CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1997, p. 117–121 Vol. 4, No. 2
1071-412X/97/$04.00+0

Improved Reactivity of Hepatitis C Virus Core Protein Epitopes in a Conformational Antigen-Presenting System

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Received 8 July 1996/Returned for modification 12 September 1996/Accepted 6 November 1996

Recent studies have identified several epitopes in the N-terminal portion of the nucleocapsid protein which are predominantly recognized by sera of patients infected with hepatitis C virus (HCV). The characterization of the sequences recognized by these antibodies and the evaluation of their reactivities have been performed mainly with synthetic peptides. However, synthetic peptides are notoriously unreliable as antigens when the immune response is directed against conformational epitopes. In order to improve the detection of antibody responses in HCV-infected patients, we have evaluated the reactivities of three immunodominant regions of the HCV core protein (residues 1 to 20, 21 to 40, and 32 to 46) displayed in a conformation-specific manner on the surface of the Flock House virus (FHV) capsid protein. The results obtained with these proteins in the analysis of 94 serum samples positive by anti-HCV enzyme-linked immunosorbent assay were then compared with those obtained with the corresponding synthetic peptides. The sequence most reactive both with the peptide and with the FHV protein was the region from residues 1 to 20, confirming the low conformational requirements for the display of these residues. On the other hand, the already reported conformational nature of residues 32 to 46 is in keeping with its observed high reactivity when displayed by the FHV recombinant protein and with the low reactivity displayed by its corresponding synthetic peptide. Finally, the high reactivity observed for the chimeric protein displaying the region from residues 21 to 40, as opposed to the results obtained with the synthetic peptide, also suggests that this sequence contains one or more conformational epitopes whose structures cannot be mimicked correctly with synthetic peptides.

Hepatitis C virus (HCV) has been identified as the major causative agent of parenterally transmitted non-A, non-B hepatitis (6). The HCV genome encodes a polyprotein (7, 13, 29) which is cleaved to yield several structural and nonstructural proteins (11) to which HCV-infected patients develop a heterogeneous immune response. The initial worldwide screening of blood for antibodies against HCV was carried out with a polyprotein (C100-3) from the NS3-NS4 region (15), but the specificity and sensitivity of this region were not considered to be sufficient (16, 24). Therefore, more sensitive assays that use antigens derived from different parts of the HCV genome have been developed (4, 8, 12, 14, 22). Recent studies have identified several epitopes in the N-terminal portion of the nucleocapsid protein which are predominantly recognized by HCV-infected patients (3, 9, 10, 21, 25, 28). Antibodies against this region are regarded to be a reliable marker of virus replication since their presence was found to be closely associated with the presence of specific mRNA (23, 28, 30, 33). The characterization of the sequences recognized by these antibodies and their reactivity has been performed mainly with the use of synthetic peptides (5, 9). However, recent studies have provided evidence that there are severe limitations in the use of synthetic peptides bound to the solid phase for the detection of antibodies against conformational epitopes (18, 19, 26). We have recently developed a new epitope-presenting system to improve the detection of antibodies against conformational epitopes. This antigen-presenting system, based on the capsid protein of the Flock House virus (FHV), has been successfully used in the presentation of several conformational epitopes of human immunodeficiency virus type 1 (2, 27, 31). Therefore, we have inserted in the FHV capsid protein three portions of the N-terminal region of the core protein of HCV and studied the reactivities of these chimeric proteins by screening 94 positive serum sample detected by a commercial anti-HCV enzyme-linked immunosorbent assay (ELISA) kit. The results were then compared with those obtained with the corresponding synthetic peptides.

