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Isolation and Characterization of Whitewater Arroyo Virus, a Novel North American Arenavirus

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Rodents are principal hosts for each of the well-characterized arenaviruses. Prior to the present study, Tamiami (TAM) virus was the sole arenavirus known to be indigenous to North America; it has been isolated only from southern Florida where its primary host is the cotton rat *Sigmodon hispidus.* Recently, arenavirus antibody was found in *Neotoma albigula* woodrats collected from the southwestern United States. The purpose of the present study was to isolate and characterize the arenavirus associated with *N. albigula.* Three isolates of a novel arenavirus (proposed name "Whitewater Arroyo," WWA) were recovered from two arenavirus antibody-positive *N. albigula* collected from Whitewater Arroyo in McKinley County, New Mexico. Two-way serologic tests indicated that WWA virus is antigenically distinct from other arenaviruses but most closely related to TAM virus. Phylogenetic analysis of nucleocapsid protein gene sequence data showed that WWA virus is a novel arenavirus that is genetically most closely related to TAM virus. The recovery of WWA virus from antibody-positive *N. albigula* suggests that WWA virus infection in this species can be chronic and thus that *N. albigula* is a reservoir host of the virus.

INTRODUCTION

The family *Arenaviridae* includes 16 antigenically related viruses classified into two groups: the Old World or lymphocytic choriomeningitis–Lassa virus complex and the New World or Tacaribe virus complex (Buchmeier *et al.,* 1995; Bowen *et al.,* 1996). Specific rodent species are principal hosts for each of the well-characterized arenaviruses (Childs and Peters, 1993). Humans become infected with arenaviruses by contact with infected rodents or infectious rodent excreta or secreta. Two Old World arenaviruses [lymphocytic choriomeningitis (LCM) and Lassa (LAS)] and four New World arenaviruses [Guanarito (GUA), Junin (JUN), Machupo (MAC), and Sabiá (SAB)] are known to cause severe disease in humans.

The genomes of viruses belonging to the family *Arenaviridae* consist of two single-stranded RNA segments, designated large (L) and small (S). The L RNA segment encodes the viral polymerase and a putative zinc-binding protein; the S RNA segment encodes the nucleocapsid (N) protein and a glycoprotein precursor (GPC) that is proteolytically cleaved to yield the envelope glycoproteins GP1 and GP2 (Southern, 1996). The results of phylogenetic analyses of S segment nucleotide sequence data are consistent with the New World–Old World division of the family *Arenaviridae* (Clegg, 1993; Bowen *et al.,* 1996) and indicate that the New World virus complex comprises three phylogenetic lineages, designated A, B, and C (Bowen *et al.,* 1996). Lineage A includes Tamiami (TAM), Flexal (FLE), Parana (PAR), and Pichindé (PIC) viruses; lineage B includes Amapari (AMA), GUA, JUN, MAC, SAB, and Tacaribe (TCR) viruses; and lineage C contains Latino (LAT) and Oliveros (OLV) viruses.

LCM virus is the only Old World arenavirus known to occur in the Americas. LCM virus was introduced into the Americas from the Old World along with its principal host, the house mouse *Mus musculus* (Childs and Peters, 1993). At the onset of the present study, TAM virus was the only New World arenavirus known to occur in North America. TAM virus is known only from Florida where its principal host is the cotton rat *Sigmodon hispidus* (Anonymous, 1970; Bigler *et al.,* 1975; Calisher *et al.,* 1970; Jennings *et al.,* 1970). Recently, cross-reactive antibody to TAM and PIC viruses was found in *Neotoma albigula* and four other *Neotoma* species collected from the western United States (Kosoy *et al.,* 1996). This finding was the first evidence for the presence of a New World arenavirus in North America outside Florida. The objective of the present study was to isolate and characterize the arenavirus associated with *N. albigula* in the western United States.

