Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T, Miyakawa Y, Tabuchi A, Tanaka J, Yoshizawa H.	Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C.	Transfusion	48(2)	286-294	2008
小方則夫	B型肝炎ウイル（hepatitis B virus: HBV）感染症対策：日本において接種可能（であった）4種HBワクチンのHBs抗体誘導能の凝集法（passive hemagglutination assay）評価と国際単位表示法（chemiluminescentimmunoassay）評価との比較検討.	臨床病理	第57巻第10号	954 - 960	2009
Kusakabe A, Tanaka Y, Mochida S, Nakayama N, Inoue K, Sata M, Isoda N, Kang JH, Sumino Y, Yatsuhashi H, Takikawa Y, Kaneko S, Yamada G, Karino Y, Tanaka E, Kato J, Sakaida I, Izumi N, Sugauchi F, Nojiri S, Joh T, Miyakawa Y and Mizokami M.	A Case-Control Study for the Identification of Virological Factors associated with Fulminant Hepatitis B.	Hepato Res	39	648-56	2009
Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, Kuramitsu T, Kawata S, Tanaka E, Izumi N, Okuse C, Kakumu S, T, Hino K, Hiasa Y, Sata M, Maeshiro T, Sugauchi F, Nojiri S, Joh T, Miyakawa Y, and Mizokami M.	Distribution of Hepatitis B Virus Genotypes among Patients with Chronic Infection in Japan Shifting toward an Increase of Genotype A.	J Clin Microbiol	47	1476-83	2009

Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M.	Genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J.	J Virol.	83	10538-47	2009
惠谷ゆり	日本で接種できる任意接種のワクチン：B 型肝炎ワクチン	チャイルドヘルス特集	第 1 巻	31-33	2010

生物学的製剤基準 2007 年版

pp２１１−２１６

「抗 HBs 人免疫グロブリン、乾燥抗 HBs 人免疫グロブリン、ポリエチレングリコール処理抗 HBs 人免疫グロブリン、乾燥ポリエチレングリコール処理抗 HBs 人免疫グロブリン」

小高　千加子

抗 HBs 人免疫グロブリン

概　説

【疾患について】　B 型肝炎（Hepatitis B）は，ウイルス肝炎の一種である B 型肝炎ウイルス（Hepatitis B Virus：HBV）の感染によって起こる．HBV は主として感染しているヒトの血液を介して感染する．また感染している血液が混入したヒトの体液などを介して感染することがある．

HBV の一過性感染を受けた人の多くは自覚症状がないまま治癒し（不顕性の感染），一部の人が急性肝炎を発症する（顕性感染）．また急性肝炎を発症した場合，まれに劇症化することがある．HBV の持続感染者（HBV キャリア）のうち約 10～15％が慢性肝炎を発症する（慢性 B 型肝炎）．慢性 B 型肝炎を発症した場合，放置すると，気がつかないうちに肝硬変，肝がんへ進展することがあるので注意が必要である．

【疾患の動向】　B 型肝炎ウイルスに感染しても，時間の経過とともに自然に体内から排除される例もしばしば見受けられる．一方，ウイルスが排除されずにキャリアとなる例があるが，これは，出生時や乳幼児期に感染した場合が多いとされており，わが国には 120～140 万人のキャリアがいると推定されている．B 型肝炎ウイルスのキャリアの率は，40 歳代以上では，どの年代でも人口の 1～2％であり大きな差はないが，30 歳代以下では，1％未満と少なく，特に制度として母子感染対策を開始した 1986 年以降に生まれた世代での新たなキャリアの発生は少ない．

【製剤について】　抗 HBs 人免疫グロブリンは，HBs 抗原陽性血液の汚染事故後の B 型肝炎や新生児の B 型肝炎の発症予防を目的として開発された特殊免疫グロブリン製剤である．

B 型肝炎（Hepatitis B）と免疫グロブリン（Immunoglobulin）のそれぞれの略語をつらねて HBIG と略称されることがある．当初は液剤の本剤のみであったが，のちに乾燥製剤である「乾燥抗 HBs 人免疫グロブリン」も承認された．

また，現在の製造工程ではヒトパルボウイルス B 19 等のウイルスを完全に不活化・除去することは困難であり，感染症伝播の危険性を完全に排除することはできない．

【適応症】

1. HBs 抗原陽性血液汚染事故後の B 型肝炎発症予防
   汚染血中の B 型肝炎ウイルス（HBV）は肝細胞で増殖するが，早期（48 時間以内，遅くとも 1 週間以内）に本剤を投与すると，血中 HBV は肝細胞に取り込まれる前に抗 HBs 抗体で中和処理される．
2. HBs 抗原陽性の母親から生まれた新生児の B 型肝炎予防（原則として，沈降 B 型肝炎ワクチンとの併用）．

用法：筋肉内注射のみに使用すること．決して，静脈内には注射してはならない．

今回の改正のポイント

1．「適応症」の記述を変更修正．
2．2.1 原血漿の一部記述の変更．
3．2.3 最終バルク及び小分について変更修正．
4．3.8 力価試験について変更修正．

解　説

2　製法：2.1　原血漿

血液製剤総則3により，HBs抗原陽性の血液は血液製剤の原材料として使用してはならないと規定されている．HBs抗原には4つのサブタイプ（adw，adr，ayw，ayr）があることが知られている．このサブタイプの分布は地域によって異なっているが，現在わが国ではadw，adrがほとんどを占めている．抗HBs人免疫グロブリンには，どのタイプにも感染防御に重要な共通抗原aに対する抗体が含まれているので，他のサブタイプのHBウイルス感染に対しても予防効果があると考えられている．

2.3　最終バルク及び小分

原画分に安定剤，等張化剤などを含む液を加えて，最終バルクを作り，分注する．この際，1mL中の抗HBs抗体価を200単位以上になるようにする（3.8 力価試験の項参照）．製剤中のたん白質濃度についての規定はない．適当な保存剤を用いることができる．保存剤の規定は人免疫グロブリンと同じである（「人免疫グロブリン」2.3 最終バルク及び小分の項（p 191）参照）．

3　小分製品の試験

保存剤を使用しない場合は，3.2を除く．

3.8　力価試験

一般試験法A「抗HBs抗体価測定法」（p 251）参照．

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乾燥抗 HBs 人免疫グロブリン

概　説

前述の抗 HBs 人免疫グロブリン（液剤）の凍結乾燥製剤である本剤は，保存剤を加えることなく製造でき，しかも有効期間が液剤の2年に対して本剤では5年と長く，長期間保存可能な製剤である．その他の点は，「抗 HBs 人免疫グロブリン」（p 211）を参照．

