JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1994, p. 2613–2615
0095-1137/94/$04.00+0

Vol. 32, No. 10

Hepatitis C Virus (HCV)-Specific In Vitro Antibody Secretion by Peripheral Blood Lymphocytes: Correlation with Progression of Disease and HCV RNA in HCV Antibody-Positive Patients

JACQUES DUCOS,[1]* ANNE-MARIE BIANCHI-MONDAIN,[1] MARIEL FRANCOIS,[2] MARC BOISSET,[1] JEAN-PIERRE VENDRELL,[1] FRANCIS BARIN,[2] ARLETTE SERRE,[1] AND DOMINIQUE LARREY[3]

Institut National de la Santé et de la Recherche Médicale (INSERM) U249 and Département d'Immunologie, Centre Hospitalier Universitaire Lapeyronie,[1] and Département d'Hépatogastroenterologie,[3] 34295 Montpellier, and Unité de Virologie, Département de Microbiologie Médicale et Moléculaire, Centre Hospitalier Universitaire Bretonneau, 37044 Tours,[2] France

Received 29 March 1994/Returned for modification 24 May 1994/Accepted 26 July 1994

Hepatitis C virus-specific in vitro antibody production (HCV IVAP) by peripheral blood lymphocytes in 53 HCV antibody-positive patients was investigated in comparison with alanine aminotransferase (ALT) levels and HCV RNA in serum samples. All 29 HCV IVAP-positive patients were HCV RNA positive; 26 had elevated ALT levels. Among the 24 HCV IVAP-negative patients, 16 were HCV RNA negative, with 12 presenting normal ALT values. These data indicate that HCV IVAP results are highly correlated ($P < 0.001$) with HCV RNA results and ALT levels. Our study indicates that HCV IVAP can be used as a novel assay in the diagnosis and pathogenesis exploration of HCV infection.

The detection of specific antibodies in serum samples is the easiest method for identifying patients who are or have been infected by the hepatitis C virus (HCV) (1). Third-generation screening and confirmatory assays with various core and protease polypeptide epitopes (4, 16) have greatly improved the sensitivity and specificity of HCV antibody detection (14, 16, 18). However, it still remains necessary to resort to molecular biology technology to determine whether an HCV antibody-positive patient is a virus carrier or has recovered from a past infection. The amplification of viral nucleic acid by PCR is a highly sensitive method to detect viral sequences in serum samples (3, 7, 8, 9, 12), although it is technically difficult. Moreover, the level of HCV RNA in serum samples has been reported to fluctuate in relation to the phase of the disease (5). Spontaneous in vitro secretion of specific antibodies by peripheral blood lymphocytes (PBL) in natural or chronic viral infections has been reported (11, 13, 15). Furthermore, a relationship between immune system activation by viral antigens and in vitro specific antibody secretion has been suggested (15, 17). The evaluation of specific HCV in vitro antibody production (IVAP) by PBL in comparison with HCV RNA and alanine aminotransferase (ALT) levels was the aim of this study. We compared the HCV antibody serological profile, HCV RNA detected by reverse transcriptase nested PCR (RT-PCR), and HCV IVAP in 53 HCV-positive untreated patients.

Blood samples from 53 patients (31 males and 22 females) were collected for routine diagnosis of HCV infection. Their median age was 47 years (range, 26 to 69 years). All patients were randomly selected on the basis of positivity for HCV antibodies in serum samples by the HCV 3.0 enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Raritan, N.J.). ELISA-positive samples were confirmed by the RIBA HCV 3.0 assay (Chiron Corporation, Emeryville, Calif.). Ten blood samples from healthy subjects (HCV antibody negative) were used as controls. HCV IVAP by patients and controls was evaluated as previously described (17), including control PBL cultures with cycloheximide. The specificities of HCV antibody content in undiluted supernatants of PBL cultures were also analyzed by the RIBA 3.0 procedure.

