Dominant glycoprotein epitope of four corners hantavirus is conserved across a wide geographical area

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A newly identified hantavirus, tentatively called Four Corners virus (FCV), was found to be the aetiological agent of a 1993 outbreak of hantavirus pulmonary syndrome (HPS) in the southwestern United States. Immunodominant epitopes of 43 and 31 amino acids were identified in the nucleocapsid protein and G1 glycoprotein, respectively. The G1 genes of different hantaviruses are highly divergent, suggesting that geographically diverse FCVs might fail to cross-react owing to antigenic drift. We now show that the immunodominant epitope of G1 is conserved among 18 FCVs from a broad geographical area, despite extensive nucleotide sequence heterogeneity. Antibodies from all 45 HPS patients, separated by more than 3000 km were shown to be reactive with the dominant G1 epitope. Evidence for limited cross-reactivity between the G1 antigen of a novel hantavirus of the cotton rat and that of FCV is presented.

Introduction

Hantaviruses comprise a genus of minus-sense RNA viruses of the family Bunyaviridae. There are at least six distinct and well-characterized groups of hantaviruses worldwide (Chu et al., 1994; Xiao et al., 1994; Yanagihara & Gajdusek, 1987). Each is primarily associated with a single species of rodent. Human infection and disease result when aerosols of rodent excreta are accidently inhaled. Hantaan virus (HTNV) and Seoul virus (SEOV) are aetiological agents for haemorrhagic fever with renal syndrome (HFRS). HTNV is associated with field mice of the genus Apodemus, whereas SEOV is associated primarily with the commensal rat Rattus norvegicus. Puumala virus (PUUV) causes nephropathia epidemica, a mild form of HFRS. Prospect Hill virus (PHV) has not been associated with human infection or disease. PUUV and PHV occur in voles of the genus Clethrionomys and in Microtus pennsylvanicus, respectively.

HFRS is an acute grippe-like illness characterized by high fever, myalgia, proteinuria, renal failure, cardiovascular instability, thrombocytopenia, leukocytosis, and haemorrhagic manifestations such as petechiae and scleral haemorrhages. The most severe form of HFRS is that caused by HTNV, with a mortality of approximately 5 to 20%.

A new form of hantavirus disease known as hantavirus pulmonary syndrome (HPS) was recognized in 1993 (Centers for Disease Control and Prevention, 1993a, b; Nichol et al., 1993). The aetiological agent was shown to be genetically (Nichol et al., 1993; Hjelle et al., 1994a) and serologically (Jenison et al., 1994) distinct from previously described hantaviruses. HPS has a mortality of approximately 60% (Centers for Disease Control and Prevention, 1994a). Unlike HFRS, renal disease and haemorrhage are absent or mild in HPS, while pulmonary oedema and cardiovascular instability are prominent (Duchin et al., 1994). Cases of HPS have been reported throughout the United States, but the states of the Four Corners region (New Mexico, Arizona, Colorado, and Utah) have had the highest caseload. Four Corners was also the site of the outbreak that led to the recognition of HPS (Centers for Disease Control and Prevention, 1993a, b). The causative virus is known commonly as the Four Corners virus (FCV), although names for specific isolates (e.g. Muerto Canyon virus, Convict Creek virus) have also been proposed. We will refer to the HPS virus as FCV pending the acceptance of another name. The predominant host for FCV is the deer mouse Peromyscus maniculatus, but high prevalence has also been observed in the related species P. truei (Nerurkar et al., 1994; Childs et al., 1994).

Cases of HPS caused by viruses with sequence similarity to FCV have been identified within the range of P. maniculatus in 14 western states (Centers for
Disease Control and Prevention, 1994a; Hjelle et al., 1994b). A possible additional non-fatal case of HPS, believed to have been caused by a novel hantavirus of the cotton rat *Sigmodon hispidus*, occurred in Florida (Centers for Disease Control and Prevention, 1994b). Definitive evidence that the Florida illness was caused by an acute hantavirus infection was lacking because no viral sequences could be amplified from the case-patient’s blood, and no IgM response could be documented at that time against any hantavirus.