MATERIAL AND METHODS

Rodent organs

Virus isolation was attempted on kidneys and/or spleens from 16 *N. albigula* whitethroat woodrats. Three

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of the woodrats were antibody-positive to TAM and PIC viruses; none were antibody-positive to LCM virus (M. Y. Kosoy, unpublished data). All 16 woodrats were collected in 1993 from McKinley County, New Mexico, for a study on the ecology of Sin Nombre virus (family *Bunyaviridae,* genus *Hantavirus*) (Childs *et al.,* 1994). At the time of capture, the internal organs of each rodent were collected aseptically and placed in separate cryovials. All specimens were stored continuously at $<-70°$ until tested for virus in the present study.

Virus isolation

Each organ was prepared for virus isolation as a 10% V/V suspension in 0.01 *M* phosphate-buffered saline (PBS), pH 7.20, containing 10.0% V/V heat-inactivated fetal bovine serum (FBS) and antibiotics. Vero E6 cell (ATCC: CRL 1586) monolayers were grown in 25-cm^2 plastic tissue culture flasks, using Eagle's minimum essential medium (EMEM) supplemented with 10.0% V/V heat-inactivated FBS, 1.5 g/l sodium bicarbonate, 0.29 mg/ml L-glutamine, and antibiotics. Monolayers were inoculated with 0.20 ml of organ suspension and maintained at 37° under EMEM supplemented with 2.0% V/V heat-inactivated FBS, 0.29 mg/ml L-glutamine, and antibiotics. Maintenance medium was changed on Day 4 or 5 postinoculation (PI). Cells were scraped from the monolayers on Day 14 PI and used to prepare cell "spots" on 12-well teflon-coated glass microscope slides; then the cultures were stored at $-70°$. Cell "spots" were air-dried, gamma irradiated (1×10^6 rads), fixed in cold acetone, and tested for arenavirus antigen by an indirect fluorescent antibody test (IFAT), using a hyperimmune mouse ascitic fluid (HMAF) to TAM virus in conjunction with a goat anti-mouse IgG fluorescein isothiocyanate conjugate (Cappel Laboratories, West Chester, PA). Infected cell cultures were thawed and virus stocks prepared as supernatant fluids (passage designation: Vero E6+1).

Hyperimmune mouse ascitic fluid preparation

HMAF to arenavirus isolate AV 9310135 from *N. albigula* was made in young adult ICR mice by using a modification of the method described by Brandt *et al.* (1967). The immunogen (a 10% W/V suspension of infected mouse brains in 0.01 *M* PBS, pH 7.20) was prepared from ICR mice inoculated intracranially at age 1 day with a 1:100 dilution of virus (passage history: Vero E6 + 3) in 0.01 *M* PBS, pH 7.20. The adult mice were inoculated intraperitoneally (IP) and subcutaneously (SQ) with 0.2 ml of Freund's complete adjuvant and 0.2 ml of immunogen, respectively, on Day 0; IP and SQ with 0.2 ml of an emulsion consisting of equal volumes of immunogen and Freund's incomplete adjuvant on Days 7, 21, and 28; and IP with Sarcoma 180 cells on Day 35 to induce ascites. Paracentesis was initiated on Day 39 and repeated every 3–4 days until a total of three taps were performed.

Enzyme-linked immunosorbent assay (ELISA)

