

1 Titration of IgG anti-GOR showed a median value of 1:20 in patients with occult
2 HCV infection with serum GOR antibody titres ranging from 1:10 to 1:80 (figure 2). In
3 patients with chronic hepatitis C the median IgG anti-GOR titre was 1:80 and titres ranged
4 from 1:40 to 1:320. Thus, GOR IgG antibody levels were significantly lower among
5 individuals with occult HCV infection compared with chronic hepatitis C patients ($P <$
6 0.001 ; figure 2). On the other hand, the analysis of IgG anti-GOR titres in sequential serum
7 samples demonstrated minor changes in IgG anti-GOR levels among GOR antibody-
8 positive patients with occult HCV infection. Similarly, there were no changes in IgG anti-
9 GOR titres among GOR antibody-positive untreated chronic hepatitis C patients within a
10 one-year period of survey (data not shown)

11 As regards the clinical, laboratory and histological characteristics, patients with
12 occult HCV infection who tested positive to IgG anti-GOR did not differ from those who
13 were GOR antibody-negative (table 1); the histological activity (average scores of necro-
14 inflammation and fibrosis) tended to be greater, although not significantly, among IgG
15 anti-GOR-positive patients with occult HCV infection (data not shown). On the other hand,
16 the percentage of infected hepatocytes (that is, cells positive to genomic HCV RNA by in
17 situ hybridization) resulted significantly greater ($P = 0.042$) in patients with occult HCV
18 infection who tested positive to IgG anti-GOR (figure 3). However, the percentage of
19 HCV-infected hepatocytes did not correlate significantly with IgG anti-GOR titres among
20 the twenty-two GOR antibody-positive patients ($r_s = 0.311$, $P = 0.19$). In patients with
21 overt chronic HCV infection the median percentage of infected hepatocytes observed by in
22 situ hybridization was 8.0 (range 2.5 – 38.6), which resulted significantly higher ($P < 0.001$)
23 compared with occult HCV infection (median of 4.0, range 0.1 – 18.0), in agreement with
24 a previous report (19).

1 With respect to rheumatoid factor, it was detected in the serum from 12 of the 110
2 (10.9%) patients with occult HCV infection, including one (4.5%) of the 22 GOR
3 antibody-positive individuals. Similarly, C-reactive protein was detectable in 15/110
4 (13.6%) patients with occult HCV infection, including 1/22 (4.5%) IgG anti-GOR-positive
5 individuals. Finally, cryoglobulins were found in 14/110 (12.7%) patients with occult HCV
6 infection; only one of them (4.5%) had IgG anti-GOR detectable.

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1 DISCUSSION

2 In this study, we have observed a 20% frequency of IgG antibody reactivity to the
3 GOR autoepitope in the serum of anti-HCV-negative patients with occult HCV infection.
4 Low IgG anti-GOR titres were found in most GOR antibody-positive individuals.
5 Importantly, IgG anti-GOR was not detected in any of the patients without HCV
6 irrespective of the etiology of the liver disease. Thus, despite repeated absence of serum
7 anti-HCV antibodies by commercial immunoassays IgG anti-GOR can be found in patients
8 with occult HCV infection. Most studies had only detected anti-GOR reactivity in HCV
9 seropositive patients (6,9,14,15,21,31). However, a few reports identified anti-HCV-
10 negative individuals who tested positive to anti-GOR (9,14) including blood donors (7,32);
11 although, these studies did not exclude the presence of occult HCV infection. In addition, it
12 has been reported that detection of anti-GOR without anti-HCV is not associated with
13 hepatitis C viremia (1). In this way, among occult HCV-infected patients HCV RNA is
14 persistently negative in serum (2).

