Viral and Host Factors in Early Hepatitis C Virus Infection

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Since 1980, the Transfusion-transmitted Viruses Study (TTVS) (1974-1980) has continued to maintain its computerized database and stored sera to enable ongoing study of new transfusion events since the 1970s. Most recently, we have used this resource to study parameters of acute hepatitis C virus (HCV) infection among 94 donor–recipient pairs in which there was transmission. In addition, frequent recipient observations permitted further characterization of the early phase of the infection's course. Donor RNA load ranged from 3.7 to 3,160,000 IU/mL. Onset of recipient viremia was judged from a total of 67 sera collected during the 4th through 8th days posttransfusion; only 2 of the 67 sera were still RNA nonreactive by that time. The recipients' latent periods to an alanine aminotransferase (ALT) elevation of ≥90 IU/L ranged from 6 to 112 days (median, 46 days) and was shorter with higher donor RNA levels. Descriptors of the recipient's illness showed several strongly positive and negative correlations. The latent period tended to be shorter in the 37% of cases that were clinically overt. Attributes of donors with genotypes 1 and non-1 and subtypes 1a and 1b did not differ significantly. Recipients with genotype 1 strains had shorter latent intervals than non-1 strains. On multivariate analysis, latent period was significantly associated (negatively) only with the highest ALT level during the first 120 days of follow-up ($P = .014$). In conclusion, host factors are more important determinants of acute HCV infection dynamics than virus-associated factors. (HEPATOLOGY 2005;42:86-92.)

In the past, the most clearly defined cases for study of acute hepatitis C virus (HCV) infection resulted from blood-borne transfusion transmission in populations followed prospectively. This approach identified not only overt cases but also the more numerous subclinical infections. In the last decade, however, screening of blood donors for antibody to HCV (anti-HCV) and then hepatitis C viremia has almost eliminated this route of infection.[1] Fortunately, databases and specimens for several past groups of prospectively followed transfusion recipients are still available.[2] One of these is the Transfusion-transmitted Viruses Study (TTVS), from which it is still possible to obtain new information about HCV infections as increasingly informative laboratory assays have been developed.[3-7]

We have identified and further characterized 94 acute transfusion-transmitted HCV cases in which an anti–HCV-negative recipient was infected by a single seropositive donor. We have examined the idea that there could be correlations between donor and recipient parameters, reflecting the replicative capacity or pathogenetic potential of the strain shared between donor and recipient. We have also attempted a thorough description of recipient events during the first 4 months of the infection.

Patients and Methods

Population. Many details of TTVS have been previously reported.[8-10] Briefly, from July 1974 through Octo-

Abbreviations: HCV, hepatitis C virus; TTVS, Transfusion-transmitted Viruses Study; ALT, alanine aminotransferase; HBV, hepatitis B virus; RT-PCR, reverse-transcription polymerase chain reaction; TMA, transcription-mediated amplification; NANB hepatitis, non-A non-B hepatitis.

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ber 1979, patients who were likely to undergo transfusion were asked at hospital admission for informed consent to participate in a study of transfusion safety. Potential subjects with hepatitis B surface antigen positivity were excluded. Follow-up continued through June 1980 with visits and serum collection every 2 to 3 weeks for 6 months. For the very early course, a serum was electively obtained 4 to 8 days after transfusion before hospital discharge. Later, if posttransfusion elevations of alanine aminotransferase (ALT) activity occurred, follow-up visits were made weekly.

Laboratory Tests During TTVS. By available assays, we excluded 10 cases of transfusion-acquired hepatitis B virus (HBV) infection; there were no cases of hepatitis A virus transmission. Serum from every donation to each recipient was assayed for HBV markers and for ALT. The recipients' pretransfusion and all posttransfusion follow-up evaluations were tested for HBV markers and ALT levels.

The ALT assay was carefully standardized across centers, using the same protocol, the same model spectrophotometer, centrally purchased reagents, and centrally prepared quality control aliquots. Enzyme activity is influenced by conditions of storage, so samples were handled on the first workday after collection.[11] The upper limit of normal (log mean plus 3 SD) for the recipient population was 44 IU/L.[10]

Two tubes of 2.5 mL sera from each donation and each recipient visit were set aside promptly for long-term storage. They were maintained at −40°C at each center before shipment to a central repository, where they have been kept at −70°C. The pertinent institutional review boards approved the present use of these sera.