An IgG antibody-capture ELISA was used to quantitatively assess antigenic relationships among the *Neotoma* arenavirus isolates and to establish the relationship of the isolates to other arenaviruses. The ELISA was done using a modification of the method described by Childs *et al.* (1994). Test and reference antigens were prepared from infected Vero E6 cell cultures; a control (comparison) antigen was prepared from uninfected Vero E6 cell cultures. Arenaviruses used to prepare reference antigens were AMA (strain BeAn 70563), FLE (BeAn 293022), GUA (INH-95551), LAT (10924), JUN (XJ-14), MAC (Carvallo), OLV (3229), PAR (12056), PIC (An 3739), SAB (SP H 114202), TCR (TRVL 11573), TAM (W-10777), and LCM (Armstrong). Test and reference antigens were optimized by box titration against the homologous "antibodies" [HMAF or hyperimmune hamster serum (HHS)]. Serial twofold dilutions of each antibody (from 1:200 through 1:409,600) were made in skim milk and tested against antigens coated on U-bottom wells in 96-well flexible assay plates (Becton–Dickinson Labware, Oxnard, CA). Goat anti-mouse IgG peroxidase conjugate (H and L chain: Boehringer-Mannheim, Indianapolis, IN) and goat anti-hamster IgG peroxidase conjugate (H and L chain; Kirkegaard and Perry, Gaithersburg, MD) were used to detect bound IgG in tests employing HMAF and HHS, respectively. Optical densities at 410 nm (OD_{410}) were measured with a Dynatech MR 5000 microplate spectrophotometer. The adjusted OD_{410} for each antibody–antigen reaction was the OD_{410} of the well coated with viral antigen less the OD_{410} of the corresponding well coated with control antigen. The endpoint titer for each antigen–antibody combination was considered to be the highest antibody dilution which yielded an adjusted OD_{410} greater than or equal to 0.40. Preliminary tests indicated that the 0.40 cut-off value was optimal with regard to test specificity. All tests were done in duplicate on the same day. The reciprocal of the geometric mean of endpoint titers for duplicate tests on each antibody–antigen combination was used for analysis. Groups of similar antigens were identified using the hierarchical cluster analysis option in SPSS for Windows, Release 6.1.3 (SPSS, Inc., Chicago, IL). This method of analysis was used previously to study serologic relationships among influenza viruses (Lee, 1968; Lee and Tauraso, 1968). In the present study, the measure of similarity between antigens was the Pearson product-moment correlation coefficient; correlation coefficients were calculated using a $\log_{10}$ transformation of the ELISA titers shown in Table 1 (values less than 200 were considered to be 100); and the clustering method was average linkage between groups.

Neutralization tests

Plaque reduction neutralization tests (PRNT) were done to determine further the serologic relationship be-

TABLE 1

ELISA Results Using Antigens Prepared from Three Unidentified *Neotoma albigula* Virus Isolates and 13 Known Arenaviruses

	Antibody[b]													
Antigen[a]	AV 9310135	TAM	PIC	PAR	FLE	OLV	LAT	GUA	AMA	JUN	MAC	TCR	SAB	LCM
AV 9310135	25,600[c]	6,400	4,525	25,600	400	200	6,400	<200	<200	<200	<200	<200	<200	<200
AV 9310040	51,200	12,800	3,200	25,600	800	200	6,400	<200	<200	<200	<200	<200	<200	<200
AV 9310041	51,200	9,051	6,400	36,203	800	200	9,051	<200	<200	<200	<200	<200	<200	<200
TAM	6,400	25,600	1,600	25,600	283	<200	3,200	<200	<200	<200	<200	<200	<200	<200
PIC	1,600	6,400	36,203	12,800	400	<200	800	<200	<200	<200	<200	<200	<200	<200
PAR	283	200	200	102,400	6,400	200	400	<200	<200	<200	<200	<200	<200	<200
FLE	200	<200	<200	6,400	12,800	<200	<200	<200	<200	<200	<200	<200	<200	<200
OLV	<200	<200	<200	1,600	<200	2,262	25,600	<200	<200	<200	<200	<200	<200	<200
LAT	<200	<200	<200	3,200	<200	400	102,400	<200	<200	<200	<200	<200	<200	<200
GUA	<200	<200	<200	200	<200	<200	1,600	18,101	1,600	1,600	1,600	800	<200	<200
AMA	<200	<200	<200	<200	<200	<200	200	6,400	6,400	6,400	3,200	3,200	<200	<200
MAC	<200	<200	<200	<200	<200	<200	200	6,400	1,600	25,600	25,600	9,051	<200	<200
JUN	<200	<200	<200	200	<200	<200	800	9,051	1,600	51,200	12,800	25,600	<200	<200
TCR	<200	<200	<200	<200	200	<200	3,200	12,800	1,600	800	6,400	25,600	<200	<200
SAB	<200	<200	<200	<200	200	<200	6,400	12,800	800	26,600	3,200	3,200	400	<200
LCM	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	12,800

[a] Test and reference antigens were infected Vero E6 cells. AV 9310135, AV 9310040, and AV 9310041 were isolated from *Neotoma albigula;* TAM, Tamiami; PIC, Pichindé; PAR, Parana; FLE, Flexal; OLV, Olivernos; LAT, Latino; GUA, Guanarito; AMA, Amapari; JUN, Junin; MAC, Machupo; TCR, Tacaribe; SAB, Sabiá; LCM, lymphocytic choriomeningitis.

