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Three Cases of Infection with Hepatitis C Virus Genotype 5 among Brazilian Hepatitis Patients

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During the course of routine genotyping of hepatitis C virus isolates by 5′ noncoding region sequencing, three samples were found to bear genotype 5-specific nucleotides. A serotyping method was subsequently applied and confirmed the finding. This is the first report of the occurrence of genotype 5 in Brazil.

Genotyping of hepatitis C virus (HCV) isolates is a tool that has been applied for epidemiological studies; this technique has indicated that genotype 1 shows a poorer response to interferon therapy than do other genotypes (8). This observation provided the basis for the introduction of HCV genotyping into the management of infected patients by providing ancillary information for therapeutic strategies.

São Paulo is the largest and most populated city in Brazil and has a prevalence of HCV of 1.8% (5). Since São Paulo is a cosmopolitan city that had in the past and is still constantly receiving groups of immigrants from all over the world, it would be conceivable to suppose that a broad representation of HCV genotypes would be found there. However, the distribution of HCV genotypes actually observed resembles that described for the United States and other, eastern countries, explicitly, a predominance of genotype 1 followed by genotype 3 and a small percentage of genotype 2. A distinctive feature is a high prevalence of genotype 3, responsible for about 30 to 40% (1) of hepatitis C cases. Recently, the occurrence of genotype 4 was also described for this population (1).

During the course of a routine analysis of HCV carriers, we detected three samples displaying a pattern of 5′ noncoding region (NCR) motifs compatible with genotype 5 infection (12). Patient 1 (C2943) is a woman born in Brazil in 1950. Anti-HCV antibody was first detected through a blood donation in 1999. In October 2001, the HCV load was 77,770,900 IU/ml; liver enzymes have always been within normal limits. She never received antiviral medication or left the country and denies a history of blood transfusion, tattooing, piercing, intravenous drug use, or other risk factors for HCV acquisition.

Patient 2 (C2434) is a man born in Brazil in 1950. He never left the country and denies a history of blood transfusion, tattooing, piercing, intravenous drug use, or other risk factors for HCV acquisition. Patient 3 (C2072) is a man born in Brazil in 1944. He never left the country and also denies a history of blood transfusion, tattooing, or piercing. He has been working as a carpenter for many years and reports having experienced a few accidents involving bleeding.

Sequencing of the 5′ NCR of HCV has become the "gold standard" for HCV genotyping. We perform this test by amplifying by reverse transcription-PCR almost the entire 5′ NCR with primers HC11 and HC18 (10) and by dideoxy cycle sequencing with a Cy5-labeled internal primer (Cy5 Thermosequenase core sequencing kit; Visible Genetics Inc., Toronto, Ontario, Canada). Sequencing products are run on an automated DNA sequencer (Long Tower; Visible Genetics). Sequence alignment is performed by using CLUSTAL W, available at the site http://www.clustalw.genome.ad.jp/. In order to confirm our DNA sequence genotyping results, serotyping was performed by use of a commercial assay (HCV Serotyping 1-6 Assay; Murex, Dartford, England).

The presence of anti-HCV antibody was assessed by use of a commercial third-generation assay (HCV EIA 3.0; Abbott, Abbott Park, Ill.), and immunoblotting (Riba HCV 3.0 SIA; Chiron Co., Emeryville, Calif.) was also performed. Viral load was determined by quantitative reverse transcription-PCR (HCV Monitor 2.0; Roche, Basel, Switzerland).

All three samples were reactive in standard anti-HCV serologic and immunoblotting tests; reactivity to the different antigenic fractions is shown in Table 1. Since genotype 5 has never been described in Brazil and is only rarely found outside South Africa, we attempted to confirm this finding by a distinct approach, i.e., serotyping by the Murex HCV assay, which is based on nonstructural protein 4 (NS4)-derived antigens. All

TABLE 1. Reactivity of the three putative genotype 5 samples on immunoblotting[a]

Patient	Immunoblotting result for:			
	c100, 5-1-1	c33c	c22	NS5
C2072	4+	4+	4+	−
C2434	2+	4+	4+	3+
C2943	3+	4+	4+	−

[a] c100, 5-1-1, c33c, c22, and NS5 are antigenic fractions. +, score of band intensity ranging from 1 to 4+; −, negative.

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FIG. 1. Alignment of the 5′ NCR sequences (positions −258 to −32) of the genotype 1a HCV prototype HC-J1 (GenBank accession number D10749), the three putative cases of genotype 5 (GenBank accession numbers AY033767 [C2072], AY033768 [C2434], and AY033769 [C2943]), and a genotype 5 isolate from Belgium (BE95; GenBank accession number L29581). At position −108, HC-J1 has a C instead of a T, which is commonly observed in most HCV isolates of all genotypes. Thus, this mutation (T→C) does not constitute a genotype 5-specific motif but constitutes an isolate-specific variation. Asterisks indicate sequence identity, and gaps indicate sequence differences.

three samples were assigned to genotype 5 by this test too. Sequence alignment of the corresponding 5′ NCR sequences against an HCV genotype 1a prototype (Fig. 1) depicts positions that are genotype 5 specific (12). The possibility of contamination is weak, since we use rigorous anticontamination measures, samples were processed with a very large time interval between them, position −236 is an A for sample C2943 and a T for the other two samples, and there are other, distinct nucleotides at positions upstream from the aligned region (data not shown).

We present here the first description of HCV genotype 5 in Brazil. The occurrence of this genotype in persons unlinked epidemiologically to South Africa, the region with the greatest incidence of genotype 5, raises the question of the source of contamination. In common with Canadian genotype 5 HCV-infected blood donors, the Brazilian patients investigated here were all more than 40 years old (9). Interestingly, in contrast to these genotype 5 cases, with no evident links to South Africa, three of four genotype 4-infected patients seen at our laboratory are originally from the Middle East, where genotype 4 is largely predominant

Genotype 5 is rarely found outside South Africa but has been described for 2 of 6,807 chronic hepatitis C patients in the United States (3) and for 16 of 358 viremic individuals in Montreal, Quebec, Canada (2). It has also been sporadically found in Belgium (13), The Netherlands (7), and Saudi Arabia (11). Thus, it seems that genotype 5 has a worldwide distribution but, except in South Africa, is always a minor component of the HCV population. Description of this rare HCV genotype in Brazil has implications for future developments of serologic and molecular screening tests, which must include a genotype 5 challenge, as the pathogenicity of this genotype is well documented (4, 6, 9).

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