INTERNATIONAL FORUM

Vox Sanguinis

Occult hepatitis B infection in blood donors

H. W. Reesink, C. P. Engelfriet, G. Henn, W. R. Mayr, G. Delage, F. Bernier, T. Krusius, A. Assal, P. Gallian, C. Corbi,
P. Morel, B. David, P. De Micco, H. Murokawa, H. Yugi, S. Hino, K. Tadokoro, Ø. Flesland, E. Brojer, M. Łętowska, G. Olim,
F. Nascimento, H. Gonçalves, L. Castro, M. Morais, S. L. Stezinar, M. Alvarez, S. Sauleda, R. González, C. Niederhauser,
M. Stolz, J.-P. Allain, S. Owusu-Ofori, R. Eglin, S. Stramer, M. Busch, D. M. Strong, J. Epstein, R. Biswas

Occult hepatitis B virus (HBV) infection is diagnosed when an HBV DNA test is positive but hepatitis B surface antigen (HBsAg) is undetectable. Occult HBV infection (OBI) may represent (i) acute infection in the window period, (ii) HBV tail-end of chronic HBV infection, (iii) persistence of replication at low level after recovery in the presence of anti-HBs, or (iv) occurrence of an escape mutant in vaccinated or unvaccinated individuals not detected by current HBsAg assays [1].

Since the introduction of sensitive HBV DNA nucleic acid amplification technique (NAT) screening in various blood transfusion organizations, the existence of such OBIs in donors has become apparent. Although donor blood in the window period of an HBV infection is known to be highly infectious, the significance of OBIs in other situations (see above) is not clear.

To obtain more information on this subject, the following questions were sent to experts in the field. We obtained 15 contributions to this forum.

In case HBV DNA NAT has been introduced in your country:

Question 1: Since when do you screen blood donations for HBV DNA by NAT and how many occult HBV infections [n/total (%)] were observed? Which technique is applied?

Question 2: Do you test individual donations or do you test in pools. Please indicate the pool size.

Question 3: Which other HBV markers were present and what was the HBV DNA level (IU/ml) in the observed occult HBV cases? Please summarize the results in a table.

Question 4: Did you perform look-back studies in recipients transfused in the past with blood products from occult HBV-infected donors? If yes, please provide the results.

Question 5: Was further subtype and sequence analysis done of the HBV in OBI donors. Please give the results.

If HBV NAT is not (yet) introduced in your country:

Question 6: Do you consider implementing HBV NAT in your centre/country, and if yes, when?

In Table 1, the results of random HBV DNA NAT screening in seven countries/centres are summarized. In the four European countries using a small pool size or individual donors for testing, a prevalence of OBI of 1 : 7500 (Slovenia) to 1 : 63 000 (Poland) was observed. In Japan, the prevalence was, over the years, 1 : 107 000. A study in Ghana showed a prevalence of 1 : 6000. OBIs in the window period were 5/31 (16%) in Poland but 40% in Japan.

Table 1 Occult hepatitis B virus (HBV) infections (OBI) in random donors screened by HBV DNA NAT

Country	Year of introduction	HBV DNA+ n/total	Prevalence of OBI	Technique(s) used	Pool size	Remarks
Poland	2005	31/1 962 036	1 : 63 000	Roche	1 : 24 (2006)	5/31 OBI were WP infections
				Chiron	1 : 6 (2007)	(1 : 490 000)
Portugal	2006	3/174 074	1 : 50 000	Roche	1 : 6	
				Chiron	ID	
Spain	2004–2006	39/1 641 891	1 : 42 000	Roche	ID	15/24 centres implemented
				Chiron	1 : 6	HBV NAT
				in house	1 : 8	
Slovenia	2007	4/30 000	1 : 7500	Chiron	ID	
Japan	1999	381/40 591 962	1 : 107 000	Roche	1 : 500 (1999)	40% WP and 60% tail-end OBI
				multiplex	1 : 50 (2000)	
					1 : 20 (2004)	
Ghana	2005/06	3/18 400	1 : 60 000	in house	?	Study only
French Antilles and La Réunion	2005	0/74 959	?	Chiron	ID	

ID, individual donor; WP, window period.

Look-back studies in Japan revealed that 11/22 (50%) blood components in the window period provoked seroconversion of HBV markers in recipients, whereas only 1/33 (3%) blood products with OBI in the tail-end stage of chronic HBV infection caused serologic evidence of HBV infection in recipients. In countries where look-back was possible on previous donations of OBI index donors, seroconversion for HBV markers in recipients was also not observed. In the majority of tail-end OBI's other HBV markers, that is, antibody to hepatitis B core antigen (anti-HBc) and/or antibody to hepatitis B surface antigen (anti-HBs) and/or antibody to hepatitis Be antigen (anti-HBe) were present.

In the USA, various studies were performed in anti-HBc reactive donors, indicating a prevalence of 0·4–1% of OBI (1 : 37–49 000) in these populations; look-back studies on previous donations were not available. The level of HBV DNA in OBI is in general low. The OBI HBV DNA levels ranged from the lower limit of detection of the NAT assay used, to approximately a factor 10–100 higher. It is therefore likely that the majority of OBIs would not be detected when a pool of more than 10–20 donor samples was used.

Several countries, such as the UK and France, do not plan to implement HBV DNA NAT screening, since cost–benefit analysis indicated that implementation of HBV DNA NAT was not cost-effective. Finland and the Netherlands plan to implement this screening test between 2007 and 2009.

In conclusion, countries in Europe, in which HBV NAT donor screening was implemented, found OBIs in 1 : 7500–63 000 donors, using individual donor or a small pool of donor samples for testing. In Japan, 1 : 107 000 OBIs were found. The majority of OBIs in Europe were tail-end chronic HBV infections with a low risk for transmitting HBV to recipients. In Japan, an endemic country for HBV infections, 40% of all OBIs detected were acute window period infections, and previous donations of these donors induced seroconversion for HBV markers in 50% of recipients. This indicates that blood safety for HBV infection is mainly improved by detection of OBIs in the window period. In areas of the world where HBV infections are highly endemic (such as Japan), HBV NAT screening in individual donor or in a small pools will improve blood safety, but in low endemic countries this still has to be proved.

Reference

1 Allain J-P: International collaborative study proposal for the characterization of occult hepatitis B virus infection identified by nucleic acid or anti-HBc screening. *Vox Sang* 2007; 92:254–257

Editors
H. W. Reesink
Sanquin Consulting Services
Plesmanlaan 125
NL-1066 CX Amsterdam
The Netherlands
E-mail: h.reesink@sanquin.nl

and
Department of Gastroenterology and Hepatology
Academic Medical Center
Amsterdam
The Netherlands
E-mail: h.w.reesink@amc.uva.nl

C. P. Engelfriet
Sanquin Research and Sanquin Diagnostic Services
Plesmanlaan 125
NL-1066 CX Amsterdam
The Netherlands
E-mail: p.engelfriet@sanquin.nl

G. Henn & W. R. Mayr

Question 1

We started our HBV NAT in 1999 and we found one OBI (0·0000006%); the donor will be tested again 6 months after the NAT-positive donation.

The technique used is a mini pool technique with a detection level of 551 IU/ml for every single donation in the pool.

Question 2

We perform our screening tests on pools of 96 samples.

Question 3

NAT – positive, HBsAG – negative, anti-HBs – negative, anti-HBc – positive and anti-HBe – marginally, positive.

The donor donated blood on 22 March 2007 (don_0). The last donation was on 15 January 2007 (don_{0-1}) and the previous donation to last one was on 30 October 2006 (don_{0-2}).

The donation don_{0-1} was given 70 days before don_0 and this was found negative for NAT [ultrasensitve single donation polymerase chain reaction (PCR)], HBsAG negative, anti-HBs negative, anti-HBc positive, anti-HBe marginally positive. The donation don_{0-2} was given 75 days before don_{0-1} and was found negative in all hepatitis B markers including PCR.

Question 4

We performed a look back-study on the recipient who was negative in all serological HBV markers and in the NAT 4 5 months after transfusion.

Question 5

No subtypes were tested.

W. R. Mayr
G. Henn
Blutspendezentrale
Österreichisches Rotes Kreuz
Wiedner Hauptstraße 32
A-1040 Wien
Austria

E-mail: gabriela.henn@roteskreuz.at;
wolfgang.mayr@roteskreuz.at

G. Delage & F. Bernier

HBV DNA NAT has not yet been introduced.

Question 6

Introduction is planned for May 2009. We are waiting for approval by our regulatory authorities of a test kit for blood donor screening.

G. Delage
Medical Affairs – Microbiology
Héma-Québec
4045 Cote-Vertu
St-Laurent (Québec) H4R 2W7
Canada
E-mail: gilles.delage@hema-quebec.qc.ca

F. Bernier
Product Qualification
Héma-Québec
4045 Cote-Vertu
St-Laurent (Québec) H4R 2W7
Canada
E-mail: france.bernier@hema-quebec.qc.ca

T. Krusius

Question 1

Currently, Finnish Red Cross Blood Service is screening donor blood samples only for hepatitis C virus (HCV) and human immunodeficiency virus (HIV) by NAT. The prevalence of HBsAg carriers and the incidence of new infections have been low in the donor population in Finland.

Question 2

Finnish Red Cross Blood Service is negotiating with one supplier to start individual donor NAT screening of all three viruses. The target is to implement individual donor NAT screening of blood donors by February 2008.

T. Krusius
Finnish Red Cross Blood Service
Helsinki
Finland
E-mail: tom.krusius@bts.redcross.fi

A. Assal et al.

Question 1

NAT of HBV DNA has not been implemented in continental France, but only in the French overseas territories (French

Antilles and Island of La Réunion). The rationale for such decision is explained in the answer to *Question 6*. In those sites, NAT is performed in an individual donation format (ID-NAT) with a triplex assay: The Procleix® Ultrio™ assay (GenProbe/Chiron). Since January 2005, date of HBV NAT implementation, 74 959 donations have been tested and no OBIs were detected.

