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Genotyping of Hepatitis C Virus in South Africa

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The six major hepatitis C virus genotypes were investigated by using samples from 79 seropositive and PCR-positive blood donors from three different regions of South Africa as well as 9 patients with chronic renal failure, 19 with liver disease, and 23 with hemophilia. PCR products of the genome were typed by restriction fragment length polymorphic analysis by *Rsa*I-*Hae*III and *Mva*I-*Hin*fI double digestion. Type 5 occurred in 40% of this population group; type 1 occurred in 33%; and types 2, 3, and 4 were found in 13.8, 7.7, and 2.3%, respectively.

The major etiological agent of posttransfusion and community-acquired non-A non-B hepatitis has been identified as hepatitis C virus (HCV) (6, 10). On the basis of sequence homology, this single-stranded positive-sense RNA enveloped virus has been provisionally classified as a separate genus of the family *Flaviviridae* (13). Although the disease associated with HCV may be benign, persistent infection may lead to liver cirrhosis and hepatocellular carcinoma (18). Comparative sequence analysis of complete HCV genomes (16) and PCR fragments from various genomic regions has shown that HCV can be grouped into distinct but related genotypes. At present, six major genotypes with numerous subtypes have been described (20). Recently, Tokita et al. (25) identified an additional three genotypes. Some genotypes have been associated with severity of disease (17) and responsiveness to interferon therapy (9, 26). The distribution of genotypes varies according to geographical area, with types 1a, 1b, 2a, 2b, and 3a being found predominantly in Europe, the United States, and the Far East (1, 4, 11, 12, 22, 24), while types 4, 5, and 6 are apparently confined to Central and North Africa and the Middle East (1, 2, 21), South Africa (1, 2, 14), and Hong Kong (1, 12, 21), respectively. The recently described types 7, 8, and 9 have thus far been found only in Vietnam (25).

This study was prompted by reports of a new genotype in South Africa, namely, type 5a, and aimed to determine the prevalence of this 5a genotype in the country.

Serum samples were collected from seropositive and PCR-positive individuals, including 36 blood donors from the Natal Blood Transfusion Service serving the Kwa Zulu-Natal region, 29 donors from the South African Blood Transfusion Service serving the former Transvaal region, and 14 donors from the Western Province Blood Transfusion Service. Serum samples were also collected from 9 patients with chronic renal failure attending two hemodialysis units in Cape Town, 23 patients with hemophilia from Red Cross Children's War Memorial Hospital and Groote Schuur Hospital in Cape Town, and 19 patients with liver disease attending the Liver Clinic at Groote Schuur Hospital.

Serum and plasma samples were screened for HCV antibodies with one of three different second-generation enzyme-linked immunosorbent assay systems, Ortho 2.0 (Ortho Diagnostic Systems, Inc., Raritan, N.J.), Abbott 2.0 (Abbott Laboratories, North Chicago, Ill.), or UBI (United Biomedical, Inc., Hauppauge, York, N.Y.). The choice of assay system was determined by the center from which the samples originated.

RNA was extracted from 200 μl of serum or plasma by the guanidinium isothiocyanate-phenol-chloroform method (5) with minor modifications. A combined reverse transcription-linked PCR was used to amplify cDNA. Five microliters of RNA was added to a thin-walled 250-μl tube (Perkin-Elmer Corp., Norwalk, Conn.) containing 1× PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$) and 0.2 μM outer primers for the 5′ noncoding region (NCR) (3). The tube was heated to 95°C for 5 min and was cooled to 20°C before the addition of 200 μM (each) dATP, dCTP, dTTP, and dGTP; 1.25 U of *Taq* DNA polymerase (Boehringer Mannheim, GmbH, Biochemica, Mannheim, Germany); 100 U of Moloney murine leukemia virus reverse transcriptase (Gibco BRL Life Technologies, Ltd., Paisley, Scotland), and 10 U of RNasin (Promega Corp., Madison, Wis.). Reverse transcription was performed at 42°C for 45 min before denaturation at 92.5°C for 20 s. Thereafter, amplification was performed for 35 cycles in a Perkin-Elmer 9600 thermal cycler, with cycling temperatures and times of 50°C for 30 s, 72°C for 45 s, and 92.5°C for 20 s. A final extension step of 72°C for 7 min was also included. Three microliters of the PCR product from the outer reaction was removed and transferred to a thin-walled tube containing fresh reagents and 1.2 μM inner primers (3) and was subjected to a further 35 cycles of amplification. The PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The size of the PCR product was 251 bp.

