

Anti-C1q Antibodies in Patients with Chronic Hepatitis C Infection

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ABSTRACT

OBJECTIVE: 1. Anti-C1q antibody, detected with high frequency in systemic lupus erythematosus (SLE) and hypocomplementemic urticarial vasculitis, may have a direct pathogenic role in complement mediated autoimmune diseases. 2. Extrahepatic autoimmune laboratory features of HCV infection include autoantibody production and the development of mixed cryoglobulinemia. 3. In this study, we investigated the prevalence of anti-C1q antibody in a population of patients with chronic HCV infection.

METHODS: Serum was obtained from a group of 50 patients with chronic HCV infection and compared to control groups of patients with SLE, rheumatoid arthritis (RA), scleroderma (PSS), Sjogren's syndrome (SS), mixed connective tissue disease (MCTD), and healthy individuals. Levels of anti-C1q IgG antibody were measured by ELISA.

RESULTS: Anti-C1q antibody was detected in 38% of HCV patients compared with 2% of healthy controls ($p < .0001$). Levels were also significantly elevated in patients with SLE (6.5%), RA (20%), PSS (15%), SS (15%) and MCTD (15%). C1q – CIC (circulating immunocomplex) levels correlated only weakly with serum anti-C1q levels.

CONCLUSION: In addition to numerous other autoantibodies, patients with chronic HCV infection exhibit increased production of anti-C1q IgG antibodies. This observation may have implications for the pathogenesis of the mixed cryoglobulinemic vasculitis syndrome of HCV.

INTRODUCTION

Infection with hepatitis C virus is associated with a variety of autoimmune phenomena. HCV patients frequently produce type II and type III mixed cryoglobulins and less commonly develop symptomatic cryoglobulinemic vasculitis (CV). There is substantial evidence that the immune complexes found within affected vessels are composed of HCV particles bound to anti-HCV antibodies, which locally activate the classical complement pathway.

Anti-C1q antibodies are highly prevalent in prototypical autoimmune disease, systemic lupus erythematosus (SLE).

In fact, anti-C1q antibodies are strongly correlated with lupus nephritis. These antibodies are also detected in hypocomplementemic urticarial vasculitis, rheumatoid vasculitis, IgA nephropathy, anti-glomerular basement membrane (anti-GBM) nephropathy, and HIV infection. Furthermore, anti-C1q antibody has been directly implicated in the pathophysiology of complement-mediated immune complex disease in an animal model of anti-GBM disease. Therefore, we hypothesized that anti-C1q antibodies may also be present in HCV infected patients, and, if so, could be important in the development of cryoglobulinemic syndromes. In this investigation, we determined the prevalence of anti-C1q antibodies in the serum of unselected patients with chronic HCV infection.

MATERIALS AND METHODS

- **Reagents:** C1q Protein was purchased from Quidel Corp. The horseradish peroxidase (HRP)-conjugated goat anti-human IgG were from Sigma (St. Louis, Mo).
- **Blood donors:**
 1. 91 healthy controls
 2. 50 patients with Chronic Hepatitis, confirmed by HCV RNA PCR testing.
 3. 130 patients with SLE:
 4. 20 patients with rheumatoid arthritis (RA)
 5. 10 mixed connective tissue disease (MCTD)
 6. 15 Scleroderma (SSc)
 7. 20 Sjogren's syndrome (SS)

8. 5 CREST (Calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia) patients

- **Assay for C1q-specific antibodies.**

1. The Costar type II microtiter plates were coated with 100 μL of 5 $\mu\text{g}/\text{mL}$ in carbonate buffer (pH 9.6) per well.
2. The plate was blocked with 150 μL of 1% BSA in PBS for 1 hour at room temperature.
3. Portions (100 μL) of serum, diluted 1:50 in 1% BSA in high salt phosphate-buffered (1 M NaCl in PB)-Tween, were added to the appropriate wells
4. The plates were incubated for 1 hour with shaking at room temperature. Following incubation, the plates were washed four times with PBS-Tween (0.05% Tween-20).
5. Portions (100 μL) of HRP-conjugated goat anti-human IgG (diluted 1:10,000) were added to the plates, incubated for 30 minutes.

6. The plates were washed and 100 μ L of TMB substrate buffer was added to all wells. The color was developed for 10 to 15 min at room temperature. The plates were read at 450 nm on an EIA reader. The calibration curve was constructed by plotting the optical density (OD) of the standards at 450 nm (y-axis) versus arbitrary EIA units (x-axis).

