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Species-Specific Variants of GB Virus A in Captive Monkeys

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Sequences from the putative 5′ nontranslated region of GB virus A were isolated from mystax, owl monkeys, and tamarins. Though sequences of isolates from each animal species are virtually identical at the nucleotide level (95%), isolates from different species are dramatically different (52 to 79% identical) and genetically cluster on this basis.

The GB hepatitis agent was initially characterized in nonhuman primates inoculated with the blood serum of a 34-year-old surgeon experiencing acute clinical hepatitis (2). Following 12 serial animal passages, two distinct viral genomes were cloned from the serum of an animal with acute hepatitis (9). On the basis of genome size and organization, GB virus A (GBV-A) and GBV-B appear to be unique members of the *Flaviviridae*, most closely related to, but distinct from, the hepatitis C virus (HCV) group (4). Subsequent studies have demonstrated that GBV-B, in the absence of GBV-A, can induce hepatitis in the tamarin animal model (7). However, no significant elevations in serum liver enzyme levels were noted in tamarins singly inoculated with GBV-A, despite persistence of the virus in serum (7). Additionally, several animals were found to have a variant of GBV-A present in their serum prior to inoculation. Sequences within the putative 5′ nontranslated region (NTR) of this variant were virtually identical to one another, though they were only 79% identical to those in the original GBV-A isolate (8). In the present study, we have identified GBV-A variants in mystax (GBV-A_{mx}), owl monkeys (GBV-A_{tri}), and tamarins (GBV-A_{lab}) not known to have been inoculated with an infectious agent. These sequences are 52 to 79% identical at the nucleotide level to GBV-A and genetically cluster on the basis of the primate species from which they were isolated.

Blood sera from *Saguinus labiatus* (tamarins), *Saguinus mystax* (mystax monkeys), and *Aotus trivirgatus* (owl monkeys) were used as source material for nucleic acids. Briefly, 25- to 50-μl volumes were extracted with the DNA/RNA isolation kit (U.S. Biochemicals, Cleveland, Ohio) or the QIAamp HCV kit (Qiagen Inc., Chatsworth, Calif.). Random hexamer-primed nucleic acids were reversed transcribed as directed by the manufacturer with the RNA PCR kit (Perkin-Elmer, Foster City, Calif.) in a volume equal to that of the initial serum. Five microliters of the reverse transcription reaction mixture was used in a 25-μl PCR mixture with 2.0 mM $MgCl_2$ and 0.5 μM (each) oligonucleotide primer (8): 5′vA-s (5′-AGGGTTCGT AGGTGGTAAATCCC-3′) and 5′vA-a (5′-TGCCACCAGG GGTCACCCGAAG-3′). Amplification was performed by "touchdown" PCR (6) for 43 cycles (94°C, 20 s; 55°C, 30 s, decreasing by 0.3°C per cycle; 72°C, 60 s), followed by 10 cycles (94°C, 20 s; 40°C, 30 s; 72°C, 60 s) and a final extension of 72°C for 10 min. PCR products were gel isolated with the QIAEX gel extraction kit (Qiagen) and sequenced with the Applied Biosystems model 373 DNA sequencer. Pairwise nucleotide sequence identities were determined with the PILEUP program of the Wisconsin Sequence Analysis Package (version 8, September 1994; Genetics Computer Group, Madison, Wis.), while the phylogenetic tree was constructed using the GeneWorks software package (version 2.45; IntelliGenetics, Mountain View, Calif.).