Study Cohort for These Analyses. In screening 5,386 donor sera by second-generation enzyme-linked immunoassay, 180 were reactive. By third-generation enzyme-linked and recombinant immunoblot assays, 162 were confirmed as anti-HCV positive. These units had been given to 138 recipients. For the present study, we excluded 31 persons from this group. These were 15 patients given more than one anti-HCV–positive unit, eight who were anti-HCV–positive before transfusion, 4 who also acquired HBV infection, 3 who lacked passive anti-HCV after transfusion, and one without residual serum in the repository. Of the remaining 107 recipients, 94 developed RNA positivity and seroconverted.[7]

All of the 94 recipients were followed throughout the first 120 days, which we have defined as the acute phase. The scheduled number of visits for all participants during this interval was 8; for the 94 infected recipients, the average number of visits was 10.8, reflecting more vigorous follow-up. The patients were usually seen at home, where the nurse–epidemiologist reviewed a list of likely symptoms and also looked at the recipient's skin and eyes. Sera were transported back to the laboratory on ice.

Recent Laboratory Testing. Donor HCV RNA was quantitated by a reverse transcription polymerase chain reaction (RT-PCR) (COBAS AMPLICOR HCV Monitor version 2.0, Roche Diagnostic Systems, Indianapolis, IN). Sera nonreactive by RT-PCR were further evaluated by use of an HCV transcription-mediated amplification (TMA) assay (Gen-Probe Inc., San Diego, CA).[12] Only one anti-HCV–positive serum was nonreactive for RNA by both RT-PCR and TMA but was associated with posttransfusion acquisition of passive anti-HCV, RNA reactivity, and later active seroconversion.

The appearance of hepatitis C viremia in recipients was evaluated by testing the first three specimens from each recipient for HCV RNA by qualitative TMA. Because of limited volume, we used a reduced input volume of 150 μL instead of the manufacturer's recommended 500 μL. The manufacturer's probit analysis of the TMA assay using this input volume showed a 90% detection limit of 37 IU/mL. In the three instances in which the first posttransfusion specimen was RNA-negative with 150 μL, we repeated the test in duplicate using 500 μL.

Genotypes and Subtypes. Because of depleted volumes of archived donor sera, genotyping was based on a recipient specimen taken 3 to 6 weeks post-transfusion. The methods for genotyping at Bayer Diagnostics (Emeryville, CA) and of subtyping at Edinburgh University have been previously described.[7]

Procedural Issues and Assumptions. During TTVS itself, the working definition of a hepatitis episode was the occurrence of an ALT value $\geq$ 90 IU/L at 15 or more days after the first transfusion, preceded or followed by a value of $\geq$ 60 IU/L within 13 to 17 days. This definition focused attention on candidate cases for non-A, non-B (NANB) hepatitis, and increased the frequency of observations to once weekly for 1 to several weeks. Clinical status was examined closely, and we sought other causes of hepatic inflammation such as anesthetic agents or potentially hepatotoxic drugs. The data for each case were reviewed by our External Advisory Committee. Four of the 94 recipients included in this analysis did not meet the TTVS criteria for NANB hepatitis. They were identified by having received an anti-HCV–positive unit; all 4 had the posttransfusion appearance of HCV RNA and active seroconversion.

The incubation period for infectious diseases is defined as the time from acquisition of the infection to the onset of relevant symptoms caused by the transmitted agent.[13] Because most hepatitis C cases are asymptomatic, it has been common practice to use the interval to the first ALT

elevation considered compatible with the onset of NANB hepatitis.[14] This use of the term, however, differs from that for other infectious agents. For hepatitis C, therefore, it seems preferable to use the term *latent period*, applicable to both asymptomatic as well as symptomatic patients. When symptoms occurred, they coincided with the ALT elevations.

The value of 90 IU/L, consistent with other studies of transfusion-associated hepatitis at that time,[15-17] was chosen to avoid overcalling the lesser elevations sometimes seen in the early post-transfusion interval. Excluding the 138 recipients intensively screened for the present study and an additional 10 persons with HBV infection alone, 1,385 persons remained without evidence for HCV, HBV, or HAV. Of these, the ALT criteria were met by 42 (3.0%) recipients. In a control group of 1,551 non-transfusion patients followed by the same protocol, 35 persons (2.2%) met the ALT criteria for NANB hepatitis. These last two sets of cases remain etiologically unexplained.