Question 2

HBV NAT is performed on individual donations in the French overseas territories only.

Question 3

Not applicable.

Question 4

Not applicable.

Question 5

Not applicable.

Question 6

NAT was introduced in France for screening HIV-1 and HCV in blood donations in July 2001, in addition to serological screening tests. [1] At that date, no commercial standardized assay was available for HBV DNA screening and the question to test or not for the genome of that virus had no to be discussed. However, as a consequence of HIV and HCV NAT implementation, the overall residual risk of transfusion-transmitted infections became mainly related to HBV. Recently, a new triplex assay that includes HBV DNA was evaluated in France, in the frame of a European multicentre study [2], rising the debate about whether to implement HBV NAT screening in blood donations or not. As a result, the decision was made not to implement HBV NAT in continental France for the following reasons:

1. France is a low endemic area for hepatitis B. The prevalence of chronic HBV carriers in the French general population is estimated to be 0·2 to 0·5% (100 000 to 150 000 chronic carriers). HBV incidence is close to 2000 to 3000 new cases of acute hepatitis B per year. A marked decrease in incidence was observed since 1994 due to improved vaccinal coverage.

Regarding the blood donor population, the overall HBsAg rate in 2005 was 1·40 per 10 000 donations (0·02 in repeat donors and 9·30 in first time donors). The residual risk of transfusion-transmitted hepatitis B (TTHBV) for the 3-year period from 2003 to 2005 was 1/1 700 000 [95% confidence interval (CI): 0–1/425 000].

2. Minipool NAT (MP-NAT) has only a little additional health benefit in comparison with the current screening strategies based on both sensitive HBsAg and anti-HBc assays used in France. The prevention of TTHBV in French blood donors relies on testing for both HBsAg and anti-HBc. The risk

of TTHBV has steadily decreased, thanks to the improvement of HBsAg screening assays. Currently, blood donations are tested by two of the most sensitive HBsAg screening assays in France as more than 50% of donations are tested by the Prism HBsAg assay (Abbott) of which sensitivity is estimated at 0·05 to 0·08 ng/ml and the rest by the Monolisa HBsAg Ultra (Biorad) (sensitivity < 1 ng/ml). Our study as well as several studies showed that HBV NAT performed on mini-pools is expected to offer only a small incremental yield in transfusion safety in comparison with serological assays combining the use of sensitive HBsAg and anti-HBc assays. [3,4] In France, we estimated that the Procleix® Ultrio™ assay would reduce the HBV window period by 15 days in ID-NAT and only 5 days in pools of eight samples. In their studies about comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection, Biswas et al. [3] showed that when compared to the most sensitive HBsAg assays, none of the MP-NAT assays would significantly close the HBV WP. They demonstrated that the most sensitive HBsAg assay detected HBV before two of the MP-NAT assays. However, the difference was not significant. In contrast, the most sensitive NAT assay evaluated in this study was estimated to close the window period by 20 days relative to the most sensitive HBsAg assay. These outcomes are consistent with those found in our study and reinforce the conviction that the yield of HBV MP-NAT would be extremely poor in non endemic areas. The issue of HBV NAT implementation in blood donation screening was addressed by Sue Stramer in a Transfusion Review editorial entitled 'Pooled hepatitis B virus DNA testing by nucleic acid amplification: implementation or not'. [5] S. Stramer concluded that 'the yield from clinical studies has been marginal or nonexistent and HBV MP NAT has been shown to have poor cost-effectiveness relative to other blood safety measures'.

3. Haemovigilance data show a very low rate of TTHBV infections. No transfusion-transmitted cases of HBV infections were reported by the hemovigilance network in France in the last 4 years. From 1985 to 2003, only four post-transfusion HBV infections were described corresponding to window period cases as demonstrated by look-back procedures including HBV DNA testing in the four HBsAg-negative archived samples of the previous donations.

4. Modest clinical impact of TTHBV infections in recipients. On the basis of 2·5 million donations collected in France per year with a residual risk of TTHBV estimated at 1 700 000 donations, and 1·2 transfused components per donation, only two recipients would receive HBV-infected blood components per year. We have applied to those figures the model described by A. Pereira in his study of health and economic impact of post-transfusion hepatitis B published in transfusion in 2003 [6]. This model predicts that 4% of patients given HBV-infective blood would have a chronic hepatitis and 0·97% would die of liver disease (including fulminant

hepatitis). In our situation, this would lead to one chronic hepatitis B every 12 to 13 years and one death as a result of the liver disease every 50 years. In these conditions, the projected yield of HBV MP-NAT is expected to be very marginal.

5. Poor cost-effectiveness. Several studies demonstrate the poor cost-effectiveness of NAT. In non-endemic areas for hepatitis B, HBV MP-NAT would provide a very small health benefit at a very high cost [6].

Conclusion

As a result of the different reasons presented above, it has been considered that the current HBV screening tests in France are sufficient to safeguard the blood supply and that the marginal yield of HBV MP-NAT does not justify its implementation in routine screening of blood donors. Although the anti-HBc assays specificity is not optimal, they represent a second safeguard, particularly for the detection of occult hepatitis B. As a consequence, anti-HBc testing further reduces the need of HBV NAT implementation. [5]

However, single-sample NAT or MP-NAT with smaller pool sizes and/or modified procedures (genome enrichment or test improvement) would be more relevant. In contrast, in the French overseas territories, where NAT is routinely performed on individual donations, the epidemiological situation is different as HBV prevalence in the general population, as well as in blood donors, is high enough to justify HBV NAT implementation.

The availability of new automated NAT systems using triplex assays and the need to renew NAT platforms could modify the current situation in the next future in continental France as HBV NAT could be a part of an overall change in NAT technology and not a reaction to a concerning health issue in transfusion.

References
1 Assal A, Coste J, Barlet V, Laperche S, Cornillot C, Smilovici W, Pillonel J, Andreu G: Application of molecular biology to blood transfusion safety: the nucleic acid testing. Transfus Clin Biol 2003; 10:217–226
2 Koppelman M, Assal A, Chudy M, Torres P, de Villaescusa RG, Reesink HW, Lelie PN, Cuypers HT: Multicenter performance evaluation of a transcription-mediated amplification assay for screening of human immunodeficiency virus-1 RNA, hepatitis C virus RNA, and hepatitis B virus DNA in blood donations. Transfusion 2005; 45:1258–1266
3 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; 43:788–798
4 Busch MP: Should HBV NAT replace HBsAg and/or anti-HBc screening of blood donors. Transfus Clin Biol 2004; 11:26–32
5 Stramer S: Pooled hepatitis B virus DNA testing by nucleic acid amplification: implementation or not. Transfusion 2005; 45:1242–1246

6 Pereira A: Health and economic impact of posttransfusion hepatitis B and cost-effectiveness analysis of expanding HBV testing protocols of blood donors: a study focused on the European union. *Transfusion* 2003; 43:192–201

A. Assal
P. Gallian
C. Corbi
P. Morel
B. David
P. De Micco
Etablissement Français du Sang
Direction Médicale et Scientifique
20 Avenue du stade de France
93218 La Plaine Saint Denis
France
E-mail: azzedine.assal@efs.sante.fr

H. Murokawa *et al.*

The Japanese Red Cross Blood Centre conducts serological tests for HBV, HCV and HIV with the agglutination method. As for serological HBV screening, we use HBsAg and a combination of anti-HBs and anti-HBc assays. While a donor with a low anti-HBc titre is accepted, a donation with a high anti-HBc titre is qualified only if its anti-HBs titre is sufficiently high to induce a protective effect. Because of the high prevalence of HBV infection in Japan, only seronegative samples are pooled for NAT screening [1]. Whenever we detect a HBV DNA-positive sample, we test it with enzyme immunoassay (EIA) for serological markers [HBsAg, anti-HBs, anti-HBc and immunoglobulin M (IgM) anti-HBc], estimate the virus load and determine the DNA sequence of S region to classify the genotypes.

Question 1
In July 1999, the Japanese Red Cross implemented NAT for HBV, HCV and HIV-1 using the multiplex real-time PCR reagents (Roche, Tokyo, Japan). For virus resolution testing, we used the nested PCR method (in-house reagents). We had screened 40 591 962 blood donations till the end of May 2007 and detected 381 (1 : 107 000) HBV DNA-positive ones without detectable HBs antigen.

Question 2
We perform NAT in pools. When we started testing, we used 500-pool system. Then, we moved to the 50-pool system in February 2000. In August 2004, we further decreased the pool size to 20.

Question 3
Results are summarized in Table 1. Virus loads were below our quantitative limit (25 IU/ml) in more than half of the anti-HBc-positive donations, while those of anti-HBc-negative donations varied in wide range.

Question 4
All donations from repeat donors received from 1997 to 2004 were subjected to a look-back study when a subsequent donation turned positive for a 50-NAT, HBsAg, or anti-HBc. Repository samples of such donations were routinely analysed by HBV ID-NAT. Of the repository tubes that had been aliquoted from the donations obtained from 1 February 2000 to 31 January 2004, a total of 15 721 were subjected to ID-NAT [2] and we identified 158 HBV ID-NAT-positive blood donations. Ninety-five (60%) of them were donated in the late stage of HBV infection and 60 (38%) in the early stage. Eleven of 22 (50%) components derived from donations in the early stage proved to have caused seroconversion of HBV markers in the recipients, whereas only 1 of 33 (3%) donations in the late stage showed serologic evidence of infection.

Question 5
Results are summarized in Table 2. Among 81 donations with anti-HBc, genotypes of 80 (99%) cases were B or C that are the major ones in Japan. Sixty-nine (85%) donors were older than 50. On the other hand, 55 genotype A donors were anti-HBc negative and number of female donor was only one. These points were the most outstanding differences between genotype A and B/C.

