血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70165	2007/11/29	70768	日本脳炎	Epidemiol Infect 2007; 135: 974-977	2004年11月から2005年2月にかけて、日本の西部に位置する広島県の野生イノシシから血清25検体を採取した。日本脳炎ウイルス(JEV)に対する抗体検査を、IgMキャプチャー及びIgG酵素免疫測定法(ELISA)、並びにプラーク減少中和試験により行った。17検体(68%)がJEV中和抗体陽性だった。中和抗体陽性検体は全てIgG-ELISA陽性だった。1検体はIgMも陽性だった。約70%の野生イノシシが抗JEV抗体陽性であることが示され、この地域のJEV感染サイクルに関与している可能性が提示された。	51
70112	2007/09/28	70553	麻疹	asahi.com 2007年4月18日	東京都や埼玉県など関東地方ではしかが流行していることが、国立感染症研究所感染症情報センターがまとめた定点調査でわかった。例年より流行は早く、人の移動が活発になる連休に向けてさらに広がることが予想されるとして、同センターは緊急情報を出して注意を呼びかけている。同センターによると、例年、はしかの発症は乳幼児に多いが、今年の流行は10代前半や大人に多いのが特徴という。	

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 7. 18	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	Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Mizoguchi H; Japanese Red Cross NAT Screening Research Group. Transfusion. 2007 Jul;47(7):1162-71.	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) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○献血者のB型肝炎ウイルス血症及び抗原血症の期間:急性期におけるオカルト(B型肝炎表面抗原陰性)感染の予備的エビデンス

背景:日本赤十字社(JRC)は、血清学検査陰性献血者の検査のため、プールの全自動化及び核酸増幅検査(NAT)システムを導入した。JRCの保管検体と複数回献血に基づき、JRCスクリーニングシステムでB型肝炎表面抗原(HBsAg)及び抗B型肝炎コア抗原抗体が陰性であったB型肝炎ウイルス(HBV)DNA陽性献血者の遡及とフォローアップ検査を行った。

試験デザイン及び方法:2000年2月1日～2003年3月31日の期間に、半自動式多重検査システム(AMPLINAT MPX test, Roche)を用いて17,314,486本を50検体プールで検査した。当該期間に328本のHBV DNA陽性献血血液が見つかった。これらの献血者のうち26名から短期間に新たな検体を入手することができ、これにより急性HBV感染におけるウイルスマーカーの動態を調べることができた。ウイルス血症及び抗原血症の検出期間は、定量PCR法(JRC)とHBsAg酵素免疫測定法(Auszyme II, AxSYM, Abbott)、化学発光免疫測定法(Abbott)から得られた結果の回帰分析から推定した。

結果:個別NAT及び20検体ミニプール(MP) NATのHBV DNAの検出可能期間の中央値はそれぞれ74日及び50日と推定され、HBsAgの検出可能期間は42日と推定された。献血者26名中6名は変異型ウイルスに感染、この6名のうち3名はウイルス量が中等度(10^4～10^5 HBV DNA copies/mL)であったにもかかわらず、観察期間中に検出限界以上のHBsAgを発現しなかった。

結論:変異ウイルスの伝播は、急性期のオカルトHBV感染を引き起こすと考えられた。HBV NATは、MPで行ったとしても、HBsAg検査よりも効果的で、HBsAgウインドウ期前後の感染献血者を排除することができる。

報告企業の意見	今後の対応
献血時の核酸増幅検査で陽性となった献血者の検体を使用して急性HBV感染におけるウイルスマーカーの動態を調べたところ、個別NAT及び20検体ミニプール(MP) NATのHBV DNAの検出可能期間の中央値はそれぞれ74日及び50日、HBsAgの検出可能期間は42日と推定され、変異ウイルスの伝播は急性期のオカルトHBV感染を引き起こすと考えられたとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。NATの精度向上についても評価・検討している。

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」
合成血-LR「日赤」
照射合成血-LR「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

Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen–negative) infection in the acute stage

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BACKGROUND: The Japanese Red Cross (JRC) implemented a fully automated pooling and nucleic acid amplification test (NAT) system for testing seronegative donations. The JRC sample repository and repeat blood donations allowed for lookback and follow-up studies of hepatitis B virus (HBV) DNA–positive donors, who tested negative for hepatitis B surface antigen (HBsAg) and anti–hepatitis B core antigen in the JRC screening system.