The genomes of hantaviruses are made up of the three RNA segments L, M and S (Elliott et al., 1991). The L segment encodes the transcriptase. The M segment encodes the envelope product, which is cotranslationally processed into the glycoproteins G1 and G2. The S segment encodes the nucleocapsid protein N. The latter is relatively conserved among different hantaviruses. One consequence of that conservation is that antibodies directed against the N protein are usually reactive with a variety of hantavirus species (Zöller et al., 1989; Wang et al., 1993b). Thus, serological assays based solely upon nucleocapsid antigens are unable to readily distinguish among closely related hantavirus species.

IgG antibodies to the G1 protein of FCV are universally present by the onset of pulmonary disease in patients with HPS (Jenison et al., 1994). Such antibody reactivity maps to a single 31 amino acid epitope of FCV G1 that shares only 13 and 15 amino acids with its nearest relatives PUUV and PHV, respectively. As a result, the PUUV and PHV G1 proteins are not reactive with antibodies from patients with HPS, in a Western blot. The high interspecies divergence of the G1 gene suggests that genetic drift among geographically diverse FCVs could result in important antigenic variation in the dominant epitope of G1.

### Methods

**Patient and rodent samples.** Serum (1 to 5 ml), lung tissue, and blood clot samples (1 to 5 g) were obtained at autopsy from patients with suspected HPS from: McKinley and Cibola Counties, New Mexico; Apache and Navajo Counties, Arizona; Montezuma County, Colorado; Kootenai County, Idaho, and from Glacier County, Montana. All patient blood samples used in this study were from case-patients confirmed to have HPS by: (i) presence of IgM and IgG antibodies to FCV N protein and IgG antibodies to the FCV G1 glycoprotein on Western blot (Jenison et al., 1994); and/or (ii) presence of FCV RNA in peripheral blood or autopsy tissue by reverse transcription (RT)–PCR analysis (Nichol et al., 1993; Hjelle et al., 1994c). Acute and convalescent serum samples from a Florida case-patient with suspected HPS were generously provided by T. Ksiazek and C. J. Peters. Solid tissues were stored at −70 °C until RNA was extracted from 200 to 500 mg tissue by standard protocols (Hjelle, 1994a). Serum samples were made 0.02% in sodium azide and stored at 4 °C. *P. manieulatus* tissue samples were obtained from wild caught animals sacrificed for plague and hantavirus surveys in Siskiyou County, Orange County, and Channel Island (Santa Barbara County, California, and from Apache County, Arizona, or from Glacier County, Montana. Animals from California were placed individually into double 1 pint freezer bags and frozen. Methods for processing rodent tissues were as described in another study involving rodent specimens from southern California (M. J. Turell, G. W. Korch, C. A. Rossi, D. Seshne, B. Enge, D. Dondero, M. Jay, G. Ludwig, D. Li, C. Schmaljohn, R. Jackson & M. Ascher, unpublished). After thawing, an aliquot of 20 to 250 μl of pulmonary tissue/blood was withdrawn by syringe after puncture of the bag and the thoracic cavity with an 18-gauge needle. The supernatant fraction (5 μl) was used at a 1:400 dilution in Western blot assays to detect antibodies against FCV N protein (Jenison et al., 1994).

For animals from Arizona and Montana, lung or liver tissues were obtained from sacrificed animals and stored frozen. A cardiac blood sample or tissue sample supernatant was used for Western blot analysis. Immune complexes were detected using a 1:1000 dilution of alkaline phosphatase-conjugated antibody to IgG of *P. leucopus* (Kirkegaard and Perry; see below for Western blot methods). The remaining portions of the tissue sample from seropositive animals were used as a source of RNA for subsequent RT–PCR studies.

