Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 August 21; 13(31): 4224-4229
World Journal of Gastroenterology ISSN 1007-9327
© 2007 *WJG*. All rights reserved.

RAPID COMMUNICATION

Spontaneous elimination of hepatitis C virus infection: A retrospective study on demographic, clinical, and serological correlates

Perdita Wietzke-Braun, Larissa Bettina Mänhardt, Albert Rosenberger, Angela Uy, Giuliano Ramadori, Sabine Mihm

Perdita Wietzke-Braun, Larissa Bettina Mänhardt, Giuliano Ramadori, Sabine Mihm, Department of Gastroenterology and Endocrinology, Robert-Koch-Str.40, 37075 Göttingen, Germany
Albert Rosenberger, Department of Genetic Epidemiology, Humboldtallee 32, Göttingen 37073, Germany
Angela Uy, Department of Microbiology, Kreuzbergring 57, Göttingen, Georg-August-Universität, Göttingen 37075, Germany
Supported by the Deutsche Forschungsgemeinschaft, SFB 402, TP C1, C6, and D3
Correspondence to: Professor Dr. Sabine Mihm, Department of Gastroenterology and Endocrinology, Georg-August-Universität, Robert-Koch-Straße 40, Göttingen 37075, Germany. smihm@med.uni-goettingen.de
Telephone: +49-551-398946 Fax: +49-551-397855
Received: 2007-04-26 Accepted: 2007-05-21

© 2007 *WJG*. All rights reserved.

Key words: Hepatitis C virus; Hepatitis; Self-limited infection; Anti-HCV antibodies; Co-infection; HCV serotype

Wietzke-Braun P, Mänhardt LB, Rosenberger A, Uy A, Ramadori G, Mihm S. Spontaneous elimination of hepatitis C virus infection: A retrospective study on demographic, clinical, and serological correlates. *World J Gastroenterol* 2007; 13(31): 4224-4229

http://www.wjgnet.com/1007-9327/13/4224.asp

Abstract

AIM: To find correlates to spontaneous clearance of hepatitis C virus (HCV) infection, this study compared individuals with self-limited and chronic infection with regard to clinical, demographic, and serological parameters.

METHODS: Sixty-seven anti-HCV positive and repeatedly HCV RNA negative individuals were considered to have resolved HCV infection spontaneously. To determine the viral genotype these patients had been infected with HCV serotyping was performed. For comparison reasons, 62 consecutive patients with chronic hepatitis C were enrolled. Cases and controls were compared stratified for age and sex.

RESULTS: Retrospective analysis showed (1) a lower humoral reactivity to HCV in patients with self-limited compared to chronic HCV-infection and (2) that younger age, history of iv drug use, and acute/post-acute hepatitis A or B co-infections, but not viral genotypes, are independent correlates for spontaneous HCV clearance.

CONCLUSION: The stronger humoral reactivity to HCV in patients with persistent infections and in those with a history of iv drug use is supposed to be due to continuous or repeated contact(s) to the antigen. Metachronous hepatitis A or hepatitis B infections might favor HCV clearance.

INTRODUCTION

Hepatitis C virus (HCV) infection represents a serious threat for the public health worldwide. The natural course of HCV infection is highly variable and ranges clinically from asymptomatic to fulminate, and with regard to the infection status from silent to self-limited to viremia[1,2]. The course of infection is certainly influenced by viral, host, or environmental parameters, or by a combination of these[1,2]. Acute hepatitis C is heralded by detectable HCV RNA in serum, usually followed by an antibody response directed against various structural and non-structural viral proteins[3,4]. Chronic HCV infection is marked by viral persistence for at least six months despite the presence of a humoral immune response. The antibodies produced either appear not to be able to neutralize the virus[5] or, as suggested, they appear delayed at a time when the humoral response is considered to be dysfunctional[6].

Recovery from HCV infection is defined by the presence of HCV-specific antibodies in the absence of detectable HCV RNA[7,8]. Initially, self-limited infections were assumed to be rare, if occurring at all[9,10]. More definite calculations revealed an overall chronic rate averaging 80% and implying a rate of self-limited infections of around 20%[3,11]. Recent studies report even higher rates of at least 50% spontaneous clearance of HCV in various populations[11]. Since some reports indicate that humoral immunity might be progressively lost in patients who had recovered from HCV infection spontaneously[8,12], self-limited virus elimination might have been underestimated.

