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Anti-Clq antibodies in patients with chronic hepatitis C infection

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ABSTRACT

Objective. *Extrahepatic autoinununе features of HCV infection include autoantibody production and the development of mixed cryoglobulinemia. Anti-Clq antibody, detected with high frequency in systemic lupus erythematosus and hypocomplementemic urticarial vasculitis, may have a direct pathogenic role in complement mediated autoimmune diseases. In this study, we investigate the prevalence of anti-Clq 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 control groups comprised of patients with SLE, rheumatoid arthritis (RA), scleroderma (PSS), Sjögren's syndrome (SS), mixed connective tissue disease (MCTD), and healthy individuals.*

Results. *Anti-Clq antibody was detected in 38% of HCV patients compared with 2% of healthy controls (p <. 000l). Levels were also significantly elevated in patients with SLE (61%), RA (20%), PSS (15%), SS (15%) and MCTD (15%).*

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

Introduction

Infection with hepatitis C virus is associated with a variety of autoimmune phenomena. HCV patients frequently produce type II and III mixed cryoglobulins and less commonly develop symptomatic cryoglobulinemic vasculitis (CV). CV is a small vessel immune complex mediated vasculitis that frequently presents with palpable purpura, peripheral nerve disease, glomerulnephritis, arthralgia/arthritis, and asthenia. 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 (1). The factors responsible for determining which patients with HCV progress from asymptomatic mixed cryoglobulinemia to active CV remain largely unknown. Anti-Clq antibodies are highly prevalent in the prototypical autoimmune disease, systemic lupus erythematosus (SLE) (2). Analogous to CV, SLE can also cause a complement mediated small vessel cutaneous vasculitis and glomerulonephritis. In fact, anti-Clq antibodies are strongly correlated with lupus nephritis (3). These antibodies are also detected in hypocomplementemic urticarial vasculitis (4), rheumatoid vasculitis (5), IgA nephropathy (6), anti-glomerular basement membrane (anti-GBM) nephropathy (7) and HIV infection (8). Furthermore, anti-Clq antibody has been directly implicated in the pathophysiology of complement mediated immune complex disease in an animal model of anti-GBM disease (9). Therefore, we hypothesized that anti-Clq antibodies may also be present in HCV infected patients, and if so, could be important in the development of cryoglobulinemic syndromes. In this initial investigation, we determined the prevalence of anti-Cl q antibodies in the serum of unselected patients with chronic HCV infection.

Materials and methods

Patients

Fifty patients with chronic hepatitis C infection, confirmed by HCV RNA PCR testing, provided serum samples as part of an IRB-approved HCV database program at the University Of Cincinnati College Of Medicine. The clinical characteristics of these patients have been published previously (10). None of the patients were receiving immunotherapy or had clinical evidence of CV. Sera were also collected from 291 control patients. This group included 91 patients without evidence of autoimmune disease (healthy controls), 130 patients with SLE, 20 patients with rheumatoid arthritis (RA), 10 patients with mixed connective tissue disease (MCTD), 10 patients with Sjögren's syndrome (SS), and 20 patients with scleroderma (PSS).

Serological assessment

Anti-Clq IgG antibody levels were determined using a solid phase ELISA (6, 7). Briefly, Costar type II microtiter plates were coated with 100 μL of human Clq (5 μg/mL) per well in carbonate buffer (pH 9.6). After incubation at 4°C overnight, the plates were washed three times with PBS-Tween-20 (PBS-T). The plates were then blocked with 150 μL of 1% BSA and 0.1% NaN_3 in PBS for 1 hour at room temperature. Because aggregated IgG could bind to the plate bound Clq, we utilized a high salt concentration (1 M NaCI) PBS-T to clear the sera of immune complexes. Portions (100 μL) of serum, diluted 1:50 in the high salt PBS-T with 1% BSA (a dilution found to be optimal and uniformly used in subsequent assays), were added to the appropriate wells; the plates were incubated for 1 hour with shaking at room temperature. Following incubation, the plates were washed four times with PBS-T. Portions (100 μL) of BRP-conjugated goat anti-human IgG (diluted 1:2,000) were added to the plates. After the plates were incubated for 30 minutes with shaking at room temperature, the plates were washed and 100 μL of TMB substrate buffer was added into all wells. The color was developed for 15 mm at room temperature. The plates were read at 450 nm on a Bio-Teck EL-800 reader.

The calibration curve was constructed by plotting the optical density (OD) of the standards at 450 nm versus arbitrary EIA units.

A standard curve was established from OD values obtained from twofold serial dilutions of a high-positive serum range from 1:50 to 1:1600. An arbitrary value of 200 units was assigned to the lowest dilution (1:1600); 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. BioTek software connected a straight line between the means of calibrator replicates. Arbitrary EIA units were used for the quantitative assay. All subsequent OD values were transformed to the EIA units from the standard curve.

Statistical analysis

Differences in anti-Clq antibody seropositivity between groups were compared by Fisher's Exact Test.