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MATERIALS AND METHODS

Recombinant FHV proteins carrying the HCV epitopes. Three HCV sequences (amino acid residues 1 to 20, 21 to 40, and 32 to 46) of the core protein were inserted in five different positions on the surface of the FHV capsid protein (2). The recombinant proteins (FHV-C1, FHV-C2, and FHV-C3) were produced in *Escherichia coli* BL21(DE3) cells by using a pET expression system (Novagen, Madison, Wis.) and were purified as described by Blum et al. (1). Figure 1 shows that residues 1 to 20 of the HCV core protein were most reactive when they were inserted in the I2 position, residues 21 to 40 were most active when they were inserted in the I3 position, and residues 32 to 46 were most active when they were inserted in the L2 position of the FHV capsid protein. These three proteins were named FHV-C1, FHV-C2, and FHV-C3, respectively, and were used in all subsequent assays. The first two synthetic peptides used in this study (Pep-C1 and Pep-C2) were synthesized by automated continuous flow solid-phase peptide synthesis by using 9-fluorenylmethoxycarbonyl chemistry in a Milligen 9050 Peptide synthesizer (Millipore Corp., Bedford, Mass.) and were purified by reverse-phase high-pressure liquid chromatography: MSTNPKPQRKTKRNTNRRPQ (Pep-C1; residues 1 to 20), DVKFPGGGQIVGGVYLLPRR (Pep-C2; residues 21 to 40). Pep-C3 (residues 27 to 59; GGQIVGGVYLLPRRGPRLGVRAT RKTSERSQPR), which contained the highly immunogenic epitope from residues 32 to 46, was kindly provided by M. U. Mondelli, IRCCS Policlinico San Matteo, Pavia, Italy. The 44-mer peptide from residues 2 to 44 of the HCV nucleocapsid protein (STNPKPQRKTKRNTNRRPQDVKFPGGGQIVGG VYLLPRRGPRLG) was kindly provided by P. Ferroni, University of Udine, Udine, Italy.

ELISA procedures. Proteins and synthetic peptides (0.2 μg/well) in 200 mM carbonate buffer (pH 9.6) were bound to a 96-well microplate (MaxiSorp; Nunc) by incubating them in the microplate overnight at 4°C. The peptides and proteins

HCV core epitopes inserted	Positions of insertion in FHV capsid protein				
	L1	L2	L3	I2	I3
C1	1.96±0.12	2.05±0.32	1.98±0.35	2.25±0.34	2.01±0.34
C2	0.95±0.11	0.86±0.23	0.79±0.32	0.96±0.15	1.13±0.25
C3	1.40±0.33	2.42±0.29	1.86±0.20	2.05±0.15	2.01±0.10

FIG. 1. Stereoribbon diagram of the FHV capsid protein showing the outer loops where the different HCV core regions, C1 (residues 1 to 20), C2 (residues 21 to 40), and C3 (residues 32 to 46), were inserted. The table shows the reactivity in an ELISA (OD_{492}) of all the FHV recombinant proteins with a panel of highly reactive HCV-positive sera (dilution, 1:100). The underlined values indicate the most reactive insertion position for each epitope.

that were absorbed were washed three times with TTBS (Tris [50 mM; pH 7.5], NaCl [500 mM], 0.1% Tween 20) (17) and were incubated with the patients' sera at room temperature for 1 h. In all assays the sera were used at a 1:100 dilution. After three washes with TTBS, horseradish peroxidase-conjugated goat anti-human immunoglobulin G (Dako) at a dilution of 1:2,000 was applied to the wells for 1 h at room temperature. After three further washes with TTBS the color was developed with *ortho*-phenylendiamine dihydrochloride (Sigma-Fast; Sigma Chemical Co., St. Louis, Mo.). The reaction was stopped after 5 min by adding 3 M sulfuric acid, and the optical density (OD) was measured at 492 nm (OD_{492}). All datum points are the means of triplicate determinations. Variations between the individual datum points were generally within 15%. Five HCV-positive serum samples highly reactive with all three epitopes were selected for testing the immunoreactivity at different dilutions (see Fig. 2).