**RT–PCR.** The RNA from about 5 mg of tissue was used for each RT–PCR reaction. Nested RT–PCR assays were performed as described (Hjelle, 1994a). Antisense primers and the outer sense primer were designed from FCV sequences previously reported (Hjelle, 1994a; GenBank accession U02471). The inner sense primer was designed from unpublished FCV sequences (B. Hjelle, F. Chavez-Giles, N. Torrez-Martinez, T. Yamada & S. Jenison, unpublished). The inner primers were designed to amplify a portion of the G1 glycoprotein (amino acids 1 to 107) that spans the G1 epitope of FCV identified previously (amino acids 58 to 88 of FCV). Outer primers had the sequence 5' TAGTAGTAGACTCCGAGAAGA 3' (sense) and 5' GGAGGAATATACATGTGCTTT 3' (antisense). After an initial reverse transcriptase step of 42 °C for 1 h, thermal cycling was performed at 94 °C, 42 °C and 72 °C for 1 min, 1 min and 1.5 min each for 30 cycles in a Perkin-Elmer model 480 thermal cycler. Three μl of the 100 μl RT–PCR reaction mixture was then used as the template for the inner primers 5' TAAAGGCTGCAGAGAATGGTAGGGTGG- GTTTGATC 3' (sense) and 5' AATTCTGATATCCATTTTGT 3' (antisense). Underlined bases comprised added restriction recognition sites for *PvuI* and *HhaI*. The bases shown in bold type in the sense primer comprise the initiating methionine codon. Cycling conditions were identical to those listed above. The 320 bp product was visualized by agarose gel electrophoresis, and the cDNA was excised from the gel. The nucleotide sequence was determined by the dideoxynucleotide method (Sequenase, U.S. Biochemicals).

**Western immunoblots.** Western blots were prepared with the recombinant G1 antigen encoded by the *rep* expression constructs pFCV-M-1275 and pFCV-M-CEx316 (Koerner et al., 1991; Jenison et al., 1994). The former encodes FCV G1 amino acids 31 to 451. The *Trp E* fusion protein encoded by pFCV-M-CEx316 is the smallest in a series of 3' to 5' nested deletions of the FCV G1 that retained antigenic activity (amino acids 31 to 88). It includes the 31 amino acids that comprise the dominant epitope of the G1 glycoprotein (amino acids 58 to 88). A Trp E protein lacking FCV sequences was applied in a third lane as a means of detecting any antibodies that might fortuitously react with the Trp E fusion backbone itself. Bacterial lysates containing the FCV G1 fusion protein or control protein were subjected to PAGE and transferred to nitrocellulose.

Fifteen μl of patient serum or autopsy blood sample was diluted in 3 ml of a Tris-buffered saline detergent solution containing 5% powdered milk and 5% *Escherichia coli* lysozyme for 4 h at room temperature with rocking. The mixture was then used to probe Western blot membranes overnight at 4 °C, then washed three times in Tris-buffered saline with detergents as described (Jenison et al., 1994). A secondary alkaline-phosphatase conjugated goat anti-human IgG
(Boehringer-Mannheim) was then used at a 1:1000 dilution in Tris-buffered milk solution for 4 h at room temperature. After three additional washes, bound alkaline phosphatase was detected with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

**Phylogenetic tree analysis.** The 274 nucleotides (nt) of the 18 FCV cDNA sequences internal to the primers used to generate the 320 nt G1 gene amplimers were aligned with the corresponding sequences of PUUV (Vapalahti et al., 1992), PHV (Parrington et al., 1991), SEOV (Arikawa et al., 1990), and HTNV (Schmaljohn et al., 1987), using the Sequid II program version 3.81 (available from D. J. Khoulfi and D. D. Rhodes, Kansas State University, Manhattan, Kansas, U.S.A.). A gl statistic of −1.25 was determined for the set of sequences, indicating that there was sufficient information in the data set to make parsimony an appropriate tool for this analysis (Hillis et al., 1994). The sequences were examined for biases in state changes using the MacClade 3.0 computer program (Maddison & Maddison, 1992; Hillis et al., 1994). Non-random state-change biases were identified favouring A to G and T to C transitions. In subsequent phylogenetic analyses, the characters were weighted according to the reciprocal of the total number of changes to each character state. Weightings for very rare changes of state were sometimes reduced to avoid violations of the triangle inequality (Maddison & Maddison, 1992). Phylogenetic analyses were performed using the heuristic search with the MULPARS option of PAUP 3.1.1 (Swoford, 1991). Branch swapping to locate the most parsimonious tree at each step used the subtree pruning and rebranching algorithm.

**Results**

**FCV nucleotide sequences show regional variation but protein sequences are relatively conserved**

The geographical range of *P. maniculatus* spans most of the western and northern United States (Baker, 1968). We determined the partial nucleotide sequence of 18 alleles of the FCV G1 gene at widely separated sites within the range of *P. maniculatus*. All sites were within states that have had clinical cases of HPS. Of the 76 cases of HPS known to us in June, 1994, 58 arose within the six states considered in this study.