[b] Antibodies to AV 9310135, TAM, PIC, FLE, OLV, GUA, AMA, JUN, MAC, TCR, SAB, and LCM viruses were hyperimmune mouse ascitic fluids. Antibodies to PAR and LAT viruses were hyperimmune hamster sera.

[c] Reciprocal of geometric mean of endpoint titers for duplicate tests. Homologous titers are underlined.

tween the *Neotoma* virus isolates and TAM virus. Serial twofold dilutions of each HMAF (starting at 1:10) were tested against a constant virus challenge. HMAFs were diluted in 0.01 *M* PBS, pH 7.20, containing 0.75% W/V bovine albumin (PBS-BA) and heat-inactivated (56° × 30 min). Tests were done in Vero cell (ATCC: CCL-81) monolayers grown in six-well plastic plates (each well 9.6 cm^2), using EMEM supplemented with 7.0% V/V heat-inactivated FBS, 1.5 g/l sodium bicarbonate, 0.29 mg/ml L-glutamine, and antibiotics. The virus challenge (approximately 100 plaque forming units/0.1 ml) was prepared in PBS-BA containing 4.0% V/V guinea pig (GP) complement (Life Technologies, Grand Island, NY). Preliminary testing indicated that 4.0% V/V GP complement in the virus diluent enhanced neutralization of virus isolate AV 9310135 by its homologous HMAF and that the same concentration of GP complement in the absence of the HMAF did not neutralize the virus. Equal volumes of virus challenge and HMAF dilution were mixed and incubated at 37° × 60 min; then 0.10 ml of each virus–HMAF mixture was inoculated onto each of two monolayers. The inoculated monolayers were incubated at 37° × 60 min and overlaid with 4.0 ml of Basal Medium Eagle (Life Technologies, Grand Island, NY) containing 1.0% W/V Seakem ME agarose (FMC Products, Rockville, ME), 2.0% V/V heat-inactivated FBS, 0.29 mg/ml L-glutamine, 1.6 mg/ml $NaHCO_3$, and antibiotics. Cultures were incubated for 6 days at 37° in a humidified atmosphere of 5% CO_2 in air before addition of a second overlay (2.0 ml per monolayer) prepared in the same manner as the first but supplemented with 0.03 mg/ml neutral red. Cultures were examined for plaques over the next 5 days. Antibody titers were determined based on 80% plaque reduction and expressed as the reciprocal of the antibody dilution before addition of the virus challenge.

Partial genomic sequence determination and analysis

Virus RNA preparation, and reverse transcription and polymerase chain reaction (PCR) amplification of virus genomic templates, were done according to the methods described by Bowen *et al.* (1996). Oligonucleotide primers 1010C (5′-TCIGGIGAIGGITGGCC-3′; I, inosine) and 1696R (5′-ACATIATGCAITCIAICAGIGCACAGTG-3′), which are reactive with all known New World arenaviruses (Bowen *et al.,* 1996), were used to amplify a region of the N protein gene of virus isolate AV 9310135 from *N. albigula.* The PCR product was extracted from the agarose gel using the Sephaglas BandPrep kit (Pharmacia Biotech, U.S.A.) and sequenced directly by the dye termination cycle sequencing technique (Applied Biosystems, Inc., Foster City, CA), using primers 1010C and 1696R. Four other primers [1223C (5′-TTGACATTGAGGGGCCACCA-3′), 1463C (5′-GGAAACTTTTTGATATGCATGG-3′), 1479R (5′-CATATCAAAAAGTTTCCTTATATC-3′), and 1242R (5′-TGGTGGCCCCTCAATGTCAA-3′)] were designed on the basis of data obtained in the first round of sequencing, and these primers were used to confirm the sequence of