Patient sera. The 94 serum samples used in this study were collected from patients with clinical and histological diagnoses of liver disease and who were all found to be anti-HCV positive with a commercial kit (second-generation ELISA; Ortho Diagnostic Systems, Raritan, N.J.) and confirmed with a RIBA II assay kit (Chiron Corporation, Emeryville, Calif.), which was used according to the manufacturer's instructions. In the RIBA II assay, the following four recombinant proteins, fused to superoxide dismutase (SOD), are adsorbed on nitrocellulose strips: C100-3, which extends from the 3′ end of the NS3 region to almost the entire NS4 region; 5-1-1, which is a 42-amino-acid fragment within the NS4 region; c33c, which is derived from NS3 region; and c22-3, which is the putative nucleocapsid protein. SOD is also present on the strips as a control.

Sera from 20 HCV-negative individuals served as controls for calculating ELISA cutoff values. The mean absorbance values for the HCV-negative samples were as follows: FHV wild-type protein, 0.11; Pep-C1, 0.15; Pep-C2, 0.07; and Pep-C3, 0.07. The cutoff values for the peptides (Pep-C1, 0.43; Pep-C2, 0.13; and Pep-C3, 0.13) and the proteins (FHV wild-type, 0.26) were calculated by considering the mean values for each reagent plus 3 times the standard deviation observed for each sample. The screening of serum samples from all 94 patients was performed at a dilution of 1:100, because this was found to be the dilution with the highest signal-to-background ratio.

RESULTS

Three well-known immunoreactive HCV core regions localized at residues 1 to 20 (C1), 21 to 40 (C2), and 32 to 46 (C3) were inserted into the five different available positions of the FHV capsid protein. In a preliminary study we used 10 patient serum samples positive by anti-HCV ELISA to select the best insertion positions for reactivity for each region. Residues 1 to 20 were found to be the most reactive when they were inserted in the I2 position, residues 21 to 40 were the most reactive when they were inserted in the I3 position, and residues 32 to 46 were the most reactive when they were inserted in the L2 position (Fig. 1).

Figure 2 shows the ELISA reactivities displayed by these three proteins with five highly reactive representative patient serum samples compared to the ones observed for equal

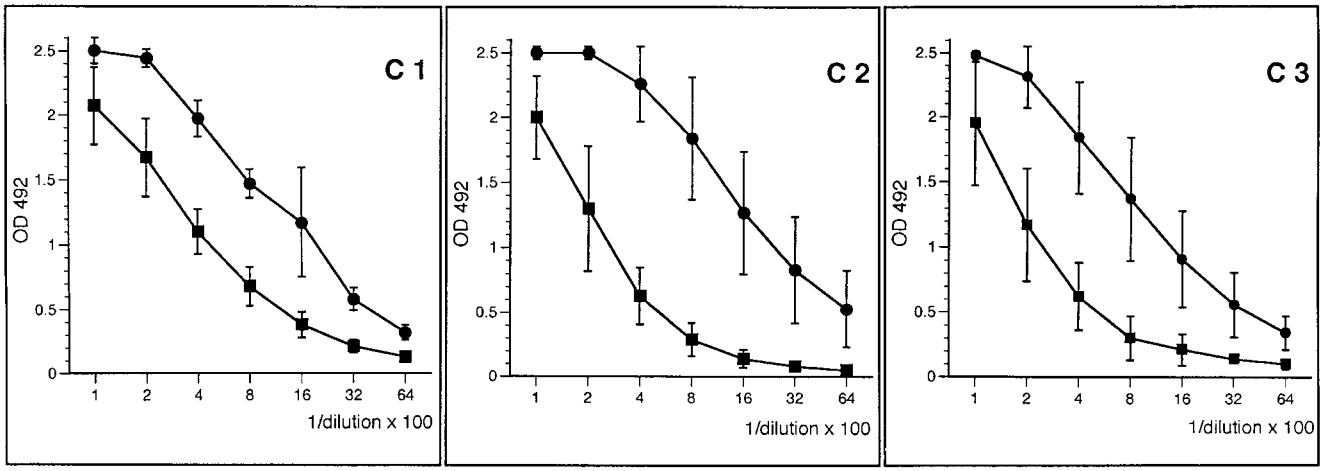

FIG. 2. Mean binding curves for five highly reactive HCV-positive serum samples at the dilutions indicated with three FHV recombinant proteins (●) and their corresponding peptides (■): C1 (region from residues 1 to 20), C2 (region from residues 21 to 40), and C3 (region from residues 32 to 46).

