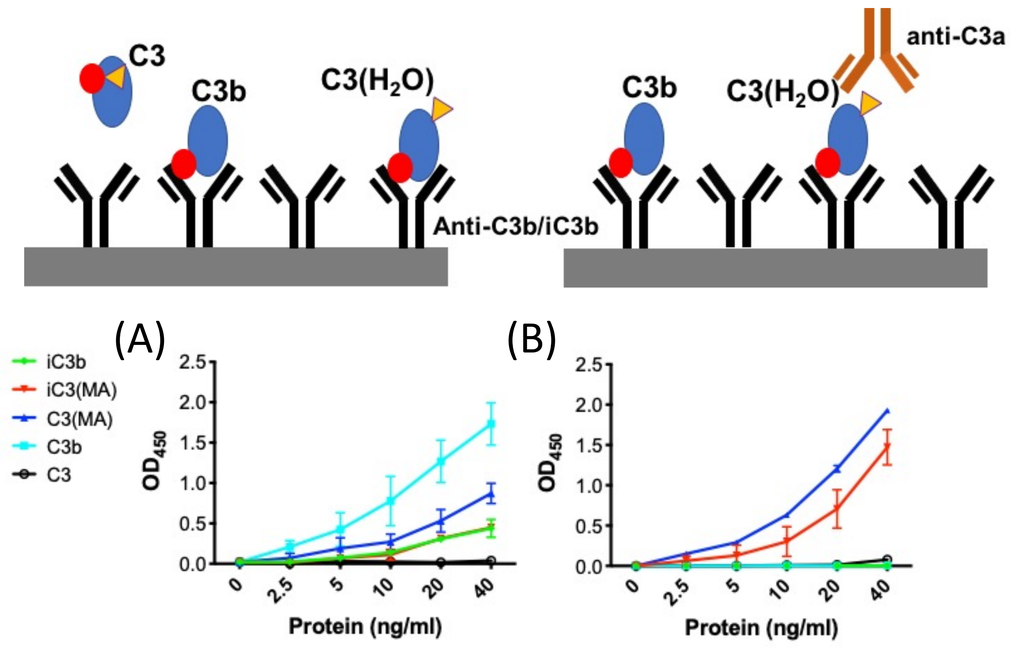
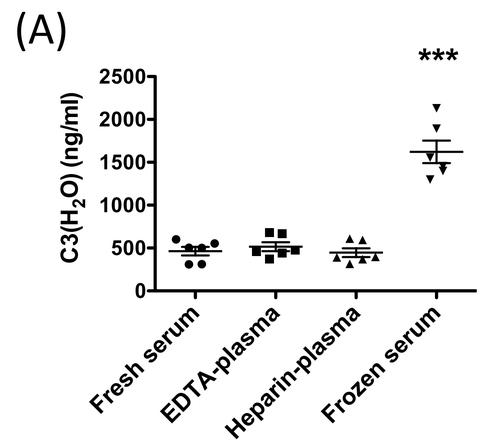
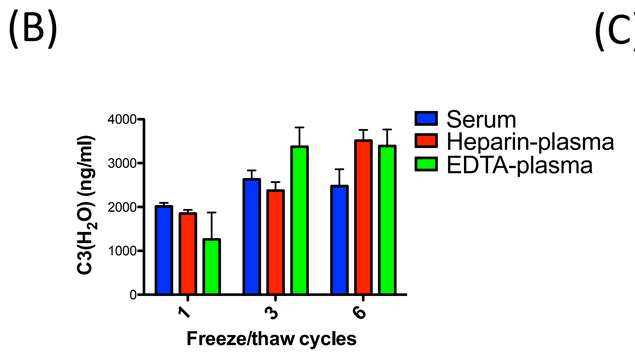


Figure 1. Specificity of ELISA shown using purified proteins.
(A) Captured proteins shown by using anti-C3 pAb for detection**. (B)** Specificity of ELISA using anti-C3a pAb. C3(MA) and iC3(MA) are detected.