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ポリエチレングリコール処理抗HBs人免疫グロブリン

概　説

ポリエチレングリコール処理抗HBs人免疫グロブリンは，静注用の製剤である．

また，現在の製造工程ではヒトパルボウイルスB19等のウイルスを完全に不活化・除去することは困難であり，感染症伝播の危険性を完全に排除することはできない．

【適応症】　HBs抗原陽性血液汚染事故後のB型肝炎発症予防．

用法：本剤は直接静注するか，又は日本薬局方生理食塩液など中性に近い補液に混じて，点滴注射する．直接静注する場合は，極めて徐々に行う．

今回の改正のポイント

1．「適応症」について記載．
2．2.2 原画分について記載．
3．2.3 最終バルク及び小分について記載．
4．3.3 免疫グロブリンG重合物否定試験について記載．
5．3.8 力価試験について記載．

解　説

1　本質及び性状

本剤は，ヒトのポリエチレングリコール処理免疫グロブリンG中の「抗HBs抗体」を含む液剤である．

2　製法：2.1　原血漿

「抗HBs人免疫グロブリン」2.1項（p 212）参照．

2.2　原画分

免疫抗体を変質させることなく，更に肝炎ウイルスその他の病原微生物を除去できる方法によって原血漿を画分し，免疫グロブリンG画分を集める．得られた画分を更にポリエチレングリコールを用いて分画し，静注可能な原画分とする．

2.3　最終バルク及び小分

「人免疫グロブリン」2.3項（p 191）参照．この際，1mL中の抗HBs抗体価を200単位以上になるようにする（一般試験法A「抗HBs抗体価測定法」（p 251）参照）．

3　小分製品の試験：3.3　免疫グロブリンG重合物否定試験

「ポリエチレングリコール処理人免疫グロブリン」3.3項参照．

3.8　力価試験

一般試験法 A「抗 HBs 抗体価測定法」（p 251）参照.

[国立感染症研究所 血液・安全性研究部：小高 千加子]

乾燥ポリエチレングリコール処理抗HBs人免疫グロブリン

概　説

本剤は，ヒトのポリエチレングリコール処理免疫グロブリンG中の「抗HBs抗体」を含む乾燥製剤であり，前述の「ポリエチレングリコール処理抗HBs人免疫グロブリン」の解説を参照されたい．

過去10年に遡って，製剤として製造されていない．

[国立感染症研究所 血液・安全性研究部：小高 千加子]

Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C

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BACKGROUND: In planning optimal hepatitis B virus (HBV) blood screening strategies, the minimum infectious dose and early dynamics of HBV need to be determined for defining the window period for HBV DNA as well as for hepatitis B surface antigen (HBsAg).

STUDY DESIGN AND METHODS: Pairs of chimpanzees were inoculated with preacute-phase inocula containing HBV of genotype A or genotype C to determine the minimum infectious dose, and two pairs of chimps infected with the lowest infectious dose of genotypes A and C were followed for HBV markers.

RESULTS: The minimum 50 percent chimpanzee infectious dose (CID_{50}) was estimated to be approximately 10 copies for genotype A and for genotype C. In the two chimps inoculated with the lowest infectious dose, the HBV DNA window was 55 to 76 days for genotype A and 35 to 50 days for genotype C, respectively. The HBsAg window was 69 to 97 days for genotype A and 50 to 64 days for genotype C, respectively. The doubling times of HBV DNA were 3.4 days (95% confidence interval [CI], 2.6-4.9 days) for genotype A and 1.9 days (95% CI, 1.6-2.3 days) for genotype C. When comparing the replication velocity of HBV DNA between the two genotypes, the doubling time of genotype C was significantly shorter than that of HBV genotype A ($p < 0.01$).

CONCLUSION: Although the CID_{50} of approximately 10 copies was similar for the two HBV genotypes, the doubling time and pre-HBV nucleic acid amplification technology (<100 copies/mL) window period in chimps infected with the lowest infectious dose seemed to be shorter for genotype C than for genotype A.

Posttransfusion infection with hepatitis B virus (HBV) has decreased dramatically since screening for hepatitis B surface antigen (HBsAg) was introduced in the early 1970s. The number of reported posttransfusion hepatitis B cases has been further reduced after screening for antibody to HBV core (anti-HBc) was implemented in the late 1980s in the United States and Japan.[1,2] Japan introduced HBV DNA screening by nucleic acid amplification technology (NAT) in minipools (MPs) in 1999. Since introduction of MP-NAT, more than 500 seronegative donations with detectable HBV DNA have been interdicted, although there are still units of blood in an early or late phase of HBV infection

ABBREVIATIONS: CID_{50} = 50 percent chimpanzee infectious dose; CLIA = chemiluminescent immunoassay; JRC = Japanese Red Cross; MP(s) = minipool(s).

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TRANSFUSION **;**:**-**.

with low viral load that can escape detection by NAT.[3] Interestingly, the lookback program of the Japanese Red Cross (JRC) demonstrated that low viral load donations in the window phase were more than 10-fold more often implicated in HBV transmission reports than were occult carriers with anti-HBc titers below the exclusion limit of the anti-HBc hemagglutination inhibition screening assay.[3] Bearing in mind the relatively high infectivity of HBV in the window phase, exact knowledge on early dynamics of HBV replication is important for residual risk estimations.[4-7] It will determine the threshold of NAT in identifying blood donors during the preacute phase of HBV infection, which is important for planning and executing evidence-based hepatitis B blood screening strategies.[7,8] In this context, the relative infectivity of HBV in the early window phase is an important factor for measuring the effect of NAT screening systems on the residual risk of HBV transmission by blood transfusion.[7-9]

Chimpanzees are the only experimental animals susceptible to human hepatitis viruses and have been very useful in transmission studies.[10] As early as the mid-1970s, it was demonstrated that blood units from HBV carriers, especially those positive for the presence of hepatitis B e antigen (HBeAg), have a tremendously high infectious potential and can transmit infection to chimps by intravenous inoculation with 1 mL of plasma diluted to $1:10^8$.[11] Now that NAT enables detection of HBV DNA in blood donors even in individual-donation format, it can be estimated by mathematical modeling what the residual risk would be depending on the minimum copy number required for infection.[9] More conservative risk modeling assumes that a single copy of HBV, if it successfully reaches a hepatocyte in susceptible hosts, may be enough to establish infection.[5] To pursue strategies for preventing HBV infections by blood transfusions, additional information on the infectivity of HBV is crucially required. It is imperative to define not only the minimum copy number of HBV DNA or number of virions required for transmission of HBV, but also the early dynamics of HBV replication in the circulation of infected hosts. This can be established more accurately in chimpanzee experiments. In this report on experimental transmission of hepatitis B in chimps, the minimum infectious dose was determined separately for HBV of genotypes A and C, and the copy number of HBV DNA for establishing infection was defined for each of them. Moreover, the doubling time and logarithmic time of HBV DNA were determined by following the viral dynamics in the preacute phase of chimpanzees who had received the minimum infectious dose of HBV.

