医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2004 年 12 月 20 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	乾燥抗D（Rho）人免疫グロブリン	研究報告の公表状況	AABB WEEKLY REPORT 10（44）3, 2004	公表国 アメリカ
販売名（企業名）	抗D人免疫グロブリン-Wf（ベネシス）			

研究報告の概要	使用上の注意記載状況・その他参考事項等
米国血液銀行協会（AABB）は会員に対し、共通の感染源に由来する A 型肝炎ウイルス（HAV）の流行に曝露された可能性のある供血者の取り扱いについて、HAV に最後に曝露された可能性がある日から 120 日間供血停止することを勧告する。また、供血者が HAV に曝露されている可能性がある場合には当該製品の回収の勧告について審議する。	2. 重要な基本的注意 (1)本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から抗D（Rho）人免疫グロブリンを濃縮・精製した製剤であり、ウイルス除去を目的として、製造工程において濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
血液製剤のHAV汚染防止に関するAABBの自主規制に関する情報である。 万一原料血漿にHAVが混入した場合、EMCのウイルスバリデーション試験成績から、製造工程において不活化・除去が十分でないと考えられるため、その事実を総合機構及び厚生労働省に報告する。	HAV が原料血漿に混入した場合にはその事実を総合機構及び厚生労働省に報告する。

At HHS, he will oversee the regulatory groundwork to set up a new Medicare outpatient prescription benefit in 2006. Some policy analysts have said he may have to push for significant cuts in Medicaid and Medicare.

Even before the nomination was announced, Sen. Byron L. Dorgan, D-N.D., threatened to block Senate confirmation of the next HHS secretary out of frustration with Bush administration opposition to legalizing importation of prescription drugs. It was not immediately clear where Leavitt stands on drug imports.

He would also come into office with an FDA that is facing criticism for its handling of this year's flu vaccine shortage and for not sufficiently monitoring the safety of the arthritis drug Vioxx. FDA's management has been accused of suppressing data on dangers of Vioxx and other drugs.

AABB Bulletin Spells Out Criteria For Donors Potentially Exposed to Hepatitis A Outbreaks

AABB Association Bulletin #04-08, issued Dec. 16, is intended to provide information to blood collection facilities for management of donors and blood products associated with known or suspected common source outbreaks of hepatitis A virus (HAV) infection.

The Association Bulletin recommends that blood collection facilities should follow the determination of their local health departments regarding potential exposure to HAV as a result of a common source outbreak. When the health department publicly notifies the community that a common source outbreak has occurred or that the potential for an outbreak exists due to the discovery of an infected food handler or other common source and describes groups of individuals who should be evaluated for receipt of immune serum globulin (Ig) injections, the local blood collection facility should defer from blood donation those individuals meeting the Ig criteria. The deferral from blood donation should be for a period of 120 days from the date of last potential exposure.

The bulletin also discusses recommendations for product retrieval when blood has been collected from individuals in a community where there may have been common source exposure to HAV.

A copy of Association Bulletin #04-08 is posted on the AABB Web site, www.aabb.org, under "Member's Area/Archives/Association Bulletins."

Correction

In last week's issue of Weekly Report, the article on *BLA (Biologics License Application) Checklists* incorrectly stated that the coalition task force will send "comments to the FDA to determine whether the new guidance follows needed validation and process controls for the products' manufacture." It should have stated that the coalition task force will submit "comments to FDA in an effort to ensure the new guidance, once finalized by the FDA, will represent reasonable recommendations for validation and process controls necessary for the manufacture of Platelets Pheresis products."