Preinoculation sera obtained from tamarins, mystax, and owl monkeys were used as a source of nucleic acids for reverse transcription. For PCR amplification, an oligonucleotide primer pair which has been previously shown to be capable of detecting sequences within the putative 5′ NTR of GBV-A variants was used (8). PCR fragments were successfully isolated from six tamarins, two mystax, and seven owl monkeys. By nucleotide comparison analyses, it was found that sequences isolated within an animal species were virtually identical (>95%) to one another (Table 1). In contrast, sequences from different animal species were significantly different (63 to 71% identity), and each of these sequences is distinct from the original GBV-A isolate, having only 61 to 75% identity within the region. Further, each of these sequences was drastically different from GBV-C (45 to 56% identity), the closest genetic relative of GBV-A (3). A phylogenetic tree generated from a multiple sequence alignment demonstrates the clustering observed in the numerical analysis in that sequences isolated from the various monkey species cluster on distinct branches of the tree (Fig. 1). The GBV-A and GBV-C sequences are the most dissimilar, while GBV-A and GBV-A_{lab} sequences are the most similar.

GBV-A variants are found in various monkey species not inoculated with infectious materials. Additionally, tamarins naturally infected with GBV-A_{lab} appear to be at least partially resistant to experimental infection with GBV-A. Of four tamarins inoculated with H205 (a source of GBV-A and GBV-B), all became infected with GBV-B, while only one became dually infected with GBV-A and GBV-B (7). Each of the three animals which appeared to be resistant to GBV-A infection had GBV-A_{lab} present in the serum prior to inoculation. In contrast, a tamarin harboring GBV-A_{lab} became infected with both GBV-A and GBV-B after being experimentally inoculated with an additional passage of H205. Thus, it may be that marginal protection against GBV-A is provided by GBV-A_{lab}, though animals challenged with high-titer GBV-A do become infected. Also of note is that infection with GBV-A_{lab} was not clinically apparent and the animals did not have elevated alanine amino transferase levels. This is also the case in tamarins

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TABLE 1. Nucleotide sequence identity of GBV-A variants

Isolate	% Identity with[a]:				
	GBV-A_{mx}	GBV-A_{tri}	GBV-A_{lab}	GBV-A	GBV-C
GBV-A_{mx}	98.79	67.31 ± 0.94 (65.7–68.8)	63.25 ± 0.65 (62.3–64.3)	61.76 ± 0.58 (61.4–62.2)	49.58 ± 0.42 (49.3–49.9)
GBV-A_{tri}		95.34 ± 4.30 (87.2–99.7)	71.02 ± 12.67 (67.9–73.2)	68.48 ± 0.67 (67.4–69.3)	56.83 ± 11.16 (55.5–58.4)
GBV-A_{lab}			95.76 ± 15.10 (93.0–98.7)	75.93 ± 0.53 (75.3–76.6)	51.51 ± 0.85 (50.1–52.6)
GBV-A					45.40

[a] Mean identities were calculated from pairwise nucleotide sequence identities as described in the text. Values in parentheses are ranges of pairwise identities.

experimentally infected with GBV-A alone (7). These data further support the conclusion that GBV-A is of nonhuman primate origin (8) and does not induce hepatitis in these animals.

The divergence observed between GBV-A variants from the different monkey species is significant. Though isolates obtained from the animals within a species are virtually identical, drastic differences are observed between isolates from different species (Table 1). On the basis of criteria for distinguishing HCV types from subtypes (1), each species-specific isolate would be considered a distinct GBV-A type (approximately 30% nucleotide divergence), except in the case of GBV-A_{lab}