STUDY DESIGN AND METHODS: From February 1, 2000, to March 31, 2003, 17,314,486 units were tested in 50-sample pools with a semiautomated multiplex assay system (AMPLINAT MPX test, Roche). During this period, 328 HBV DNA–positive donations were found. From 26 of these donors, sequential samples were available at short intervals. This enabled us to examine the dynamics of viral markers in acute HBV infection. The length of detectable periods of plasma viremia and antigenemia were estimated by regression analysis from the results obtained in the quantitative polymerase chain reaction assay (JRC) and HBsAg enzyme immunoassay (Auszyme II, AxSYM, Abbott) and chemiluminescence immunoassay (Abbott).

RESULTS: The median length of detectable HBV DNA in individual donation and 20-sample minipool (MP) NAT format was estimated to be 74 and 50 days, respectively, whereas the median length of detectable HBsAg was estimated to be 42 days. Six of the 26 donors were infected with mutant viruses, and 3 of these 6 donors did not develop detectable HBsAg during the entire observation period, despite a moderately high viral load of 10^4 to 10^5 HBV DNA copies per mL.

CONCLUSION: Transmission of mutant virus may cause occult HBV infection in the acute stage. HBV NAT, even in MP configuration, is more effective than HBsAg testing and capable of interdicting infected donors in the pre- and post-HBsAg window periods.

Studies on the early dynamics of hepatitis B viremia and antigenemia have placed an emphasis on comparing the sensitivity of hepatitis B virus (HBV) nucleic acid amplification test (NAT) methods and hepatitis surface antigen (HBsAg) assays in detecting early HBV infection with seroconversion panels.[1-4] We previously reported the dynamics of HBV DNA and HBsAg of genotypes A, B, and C in both the early increasing phase and the later decreasing phase of viremia with lookback and follow-up samples of 50 minipool (MP)-NAT–reactive donations.[5] With slight variation in the kinetics between the genotypes, a median HBV doubling time of 2.6 days was calculated in the ramp-up phase, which was the same as reported by Biswas and coworkers,[1] and the $t_{1/2}$ in the decreasing phase was 1.6 days. Regression analysis showed that the mean viral load at the HBsAg seroconversion point in chemiluminescence immunoassay (CLIA) was 2100 copies per mL.[5,6] A few HBsAg CLIA–negative donations, however, were found with relatively high viral roads between 10,000 and 100,000 copies per mL, which would be expected to be CLIA HBsAg–positive. To obtain a more accurate picture of the differences in

ABBREVIATIONS: CLIA = chemiluminescence immunoassay; HI = hemagglutination inhibition; ID = individual donation; JRC = Japanese Red Cross; MP = minipool; s/co = signal-to-cutoff.

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early dynamics of HBV DNA and HBsAg relative to anti-hepatitis B core antigen (HBc) and alanine aminotransferase (ALT) response levels in the acute stage of HBV infection, we selected 26 blood donors of whom multiple sequential samples were available at relatively close intervals. This enabled us to compare the duration of detectable HBsAg with that of HBV DNA in both MP-NAT and individual donation (ID)-NAT format. Hereby not only the pre-HBsAg window period, but also the post-HBsAg window period was taken into account. This latter "core window period" is of less concern in Japan, because since 1989 all blood donations are tested for anti-HBc, but may be relevant for other highly endemic areas of hepatitis B in Asia, where anti-HBc testing is not performed. In this study special attention is given to a few donors with a relatively high viral load that remained HBsAg-negative during the entire follow-up period to anti-HBc seroconversion.

MATERIALS AND METHODS

MP-NAT

The NAT screening system in Japan was reported previously by Mine and colleagues.[7] In brief, all blood donations in the Japanese Red Cross (JRC) were from volunteers screened with a questionnaire survey administered by JRC blood centers throughout Japan. These donations were screened by serologic tests.[5-10] Serologically positive and elevated-ALT (>60 IU/mL) samples were excluded from NAT screening. NAT screening was carried out with a 50-sample pool with a multiplex (MPX) reagent (AMPLINAT MPX, Roche Diagnostic, Tokyo, Japan) specially ordered by JRC. The 95 percent HBV DNA detection limit of the AMPLINAT MPX test system was found to be 30 (range, 22-60) copies per mL based on a plasma standard quantified with an HBV RNA viral load assay (AMPLINAT MPX, Roche)[11] and was found to be 15 IU/mL (60 copies/mL) according to validation studies with the WHO standard by JRC.