Table 1 Number of the occult HBV cases

HBV DNA (IU/ml)	Anti-HBc − Anti-HBs −	+	Anti-HBc + Anti-HBs −	+
< 25	18	1	21	21
25–75	32	3	7	10
75–250	53	1	11	4
250–750	82	0	3	2
> 750	107	3	1	1
Sum	292	8	43	38

Table 2 Genotypes of the occult HBV cases

Genotype	Anti-HBc − Anti-HBs −	+	Anti-HBc + Anti-HBs −	+
A	52	3	0	0
B	32	2	12	16
C	206	3	31	21
D	0	0	0	1
H	2	0	0	0
Sum	292	8	43	38

References

1 Yugi H, Hino S, Satake M, Tadokoro K: Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology in Japan. *Vox Sang* 2005; 89:265

2 Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K: Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. *Transfusion* 2007; 47:1197–1205

H. Murokawa
H. Yugi
S. Hino
K. Tadokoro
Department of Nucleic Acid Amplification Testing
Central Blood Institute
Japanese Red Cross Blood Service
Nakarokugo 3-30-1
Oota-ku
Tokyo 144-0055
Japan
E-mails: h-murokawa@bs.jrc.or.jp; yugi@bs.jrc.or.jp;
s-hino@bs.jrc.or.jp; k-tadokoro@bs.jrc.or.jp

Ø. Flesland

HBV-NAT is not yet introduced in Norway.

Implementing HBV NAT has not been considered for cellular products. It may be introduced for plasma for fractionation, depending on a new contract with our fractionation partner. Our present contract expires in June 2009. HCV NAT was introduced in April 2000. From May 2007, it is no longer a requirement for cellular products. The contract with our fractionation partner still requires HCV NAT for plasma that is sent for fractionation. If the HCV NAT requirement is successfully renegotiated before June 2009, the result may include other tests, such as HBV NAT. HBsAg is done on all donations. Anti-HBc is done in new donors and in repeat donors when it is more than 12 months since the previous donation. From January 2007, this is changed to new donors and repeat donors when it is more than 6 months since the previous donation. The reason is that we found that donors that had been in self-imposed quarantine sometimes had seroconverted for anti-HBc, but were HBsAg negative. Because most quarantine periods are 6 months, it was decided to retest anti-HBc when the time period between two donations was more than 6 months. Norwegian donors donate on average 2·4 times per year and with this new guideline approximately 50% of the donors will be anti-HBc tested at each donation. In some respects, this testing policy will compensate for the lack of HBV NAT.

Ø. Flesland
Laboratory Centre
Asker and Baerum Hospital
NO-1309 Rud
Norway
E-mail: oystein.flesland@sabhf.no

E. Brojer & M. Łętowska

Question 1

HBV DNA NAT is performed in Poland since 2005 on samples seronegative for HBsAg, anti-HCV and anti-HIV. After testing of 1 962 036 donations, we identified 31 HBV DNA-positive/HBsAg-negative donors. According to the results of follow-up and look-back analysis by molecular and serological tests, four were identified as window period donations (frequency ~1/490 000; 0·0002%), one as secondary window period and 26 as OBI (frequency ~1/78 000; 0·0013%). NAT is performed in molecular biology laboratories in 11 regional blood transfusion centers. Two alternative techniques are used: (i) based on PCR – Cobas Ampliscreen test (Roche Diag) or (ii) based on TMA – Procleix ULTRIO test (Chiron).

Question 2

Until the end of 2006, two alternative systems were used: (i) individual donation testing by Procleix Ultrio or (ii) 24-donation pool testing using Cobas Amplicor. Since 2007, the pool size was decreased to six donations and the method of pool testing was changed to Cobas S201 system (based on real-time PCR technology).

Question 3

No.	Level of HBV DNA	Anti–HBc/anti–HBc/anti–HBe
1	1.82×10^3	-/+/-
2	< 100	-/+/-
3	< 100	-/+/-
4	< 100	-/+/-
5	< 100	-/+/-
6	2.6×10^4	-/+/-
7	< 100	-/+/-
8	5·28	-/+/-
9	3.25×10^2	+/-/-
10	1.14×10^7	-/+/+
11	< 100	-/+/+
12	< 100	-/+/+
13	< 10	-/+/+
14	1.8×10^3	-/+/+
15	< 100	-/+/+
16	Pos	-/+/+
17	< 10	+/+/-
18	< 100	+/+/+
19	Pos	Not tested/+/-
20	Pos	Not tested/+/+
21	< 10	Not tested/+/+
22	< 10	Not tested/+/+
23	< 10	Not tested/+/-
24	Pos	-/+/nt
25	160	+/+/-
26	> 20	+/+/+

Question 4

The look-back procedure is obligatory. Hospitals, where previous donations of occult HBV donors were transfused, are informed of HBV DNA detection in the next bleed of the repeat donor. If possible, the recipients are contacted by hospital staff and the HBV markers (HBsAg, HBV DNA and anti-HBc) are tested. In the reference laboratory (Institute of Haematology and Transfusion Medicine, Warsaw, Poland), the archive samples of occult HBV repeat donors are tested for HBV DNA in single donation format.

There were 23 repeat donors with occult HBV. In 10 out of 42 available archive samples, HBV DNA was detected and in the rest it was not detected. In four recipients, the studies of HBV markers after transfusion were performed and the results are as follows: (i) two patients obtained HBV DNA-positive donation containing 198 IU/ml; one of them (immunodeficient; vaccinated for HBV) obtained platelet concentrate and the other (immunocompetent, probably not vaccinated) obtained red blood cell concentrate; (ii) both recipients were HBV DNA, HBsAg and anti-HBc negative after transfusion; (iii) they had anti-HBc performed after transfusion - 380 days and 384 days, respectively. HBV markers were also not detected in the two patients transfused with red blood cells prepared from previous donations of two other occult HBV donors. In these cases, the examination of archive samples confirmed the HBV DNA negativity of the donors at the time the donations were transfused. However, those donations were anti-HBc positive.

Summing up, according to our observations, the HBV infection was not transmitted by transfusion of red blood cell and platelet concentrate from donor with ~200 IU HBV DNA/ml and from HBV DNA-negative/anti-HBc-positive donors in which the HBV DNA was detected in the next donation.

Question 5

The subtype and sequence analysis of five cases was done in our laboratory and now the studies are continued in cooperation within HBV Safety Study Group in International Society for Blood Transfusion (ISBT) Transfusion Transmitted Infections (TTI) Working Party. The HBV genotype distribution is 60% - genotype A; 40% genotype D. The HBV DNA from two vaccinated donors showed mutant features. In one genotype A-infected donor, G145A substitution was present. The sequence of the second one strain (genotype D) was highly unusual and contained D144G mutation in the fourth loop and a substitution of cysteines at position 124 and 139. Our preliminary results are described in Hepatology [1].

Reference

1 Brojer E, Grabarczyk P, Liszewski G, Mikulska M, Allain J-P, Letowska M: Characterisation of HBV DNA positive/HBsAg negative blood donors identified in the Polish NAT screening program. Hepatology 2006; 44:1666–1674

E. Brojer
M. Łętowska
Institute of Haematology and Transfusion Medicine
Gandhi 14
02-776 Warsaw
Poland
E-mails: ebrojer@ihit.waw.pl; letowska@ihit.waw.pl

G. Olim et al.

Question 1

Blood donations have been screened for HBV DNA NAT in the three regional blood centers of the Portuguese Blood Institute since July 2006, and was fully implemented in October 2006. Three out of 174 074 (0·0017%) OBIs were observed.

The techniques applied are: Procleix – Ultrio Assay (two positives in 123 834 donations) and MPX – Roche (one positive in 50 240 donations).

Question 2

Individual donations were tested by Procleix – Ultrio Assay; pools of six samples were tested by MPX Roche.

Question 3

	HBsAg	HBcAc	HBsAc	HBcAc IgM	HBeAg	HbeAc	HBV/DNA level
Sample 1	Negative	Reactive	Reactive	Negative	Negative	Negative	?
Sample 2	Negative	Reactive	Reactive	Not tested	Not tested	Not tested	?
Sample 3	Negative	Negative	Not tested	Not tested	Negative	Negative	53 IU/ml

Question 4

The donor of sample 1 was a first-time donor. Blood components were discarded. The donor of the sample 2 was a regular donor who was tested negative in the previous donation, 4 months before.

Question 5

In sample 3, sequence analysis was done 30 days later and HbsAg was positive.

G. Olim
F. Nascimento
H. Gonçalves
L. Castro
M. Morais
Instituto Português do Sangue, IP
Parque de Saúde de Lisboa
Av. do Brasil, N.O 53 - Pavilhão 17
1749-005 Lisboa
Portugal
E-mail: dirips@ips.min-saude.pt
Web: www.ipsangue.org

S. L Stezinar

Question 1

Mandatory NAT screening for HBV DNA was implemented in Slovenia in February 2007. During the 5-month period 30 000 donations were screened and HBV DNA was detected in four donations (0·013%). The observed yield is 1/7500.

The technique in use for screening is Procleix Tigris System and the assay used is Procleix Ultrio Assay (Chiron/Novartis).

Question 2

Testing is performed on individual donations.

S. L. Stezinar
Department for Diagnostic Services
Blood Transfusion Centre of Slovenia
Šlajmerjeva 6
SI- 1000 Ljubljana
Slovenia
E-mail: snezna.levicnik@ztm.si

M. Alvarez, S. Sauleda & R. González

Question 1

In Spain, HBV DNA screening is not mandatory. Therefore, only 15 of the 24 blood centres have implemented HBV NAT. Between January 2004, when the first centre started HBV NAT, and December 2006, 39 OBIs have been observed, out of 1 641 891 screened donations. As December 2006, the applied techniques are: in-house PCR, with enrichment extraction, in one centre, commercial PCR (Roche Molecular Systems, Mannheim, Germany) in six centres and Transcription-Mediated Amplification (TMA, Chiron Corporation, Emeryville, CA, USA) in eight centres.

