Ultrio and 20 copies/mL for AmpliScreen). Ultrio detected all six HBV genotypes (A-G) at 100 copies per mL or lower. Of the 2912 blood donation pools tested by Ultrio, no yield samples for any agent were identified. There were five false-positive pools (0.17%) and 11 donations that had concordant reactive serology and NAT results. The unresolved pool reactive rate was comparable to the AmpliScreen test at 0.16 percent. There were no invalid assays (in contrast to the AmpliScreen assay reporting an invalid assay rate of 3.92%[13]); with the automated pipetting system, the invalid sample rate was 0.19 percent (this variable was not reported for AmpliScreen). A window-period reduction of 14 days (range, 11-18 days) was observed when seroconversion panels were tested by ID NAT; the window-period reduction decreased to 6 days (range, 3-8 days) in pools of 8 and 3 days (range, 0-5 days) in pools of 16. No window-period reduction was observed in a pool of 24 with PRISM being more sensitive in about half of the panels tested. Finally, comment should be made regarding the comparability of Ultrio HIV-1 and HCV sensitivity to the Procleix HIV-1/HCV combined assay (Gen-Probe/Chiron). The 95 percent detection limits for HIV-1 in the study by Koppleman and coworkers were 65 copies per mL (range, 41-119 copies/mL) for Ultrio versus 31 copies per mL (range, 20-52 copies/mL) for the HIV-1/HCV combined assay in a previous study. For HCV, the 95 percent detection limits for Ultrio were 86 copies per mL (range, 50-204 copies/mL) and 85 copies per mL (range, 64-118 copies/mL) for the HIV-1/HCV combined assay, again in the current versus earlier study. Although the HIV-1 point estimates were qualitatively different, this difference was not significant because the 95 percent confidence intervals overlapped. In the United States, however, further evaluation is necessary to confirm this finding and determine its clinical relevance prior to licensure. Koppleman and coworkers concluded that a two- to five-fold decrease in the residual risk can be expected by the use of HBV MP NAT if the test includes a prior virus enrichment step, if smaller pool sizes are used (fewer than eight donations), or if ID NAT is used.[14]

The sensitivity of MP NAT for HBV remains only a few days better, dependent on pool size, than newer assays for HBsAg. Because window-period differences of 1 day translate to 1.4 additional HBV infections detected per 10 million donations screened,[11] the greatest benefit from HBV NAT would occur in the ID NAT format. ID NAT can detect HBV infection 25 to 36 days earlier than the currently licensed HBsAg assays and is more sensitive than HBsAg.[11] The FDA had planned to increase the sensitivity of licensed HBsAg assays by requiring these assays to be able to detect a lower concentration of HBsAg on the CBER HBsAg Lot Release Panel. Although a draft guidance document was released in April 2002, it was withdrawn. The FDA discussed the possibility of a technology neutral approach in which a sensitivity level for detection of HBV infection could be set; the standard then could be met by either MP NAT or a highly sensitive HBsAg assay or other equivalent technology.[21]

The impact of MP NAT for HBV is expected to be marginal largely owing to the existence of sensitive tests for HBsAg and the availability of even more sensitive HBsAg tests expected in the near future. In addition, in the United States, the need for HBV NAT is reduced further by the existence of anti-HBc screening. The yield from clinical studies has been either marginal or nonexistent and HBV MP NAT has been shown to have very poor cost-effectiveness relative to other blood safety measures; this is coupled with the fact that there has been an absence of reported cases of transfusion-transmitted HBV. Therefore, a true benefit has yet to be established for this test, at least in the MP format. The desire to implement will be negatively impacted further, especially for tests in the triplex format, if there is deterioration in any test characteristic including HIV-1 or HCV sensitivity, or test specificity. It is also very important that the implementation of HBV MP NAT be voluntary, that is, that the availability of technology should not mandate its use. Finally for those who implement HBV MP NAT, careful analysis of yield cases compiled into a national reporting system to include associated donor demographic and laboratory test characteristics would be extremely useful in planning for the future.