both strands of the PCR product. Sequence compilation, alignment, and analyses were performed using the Wisconsin Sequence Analysis Package, Version 8.1 (Genetics Computer Group, Inc., Madison, WI). The sequences used in the analyses included JUN [GenBank Accession No. D10072 (Ghiringhelli *et al.,* 1991)], MAC [X62616 (Griffiths *et al.,* 1992)], PIC [K02734 (Auperin *et al.,* 1984)], SAB [U41071 (Gonzalez *et al.,* manuscript submitted)], TCR [M20304 (Franze-Fernandez *et al.,* 1987)], AMA [U43685 (Bowen *et al.,* 1996)], FLE [U43687 (Bowen *et al.,* 1996)], GUA [U43686 (Bowen *et al.,* 1996)], LAT [U43688 (Bowen *et al.,* 1996)], PAR [U43689 (Bowen *et al.,* 1996)], TAM [U43690 (Bowen *et al.,* 1996)], LCM [Armstrong strain: M20869 (Salvato *et al.,* 1988)], LCM [WE strain: M22138 (Romanowski *et al.,* 1985)], Lassa [(LAS) Josiah strain: J04324 (Auperin *et al.,* 1989)], LAS-GA391 [Nigerian strain X52400 (Clegg *et al.,* 1991)], and Mopeia [(MOP) strain: M33879 (Wilson and Clegg, 1991)] viruses. A Fitch–Margoliash phylogenetic analysis was carried out using programs of the PHYLIP 3.5c package as described by Bowen *et al.* (1996).

Safety

All work with potentially infectious rodent tissues was performed in a laminar flow biosafety hood in a biosafety level-3 (BSL-3) laboratory; preparation of HMAF against virus isolate AV 9310135 was done in a BSL-4 laboratory.

RESULTS

Virus isolation

Three arenavirus isolates were recovered from two of 16 *N. albigula* woodrats collected from Whitewater Arroyo in McKinley County, New Mexico. Both virus-positive woodrats were antibody-positive against TAM and PIC viruses (M. Y. Kosoy, unpublished data). One virus isolate each was obtained from a kidney (specimen No. 9310135) and the spleen (No. 9310041) from a 105-g male *N. albigula;* one isolate was obtained from a kidney (No. 9310040) from a 97-g male *N. albigula.* Virus isolation attempts (through passage Vero E6 + 3) initiated with splenic tissue from the smaller woodrat were unsuccessful. In each successful isolation attempt, no cytopathic effect was observed in the Vero E6 cell monolayer inoculated with the original organ suspension, and intracytoplasmic inclusions of virus antigen were detected by IFAT in cells from that monolayer. In Vero cell monolayers maintained under a semi-solid agar overlay containing neutral red, each isolate (passage history: Vero E6 + 3) produced indistinct plaques 1.5–2.0 mm in diameter on Day 8 PI.

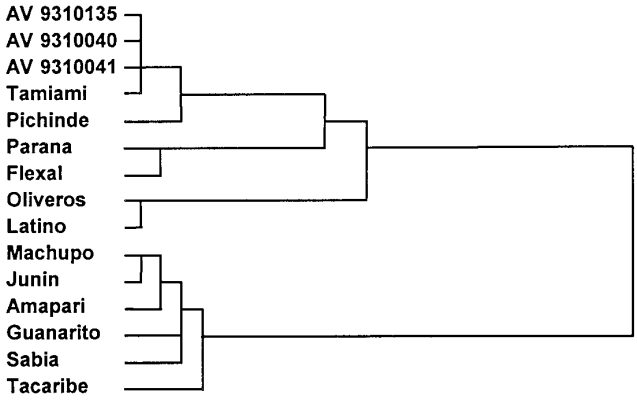

FIG. 1. Dendrogram showing grouping of New World arenavirus ELISA antigens as determined by cluster analysis (average linkage between groups) of data contained in Table 1. AV 9310135, AV 9310040, and AV 9310041 denote the three strains of Whitewater Arroyo virus isolated from *Neotoma albigula.*

TABLE 2

Results of Neutralization Tests Using Three Unidentified *Neotoma albigula* Virus Isolates and Tamiami Virus

		Antibody[b]	
Virus[a]	Strain	AV 9310135	Tamiami
?	AV 9310135	40	<10
?	AV 9310040	40	10
?	AV 9310041	40	<10
Tamiami	W-10777	<10	160

[a] ?, unidentified arenavirus isolate from *Neotoma albigula.*

[b] Reciprocal titer. Homologous titers are underlined. The ELISA titer of the antibody to virus isolate AV 9310135 was 1:25,600 (Table 1).