MATERIALS AND METHODS

Chimpanzees

Six chimps entered this study. Their age, sex, and weight, as well as the HBV inocula that they received are listed in Table 1 along with the infection outcome. Every chimp was kept in an individual cage and received humane care in accordance with all relevant requirements for the use of primates in an approved institution. None of the six chimps had serologic or molecular biologic evidence of past or present HBV infection prior to the inoculation. They were also not infected with hepatitis C virus (HCV) and human immunodeficiency virus type-1. Chimps were inoculated intravenously while they were under anesthesia by intramuscular injection with ketamine hydrochloride. After the inoculation, serum samples were obtained once a week or more frequently as required, until 16 weeks or longer. They were tested for HBV DNA, HBsAg, anti-HBc, anti-HBs, and alanine aminotransferase as well as aspartate aminotransferase.

Inocula containing HBV

The chimpanzees received four kinds of inocula (Table 1). Inocula I and III were fresh-frozen plasma (FFP) units from blood donors acutely infected with HBV genotypes A and C, respectively. Inocula II and IV were plasma samples from chimps infected with inoculum I of genotype A and inoculum III of genotype C, respectively (see Figs. 1A and 1B). Inocula II and IV were: 1) recovered in the preacute phase of HBV infection before immune responses of the host had developed; 2) positive for the presence of HBV DNA in the highest titer before anti-HBc developed; and 3) taken with utmost care to maintain the infectious activity and avoid attenuation during serial processing from blood collection until storage. Immediately after blood drawing

TABLE 1. Six chimpanzees and HBV inocula and HBV infection outcomes

Chimpanzee	Age, sex, weight	HBV DNA copies	Outcome
Inoculum I: FFP from a human donor in the preacute phase of HBV infection of genotype A			
1 Chimp 246	13 years, male, 60.7 kg	1 mL (6.9×10^4 copies/mL)	Infected
Inoculum II: Preacute-phase plasma of Chimp 246 containing HBV (2.6×10^6 copies/mL)			
2 Chimp 272	9 years, male, 58.7 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	8 years, male, 51.4 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
4 Chimp 280	8 years, male, 39.4 kg	1 mL ($1:10^5$ dilution)	Infected
Inoculum III: FFP from a human donor in the preacute phase of HBV infection of genotype C			
2 Chimp 272	Reinoculation	5 mL (5.3×10^5 copies/mL)	Infected
Inoculum IV: Preacute-phase plasma of Chimp-272 containing HBV (3.0×10^6 copies/mL)			
5 Chimp 269	11 years, male, 62.5 kg	1 mL ($1:10^6$ dilution)	Not infected
6 Chimp 285	7 years, male, 41.1 kg	1 mL ($1:10^6$ dilution)	Not infected
5 Chimp 269	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
6 Chimp 285	Reinoculation	1 mL ($1:10^5$ dilution)	Infected

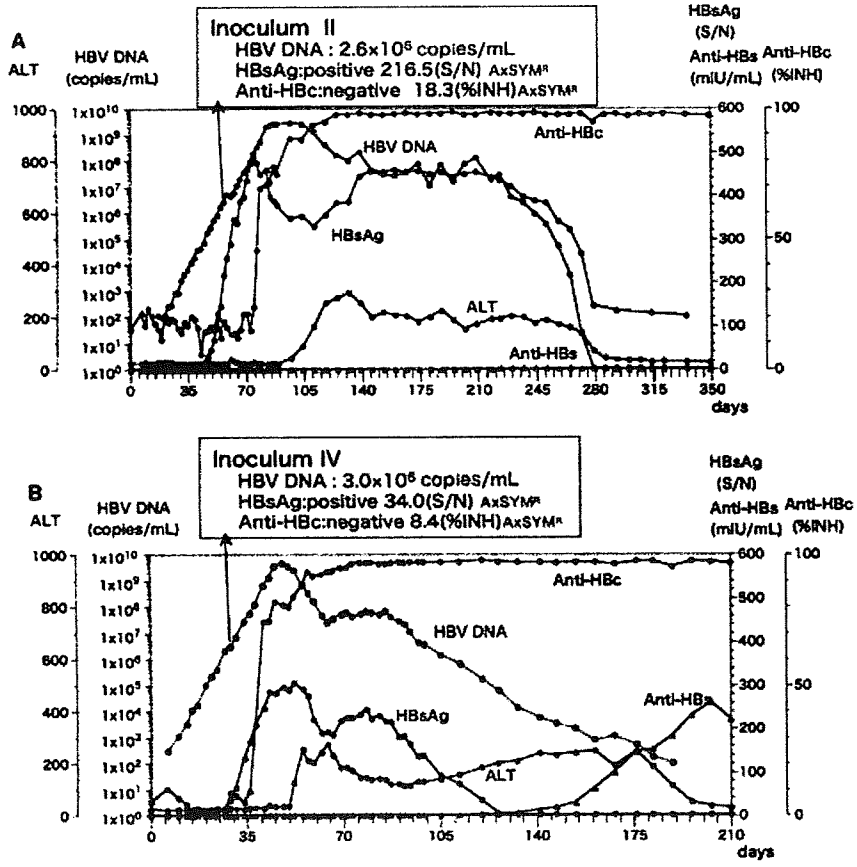

Fig. 1. Time course of HBV serum markers in chimps used as a source of inoculation of HBV genotype A and genotype C. (A) Chimp 246 was inoculated with human plasma of HBV genotype A. Inoculum II for chimp infectivity studies was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 2.6×10^6 copies per mL and the HBsAg response had increased to a signal-to-noise (S/N) ratio of 216.5 (cutoff S/N = 2.0). (B) Chimp 272 was inoculated with human plasma of HBV genotype C. Inoculum IV was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 3.0×10^6 copies per mL and HBsAg had increased to an S/N ratio of 34.0.

from chimps, plasma samples were separated. They were divided into 15 tubes in 1-mL aliquots, snap-frozen in liquid nitrogen, and kept in a deep freezer at –80°C until used for transmission experiments. For each experiment, the plasma in one tube was thawed gently by immersing it in a water bath at 37°C, and the required amounts were used.

Laboratory tests

HBsAg, anti-HBc, and antibody to HBsAg (anti-HBs) were determined by chemiluminescent immunoassay (CLIA) with commercially available kits (AxSYM, Abbott Japan, KK, Tokyo, Japan), with the index of 2.0 (signal-to-noise [S/N]), 50 percent inhibition, and 5.0 mIU per mL as cutoff values, respectively. Qualitative assay for HBV DNA was performed by polymerase chain reaction (PCR) with primers deduced from the S region of HBV DNA.[12] HBV DNA was quantitated by the PCR (TaqMan, Roche Diagnostics KK, Tokyo, Japan) with a sensitivity of 100 copies per mL. Quantitative assays for HBV DNA were performed simultaneously for an accurate comparison of data.