Serologic screening tests

The JRC criterion for a serologically positive result is HBsAg positivity by reverse passive hemagglutination assay (sensitivity, 2 ng/mL) in the first screening and confirmed by enzyme immunoassay (EIA; AxSYM, Abbott Laboratories, Abbott Park, IL) or a high titer of the anti-HBc ($\geq 2^5$) without the antibody to HBsAg (anti-HBs; 200 mIU/mL). The titer of total (immunoglobulin G [IgG] + immunoglobulin M [IgM]) anti-HBc was determined by hemagglutination inhibition (HI) assay.[8] ALT was measured by machinery (ACA 5200, Olympus Co., Tokyo Japan) with reagents of transaminase HR-II (Wako Co., Tokyo, Japan).

Samples for studies

A total of 328 samples, collected from February 1, 2000, to March 31, 2003, were found positive by 50-pool NAT. Of these 328 donors, 26 had sampling intervals of less than 31 days and could be followed-up or looked back for both the increasing and the decreasing phase of viral load. Donors with sampling intervals longer than 32 days were excluded from analysis, because there might be a chance the viremia phase is missed by the sampling error (the shortest interval of HBV-DNA levels above 1200 copies/mL was 26 days) (Table 2, figures in bold).

Aliquots of all blood donations were stocked from September 1996 below –30°C for 10 years in two facilities in Japan.[7] When the donations were found to be HBV DNA–positive by pool-NAT (NAT-positive), the last stocked samples of the same donor were looked back by individual NAT. Of six donors, samples were obtained at short intervals with informed consent from hospitals, which enables us to study the course of serum markers in the recovery phase.

Serologic and individual NAT analyses of pool NAT-positive, follow-up, and lookback samples

Anti-HBc IgM was detected with EIA (IMxHBc-M, Abbott Laboratories). HBsAg detection by EIA was carried out with HBsAg testing by overnight method (Auszyme II, Abbott Laboratories, until October 2002; and AxSYM HBsAg, Abbott Laboratories, was adopted thereafter). Some samples were retested by CLIA (PRISM and Architect, Abbott Laboratories). Anti-HBs levels were measured by passive hemagglutination assay (JRC in-house reagent) and EIA (AxSYM, Abbott Laboratories). Data were arranged according to the manufacturer's instructions. The qualitative and quantitative detections of HBV DNA by individual NAT were reported previously by Minegishi and coworkers.[6]

The genotypes of HBV and precore or core promoter mutations were determined and characterized according to Okamoto and coworkers.[12,13] The lengths of viremia (HBV DNA load greater than 60 or 1200 copies/mL) and antigenemia were measured directly from log-linear lines of each of the 26 figures, because the slope of ramp-up curve was peculiar to each sample as shown in Figs. 1 through 3.

Statistical analysis

For estimation of the length of HBV DNA MP-NAT– and ID-NAT–detectable periods, we had to make a number of assumptions: 1) The increase and decrease in HBV DNA viral load and HBsAg signal-to-cutoff (s/co) follows a log-linear relationship with time.[5] 2) Taking into account the present uncertainty in standardizing HBV DNA assays in