Although the yield of MP NAT for HBV is expected to be low in the United States, the yield may be greater in donor populations in developing countries where HBV is highly endemic. In many of these countries, anti-HBc testing is not performed, because the donor loss is considered to be too great. Countries that have implemented HBV NAT including Japan and Germany have reported HBV NAT yields of 1 in 145,000 to 1 in 1.5 million, respectively.[22,23] Whether MP NAT for HBV would be a cost-effective strategy for donor screening in other countries can only be determined by future studies.

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				2005. 6. 22	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Glynn SA, Wright DJ, Kleinman SH, Hirschkorn D, Tu Y, Heldebrant C, Smith R, Giachetti C, Gallarda J, Busch MP. Transfusion. 2005 Jun;45(6):994-1002.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			米国

研究報告の概要

○C型肝炎ウイルス感染初期におけるウイルス血症の動態
【背景】C型肝炎ウイルス(HCV)の感染初期のウイルス増殖動態を明らかにすることは、ウイルス病原性や、輸血などによる急性感染における二次伝播の可能性に対する理解を深めるために重要である。
【実験デザイン及び方法】512プールNATを用いたNATスクリーニングによりHCV-RNA陰性から陽転したか、RNA検出後に抗体が検出されるまで追跡調査した77名から供血された原料血漿について、個別NAT及びHCV-RNAの定量を行った。
【結果】指数増殖が起きるramp-up phase中の倍化時間は10.8時間であった(95%CI 9.9-12.0)。50個のパネル中37個でramp-up phase前に断続的なウイルス血症が観察され、検出可能な血液中ウイルスの存在は、最も早いもので推定されるramp-upの開始63日前に生じていた。ramp-upとセロコンバージョンの間に生じるプラトー期または高ウイルス血症期は、56.3日続くと推定された(95%CI 44.8-67.8)。
【結論】低レベルの断続的なウイルス血症は、通常セロコンバージョン前に起きるウイルス量の指数増殖期及び高力価プラトー期ウイルス血症の2ヵ月前に起こり得る。低レベルのウイルスが存在する血漿の輸血により、受血者がHCVに感染するかどうかを評価するための動物接種試験(感染実験)が進行中である。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
照射合成血「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
C型肝炎ウイルスの感染初期において、低レベルの断続的なウイルス血症は、通常セロコンバージョン前に起きるウイルス量の指数増殖期及び高力価プラトー期ウイルス血症の2ヵ月前に起こり得るとの報告である。	HCVの感染初期におけるウイルスの感染性は不明である。今後も引き続き、HCV感染に関する新たな知見及び情報の収集に努める。

7

Dynamics of viremia in early hepatitis C virus infection

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BACKGROUND: It is important to characterize viral dynamics in early hepatitis C virus (HCV) infection to further our understanding of viral pathogenesis and the potential for secondary transmission in acute infection through blood transfusion or other routes.

STUDY DESIGN AND METHODS: Serial units given by 77 source plasma donors who had evolved from HCV RNA–negative to HCV RNA–positive by nucleic acid amplification technology (NAT) screening with 512-unit pool-NAT or were followed from RNA detection to antibody conversion were tested by individual NAT and quantitative RNA assays.

RESULTS: During the ramp-up phase when exponential growth occurs, HCV viral load doubled every 10.8 hours (95% confidence interval [CI], 9.9-12.0). Intermittent viremia was observed before the ramp-up phase in 37 of 50 panels with the earliest detectable viremic bleed occurring 63 days before the estimated onset of ramp-up. The plateau phase or high-titer viremic period that occurs between ramp-up and seroconversion was estimated to last 56.3 days (95% CI, 44.8-67.8).