医薬品
医薬部外品　研究報告　調査報告書
化粧品

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
				2005.1.28	該当なし	
一般的名称	解凍人赤血球濃厚液		研究報告の公表状況	J Gen Virol 2005; 86: 595-9.	公表国 日本	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
日本赤十字社は 1999 年 7 月 1 日より核酸増幅検査（NAT）を実施し、献血者における B 型肝炎ウイルス（HBV）、C 型肝炎ウイルス、ヒト免疫不全ウイルス 1 のスクリーニングを行っている。日本で遺伝子型が H の HBV 事例が初めて発見された。NAT 陽性であったこの時の献血血液の HBV の血清学的マーカーは検出されなかったので、感染初期のウィンドウ・ピリオドに献血が行われていた可能性がある。3215 bp の完全なゲノム配列が決定され、米国ロスアンジェルス由来の genotype H 株（LSA2523）と 99.3%の相同性が認められた。また、遺伝子型 A から H すべてに保存されている HBs 抗原にあるロイシン・ジッパー・モチーフが認められた。	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「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
本邦の献血者において、遺伝子型が H の HBV 事例が初めて発見され、現行の NAT スクリーニングで検出できたとの報告である。	引き続き情報の収集に努める。

Journal of General Virology (2005), 86, 595–599 DOI 10.1099/vir.0.80732-0

Short Communication

Characterization of genotype H hepatitis B virus strain identified for the first time from a Japanese blood donor by nucleic acid amplification test

Hitoshi Ohnuma,[1] Akira Yoshikawa,[1] Hideaki Mizoguchi[1], Hiroaki Okamoto[2] and the JRC NAT Screening Research Group

Correspondence
Akira Yoshikawa
yoshikawa@saitama.bc.jrc.or.jp

[1]Saitama Red Cross Blood Center, 1370-12, Takahagi, Hidaka, Saitama-ken 350-1213, Japan

[2]Division of Virology, Department of Infection and Immunity, Jichi Medical School, Tochigi-ken 329-0498, Japan

Received 28 October 2004
Accepted 9 December 2004

The Japanese Red Cross has been conducting a nucleic acid amplification test (NAT) screening for hepatitis B virus (HBV), hepatitis C virus and human immunodeficiency virus 1 among blood donors since July 1 1999. The first case of HBV genotype H was found and reported in Japan. Serological markers of HBV were not detected in this NAT-positive donation. It may be that the positive donation was in the serological window period at the early stage of infection. The complete genome of 3215 nt was sequenced, and the sequence had 99·3 % homology with the strain from Los Angeles, USA (LSA2523). Here, a leucine zipper motif was found in the region of the HBV surface antigen conserved through genotypes A–H.

Hepatitis B virus (HBV) has been classified into seven genotypes A–G. However, recently genotype H, which is phylogenetically closely related to genotype F, has been reported (Arauz-Ruiz *et al.*, 2002). These genotypes of HBV show a distinctive geographical distribution and a relevance to clinical severity (Mayert *et al.*, 1999; Kobayashi *et al.*, 2002; Locarnini, 2002). They are important in epidemiological studies, analysis of modes of infection and medical treatment.

On July 1 1999, the Japanese Red Cross (JRC) started a nationwide screening by using the nucleic acid amplification test (NAT) of pools of 500 sera. From February 1 2000, the pool size was reduced to 50 (Mine *et al.*, 2003). By March 31 2003, 19 454 693 units in the 500- or 50-sample pools had been tested using NAT screening. During this period, 349 HBV DNA-, 53 hepatitis C virus RNA- and 6 human immunodeficiency virus type 1 RNA-positive donors were found.

The NAT screening system in Japan has been reported previously (Ohtake & Nishioka, 2000; Mine *et al.*, 2003; Minegishi *et al.*, 2003). Samples that are serologically positive and have elevated alanine aminotransferase (>60 IU l^{-1}) are excluded from NAT screening. All voluntarily donated blood in the JRC is qualified by a questionnaire administered by the JRC blood centres.

HBV DNA loads were calculated from the working curve (10^7, 10^6, 10^5, 10^4, 10^3, 10^2 copies ml^{-1}) produced by domestic standard samples that were prepared based on the international standard samples (NIBSC). Quantification was carried out using Sequence Detector version 1.7 (PE Applied Biosystems). The data show the mean of quadruplicate tests. The results have already been reported (Minegishi *et al.*, 2003).