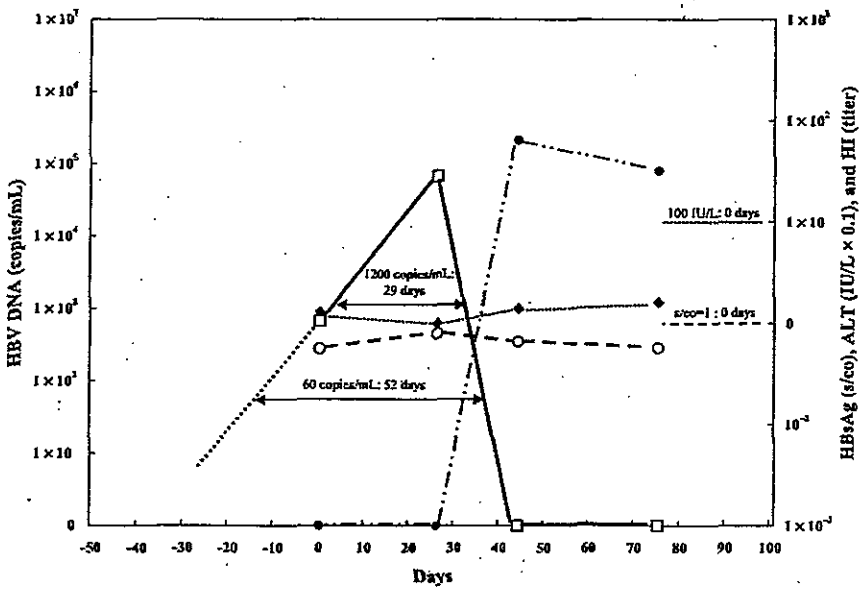

Fig. 1. Course of occult HBV infection. The viremia period with HBV DNA loads greater than 1200 copies per mL is 29 days. HBV DNA is first detected by MP-NAT on Day 0. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day 0 (straight line). Undetectable HBV DNA load was assumed 1 copy per mL (intrapolation). The viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 52 days (shown in italics). The s/co (S/N/2) value of HBsAg EIA (○; AxSYM, Abbott Laboratories) level remained below one during observation. The ALT (◆) level also remained less than 100 IU per L during observation even after anti-HBc titer (HI, ●) increased. The maximum HI titer is 2^6. The donor whose results are shown in Fig. 1 corresponds to Donor 3 in Tables 1-3.

copies per mL, we assumed a 95 percent detection limit of the AMPLINAT system in ID-NAT of approximately 60 copies per mL. For the current 20-donation MP-NAT system in use, we therefore assume a cutoff HBV DNA level of 1200 copies per mL above which HBV DNA is detected with greater than 95 percent certainty. 3) AMPLINAT-reactive donations below the quantification limit in the JRC quantitative polymerase chain reaction assay were assumed to have a viral load of 60 copies per mL. In fact, these samples could also have had a lower viral load around the 50 percent detection limit and therefore the observed periods might be longer when estimated at the approximately 60 copies per mL cutoff level (Table 2, figures in lightface text). For the HBV DNA–detectable periods that were determined by extrapolation (Table 2, figures in italics), however, the estimates of HBV DNA–detectable periods may have been shorter, because intervals could be two to three doubling times (5-8 days) or half-times (3-5 days) longer at the 50 percent AMPLINAT detection limit. 4) An AMPLINAT-negative sample in the previremic or the early recovery phase that was required for estimating the slope of the decreasing viral load was assumed to have a concentration of 1 copy per mL. For estimation of the length of periods of detectable HBV DNA above 1200 and 60 copies per mL, this is a conservative estimate, because AMPLINAT may have failed to detect higher HBV DNA levels in the range between the 50 to 95 percent detection limit (estimated, 10-60 copies/mL). If this was the case, HBV DNA levels would increase or decrease slower and the HBV DNA–detectable period above 60 copies per mL would be estimated to be shorter. The U test and Yates chi-square test were used for statistical analysis.

RESULTS

Of 328 NAT-positive donations, 26 were from donors who could be followed-up in both increasing and decreasing phases of HBV DNA load. The data of the 26 donors including genotype, subtype, mutation, sex, age, sample numbers, and period from ID-NAT–positive (>60 copies/mL) to anti-HBs seroconversion are shown in Table 1. The lengths of the viremia and antigenemia, peak HBV loads, ALT levels, HI titers (total IgG + IgM anti-HBc), and periods from pool NAT-positive (>1200 copies/mL) day to the HBV DNA load peak and the ALT level or HI titer peak are shown in Table 2. Data shown in Tables 1 and 2 are arranged according to the lengths of the HBsAg EIA–positive period. There are data sets of different quality in Table 2 (data in bold, lightface, and italics) as shown in the footnote of Table 2. For statistical comparison between HBV DNA– and HBsAg-detectable periods we take those donors into account that have bold figures for both HBsAg and HBV-DNA (Donors 5, 10, 15, 19, 21, and 24). The length of viremia (>1200 copies/mL; MP-NAT) was longer than that of antigenemia in Donors 5, 10, and 15 and length of antigenemia was longer than that of viremia in Donors 19, 21, and 24. Peak HBV DNA loads of Donors 19, 21, and 24 were higher than those of Donors 5, 10, and 15 (U test, $p < 0.05$). Median length of viremia (>1200 copies/mL) was 62 days (range, 26-99 days) and that of antigenemia was 56 days (range, 23-129 days). When we take into account donors with bold and regular printed data ($n = 15$; intrapolation of assumption, <100 copies/mL: 60 copies/mL, undetectable: 1 copy/mL), 10 donors had longer viremia (>1200 copies/mL) than antigenemia periods, one had same period and four had longer antigenemia than viremia (>1200 copies/mL). Median length of viremia (>1200 copies/mL) was 58 days (range,