Question 2

We test individual donations (seven centres) and pools. The pool size is 48 donations (in-house PCR), 8 units (one centre) and 6 units (six centres).

Question 3

Procleix Ultrio (S/CO)	Procleix	Ultrio	HIV-RNA	HCV-RNA	HBV-RNA	HBsAg PRISM (S/CO)	Anti-HBc AxSYM (S/CO)	Anti-HBs AxSYM	HBV DNA kvan. (Roche Amplicor Quant)
1 + (11·53)	+ (13·47)	+ (12·11)	− (0·09)	− (0·82)	+ (18·5)	− (0·29)	+ (0·061)	− (0·2)	Not detected
2 + (13·72)	+ (2·18)	− (0·32)	− (0·25)	− (0·27)	− (0·28) + (14·10)	− (0·33)	+ (0·051)	+ (41 IU/l)	Not detected
3 + (14·74)	+ (12·93)	+ (11·35)	− (0·27)	− (0·07)	+ (23·08)	− (0·28)	+ (0·059)	−	+ < 60 IU/ml
4 + (6·77)	+ (14·29)	+ (13·70)	− (0·33)	− (0·11)	+ (19·80)	− (0·26)	+ (0·063)	− (0·5)	+ 491 IU/ml

S/CO, signal/cat off; +, reactive; −, non-reactive.

Question 4

Look-back studies in recipients transfused in the past with blood components from occult HBV-infected donors are in progress. Thus far, there are no available results.

Question 5

We plan to do the subtyping and sequence analysis of the occult HBV-infected donations. At the moment, there are no results available.

Question 3

From 22 OBIs, the results of other HBV markers were: HBcAb - positive, HBsAb - positive in 16 cases and HBcAb - positive, HBsAb - negative in six cases [1,2].

In eight of these 22 OBIs, in which DNA HBV was detected by NAT, the results of nested PCR were positive in four cases and negative in the other four cases [2]. The sensitivity of this nested PCR is 180 IU/ml. In other six from these 22 OBIs [1], the DNA level was around 25 IU/ml.

Question 4

Look-back studies are not systematically performed. Two recipients transfused with one red cell concentrate and one platelet concentrate from donors with OBI were negative for HBcAb 1 year later transfusion [1]. There was also no evidence of transmission in a liver transplant patient that received a red cell concentrate from a donor with OBI [3].

Question 5

According to the results from a recent work [4], four donors, all infected with HBV genotype D, presented multiple scape mutations in the S region of the viral genome. In another work [2], two donors were infected by HBV A genotype (adw2 subtype) and one donor was infected by HBV D genotype, ayw3 subtype.

References

1 Sauleda S, Maymó RM, Piron M, Candotti D, Allain JP, Hernández JM, Puig Ll, Casamitjana, N: Serological and molecular markers of hepatitis B virus in Spanish blood donors: evidence of occult Hepatitis B. Vox Sang 2006; 91 (Suppl. 3):63
2 González R: Prevalence of Hepatitis B Virus Infection in Blood Donors of Madrid. Molecular Epidemiology of the Virus. Madrid, Universidad Complutense, 2007 (in Spanish)
3 Ontañon A, Arroyo JL, Romon I, Amunarriz C, Hermosa V: Which individual donation (ID)-NAT test algorithm has to be followed for optimal specificity and maximum recognition of blood donors with occult HBV infection? Vox Sang 2007; 93 (Suppl. 1):159
4 Torres P, Nieto S, García-Carpio R, Ruiz M, Rodriguez MA, Barbolla L, Zaajier H, Koppelman M, Lelie N: Occult HBV infections in the acute and chronic stage interdicted by transcription mediated amplification (TMA) testing of small donor pools. Vox Sang 2007; 93 (Suppl. 1):138

M. Alvarez
Centro de Transfusión de Alicante
Ctra. N-332
Km 113
03550 San Juan
Alicante
Spain
E-mail: alvarez_man@gva.es

S. Sauleda
Banc de Sang i Teixits
P. Vall d'Hebron 119-129
08035 Barcelona
Spain

R. González
Centro de Transfusión de la Cruz Roja
C/ Juan Montalvo, n° 3.
28040 Madrid
Spain

C. Niederhauser & M. Stolz

Question 1

At present, the routine screening algorithm for HBV in blood donations in Switzerland relies solely on serological tests for HBsAg. Because it is well-known that the risk of transfusion-transmitted HBV still remains significant despite these tests, a study was designed to determine the prevalence of anti-HBc 'confirmed' positive donations and to evaluate whether routine anti-HBc and/or HBV NAT assays may provide additional safety to blood products [1]. For this purpose, 18 143 blood donations were screened initially for HBsAg, anti-HBc and with HBV NAT in minipools of 24 donations [detection limit: 240 copies (45 IU)/ml per individual donation]. Those samples that were repeatedly reactive in the initial anti-HBc assay were verified with two additional anti-HBc assays, as well as, with an anti-HBs assay and in single-donation HBV NAT [detection limit: 12 copies (2·4 IU)/ml per individual donation].

In our study, no occult HBV-infected donor was detected. Of the 262 'confirmed' anti-HBc-positive donations none were positive for HBV DNA in single donation PCR assay. In contrast to our findings, it has been shown that occult hepatitis cases can be detected in the donor population [2,3]. If a look-back procedure is taken as a basis for determining the occurrence of transfusion-transmitted hepatitis B infection, none appears to have occurred as a consequence of an occult HBV case in Switzerland within the last 10 years.

Question 2

In our study, screening in minipools of 24 donations was performed. One millilitre (1 ml) was used for the nucleic acid extraction procedure. Extraction was performed with the QIAamp 96 Virus BioRobot testkit (Qiagen, Hilden, Germany) adapted for use on a Tecan pipetting machine. Fifty microlitre of DNA extract was mixed with 50 µl of prepared mastermix and subjected to PCR, followed by DNA detection on the COBAS Amplicor test system (Roche Diagnostics, Rotkreuz, Switzerland).

Commencing in July 2007, we will begin a follow-up study whose aim is to screen for HBV infection in individual donations with the Tigris Procleix system over the following 1.75 years. It is estimated that 300 000 donations will be screened, which accounts for approximately 50–60% of the total Swiss donations during this time period.

Question 3

As stated above, as yet no occult HBV cases have been observed in Switzerland. However, an effort was recently initiated to search for such cases by screening anti-HBc-positive but HBsAg-negative blood donations for the presence of HBV DNA. Seventy-four such donations were identified and tested in single donation HBV NAT, as well as further serological tests. One donation from a repeat donor was HBV DNA positive

though probably with a low viral load. The quantification was not performed as not enough material was available. The anti-HBs concentration was > 1000 IU/ml and the anti-HBc was confirmed with two additional anti-HBc assays targeting different regions of the HBc antigen. Subsequently, the samples from the existing serum bank were followed up. In total 21 consecutive serum bank samples from this donor were screened for HBV NAT, HBsAg, anti-HBc and anti-HBs. The last 11 samples were all anti-HBc reactive and had anti-HBs titres of > 1000 IU/ml. Besides the last donation, three additional samples of the 21 were also HBV DNA positive. From the corresponding four erythrocyte concentrates, two were not transfused to patients and two were sent abroad within a humanitarian project. Unfortunately, we were incapable of obtaining information on the recipients of these blood products.

In conclusion, the introduction of HBV NAT in minipools shows only marginal benefits as the test is too insensitive. This is the case in both the early, as well as in the chronically carrier phase of the infection. If, on the other hand, the introduction of anti-HBc testing is chosen as an additional marker in the blood donation screening, it is imperative that more specific anti-HBc assays are developed in order to prevent an unacceptably high deferral rate of anti-HBc 'false'-positive donors.

Question 4

Generally speaking, in Switzerland it is relatively easy to obtain data for look-back procedure from the side of blood transfusion services, as it is mandatory to store at least 0·9 ml plasma at −30 °C of each donation for a minimum of 5 years. However, in the hospitals the situation is quite the opposite. Here unfortunately rarely are any pre- and post-operation samples available. In Switzerland, during the last 10 years proven HBV transfusion-transmitted cases have rarely been observed. In the majority of these cases, the donors had a very recent HBV infection and therefore transfusion-transmitted infections were most likely due to window cases.

Question 5

So far, we have had insufficient sample material from the donor and in particular the recipient to perform an adequate DNA analysis and subtyping of these HBV cases. During our ongoing study over the next 1.75 years, one of our major goals will focus on the collection of enough material from recipients who later exhibited signs of a recently acquired HBV infection.

References

1 Niederhauser C, Graziani M, Stolz M, Tinguely C, Mansouri Taleganhi B, Schneider P: Blood donor screening: how to decrease the risk of transfusion transmitted hepatitis B virus? *Swiss Medical Weekly* (in press)
2 Glynn SA, Kleinman SH, Wright DJ; Busch MP for the REDS group: International application of the incidence rate/WP model. *Transfusion* 2002; 42:966–972
3 Allain JP: Occult hepatitis B virus infection. *Transfus Clin Biol* 2004; 1:18–25

C. Niederhauser
M. Stolz
Blood Transfusion Service SRC Berne
Murtenstrasse 133
3008 Berne
Switzerland
E-mails: christoph.niederhauser@bsd-be.ch;
martin.stolz@bsd-be.ch

J.-P. Allain & S. Owusu-Ofori

HBV transmission remains the higher risk of viral transmission to blood component recipients in the UK; a risk estimated to 1 : 60 000–250 000 [1]. Since the mid-1990s, data indicating that the prevalence of anti-HBc was approximately 0·4% have been provided as well as evidence that screening for this serological marker would be effective because most transmitted HBV infections were retrospectively found originating from donations carrying this marker [2]. As a result, screening for anti-HBc has been discussed for many years, but is still not implemented. Clearly, screening for such marker would not cover the presumably rare cases of HBV window period whose incidence has not been evaluated [1]. However, the final decision for anti-HBc screening is imminent.