CONCLUSIONS: Intermittent low-level HCV viremia can occur as much as 2 months before the periods of exponential increase in viral load and the high-titer plateau-phase viremia that usually precede seroconversion. Animal inoculation studies are in progress to evaluate if transfusion of low-level viremic plasma can transmit HCV infection.

Studying the dynamics of early hepatitis C virus (HCV) infection is crucial to our understanding of HCV transmission, pathogenesis, and determinants of viral clearance or persistence. Studies of transfusion hepatitis cases,[1,2] health-care workers with accidental needlestick exposure,[3] and experimentally inoculated chimpanzees[4,5] have shown that high-level viremia usually occurs within 1 to 2 weeks of transfusion or inoculation. This high-titer viremic period, often referred to as the plateau phase, appears to persist for 40 to 60 days before alanine aminotransferase (ALT) levels become elevated (40-50 days) and/or antibody seroconversion occurs (50-60 days).[1,6] It is, however, unclear if these findings are generalizable to those transmission events that may not involve such high-viral-load parenteral exposures.

Further, HCV-seroconverting plasma donor panels have demonstrated that a period of exponential growth in

ABBREVIATIONS: d = discriminatory; TMA = transcription-mediated amplification.

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This work was supported by the National Heart, Lung and Blood Institute Contracts N01-HB-97077 (superseded by N01-HB-47114), -97078, -97079, -97080, -97081, and -97082 through the Retrovirus Epidemiology Donor Study. Data on plasma donor panels were provided by the Alpha Therapeutic Corporation and the National Genetics Institute and testing reagents by the manufacturers of the NAT assay systems (Roche Molecular Systems and Gen-Probe, Inc.).

Received for publication October 5, 2004; revision received November 16, 2004, and accepted November 16, 2004.

doi: 10.1111/j.1537-2995.2005.04390.x

TRANSFUSION 2005;45:994-1002.

viral load, termed the ramp-up phase, precedes the high-titer viremic plateau phase.[6,7] Serum HCV RNA appears to increase extremely rapidly in this ramp-up phase, with estimated mean viral doubling times ranging from 10.8[7] to 1.7 hours.[1,6] The variation in doubling time estimates across studies may stem from not having applied consistent criteria to delineate when ramp-up starts and ends. The period preceding the ramp-up phase, that is, the pre-ramp-up period, has also not been well characterized. Nübling and colleagues[7] recently reported that, similar to human immunodeficiency virus (HIV)[8] and hepatitis B virus,[9] very-low-titer viremia may intermittently occur in this early phase. The frequency and temporal distribution of such intermittent viremic episodes, however, have not been well defined, and it is possible that these occurrences represent an early ramp-up rather than a distinct pre-ramp-up phenomenon. Finally, the presence of low-level viremia in the pre-ramp-up phase, if confirmed, could have potential implications on the risks of secondary transmission through blood transfusions because current risk estimates assume that the pre-ramp-up phase is of short duration and nonviremic (thus not infectious);[10] if pre-ramp-up viremia occurs frequently after community exposures and is infectious via the blood transfusion route, these risk estimates will need to be modified.

To enhance our understanding of HCV viral dynamics in pre-ramp-up, ramp-up, and plateau phases, we applied highly sensitive qualitative and quantitative HCV RNA amplification assays to serial samples from source plasma donors who had evolved from being RNA-negative to RNA-positive and in some instances from being RNA-positive to antibody-positive. This report thus provides a comprehensive and detailed evaluation of the viremic events associated with primary HCV infection through seroconversion.