- **Establishment of the arbitrary EIA Units:** A standard curve was established from OD values obtained from two-fold serial dilutions of a high-positive serum (range from 1:50 to 1:1600). An arbitrary value of 200 units was assigned to the highest concentration (1:50) and a value of 0 unit was assigned to the buffer only. The standard curves were derived by computer-assisted data reduction with the four-parameter function.
- **Interferences.** To investigate whether there was any interference of the anti-C1q autoantibodies, five specimens were spiked with different

concentrations of bilirubin (10 and 20 mg/dL), hemoglobin (208 and 416 mg/dL) and triglycerides (1000 and 2000 mg/dL).

- **Detection of C1q-Immune complexes.** Serum C1q-CIC levels were determined by a commercial EIA kit (Binding Site Ltd, England).

RESULTS

- Two out of 91 healthy control donors (2%) had low positive levels of anti-C1q antibody. The specificity is 98%.
- Eighty of the 130 (61.5%) SLE patients had anti-C1q antibodies. 10 patients with a history of lupus nephritis had a slightly higher frequency (70%).
- In the other patient groups, individuals positive for C1q antibodies were 15% (3/20) for RA-patient, 20% (4/20) for SS-patients, 20% (3/15) for Scleroderma-patient and 20% (2/10) for MCTD-patients, respectively. There was no C1q antibody detected in patients with CREST (Figure 1 and table 1).
- 38% patients (19/50) with chronic HCV infection had serum anti-C1q IgG antibody (Figure 1). Twelve of 50 HCV patients (24%) had C1q circulating immunocomplexes.
- In these HCV patients, IgG rheumatoid factor (RF), IgM-RF and dsDNA

antibody were present in 52%, 26% and 0%, respectively (data not shown).

DISCUSSION

- Many patients with chronic HCV exhibit a broad spectrum of antibodies which may include polyclonal or monoclonal rheumatoid factors and cryoglobulins. There is growing evidence to support the hypothesis that due to chronic antigenic stimulus, and probably under the direct influence of the hepatitis C virus on immune cells, a progressive lympho-proliferative process develops leading to cryoglobulinemic vasculitis.
- These antibodies are frequently detected in the serum of lupus patients, and have also been found in other conditions in which complement activation plays a key role in disease pathogenesis.
- In the murine kidney, anti-C1q antibody did not appear to deposit in the glomerulus in the form of circulating immune complexes; rather, anti-C1q antibody bound to the C1q that was present in anti-glomerular basement

membrane (GBM) antibody immune complexes located in the mouse kidney. Based on this data, one could speculate that anti-C1q antibody may play a role in the pathogenesis of disparate, complement mediated disease processes.

- We found a prevalence (38%) of anti-C1q antibody in HCV infected patients. The result is lower than another report (Tsai et al 1995) that found 124 of 173 (72%) HCV patients had C1q-CIC. Our data confirms that there is production of anti-C1q antibodies in patients with chronic HCV infection, paving the way for further study of its possible pathogenic role in patients with cryoglobulinemic vasculitis. We hypothesize a role for this antibody in the vasculitic complications of HCV.
- Our data has shown elevated levels of the IgG antibodies in SLE patients. The modified method (using high salt buffer diluted samples) can increase the specificity of this detection. It may be used to distinguish SLE patients from

other autoimmune diseases. (Table.1). This data shows that anti-C1q autoantibodies are strongly associated with renal involvement in SLE.

- The incidence of anti-C1q antibodies is highly variable between these diseases. The incidence of anti-C1q in RA and Sjogren's syndrome varied between 3 and 13%, and is nearly 94 and 100%. But, we found 15 – 20% positive rates in other autoimmune diseases such as RA, MCTD, and SSc. Significant differences were compared with SLE patients ($P < 0.05$). We conclude that IgG anti-C1q antibody assay is more specific in SLE patients than in other autoimmune diseases.
- However, while a large proportion of HCV patients will produce RF and cryoglobulins, cryoglobulinemic vasculitis remains relatively unusual. Factors triggering the evolution of a “benign” asymptomatic cryoglobulinemia to a symptomatic vasculitis are largely unknown. We hypothesized that anti-C1q antibodies could be one of these factors.