Because the alternative to anti-HBc screening to improve the HBV blood safety is NAT, this option has been examined. There are two commercial triplex assays simultaneously detecting the genomes of HCV, HIV and HBV at a cost not significantly superior to HCV alone or HCV and HIV NAT as currently partially implemented in England. However, these assays are applied to pools of 48 plasma samples and there is substantial evidence indicating that, in such conditions, the HBV DNA yield would be minimal if any improvement to blood safety, inferior to anti-HBc. While the cost-effectiveness of anti-HBc makes the health authorities reluctant to implement this assay, the option of NAT screening in individual donations or in pools of six samples is clearly more expensive and not an option at the present time.

More interesting to the discussion is the collaborative pilot studies and ultimately routine implementation of an in-house triplex NAT in a high endemic country of West Africa in a 900-bed teaching hospital regional blood centre in Ghana. Ghana is one of the countries of the world where HBV infection prevalence is the highest as HBsAg is present in 15% of the population by age 16. By age 40, nearly 100% of the population carry anti-HBc, indicating contact with the virus [3]. Preliminary studies have shown that the HBV genotype E viral load distribution was massively skewed towards

low values, below 10E4 IU/ml, prolonged by a tail of occult HBV at a frequency of 1 : 62 blood donations [4]. The vast majority of these occult infections carried a viral load below 500 IU/ml, making it difficult to detect in plasma pools of any size. It was shown that pools of 10 plasma samples failed to detect approximately 50% of occult HBV present in individual plasma samples [4]. More recently, we have further studied nine cases of occult HBV genotype E and found that approximately 50% of them circulated in donors carrying anti-HBs, the rest carrying anti-HBc only [5]. At present, there is no compelling evidence that occult HBV with viral load below 100 IU/ml in the presence of anti-HBc only or anti-HBc and anti-HBs be infectious. There are no published cases of transmission by transfusion when anti-HBs is present and very few in case of anti-HBc only [6,7].

As a result of these preliminary studies and infectivity data from the literature, the Komfo Anokye Teaching Hospital transfusion committee proposed to the administration of the hospital to implement in-house triplex NAT for HCV, HIV and HBV in pools of 10 plasmas at a cost of $ 4/unit increasing the cost of a blood unit supported by patients from $12 to $16. This proposal was accepted and screening of donations collected after pre-donation screening with rapid tests for anti-HIV, anti-HCV and HBsAg yielded a negative result was implemented in February 2005 [5]. Two senior members of the blood centre staff had received a specific training for 2 and 4 weeks, respectively, at the Cambridge molecular virology laboratory. Supply of equipment and reagents was ensured by the Cambridge blood centre, although these costs were included in the cost per unit and supported by the hospital.

Between February 2005 and December 2006, 18 400 units have been screened. Twenty-one of 1840 pools reacted for HBV DNA of which 10 were confirmed by identifying a single infected unit. Seven of the positive units corresponded to testing error and three to occult HBV. The yield compared to EIA screening was therefore 1 : 6133 units but the safety gain was 1 : 1840. The viral load of these occult HBV has not been quantified.

The clinical relevance of HBV genomic screening, contrary to HIV and HCV RNA, needs to be confronted to the HBV condition of recipients. In Kumasi, 31% of the blood usage is paediatric (mostly for acute primary malarial infection), 80% before age 5. In this age range, susceptibility to developing chronic HBV infection is > 50% and preliminary data indicate that 75% of these children have not been in contact with HBV. As a result, approximately 45% of the transfused children receiving an HB-infected transfusion will develop chronic hepatitis B. In adults, approximately 15% of recipients do not carry HBV markers and should be considered at risk of acute infection and subsequent recovery. It can be concluded that by identifying 10 infectious units identified, two

to three chronic infections are averted. The cost-effectiveness of this result is being calculated.

The local conditions in Ghana prevent the follow-up of recipients unless they return to the hospital. There is, therefore, no look-back possible although, in the context of the BOTIA European study aiming to constitute a large donor-recipient sample repository, samples from Ghana are included and will provide some data in the future. The genotype of HBV in Ghana, HBsAg positive or occult has been determined and 95% are genotype E, the remaining 5% being genotype A1 or D. In the sequencing analyses, a surprisingly high frequency of abnormal core mutations was found, preventing the translation of normal capsid protein. In an area where HBV vaccine was very rare before the systematic vaccination of neonates in 2003, there was no evidence of mutants in the 'a' region of the S protein, suggesting that escape mutation is not a mechanism of HBV persistence in these areas.

References

1 Soldan K, Gay NJ, Allain JP, Llewelyn C, Jones C, Reeves I, Ramsay M: The prevalence of hepatitis B infection in adults with no recognised increased risk of infection. J Infect 2000; 41:198–199

2 Allain JP, Hewitt PE, Tedder RS, Williamson LM: Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol 1999; 107:186–195

3 Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giachetti C, Shyamala V, Yeboah F, Anokwa M, Owusu-Ofori S, Opare-Sem O: The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. Blood 2003; 101:2419–2425

4 Owusu-Ofori S, Temple J, Sarkodie F, Anokwa M, Candotti D, Allain JP: Pre-donation screening of blood donors with rapid tests: implementation and efficacy of a novel approach to blood safety in resource-poor settings. Transfusion 2005; 45:133–140

5 Zahn A, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, Allain JP: Molecular characterization of occult hepatitis B strains in genotype E-infected subjects. J Gen Virol 2008: in press

6 Gerlich WH: Breakthrough of hepatitis B virus escape mutants after vaccination and virus reactivation. J Clin Virol 2006; 36 (Suppl. 1):S18–S22

7 Satake M, Taira R, Yugi I, Hino S, Kanemitsu K, Ikeda H, Todokoro K: Infectivity of blood components with low HBV DNA levels identified in a look back program. Transfusion 2007; 47:1197–1205

J.-P. Allain
Division of Transfusion Medicine
East Anglia Blood Centre
Department of Hematology
University of Cambridge
Long Road
Cambridge CB2 2PT
UK
E-mail: jpa1000@cam.ac.uk

Table 1 Observed microbial risks to blood safety in the UK TTIs reported to Serious Hazards of Transfusion (SHOT), 1995–2005

Year of transfusion	Pre-1997	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total	Deaths
Infection												
HAV	1 (1)	–	–	–	1 (1)	–	–	–	–	1 (1)	3	–
HBV	3 (3)	1 (1)	1 (1)	2 (3)	1 (1)	–	1 (1)	1 (1)	–	1 (1)	11	–
HCV	1 (1)	1 (1)	–	–	–	–	–	–	–	–	2	–
HIV	1 (3)	–	–	–	–	–	1 (1)	–	–	–	2	–
HEV							–	1 (1)	–		1	–
HTLVI	2 (2)	–	–	–	–	–	–	–		–	2	–
Bacteria	2 (2)	3 (3)	4 (4)	4 (4)	7 (7)	5 (5)	1 (1)	3 (3)		2 (2)	31	7
Malaria	–	1 (1)	–	–	–	–	–	1 (1)	–	–	2	1
vCJD/prion	1 (1)	1 (1)	–	1 (1)	–	–	–	–	–		3	–
Total	11 (13)	7 (7)	5 (5)	7 (7)	9 (9)	5 (5)	3 (3)	5 (5)	1 (1)	4 (4)	57	8

S. Owusu-Ofori
Transfusion Medicine Unit
Komfo Anokye Teaching Hospital
Kumasi
Ghana

R. Eglin

HBV NAT is not introduced in National Blood Service (NBS) UK at present.

Question 6

There are no plans to introduce HBV NAT screening of donors into the NBS UK. The reasoning behind this is that there have been very few TTIs identified with HBV in the UK over the past 10 years (see Table 1).

R. Eglin
National Blood Service
Colindale Avenue
London NW9 5BG
UK
E-mail: roger.eglin@nbs.nhs.uk

S. Stramer et al.

Question 1

Two HBV DNA tests were licenced by the Food and Drug Administration (FDA) since 2005 for potential screening of blood donors in the USA. However, FDA has not required or recommended HBV NAT screening, because studies have indicated that its implementation in the minipool testing format would result in only a marginal increase in blood safety compared to HBsAg testing for potentially infectious window-period donations [1,2] and because large-scale use in the individual donation testing format currently is not feasible. Additionally, anti-HBc testing has been in place for

transfusible blood in the USA since the mid-1980s limiting the estimated value of HBV DNA NAT for detection of otherwise 'occult', that is, HBsAg-negative, HBV in chronic infections.

The Roche COBAS AmpliScreen HBV test was licenced with a donor screening claim in April 2005, but, to date, the test has been implemented only by a small number of blood collection centers. The yield from the Roche extended clinical trials as well as their post licensure experience both demonstrated a yield of about 1 : 300 000 of HBV DNA positive, HBsAg and anti-HBc non-reactive donations. This yield has remained constant since licensure. However, with the more recent licensure and increasing use of more sensitive HBsAg assays, the HBV DNA-positive yield will require reassessment. In an attempt to determine the impact of more sensitive HBsAg testing, the available surplus HBV DNA positive yield samples from the use of the Roche assay have been tested by PRISM HBsAg; of a total of eight available samples, three (38%) were reactive and confirmed positive for HBsAg.

The Gen-Probe/Chiron Ultrio Assay (triplex for HIV RNA, HCV RNA and HBV DNA) was licenced in October 2006 for HBV DNA detection using the semi-automated (eSAS) platform, and for use on the automated TIGRIS platform in May 2007. The Ultrio Assay is not labelled for donor screening since 'yield' samples, that is, HBV DNA-positive samples that were negative for HBsAg and anti-HBc, were not detected in pre-licence clinical trials. Larger-scale studies are ongoing to determine whether 'yield' samples can be found when donors are screened with this assay in minipools of 4, 8 and 16.