The genotypes of HBV are classified based on an intragroup nucleotide divergence of up to 4·2 % of the S-gene sequences or in some cases up to 8·0 % of complete genomes (Norder *et al.*, 1992, 1993, 1994; Okamoto *et al.*, 1988). Precore mutation (from G to A at nt 1896) or core promoter mutations (from A to T at nt 1762 and/or from G to A at nt 1764) were detected and characterized using the methods of Okamoto *et al.* (1990, 1994). Out of 349 HBV NAT-positive donors, 17 had precore mutants (genotype B, 7 donors; genotype C, 10 donors), 31 had core promoter mutants (genotype A, 2 donors; genotype C, 29 donors) and 13 had mutants with both precore and core promoter mutations (genotype B, 1 donor; genotype C, 12 donors). Sequencing was carried out directly by using a BigDye Terminator Cycle Sequencing kit and ABI Prism 3100 Genetic Analyser (PE Applied Biosystems). To analyse the sequences, Sequencher Mac version 4.1 (Hitachi Software Engineering) or GENETYX-MAC version 9.0 (Software Development) was used.

In Japan, genotypes C and B have been dominant among HBV-viraemic patients. However, recently genotype A has increased in prevalence (Kobayashi *et al.*, 2002; Koibuchi *et al.*, 2001; Orito *et al.*, 2001). Out of 349 HBV NAT-positive donations, there were 40 cases of genotype A

The GenBank/EMBL/DDBJ accession number for the sequence reported in this paper is AB179747.