MATERIALS AND METHODS

HCV-positive plasma donor panels

To study viral dynamics of early HCV infection, we analyzed data from two sets of anonymized HCV panels whose construction is detailed in the Appendix. The first set included 55 panels with each panel consisting of serial units given in 1997 to 1999 by source plasma donors who had evolved from being HCV RNA–negative to RNA-positive by the HCV-reverse transcriptase (RT)-polymerase chain reaction (PCR) assay (UltraQual, National Genetics Institute, Los Angeles, CA), conducted on pools of 512 samples. These 55 panels were selected from a group of 77 potential panels because they included samples collected before the first RT-PCR–positive unit and were therefore informative to an analysis of early replication dynamics. The 55 plasma donors gave a total of 629 units (median, 11 units/panel; range, 4 and 21 units/panel, respectively) with a mean of 4.9 days between collections. Each sample had also been tested for ALT. Data on risk factors, genotypes, subtypes, or postseroconversion information were not available.

We further characterized these panels by quantifying viral loads (copies/mL) with a PCR assay (COBAS Amplicor HCV Monitor, Version 2.0, Roche Molecular Systems, Pleasanton, CA) with quantification limit of 600 IU per mL. Samples with high viral load (>1 $\times 10^6$ copies/mL) on initial neat testing were diluted 1:100 and retested by COBAS Amplicor HCV Monitor, Version 2.0, PCR to get accurate viral load measurements during the early plateau phase. For all bleeds in which RNA was below quantification, testing in four replicates by individual donation nucleic acid amplification testing (NAT) was conducted at Gen-Probe Inc. (San Diego, CA) with a discriminatory(d)-HCV assay (Procleix, Gen-Probe, Inc.) based on transcription-mediated amplification (TMA)[11] (this assay was conducted in singlicate for three bleeds with insufficient volume). In our analysis, we considered all information on units collected as early as 71 days before and as late as 43 days after RNA was first quantified. As controls we included 55 samples derived from serial plasma units from five donors not infected with HCV (these donors were infected with HIV); these units had been processed and aliquoted in the same laboratory during the same period with the same procedures as were used for the HCV-infected donor panels. These 55 control samples were sent to Gen-Probe under code interspersed with the specimens from the HCV-infected donors; HCV d-TMA was negative on all control samples, indicating lack of contamination during unit aliquoting and the specificity of the HCV d-TMA assay.

To characterize the mean duration of the preseroconversion viremic plateau phase, we included in our analysis the serial data obtained on the 55 panels described above and on a second set of panels representing 22 plasma donors. These 22 donors were followed at regular intervals after detection of their HCV infection by quantitative RT-PCR with 17 donors having serial bleed data through a third-generation HCV enzyme immunoassay (EIA) antibody seroconversion. ALT levels were also available on most bleeds. These data collected in 1997 allowed us to more thoroughly evaluate the plateau phase because information obtained for the plateau phase on the 55 panels was incomplete (only 1 of the 55 panels perchance had data until seroconversion occurred). The study protocol was approved by the UCSF Committee on Human Research.

Viral dynamics in the ramp-up phase

To estimate how rapidly, on average, viral load increased in the ramp-up phase, we first needed to select, for each panel, the bleeds collected when viral load was increasing exponentially. Although viral load can increase in pre-

ramp-up and in the plateau phase, the rate of increase should by definition be smaller than that observed in the ramp-up phase. Assuming that the first RNA quantifiable bleed ("Time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, we calculated the rate of increase in log viral load observed 1) between the bleed preceding the Time 0 bleed and the Time 0 bleed and 2) between the Time 0 bleed and the following bleed, and classified bleeds associated with higher rates of increase in viral load as ramp-up bleeds. This selection process is detailed in the Appendix, which also describes the method used to impute viral loads for units that were positive on one of four, two of four, three of four, or four of four TMA replicates as 8.9, 13.2, 19.6, and 119.2 copies per mL, respectively. Information on log viral load and collection days for the selected ramp-up bleeds was then entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc., Cary, NC).[12] The mean viral load doubling time during ramp-up was then calculated as log(2)/mean slope.