To find correlates to spontaneous viral clearance, this study compares a group of 67 HCV-infected individuals who overcame infection spontaneously to a group of 62 chronic HCV-infected patients with regard to demographic, clinical, and serological findings, retrospectively. The diagnosis of an HCV infection that had been resolved by the host spontaneously (presence of HCV antibodies) was ascertained by a second, independent assay which detects antibodies to four viral HCV proteins. By applying this assay, the humoral reactivity to HCV was found to be lower in individuals with self-limited than in individuals with chronic HCV infection. Further findings showed that the ability to clear HCV spontaneously was independently associated with younger age, history of iv drug use, and acute/post-acute hepatitis A virus (HAV) or hepatitis B virus (HBV) co-infections at time of diagnosis of HCV, but not with HCV genotypes.

MATERIALS AND METHODS

Study population

From 1992 to 2000, a total of 67 patients who were positive for anti-HCV antibodies, but who repeatedly lacked detectable levels of HCV RNA, visited the out-patient liver department. These patients were regarded to as those with a self-limited HCV infection. To ascertain the diagnosis of a former HCV infection, the presence of anti-HCV antibodies was tested by a second, independent immunoassay (see below). For comparison reasons, a randomly selected group of 62 chronic HCV-infected patients of the same period were enrolled. Chronic infection was proven by the presence of HCV antibodies and serum HCV RNA over a period of at least six months. Liver disease was confirmed histopathologically. None of the patients were previously treated with an antiviral therapy. All patients had an anti-human immunodeficiency virus antibody seronegative status. The study was approved by the local ethical committee and conformed to the ethical guidelines of the 2000 Declaration of Helsinki, and informed consent was obtained from each patient.

Detection of anti-HCV antibodies

All sera were screened for the presence of anti-HCV antibodies using a third-generation microparticle enzyme immunoassay (HCV version 3.0 Axsym, Abbott Laboratories, Chicago, IL, USA). For confirmation reasons, a second assay was applied which also discriminates between different antibody specificities (CHIRON RIBA HCV 3.0 SIA, Ortho Diagnostic Systems Inc., Raritan NJ, USA). This enzyme immunoblot assay, in brief, utilizes the recombinant HCV encoded antigens c33c and NS5 derived from the NS3 and NS5 region of the virus and three synthetic peptides, c100p, 5-1-1p, and c22p, corresponding to the NS4 and the core encoding regions, respectively. The test is regarded positive when reactivity to at least two antigens is obtained, or indeterminate, if the sample shows reactivity to one antigen only. Above that, antibody reactivity can be scored by comparing the intensity of each HCV band to the intensity of two internal control bands on a scale from 0 to 4.

Detection of HCV RNA and determination of HCV geno- and serotypes

Serum RNA was isolated using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). One third of the final eluate was reverse transcribed and subjected to a highly sensitive nested PCR protocol as described[13]. In HCV RNA positive sera, virus genotyping was performed by using the Innolipa HCV Ⅱ line probe assay (Innogenetics, Ghent, Belgium). Sera from patients with non-detectable HCV RNA, were tested for type-specific antibodies to HCV genotypes 1, 2, 3, 4, 5, and 6 by using the MUREX HCV Serotyping 1-6 Assay (Abbott, Wiesbaden, Germany)[14,15].

Serologic testing for HAV, HBV, Epstein-Barr virus (EBV), and cytomegalovirus (CMV) infections

Antibodies directed against HAV (IgM and IgG) were tested using the Anti-HAV assay (Roche Diagnostics GmbH, Mannheim, Germany) performed on Elecsys® 2010/Modular Analytics E170. Serologic testing for HBV infection status was carried out on the Axsym®system (Abbott. Laboratories, Chicago, IL, USA) by determining anti-HBs (AUSAB®), anti-HBe (Anti-Hbe 2.0), HBeAg (HBE 2.0), anti-HBc (CORE), anti-HBcIgM (CORE-M), and HBsAg (HbsAg V2). HBV-specific DNA was detected as described[16]. Previous or recent contacts to EBV or CMV were assessed using commercially available test kits.