Calculation for doubling time and logarithmic time of HBV DNA

To calculate the doubling time and the logarithmic time (time for reaching 10 times the amount) of HBV DNA at ramp-up after the infection, HBV DNA copy numbers were evaluated statistically by log linear regression analysis. The comparison of regression slope (the doubling time and the logarithmic time) between HBV genotypes was evaluated by growth curve analysis (Vonesh-Carter-Ohtaki method).[13,14]

RESULTS

Inocula and copy numbers of HBV genotype A or genotype C recovered from chimpanzees in the preacute phase of infection

Chimp 246 was injected intravenously with 1 mL of FFP from a blood donor in the preacute phase of HBV infection (Table 1); the donor had been screened by NAT at a JRC Blood Center. His plasma sample contained 6.9×10^4 copies per mL of HBV DNA genotype A and was positive for the presence of HBsAg but negative for the presence of anti-HBc (inoculum I). Plasma was harvested from Chimp 246, in the preacute phase of HBV infection 57 days after inoculation (inoculum II). It contained 2.6×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1A).

Likewise, Chimp 272 was injected intravenously with 5 mL of FFP from a blood donor in the preacute phase of HBV infection who had been screened by NAT at JRC (Table 1). It contained 5.3×10^5 copies per mL of HBV DNA genotype C and was positive for the presence of HBsAg but negative for the presence of anti-HBc and anti-HBs (inoculum III). Thus, Chimp 272 was inoculated with

2.7×10^6 copies of HBV genotype C. The preacute plasma sample was collected from Chimp 272 29 days after challenge (inoculum IV). It contained 3.0×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1B).

Estimates of HBV DNA copy numbers in serial 1-in-10-fold dilutions and inocula below the HBV NAT detection limit

Serial 1-in-10 dilutions of inoculum II of genotype A were prepared in preinoculation serum sample from each chimp (e.g., Chimp 272, Chimp 279, and Chimp 280, respectively). Dilutions were delivered to three tubes each in 1-mL aliquots and snap-frozen in liquid nitrogen. Concentration of HBV DNA was determined in one of the three tubes in each dilution so as to guarantee copy numbers of HBV DNA in the other two vials that were inoculated into chimps. These samples had been stored in a deep freezer at –80°C until inoculation.

Table 2 shows the measured HBV DNA concentrations in 1-in-10 dilutions of inoculum II (genotype A). The quantitative HBV DNA results starting from 2.6×10^6 copies per mL in the undiluted sample varied between 2.0×10^5 to 2.3×10^5, 2.0×10^4 to 2.4×10^4, 1.6×10^3 to 2.0×10^3, and 1.7×10^2 to 2.8×10^2 copies per mL, respectively, in the 1:10, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions. These quantitative results are an indication of the accuracy of the dilution and assay procedure. On the premise that dilutions beyond $1:10^4$ had been performed properly, further dilutions to $1:10^5$ and $1:10^6$ would have contained 16 to 28 and 1.6 to 2.8 HBV DNA copies per mL (ranges estimated by variations of HBV DNA measurements in lower dilutions), respectively, although they were below the detection limit of the PCR method used.

Likewise, serial 1-in-10 dilutions of inoculum IV (genotype C) were prepared in the plasma sample from Chimp 269 and Chimp 285. HBV DNA in 3.0×10^6, 3.5×10^5 to 3.8×10^5, 3.6×10^4 to 3.9×10^4, 3.6×10^3 to 4.6×10^3, and 4.3×10^2 to 4.6×10^2 copies per mL were detected in the original serum samples at 1:10, $1:10^2$, $1:10^3$, and $1:10^4$ dilutions thereof, respectively (Table 3). Thus, further experiments were performed on the assumption that serial dilutions of $1:10^5$ and $1:10^6$ of inoculum IV would have contained 35 to 46 and 3.5 to 4.6 HBV DNA copies per mL, respectively.

Determination of the minimum copy number required for transmission of HBV genotype A or genotype C to chimpanzees

When Chimp 272 and Chimp 279 were inoculated intravenously with 1.0 mL of inoculum II diluted $1:10^6$ (equivalent to 1.6 to 2.8 copies of HBV DNA in an in vitro assay), HBV infection did not develop in either of them during monitoring for 119 days (17 weeks) and thereafter. Chimp 279 was then rechallenged with 1.0 mL of inoculum II diluted $1:10^5$ (equivalent to 16-28 copies). He then became infected and developed HBV DNA in his serum 55 days (8 weeks) after the inoculation. Chimp 280 was also inoculated intravenously with 1.0 mL of inoculum II diluted $1:10^5$ (equivalent to 16 to 28 copies of HBV DNA). He developed HBV DNA in the circulation 76 days (11 weeks) after infection. In view of the incubation period of 55 to 76 days (8-11 weeks) for $1:10^5$ dilution of inoculum II, HBV infection would probably not have occurred in chimps who received $1:10^6$ dilution if they had been followed longer than 119 days (17 weeks).

Chimp 269 and Chimp 285 were inoculated with 1.0 mL of inoculum IV diluted $1:10^6$ (equivalent to 3.5-4.6 copies of HBV DNA in an in vitro assay). During follow-up

TABLE 2. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype A (inoculum II)*

		Serial dilutions in preinoculation serum samples of each chimpanzee					
Chimpanzee	Undiluted	1:10	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^6$
272	2.6×10^6	2.3×10^5	2.0×10^4	2.0×10^3	1.7×10^2	Not done	<100
279	2.6×10^6	2.0×10^5	2.4×10^4	2.0×10^3	2.4×10^2	<100	<100
280	2.6×10^6	2.3×10^5	2.3×10^4	1.6×10^3	2.8×10^2	<100	Not done

* Data are reported as copies per mL.

TABLE 3. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype C (inoculum IV)*

		Serial dilutions in preinoculation serum of each chimpanzee					
Chimpanzees	Undiluted	1:10	$1:10^2$	$1:10^3$	$1:10^4$	$1:10^5$	$1:10^6$
Chimp 269	3.0×10^6	3.8×10^5	3.9×10^4	3.6×10^3	4.6×10^2	<100	<100
Chimp 285	3.0×10^6	3.5×10^5	3.6×10^4	4.6×10^3	4.3×10^2	<100	<100

* Data are reported as copies per mL.

for 112 days (16 weeks), however, no HBV infection occurred in either of them. Subsequently, they were rechallenged with 1.0 mL of inoculum IV diluted 1:10^5 (equivalent to 35-46 copies of HBV DNA) 17 weeks after the initial inoculation. They developed HBV DNA in the circulation 35 and 50 days thereafter, respectively, indicating that both of them were infected. Therefore, the 50 percent chimp infectious dose (CID_{50}) for both genotype A and genotype C lies between the lowest infectious dose of approximately 30 copies and the subinfectious dose of approximately 3 copies or at approximately 10 HBV DNA copies.

HBV infection resolved in all six chimps and they never became carriers. Within a few weeks after the peak HBV DNA titer was reached, serum levels of transaminase increased slightly, within 3 times the upper limit of normal.