Statistical Methods. The analyses used SAS 9.1 programs (SAS Institute, Cary, NC). Statistical procedures included the Wilcoxon test, Spearman rank-sum correlation, and multiple linear regression.

Results

Donor Characteristics. Of the 94 implicated donors, 80 (85%) were male, with a median age (interquartile range) of 26 years (22 and 33 years); for the 14 women, the median age was 24.5 years (22 and 30 years). ALT at donation was $>$ 44 IU/L for 33 men (41%) and 6 women (43%).

Eighty-three implicated donations (88%) were reactive for RNA by quantitative RT-PCR; their values ranged from 199 to 3,160,000 IU/mL, with a median of 56,200. For analysis, the 10 additional donations reactive only by TMA were assumed to have 37 IU/mL. One donor serum nonreactive by both RT-PCR and TMA but associated with development of recipient HCV viremia and seroconversion was assumed to have 3.7 IU/mL. Using this set of values as continuous, there was no association of HCV RNA level with the geographical location or donor's sex, but donor age group was positively correlated ($r = 0.342$, $P = .001$). There was no correlation between the donor HCV RNA level and ALT activity ($r = 0.066$, $P = .527$).

Recipient Characteristics. Of the 94 recipients, 47% were men, ranging in age from 26 to 77 years with a median of 54 years. The 50 women ranged in age from 17 to 75 years with a median of 48. The major medical conditions causing hospitalization covered a wide range of routine surgical procedures. By definition of the study population, all were hepatitis B surface antigen negative and subsequently shown to be anti-HCV negative before the implicated transfusion. Pre-transfusion serum ALT had a median of 11 IU/L, and the 5th and 95th percentiles were 1 and 31 IU/L.

Potential Correlations Between Donor and Recipient Characteristics. To investigate the dynamics of early HCV replication, we tested the first three posttransfusion specimens for RNA. Of 94 recipients, 91 had RNA positivity in the first serum obtained 1 to 27 days (median, 7 days) after the implicated transfusion. The first sera obtained from the three remaining recipients were RNA negative (at days 1, 7, and 8).

These three initially RNA-negative sera were confirmed as negative when TMA was repeated in duplicate using 500 μL input serum. All three, however, were anti-HCV reactive, indicating passively acquired antibody. The second and third sera were obtained from days 8 through 73 (respective medians, 14 and 29 days); all were RNA-positive. None of the recipients became RNA-negative within the acute phase as we have defined it. Subsequent follow-up from 4.9 to 36.7 months (median, 9.5 months) after the implicated transfusion showed that 24 (26%) of the 94 became RNA-negative by the last 1 to 6 visits.

We also considered whether a large donor inoculum would result in early RNA positivity. The recipient RNA-negative at day 1 had been transfused with the only donor unit that had been RNA-negative by 3 assays[7]; this unique case is not considered further in this section. Otherwise, a first serum was available within 4 to 8 days posttransfusion for 67 recipients (71%). Thirteen of these had donor viral loads $<$ 370 IU/mL, including the two who were RNA negative at day 7 or 8; the other 11 were all RNA positive by day 8 posttransfusion. The remaining 54 recipients were exposed to inocula with $\geq$ 370 IU/mL, and all tested RNA positive by day 8. The association between size of inoculum ($<$370 IU vs. $\geq$370 IU) and early detection of acute viremia was statistically significant (exact $P = .044$).

The latent period from infection to an ALT value $\geq$ 90 IU/L ranged from 6 to 119 days, with a median of 46 and a mode of 42 days. The size of the donor's inoculum was negatively correlated with the length of the latent period (Table 1). Higher donor viral loads were associated with an increased occurrence of symptoms, but at a borderline level ($P = .064$).

Donor ALT level (Table 1), as a possible index of an individual HCV strain's pathogenicity, was not correlated with the latent period, nor with the interval from the first elevated ALT (initial) to the highest ALT level. It was correlated positively, however, with the level of the first

Table 1. Spearman Correlation Coefficient (r) and Significance Probability (*P*) of Donor's HCV RNA Level and ALT Activity and the Recipient's Acute Course

	Donor Index	
Recipient ALT Descriptor	HCV RNA	ALT Activity
Latent period* (days)	r = −0.326	r = −0.162
	P = .002	*P* = .127
Interval initial to highest ALT (days)	r = 0.131	r = −0.016
	P = .219	*P* = .538
Level of first ALT ≥ 90*	r = 0.040	r = 0.297
	P = .706	*P* = .004
Level of peak ALT	r = .093	r = 0.199
	P = .385	*P* = .060

* Defined by the first ALT value ≥ 90 IU/L.