Statistical analysis

To prevent bias data were stratified by age ($\leqslant$ 42 years; > 42 years) and sex. Comparing cases and controls, Mantel-Haenszel tests were performed for qualitative data, and linear models were applied to quantitative data. If necessary, log-transformations were performed to achieve normal distributed values; means and limits of confidence intervals are presented re-transformed.

To overcome the problem of multiple testing in sets of correlated parameters belonging to the same topic (e.g. alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltranspeptidase (γ-GT)), permutation tests were performed by randomly allocating study participants as cases or controls. The empirical quantile q of numbers of permutations with the same or more significant statistical test (test statistics exceeding the observed values) was regarded as estimator. The upper 95% confidence limit of q, assuming a binomial distribution, was used as a conservative overall *P*-value.

Exact conditional logistic regressions were performed on the result of serological tests for both approaches to estimate the impact of HAV/HBV co-infections and the history of iv drug use on the outcome of infection.

The local level of significance was set to a screening level of $\alpha = 0.05$. All calculations were performed with SAS 9.2.

RESULTS

Sera from patients, who were tested positive for anti-HCV antibodies by a third-generation microparticle enzyme immunoassay, were subjected to an independent immunoassay. This assay allows the detection of antibodies to four different HCV proteins and a semi-quantitative estimate

Table 1 Frequency of reactivity to HCV proteins in individuals with self-limited and chronic HCV infection

Antibody to	Self-limited HCV infection (n = 55)	Chronic HCV infection (n = 58)	Level of significance
	Qualitative reactivity (n_{pos}/n_{neg}) Quantitative reactivity(mean ± SD)		
Overall	49[1]/2	56/2	P = 0.2073
	8.79 ± 5.04	13.07 ± 4.14	P < 0.0001
C22p (core)	46/9	56/2	P = 0.0737
	2.80 ± 1.58	3.74 ± 0.92	P = 0.0003
C33c (NS3)	50/5	56/2	P = 0.4142
	2.74 ± 1.47	3.67 ± 0.95	P = 0.0002
C100p + 5-1-1p (NS4)	42/13	53/5	P = 0.0681
	2.09 ± 1.62	3.31 ± 1.29	P < 0.0001
NS5 (NS5)	20/35	43/15	P = 0.0002
	1.11 ± 1.63	2.35 ± 1.76	P = 0.0002

All tests were stratified for sex and age; [1]4 patients classified as indeterminate.

of their reactivity (scored from 0 to 4). Analysis was performed with 55 or 58 patients with self-limited or chronic HCV infections, respectively. Analysis revealed reactivity or an indeterminate result in 53 of 55 patients (96.4%) with self-limited infection and in 56 of 58 patients (96.6%) with chronic hepatitis C. Thus, the overall qualitative assay reactivity (test regarded as positive or negative) was comparably high for both groups (P = 0.2073). The number of antigens patients' sera were reactive to, however, were lower in patients who had overcome infection than in patients with chronic hepatitis C (e.g. 29% of patients with a self-limited infection showed 4 positive tests compared to 74% of chronic hepatitis C patients, P = 0.0001, data not shown). Moreover, the overall quantitative assay reactivity, given as the sum of reactivity scores, is significantly different between the groups (P < 0.0001). Patients with chronic hepatitis C reached a mean score of 13.07, being close to the maximum of 16, whereas patients with self-limited HCV infection showed a mean score of 8.79 (Table 1). This decreased reactivity in patients with self-limited compared to those with chronic infection was seen for each of the four antigens. Since the demographic comparison revealed that individuals with a self-limited HCV infection were significantly younger than those with a chronic infection at the time of diagnosis, and the proportion of males is, although not reaching statistical significance (P = 0.1197), higher (Table 2), these results were obtained after stratification for age and sex.