Although HBV NAT testing of US blood donors is not routine, anti-HBc testing is performed by all US blood centers and several studies have been conducted in which sensitive HBV NAT has been performed on anti-HBc-reactive donations that lacked detectable HBsAg. These studies have yielded data regarding the frequency of so-called 'occult' HBV infections. Table 1 presents the results of the three published US studies, which detected HBV DNA in 0·4% to 1% of

Table 1 Detection of HBV DNA in HBsAg-negative/anti-HBc-positive blood donor units in three US studies [3]

Study	Number of donations tested	HBV DNA positive		Rate of HBV DNA positivity in otherwise suitable units	Follow-up	Reference
		n	%			
1	395	4[a]	1·01	1 : 49 000	None	[4]
2	3000	19	0·63	1 : 37 000	None	[5,6]
3	2900	12	0·41	1 : 48 942	Six (all individual donor NAT non-reactive; three anti-HBs positive)	[2]

[a]Only negative or low-level antibody to HBsAg (≤ 100 milli IU/ml) samples were tested for HBV DNA.

anti-HBc-only units yielding rates of OBIs ranging from 1 in 37 000 to 1 in 49 000 donations.

In addition to these published studies, since May 2004, the American National Red Cross (ARC) has tested every anti-HBc repeatedly reactive donation for HBV DNA. These studies either used the UltraQual HBV test at National Genetics Institute (NGI), either in minipools of 16 or individually (donation sensitivity ranged from 3·1 to 50 copies/ml), or more recently by testing each individual donation using the licenced Roche COBAS AmpliScreen HBV test and the 1 ml multiprep procedure (5 IU/ml or approximately 20 copies/ml sensitivity). Over the 3 years since this testing was implemented, approximately 63 000 anti-HBc-reactive donations have been tested for the presence of HBV DNA. Of those, 1080 (2%) were HBsAg and DNA positive, 1874 (3%) were HBsAg non-reactive but DNA reactive, and the remaining 59 797 (95%) were HBsAg and HBV DNA non-reactive (of which 12 584, or 21%, were from donors who were anti-HBc reactive on two occasions and therefore the donors were indefinitely deferred consistent with current FDA recommendations).

All HBsAg non-reactive donors were invited for follow-up testing. Of the DNA-positive donors at index, only 11% participated in follow-up studies and of those, 125 of 200 (62%) had serological evidence of past HBV infection with either persistent anti-HBc or anti-HBs reactivity; however, only three of the 125 still tested DNA positive for up to 4 months following the index donation. The remaining donors in follow-up had either received the HBV vaccine (11 of 200 or 6%) or had likely false-positive DNA results (64 of 200 or 32%). The source of false positivity was undoubtedly related to the mixing of HBsAg-confirmed positive samples with HBsAg-non-reactive samples during HBV DNA testing, because the false positivity was eliminated when the HBsAg-positive samples were no longer submitted for DNA testing. The HBV DNA-positive rate observed in anti-HBc-reactive donations in this study, adjusting for likely false positivity, is 0·96%, or 1 in 29 000 for otherwise suitable units (and is within the range of 0·4 to 1% and 1 : 37 000–1 : 49 000 reported in Table 1). Thus, this large-scale prospective study, together

with the three smaller studies, validates that approximately 1% of anti-HBc-reactive, HBsAg-negative donors are HBV DNA positive.

Of the DNA-negative donors at index, again only 10% participated in follow-up and of those, 815 of 1254 (65%) had serological evidence of HBV infection on follow-up, with one donor having DNA positivity at 4 and 6 months following index donation testing. The 10 to 11% participation rate in the follow-up studies reflects the lack of interest of donors in taking part in such studies, even though it might lead to their reinstatement.

Thus, following this extensive evaluation, approximately two-thirds of anti-HBc reactivity at index could be corroborated by HBV markers (either persistent anti-HBc or anti-HBs reactivity) observed at follow-up. However, only four of 1454 (0·3%) donors could be documented to have persistent OBI. Upon further testing of 410 retrieved plasma units from a sampling of the index donors who tested HBV DNA positive on pilot tube plasma from the index donation (not associated with the same donors who enrolled in follow-up studies), only 10 HBV DNA positive results could be reproduced, of which nine were HBV DNA reactive by multiple assays (NGI and Roche COBAS AmpliScreen, multiprep). (The other 400 plasma units could not be corroborated to have had HBV DNA positive results and these were likely to have been false positives.) This increased the rate of occult HBV in this population to 2·4%; of interest was that five of the 10 HBV DNA positive samples had reactivity when using an HBsAg assay (PRISM) with 10-fold increased sensitivity relative to the FDA licenced test originally used in the studies. (Additional ARC data are cited below as results of 'Study 4.')

Question 2

The Roche HBV test is licenced in the USA to screen individual samples and pools composed of equal aliquots of not more than 24 individual samples in conjunction with licenced HBsAg and anti-HBc tests. The Gen-Probe/Chiron Ultrio Assay is licenced in the USA to screen individual samples and pools composed of equal aliquots of not more than 16

individual samples in conjunction with licenced HBsAg and anti-HBc tests; this test does not have an HBV DNA screening claim in the USA because 'yield' samples (HBV DNA positive samples negative for HBsAg and anti-HBc) were not found in preapproval studies.

Question 3

Table 2 Additional HBV markers and viral load in HBsAg-negative/anti-HBc-reactive donations in the four US studies

Study	Additional HBV markers	Viral load (copies/ml)
1	n = 4; all anti-HBs-negative/low level positive; 1 of 4 HBsAg positive by more sensitive HBsAg assay (PRISM)	10-100
2	n = 19; 1 anti-HBc IgM positive; anti-HBs not tested	11 units < 100 8 units 100-500
3	n = 12; 3 anti-HBs positive; all negative using HBsAg EIAs in use at the time of study	≤ 1200 (11/12 individual donor NAT positive only)
4	n = 125 of 200 (62%) anti-HBc and/or anti-HBs reactive at follow-up; four with persistent DNA positivity at 4-6 months	< 100-300

Question 4
No lookback was performed in any of these studies.

Question 5
To date, no subtyping or sequencing data are available; collaborative studies are underway.

References
1 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; 43:788-798
2 Kleinman SH, Strong DM, Tegtmeier GG, Holland PV, Gorlin JB, Cousins C, Chiacchierini RP, Pietrelli LA: Hepatitis B virus (HBV) DNA screening of blood donations in minipools with the COBAS AmpliScreen HBV test. *Transfusion* 2005; 45:1247-1257
3 Kuhns MC, Busch MP: New strategies for blood donor screening for hepatitis B virus. *Molecular Diagnostic Therapy* 2006; 10:77-91
4 Kleinman SH, Kuhns MC, Todd DS, Glynn S, McNamara A, Demarco A, Busch MP: Frequency of HBV DNA detection in US blood donors positive for ant-HBc: implications for transfusion transmission and donor screening. *Transfusion* 2003; 43:696-704
5 Busch MP: Should HBV DNA NAT replace HBsAg and/or anti-HBc screening of blood donors? *Tranfus Clin Biol* 2004; 11:26-32
6 Stramer SL: Pooled hepatitis B virus DNA testing by nucleic acid amplification: implementation or not. *Transfusion* 2005; 45:1242-1246
7 Stramer SL, Foster GA, Notari EP: HBV DNA PCR positive rates of antibody to hepatitis B core (anti-HBc) repeat reactive (RR) donors: implications for reentry protocols. *Transfusion* 2006; 46(9S):21A

R. Biswas
FDA
HFM-100
1401 Rockville Pike
Rockville
MD 20852-1448
USA
E-mail: robin.biswas@fda.hhs.gov

C型肝炎に関する最近の情報

国立感染症研究所ウイルス第二部第四室
主任研究官　相崎 英樹

鈴木哲朗[1]，多田有希[2]，岡部信彦[2]，脇田隆字[1]
1) 国立感染症研究所ウイルス第二部，2) 同感染症情報センター

はじめに

1988年，米国カイロン社が輸血後非A非B型肝炎の原因ウイルスとして，C型肝炎ウイルス（HCV）の遺伝子のクローニングに成功した。その後，このウイルスに対する各種診断技術が開発され，血液スクリーニングに導入されたため，輸血によるC型肝炎の発生は激減した。しかしながら，現在我が国には150万人以上，全世界にも約1.7億人もの感染者が存在すると推定されており，肝硬変，肝癌と進行する可能性もあり，公衆衛生上最も重要な病原ウイルスの1つである。本稿では，C型肝炎に関する最近の話題とそれの理解に必要な病気の特徴について述べたい。

C型肝炎の最新の話題

最近，（1）薬害C型肝炎訴訟と（2）肝炎患者の個人情報が厚労省の地下倉庫に放置されていた，という2つの血液製剤によるC型肝炎問題が連日ニュースで大きく取り上げられている。（1）の薬害C型肝炎訴訟は，HCVに汚染された血液製剤フィブリノーゲンを止血剤として投与されたことで，HCVに感染したとして患者171人が国と製薬会社を相手取って総額100億円を超える損害賠償を求めた訴訟であり，2002年10月に東京・大阪から始まり，全国の5ヵ所の集団訴訟に広がった。5つの地裁とも判決は製薬会社の責任を認め，このうち4つの地裁では国の責任も指摘されている。（2）は，2002年8月に血液製剤フィブリノーゲンを投与されC型肝炎に感染した可能性がある418人のリストを旧三菱ウエルファーマ（現田辺三菱製薬）が厚労省に報告したものの，厚労省と製薬会社のいずれも患者本人に検査や治療を呼びかけてこなかったことから，「治療機会を奪われた」ということで問題となっている。最近，こうした状況下で大阪高裁が患者と国・製薬会社の双方に和解を勧告したことから，与野党がそれぞれウイルス性肝炎治療の患者支援策を打ち出し，一気に長年の懸案解決に向けた動きが始まっている。

疫 学

我が国のC型肝炎，肝硬変，肝癌患者の数は約50万人，一方症状が現れていない「無症候性キャリア」は110～140万人と推定されている。全国の日赤血液センターにおける初回献血者のデータに基づく2000年時点のHCV抗体陽性率は，年齢が上がるとともに増え，60～69歳で3.38%である。