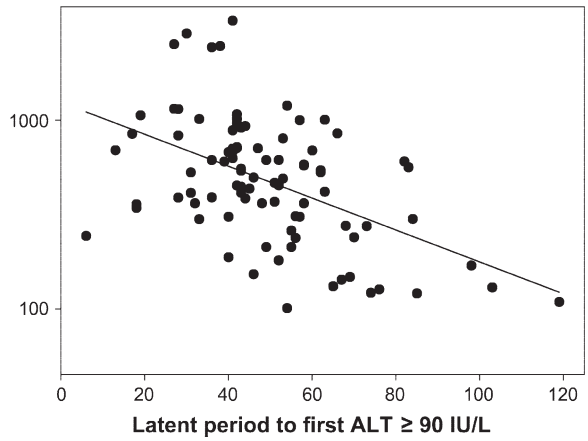

Fig. 1. The length of the latent period from transfusion to an ALT value ≥ 90 IU/L in relation to highest alanine aminotransferase (ALT) value during the acute phase of hepatitis C infection.

elevated ALT, and also correlated positively at a borderline level with the highest ALT value.

Recipients' Acute Course. There were no differences in the distribution of latent periods by geographical location or sex. Persons younger than 50 years had a shorter latent period (median, 40 days) than older individuals (median, 51 days) ($P = .040$).

The interrelationships among the four ALT descriptors (latent period, interval from initial elevated to the highest ALT, level of first ALT ≥90 IU/L, and level of highest ALT) were used to characterize the patient's acute infection (Table 2). The latent period was negatively correlated at a very significant level with the highest ALT; thus, the shorter the latent period, the higher was the maximal ALT (Fig. 1). The interval from the initial elevation to the highest ALT was also highly negatively correlated with the level of the first ALT ≥ 90 IU/L, whereas the level of the initial ALT was positively correlated with that of the highest ALT.

The first ALT elevation of ≥90 IU/L ranged from that value to 2,880 IU/L, with a median (interquartile range) of 204 IU/L (122 and 368 IU/L). For 22 recipients (24% of 90 meeting the NANB case definition), the initial elevation was higher than any seen subsequently within 4 months of infection.

Information on the occurrence of symptoms and jaundice was available for 90 of the 94 recipients, including the four asymptomatic recipients who did not meet the ALT criteria for NANB hepatitis. Fifty-seven (63%) were asymptomatic. Fourteen (16%) recipients had symptoms without jaundice, and 19 (21%) recipients had both symptoms and icterus. The frequency of clinical illness did not differ by sex or age. The range of latent periods for overt and subclinical cases were the same, but the median (interquartile ranges) was 41 days (33 and 47 days) for clinical cases and 52 days (43 and 62 days) for the subclinical cases ($P = .001$).

It is generally accepted that the occurrence of symptoms and jaundice indicates more severe hepatic damage in the acute phase, as reflected by the maximum value for the ALT assay. Table 3 shows the association of highest ALT level during the first 4 months in relation to clinical manifestations. The highest levels for the icteric recipients ranged from 275 to 3,365 IU/L, with a median of 847; for the anicteric but symptomatic, from 109 to 2,435 IU/L with a median of 648; and for the asymptomatic, from 12

Table 2. Spearman Correlation Coefficient (r) and Significance Probability (*P*) of Alanine Aminotransferase (ALT) Descriptors During Acute Hepatitis C Virus Infection

Descriptor	Interval Initial to Highest ALT (days)	Level of First ALT ≥ 90 (IU/L)	Level of Highest ALT (IU/L)
Latent period* (days)	−0.238	−0.193	−0.465
	.048	.069	<.001
Interval initial to highest (days)	–	−0.399	0.134
		<.001	.208
Level of first ALT ≥ 90 (IU/L)	–	–	0.501
			<.001

* Defined by the first ALT value ≥ 90 IU/L.