Eight of the 18 sequences were from humans with HPS, whereas seven and three were from seropositive *P. maniculatus* and *P. truei*, respectively. G1 sequences included five from *P. maniculatus* from the far western United States (California). Two of those were from far northern California (Siskiyou County); one was from far southern California (Orange County), and two were whereas those from *P. maniculatus* are preceded by 'PM', and those from *P. truei* are preceded by 'PT'. The state designation follows (AZ, Arizona; CA, California; CO, Colorado; ID, Idaho; MT, Montana; NM, New Mexico). Sequences from California sites are followed by a regional designation (S, Siskiyou County; Or, Orange County; Ch, Channel Island). PHV, PUUV, SEOV and HTNV are indicated by PH-1, PUU, SEO and HTN, respectively. The nucleotide sequence of the Florida virus FL-1 G1 gene (*S. hispidus*) is not available. The inset below is a map of the western United States, defining more precisely the sites from which viral sequences were obtained.
from Channel Island, located about 30 km off the coast of mainland Santa Barbara County. The Siskiyou County mice were separated by about 1000 km from the Channel Island and Orange County mice (Fig. 1, inset). Sequences from the north-central United States were obtained from the autopsy lung tissue of case-patients from Idaho and Montana, and from a *P. maniculatus* from northern Montana. Those patients and rodent lived within 250 km of each other, but approximately 1000 km from the closest California deer mice studied. The Colorado, Arizona and New Mexico FCV sequences were obtained from the lung tissue of case-patients (six sequences), *P. maniculatus* (one sequence) or *P. truei* (three sequences). These sequences came from a broad range of sites in and around the Four Corners region, but most were approximately 1500 km from the north-central case-patients and 1000 to 1300 km from the various California sites.

In general, nucleotide sequence distance of viruses from nearby sites were closely related to one another. For example, a nucleotide sequence of 3.6% was noted in comparisons of the two Siskiyou County *P. maniculatus* viruses, which were trapped within 1 km of one another. A remarkable exception is the considerable nucleotide sequence distance observed in comparisons between the two Channel Island *P. maniculatus* sequences and the sequence obtained from the Orange County *P. maniculatus* (Fig. 1). The Channel Island sequences (which differed from each other by 2.6%), differed by approximately 18% from that of the Orange County sequence, despite a geographical separation of only about 160 km. Those sequences from FCVs separated by 1000 km or more differed by 8.8 to 17%. The distances between any of the *Peromyscus* or HPS viruses and the nearest relative among the hantaviruses (PUUV) ranged from 42 to 47%.

Phylogenetic tree analysis showed that the FCV nucleotide sequences derived from the various geographical regions were, in general, reflective of the geographical region from which they were derived (Fig. 1). The Arizona, New Mexico and Colorado sequences were grouped into one extended clade, regardless of whether they came from humans or either of two *Peromyscus* species. The Montana and Idaho sequences were organized into another clade (which also encompassed several California sequences), and the mainland California sequences were phylogenetically related. The Channel Island, California sequences formed an outgroup in comparison to the mainland sequences.

With the exception of the three southern California amplimers, all of the amplimers studied encoded predicted protein sequences with only three residues displaying any variation (only residue 98 was variable in more than one of the 15 other predicted protein sequences). The Orange County and Channel Island California amplimers encoded protein sequences differing by three to five amino acid residues from the prototype 3H226 (NM-1) sequence. The five variations between the Ch2 (Channel Island) protein and the prototype sequence of 3H226 included four conservative substitutions (I to V at residue 40; L to F at residue 42; T to S at residue 43; and T to S at residue 98). However

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**Table 1. Geographical locations and Western blot intensities of serum IgG reactivity to the G1 glycoprotein of FCV in patients with HPS**

<table>
<thead>
<tr>
<th>State</th>
<th>Number</th>
<th>Intensity (0 to 4)</th>
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<tbody>
<tr>
<td>Arizona</td>
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<td>Florida</td>
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<td>Trace</td>
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<tr>
<td>Idaho</td>
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<td>2</td>
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<tr>
<td>Montana</td>
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<td>1</td>
</tr>
<tr>
<td>New Mexico</td>
<td>9†</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>2</td>
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<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Wyoming</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
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* Scored at 1:200 serum dilution according to Jenison et al. (1994).
† Includes two patients with remote infection (1985 and 1975).
all variation of the G1 glycoprotein sequence was outside of the 31 amino acid epitope (amino acids 58 to 88) identified previously (Jenison et al., 1994).