ELISA

Endpoint titers for duplicate tests on each antibody–antigen combination were 95.1% concordant; all differences between duplicate tests were less than fourfold. In the ELISA, the reactivity profiles of the three *Neotoma* virus antigens were quite similar to each other; similar to but distinct from the reactivity profiles of TAM, PIC, PAR, and FLE virus antigens (in order of decreasing similarity); and distinct from the reactivity profiles of eight other New World arenavirus antigens and LCM virus antigen (Table 1; Fig. 1). These findings suggest that the three *Neotoma* arenavirus isolates are strains of a novel virus that is antigenically most closely related to TAM virus. The two-way fourfold differences in reactivity between TAM virus antigen and antigen prepared from isolate AV 9310135 suggest that the *Neotoma* isolates represent an arenavirus that is distinct from TAM virus.

PRNT

In the PRNT, the three virus isolates from *N. albigula* were very similar to each other and distinct from TAM virus (Table 2).

Analyses of virus genomic sequences

A 616-base-pair DNA fragment was amplified from the N protein gene of WWA virus strain AV 9310135 and

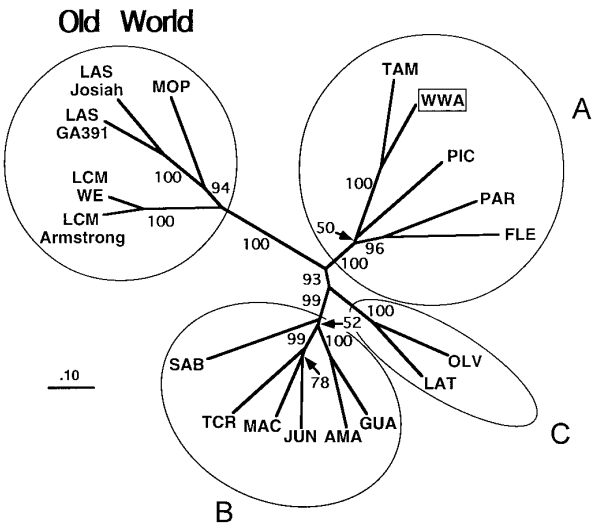

FIG. 2. Phylogeny inferred by Fitch–Margoliash analysis of partial N protein gene sequences of Whitewater Arroyo (WWA) virus and other arenaviruses. Branch lengths are proportional to distance with transversions weighted 2:1 over transitions. The scale bar indicates a 10% weighted difference. Numbers indicate the percentage of 1000 bootstrap replicates that support each labeled interior branch. Circles denote the Old World virus complex and the three lineages within the New World virus complex which are labeled A, B, and C. The position of WWA virus is indicated by boxed text.

sequenced. The sequence of that fragment was deposited into the GenBank sequence database under Accession No. U52180. The WWA virus strain AV 9310135 sequence displayed 73.1% nucleotide (nt) identity and 80.5% amino acid (aa) identity (205 amino acids) with the corresponding sequences of TAM virus, and a lesser degree of identity with other arenaviruses. This degree of relatedness is slightly less than that observed for other pairs of closely related New World arenaviruses: JUN and MAC (75.9% nt and 85.7% aa identity), GUA and AMA (75.4% nt and 85.7% aa identity), and OLV and LAT (74.1% nt and 81.4% aa identity) (Bowen *et al.,* 1996). The Fitch–Margoliash analysis indicated that WWA virus is a novel member of lineage A within the New World serocomplex (Bowen *et al.,* 1996) and that WWA virus represents a sister taxon to TAM virus (Fig. 2). Bootstrap support for monophyly of TAM and WWA viruses was 100%. Collectively, the analyses of nt sequence data indicate that WWA virus strain AV 9310135 is a unique arenavirus and thus are concordant with the conclusions derived from the serologic analyses.

DISCUSSION

The results from serologic tests and analyses of partial N protein gene sequence data indicated that WWA virus is a novel arenavirus that is most closely related to TAM virus. The name "Whitewater Arroyo" (WWA) is proposed to denote the geographic origin of the first isolates of this virus; and isolate AV 9310135 is designated as the prototype strain of WWA virus. The isolation of WWA virus from *N. albigula* woodrats collected from New Mexico is the first unequivocal evidence for the presence of a New World arenavirus in the western United States.