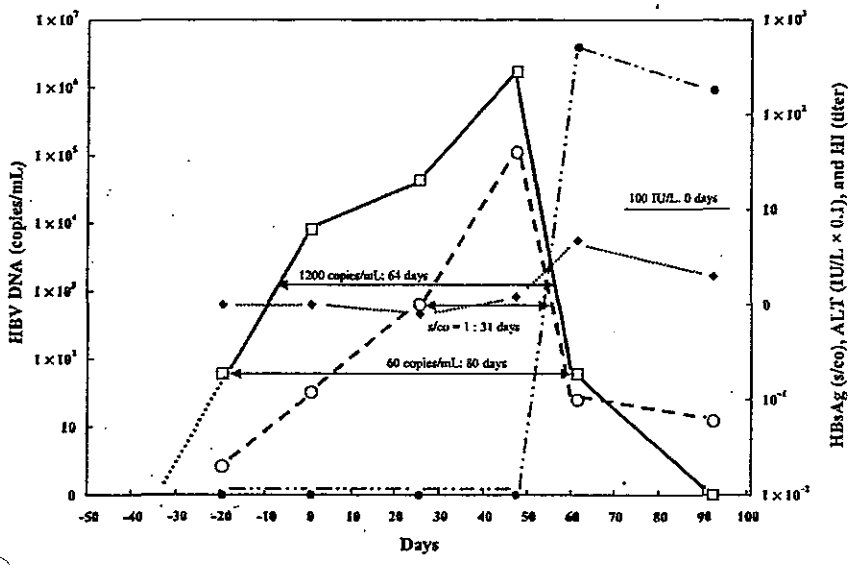

Fig. 2. Course of a case that ALT levels remained less than 100 IU per L. The viremia period with HBV DNA loads greater than 1200 copies per mL is 64 days. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day –20 (straight line). HBV DNA load below 100 copies per mL was assumed 60 copies per mL. The viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 80 days. The HBsAg EIA (○, Auszyme II, Abbott Laboratories)-positive period is 31 days. The s/co datum of Day 47 is greater than 40. Maximum HI titer is 2^9. The donor whose results are shown in Fig. 2 corresponds to Donor 10 in Tables 1 through 3. (◆) ALT; (●) HI.

26-108 days) and that of antigenemia was 48 days (range, 23-129 days). When we compare the detectable length between viremia and antigenemia of all data (n = 26) including italic (slopes are based on assumption of timing of viremia peak and estimates are obtained by extrapolation), median length of viremia (>1200 copies/mL) was 50 days (range, 23-116 days), and that of antigenemia was 42 days (range, 0-175 days; U test, not significant). Eight donors had longer HBsAg-detectable period than viremia period above 1200 copies per mL. In contrast, median length of viremia above 60 copies per mL (ID-NAT; most of them were italic because of the estimation by extrapolation) was 74 days (range, 43-213 days) and all donors had longer viremia period than HBsAg-detectable period (U test, $p < 0.01$).