TABLE 1. Comparison of reactivities observed in 94 anti-HCV-positive serum samples with FHV recombinant proteins and their corresponding synthetic peptides

OD_{492}	No. (%) of serum samples reactive with:					
	FHV-C1	Pep-C1	FHV-C2	Pep-C2	FHV-C3	Pep-C3
Less than the cutoff[a]	7 (8)	15 (16)	7 (8)	26 (18)	4 (4)	17 (18)
Greater than the cutoff (<0.5)[a]	8	11	10	46	7	50
0.5–1	23	38	31	19	27	26
>1–1.5	26	25	35	3	42	1
>1.5	30	5	11	0	14	0
Total positive	87 (92)	79 (84)	87 (92)	68 (72)	90 (96)	77 (82)
Mean ± SD OD	1.18 ± 0.56	0.85 ± 0.43	1.04 ± 0.47	0.36 ± 0.31	1.13 ± 0.43	0.42 ± 0.3

[a] The mean cutoff absorbance values were as follows: FHV wild-type protein, 0.11; Pep-C1, 0.15; Pep-C2, 0.07; and Pep-C3, 0.07.

amounts of their corresponding synthetic peptides. The results indicate that at increasing dilutions the reactivities of the recombinant proteins were greater than the ones observed for the synthetic peptides. Only Pep-C1 was capable, at low dilutions, to give immunoreactivities comparable to those of the FHV-C1 protein, whereas increased differences in immunoreactivity were observed between Pep-C3 and Pep-C2 and their corresponding chimeric proteins (FHV-C3 and FHV-C2, respectively).

Sera from 94 anti-HCV ELISA-positive patients were then examined for their reactivities against each of the three recombinant proteins (FHV-C1, FHV-C2, and FHV-C3) in parallel with those against the three corresponding synthetic peptides (Pep-C1, Pep-C2, and Pep-C3). The frequency distribution of the ELISA OD values for each of the chimeric proteins and their corresponding peptides are reported in Table 1. The most reactive and most recognized proteins were FHV-C3 (96%) and FHV-C1 (92%), with mean absorbances of 1.13 ± 0.43 and 1.18 ± 0.56, respectively. The chimeric protein FHV-C2 was also recognized by most serum samples (92%), although with slightly lower mean absorbance values (1.04 ± 0.47) (Table 1). A markedly different pattern was observed with the three peptides, and only Pep-C1, which was reactive in 84% of the cases, displayed high reactivities. Pep-C2 was the peptide least able to detect antibodies against the region from residues 21 to 40, with 72% of the serum sample scoring positive. This result is even more impressive by considering that 46 of the 68 positive serum samples (68%) had reactivities just above the cutoff value. Finally, Pep-C3 yielded a result similar to that for Pep-C2, recognizing 82% of the serum samples, with a low mean absorbance of 0.42 ± 0.3.

Table 2 groups the reactivities of the 94 serum samples against the three recombinant proteins and compares the results with those observed for the three peptides. Overall, it can be noted that the use of peptides failed to give a positive result for 10 serum samples (12%), while the three proteins failed to give a positive result for only 2 serum samples (2%). Interestingly, one of these two nonreactive serum samples (OD_{492}s: for Pep-C1, 0.07; for Pep-C2, 0.07; and for Pep-C3, 0.04) was found to be reactive (OD_{492}, 0.68) in an ELISA with a 44-mer peptide comprising residues 2 to 44 of the core protein. Moreover, it must be noted that while 82 (87%) serum samples were reactive with all three proteins, only 62 (67%) serum samples were found to be reactive with all three peptides.