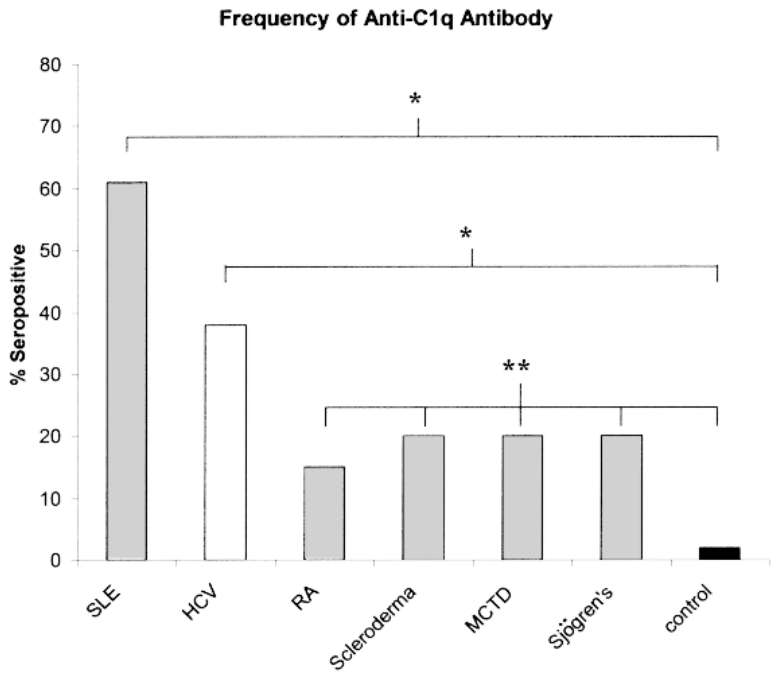

Fig. 1. Compared to healthy control populations, patients with connective tissue disease and HCV infection have significantly elevated frequency of anti-C1q antibodies.
*p <.0001
**p <.001

Results

The high specificity of the assay was confirmed by the low seropositivity rate in the 91 healthy control donors (2%). As expected, most patients with SLE had anti-Clq antibody (61%); patients with a history of lupus nephritis had a slightly higher frequency (70%). Anti-Clq antibody was present in a statistically significant minority of patients with other connective tissue disease including RA (15%), PSS (20%), SS (20%) and MCTD (20%). Interestingly, we found that 38% of 50 patients with chronic HCV infection had serum anti-Clq IgG antibody (Fig 1). In these same patients, IgM rheumatoid factor and dsDNA, detected by ELISA, were present in 52% and 0%, respectively (data not shown). We used the high salt concentrated buffer to remove circulating immune complexes from the sera that was tested for the anti-Clq antibodies. This method, while efficient at clearing CIC from sera, could be expected to decrease anti-Clq antibody levels in the sera by removing anti-Clq antibody bound to Clq containing immune complexes, decreasing the sensitivity of the ELISA, and leading to an underestimate of the true anti Clq antibody seropositive rate.

Discussion

Many patients with chronic HCV exhibit a broad spectrum of antibodies which may include polyclonal or monoclonal rheumatoid factors and cryoglobulins; there is a growing body of 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 lymphoproliferative process develops leading to cryoglobulinemic vasculitis, and rarely culminating in non-Hodgkin's B cell lymphoma (12). 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 cryoglobuline-

mia to a symptomatic vasculitis are largely unknown. We hypothesized that anti-Clq antibodies could be one of these factors. These antibodies are frequently detected in the serum of lupus patients (2, 3), and have also been found in other conditions in which complement activation plays a key role in disease pathogenesis (4, 6, 7).

In their recently published studies in a complement dependent model of murine anti-GBM nephritis, Trouw *et al.* demonstrated that the addition of anti-Clq antibodies to subnephritic doses of anti-GBM antibody resulted in acute glomerulonephritis (9). In this model, GN did not occur in Clq or C3 deficient mice, suggesting that the anti-Clq antibody induced GN through amplification of the early events in the complement cascade. Furthermore, anti-Clq antibody did not appear to deposit in the glomerulus in the form of circulating immune complexes; rather, anti-Clq antibody bound to the Clq that was present in anti-GBM antibody immune complexes located in the mouse kidney. Based on this data, one could speculate that anti-Clq antibody may play a role in the pathogenesis of disparate complement mediated disease processes. Our data confirms that there is production of anti-Clq antibodies in patient with chronic HCV infection, paving the way for further study of a possible role in the pathogenesis of cryoglobulinemic vasculitis.

A major weakness of this study is that none of our patients, including those with anti-Clq antibody, had cryoglobulinemic vasculitis. The retrospective nature of the analysis precludes measurement of cryoglobulins or complement levels in the stored serum. Most of these patients subsequently received immunotherapy, likely altering the natural history of their disease, and rendering prospective study of this cohort unlikely to be informative. Therefore, more definitive analysis of the importance of anti-Clq antibodies in patients with HCV infection, particularly in patients with cryoglobulinemic vasculitis, will require future studies in well characterized groups of HCV patients.

Conclusion

In this study, we demonstrate for the first time that anti-Clq antibody should be added to the list of autoimmune phenomena attributable to chronic HCV infection. Based on the emerging evidence of anti-Clq antibody in the pathogenesis of certain autoimmune disease processes, we postulate a potential mechanistic role for anti-Cl q antibodies in cryoglobulinemic vasculitis.

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