HBsAg was not detected during observation in three donors: "occult" HBV infection (Donors 1-3 in Tables 1-3). One of such donors (Donor 3) is shown in Fig. 1. The three donors showed peak HBV DNA loads of less than 10^5 copies per mL and peak ALT levels less than 100 IU per L. The data of these three cases are as follows: genotype C, subtype adr, and precore or core promoter mutants (Tables 1-3 and Fig. 4). The maximum viral loads of Donors 4, 5, and 8 were less than 10^5 copies per mL (8,200-80,000 copies/mL) but periods of detectable HBsAg were 11 to 28 days, although the maximum viral loads of Donors 1 through 3 were 20,000 to 68,000 copies per mL and HBsAg was undetectable (Tables 2 and 3). To examine the difference, we compared the DNA sequences of core promoter and precore region (nucleotides 1699-1780 and 1885-1919) and surface region (nucleotides 535-594) between Donors 1 to 5 and 8 (Fig. 4). The mutations in core-promoter region (nucleotides 1762 and 1764; Donor 2) and precore region (pC-28; Donors 1 and 3) were observed in "occult" cases. No difference was observed in sites of escape mutations except for S-129 (Q129N) of Donor 1. Because Donor 1 had no clear evidence of anti-HBc (HI:2^5, anti-HBc[IgM]:negative) and anti-HBs seroconversion, we could not exclude the possibility that Donor 1 was a late-stage typical occult carrier. Donors 2 and 3, however, showed clear seroconversion to anti-HBc(IgM) and anti-HBs and could be classified as "occult HBV infections in acute stage" because the viral loads reached levels of 29,000 and 68,000 copies/mL, whereas HBsAg response remained at or below cutoff level (Table 3).

Although we had already reported that the precore and core promoter mutations affect the doubling time of HBV DNA loads,[5] the effect of the mutations on HBsAg production is unclear. When comparing peak viral loads and HBsAg detection in mutant and wild-type infections, there were significant differences between them (peak HBV DNA loads, $p < 0.05$; length of HBsAg detection periods, $p < 0.01$; U test). The nucleic acids from nucleotide 1374 to nucleotide 1838 encode X protein. Mutations at nucleotide 1719 and nucleotide 1721 change the amino acids from leucine to valine (x-116), nucleotide 1762 change the amino acid from lysine to methionine (x-130), and nucleotide 1764 change the amino acid from valine to isoleucine (x-131). Though the function of X protein has not been established, mutations of X-protein might affect the production of HBsAg.[14] Mutations in core promoter or precore, however, were found in Donors 7, 11, and 13 whose HBsAg detectable periods were 28 to 40 days. We should compare the sequence of the other regions between Donors 1 to 5, 7, 8, 11, and 13.

The peak ALT level was maintained below 100 IU per L in 12 donors (Table 2). One of these 12 cases is shown in Fig. 2 (Donor 10). About 5 donors with the shortest intervals (Donors 19, 21, 22, 24, and 26; Table 2), course of markers (ALT/anti-HBc vs. HBV-DNA timing analysis) were compared. The peak viral load preceded the peak