Table 3. Relation of Recipient's Peak ALT Within 120 Days After the Implicated Transfusion With Presence of Symptoms With or Without Icterus During Same Interval

	Number of Recipients* With:		
Peak ALT Level (IU/L)	No Symptoms	Symptoms Only	Symptoms and Icterus
<400	28	4	1
400–799	22	5	7
800–3,365	7	5	11

* Omits four recipients for whom the data were missing.

to 1,195 IU/L with a median of 389. Rank-sum analysis of ALT values among recipients with overt infections compared with those with subclinical cases was statistically very significant ($P < .001$).

The total number of transfusions given to recipients (including the implicated one) ranged from 1 to 14 (median, 2 units). The total number of transfusions did not influence any of the parameters discussed above except the interval to the highest ALT, which was positively correlated at a borderline level ($r = 0.293$, $P = .055$).

Relationship of Genotypes and Subtypes of Donor/Recipient Clusters to Other Infection Parameters. We obtained the attributed donor HCV genotypes (based on early recipient samples) for 90 (96%) of the 94 donors. Sixty-nine (77%) were genotype 1, with 36 of subtype 1a, 31 of subtype 1b, and 2 not subtyped. Twenty-one (23%) were non-1 genotypes, of which five were subtype 2a/2c, 13 were 2b, and three were 3a. No statistically significant differences were found with respect to geographical area, sex, or age between those with genotype 1 and non-1, nor subtypes 1a and 1b. The distribution of donor ALT values and RNA levels did not differ between genotype 1 and non-1 strains ($P = .786$). Donors with lb subtype had somewhat higher RNA levels (median, 37,000 IU/mL) than donors with 1a (median, 5,870 IU/mL), but this difference was not significant ($P = .124$). The 1b donors had ALT values (median, 48 IU/L) above those for donors infected with the 1a subtype (median, 30 IU/L), but the difference was only of borderline significance ($p = 0.056$).

The recipients' latent periods for genotype 1 (median, 36 days) were significantly shorter than for non-1 genotypes (median, 45 days) ($P = .018$) (Fig. 2). There was no significant difference in latency between subtypes 1a and 1b ($P = .230$). The highest ALT value for the 69 recipients having genotype 1 ranged from 97 to 3,365 IU/L, with a median of 529 IU/L; that of the 21 non-1 genotypes ranged from 122 to 2,475 IU/L with a median of 451 IU/L. These distributions did not differ significantly ($P = .103$). Comparing recipients with subtypes 1a and 1b, neither the latent periods nor highest ALT levels differed. No association of genotypes or subtypes with symptoms was seen.

Multivariate Analysis for Associations of the Latent Period. Univariate analyses found 5 characteristics of the recipients' infection that were associated with the length of the latent period: the donor's viral load, the genotype (type 1 vs. non-1), the recipient's age (<50 years vs. ≥50 years), the maximal ALT level, and the occurrence of symptoms. Multivariate analysis of these variables showed that only maximal recipient ALT level was significantly associated with time to ALT elevation ($P < .014$), although the genotypic dichotomy approached significance ($P = .070$). The variance in the length of the latent period explained by this model (r^2) was 0.144.