CONCLUSION

- The concentrations of autoantibody IgG against C1q are significantly increased in SLE and HCV-infected patients compared to other autoimmune diseases.
- Therefore, more definitive analysis of the importance of anti-C1q antibodies in patients with HCV infection, particularly in patients within whom cryoglobulinemic vasculitis develops, will require future studies in well characterized groups of HCV patients.

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Table 1. Anti-C1q Prevalence in Different Groups

Groups	N	Mean \pm SD	Positive	
HCV Infection	50	16.6 \pm 31.3	19/50 (38%)	
SLE	130	36.2 \pm 43.6	80/130 (61.5%)	
RA	20	13.7 \pm 22.5	3/20 (15%)	
Sjogren's syndrome	20	9.8 \pm 9.7	4/20 (20%)	
Scleroderma	15	9.1 \pm 6	4/15 (20%)	
MCTD	10	11.8 \pm 13.6	2/10 (20%)	
CREST	5	5.0 \pm 2.9	0/5	
Healthy Donors	92	4 \pm 3	2/92 (2%)	

Frequency of Anti-C1q Antibody

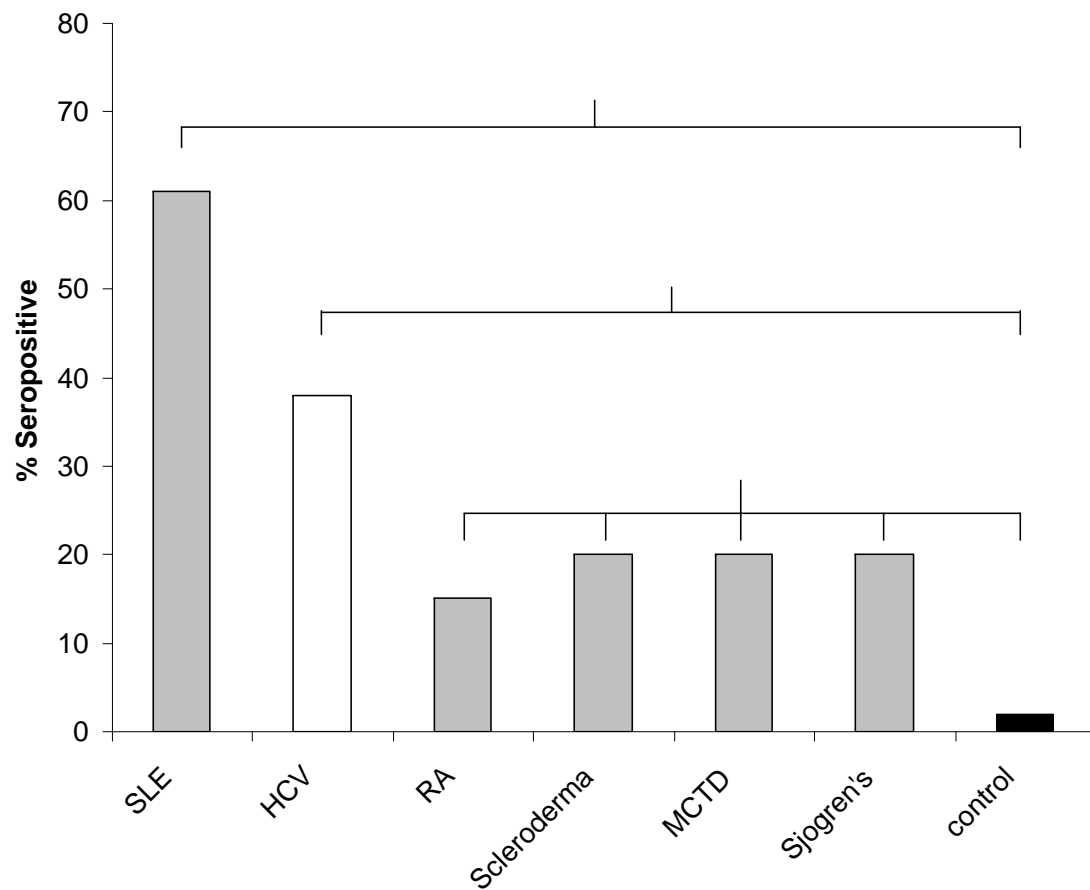


Figure 1. Compared to healthy control populations, patients with connective tissue disease and HCV infection have significantly elevated frequency of anti-C1q antibodies.

* $p < .0001$

** $p < .001$