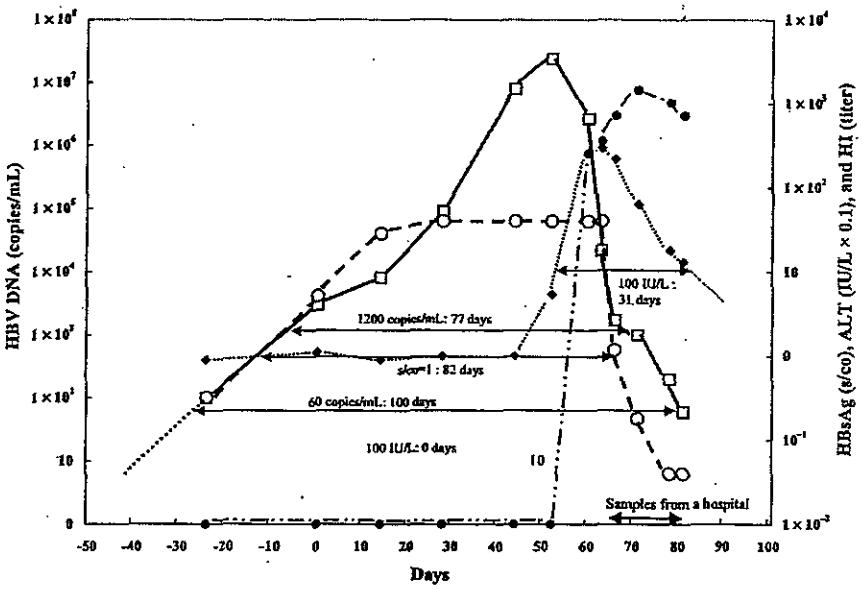

Fig. 3. Course of a symptomatic HBV infection. The viremia period with HBV DNA loads greater than 1200 copies per mL is 77 days. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day –24 (straight line). HBV DNA load below 100 copies per mL was assumed to be 60 copies per mL. The length of viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 110 days (shown in italics). The length of the HBsAg EIA (○; Auszyme II, Abbott Laboratories)-positive period is 82 days, longer than the viremia period with HBV DNA loads greater than 1200 copies per mL (77 days). The s/co data on Days 29, 45, 53, 61, and 64 are greater than 40. The ALT (◆) level on Day 63 is 2973 IU per L. The estimated line of ALT is drawn (thick dotted line) from the log-linear line (thin dotted line) on Day 81. The symptomatic period with ALT levels more than 100 IU per L is estimated to be 31 days (shown in italics). Maximum HI (●) titer is $2^{10.5}$. After Day 63, samples were obtained from a hospital with informed consent. The donor whose results are shown in Fig. 3 corresponds to Donor 21 in Tables 1 through 3.

ALT and anti-HBc level with approximately 2 weeks (ALT, 14, 11, 12, 23, and 13 days; anti-HBc, 14, 19, 12, 16, and 18 days; Table 2, bold). The case of Donor 21 is shown in Fig. 3. The lengths from individual NAT-positive (>60 copies/mL) day to anti-HBs seroconversion day (>10 mIU/mL) could be measured in 17 donors, ranges from 33 to 188 days (Table 1).

DISCUSSION

The JRC has established a sample repository of all donations, which enables doing valuable lookback studies on donors that were found AMPLINAT reactive in the JRC MP screening system. In a previous report, we have already described the dynamics of viremia in donors that were identified by the JRC MP-AMPLINAT screening system and of whom follow-up samples were available in either the increasing phase or the decreasing phase of viremia.[5] The data, however, did not allow for accurate timing of the peak of viremia in most of the donors and therefore the total length of the AMPLINAT-detectable period could not be reliably established. From a blood safety perspective comparison of the total lengths of the periods of detectable HBsAg and HBV DNA in either MP- or ID-NAT format is of interest. To be able to estimate these periods we selected 26 donors of whom samples were available in both the increasing and decreasing phase of viremia and whose sampling intervals were less than 1 month (the shortest time of detectable viremia found in our donor follow-up studies). The follow-up data were used to estimate the time periods where the viral load was higher than 1200 copies per mL (the estimated 95% detection limit of the current 20-donation MP-AMPLINAT system of JRC) and above 60 copies per mL (the estimated 95% detection limit of AMPLINAT in ID-NAT format). A similar calculation method was followed to estimate HBsAg-detectable periods based on the assumption that HBsAg s/co values follow a similar log-linear relationship with time in the ramp-up phase and recovery phase as assumed for HBV DNA donors (which assumption may be not totally correct). When we based our calculations on the most reliable data of the subset of six donors, the median MP-NAT–detectable period (>1200 copies/mL) was 62 (range, 26-109) days, whereas the ID-NAT–detectable period (>60 copies/mL; not so reliable data as ID-NAT) was 84 (range, 45-155) days. These periods were longer than the median HBsAg-detectable periods, which in the same donors was found to be 56 (range, 23-129) days. A similar result was found when all data were taken into account including the calculated periods based on the subset of donors in whom the dynamics of viremia were based on assumptions of viral load in some of the samples (see Results, Table 2).

Interestingly, we found 3 of the 26 donors (11.5%) who during the entire course of acute infection never developed HBsAg levels above the cut of CLIA and EIA, whereas HBV-DNA levels in these 3 donors reached levels as high as 20,000, 29,000, and 68,000 copies per mL. We are tempted to classify these cases as occult HBV infection in the acute phase, because normally the HBsAg CLIA assay becomes detectable in acute infection when HBV DNA reaches a concentration above approximately 2,000 copies per mL.[1,3,6,4] So far, occult HBV infection has