PBL cultures from 29 (54.7%) of the 53 HCV serum antibody-positive patients (20 males and 9 females) showed spontaneous HCV IVAP by RIBA 3.0 (Fig. 1). The cell cultures from the other 24 HCV antibody-positive patients and all those from healthy controls were negative for HCV IVAP. We divided the 29 IVAP-positive patients into three groups (Table 1). Serum samples from 24 patients (group A) exhibited four bands corresponding to core, NS3, NS4, and NS5 antigens. Serum samples from four patients (group B) showed three bands corresponding to core, NS3, and NS4 antigens. One patient (group C) showed two bands in serum samples (core and NS3 antigens). Among the 29 HCV antibody-secreting patients, 12 (41.4%) showed the same pattern in supernatants of PBL as in the corresponding serum samples (group A, 8 patients; group B, 3; group C, 1; the other 17 patients showed the lack of one or more HCV antibody specificities in PBL supernatants compared with those in the corresponding serum samples. Reactivity to HCV antigens in serum samples was correlated with HCV IVAP as assessed by the number of bands on the RIBA 3.0 assay: 24 (68.6%) of 35 serum samples that presented four bands, compared with 5 (27.8%) of 18 that were reactive with three or fewer antigens, were found to be IVAP positive ($P < 0.01$). The positive predictive value of a profile of four positive bands by RIBA 3.0 was 69.8%. On the other hand, IVAP positivity was not found to be associated with the presence or absence of serum antibodies against core, NS3, NS4, or NS5 antigens.

RT-PCR was used to detect a 5′ noncoding HCV RNA sequence in serum samples by following the protocol previously described (6). All serum samples were tested twice. A negative sample as a control for contamination and specificity and a positive sample as a control for efficiency were included in every PCR run. All samples were tested for ALT with the Ektachem automate (Kodak Diagnostics, Les Ulis, France).

* Corresponding author. Mailing address: Departement d'Immunologie, Centre Hospitalier Universitaire Lapeyronie, 34295 Montpellier, France. Phone: 3367338340. Fax: 3367338963.

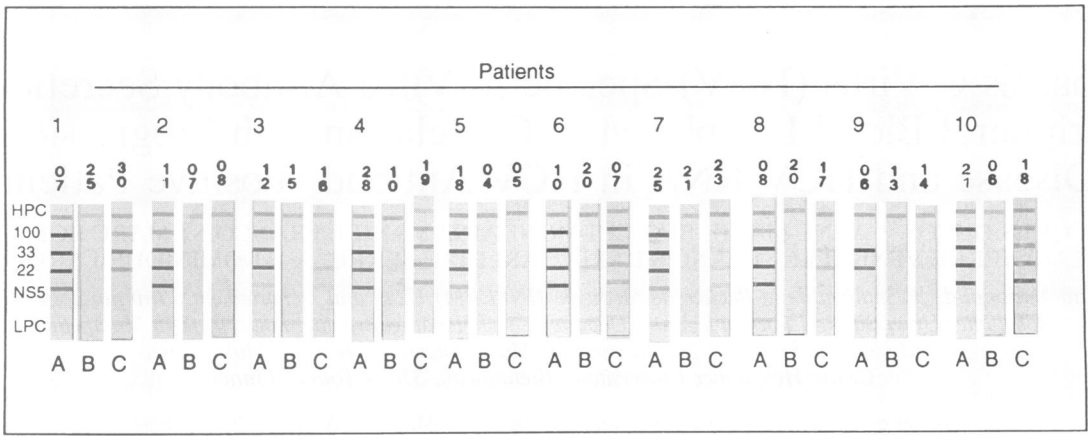

FIG. 1. Representative patterns of RIBA 3.0 assays with serum samples (A) and corresponding PBL culture supernatants from HCV-infected patients in the presence (B) or absence (C) of cycloheximide. HPC, high-positive control; LPC, low-positive control. Lanes 1, 3, 4, 6 to 8, and 10, IVAP-positive patients; lanes 2, 5, and 9, IVAP-negative patients.

The ALT level was used to estimate disease activity. Liver disease was considered to be active for ALT values 1.5 times greater than the upper limit of normal values.