TABLE 2. Grouped reactivities of the 94 anti-HCV ELISA-positive serum samples according to the combinations of recognized recombinant proteins and synthetic peptides

Peptide reactivity	No. of reactive serum samples with:						
	FHV-C1.2.3	FHV-C1.2	FHV-C2.3	FHV-C1.3	FHV-C1	FHV-C3	None
Pep-C1.2.3	62						
Pep-C2.3	2		2				
Pep-C1.3	7			1			
Pep-C1	6				1		
Pep-C3	3						
None	2	1	2	1	1	1	2

DISCUSSION

The FHV antigen-presenting system has already demonstrated its ability to display human immunodeficiency virus type 1 conformational epitopes (2, 27, 31). Therefore, we used the FHV system to display on the external surface of FHV three immunodominant sequences of the N-terminal portion of the HCV core protein. The aim of this work was to improve the detection of antibodies against these regions by providing the conformational requirements necessary to mimic the original antigenic conformations. The analysis of 94 serum samples positive by anti-HCV ELISA indicated that all three recombinant proteins were more reactive than their corresponding synthetic peptides. This fact is even more striking by taking into account that in all assays, the synthetic peptides were present in 20-fold molar excess. The similar reactivities observed for both the FHV-C1 protein and Pep-C1 (Table 1) confirm the immunodominance of this region in HCV-infected individuals (3, 20, 32) and the linear nature of this epitope (9). Nonetheless, the dilution curve shows that the antibodies in the positive sera bind with a slightly higher immunoreactivity to the recombinant protein than to the synthetic peptide (Fig. 2). A possible explanation for this could be that the binding of the peptide can sometimes occlude key antigenic amino acids at the peptide-plastic interphase, thus lowering its reactivity (19).

The degree of reactivity of patient sera against the region from residues 21 to 40 is still controversial in comparison with previous observations (3), obtained by assays performed with a synthetic peptide, indicating that only 58% of the infected sera contained antibodies against this sequence. However, recent studies have shown that the region from residues 21 to 40 is also known to contain at least two B-cell epitopes. Sallberg et al. (25) identified an antibody binding site at residues 29 to 34, and Siemoneit et al. (28) isolated a monoclonal antibody specific for the sequence from residues 34 to 39 which was subsequently found to be recognized by 80% of the serum samples which tested RIBA positive for c22-3 and c33c. The high re-

activity observed with the FHV-C2 protein compared to the very poor reactivity of its corresponding synthetic peptide suggests that this region is indeed highly immunogenic in HCV-infected patients and that the conformational requirements for its correct display are poorly met by synthetic peptides. This conclusion is supported by the observed much higher immunoreactivity of patients' antibodies for the recombinant protein FHV-C2 as opposed to that for the synthetic peptide (Fig. 2).

Analogous results were obtained for the region from residues 32 to 46 both in the serum dilution experiment and in the screening, where the mean absorbance values observed for the FHV-C3 chimeric protein were much higher than those observed for its corresponding peptide (Table 1 and Fig. 2). This is in keeping with the already proposed conformational nature of this particular epitope (3). Moreover, it must be noted that the peptide used for the detection of antibodies against the C3 region (Pep-C3) was considerably longer (residues 27 to 59) than the sequence inserted in the FHV protein (residues 32 to 46). The reason for this was due to the already described weak reactivity of the synthetic peptide from residues 32 to 46 when it was bound to the solid phase with a monoclonal antibody specific for this sequence (3).