Viral dynamics in the pre-ramp-up phase

To ensure that we were evaluating the viral dynamics of the pre-ramp-up phase rather than an *early ramp-up phenomenon*, we conservatively determined a day when ramp-up started. As shown in Fig. 1, the ramp-up start day was determined by back calculating, with each donor's specific rate of increase in log viral load during ramp-up, the day on which viral load would have been 1 copy per 20 mL or 0.05 copies per mL. This low viral concentration is consistent with the numbers of copies per mL that would be present in a unit of red blood cells (RBCs) that contains just one HCV viral copy. This approach allowed us to be reasonably certain that we had not included a ramp-up bleed in our pre-ramp-up classification (see Appendix). We then selected all units given by plasma donors in the pre-ramp-up phase (i.e., before the conservatively estimated ramp-up start day) and categorized these units as viremic (one of four to four of four TMA-positive results) or as nonviremic (zero of four TMA-positive). We used a continuous time Markov chain with two states (viremic and nonviremic) to estimate the mean viremic and nonviremic periods.[13]

Viral dynamics in the plateau phase

To estimate the mean duration of the preseroconversion viremic plateau phase, defined here as the minipool NAT-positive period occurring between ramp-up and seroconversion (Fig. 1), we evaluated all plateau-phase data from 77 panels (see Appendix for selection of plateau phase bleeds). The mean plateau phase duration was estimated with PROC LIFEREG (SAS Institute Inc.),[12] assuming a normal distribution (although a normal distribution was theoretically not plausible, other plausible distributions all yielded estimates very similar to the normal distribution based estimate). The log viral loads during the plateau phase were assessed with a repeated-measures regression model to estimate the mean viral load during the plateau phase.[12]

ALT and correlation with viral load in the plateau phase

We evaluated the distribution of ALT levels in each phase of primary HCV infection and calculated the intra- and intersubject correlations between ALT and RNA levels observed in the plateau phase with a stratified Spearman (PROC FREQ, SAS Institute Inc.)[12] and a Spearman (PROC CORR)[12] correlation, respectively. The latter analysis was

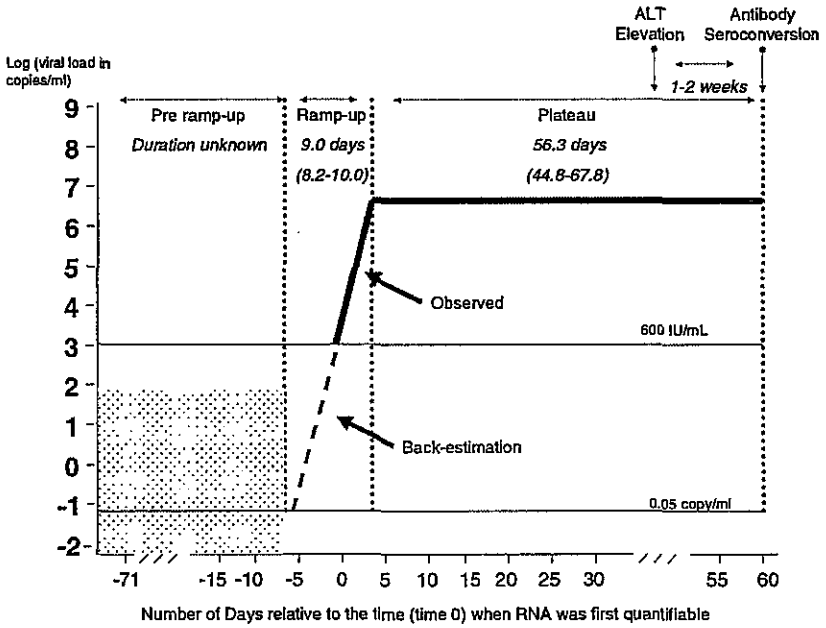

Fig. 1. Pre-ramp-up, ramp-up, and plateau phases of HCV viremia. The pre-ramp-up phase (period preceding the ramp-up phase) is of unknown duration whereas the ramp-up phase (period of exponential growth in viral load) was estimated to last a mean of 9.0 days (95% CI, 8.2-10.0 days). The mean duration of the high-titer viremic plateau phase (the minipool NAT-positive period occurring between ramp-up and seroconversion) was estimated to be 56.3 days (95% CI, 44.8-67.8).

restricted to the plateau phase because significant ALT elevations were only observed in that phase.