Further analyses, which were also adjusted for age and sex, revealed significant lower levels of serum ALT, AST, and γ-GT activity in patients who overcame infection than those with a chronic infection. The source of infection was also found to be significantly different between both groups (P = 0.0089). The majority of individuals with a self-limited HCV infection had a history of iv drug use (79%), whereas the most frequent source of known modes of infection among chronic hepatitis C patients was the administration of blood transfusions, blood products, or dialysis (47%). Moreover, both groups differ in the status of HAV and/or HBV infections (P < 0.0001). This is primarily due to the fact that the group with self-limited HCV infection does, in contrast to the group with chronic infection, contain a considerable number of patients with acute or post-acute HAV or HBV infections (33% *vs* 0%). Both groups had a similar incidence of EBV or CMV infections.

With respect to viral genotype patients have or had been infected with, HCV RNA positive sera from persistently infected individuals were tested for viral genotypes directly using a sequence-based technique, whereas HCV RNA negative sera from individuals who overcame the infection were tested for type-specific antibodies to HCV (Table 2). By comparing both groups, we found no significant difference in HCV genotype distribution (P = 0.2283). Nevertheless, the number of HCV type 3 infected patients was slightly higher in those with self-limited compared to those with persistent infections (29% *vs* 12%).

By applying a regression model, an overall lower reactivity against HCV antigens in patients with self-limited infection was confirmed (P = 0.0012). By considering HCV type 1 infected individuals separately, those with a history of iv drug use were found to show higher reactivity to HCV antigens than those without. This finding is valid, both for patients with self-limited and persistent infections (Figure 1). Quantitative reactivity appears not to be affected by the outcome or the source of infection in patients with HCV types 2 or 3 (P = 0.9512).

DISCUSSION

This retrospective study identified 67 individuals over a period of eight years who tested positive for anti-HCV antibodies, but who repeatedly lacked detectable levels of HCV RNA. These patients were considered as those with a previous HCV infection who had spontaneously eradicated the virus. To our knowledge, this series represents the largest single-center cohort of patients with self-limited HCV infection.

Our study revealed a lower humoral immunity against HCV in patients with self-limited infections compared to those with persistent infections (Table 1 and Figure 1). This finding, which is in line with an observation by others[17], might reflect the fact that the humoral response, such as cellular responses, declines in magnitude over time because of the absence of the pathogen[18]. This could explain that two of our patients with self-limited HCV infection showed no reactivity when tested against four individual HCV encoded proteins in a recombinant immunoblot assay. Support for this notion is also provided by a direct comparison of humoral and cellular responses that was possible in a cohort of women who were accidentally exposed to HCV[8]. Besides a decline of both humoral and cellular responses, this study revealed cellular responses to be detectable over a longer period than humoral responses.

By comparing both groups to each other, differences in clinical and demographic parameters as serum activities of liver-specific enzymes, age, sex, the source of infection, and the status of HAV/HBV co-infections also became evident (Table 2). HAV/HBV co-infections have been recently described as a correlate of spontaneous HCV clearance among people with hemophilia[19]. Since HBV and HCV have the same transmission routes, co-infections due to a simultaneous or a superinfection may occur and

Table 2 Demographic, clinical, and virological characteristics of patients with self-limited and chronic HCV infection