Finally, the data in Table 2 indicate that there is close agreement between the results obtained with our chimeric proteins and those obtained with the commercially available ELISA kit, with only two false-negative serum samples (2%) being found. The fact that one of the two negative serum samples was reactive with a synthetic peptide comprising residues 2 to 44 implies the existence of one or more immunoreactive epitopes in the joining regions not covered by our recombinant proteins, and further work could be aimed at characterizing these epitopes. The lack of reactivity even with this 44-mer peptide of the second serum sample could be due to the presence in this patient of antibodies against rarely represented immunoreactive epitopes beyond the regions studied (5, 9). Interestingly, the commercially available kit contains not only a great portion of the nucleocapsid protein (residues 1 to 150) but also several portions of the NS3 and NS4 regions. By comparison, our chimeric proteins span only the first 46 residues of the nucleocapsid protein.

The results presented in this report confirm that the entire N-terminal portion of the HCV core protein is one of the major targets for the immune response in infected individuals and highlights the growing usefulness of antigen-presenting systems for the correct display of important conformational epitopes.

ACKNOWLEDGMENTS

This study was supported in part by a grant (93.01078 PF.70) from the Consiglio Nazionale delle Ricerche, Progetto Finalizzato Biotecnologie e Biostrumentazione, Contratto di Ricerca.