RESULTS

Figure 2 presents serial RNA values for 55 plasma donors who met the selection criteria for analysis of pre-ramp-up and ramp-up viremia. Low-level intermittent viremia was detected in the "late" pre-ramp-up period followed by the ramp-up period of exponential growth in viral load. As schematized in Fig. 1, the ramp-up phase was then followed by a high-titer viremic plateau phase preceding HCV antibody seroconversion. During this plateau phase, viral loads fluctuated between 4.1×10^4 and 7.2×10^7 copies per mL, with a mean level of 3.8×10^6 copies/mL. The mean duration of the plateau phase was estimated to be 56.3 days (95% confidence interval [CI], 44.8-67.8) among the 77 panels (the 55 panels shown in Fig. 2 and 22 panels with additional plateau-phase information). The plateau phase lasted between 30 and 65 days about 75 percent of the time, with less than 10 percent of subjects having a plateau phase exceeding 100 days.

Figure 3 shows how log viral load for the selected ramp-up bleeds linearly increased during the ramp-up phase with viral load doubling every 10.8 hours (95% CI, 9.9-12.0). The ramp-up phase was conservatively estimated to begin 6.1 days before the time when RNA was first quantifiable (600 IU per mL; Fig. 1). Fifty of the 55 donors had given at least 1 unit in the pre-ramp-up phase, that is, before their estimated cutoff date of 0.05 copies per mL. Of the 225 units given in the pre-ramp-up phase, 108 were found to be TMA-reactive on at least one of four replicates (deemed viremic) whereas 117 units (and all 55 control HCV-uninfected donor units) were nonreactive by TMA on four of four replicates (deemed nonviremic). Six panels with viremic pre-ramp-up units had undergone additional testing by d-TMA and by an HCV PCR (COBAS AmpliScreen, Roche Molecular Systems, Pleasanton, CA) for the purposes of another study (from 3 to 23 additional replicate test results were available). The presence of low-titer viremia in the pre-ramp-up phase was confirmed in five of the six panels with 48 percent of the viremic bleeds testing reactive on at least one additional RNA test. Figure 4 shows, for each donor, the TMA reactivity of each unit given in the pre-ramp-up phase. We found that intermittent TMA reactivity occurred in the pre-ramp-up phase for at least 37 of 50 donors (possibly more since intermittent TMA reactivity may be missed because bleeds were not obtained on every day of the pre-ramp-up phase). In our observation period, the earliest detectable viremic bleed occurred an estimated 63 days before onset of the estimated 0.05 copy per mL ramp-up period. Of the TMA-reactive bleeds, 37, 18, 22, and 23 percent were reactive on one, two, three, and four of four TMA replicates, respectively; four of four or three of four reactive TMA replicates were as likely to occur in the early or later part of the pre-ramp-up phase ($p = 0.53$). The Markov chain model indicated that periods of detectable viremia lasted on average 5.3 days and alternated with periods of nonviremia lasting on average 5.5 days.