HCVの感染経路としては，感染血液の輸血，経静脈的薬物濫用，入れ墨，針治療，観血的医療行為などが考えられる。母子感染は妊婦がHCV-RNA陽性の場合，出生児が感染する確率は10%程度と言われている。また，血液透析に伴うHCV新規感染の発生は平均年率2%程度の頻度あるといわれ，歯科診療における潜在的な感染の可能性も示唆されている。ニュースで取り上げられている血液製剤フィブリノーゲンを投与された約28万人のうち感染者は約1万人と推計されている。我が国のC型肝炎患者のうち，輸血歴を有するものは3～5割程度にすぎず，多くの患者で感染経路は不明である。

HCV感染に伴って急性肝炎を発症した後，30～40%ではウイルスが検出されなくなり，肝機能が正常化するが，残りの60～70%はHCVキャリアになり，多くの場合，急性肝炎からそのまま慢性肝炎へ移行する。慢性肝炎から自然寛解（治療なしでウイルスが消える）する確率は0.2%と非常に稀で，10～16%の症例は初感染から平均20年の経過で肝硬変に移行する。肝硬変の症例は，年率5%以上と高率に肝細胞癌を発症する。肝癌死亡総数は年間3万人を越え，いまだに増加傾向にあるが，その約8割がC型肝炎を伴っている。

現行のスクリーニングシステム実施下では，輸血その他の血液製剤による新たなC型肝炎の発生は限りなくゼロに近づいている。現在，米国では薬物濫用者を中心に年間25,000人の新たなHCV感染者が発生しているが，日本ではHCVによる新たな急性肝炎の発症は2001年以降年間40～70人程度と大変少なく抑えられている。以上から，国のC型肝炎対策の基本は，多くの国民に対してC型肝

炎ウイルス検査を行い，早期に感染の有無を確認し，感染者に対して適切な治療を行うことと考えられている。さらに，上記のような病気について正しい知識を普及させることは，感染者の就業・入所・入学等に伴う偏見・差別等を防ぐためにも重要である。

臨床症状

C型肝炎では全身倦怠感に引き続き，比較的徐々に食欲不振，悪心・嘔吐，右季肋部痛，上腹部膨満感，濃色尿などが見られるようになる。一般的に，C型肝炎ではA型やB型肝炎とは異なり，劇症化することは少なく，黄疸などの症状も軽い。慢性肝炎ではほとんどが無症状で，倦怠感などの自覚症状を訴えるのは2～3割にすぎない。肝硬変で非代償期まで進行すると黄疸，腹水，浮腫，肝性脳症による症状である羽ばたき振戦（手指が震える），意識障害などが出現するようになる。肝細胞癌を合併すると，初期は無症状であるが末期になると肝不全に陥り，他の癌と同様に悪液質の状態となる。以上のように，C型肝炎の問題点は症状が全くない潜伏期間が20～30年に及ぶこともあるため，治療の機会がなく悪化させるケースが少なくないことである。

診断

C型肝炎のもうひとつの問題点は，HCVに感染していても肝機能検査では正常を示すことが多いことである。そこで，HCV感染の有無を判定する方法としては，HCV血清抗体の検出と核酸・抗原の検出の2種類が用いられている。一般的には，初めにHCV抗体検査が行われる。この抗体検査で陽性となった場合，（1）HCVに感染しているキャリア状態，（2）過去に感染し，現在ウイルスは排除された状態，の2つの可能性が考えられる。このようなHCVキャリアと感染既往者とを適切に区別するため，HCV-RNAの検出を行う。また，急性C型肝炎においてもHCV抗体の陽性化には感染後通常1～3ヵ月を要するため，この時期の確定診断にはHCV-RNA定性検査が行われる。

治療

2002年に発足したC型肝炎等緊急総合対策では，保健所，老人保健，政府管掌健康保険等による肝炎ウイルス検査を導入し，ハイリスクグループ（1992年以前に輸血を受けた者，輸入およびそれと同等のリスクを有する非加熱血液凝固因子製剤を投与された者，1994年以前にフィブリノーゲン製剤（フィブリン糊を含む）を投与された者，大きな手術・臓器移植を受けた者，薬物濫用者，入れ墨・ボディピアスをしている者，その他過去に健康診断等で肝機能異常を指摘されているにも関わらず，その後肝炎検査を実施していない者等）を重点対象としつつ，一定年齢以上の全ての国民を対象にC型肝炎検査を行う体制が構築された。しかしながら，健診の受診率がそれほど高くなく，実際老人保健事業のC型肝炎ウイルスの節目検診で25～30％の受診率であった。また，その検診で要精密となった者のうち実際に二次医療機関を受診したのは8割程度，さらに，二次医療機関でも専門の医療機関を受診された方はその約半分という状況で，健診と治療連携における課題がある。

C型肝炎の治療の中心はインターフェロン（IFN）である。従来の単独投与に加え，2001年からリバビリンとの併用療法に医療保険が適用されるようになり，2002年からIFNの保険適用上の投与期間の制限が撤廃，2003年からペグインターフェロン，2005年からIFN自己注射承認，2006年から代償性肝硬変もIFNの適応，と治療法は年々進歩している。一般に，IFNによってHCVが排除されるのは30％程度，リバビリンとの併用療法の場合で約40％と言われているが，IFN療法でウイルスを排除できなかった場合でも，肝炎の進行を遅らせ，肝癌の発生を抑制，遅延させる効果が期待できる。

以上のように治療法は進歩しているが，実際には適切な治療法が選択されない場合や中断してしまうという問題点がある。そこで，二次医療圏に1ヵ所程度の専門医療機関，都道府県に1ヵ所の肝疾患診療連携拠点病院というものを置き，かかりつけ医と専門医療機関の連携を進め，治療水準の均てん化（全国どこでも一定以上の同じ水準の治療を受けられること）を計りつつ，高度専門的集学的な治療を適応できる医療機関の確保を目指している。また，IFN治療は，治療費が月に約7万円と高額で患者にとって大きな負担になっているが，IFN治療で癌患者が減り医療費抑制にもつ

ながることが知られているので，検査費だけでなく治療費にも公的助成することが検討されている。

まとめ

ここ十数年の間にHIV，HCV，SARS等が新たに発見され，さらに現在鳥インフルエンザの脅威が叫ばれている。HIV，HCVでは血液製剤による感染を防ぐ有効な対策が講じられるのが遅れ，被害が広がったと問題になっている。また，分子生物学の発達によって新たな治療法の開発が試みられている。最近では，インフルエンザ治療薬タミフルもその副作用から「薬害」ではないかと問題視されている。このように生命科学や医学研究の発達によって，より医療の現場は一層複雑になって

きている。一方，今後も未知の感染症や治療法の想定外の副作用などが発生する恐れもあり，そのような状況で我々は如何に準備していけば良いか，大変難しい問題である。筆者は医学生時代に「病気を診ずして病人を診よ」と教わってきたことを覚えている。これは，医療者は疾病そのものの診断や治療だけにとらわれることなく，病をもっている人の心の痛みをよく理解し，患者さんを全人的に診て治療することの重要性を訴えているものである。国，製薬会社，医療関係者，マスコミなどが患者の視点に立ってこのような精神を実践することで，新しい事態により早く的確に対応できると思われる。

第15回日本CT検診学会学術集会

イノベーション：CT検診
～肺がん早期検出から禁煙支援へ，さらにメタボ対策まで～

1．会期： 2008年2月15日（金）・16日（土）
2．会場： 亀戸文化センター・カメリアホール　東京都江東区亀戸2-19-1カメリアプラザ5階
　　　　　　 TEL: 03-5626-2121（代表）　FAX: 03-5626-2120
3．会費： 参加費　5,000円　　懇親会　3,000円
4．学術集会：
　　1）シンポジウム　　『コンピュータ支援診断とCT検診』・『禁煙支援とCT検診』・
　　　　　　　　　　　　『肺がんCT検診のための人材確保：教育・認定』
　　2）パネルディスカッション　　『特定健診および特定保健指導とCT検診』
　　3）教育講演　　　　『内臓脂肪蓄積とがんのプロモーション』（仮題）
　　4）一般演題・ポスター展示
　　　　肺がん検診成績・肺がん検診精度・アスベスト関連，小型肺がんの診断および治療・呼吸
　　　　器以外のCT検診，医用工学・CAD・認定制度など
5．懇親会： 2008年2月15日（金）18:00～

- -

詳細は学会ホームページをご覧下さい。
　　会長　中川　徹
　　　　株式会社日立製作所・日立健康管理センタ　放射線診断科
　　　　〒317-0076　茨城県日立市会瀬町4-3-16　TEL：0294-34-1020　　FAX：0294-35-7100
　　　　E-mail：tohru.nakagawa.rh@hitachi.com　学会ホームページhttp://www.jscts.org

ELSEVIER

Hepatitis C viral life cycle ☆

Tetsuro Suzuki *, Koji Ishii, Hideki Aizaki, Takaji Wakita

Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

Abstract

Hepatitis C virus (HCV) has been recognized as a major cause of chronic liver diseases worldwide. Molecular studies of the virus became possible with the successful cloning of its genome in 1989. Although much work remains to be done regarding early and late stages of the HCV life cycle, significant progress has been made with respect to the molecular biology of HCV, especially the viral protein processing and the genome replication. This review summarizes our current understanding of genomic organization of HCV, features of the viral protein characteristics, and the viral life cycle.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Hepatitis C virus; Translation; Polyprotein processing; RNA replication; Viral assembly

Contents

1. Introduction

Since its discovery in 1989, representing a turning-point in the search for infectious agents associated with post-transfusion non-A, non-B hepatitis, hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease and affects approximately 200 million people worldwide at the present time [1–3]. Persistent infection with HCV is associated with the development of chronic hepatitis, hepatic steatosis, cirrhosis, and hepatocellular carcinoma [3–8]. In general, people with chronic hepatitis C are relatively asymptomatic and have few, if any, clinical manifestations prior to the development of cirrhosis.