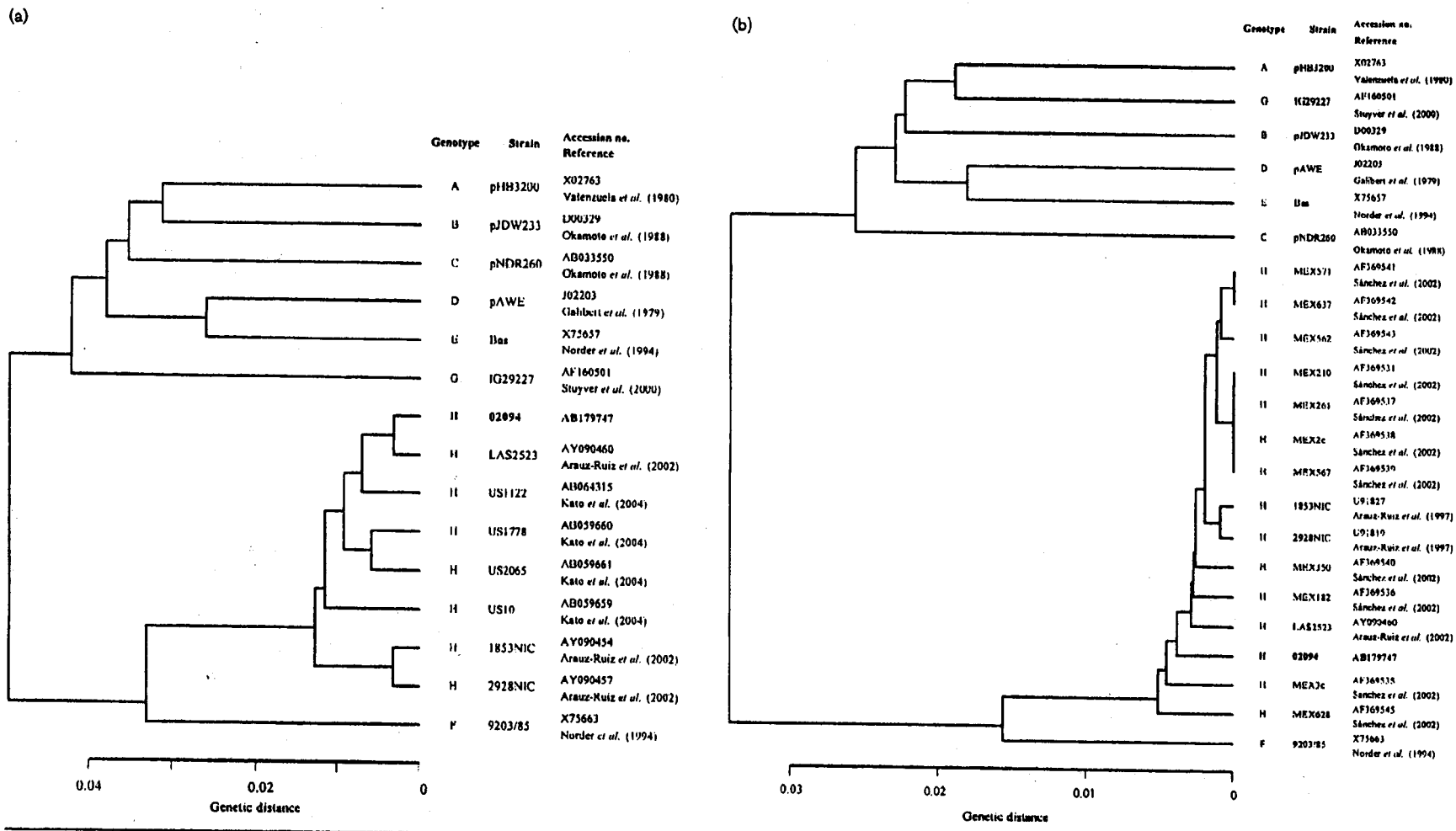

Fig. 1. (a) Dendrogram of hepatitis B virus genotypes A–H, based on 15 complete nucleotide sequences. Fourteen strains (without 02094; indicated in bold) were retrieved from GenBank/EMBL/DDBJ database. The dendrogram was constructed by the UPGMA method (unweighted pair-group method with arithmetic mean) (Nei, 1987). Phylogenetic analysis of the complete genome (3215 nt) of the strain 02094 identified it as a new genotype H, which was separated at the branch of genotype F. Complete genomes of 3215 nt were sequenced, and the sequence was found to have 99·3% homology with the LSA2523 strain. Genetic distance is indicated below the dendrogram. (b) Dendrogram of hepatitis B virus genotypes A–H, based on the S region. Twenty-one strains (without 02094; indicated in bold) were retrieved from GenBank/EMBL/DDBJ database. The dendrogram was constructed by the UPGMA method (Nei, 1987). Genetic distance of genotype H is within 0·0039. Genetic distance is indicated below the dendrogram.

(11·5 %), 39 cases of genotype B (11·2 %), 264 cases of genotype C (75·6 %), 5 cases of genotype D (1·4 %) and 1 case of genotype H (0·3 %). Genotypes E, F and G were not detected by NAT.

Genotype H was recognized for the first time in Japan by comparing the full sequence (3215 nt) of NAT-positive sample (02094) against LSA2523 (Arauz-Ruiz *et al.*, 2002). To determine the full sequence of HBV DNA genotype H, three overlapping regions were amplified and subjected to sequence analysis: nt 1–668, nt 479–1796, nt 1698–2381, and nt 2332–3215. The nucleotide sequence identity between 02094 and LSA2523 was found to be 99·3 %. The dendrogram of the strain 02094 was generated using reference strains of genotypes A–G and seven strains of genotype H made available in GenBank/EMBL/DDBJ through UPGMA methods (Nei, 1987) using GENETYX-MAC version 9.0 (Fig. 1a). Phylogenetic analysis performed on the S gene of genotypes A–H, including the 14 genotype H S-gene sequences available in GenBank/EMBL/DDBJ, is shown in Fig. 1(b).

Genotype H HBV was recovered from a blood sample donated on 9 October 2002 from a 52-year-old Japanese man. We could not obtain any additional information about the source of infection from the donor (02094). However we found, from the interview sheet, that this subject had not been abroad during the past year, which suggested that he had contracted infection of genotype H HBV in Japan. None of the serological markers of HBV was detected in the sample of 02094. HBV DNA load was $2{\cdot}6 \times 10^3$ copies ml^{-1}. The last of the stocked sample, which was collected 53 days before the NAT-positive donation (02094), proved to be PCR-negative. This may be due to the fact that the NAT-positive donation was in the serological window period at an early stage of infection. From the sequence of precore and core promoter regions, the strain 02094 proved to be a wild-type. The subtype of the strain 02094 was *adw*, because the codons 122 and 160 in the S region were both lysine (Okamoto *et al.*, 1987a, b).