Replication velocity of HBV DNA in the preacute phase of infection

Doubling time and logarithmic time of HBV genotype A

Figure 2A illustrates the appearance of HBV genotype A in the circulation, when HBV DNA reached more than 10^2 copies per mL, as well as its early dynamics in Chimp 246, Chimp 279, and Chimp 280 during the preacute phase of exponential replication. HBV DNA emerged in the circulation earlier in Chimp 246 than the other two chimps, but

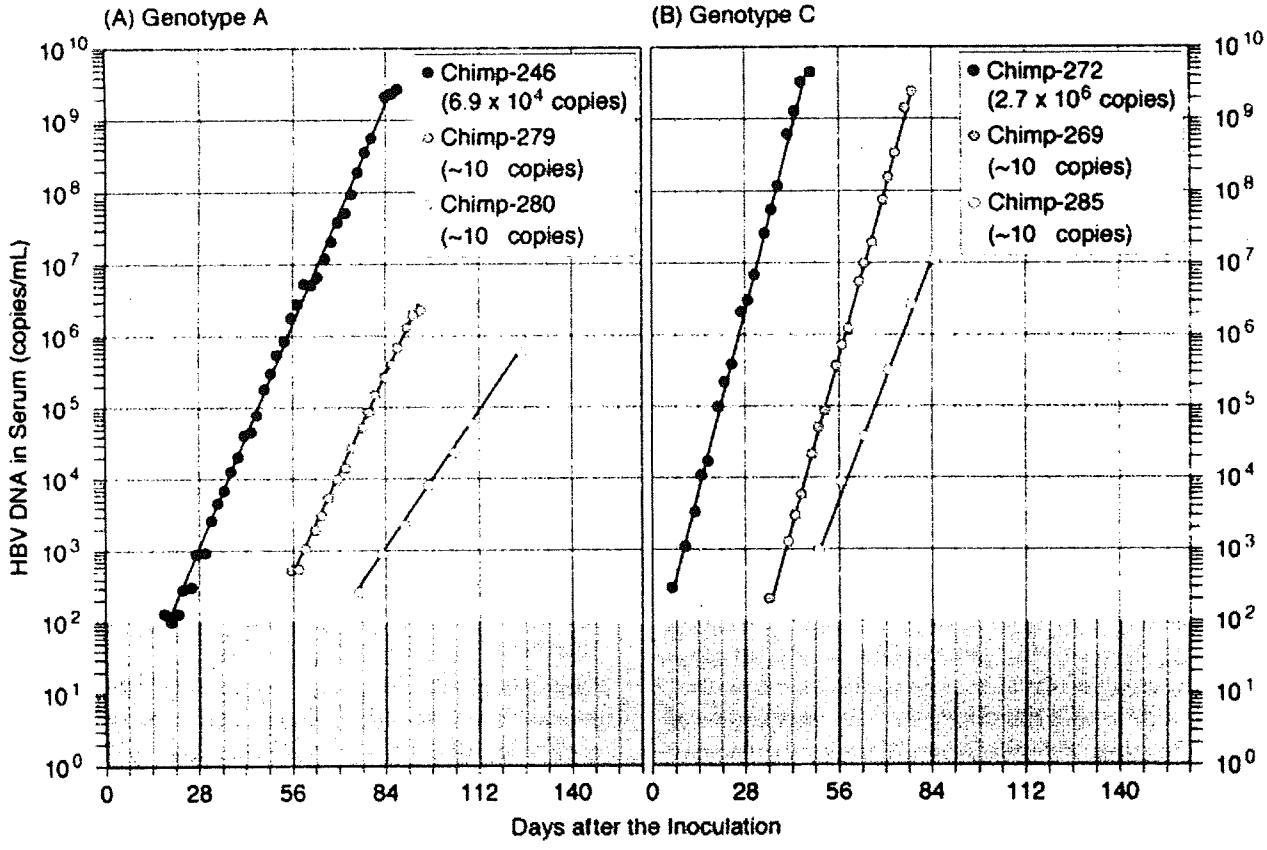

Fig. 2. Log-linear increase of HBV DNA in the circulation of chimpanzees during the early exponential replication phase. (A) Dynamics in the early ramp-up phase of viral DNA for three chimps inoculated with HBV genotype A: one chimp (Chimp 246) received 1 mL of human plasma containing 6.9×10^4 copies and the other two chimps (Chimps 279 and 280) received 1 mL of a 100,000 dilution of chimp plasma taken in the HBsAg ramp-up phase just before appearance of anti-HBc, which dilution contains a measured amount of 16 to 28 copies. (B) Graph summarizes the viral load dynamics for three chimpanzees inoculated with HBV genotype C: one chimp (Chimp 272) received 5 mL of human plasma with 2.7×10^6 copies of HBV DNA and the two other chimps (Chimps 269 and 285) received a measured amount of 35 to 46 copies (1:100,000 dilution) of preacute-phase chimpanzee plasma. Shaded areas are below the detection limit of NAT (<100 copies/mL). Only the phase of exponential replication is shown, and HBV DNA decreased after it reached peak values of 5.7×10^5 to 2.8×10^9 copies per mL in three chimps inoculated with HBV genotype A and 1.1×10^7 to 4.6×10^9 copies per mL in three chimps inoculated with HBV genotype C.

this animal had received more than a 1000-fold larger amount of copies of HBV than the other two chimps. Despite the 1000-fold higher infectious dose, the log-linear increase of HBV DNA in Chimp 246 was the same as in Chimp 279, who had received the minimum infectious dose. In Chimp 246, HBV DNA replicated exponentially from 21 to 97 days (3-13 weeks) until it peaked and then declined. Even though the same minimum infectious dose of HBV was inoculated, Chimp 279 developed detectable HBV DNA about 21 days (3 weeks) earlier than Chimp 280, in whom HBV replicated slightly slower. Despite differences in HBV doses and individual variation, the replication velocity was constant for HBV genotype A in the preacute phase of infection, before innate immune responses of the host developed, while the virus replicated at an exponential rate. The doubling time and the logarithmic time, in the early exponential viral replication phase, were calculated to be 2.7 to 4.4 and 9.0 to 14.7 days, respectively (see Table 4).

TABLE 4. Estimated doubling times and logarithmic times for HBV genotypes A and C with log-linear and growth-curve analysis

Genotype	Doubling time (days)	Logarithmic time (days)	y = a × exp(b × x) a	b	R^2
Genotype A					
Chimp 246	2.71	9.01	0.8491	0.2556	0.997
Chimp 279	3.05	10.14	0.0015	0.2271	0.998
Chimp 280	4.43	14.73	0.0022	0.1563	0.999
Genotype C					
Chimp 272	1.68	5.58	0.2074	0.413	0.998
Chimp 269	1.79	5.96	0.0002	0.3863	0.999
Chimp 285	2.5	8.31	0.0009	0.2771	0.997

TABLE 5. Comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve

Genotype	Doubling time (95% CI), days	Logarithmic time (95% CI), days	y = a × exp(b × x) a*	b (95% CI)	p Value
A	3.44 (2.64-4.89)	11.42 (8.80-16.26)	2.299	0.2017 (0.14-0.26)	<0.01
C	1.9 (1.63-2.27)	6.3 (5.41-7.54)	2.299	0.3654 (0.31-0.43)	

* To evaluate the difference of "b" (that is, slope) between the two genotypes, the growth curve model is assuming that "a" is identical.[13]

Doubling time and logarithmic time of HBV genotype C

The replication velocity in the preacute phase of infection in chimpanzees inoculated with genotype C inocula was faster than in the chimps infected with HBV of genotype A (Fig. 2B). Again, slight variation in log-linear increase of HBV DNA was found, and HBV DNA appeared in serum earlier in Chimp 272 who was inoculated with a 100,000-fold higher infectious dose than was administered to Chimps 269 and 285. As seen in the chimps inoculated with HBV genotype A, HBV genotype C increased in a log-linear fashion in the absence of host immune responses. Doubling times of HBV DNA in the circulation of Chimp 272, Chimp 269, and Chimp 285 were calculated to be 1.7 to 2.5 days and logarithmic times were 5.6 to 8.3 days as determined with the regression formula shown in Table 4.

When comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve, the difference was significant ($p < 0.01$, Table 5). That is, the doubling time of replications of HBV DNA with genotype A was estimated to be 3.44 days (95% confidence interval [CI], 2.64-4.89 days) and the logarithmic time was estimated to be 11.42 days (95% CI, 8.80-16.26 days). By contrast, those with HBV genotype C were estimated to be 1.90 days (95% CI, 1.63-2.27 days) and 6.30 days (95% CI, 5.41-7.54 days), respectively.

TABLE 6. Window periods before HBV DNA and HBsAg developed in the circulation of chimpanzees inoculated with the minimum infectious dose of genotype A or genotype C

HBV inoculated	Chimp infected	Markers of HBV infection HBV DNA (days)	HBsAg (days)
Genotype A	279	55	69
	280	76	97
Genotype C	269	35	50
	285	50	64

Window periods of HBV DNA and HBsAg in chimpanzees inoculated with the minimum infectious dose of HBV

After inoculation, the time before HBV DNA becomes detectable in the circulation by the single-sample NAT (with a sensitivity of 10^2 copies/mL) and the time before HBsAg was detected by CLIA after inoculation are listed in Table 6. The HBV DNA (<100 copies/mL) NAT window was 55 and 76 days, respectively, in Chimp 279 and Chimp 280 inoculated with the lowest infectious dose of HBV genotype A (approx. 30 copies). These NAT window periods were longer than the 35 and 50 days, respectively, found in Chimp 269 and Chimp 285 inoculated with the lowest infectious amounts of HBV genotype C (approx. 30 copies). Likewise, the HBsAg window was longer in Chimp 279 and Chimp 280 infected with genotype A than in Chimp 269 and Chimp 285 infected with genotype C (69 and 97 days, respectively, vs. 50 and 64 days, respectively).

DISCUSSION

Animal models sensitive to human hepatitis viruses offer robust advantages in obtaining basic data of viral infectivity.[10] By experimental infection of chimps with HCV, we have been able to determine the minimum infectious dose of HCV required for establishing infection.[15,16] The doubling time of HCV was determined to be 6.3 to 8.6 hours in two chimps inoculated with the minimum infectious dose of approximately 10 copies of HCV RNA. During the first 5 days after inoculation, HCV RNA did not increase above the NAT detection limit of 10^2 copies per mL in the circulation.[16] It would not be possible to detect HCV infection during the initial few days after exposure, even if 1-mL samples were used for individual NAT.

In this study, we have determined the minimum infectious dose for two standardized inocula containing defined copy numbers of HBV DNA. They were plasma passages of HBV in chimps harvested during the preacute phase of infection and had been processed with the utmost care for maintaining infectious activity. The minimum infectious dose of HBV or the dose where 50 percent of the chimps would be infected lies between 1-in-1 million and 1-in-100,000 dilution of the original inocula and is estimated to be of the order of 10 copies, as was the case for HCV.[15] On the basis of HBV DNA concentrations measured in serial dilutions of inocula (Tables 2 and 3), the minimum infectious dose can be determined to be 16 to 28 copies for HBV genotype A and 35 to 46 copies for HBV genotype C.

There are two definitions of the minimum infectious dose of HBV. Theoretically, it is a single copy of HBV. Not all HBV virions entering the circulation of recipients, however, will succeed in reaching hepatocytes, because some of them are phagocytized by circulating macrophages and Kupffer cells in the sinusoids of the liver. In a mathematical window-phase risk model, Weusten and colleagues[9] have proposed a minimum infectious dose approximately 10 copies of HBV, on the basis of the CID_{50}.[17-19] Recently the inocula derived from chronic HBV carriers used in older chimpanzee studies[17,18] were requantified by Hsia and coworkers[20] with real-time TaqMan PCR. The estimated HBV copy number per CID_{50} (geq) was 169 for genotype A *adw*, 78 for genotype D *ayw*, and 3 for genotype C *adr*, calculated by mathematical division, respectively. These viral load data, performed on cryopreserved aliquots from an inocula derived from a chronic HBV carrier (i.e., HBsAg- and anti-HBc–positive), were derived retrospectively several decades after the chimp titration studies. These results are different from the results obtained in our study, where the inocula was derived from the early ramp-up phase of viremia (HBsAg is positive but anti-HBc is negative) and the chimp titration and viral load analyses were performed prospectively. Hence, the minimum infectious dose defined as a single copy, proposed on a theoretical basis, would deserve revisiting in practical HBV infections. The window period of HBV infection changes with the size of the inoculum. The more copies of HBV inoculated therefore the shorter the incubation period in experimental transmission studies in chimps.[11] An inverse correlation is reported, also, between time before HBsAg appears in serum and the HBV dose in human beings.[21] In accordance with these reports, we also found that the NAT window was shorter in chimps receiving larger sizes of inocula both for genotypes A and C (Fig. 2). The NAT (<100 copies/mL) window period was approximately 1 week with an inoculum of 2.7×10^6 copies of genotype C, approximately 3 weeks with 6.9×10^4 copies of genotype A, 5 to 7 weeks when inoculating 35 to 46 copies of genotype C, and 8 to 11 weeks when inoculating 16 to 28 copies of HBV genotype A, while no infection was observed during 16 to 17 weeks of observation with an inocula of approximately 3 copies of genotype A or B. Theoretically, HBV infection might have become detectable after 17 weeks, but this is unlikely when extrapolating the data above. Inoculation with HBV in large amounts, as happens with transfusion with HBsAg-positive blood units, has been largely excluded since introduction of HBsAg testing in 1972. Barker and Murray[21] have shown that inoculation of lower infectious doses of HBV in the range of 10^4 to 10^7 diluted icteric plasma no longer caused clinical hepatitis in healthy individuals, while infection still occurred with up to a 10^7 diluted inoculum, as detected by an HBsAg complement fixation test. Our study showed that HBV DNA levels increase 6.5×10^3 to 2.2×10^5 copies per mL at the time of the first HBsAg-reactive sample in six chimpanzees in whom blood samples were taken at intervals of 2 to 7 days. These amounts are enough to cause clinical hepatitis B.[21] Indeed, Satake and coworkers[3] found that transmission of 5,000 to 50,000 copies of HBV by blood components with a low viral load in the pre-MP-NAT window phase could cause clinical hepatitis B. Transfusion-transmitted HBV after introduction of individual-donation or small-pool NAT (<10) is still possible, but would involve relatively low infectious doses of HBV of approximately 10 to 100 CID_{50}.