HCV is a small, enveloped RNA virus belonging to the *Hepacivirus* genus of the *Flaviviridae* family, which also includes several classical flaviviruses, including dengue virus and yellow fever virus, as well as pestiviruses, such as bovine viral diarrhea virus and the unassigned GB viruses [9,10]. This review summarizes our current understanding of genomic organization of HCV, as well as features of the viral protein characteristics, and the viral life cycle.

2. Genomic organization

The HCV genome consists of a single-stranded positive-sense RNA of approximately 9.6 kb, which contains an open reading frame (ORF) encoding a polyprotein precursor of approximately 3000 residues flanked by untranslated regions (UTRs) at both ends [11]. The precursor is cleaved into at least 10 different proteins: the structural proteins Core, E1, E2 and p7, as well as the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B (Fig. 1).

An important feature of the HCV genome is its high degree of genetic variability [12,13]. Mutation rates, however, vary in different regions. The E1 and E2 regions are the most variable, while the 5'UTR and terminal segment of the 3'UTR have the highest degree of sequence conservation among various isolates. The 5' UTR, which is ~341 nucleotide (nt) in length, contains an

internal ribosomal entry site (IRES), which is essential for cap-independent translation of viral RNA, from which four highly structured domains (domains I-IV) are produced (Fig. 1) [14–19]. These are largely conserved among HCV and related viruses [15,16]. As with other RNA viruses with IRES-mediated expression, the HCV 5'NTR is thought to contain determinants for translation, as well as cis-acting elements for RNA replication. It has been shown that (i) the sequence upstream of the IRES is essential for viral RNA replication, (ii) sequences within the IRES are required for high-level HCV replication, and (iii) the stem–loop domain II of the IRES is crucial for replication [20]. A recent study has revealed that the 5'UTR is capable of binding to a liver-specific microRNA, miR-122, resulting in enhanced HCV RNA replication [21]. (Fig. 2).

The 3'UTR varies between 200 and 235 nt in length, including a short variable region, a poly(U/UC) tract with an average length of 80 nt, and a virtually invariant 98-nt X-tail region [22–24] The X region forms three stable stem–loop structures that are highly conserved among all genotypes and, as a result, the HCV genome likely ends with a double-strand stem structure. It appears that the 3'X region, as well as the 52 nt upstream of the poly(U/C) tract, are crucial for RNA replication, while the remainder of the 3'UTR plays a role in enhancement of replication [25,26].

To date, hepaciviruses are divided into six principal genotypes of HCV that differ in their nucleotide sequences by 31–34%, and in their amino acid sequences by ~30%. HCV, like many other RNA viruses, circulates in infected individuals as a population of diverse but closely related variants referred to as quasispecies [12]. HCV heterogeneity is primarily due to a high error rate of the RNA-dependent RNA polymerase encoded by the NS5B gene. The existence of different quasispecies of the HCV genome appears to contribute to viral persistence. It has been shown that patients with chronic hepatitis C have greater genetic complexity in terms of the population of quasispecies they possess than patients with spontaneous clearance [13]. During the course of chronic infection, random genetic drift steadily induces the development of quasispecies primarily due to changes in the
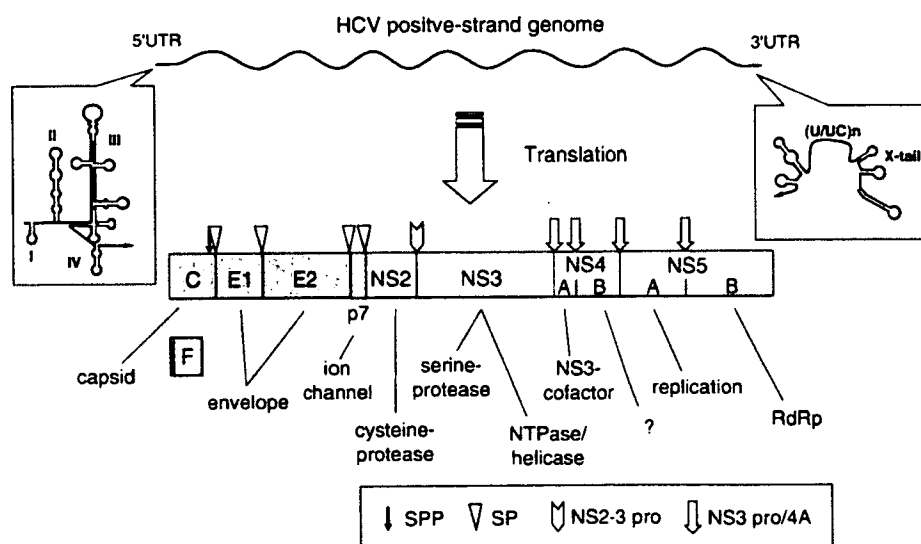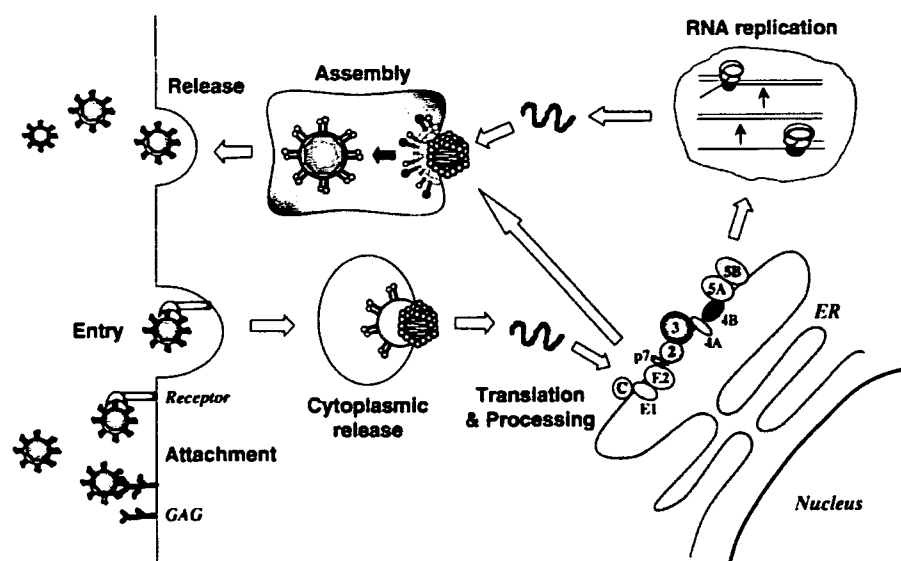

Fig. 1.

Fig. 2.

hypervariable region 1, involving the 27 N-terminal 27 residues of the E2 envelope protein [27–29].

3. Features of the viral proteins

3.1. Core protein

The HCV core protein, which is derived from the N-terminus of the polyprotein, most likely forms the viral nucleocapsid given similarities between its position and that of sequences encoding viral nucleocapsids in other flavivirus genomes. The amino acid sequence of the core protein is highly conserved among different HCV strains, compared with other HCV proteins. HCV core protein has been extensively used in a number of serologic assays since anti-core antibodies are highly prevalent among HCV-infected individuals. Although several core proteins of varying molecular weights have been identified [30–33], the core protein is released as a 191-residue precursor of 23 kDa and further processing yields the predominant form of 21 kDa. The N-terminal domain of the core protein is highly basic, while its C-terminus is hydrophobic. Several groups have reported a complex intracellular localization of the core protein [30,33–42]. The core protein is primarily detected in the cytoplasm, in association with the endoplasmic reticulum (ER), lipid droplets, and mitochondria. In some studies, a fraction of the core protein has also been found in the nucleus.

The ubiquitin–proteasome pathway, a major route by which selective protein degradation occurs in eukaryotic cells, is involved in post-translational modification of the core protein [32,43–45]. An initial report indicated that processing at the carboxyl-terminal hydrophobic domain of the core protein produced efficient polyubiquitylation and proteasomal degradation [32]. Recently, ubiquitin ligase E6AP has been identified as an HCV core-binding protein that enhances ubiquitylation and degradation of mature, as well as carboxyl-terminus truncated-core protein, and it has been suggested that E6AP-dependent degradation of the core protein is common to a

variety of HCV isolates and plays a critical role in the HCV life cycle [45].

The core protein is likely multifunctional and essential for viral replication, maturation, and pathogenesis. It is involved not only in formation of the HCV virion, but also has a number of regulatory functions, including modulation of signaling pathways, cellular and viral gene expression, cell transformation, apoptosis, and lipid metabolism [reviewed in 46].

3.2. E1 and E2 envelope proteins

The E1 and E2 proteins are essential components of the virion envelope and are necessary for viral entry. These glycosylated proteins extend from aa 192–383 (E1) and from aa 384–746 (E2) of the polyprotein, and have molecular weights of 33–35 and 70–72 kDa, respectively [47]. Along the precursor polyprotein, it has been suggested that the C-terminal transmembrane domains of E1 and E2 form hairpin structures that pass through the membrane twice, thereby allowing processing by a signal peptide in the ER lumen [48]. Upon signal peptidase cleavage, the C-termini are thought to translocate into the cytoplasm in order to generate the type I membrane topology of mature E1 and E2. Mature E1 and E2 remain noncovalently associated, interacting in part through their C-terminal transmembrane domains, which also mediates retention of the E1–E2 complex in the ER. It has recently been demonstrated that, in addition to this conventional type I membrane topology, E1 protein also adopts a polytopic topology, in which the protein twice spans the ER membrane with an intervening cytoplasmic loop spanning aa 288–360 [49].

3.3. p7 protein

The p7 protein is a small (63 aa) hydrophobic polypeptide that adopts a double membrane-spanning topology. This protein is essential for the production of infectious virions in vivo [50] and may belong to a small protein family of viroporins, which are known to enhance membrane permeability. It has been revealed