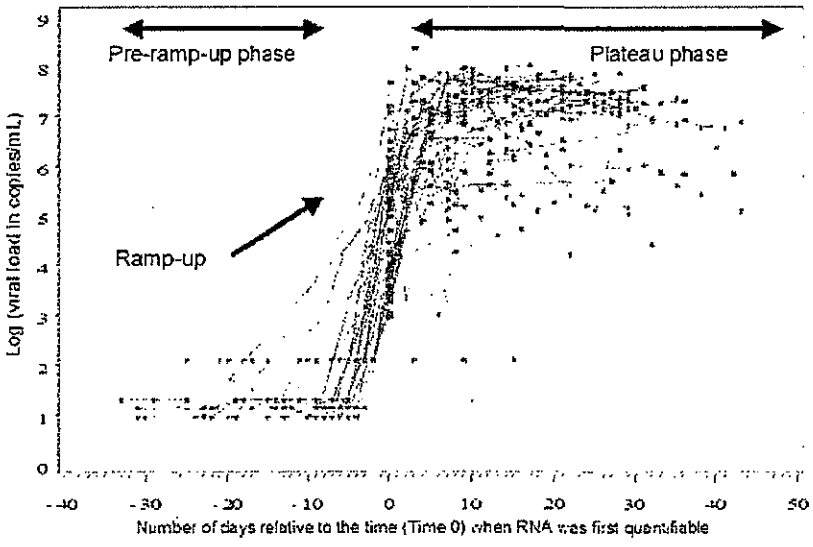

Fig. 2. Viral RNA levels for selected bleeds around the ramp-up phases from 55 plasma donor panels. Time 0 for each panel was defined as the first bleed date that had a quantifiable RNA level (≥600 IU/mL). For units collected before Time 0, only imputed viral load data on positive d-HCV TMA units that were collected after an RNA-negative unit (i.e., a unit that was negative by d-HCV TMA on all four replicates) were included. Hence, Fig. 2 does not show viral load for positive d-HCV TMA units that preceded RNA-negative units.

Except for two ramp-up bleeds with slight ALT elevations (46 and 55 IU/L, respectively), ALT levels were within normal limits (<35 IU/L) in the pre-ramp-up and ramp-up periods (median of 11 and 10 IU/L, respectively). In the pre-ramp-up phase, ALT levels were similar for viremic and nonviremic units (median, 11 IU/L). The median ALT level was 16 IU per L in the plateau phase (maximum of 352 IU/L). Nineteen percent (61 of 319 plateau phase ALT results) of ALT results were outside normal limits (>35 IU/L),[14] with 36 percent (28 of 77 subjects) of subjects having at least one ALT result of greater than 35 IU per L during the plateau phase. There was a slight tendency for each donor's ALT and RNA levels to

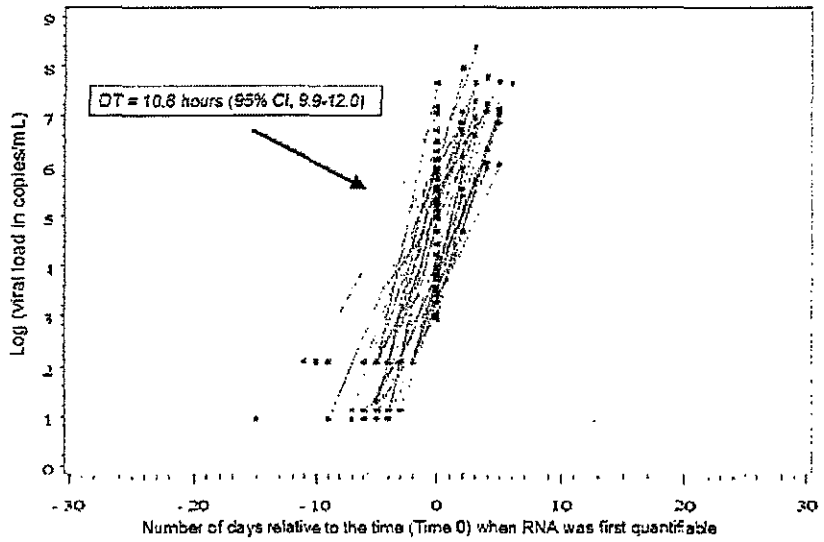

Fig. 3. Viral load in the ramp-up phase. Log viral load for the selected ramp-up bleeds linearly increased during the ramp-up phase with viral load doubling every 10.8 hours (95% CI, 9.9-12.0).