TABLE 1. Data of 26 HBV NAT–positive donors

Donor	Genotype	Subtype	Wild/ mutant*	Age (years)	Sex	Number of serial samples		Observation periods§ (days)	Sero-conversion‖ (days)
						Lookback†	Follow-up‡		
1	C	adr	M(C)¶	55	Male	0	5	105	—
2	C	adr	M (pro)	20	Female	1	3	92	62
3	C	adr	M (C)	48	Female	0	4	75	75
4	C	adr	W	24	Female	0	5	59	59
5	C	adr	W	23	Female	0	4	59	59
6	C	adw	W	21	Female	0	4	55	55
7	C	adr	M (C)	19	Female	0	3	33	—
8	C	adr	W	24	Female	1	4	99	33
9	C	adr	W	25	Male	2	3	91	75
10	C	adr	W	24	Female	1	5	112	112
11	C	adr	M (C&pro)	24	Female	0	3	44	85**
12	C	adr	W	26	Female	1	3	75	75
13	C	adw	M (pro)	36	Female	0	4	43	43
14	A	adw	W	31	Male	0	3	56	56
15	C	adr	W	24	Male	0	4	56	—
16	C	adr	W	18	Female	0	4	52	—
17	C	adr	W	26	Male	0	4	74	74
18	C	adr	W	29	Male	0	5	105	—
19	C	adr	W	25	Female	0	13	231	188
20	C	adr	W	47	Male	0	5	103	—
21	C	adr	W	23	Female	1	11	105	145**
22	B	adw	W	23	Female	0	21	134	186**
23	C	adr	W	22	Male	0	7	145	—
24	C	adr	W	27	Male	2	14	149	177**
25	B	adw	W	30	Male	0	5	105	—
26	C	adr	W	31	Male	0	20	224	—

* (C) = precore mutation; (pro) = core-promoter mutation.
† Number of looked-back (stored) samples.
‡ Number of followed-up samples including 50 pool NAT-positive sample.
§ Intervals of blood collection are less than 31 days. Looked-back (individual NAT negative) days were included.
‖ Length from individual NAT-positive day to HBsAb seroconversion (more than 10 mIU/mL) days. HBsAb levels (10 mIU/mL) were determined by EIA (AxSYM, Abbott Laboratories). — = HBsAb (more than 10 mIU/mL) was not detected during observation except for Donor 1. Anti-HBs of Donor 1 could not be tested by AxSYM (Abbott) as shown in Table 3.
¶ In addition to precore mutation, a mutation of surface region (Q129N) was observed as shown in Fig. 4.
** Some donors come to check their HBsAb seroconversion and disappearance of HBsAg after a long interval.

been defined as HBsAg-negative, but persistent HBV DNA–positive infection in a late stage, either tail end chronic carriers who over a prolonged period of time no longer produce detectable HBsAg or recovered individuals with recurrent virus replication after anti-HBs levels, have dropped to low titers.[15,16,17] The HBV DNA–positive, but HBsAg-negative infections found in the pre- and post-HBsAg window phases during acute infection (as shown in this study) should of course not be confused with the definition of occult infection. This is the normal pattern of acute infection that we have observed in 20 donors infected with wild-type virus in this study (Tables 1 and 2). In 6 donors, however, we have been able to identify core promoter or precore mutations that typically develop over time in chronic infection and are associated with seroconversion from HBeAg to anti-HBe and with lower HBV DNA and HBsAg levels.[18] We already reported that precore mutations were associated with longer viral doubling times in our acutely infected donors.[5] Most likely, these mutations already had developed in chronic carriers of the HBV genotype C strains that were transmitted to these donors. In the present study, 3 of 6 donors infected with mutant virus did not produce detectable HBsAg, whereas all 20 donors infected with wild-type virus did (Yates chi-square test, $p < 0.01$).

Therefore, the presence of core promotor and precore mutations in the transmitted virus may have effects on the HBV DNA and HBsAg expression in the recipient and be associated with a higher risk of an occult (HBsAg-negative) course or fulminant hepatitis[19-21] in the acute stage. Infection with these mutations, however, is not predictive for an occult course in the acute phase, because we saw three donors infected with mutant virus (Donors 7, 11, and 13) that developed detectable HBsAg for an estimated period of 28 to 40 days. So it may be that also host factors play a role or it may be that mutations in other regions of the genome (e.g., pre-S region) account for defect in HBsAg expression. To examine this we have to sequence other regions of the genome.

In one of the three donors with occult HBV infection (Donor 1), we also were able to identify a mutation in the S region of the genome (Q129N) at an amino acid