	Self-limited HCV infection (n = 67)	Chronic HCV infection (n = 62)	Level of significance
Sex (M/F)	50/17 (75%/25%)	36/26 (58%/42%)	P = 0.1197
Age (mean ± SD) M	38 ± 9.6	48 ± 12.4	P < 0.0001[13]
F	38 ± 10.5	51 ± 10.7	
ALT (U/L)[1]	15.2 (11.9-19.5)	46.7 (45.6-47.9)	P < 0.0001[14]
Over normal	22 (33%)	52 (84%)	
AST (U/L)[2]	11.8 (9.9-14.1)	28.7 (24.0-34.3)	P < 0.0001[14]
Over normal	15 (22%)	43 (69%)	
γ-GT (U/L)[3]	15.5 (11.7-20.5)	24.8 (18.7-32.9)	P = 0.0228[14]
Over normal	19 (28%)	32 (52%)	
Source of infection (count)			
iv drug use	44 (79%)[4]	14 (41%)	
Blood transfusion/dialysis	6 (11%)	16 (47%)	P = 0.0089[14]
Others	6 (11%)	4 (12%)	
Unknown	11 (-)	28 (-)	
HAV/HBV infections (count)			
None of both	21 (34%)	19 (31%)	
Previous HAV or HBV infection	20 (33%)[5]	42 (69%)[6]	P < 0.0001[7,14]
At least one acute/post-acute infection[8]	20 (33%)[9]	-	
Vaccination	6 (-)	1 (–)	
Unknown	-	-	
EBV infection (count)			
Previous	39 (95%)[10]	21 (100%)	
None	2 (5%)	-	P = 0.4292[14]
Unknown	26 (-)	41 (-)	
CMV infection (count)			
Previous	26 (76%)	16 (84%)	
Nnone	8 (24%)	3 (16%)	P = 0.3605[14]
Unknown	33 (-)	43 (-)	
HCV genotype (1a-1b[11]- both/2b/3a[12]/other)		11-35-2/3/6/1 83%/5%/10%/2%	P = 0.2283[14]
HCV serotype (1/2/3/other)	21/1/7/2 67%/3%/23%/6%		

[1]Upper normal limit is 23 U/L for males, and 19 U/L for females; [2]Upper normal limit is 19 U/L for males, and 15 U/L for females; [3]Upper normal limit is 28 U/L for males, and 18 U/L for females; [4]The amount was larger in men younger than 42 yr (93%) than in the older (57%); [5]Previous HAV (n = 5), previous/chronic HBV (n = 6/n = 5), previous HAV and HBV (n = 4); [6]Previous HAV (n = 17), previous HBV (n = 10), previous HAV and HBV (n = 13), previous HAV and chronic HBV (n = 2); [7]Comparison of self-limited and persistent infection regarding the number of none versus previous HAV/HBV infections revealed a lack of significance (P = 0.1892); [8]Diagnosis of acute infections with HAV or HBV: presence of IgM antibodies; diagnosis of post-acute infections with HAV or HBV: decreasing transaminase activities, increasing anti-HBcIgG or anti-HAV-IgG antibodies, decreasing anti-HBcIgM or anti-HAV-IgM antibodies; [9]18/20 patients (90%) had a history of iv drug use; [10]3/39 patients with acute EBV infection; [11]HCV genotype 1b was found in 70% older than 42 yr, but only in 33% of the younger patients (P = 0.0089); [12]HCV genotype 3 was more frequent in individuals with a history of iv drug use than in non-drug users (23% *vs* 8%, P = 0.5787); [13]Stratified for sex; [14]Adjusted for age and sex, retransformed from ln-transformation.

even persist in the same patient. De Mitri *et al*[20] found that HBV can suppress HCV replication in half of the patients co-infected with HBV and HCV. The sequence of infections has been regarded to be important in the clinical course. Unfortunately, we do not know whether HCV infection was cleared during the acute episode of HAV or HBV infection or whether it might have been already cleared before. However, from a study by Sagnelli *et al*[21], it is known that patients having been simultaneously infected with both HBV and HCV recovered from HBV infection, but progressed to HCV-related chronic hepatitis, as happens in most cases with single HBV or HCV infection[]. Superinfection of chronic hepatitis C patients with HBV, on the other hand, led to a recovery from HCV infection in half of the patients[21]. Moreover, simultaneous clearance of preexisting persistent HCV infection during an acute HBV superinfection has been described for some cases by us and others[22-24]. In fact, 26% of our individuals with self-limited HCV infection had acute or postacute HBV co-infection at time of HCV diagnosis was made compared to none of the chronic HCV-infected group. Thus, our data support the hypothesis that acute HBV infection in HCV chronic carriers might lead to an eradication of the HCV infection. Superinfection of chronic hepatitis B patients with HCV, on the other hand, has been reported to be clinically severe and long-term prognosis to be worse in terms of the development of liver cirrhosis and hepatocellular carcinoma[25,26]. Four individuals with a self-limited HCV infection presented with acute or postacute HAV superinfection at time of HCV diagnosis was made. HAV was self-limited and the clinical course benign in accordance with Sagnelli *et al*[27], although conflicting results are known from the literature (reviewed in[28]). Our finding is also in line with a recent study from Germany showing an association of HAV superinfection and decreased HCV replication which was suggested to lead to recovery from HCV in some individuals[29].