15 The frequency of IgG anti-GOR in occult HCV infection was significantly lower
16 compared with a 63.6% GOR IgG-antibody reactivity found in patients with chronic
17 hepatitis C, which is similar to the frequency reported by several authors in patients with
18 overt HCV infection (10,12,14,16,21). Also, anti-GOR levels were greater in chronic
19 hepatitis C compared with occult HCV infection. We have reported recently that sera from
20 some patients with occult HCV infection may demonstrate a positive reaction against HCV
21 non-structural proteins on immunoblot assays suggesting a very low level of specific
22 antibody production (23). In chronic hepatitis C, the presence of antibodies reactive to the
23 host-derived antigen GOR is not merely due to sequence homology but to cross-reactivity

1 at the molecular level because of conservation of residues essential for antibody binding
2 (34). Thus, de novo infection with HCV after liver transplantation produces an increase in
3 IgG anti-GOR likely due to increased viral load and replication under immunosuppression
4 indicating that the immune response to GOR autoantibody is triggered by HCV (24).

5 The low level of IgG anti-GOR antibodies detected in occult HCV infection may
6 reflect not only exposure to HCV (22), but also an ongoing productive HCV infection
7 within the liver (2). Indeed, HCV replication has been demonstrated in peripheral blood
8 mononuclear cells from occult HCV-infected patients as well (3). This may result in
9 discrete amounts of antigen production and then presentation to antibody-producing cells.
10 Interestingly, the percentage of infected hepatocytes resulted significantly greater in
11 patients with occult HCV infection who tested positive to IgG anti-GOR. The
12 mechanism(s) that regulate humoral immune responses during occult HCV infection are
13 not well known. In humans the GOR (GOR47-1) gene product cannot be translated into a
14 protein (8) and so antibody responses to GOR and HCV may be independently regulated as
15 suggested in chronic hepatitis C (11). In patients with chronic hepatitis C anti-HCV
16 antibodies usually persist for decades; although, these may eventually disappear after
17 recovery from HCV infection (29,30).

18 Among individuals with occult HCV infection, the subset of GOR IgG antibody-
19 positive patients did not show a different clinical background compared with their IgG
20 anti-GOR-negative counterparts (9). However, a greater number of IgG anti-GOR-positive
21 patients had signs of necro-inflammation, which is similar to patients with chronic hepatitis
22 C, in whom reactivity to GOR had been correlated with liver disease activity (21).
23 Nevertheless, compared with chronic hepatitis C occult HCV infection seems to be a less

1 aggressive form of the disease caused by the hepatitis C virus (19); although, liver cirrhosis
2 is present in around 4% of these patients.

3 Finally, rheumatoid factor, C-reactive protein and/or cryoglobulins were detected in
4 the serum of 10-14% of occult HCV-infected patients. Frequencies of such factors were
5 lower than those commonly found in chronic hepatitis (25), suggesting that this may reflect
6 differences in the host response to HCV between occult HCV and chronic hepatitis C
7 patients. In addition, the presence of these factors was not associated with the GOR IgG
8 antibody status. These data are in line with the notion that the significance of GOR is little
9 during triggering of autoimmune phenomena by HCV and thus GOR is unlikely a marker
10 of induced autoimmunity as already reported in chronic HCV infection (13). Indeed,
11 histological features of autoimmune disease were absent in all patients.

12 *In conclusion, we have found that sera from 20% of the patients with occult HCV*
13 *react with the GOR autoepitope on enzyme immunoassays; although, this frequency is*
14 *lower compared to GOR reactivity in patients with chronic hepatitis C. Because IgG anti-*
15 *GOR is not detected in patients with HCV-unrelated liver disease detection of IgG*
16 *antibodies to the GOR seems to reflect cross-recognition with viral sequences during*
17 *occult HCV infection, even in the absence of detectable HCV-specific antibodies using*
18 *commercial tests. Testing for IgG anti-GOR might be used to screen HCV RNA-negative*
19 *patients and thus help in identifying at least a subset of occult HCV infection without*
20 *performing a liver biopsy. Nevertheless, even after implementation of IgG anti-GOR*
21 *testing the majority of patients would still need a liver biopsy for accurate diagnosis of*
22 *occult HCV infection.*

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