REFERENCES

1. **Blum, P., M. Velugan, N. Lin, and A. Matin.** 1992. DnaK-mediated alterations in human growth hormone protein inclusion bodies. Bio/Technology **10:**301–304.
2. **Buratti, E., S. G. Tisminetzky, E. S. Scodeller, and F. E. Baralle.** 1996. Conformational display of two neutralizing epitopes of HIV-1 gp41 on the Flock House virus capsid protein. J. Immunol. Methods **197:**7–18.
3. **Cerino, A., P. Boender, N. La Monica, C. Rosa, W. Habets, and M. U. Mondelli.** 1993. A human monoclonal antibody specific for the N-terminus of the hepatitis C virus nucleocapsid protein. J. Immunol. **151:**7005–7015.
4. **Chien, D. Y., Q.-L. Choo, A. Tabrizi, C. Kuo, J. McFarland, K. Berger, C. Lee, J. R. Shuster, T. Nguyen, D. L. Moyer, M. Tong, S. Furuta, M. Omata, G. Tegtmeier, H. Alter, E. Schiff, L. Jeffers, M. Houghton, and G. Kuo.** 1992. Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeric polyprotein to capture circulating antibodies: reevaluation of the role of HCV in liver disease. Proc. Natl. Acad. Sci. USA **89:**10011–10015.
5. **Ching, W.-M., C. Wychowski, M. J. Beach, H. Wang, C. L. Davies, M. Carl, D. W. Bradley, H. J. Alter, F. M. Feinstone, and W.-K. Shih.** 1992. Interaction of immune sera with synthetic peptides corresponding to the structural protein region of hepatitis C virus. Proc. Natl. Acad. Sci. USA **89:**3190–3194.
6. **Choo, Q.-L., G. Kuo, A. J. Weiner, L. R. Overby, D. W. Bradley, and M. Houghton.** 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B hepatitis genome. Science **244:**359–362.
7. **Choo, Q.-L., K. H. Richman, J. H. Han, K. Berger, C. Lee, C. Dong, C. Gallegos, D. Coit, A. Medina-Selby, P. J. Barr, A. J. Weiner, D. W. Bradley, G. Kuo, and M. Houghton.** 1991. Genetic organization and diversity of the hepatitis C virus. Proc. Natl. Acad. Sci. USA **88:**2451–2455.
8. **Claeys, H., A. Volkaerts, H. De Beenhouwer, and C. Vermylen.** 1992. Association of HCV carrier state with the occurrence of HCV-core antibodies. J. Med. Virol. **36:**259–264.
9. **Ferroni, P., G. Mascolo, M. Zaninetti, D. Colzani, F. Pregliasco, M. Pirisi, F. Barbone, and V. Gasparini.** 1993. Identification of four epitopes in hepatitis C virus core protein. J. Clin. Microbiol. **31:**1586–1591.
10. **Goeser, T., H. M. Muller, J. Ye, E. Pfaff, and M. Theilmann.** 1994. Characterization of antigenic determinants in the core antigen of the hepatitis C virus. Virology **205:**462–469.
11. **Houghton, M., A. Weiner, G. Kuo, and Q.-L. Choo.** 1991. Molecular biology of hepatitis C viruses: implications for diagnosis, development, and control of viral disease. Hepatology **14:**381–388.
12. **Katayama, T., T. Mazda, S. Kikuchi, S. Harada, Y. Matsuura, J. Chiba, H. Ohba, I. Saito, and T. Miyamura.** 1992. Improved serodiagnosis of non-A, non-B hepatitis by an assay detecting antibody to hepatitis C virus core antigen. Hepatology **15:**391–394.
13. **Kato, N., M. Hijikata, Y. Ootsuyama, M. Nagakawa, S. Ohkoshi, T. Sugimura, and K. Shimotohno.** 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A non-B hepatitis. Proc. Natl. Acad. Sci. USA **87:**9524–9528.
14. **Khanna, A., and R. Ray.** 1995. Hepatitis C virus core protein: synthesis, affinity purification and immunoreactivity with infected human sera. Gene **153:**185–189.
15. **Kuo, G., Q.-L. Choo, H. J. Alter, G. L. Gitnik, A. G. Redeker, R. H. Purcell, T. Miyamura, J. L. Dienstag, M. J. Alter, C. E. Stevens, G. E. Tegtmeier, F. Bonino, M. Colombo, W. S. Lee, C. Kuo, K. Berger, J. R. Shuster, L. R. Overby, D. W. Bradley, and M. Houghton.** 1989. An assay for circulating antibodies to a major etiologic virus of human non-A non-B hepatitis. Science **244:**362–364.
16. **Miyamura, T., I. Saito, T. Katayama, S. Kikuchi, A. Tateda, M. Houghton, Q.-L. Choo, and G. Kuo.** 1990. Detection of antibody against antigen expressed by molecularly cloned hepatitis C virus cDNA: application to diagnosis and blood screening for post-transfusional hepatitis. Proc. Natl. Acad. Sci. USA **87:**983–987.
17. **Mohammed, K., and A. Esen.** 1989. A blocking agent and a blocking step are not needed in ELISA, immunostaining dot-blots and Western blots. J. Immunol. Methods **117:**141–145.
18. **Moore, J. P.** 1993. The reactivities of HIV-1+ human sera with solid-phase V3 loop peptides can be poor predictors of their reactivities with V3 loops on native gp120 molecules. AIDS Res. Hum. Retroviruses **9:**209–217.
19. **Moore, J. P., Y. Cao, A. J. Conley, R. Wyatt, J. Robinson, M. K. Gorny, S. Zolla-Pazner, D. D. Ho, and R. A. Koup.** 1994. Studies with monoclonal antibodies to the V3 region of HIV-1 gp-120 reveal limitations to the utility of solid-phase peptide binding assays. J. Acquired Immunodefic. Syndr. **7:**332–339.
20. **Nagayama, R., K. Miyake, F. Tsuda, and H. Okamoto.** 1994. IgM antibody to a hepatitis C virus core peptide (CP14) for monitoring activity of liver disease in patients with acute or chronic hepatitis C. J. Med. Virol. **42:**311–317.
21. **Nasoff, M. S., S. L. Zebedee, G. Inchauspe', and A. M. Prince.** 1991. Identification of an immunodominant epitope within the capsid protein of hepatitis C virus. Proc. Natl. Acad. Sci. USA **88:**5462–5466.
22. **Nishizono, A., M. Hiraga, K. Mifune, H. Terao, T. Fujioka, M. Nasu, T. Goto, J. Misumi, M. Moriyama, and Y. Arakawa.** 1993. Correlation of serum antibody titres against hepatitis C virus core protein with clinical features by Western blot (immunoblot) analysis using a recombinant vaccinia virus expressing system. J. Clin. Microbiol. **31:**1173–1178.
23. **Ohkoshi, S., M. Watanabe, K. Kuwana, H. Tawaraya, T. Kamimura, and H. Asakura.** 1993. Clinical evaluation of the antibody against core protein of hepatitis C virus. Gastroenterol. Jpn. **28:**80–83.
24. **Saito, M., A. Hasegawa, T. Kashiwakuma, M. Kohara, M. Sugi, K. Miki, T. Yamamoto, H. Mori, Y. Ohta, E. Tanaka, K. Kiyosawa, S. Furuta, M. Wakashima, S. Tanaka, and H. Hattori.** 1992. Performance of an enzyme linked immunosorbent assay system for antibodies to hepatitis C virus with two new antigens (c11/c7). Clin. Chem. **38:**2434–2439.
25. **Sallberg, M., P. Pumpen, Z. X. Zhang, P. Lundholm, I. Gusars, U. Ruden, B. Wahren, and L. O. Magnious.** 1994. Locations of antibody binding sites within conserved regions of the hepatitis C virus core protein. J. Med. Virol. **43:**62–68.
26. **Schutten, M., J. P. M. Langedijk, A. C. Andeweg, R. C. Huisman, R. H. Meloen, and A. D. M. E. Osterhaus.** 1995. Characterization of V3 domain specific neutralizing human mAb that preferentially recognizes non-syncyti-