In the chimps inoculated with approximately 30 copies of HBV, the NAT window was determined by individual-donation NAT having a sensitivity of 10^2 copies per mL, while the HBsAg window was established by CLIA with the highest sensitivity presently available.[5,12] The NAT window was 55 to 76 days and HBsAg window was 69 to 97 days, respectively, in Chimp 279 and Chimp 280 who had been inoculated with approximately 30 copies of HBV genotype A. In contrast, the NAT window was 35 to 50 days and the HBsAg window was 50 to 64 days, respectively, for Chimp 269 and Chimp 285 inoculated with approximately 30 copies of HBV genotype C. Thus, neither

the NAT nor the HBsAg window phases overlapped between minimum-dose infections of HBV genotypes A and C; they were longer for genotype A than genotype C. It may be that the NAT window is longer for genotype A, prevalent in Western countries, than genotype C common in Japan. It cannot be excluded, however, that the results observed in our inoculation studies with a limited number of chimpanzees were influenced by the host rather than the genotype of the virus. The duration of the NAT and HBsAg windows are influenced at least by three factors: 1) the infectious dose, 2) individual variation among recipients, and 3) distinct HBV genotypes.

We found the replication velocity of HBV in the preacute phase of infection remarkably different between genotypes A and C. From three chimps infected with HBV genotype A, the doubling time was estimated to be 3.44 days (95% CI, 2.64-4.89 days) and the logarithmic time 11.42 days (95% CI, 8.80-16.26 days). From three chimps infected with HBV genotype C, the doubling time was estimated to be 1.90 days (95% CI, 1.63-2.27 days), and the logarithmic time 6.30 days (95% CI, 5.41-7.54 days). Also in chimeric mice with the liver replaced by human hepatocytes, genotype A was found to replicate much slower than genotype C in the initial weeks of HBV infection.[22]

The replication velocity of HBV in the circulation, indicated by the viral doubling time, is an important factor when calculating the window-period reduction provided by NAT screening systems. Biswas and colleagues[5] calculated a doubling time of 2.56 days (95% CI, 2.24-2.97 days) based on a seroconversion panel of 23 HBV infections. Yoshikawa et al.[4] followed 93 donors in preacute phase HBV infections who had been identified by the routine NAT screening program on 50-MPs at JRC Blood Centers. They estimated a median doubling time of HBV at 2.6 days (range: 1.3-15.2).

Kleinman and Busch[7] have assessed the HBsAg window period based on the HBV doubling time of 2.56 days documented by Biswas and colleagues.[5] They estimated an HBsAg window at 38.3 days (95% CI, 33.0-43.7 days) by the CLIA HBsAg seroconversion point at a concentration of 1650 copies per mL, while Minegishi and coworkers[12] determined the HBsAg seroconversion point at 2100 copies per mL. We found the HBsAg seroconversion with AxSYM occurred when the HBV DNA concentration reached a level of 6.5×10^3 to 2.2×10^5 in six chimpanzees. The differences in HBV levels at HBsAg seroconversion in CLIA may be related to the genotype, but also could reflect differences in the calibration of HBV quantitative assays in genome copies.

It is not known if the chimpanzee model is as susceptible for HBV infection as human beings. As a result, the minimum dose of HBV for transmitting infection to man is, in fact, not precisely known. Nevertheless, a minimum human infectious dose of approximately 10 HBV DNA copies, as indicated by our chimpanzee infectivity experiments, seems a reasonable assumption for modeling the HBV transmission risk in the pre-HBV-NAT window period.

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REFERENCES

1. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet 1991;338:1040-1.
2. Kojima M, Shimizu M, Tsuchimochi T, Koyasu M, Tanaka S, Iizuka H, Tanaka T, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Posttransfusion fulminant hepatitis B associated with precore-defective HBV mutants. Vox Sang 1991; 60:34-9.
3. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low HBV-DNA levels identified in a look back program. Transfusion 2007;47:1197-205.
4. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
5. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
6. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45: 254-64.
7. Kleinman SH, Busch MP. Assessing the impact of HBV NAT on window period reduction and residual risk. J Clin Virol 2006;36 Suppl 1:S23-9.
8. Yugi H, Mizui M, Tanaka J, Yoshizawa H. Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing—Japanese experience. J Clin Virol 2006;36 Suppl 1:S56-64.

9. Weusten JJ, van Drimmelen HA, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion 2002;42:537-48.
10. Prince AM, Brotman B. Perspectives on hepatitis B studies with chimpanzees. ILAR J 2001;42:85-8.
11. Shikata T, Karasawa T, Abe K, Uzawa T, Suzuki H, Oda T, Imai M, Mayumi M, Moritsugu Y. Hepatitis B e antigen and infectivity of hepatitis B virus. J Infect Dis 1977;136: 571-6.
12. Minegishi K, Yoshikawa A, Kishimoto S, Yugi H, Yokoya N, Sakurada M, Kiyokawa H, Nishioka K. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang 2003;84: 287-91.
13. Ohtaki M, Satoh K, Kanda T, Fujikoshi Y. Local ridge estimate using random coefficient curve models for analyzing repeated measurements. J Jpn Stat Soc 2007;36:177-84.
14. Vonesh EF, Carter RL. Efficient inference for random-coefficient growth curve models with unbalanced data. Biometrics 1987;80:642-50.
15. Katayama K, Kumagai J, Komiya Y, Mizui M, Yugi H, Kishimoto S, Yamanaka R, Tamatsukuri S, Tomoguri T, Miyakawa Y, Tanaka J, Yoshizawa H. Titration of hepatitis C virus in chimpanzees for determining the copy number required for transmission. Intervirology 2004;47:57-64.
16. Tanaka J, Katayama K, Kumagai J, Komiya Y, Yugi H, Kishimoto S, Mizui M, Tomoguri T, Miyakawa Y, Yoshizawa H. Early dynamics of hepatitis C virus in the circulation of chimpanzees with experimental infection. Intervirology 2005;48:120-3.
17. Berninger M, Hammer M, Hoyer B, Gerin JL. An assay for the detection of the DNA genome of hepatitis B virus in serum. J Med Virol 1982;9:57-68.
18. Ulrich PP, Bhat RA, Seto B, Mack D, Sninski. J, Vyas GN. Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees. J Infect Dis 1989;160:37-43.
19. Prince AM, Stephan W, Brotman B. Beta-propiolactone/ ultraviolet irradiation: a review of its effectiveness for inactivation of viruses in blood derivatives. Rev Infect Dis 1983; 5:92-107.
20. Hsia CC, Purcell RH, Farshid M, Lachenbruch PA, Yu MY. Quantification of hepatitis B virus genomes and infectivity in human serum samples. Transfusion 2006;46:1829-35.
21. Barker LF, Murray R. Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Am J Med Sci 1972;263:27-33.
22. Sugiyama M, Tanaka Y, Kato T, Orito E, Ito K, Acharya SK, Gish RG, Kramvis A, Shimada T, Izumi N, Kaito M, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. Hepatology 2006;44:915-24. ◘