Between the 02094 strain obtained in the present study and the seven other reported strains of the same genotype (genotype H), there were differences ranging from 24 to 84 nt and from 10 to 47 aa in the entire genome. Of note, the 02094 isolate differed from the 1853NIC, 2928NIC, LAS2523, US2065 and US1122 by only 0–4 aa, but differed from the US10 and US1778 isolates by 13 or 20 aa in the core protein.

Amino acid sequences of the S region (226 aa, 678 nt; 15 strains) were compared between strain 02094 and known strains (Table 1, Fig. 2.). The characteristic amino acids of the S region in genotype F and H were Val^{18}, Leu^{61}, Glu^{178}, Cys^{183}, Leu^{193}, Ile^{198}, Cys^{206}, Cys^{220} and Ser^{225}. The remarkable amino acids in genotype H were Val^{44}, Pro^{45}, Gly^{47} and Ala^{224} (Fig. 2). Specific amino acid in strain 02094 was K^{30}.

There was a conserved region, except for one mutation F85C among genotypes A–H, from codon 69 to 109 reported to be the hydrophobic region, which was the domain of ER-membrane penetration (Mangold & Streeck, 1993). Within this region we found a leucine zipper motif: L-X(6)-L-X(6)-L-X(6)-L (Landschulz *et al.*, 1988) (Fig. 2). This motif may be acting to assemble HBsAg, though the motif has been reported as being nucleic acid-binding and as a eukaryotic transcriptional regulatory motif. On the other hand, the common determinant 'a' region from codon 124 to 147 is rather varied, and important in neutralizing antibodies.

Table 1. Different number of nucleotides and amino acids between the strain 02094 (not shown) and the seven other reported strains of the same genotype (genotype H)

Parentheses show the percentile identity between 02094 and the other strains.

Strain	Nucleic acid	Amino acid						
	Full length 3215 nt	Envelop protein			Core		X	Polymerase
		pre-S1 119 aa	pre-S2 55 aa	S 226 aa	pre-C 29 aa	Core 183 aa	154 aa	843 aa
LAS2523	24 (99·3)	0	0	2	0	0	0	8
US1122*	49 (98·5)	2	0*	3	0	4	9	23*
US1778	67 (97·9)	3	1	1	0	13	3	19
US2065	52 (98·4)	1	0	2	0	2	3	15
US10	67 (97·9)	1	0	6	1	20	2	17
1853NIC	84 (97·4)	0	1	1	0	0	8	24
2928NIC	77 (97·6)	0	2	1	0	1	5	17

*Full-length sequence of US1122 consisted of 3206 nt. The pre-S2 region encodes 52 aa and polymerase gene codes for 840 aa.