Antibodies from geographically diverse case-patients with HPS are reactive with the G1 epitope of the prototype (3H226) FCV sequence

In previous studies, expression of overlapping M segment clones spanning nearly the entirety of the FCV G1/G2 open reading frame revealed only a single dominant epitope, near the amino terminus of G1 (Jenison et al., 1994). Subsequent studies, in which the remaining portions of the G1 and G2 proteins were expressed, confirmed that no additional common linear epitopes are present in the amino-terminal portion of G1 or the carboxy-terminal portion of G2 (B. Hjelle, F. Chavez-Giles, T. Yamada & S. Jenison, unpublished). Serum samples from 25 case-patients with acute HPS from New Mexico, Arizona and Colorado were all previously found to have IgG antibodies with reactivity to the dominant G1 epitope.

Since that time, an additional 20 serum samples from patients with HPS have been studied. All 44 HPS patients from the western United States have proved to have antibodies to the G1 glycoprotein (Table 1). In Fig. 2, serum samples from the AZ-1, MT-1 (Montana), ID-1 (Idaho) and FL-1 (Florida) HPS case-patients were tested for IgG antibodies against the recombinant G1 antigen from New Mexico case-patient 3H226. All had reactivity against a large fusion protein containing 425 amino acids of FCV G1, as well as reactivity against a fusion protein containing a small epitope of FCV G1 (amino acids 31 to 88). The reactivity of the FL-1 case-patient (CDC, 1994b) was the weakest of the three, and the weakest of the HPS patients examined to date (Jenison et al., 1994; S. Jenison, C. Morris & B. Hjelle, unpublished). The reactivity of any G1 antibodies that might arise during infection with any of the mouse viruses described herein cannot be assessed, since only humans produce G1 antibodies detectable by Western blot analysis (S. Jenison, C. Morris, T. Yamada & B. Hjelle, unpublished).

Evidence that the Florida case-patient was infected in Florida

It was originally reported that the HPS case-patient from Florida lacked evidence for an IgM response against FCV or other hantaviruses (Centers for Disease Control and Prevention, 1994b). Although his clinical syndrome was compatible with HPS, the patient’s previous residence in an FCV-endemic state left open the possibility that his IgG antibodies were acquired after a previous infection, and that his HPS-like condition was not caused by a hantavirus from Florida.

Using a Western blot assay, we identified a readily detectable IgM reactivity to the FCV nucleocapsid in the Florida case-patient using a serum sample drawn on day 7 relative to hospitalization (Fig. 3). That IgM reactivity was absent by the time a convalescent serum sample was drawn on day 83. These data suggest that the Florida
case-patient was, in fact, acutely infected with an FCV-like virus at the time of his illness.

Discussion

The divergence among different FCV sequences we observed suggests that FCV has been present in North America for a considerable length of time. The phylogenetic analysis indicates a geographical specificity for viral sequences obtained from animals or HPS case-patients. This finding suggests that FCV could be an older virus, and not the result of a single recent and massive epizootic event or emergence of a new reassortant FCV in *P. maniculatus* across the western United States. This observation is compatible with a previous phylogenetic analysis that suggests that the FCV M segment and S segment have co-evolved over many years (Hjelle et al., 1994a). Recently, a case-patient who recovered from an HPS-like illness in 1975 was found to have IgG antibodies directed against the FCV nucleocapsid and G1 glycoprotein in a serum sample obtained in 1993 (Wilson et al., 1994). Thus, we can conclude that FCV or an FCV ancestor has probably been in North America since at least 1975.

The sequences obtained from deer mice from Channel Island showed considerable phylogenetic distance from those of any of the other (mainland) sequences (Fig. 1). There was a remarkable distance (18 and 5-5% amino acid distance) in a pairwise comparison with the sequence from Orange County, California, despite the slight geographical distance (160 km) separating the two sites. These data are compatible with the existence of a genetically isolated group of FCV-like viruses that have been able to diverge from the mainland viruses over an extended period of time.