C3(H_2O) level and generation in human serum and plasma



Fresh serum= clot 30 min at RT, centrifuge , never frozen



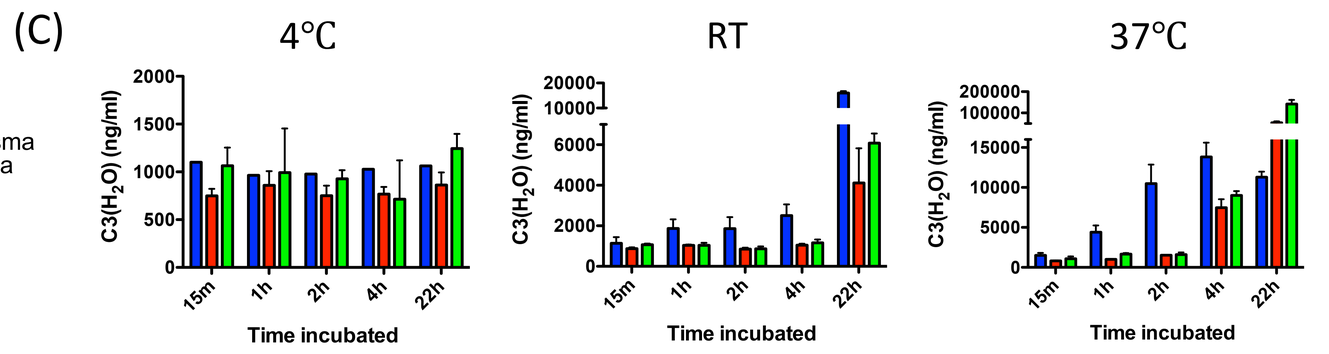
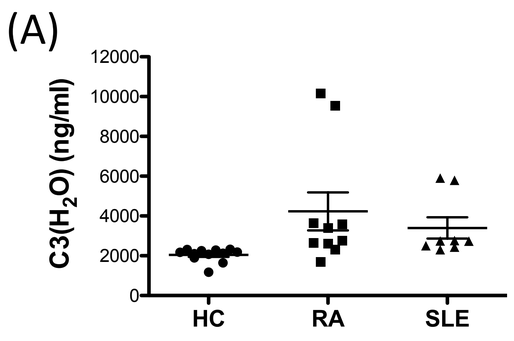
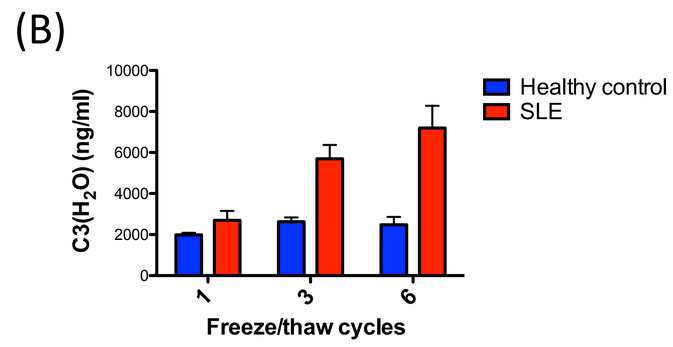
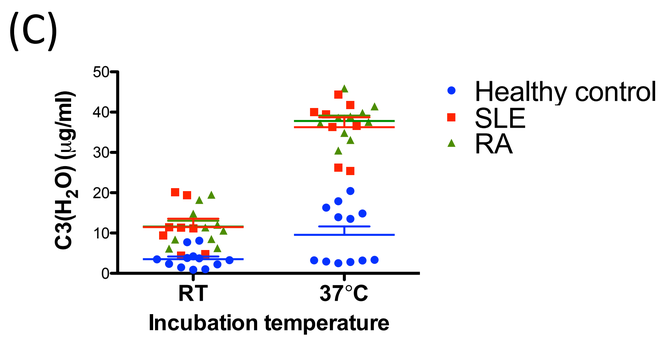


Figure 2. (A) C3(H_2O) was measured in human serum and plasma from healthy adults; n=3 donors in duplicate, *** $p < 0.001$ vs. fresh serum. **(B)** Samples were subjected to 1, 3 or 6 freeze/thaw cycles and the C3(H_2O) content was measured; n=3. **(C)** Samples were incubated for the indicated time at 4°C, room temperature (RT) or 37°C and then stored at -80°C prior to analysis; n=3.

C3(H_2O) is elevated in SLE







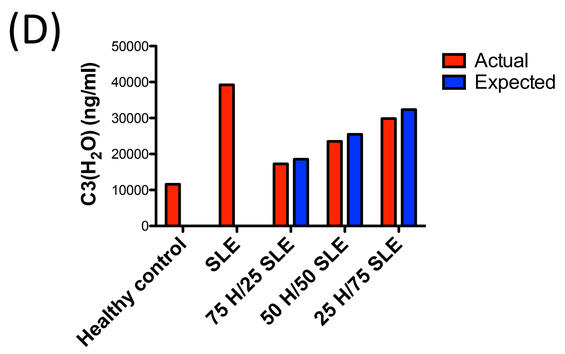


Figure 3. C3(H_2O) is elevated in patient sera. (A) C3(H_2O) levels were measured in frozen samples from patients and compared to healthy donor levels; n=4-8 (in duplicate). **(B)** Serum from SLE patients was subjected to 1, 3, or 6 freeze/thaw cycles prior to measuring C3(H_2O) content; n=3. **(C)** Serum from healthy donors or patients with SLE were incubated at RT or 37°C for 6 h before C3(H_2O) was measured; n=4-6. **(D)** Healthy control (H) and SLE sera were mixed at the indicated dilutions, incubated for 4 h at 37°C and C3(H_2O) assessed. SLE sera did not drive C3(H_2O) generation of HC C3.

Summary Table

	Fresh serum	1 F/T	3 F/T	6 F/T	6h 37°C
HC	463 (± 51)	1999 (±111)	2630 (±205)	2480 (± 381)	9581 (±2050)
SLE	ND	3399 (±538)	5698 (±676)	7192 (±1090)	36,270 (±2460)

C3(H_2O) levels in ng/ml ± SE. ND= no data

Conclusions

Both SLE and RA are associated with elevated C3(H_2O) levels following *in vitro* incubation compared to healthy controls. These data suggest that the potential for C3(H_2O) formation in SLE and RA patients are higher compared to healthy controls, which could support additional complement activation or utilization of C3(H_2O) in other pathways such as intracellular activation in immune cells. Thus, in addition to a possible diagnostic tool for pathogenic autoimmunity, these data may suggest novel mechanisms of how complement could drive symptomatic autoimmune disease.

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