The comparison between HCV RNA, ALT levels, and HCV IVAP demonstrated that all 29 (54.7%) IVAP-positive patients were positive for the presence of HCV RNA in serum samples as detected by RT-PCR. Twenty-six of them (89.7%) showed ALT values greater than 1.5 times the upper limit of normal values, whereas three (10.3%) of them presented normal ALT values. Twenty-four of the fifty-three patients (45.3%) were HCV IVAP negative. Sixteen of them were HCV RNA negative in serum samples, with twelve (75%) showing normal ALT values. The other eight were HCV RNA positive in serum samples, with six (79%) showing ALT values greater than 1.5 times the upper limit of normal values. In contrast, 2 of the 24 IVAP-negative patients were HCV RNA positive with normal ALT values and 4 patients were HCV RNA negative with elevated ALT levels. These data indicate that the IVAP results were associated with the serum HCV RNA results ($P < 0.001$) and increased ALT levels ($P < 0.001$).

TABLE 1. Comparison of HCV antibody specificities in serum samples and corresponding PBL culture supernatants

Patient group and no.	Reactivity							
	Serum samples				Supernatants			
	Core	NS3	NS4	NS5	Core	NS3	NS4	NS5
A								
8	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	0
2	+	+	+	+	+	+	0	+
1	+	+	+	+	+	0	+	+
3	+	+	+	+	+	+	0	0
3	+	+	+	+	+	0	+	0
B								
3	+	+	+	0	+	+	+	0
1	+	+	+	0	+	+	0	0
C, 1	+	+	0	0	+	+	0	0

Compared with the HCV RNA results, the IVAP technique was characterized by a sensitivity of only 64.4% but a specificity of 100%.

In this investigation, we found spontaneous, specific, and active HCV antibody secretion in 54.7% of the HCV antibody-positive patients. Notably, in supernatants of PBL cultures, we never detected HCV antibodies that had not been detected in the corresponding serum samples. Löhr et al. (13) previously reported that PBL secreting specific HCV antibodies already seem to be maximally activated in vivo. Our results demonstrated a good correlation between antibody secretion and viremia in HCV infection, concording with results obtained for other viral infections (15, 17). On the basis of these analyses, we propose that the active secretion of HCV antibodies by PBL reflects interaction of the immune system with HCV antigens and may be linked to HCV replication and liver dysfunction. IVAP by lymphocytes might reflect persistent antigenic stimulation of the immune system induced by viral replication. The HCV IVAP test, in addition to HCV PCR, might give supplementary information about disease activity compared with serological analysis. On the other hand, even though a majority (66%) of IVAP-negative patients showed negative HCV RNA results, 33% of them were HCV RNA positive, with elevated ALT levels in most cases (six of eight). This weak sensitivity might be related to the limits of the RIBA 3.0 test that only partially reflects the diversity of naturally secreted antibodies. In previous studies (2, 6, 10), a higher reactivity to HCV peptides, as assessed by the number of positive bands on the RIBA 3.0, was reported to be correlated with HCV RNA in serum samples. Our results indicate that the detection of four RIBA 3.0 proteins was often associated with HCV IVAP positivity, even though HCV IVAP positivity was not associated with specificity for a particular antibody. This investigation highlights the relationship between HCV IVAP and viremia and suggests that it would be interesting to use HCV IVAP with patients under treatment during the course of chronic active hepatitis C.

We are indebted to S. L. Salhi for editorial revision of the manuscript. We thank M. Moine and N. Thomas for technical assistance.

REFERENCES

1. **Alter, H. J., R. H. Purcell, J. W. Shih, J. C. Meldoper, M. Houghton, Q. L. Choo, and G. Kuo.** 1989. Detection of antibody to

hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N. Engl. J. Med. **321:**1494–1500.
2. **Cardoso, M. S., K. Koerner, S. Epple, and B. Kubanek.** 1993. Prevalence of HCV RNA-positive blood donors and correlation to ELISA and RIBA status. Ann. Hematol. **66:**147–151.
3. **Cha, T.-A., J. Kolberg, B. Irvine, M. Stempien, E. Beall, M. Yano, Q.-L. Choo, M. Houghton, G. Kuo, J. H. Han, and M. S. Urdea.** 1991. Use of a signature nucleotide sequence of hepatitis C virus for detection of viral RNA in human serum and plasma. J. Clin. Microbiol. **29:**2528–2534.
4. **Craske, J., W. K. Paver, and D. Farmer.** 1993. An algorithm for confirming screen reactivity in blood donors in enzyme immunoassays for antibodies to hepatitis C virus. J. Immunol. Methods **160:**227–235.
5. **Farci, P., H. Alter, D. Wong, R. Miller, J. Shih, B. Jett, and H. Purcell.** 1991. A long term study of hepatitis C virus replication in non-A, non-B hepatitis. N. Engl. J. Med. **325:**98–104.
6. **François, M., F. Dubois, D. Brand, Y. Bacq, C. Guerois, C. Mouchet, J. Tichet, A. Goudeau, and F. Barin.** 1993. Prevalence and significance of hepatitis C virus (HCV) viremia in HCV antibody-positive subjects from various populations. J. Clin. Microbiol. **31:**1189–1193.
7. **Garson, J. A., C. Ring, and P. Tuke.** 1991. Improvement of HCV genome detection with "short" PCR products. Lancet **338:**1466–1467.
8. **Garson, J. A., C. Ring, P. Tuke, and R. S. Tedder.** 1990. Enhanced detection by PCR of hepatitis virus RNA. Lancet **336:**878–879.
9. **Garson, J. A., R. S. Tedder, M. Briggs, P. Tuke, J. A. Glazebrook, A. Trute, J. A. Barbara, M. Contreras, and S. Aloysius.** 1990. Detection of hepatitis C viral sequences in blood donations by "nested" polymerase chain reaction and prediction of infectivity. Lancet **335:**1419–1422.
10. **Gretch, D., W. Lee, and L. Corey.** 1992. Use of aminotransferase, hepatitis C antibody, and hepatitis C polymerase chain reaction RNA assays to establish the diagnosis of hepatitis C virus infection in a diagnostic virology laboratory. J. Clin. Microbiol. **30:**2145–2149.
11. **Indraccolo, S., R. Zamarchi, M. L. Veronese, M. R. Mazza, M. Mion, A. Veronesi, M. Panozzo, M. Colombatti, A. Barelli, P. Rochetto, A. Amadori, and L. Chieco-Bianchi.** 1993. Standardization of in vitro synthesis and detection of HIV-1-specific antibodies. J. Immunol. Methods **157:**105–115.
12. **Lazizi, Y., E. Elfassi, and J. Pillot.** 1992. Detection of hepatitis C virus sequences in sera with controversial serology by nested polymerase chain reaction. J. Clin. Microbiol. **30:**931–934.
13. **Löhr, H., B. Fleischer, G. Michel, S. Rossol, G. Hess, K. H. Meyer zum Büschenfelde, and M. Manns.** 1993. Hepatitis C virus antibody secretion in vitro by peripheral blood lymphocytes. J. Hepatol. **14:**112–117.
14. **Pawlotsky, J.-M., A. Fleury, V. Choukroun, L. Deforges, F. Roudot-Thoraval, P. Aumont, J. Duval, and D. Dhumeaux.** 1994. Significance of highly positive c22-3 "indeterminate" second-generation hepatitis C virus (HCV) recombinant immunoblot assay (RIBA) and resolution by third-generation HCV RIBA. J. Clin. Microbiol. **32:**1357–1359.
15. **Segondy, M., J. P. Vendrell, J. Reynes, M. F. Huguet, B. Albat, J. Ducos, J. C. Nicolas, and A. Serre.** 1990. Cytomegalovirus-specific B cell activation as a potential marker for the diagnosis of cytomegalovirus infection. Eur. J. Clin. Microbiol. Infect. Dis. **9:**745–750.
16. **Uyttendaele, S., H. Claeys, W. Mertens, H. Verhaert, and C. Vermylen.** 1994. Evaluation of third-generation screening and confirmatory assays for HCV antibodies. Vox Sang. **66:**122–129.
17. **Vendrell, J. P., M. Segondy, J. Ducos, J. Reynes, M. F. Huguet, J. C. Nicolas, and A. Serre.** 1991. Analysis of the spontaneous in vitro anti-HIV-1 antibody secretion by peripheral blood mononuclear cells in HIV-1 infection. Clin. Exp. Immunol. **83:**197–202.
18. **Vernelen, K., H. Claeys, H. Verhaert, A. Volckaerts, and C. Vermylen.** 1994. Significance of NS3 and NS5 antigens in screening for HCV antibodies. Lancet **343:**853.