Historically, a variety of serologic methods, including the PRNT and ELISA, have been used to distinguish individual arenaviruses. The PRNT was used only selectively in the present study because neutralizing antibodies to FLE, LAT, OLV, PIC, and SAB viruses were not available. The present study is the most comprehensive characterization of New World arenaviruses by ELISA. The hierarchical cluster analysis of $\log_{10}$-transformed ELISA data identified three groups of similar antigens (TAM, PIC, FLE, and PAR; OLV and LAT; and AMA, GUA, JUN, MAC, TCR, and SAB). Interestingly, these three groups correspond to the three phylogenetic lineages identified by Bowen *et al.* (1996) using N protein gene sequence data. The similarity between the serologic and genetic data suggests that the evolution of epitopes reactive in the ELISA has paralleled the evolution of the arenavirus N protein gene. Whether the ELISA-reactive epitopes were associated primarily with the N protein is not known.

The dominant feature of the well-characterized arenaviruses is their ability to establish chronic infections in specific rodent hosts; and chronically infected rodents appear to be crucial for long-term persistence of arenaviruses in nature (Childs and Peters, 1993). The two virus-positive woodrats in the present study had high-titered antibody to both TAM and PIC viruses (M. Y. Kosoy, unpublished data). Assuming that WWA virus-stimulated production of the arenavirus antibody in both woodrats, the recovery of infectious virus from these rodents suggests that *N. albigula* can be chronically infected with WWA virus and thus may be a reservoir host of the virus.

Childs and Peters (1993) advanced the hypothesis that the evolution of arenaviruses is closely linked to that of their respective rodent hosts. Based on analyses of mitochondrial DNA nucleotide sequence data (Sullivan *et al.,* 1995), *N. albigula* and *S. hispidus* represent a single ancestral lineage in the family Muridae, subfamily Sigmodontinae. This finding suggests that the present WWA virus-*N. albigula* and TAM virus-*S. hispidus* associations represent a long-term shared evolutionary relationship between the family *Arenaviridae* and the murid subfamily Sigmodontinae. Alternatively, the present virus–rodent associations could have resulted from horizontal transmission of TAM virus from *S. hispidus* to *N. albigula* (or WWA virus from *N. albigula* to *S. hispidus*) in a more recent time.

The rodent *Calomys callosus* is the principal host for MAC and LAT viruses in Bolivia and Brazil, respectively; and the rodent *Calomys musculinus* is the principal host for JUN virus in Argentina (Childs and Peters, 1993). Thus, a single rodent species as well as congeneric rodent species can be naturally associated with multiple arenaviruses. In the western United States, arenavirus anti-

body has been found in five *Neotoma* species: *N. albigula* collected from Arizona, Colorado, and New Mexico; *N. fuscipes* and *N. lepida* from California; *N. mexicana* from Arizona, New Mexico, and Utah; and *N. stephensi* from Arizona and New Mexico (Kosoy *et al.,* 1996). Thus, arenavirus isolates from each of these five *Neotoma* species and from *Neotoma* rodents collected from different localities need to be characterized in order to elucidate the relationship between the rodent genus *Neotoma* and the virus family *Arenaviridae.*

Six arenaviruses are known to be pathogenic in humans. GUA, JUN, MAC, and LAS viruses are important agents of viral hemorrhagic fever syndrome in Venezuela, Argentina, Bolivia, and West Africa, respectively (Childs and Peters, 1993); SAB virus was incriminated in two cases of severe laboratory-associated illness (Coimbra *et al.,* 1994, Barry *et al.,* 1995) and a fatal case of hemorrhagic fever in Brazil (Coimbra *et al.,* 1994); and LCM virus is an etiologic agent of acute, febrile, central nervous system disease (Childs and Peters, 1993). At least two of these viruses (LAS and LCM) are also abortifacients and/or teratogens (Peters *et al.,* 1996; Sheinbergas, 1976). Whether WWA virus is pathogenic in humans remains to be investigated.

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