um-inducing HIV-1 strains. J. Gen. Virol. **76:**1665–1673.
27. **Scodeller, E. A., S. G. Tisminetzky, F. Porro, M. Schiappacassi, A. De Rossi, L. Chiecco-Bianchi, and F. E. Baralle.** 1995. A new epitope presenting system displays a HIV-1 V3 loop sequence and induces neutralizing antibodies. Vaccine **13:**1233–1240.
28. **Siemoneit, K., M. da Silva Cardoso, A. Wopl, S. Epple, H. Wintersinger, K. Koerner, and B. Kubanek.** 1994. Isotype-specific immune response to a single hepatitis C virus core epitope defined by a human monoclonal antibody: diagnostic value and correlation to PCR. Ann. Hematol. **69:**129–133.
29. **Takamizawa, A., C. Mori, I. Fuke, S. Manabe, S. Murakami, J. Fujita, E. Onishi, T. Andoh, I. Yoshida, and H. Oyakama.** 1991. Structure and organization of the hepatitis C virus genome isolated from human carriers. J. Virol. **65:**1105–1113.
30. **Tanaka, E., K. Kiyosawa, Y. Nakatsuji, Y. Inoue, T. Miyamura, J. Chiba, and S. Furuta.** 1993. Clinical significance of antibodies to non-structural and core proteins of hepatitis C virus in posttransfusion hepatitis patients during long-term follow-up. J. Med. Virol. **39:**318–324.
31. **Tisminetzky, S. G., E. A. Scodeller, P. Evangelisti, Y. Chen, M. Schiappacassi, F. Porro, F. Bizik, T. Zacchi, G. Lunazzi, S. Miertus, and F. E. Baralle.** 1994. Immunoreactivity of chimeric proteins carrying the HIV-1 epitope IGPGRAF. Correlation between predicted conformation and antigenicity. FEBS Lett. **353:**1–4.
32. **Waumans, L., H. Claeys, H. Verhaert, W. Mertens, and C. Vermylen.** 1992. Hepatitis C virus confirmation in blood donor screening. Vox Sang. **927:**1–5.
33. **Yeh, C. T., C. M. Han, S. Y. Lo, J. H. Ou, K. D. Fan, I. S. Sheen, C. M. Chu, and Y. F. Liaw.** 1994. Early determination of anti-HCc antibody in acute hepatitis C virus (HCV) by Western blot (immunoblot) using a recombinant HCV core protein fragment. J. Clin. Microbiol. **32:**2235–2241.