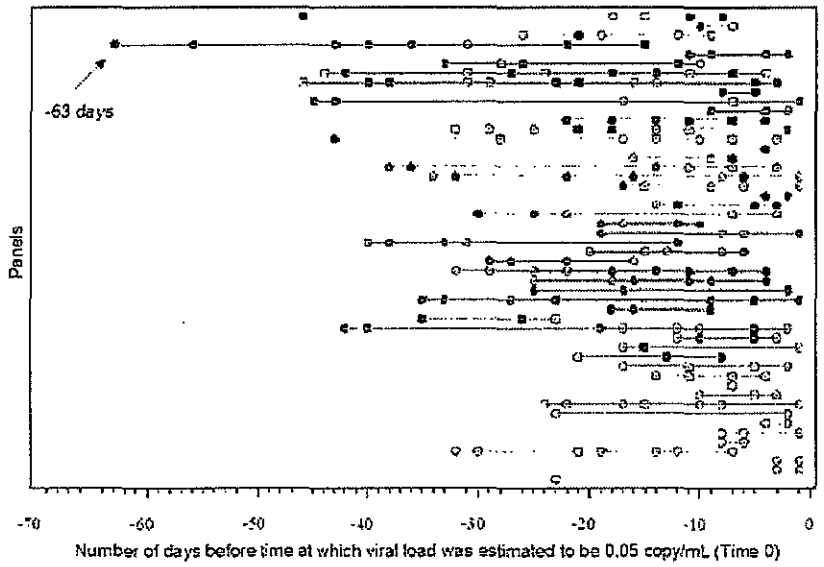

Fig. 4. TMA status of each panel's bleed(s) in the pre-ramp-up phase. Each line represents a donor and circles represent bleeds by the donor: (●) a unit that was TMA-reactive (one of four to four of four) and (○) a unit that did not react by TMA (zero of four).

increase or decrease together from visit to visit (intrasubject correlation of 0.05, $p = 0.004$). There was, however, no significant correlation between maximum ALT and RNA values in the plateau phase (intersubject correlation of 0.08, $p = 0.46$).

DISCUSSION

Our findings demonstrate that in source plasma donors with community-acquired HCV infections, intermittent low-level HCV viremia (serum RNA levels estimated at <120 copies/mL, the estimated viral copy number if four of four TMA replicates were positive—see Materials and methods and Appendix) can occur as much as 2 months before the ramp-up period. This ramp-up period (that is, period of rapid increase in circulating RNA levels) was followed by a period of high-titer viremia characterized by circulating RNA levels ranging from 4.1×10^4 to 7.2×10^7 copies per mL; the duration of this plateau phase (time from HCV RNA levels exceeding 4.0×10^4 copies/mL to EIA seroconversion) averaged 56 days. Elevations in ALT levels, if present, were primarily observed during the plateau phase and were commonly predictive of seroconversion within the next 1 or 2 weeks; however, seroconversion appeared to take place without ALT elevation in a majority of plasma donors. ALT levels did not appear to be strongly correlated with serum RNA levels, supporting the hypothesis that HCV is probably not directly hepatocytopathic.[2,3,15,16] Studies of patients who acquired hepatitis after transfusion of HCV-seropositive blood components have also found that seroconversion appears to follow a period of high-titer viremia lasting nearly 2 months (58 days),[1,2] and Thimme and colleagues[3] observed a similar phenomenon in four health-care workers evaluated within 2 weeks of an accidental needle injury. High RNA levels were usually observed within 1 to 2 weeks of transfusion or inoculation in these cases.[1,3] One previous study of plasma donor seroconversion panels also confirmed the presence of this high-titer viremia and permitted detailed evaluation of the kinetics preceding this period.[7] With data from 25 recently infected plasma donors, Nübling and colleagues[7] estimated that viral load doubled every 10.8 hours in the ramp-up phase, similar to our findings.

The period immediately after exposure and preceding ramp-up viremia, often referred to as the "eclipse" phase,