In accordance with previous epidemiological studies on

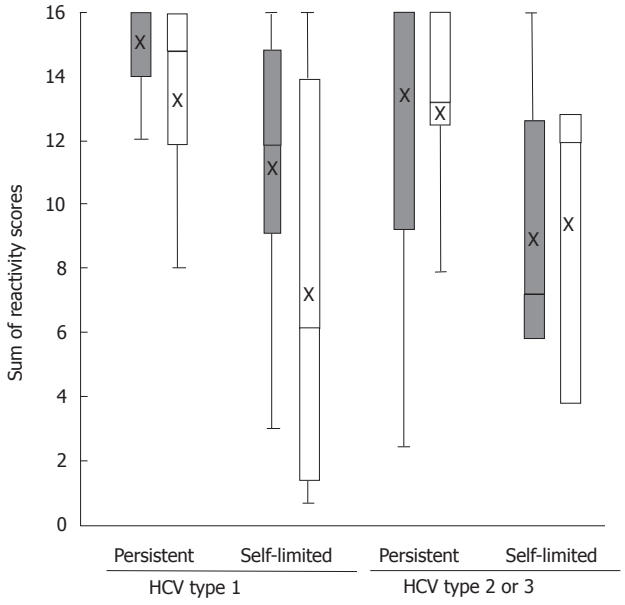

Figure 1 Serological reactivity to HCV encoded proteins with respect to virus type, the putative source and the outcome of infection. Grey indicates iv drug use and white indicates iv non-use. Mean values are indicated by x, medians by -, boxes show the interquartil range (central 50% of observations) and whiskers show the largest and lowest observed value (if not identified as outlier). By applying a regression model, an overall lower reactivity in patients with self-limited infection could be confirmed (P = 0.0012). Quantitative reactivity appears not to be affected by the outcome or the source of infection in patients with HCV types 2 or 3 (P = 0.9512).

viral genotype distribution among chronic hepatitis C patients from Germany, HCV 1b was the predominant genotype among the patients studied, especially in those older than 42 years[30-32]. Since HCV type 1 also predominates among patients with self-limited infections, type 1 infected individuals obviously are quite able to clear an HCV infection (Table 2). This is in line with two studies on 22 and 24 patients with a self-limited HCV infection who were also infected with HCV type 1 (59% and 50%) in a considerable number[33,34].

Active iv drug users have been described to have a high incidence of re-infection with HCV, and a relationship between antibody titer and the presence of the antigen has been assumed[35,36]. Our study thus strengthens this view (Figure 1), although we do not have reliable anamnestic information on the actual status of drug consumption. Apart from that, drug use itself, or even drug use in the past might have significant effects on immune functions including viral persistence[37]: On the one hand, opioids have been shown to inhibit the expression of antiviral cytokines as IFN-α and IFN-γ. On the other hand, particularly HCV replication has been shown to be enhanced both by morphine and by morphine withdrawal in a replicon system[38].

Taken together, our study provides an extensive clinical and demographic characterization of a group of 67 patients who were enrolled over a period of eight years and who can be considered to have spontaneously resolved an infection with HCV. Major findings are (1) a stronger humoral immunity to HCV in patients with chronic hepatitis C and in those with a history of iv drug use and (2) that younger age, history of iv drug use, and an acute HAV or HBV co-infection, but not viral genotypes are independent correlates for spontaneous HCV clearance.

ACKNOWLEDGMENTS

The authors thank all the physicians in the Division of Gastroenterology who were involved in the care and control of patients for their kind cooperation.