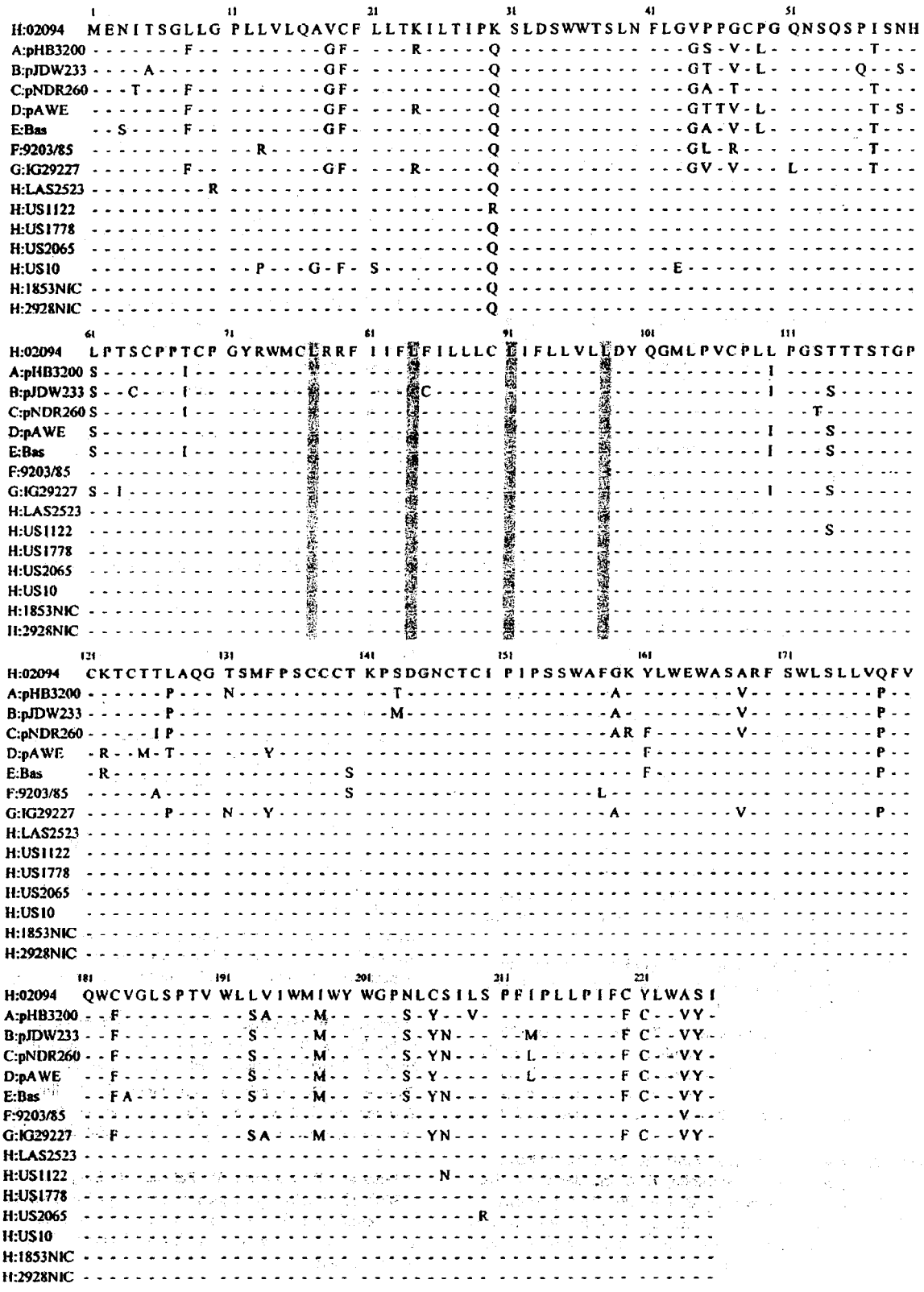

Fig. 2. Deduced amino acid sequences of the S gene of HBV genotypes A–H. Fourteen strains (without 02094) were retrieved from GenBank/EMBL/DDBJ. The positions of leucine that formed the leucine zipper motif (Landschulz *et al.*, 1988) are shaded.

Acknowledgements

This study was carried out with the collaboration of members of the JRC Blood Services Department, JRC NAT centres in Tokyo, Chitose and Fukuchiyama and the JRC blood centres throughout Japan. We gratefully acknowledge the guidance and support of the JRC President, Mr S. Fujimori; the Executive Counselor, Dr K. Tadokoro; the JRC Councillor, Dr T. Kusakari and Dr K. Nishioka in the JRC Blood Services Department and emeritus professor Dr M. Mayumi, Jichi Medical School.

References

Arauz-Ruiz, P., Norder, H., Visona, K. A. & Magnius, L. O. (1997). Molecular epidemiology of hepatitis B virus in Central America reflected in the genetic variability of the small S gene. *J Infect Dis* **176**, 851–858.

Arauz-Ruiz, P., Norder, H., Robertson, B. H. & Magnius, L. O. (2002). Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* **83**, 2059–2073.

Galibert, F., Mandart, E., Fitoussi, F., Tiollais, P. & Charnay, P. (1979). Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in *E. coli*. *Nature* **281**, 646–650.

Kato, H., Gish, R. G., Bzowej, N. & 9 other authors (2004). Eight genotypes (A-H) of hepatitis B virus infecting patients from San Francisco and their demographic, clinical, and virological characteristics. *J Med Virol* **73**, 516–521.

Kobayashi, M., Arase, Y., Ikeda, K. & 10 other authors (2002). Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. *J Gastroenterol* **37**, 35–39.

