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

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

## DISCUSSION

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

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

- 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).

The low level of IgG anti-GOR antibodies detected in occult HCV infection may reflect not only exposure to HCV (22), but also an ongoing productive HCV infection within the liver (2). Indeed, HCV replication has been demonstrated in peripheral blood mononuclear cells from occult HCV-infected patients as well [3]. This may result in discrete amounts of antigen production and then presentation to antibody-producing cells. Interestingly, the percentage of infected hepatocytes resulted significantly greater in patients with occult HCV infection who tested positive to IgG anti-GOR. The mechanism(s) that regulate humoral immune responses during occult HCV infection are not well-known. Incluments the GOR (GOR47-1) gene product cannot be translated into a protein (8) and so antibody responses to GOR and HCV may be independently regulated as suggested in chronic hepatitis C (11). In patients with chronic hepatitis C anti-HCV

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

antibodies usually persist for decades; although, these may eventually disappear after

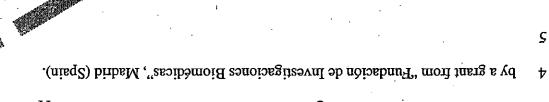
recovery from HCV infection (29,30).

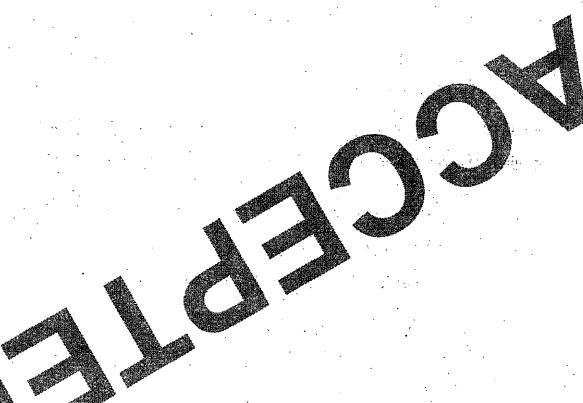
- 1 aggressive form of the disease caused by the hepatitis C virus (19); although, liver cirrhosis
- 2 is present in around 4% of these patients.
- Finally, rheumatoid factor, C-reactive protein and/or cryoglobulins were detected in
- 4 the serum of 10-14% of occult HCV-infected patients. Frequencies 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
- 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 react with the GOR autoepitope on enzyme immunoassays; although, this frequency is lower compared to GOR reactivity in patients with chronic hepatitis C. Because IgG anti-GOR is not detected in patients with HCV-unrelated liver disease detection of IgG 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 occult HCV infection. 22

## VCKNOMFEDGWEALS

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## REFERENCES

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1.

- 1. Baath, L., and A. Widell. 1994. Anti-GOR without anti-HCV core is not associated with hepatitis C viremia. Vox Sang. 66:151.
- Castillo, I., M. Pardo, J. Bartolomé, N. Ortiz-Movilla, E. Rodríguez-Iñigo, S. de
   Lucas, C. Salas, J.A. Jiménez-Hefferman, A. Pérez-Mota, J. Grans, J.M. López
   Alcorocho, and V. Carreño. 2004. Occult hepatitis C virus infection in patients in
   whom the etiology of persistently abnormal results of liver-function tests is unknown.
- 9 J. Infect. Dis. 189:7-14.
- 3. Castillo, I., E. Rodríguez-Inigo, J. Bartolomé, S. de Lucas, N. Ortíz-Movilla,
  J.M. López-Alcorocho, M. Pardo, and V. Carreño. 2005. Hepatitis C virus
  replicates in peripheral blood mononuclear cells of patients with occult hepatitis C
  urus infection. 6nt 54:682-685.
- 4. Dries, V., Il von Both, M. Muller, G. Gerken, P. Schirmacher, M. Odenthal, R. Bartenschlager, U. Drebber, K.H. Meyer zum Buschenfeld, and H.P. Dienes.

  16 1999. Detection of hepatitis C virus in paraffin-embedded liver biopsies of patients negative for viral RNA in serum. Hepatology 29:223-229.
- 5. Ferri, C., A.L. Zignego, and S.A. Pileri. 2002. Cryoglobulins. J. Clin. Pathol.
  55:4-13.
- 6. Hayashi, J., K. Nakashima, Y. Kishihara, M. Ohmiya, E. Yoshimura, M. Hirata, and S. Kashiwagi. 1993 Improved detection of antibodies to hepatitis C virus by the second-generation assay in patients with chronic non-A, non-B liver disease. J. Infect. 26:287-294.

- 7. Irving, W.L., S. Day, D. Bennett, R.P. Eglin, P. Flanagan, P. Nuttall, and V.
- 2 James. 1993. Use of anti-GOR testing in the screening of blood donors for
- 3 hepatitis C virus infection. Vox Sang. 65:38-41.
- 8. Koike, R., T. Iizuka, T. Watanabe, and N. Miyasaka. 2001. The GOR gene
- 5 product cannot cross-react with hepatitis C virus in humans. Clin. Exp. Immunol.
- 6 **124**:429-434.
- 9. Kurosaki, M., N. Enomoto, C. Sato, N. Sakamoto, Y. Hoshino, H. Haritani,
- and F. Marumo. 1993. Correlation of plasma hepatitis virus RNA levels with
- 9 serum alanine aminotransferase in non A, non B chronic liver disease. J. Med.
- 10 Virol. 39:246-250.
- 10. Lau, J.Y., G.I. Davis, E. Opito, K.P. Qian, M. Mizokami. 1993. Significance of
- antibody to the host cellular generaterived epitope GOR in chronic hepatitis C virus
- 13 infection. J. Hepatol 17 253-257.
- 11. Lohr, H.H., G. Gerken, G. Michel, H.B. Braun, and K.H. Meyer zum
- Buschenfelde. 1994. In vitro secretion of anti-GOR protein and anti-hepatitis C
- virus antibodies in patients with chronic hepatitis C. Gastroenterology 107:1443-
- 17 1448.
- 12. Maggi, F., M.L. Vatteroni, M. Pistello, C.M. Avio, N. Cecconi, F. Panicucci,
- and M. Bendinelli. 1995. Serological reactivity and viral genotypes in hepatitis C
- virus infection. J. Clin. Microbiol. 33:209-211.
- 21 13. McFarlane, B.M., C. Bridger, C.J. Tibbs, M.G. Saleh, A. Fuzio, G. Verucchi,
- L. Attard, A. Boschi, F. Chiodo, I.G. McFarlane, and R. Williams. 1994. Virus-
- induced autoimmunity in hepatitis C virus infections: a rare event. J. Med. Virol.
- 24 **42**:66-72.