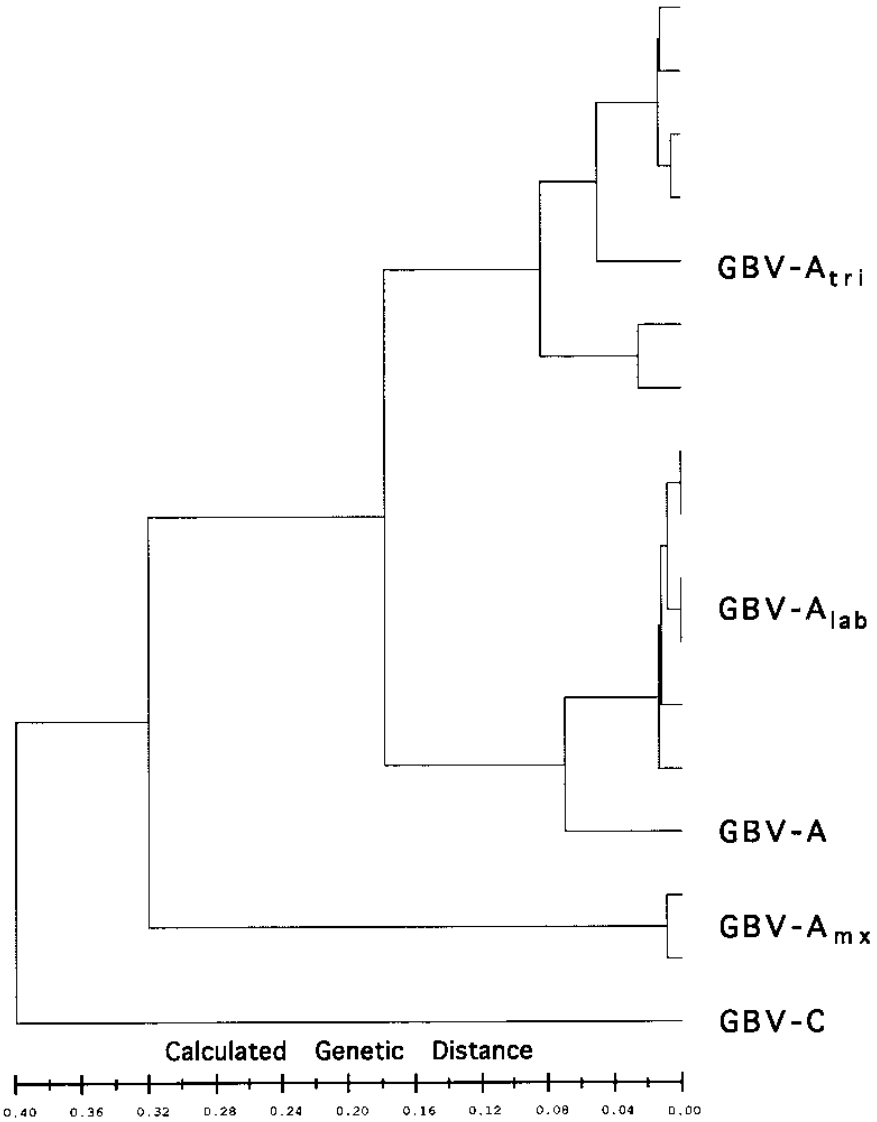

FIG. 1. Phylogenetic tree of species-specific GBV-A variants. Putative 5′ NTR sequences isolated from owl monkeys, tamarins, and mystax were aligned with GBV-A and GBV-C and the phylogenetic tree was constructed by the unweighted pair-group method with arithmetic means as described in the text. The calculated genetic distance is the number of substitutions per position in the multiple sequence alignment.

and GBV-A; GBV-A_{lab} would be considered a subtype of GBV-A (approximately 20% nucleotide divergence). An important distinction is that these criteria for HCV were established on the basis of comparative analyses within coding regions of the virus, whereas the sequences in the current analysis are from the putative 5′ NTR of GBV-A. The observed differences within the HCV 5′ NTR are not sufficient for reliable genotypic discrimination (1) and are at most 9% divergent. This is in contrast to the apparent genotypic distinctions that have been made within the 5′ NTR of GBV-C, though the maximum sequence divergence in this particular instance is approximately 17% (5). Regardless of genotypic distinction, the GBV-A variants from different monkey species are remarkably different and cluster on this basis (Fig. 1). Extension of these species-specific GBV-A variants to genome length will provide valuable insight into any further diversity between these viruses, as well as the original GBV-A isolate.

Nucleotide sequence accession number. The nucleotide sequences of GBV-A and GBV-C can be accessed in GenBank under U22303 and U36380, respectively.

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