HCV typing was performed by the method of McOmish et al. (12). Briefly, 5-μl aliquots of the nested PCR product were digested with (i) 1 U each of *Rsa*I and *Hae*III and (ii) 1 U each of *Mva*I and *Hin*fI (Boehringer Mannheim, GmbH, Biochemica) in the appropriate buffer for 2 h at 37°C. The digested products were separated by electrophoresis on a 13% polyacrylamide gel at 8 W for 2 h. The DNA fragments were visualized by silver staining, which is a sensitive and rapid method and obviates the need to use a radioactively labelled PCR product (19) (Qiagen protocol; Qiagen Inc., Chatsworth, Calif.). The banding patterns were compared with those described by McOmish et al. (12).

Figure 1A shows the banding pattern consistently produced by type 5. Type 1 produced two different electropherograms (Fig. 1A): the pattern corresponding to McOmish bA was most commonly found, while that of McOmish aA was seen in only one case. Type 2, 3, and 4 cleavage patterns are seen in Fig. 1B.

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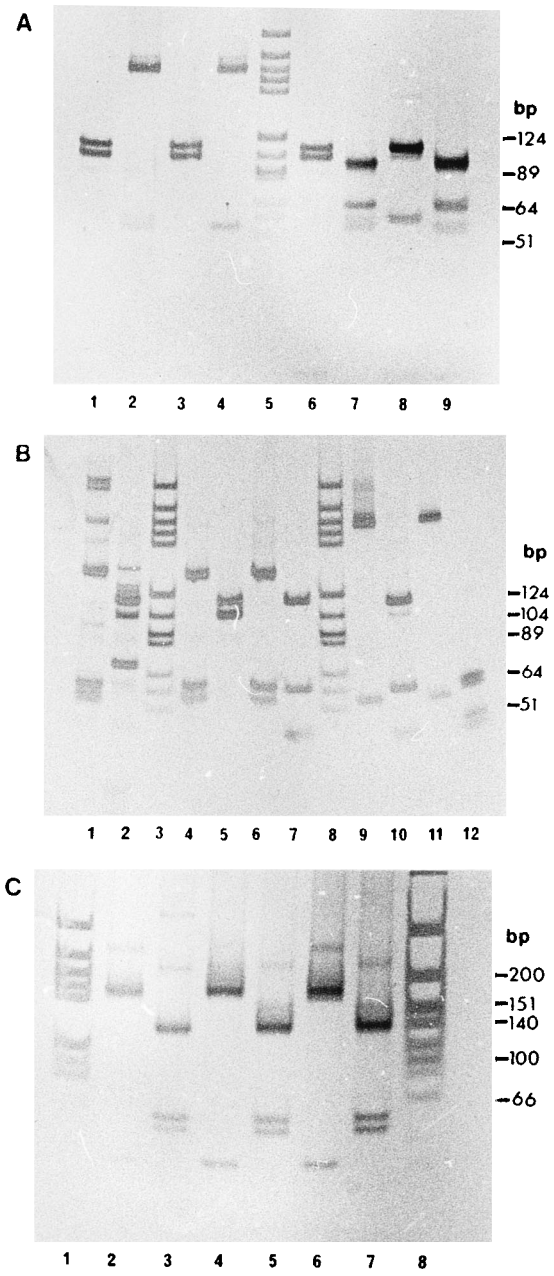

FIG. 1. Genotyping of HCV by RFLP analysis with visualization of DNA products by silver staining. (A) HCV type 5 (lanes 1 to 4) and type 1 (lanes 6 to 9). Lanes 1, 3, 6, and 8, *Rsa*I-*Hae*III double digests of the 5′ NCR PCR product; lanes 2, 4, 7, and 9, *Mva*I-*Hin*fI double digests of the PCR product; lane 5, DNA molecular weight marker V (Boehringer Mannheim). (B) HCV type 3 (lanes 1 and 2), type 4 (lanes 4 to 7), and type 2 (lanes 9 to 12). Lanes 1, 4, 6, 9, and 11, *Mva*I-*Hin*fI double digests of 5′ NCR PCR products; lanes 2, 5, 7, 10, and 12, *Rsa*I-*Hae*III double digests of 5′ NCR PCR products; lanes 3 and 8, DNA molecular weight marker V (Boehringer Mannheim). (C) Unknown genotype. Lanes 2, 4, and 6, *Rsa*I-*Hae*III double digests of the 5′ NCR PCR products (172, 44, 26, and 9 bp); lanes 3, 5, and 7, *Mva*I-*Hin*fI double digest of the 5′ NCR PCR product (142, 58, and 53 bp); lane 1, DNA molecular weight marker V (Boehringer Mannheim); lane 8, ϕX174 DNA-*Hin*fI marker (Promega). DNA product sizes are marked at the right of each panel.