研究

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国内で販売されている抗HBs抗体定量用体外診断用医薬品の評価：国内標準品を用いた検討*

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〔SUMMARY〕 国内で販売されている8種類の抗HBs抗体測定キットを用いて，抗HBsヒト免疫グロブリン(以下，抗HBs抗体)国内標準品の希釈系列検体を測定した．6種類のキットにおいてはほぼ期待される測定値を示した．しかしながら，期待値に比較して約0.5倍と1.5倍の測定値を示すキットが各1種類あった．このような乖離の原因を調査しその修正が行われた結果，1種類のキットは新規申請に向けて開発中であり，もう一種類のキットでは一部変更の手続きが行われている．これにより今後はHBV感染防御の基準と考えられる抗体価の“10 mIU/ml”という数値について，いずれのキットを用いてもほぼ均一な定量が可能になることが期待される．

〔KEYWORDS〕 HBV，抗HBs抗体測定キット，抗HBs抗体国内標準品

緒言

HBV(hepatitis B virus)感染者数は全国民の約2%にも及ぶと推定されており，B型肝炎は「国民病」とも言われている．HBV感染防御については，安全で副作用の少ないワクチンが既に開発され，わが国ではハイリスクグループおよびHBV感染者から出産した児に投与され効果をあげている．HBV感染防御に有効な抗HBs抗体価については，WHO(世界保健機関)やCDC(米国疾病予防管理センター)では10 mIU/mlという数値を提唱している[1~3]．日本国内においても同様な基準を採用するかについては議論の余地があるが，それ以前の問題として，現在国内で使用されている様々な抗HBs抗体測定キット(定量キット)が，お互いに乖離がない測定値を提供できるかについての検証がなされていない．本研究では，抗HBs抗体国内標準品(WHO国際標準品を用いて値付けしたもの)の希釈系列検体を，国内で販売されている8種類の測定キットを用いて測定し，その値を比較検討した．

材料および方法

1. 抗HBs抗体国内標準品

原料はグラクソスミスクライン社製ENGERIX-B®(HBs抗原のサブタイプはadwと考えられる)の接種により抗体価を高めた国外血由来の血漿である．内容物は白色粉末凍結乾燥品で，1バイアル(含湿度0.5%)を1.0 ml注射用水で溶解したとき320 IU/mlとなる．またpH＝6.94，浸透圧＝1.65，蛋白質含量＝29.2 mg/ml，免疫グロブリンG含量＝99.6%であり，HBsAg，HAV-RNA，HBV-DNA，HCV-RNA，HIV-1-RNAはすべて陰性である．なお本標準品の力価は国際標準品(WHO 1st, 1977)を基にして値付けされ，2001年4月より国立感染症研究所から供与され

* Evaluation of anti-HBs antibody assay kits commercially available in Japan by utilizing the national standard

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表　使用した抗 HBs 抗体測定キットの名称，測定方法，および抗体検出に用いた HBs 抗原のサブタイプ

Kit No.	メーカー名	製品名	原理/方法
1	アボット・ジャパン	アキシム オーサブ・ダイナパック	EIA
2	アボット・ジャパン	アーキテクト・オーサブ	CLIA
3	アボット・ジャパン	IMx オーサブ・ダイナパック	EIA
4	オーソ・クリニカル・ダイアグノスティックス	ビトロス HBs 抗体	CLEIA
5	東ソー	E テスト「TOSOH」II (HBsAb)	EIA
6	デイドベーリング	エンザイグノスト Anti-HBs II	EIA
7	富士レビオ	ルミパルス II HBsAb	CLEIA
8	和光純薬	スフィアライト HBs 抗体	CLEIA

CLIA：chemiluminescent immunoassay(蛍光免疫法).
EIA：enzyme immunoassay(酵素免疫法).
CLEIA：chemiluminescent enzyme immunoassay(蛍光酵素免疫法).

ている.

2. 希釈系列作成手順

（1）抗 HBs 抗体国内標準品(320 IU/vial) 1 バイアルを 1 ml の精製水で溶解した.

（2）標準品溶液 0.1 ml に検体希釈液 9.9 ml を加え均一な溶液にした(3,200 mIU/ml).

（3）3,200 mIU/ml の溶液を 5 倍希釈(溶液 1 ml＋検体希釈液 4 ml) して 640 mIU/ml の抗体液(5 ml)を作成した.

（4）以下，順次 2 倍希釈系列を作成した. (2.5 ml＋2.5 ml) 系列：0，10，20，40，80，160，320，640(mIU/ml).

3. 抗 HBs 抗体測定キット

抗 HBs 抗体測定に使用したキットは，表に示した 8 種類である．測定は各検体につき 3 重測定で行った.

結果

図 1 は各キットでの抗 HBs 抗体国内標準品希釈系列検体の測定結果である．いずれのキットも測定範囲内(0～640 mIU/ml)で期待値と測定値の関係において非常に良好な直線性が得られた(r^2＝0.98～1.00)．しかし，キット #7 と #8 では直線の傾きがそれぞれ 1.7，および 0.56 と理想である 1.0 からの明らかな乖離がみられた．そこで，対表示値を算定し，それらの結果を図 2 にまとめた．ここでの対表示値とは，各検体の抗体濃度(期待値)と測定値の比を，各濃度ごとに計算しそれらを平均した値のことである．したがって理想的な対表示値は 100 となる.

図 2 に示したように，#1～#6 のキットではおおよそ 120 前後の対表示値を示しているが，図 1 の結果から予想されたように，キット #7，#8 の対表示値はそれぞれ 169.2 および 50.1 であり，100 からの乖離が大きかった．なお WHO や CDC が提唱している，HBV 感染防御に有効な免疫獲得の基準と考えられる 10 mIU/ml の低濃度検体を用いた測定値を比較したところ，図 2 に示した「対表示値」と良く相関していることが確認された．このような測定値乖離の原因について，それぞれのメーカーと協議した結果，以下の結論に達した．#7 においてはキットを製造する際の検討に，WHO 国際標準品の抗 HBs 抗体を基準にしていなかったことが原因であると結論された．そこで現在新たなキットが開発され，それを用いた測定では対表示値が 117.2 となった(未発表データ)．また #8 においては，乖離の原因は不明であるが，WHO 国際標準品を用いて見直しを行い，再測定の結果，対表示値が 88.4 となった(未発表データ，なおこのキットは現在一部変更申請中である).

考察

現在国内における抗 HBs 抗体価の表示には，凝集法による「管数」表示と，EIA (enzyme immunoassay)，CLIA (chemiluminescent immuno-