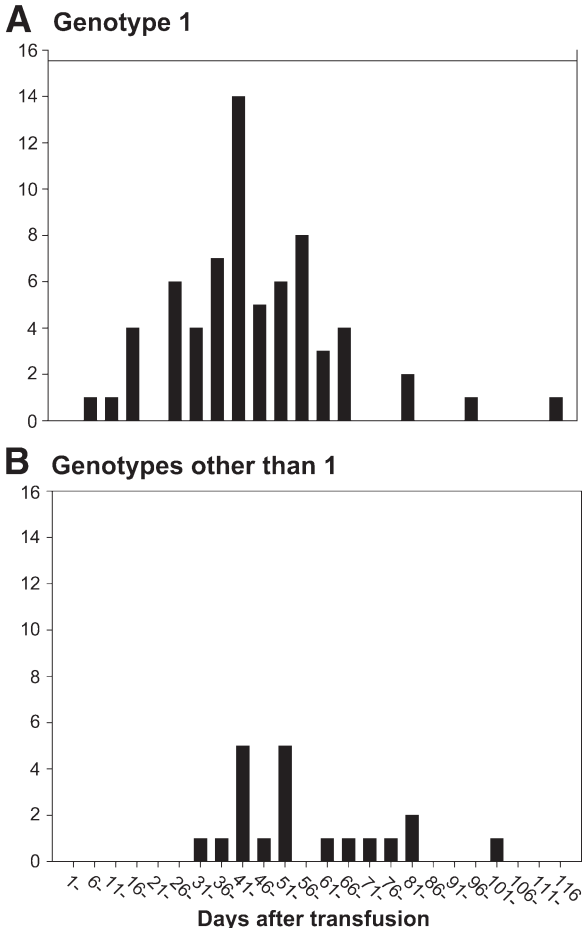

Fig. 2. Latent period of hepatitis C from transfusion to an ALT ≥ 90 IU/L for type 1 and non-1 genotypes.

Discussion

Because this report relies on HCV RNA values obtained more than 20 years after the specimens were first stored, the question of RNA degradation during this time must be addressed. We have previously discussed this issue in our report on donor–recipient transmission.[7] There was excellent correspondence between detection of RNA in the donor and whether transmission occurred. Furthermore, testing other specimens stored under less favorable conditions for a longer time still gave the expected 67% RNA detectability in persons with anti-HCV-positivity.[18] Consequently, we believe that the losses of RNA in TTVS are negligible. A recent study of plasmas stored at −20°C and −70°C indicates no significant loss.[19]

The data indicate that the recipient's viremia becomes detectable within the first week after transfusion, as has been reported previously for chimpanzees and hu-

mans.[20,21] Immediately after transfusion, the recipient experiences a period during which HCV RNA concentration in plasma quickly decreases. This may be attributable to receptor binding in the liver. This effect is compatible with the eclipse phase easily demonstrable for many viruses in cell culture.[22] Applicability to HCV is supported by kinetic studies of HCV by Garcia-Retortillo and her Barcelona colleagues before, during, and after liver transplantation in 20 HCV-infected patients.[23] In 19 of these cases, an abrupt decrease occurred in the level of viremia when the host's blood perfused the graft. This lower level persisted for 8 to 24 hours.

In the present study, an unscheduled specimen was collected in one case by happenstance on the day after transfusion of an HCV-contaminated unit. It was RNA-negative on repeated testing. In this instance, whether the absence of very early viremia is entirely compatible with the eclipse phase, or the fact that RNA had been undetectable in the donor serum, or both, is difficult to determine.

The eclipse phase is followed by a period of logarithmic growth. Glynn et al. used serial samples from frequent plasma donors who acquired HCV infection to study this phase of viral replication.[24] They estimated the doubling time to be 10.8 hours (95% CI 9.9-12.1 hours). Estimates of doubling time are also available from the Barcelona investigators.[23] They were able to estimate this parameter in only nine cases in which there was a rapid increase in viral load after the eclipse phase. The values ranged relatively widely, from 7.3 to 34.6 hours, with a median of 11 hours. Notice, however, that in 11 cases in that series the level of the subsequent viremia either did not exceed that before transplantation or was lower. In the current study, 97% of cases were RNA-positive when a serum was taken on the 4th to the 8th day of infection. The two cases of RNA negativity at day 7 and day 8, however, are compatible with the doubling time being sometimes longer than was estimated above, which may be biologic and/or related to the relatively low level of viremia in the inoculating units.

A second descriptor possibly related to the size of the inoculum is the recipient's latent period to an ALT value of $\geq$90 IU/L. A statistically significant association was seen by univariate analysis, but not by multivariate analysis. The latter result fits the finding of a correlation coefficient (r) between inoculum and latent period of only 0.326. Nothing suggested that inoculum size influenced the other 3 ALT descriptors used as indices of acute severity (interval from initial elevated to the highest ALT, level of first ALT $\geq$ 90 IU/L, and level of highest ALT).

No variation during the acute phase has been associated with genotypes or subtypes.[25,26] Our work is in agreement, with the exception that the latent period was significantly longer for the 21 non-1 genotype infections. This association, however, was not found to be significant on multivariate analysis.

In summary, our data indicate that the viral strain and the amount of the inoculum influence the course of acute hepatitis C, but only very modestly. For the latter, this is contrary to the intuitive concept that a larger inoculum results in more severe infection. The generally high correlations among the four recipient ALT descriptors suggest that they are measuring the same underlying phenomena, which is probably related to the vigor of the host's immune response. Thus, the data support the idea that variability in the host is much more important than that in the virus for determining course.

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