REFERENCES

1 **Piasecki BA**, Lewis JD, Reddy KR, Bellamy SL, Porter SB, Weinrieb RM, Stieritz DD, Chang KM. Influence of alcohol use, race, and viral coinfections on spontaneous HCV clearance in a US veteran population. *Hepatology* 2004; **40**: 892-899
2 **Seeff LB**, Hoofnagle JH. National Institutes of Health Consensus Development Conference: management of hepatitis C: 2002. *Hepatology* 2002; **36**: S1-S2
3 **Hoofnagle JH**. Course and outcome of hepatitis C. *Hepatology* 2002; **36**: S21-S29
4 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-1406
5 **Kaplan M**, Gawrieh S, Cotler SJ, Jensen DM. Neutralizing antibodies in hepatitis C virus infection: a review of immunological and clinical characteristics. *Gastroenterology* 2003; **125**: 597-604
6 **Logvinoff C**, Major ME, Oldach D, Heyward S, Talal A, Balfe P, Feinstone SM, Alter H, Rice CM, McKeating JA. Neutralizing antibody response during acute and chronic hepatitis C virus infection. *Proc Natl Acad Sci USA* 2004; **101**: 10149-10154
7 **Strader DB**, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147-1171
8 **Takaki A**, Wiese M, Maertens G, Depla E, Seifert U, Liebetrau A, Miller JL, Manns MP, Rehermann B. Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 2000; **6**: 578-582
9 **Alter MJ**, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999; **341**: 556-562
10 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52
11 **Micallef JM**, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; **13**: 34-41
12 **Cramp ME**, Carucci P, Rossol S, Chokshi S, Maertens G, Williams R, Naoumov NV. Hepatitis C virus (HCV) specific immune responses in anti-HCV positive patients without hepatitis C viraemia. *Gut* 1999; **44**: 424-429
13 **Mihm S**, Hartmann H, Fayyazi A, Ramadori G. Preferential virological response to interferon-alpha 2a in patients with chronic hepatitis C infected by virus genotype 3a and exhibiting a low gamma-GT/ALT ratio. *Dig Dis Sci* 1996; **41**: 1256-1264
14 **Simmonds P**, Rose KA, Graham S, Chan SW, McOmish F, Dow BC, Follett EA, Yap PL, Marsden H. Mapping of serotype-specific, immunodominant epitopes in the NS-4 region of hepatitis C virus (HCV): use of type-specific peptides to serologically differentiate infections with HCV types 1, 2, and 3. *J Clin Microbiol* 1993; **31**: 1493-1503
15 **Bhattacherjee V**, Prescott LE, Pike I, Rodgers B, Bell H, El-Zayadi AR, Kew MC, Conradie J, Lin CK, Marsden H. Use of NS-4 peptides to identify type-specific antibody to hepatitis C virus genotypes 1, 2, 3, 4, 5 and 6. *J Gen Virol* 1995; **76** (Pt 7): 1737-1748
16 **Heermann KH**, Gerlich WH, Chudy M, Schaefer S, Thomssen