Koibuchi, T., Hitani, A., Nakamura, T., Nojiri, N., Nakajima, K., Jyuji, T. & Iwamoto, A. (2001). Predominance of genotype A HBV in an HBV-HIV-1 dually positive population compared with an HIV-1-negative counterpart in Japan. *J Med Virol* **64**, 435–440.

Landschulz, W. H., Johnson, P. F. & Mcknight, S. L. (1988). The leucine zipper: a hypothetical structure common to a new class of DNA-binding proteins. *Science* **240**, 1759–1764.

Locarnini, S. A. (2002). Clinical relevance of viral dynamics and genotypes in hepatitis B. *J Gastroenterol Hepatol* **17**, S322–S328.

Mangold, C. M. T. & Streeck, R. E. (1993). Mutational analysis of cysteine residues in the hepatitis B virus small envelope protein. *J Virol* **67**, 4588–4597.

Mayerat, C., Mantegani, A. & Frei, P. C. (1999). Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? *J Viral Hepat* **6**, 299–304.

Mine, H., Emura, H., Miyamoto, M. & 7 other authors (2003). High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. *J Virol Methods* **112**, 145–151.

Minegishi, K., Yoshikawa, A., Kishimoto, S., Yugi, H., Yokoya, N., Sakurada, M., Kiyokawa, H., Nishioka, K. & the Japanese Red Cross NAT Screening Research Group (2003). Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. *Vox Sang* **84**, 287–291.

Nei, M. (1987). Phylogenetic trees. In *Molecular Evolutionary Genetics*, pp. 287–326. Edited by M. Nei. New York: Columbia University Press.

Norder, H., Hammas, B., Löfdahl, S., Couroucé, A. M. & Magnius, L. O. (1992). Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J Gen Virol* **73**, 1201–1208.

Norder, H., Hammas, B., Lee, S. D., Bile, K., Couroucé, A. M., Mushahwar, I. K. & Magnius, L. O. (1993). Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *J Gen Virol* **74**, 1341–1348.

Norder, H., Couroucé, A. M. & Magnius, L. O. (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* **198**, 489–503.

Ohtake, K. & Nishioka, K. (2000). Nucleic acid amplification testing of hepatitis B virus. *Lancet* **355**, 1460.

Okamoto, H., Imai, M., Miyakawa, Y. & Mayumi, M. (1987a). Site-directed mutagenesis of hepatitis B surface antigen sequence at codon 160 from arginine to lysine for conversion of subtype determinant from r to w. *Biochem Biophys Res Commun* **148**, 500–504.

Okamoto, H., Imai, M., Tsuda, F., Tanaka, T., Miyakawa, Y. & Mayumi, M. (1987b). Point mutation in the S gene of hepatitis B virus for a d/y or w/r subtypic change in two blood donors carrying a surface antigen of compound subtype adyr or adwr. *J Virol* **61**, 3030–3034.

Okamoto, H., Tsuda, F., Sakugawa, H., Sastrosoewignjo, R. I., Imai, M., Miyakawa, Y. & Mayumi, M. (1988). Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* **69**, 2575–2583.

Okamoto, H., Yotsumoto, S., Akahane, Y. & 7 other authors (1990). Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. *J Virol* **64**, 1298–1303.

Okamoto, H., Tsuda, F., Akahane, Y., Sugai, Y., Yoshiba, M., Moriyama, K., Tanaka, T., Miyakawa, Y. & Mayumi, M. (1994). Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigens. *J Virol* **68**, 8102–8110.

Orito, E., Ichida, T., Sakugawa, H. & 11 other authors (2001). Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* **34**, 590–594.

Sánchez, L. V., Maldonado, M., Bastidas-Ramirez, B. E., Norder, H. & Panduro, A. (2002). Genotypes and S-gene variability of Mexican hepatitis B virus strains. *J Med Virol* **68**, 24–32.

Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R. F. & Rossau, (2000). A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* **81**, 67–74.

Valenzuela, P., Quiroga, M., Zalvidar, J., Gray, P. & Rutter, W. J. (1980). The nucleotide sequence of the hepatitis B viral genome and the identification of the major viral genes. In *Animal Virus Genetics*, pp. 57–70. Edited by B. N. Fields, R. Jaenisch. & C. F. Fox. New York: Academic Press.