Samples from four patients (three of whom were blood donors) which were typed with *Mva*I-*Hin*fI as either type 3 or 4 showed an unusual restriction pattern on digestion with *Rsa*I-*Hae*III (Fig. 1C). It was therefore not possible to distinguish between these types. McOmish et al. (12) and Dusheiko et al. (7) have reported similar anomalous restriction fragment length polymorphism (RFLP) banding patterns for a few patients from Egypt and Iraq. Bands produced by *Rsa*I-*Hae*III digestion were larger than expected because of the loss of a normally conserved *Rsa*I site within the 5′ NCR with an A → T or A → C change at position −176 (12). Sequence analysis of cloned PCR products from three of the four cases identified in this study confirmed the loss of the *Rsa*I site. However, the base change at this site was a T → A substitution at position −177 (results not shown). Further sequence analysis of other regions of this genome needs to be done to determine if it is a subtype of type 3 or 4, or an entirely new type.

Of the 130 samples examined, 51 (39%) were identified as type 5 and 43 (33%) were identified as type 1 (Table 1). These two genotypes represent 72% (57 of 79) of the samples from the blood donor population and 89% (17 of 19) of the samples from patients with liver disease. Thus, type 5 may be associated with severe liver disease, as has been found with type 1b (17). However, many of the other known genotypes have also been found in patients with severe disease (8) and the prevalence of type 5 may simply reflect the predominant genotype of the region.

Until recently, type 5 was reported to occur only in South Africa, but Stuyver et al. (23) have now identified type 5 in Gabonese and Benelux serum samples. Thus, the distribution of type 5 may not be as restricted as previously reported. It is noteworthy, however, that no type 5 was found in samples from Egypt (1), Zaire (12, 20), and Burundi (22). In all of these countries, type 4 was most commonly found, indicating its widespread distribution in North and Central Africa. We have shown that type 4 rarely occurs in South Africa (3 of 130 samples) (Table 1). No blood donors were infected with type 4, and patients with chronic renal failure, who received on average 22 blood transfusions, were infected with types 1 and 5 only, reflecting the predominance of these genotypes in the blood donor population in South Africa. Type 4 was seen only in the hemophiliac population. The reason for this is unclear but may be related to the number of pooled blood products administered to this group.

Type 1 was found to be the second most common genotype in South Africa (33%) (Table 1). Previous studies have shown that type 1 has a global distribution (8) but, with the exception of one case in Ghana (7), has not been found in Africa. Ours is the first report of a high prevalence of type 1 in Africa. The introduction of type 1 into South Africa may have been brought about by travel of infected individuals between Europe and the United States and South Africa or importation of blood products from these countries. The recent description of types 7, 8, and 9 indicates over 96% homology in the 5′ NCR to type 1 (although other regions of the genome are very different) (25), which could result in the generation of the same RFLP pattern as type 1. However, type-specific PCR (15) of a random selection of samples identified in this study by RFLP analysis as type 1 showed all to be either type 1a (57 bp) or type 1b (144 bp). The identification of type 1 by RFLP probably reflects the true prevalence of this genotype in the region.

In this study, HCV types 2 and 3 were found in 13.8 and 7.7% of cases, respectively (Table 1). Although there was an apparent higher prevalence of type 3 in Kwa Zulu-Natal blood donors (Natal Blood Transfusion Service, 17%) than in blood donors from the northern and southwestern parts of the country (South African Blood Transfusion Service, 3%; Western Province Blood Transfusion Service, 7%), this difference was not statistically significant.

It has previously been shown that patients infected with type

TABLE 1. Distribution of HCV genotypes in the different risk groups

Risk group[a]	No. of samples	No. (%) of samples with HCV genotype						
		1	2	3	4	5	6	Unknown
Blood donors								
WPBTS	14	4 (28.5)	1 (7.1)	1 (7.1)	0	7 (50)	0	1 (7.1)
NBTS	36	11 (30.5)	4 (11.1)	6 (16.6)	0	13 (36.1)	0	2 (5.5)
SABTS	29	11 (37.9)	5 (17.2)	1 (3.4)	0	11 (37.9)	0	1 (3.4)
Patients								
CRF	9	5 (55.5)	0	0	0	4 (44.4)	0	0
Haemo	23	4 (17.4)	7 (30.4)	2 (8.6)	3 (13.4)	7 (30.4)	0	0
CLD	19	8 (42.1)	1 (5.2)	0	0	9 (47.4)	0	1 (5.2)
Total	130	43 (33)	18 (13.8)	10 (7.7)	3 (2.3)	51 (39.2)		5 (3.8)

[a] CLD, chronic liver disease; Haemo, hemophiliac; CRF, chronic renal failure; WPBTS, Western Province Blood Transfusion Service; NBTS, Natal Blood Transfusion Service; SABTS, South African Blood Transfusion Service.

1 respond poorly to therapy compared with those infected with type 2 (9, 26). Since type 5 obviously has a high prevalence in the South African community, it will be important to determine the response of this genotype to interferon therapy. Further, in order to determine if type 5 can be considered an African genotype (23) like type 4, prevalence studies in other African countries, especially in southern Africa, need to be undertaken.

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