No pair of FCV G1 gene amplimers encoded a protein sequence differing by more than five amino acids out of 91 (5-5%). None of the variation was within the 31 amino acid domain that comprises the dominant epitope of the G1 glycoprotein. Like HTNV (Schmaljohn et al., 1988; Xiao et al., 1993), FCV appears to be under strong evolutionary pressure to conserve its amino acid sequence in the face of considerable genetic drift at synonymous positions. However, in comparisons among different hantavirus species, the G1 glycoprotein is subject to an unusually large degree of divergence, relative to the N protein and the G2 glycoprotein (Schmaljohn et al., 1987; Antic et al., 1992; Vapalahti et al., 1992; Parrington et al., 1993; Hjelle et al., 1994a). The known genetic and antigenic conservation of the N protein made it seem less likely that it would demonstrate geographical sequence variation that would result in significant antigenic variation within the FCV species (Wang et al., 1993b).

We chose to use the portion of the M segment encoding the amino-terminus of the G1 glycoprotein for phylogenetic studies, because that region specifies an immunodominant and species-specific linear epitope of FCV. We wished to evaluate whether any genetic diversity we identified in the G1 glycoprotein gene of FCV could result in an alteration in its antigenic properties. We found that the predicted primary sequence of the epitope was not altered in FCV amplimers obtained from viruses separated by distances of many hundred kilometres. The sites from which we obtained FCV sequences span a significant proportion of the range of FCV's host species, *P. maniculatus*. Furthermore, IgG antibodies from the Idaho and Montana HPS case-patients were reactive with a G1 protein encoded by the M segment of a New Mexico virus (Fig. 2). Those patients were infected at sites well removed from most of the other 43 HPS case-patients from whom serum samples were available. Most of the other patients came from the Four Corners region of the southwestern United States. All 44 case-patients from the western United States had readily detectable antibodies against the FCV G1 glycoprotein (Jenison et al., 1994; S. Jenison, N. Torrez-Martinez, C. Morris, T. Yamada & B. Hjelle, unpublished).

Unlike humans, rodents infected with FCV do not develop detectable antibodies to the G1 glycoprotein, by Western blot analysis (S. Jenison, C. Morris, T. Yamada & B. Hjelle, unpublished). We therefore cannot assess the functional conservation of the G1 glycoprotein epitope in rodents, but must rely upon genetic analysis of the G1 gene to determine whether the epitope is conserved.

The hantavirus infection that struck a patient in Florida in October, 1993 was never identified as such at the molecular level because circulating viral RNA was cleared by the patient by the time his disease was recognized as HPS. A hantavirus, said to be different from FCV, was subsequently identified in cotton rats (*S. hispidus*) trapped near the site of the case (Centers for Disease Control and Prevention, 1994b).

Patients infected with FCV produce antibodies to a linear epitope of the FCV G1 glycoprotein that do not recognize the G1 glycoproteins of other hantaviruses; nor do patients with infections caused by hantaviruses other than FCV produce antibodies that cross-react with FCV G1 (Jenison et al., 1994; B. Hjelle, S. Jenison, N. Torrez-Martinez & H. W. Lee, unpublished). The presence of detectable antibodies to FCV G1 glycoprotein in the serum of the Florida patient suggests that his infection was caused by a virus with more similarity to FCV than that of PUUV or PHV. This inference is supported by the intense IgG response and moderate IgM reactivity to the FCV N protein (Fig. 3). We do not
have access to cellular blood components from the Florida case-patient and are thus unable to perform studies to examine the sequence relationships between the *S. hispidus* virus and FCV (Hjelle et al., 1994c). However, we consider the weak seroreactivity of the HPS case patient with FCV G1 to be most compatible with a partial conservation between the G1 epitope of FCV and the *S. hispidus* virus.

The conservation of the immunodominant glycoprotein epitope of FCV across a wide geographical area supports the use of that epitope in the serological diagnosis of FCV. An additional possible consequence of this finding could be apparent if vaccine development is contemplated for FCV. In general, antibodies directed against the glycoprotein antigens of hantaviruses are neutralizing (Arikawa et al., 1989, 1992; Chu et al., 1993; Lundkvist & Niklasson, 1992; Wang et al., 1993a; Yoshimatsu et al., 1992). While antibodies against the G1 epitope identified previously (Jenison et al., 1994) are not known to be neutralizing, its immunodominance in vivo and conservation across a significant portion of the range of *P. maniculatus* makes it a promising target for further study.

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