R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. *J Clin Microbiol* 1999; **37**: 68-73
17 **Umemura T**, Wang RY, Schechterly C, Shih JW, Kiyosawa K, Alter HJ. Quantitative analysis of anti-hepatitis C virus antibody-secreting B cells in patients with chronic hepatitis C. *Hepatology* 2006; **43**: 91-99
18 **Semmo N**, Barnes E, Taylor C, Kurtz J, Harcourt G, Smith N, Klenerman P. T-cell responses and previous exposure to hepatitis C virus in indeterminate blood donors. *Lancet* 2005; **365**: 327-329
19 **Zhang M**, Rosenberg PS, Brown DL, Preiss L, Konkle BA, Eyster ME, Goedert JJ. Correlates of spontaneous clearance of hepatitis C virus among people with hemophilia. *Blood* 2006; **107**: 892-897
20 **De Mitri MS**, Morsica G, Cassini R, Bagaglio S, Andreone P, Bianchi G, Margotti M, Bernardi M. Genetic variability of hepatitis C virus in HBV/HCV co-infection and HCV single-infection. *Arch Virol* 2005; **150**: 261-271
21 **Sagnelli E**, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; **36**: 1285-1291
22 **Gruener NH**, Jung MC, Ulsenheimer A, Gerlach TJ, Diepolder HM, Schirren CA, Hoffmann R, Wachtler M, Backmund M, Pape GR. Hepatitis C virus eradication associated with hepatitis B virus superinfection and development of a hepatitis B virus specific T cell response. *J Hepatol* 2002; **37**: 866-869
23 **Jessner W**, Strasser M, Graziadei I, Berr F, Vogel W. Sustained remission of chronic hepatitis C after acute hepatitis B superinfection. *Scand J Infect Dis* 2006; **38**: 818-821
24 **Wietzke P**, Schott P, Braun F, Mihm S, Ramadori G. Clearance of HCV RNA in a chronic hepatitis C virus-infected patient during acute hepatitis B virus superinfection. *Liver* 1999; **19**: 348-353
25 **Liaw YF**, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. *Gastroenterology* 2004; **126**: 1024-1029
26 **Sagnelli E**, Coppola N, Marrocco C, Onofrio M, Sagnelli C, Coviello G, Scolastico C, Filippini P. Hepatitis C virus superinfection in hepatitis B virus chronic carriers: a reciprocal viral interaction and a variable clinical course. *J Clin Virol* 2006; **35**: 317-320
27 **Sagnelli E**, Coppola N, Pisaturo M, Pisapia R, Onofrio M, Sagnelli C, Catuogno A, Scolastico C, Piccinino F, Filippini P. Clinical and virological improvement of hepatitis B virus-related or hepatitis C virus-related chronic hepatitis with concomitant hepatitis A virus infection. *Clin Infect Dis* 2006; **42**: 1536-1543
28 **Vento S**, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G, Ferraro T, Concia E. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998; **338**: 286-290
29 **Deterding K**, Tegtmeyer B, Cornberg M, Hadem J, Potthoff A, Boker KH, Tillmann HL, Manns MP, Wedemeyer H. Hepatitis A virus infection suppresses hepatitis C virus replication and may lead to clearance of HCV. *J Hepatol* 2006; **45**: 770-778
30 **Berg T**, Hopf U, Stark K, Baumgarten R, Lobeck H, Schreier E. Distribution of hepatitis C virus genotypes in German patients with chronic hepatitis C: correlation with clinical and virological parameters. *J Hepatol* 1997; **26**: 484-491
31 **Mihm S**, Fayyazi A, Hartmann H, Ramadori G. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. *Hepatology* 1997; **25**: 735-739
32 **Driesel G**, Wirth D, Stark K, Baumgarten R, Sucker U, Schreier E. Hepatitis C virus (HCV) genotype distribution in German isolates: studies on the sequence variability in the E2 and NS5 region. *Arch Virol* 1994; **139**: 379-388
33 **Schulze zur Wiesch J**, Lauer GM, Day CL, Kim AY, Ouchi K, Duncan JE, Wurcel AG, Timm J, Jones AM, Mothe B, Allen TM, McGovern B, Lewis-Ximenez L, Sidney J, Sette A, Chung RT, Walker BD. Broad repertoire of the CD4+ Th cell response in spontaneously controlled hepatitis C virus infection includes dominant and highly promiscuous epitopes. *J Immunol* 2005; **175**: 3603-3613
34 **Gerlach JT**, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, Schraut WW, Schirren CA, Waechtler M, Backmund M, Pape GR. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. *Gastroenterology* 2003; **125**: 80-88
35 **Mehta SH**, Cox A, Hoover DR, Wang XH, Mao Q, Ray S, Strathdee SA, Vlahov D, Thomas DL. Protection against persistence of hepatitis C. *Lancet* 2002; **359**: 1478-1483
36 **Grebely J**, Conway B, Raffa JD, Lai C, Krajden M, Tyndall MW. Hepatitis C virus reinfection in injection drug users. *Hepatology* 2006; **44**: 1139-1145
37 **Vallejo R**, de Leon-Casasola O, Benyamin R. Opioid therapy and immunosuppression: a review. *Am J Ther* 2004; **11**: 354-365
38 **Li Y**, Zhang T, Douglas SD, Lai JP, Xiao WD, Pleasure DE, Ho WZ. Morphine enhances hepatitis C virus (HCV) replicon expression. *Am J Pathol* 2003; **163**: 1167-1175

S- Editor Zhu LH **L- Editor** Rippe RA **E- Editor** Li JL