Antigenic and Genetic Properties of Viruses Linked to Hemorrhagic Fever with Renal Syndrome

Abstract. *Hemorrhagic fever with renal syndrome (HFRS) comprises a variety of clinically similar diseases of viral etiology that are endemic to and sporadically epidemic throughout the Eurasian continent and Japan. Although HFRS has not been reported in North America, viruses that are antigenically similar to HFRS agents were recently isolated from rodents in the United States. Examination and comparison of eight representative isolates from endemic disease areas and from regions with no known associated HFRS indicate that these viruses represent a new and unique group that constitutes a separate genus in the Bunyaviridae family of animal viruses.*

Epidemic and endemic human diseases collectively known as hemorrhagic fever with renal syndrome (HFRS) (*1*) are common to much of the Eurasian continent and Japan. Korean hemorrhagic fever (KHF), epidemic hemorrhagic fever (EHF), and nephropathia epidemica (NE) are familiar examples of more than 150 synonyms for these clinically similar diseases (*2*). Isolation of Hantaan virus (HTN), the presumed etiologic agent of KHF, from a Korean striped field mouse (*3*) and its subsequent adaptation to cell culture (*4*) afforded the opportunity for laboratory investigation of one of these elusive viruses. HTN-related agents are widely distributed throughout much of the world, as indicated by seroepidemiologic surveys and case reports. There are several hundred cases each year in the Republic of Korea, with approximately 5 percent fatality. China reported 30,000 cases requiring hospitalization in 1980 and 42,000 cases in 1981, with 7 to 15 percent fatality, and 11,000 cases have been reported in the far-eastern Soviet Union in the past 5 years. Human infection by association with laboratory rats at 19 medical centers in Japan has resulted in 116 cases and one death since 1976 (*1*). HFRS continues to represent a major threat to human health. We report here the antigenic and genetic relations among HFRS-associated and related viruses. Viruses examined represent the currently recognized host range and a wide geographic distribution of these agents. The relations suggest that a new and separate genus of Bunyaviridae is required to accommodate HTN and related viruses, and we propose that this genus be named *Hantavirus* after its prototype member.

Previous analyses of the HTN genome (*5, 6*) were consistent with morphological observations (*7, 8*), which suggested that HTN most closely resembles viruses classified in the family Bunyaviridae and complies with all the criteria required for inclusion in the family. However, the absence of a demonstrable serologic relation between HTN and existing members of the Bunyaviridae, as well as unique genetic and epidemiologic characteristics, precluded its classification in any established genus (*6*). Significantly, other viruses found to be serologically related to HTN by immunofluorescence have recently been isolated from rodents in endemic regions as well as in areas with no known HFRS (*9–15*); selected viruses from both were included in this study.

Rodents are the principal natural hosts of HTN-like viruses, and, although infections may persist for the life of the animal, no overt virus-induced pathology has been reported to our knowledge. Unlike most other Bunyaviridae, which depend on arthropod vectors for transmission, HFRS-associated viruses appear to be directly transmitted via aerosolized urine, feces, and saliva of infected rats (*Rattus*), mice (*Apodemus*), and voles (*Clethrionomys* and *Microtus*) (*16*). Two isolates included in this study were directly associated with human illness: Lee (LEE) virus, isolated from a KHF patient by passage of viremic blood into *Apodemus* (*3*); and Sapporo rat virus (SR-11) (*13*), isolated from a laboratory rat at a Japanese medical school after an outbreak of EHF among animal handlers (Table 1). Other viruses obtained from endemic disease areas are suspected eti-

Table 1. Virus isolates related to HFRS. Isolates were propagated in a cloned line of Vero cells (Vero E6; ATCC C1008). Growth curves for each virus revealed that maximum infectious virus release varied among isolates and ranged from 7 days after infection (SR-11) to 22 days (PA). Seed stocks were prepared from cell culture supernatants harvested at times of maximum virus release and ranged from 1×10^5 plaque-forming units (PFU) per milliliter (GP) to 2×10^7 PFU/ml (HTN). All subsequent experiments were performed with one additional passage of these infectious inocula in Vero E6 cells.

Virus	Host	Location	Disease	Reference
HTN	*Apodemus*	Korea	KHF	(*3, 4*)
LEE	Human	Korea	KHF	(*3*)
UR	*Rattus*	Korea	KHF	(*15*)
TCH	*Rattus*	Louisiana	?	(*11*)
GP	*Rattus*	Pennsylvania	?	(*9, 10*)
PH	*Microtus*	Maryland	?	(*12*)
SR-11	*Rattus*	Japan	EHF	(*13*)
PA	*Clethrionomys*	Finland	NE	(*14, 17*)

Table 2. Antigenic relations of HFRS-related viruses: RIA titers ($\times 10^3$). Infected Vero E6 cell monolayers were scraped, pelleted at 2000*g*, and washed twice in physiological saline. Cells were lysed with 1 percent Triton X-100 in 0.1*M* tris-HCl (*p*H 7.4) and 0.4*M* NaCl, and nuclei were removed by centrifugation at 2000*g*. Supernatant lysates were used as antigen for solid-phase RIA (*23*). Antisera were prepared by infection of six adult Wistar rats with each virus. All animal experiments were performed under conditions of high-level containment to preclude virus transmission between cages and minimize the risk of exposure. Rats were bled 28 days after infection and individual rat sera were screened by RIA to determine the presence of antibody to homologous and heterologous viruses. Pools of six antisera to each virus were used for all subsequent experiments. Half-logarithmic serum dilutions were assayed with a constant concentration of each antigen, and RIA titers were determined as the reciprocal of the highest dilution resulting in at least 2.5 times the background ^{125}I counts bound to uninfected cell lysate controls. Asterisks indicate one-way antigenic homology and daggers two-way homology.

Serum pools	Infected cell lysates							
	HTN	LEE	UR	TCH	GP	SR-11	PH	PA
HTN	100*	30	30	30	30	30	10	3
LEE	100*	100*	30	30	30	30	10	10
UR	100*	10	100†	100†	100†	100†	10	10
TCH	100*	30	100†	100†	100†	100†	10	10
GP	100*	100*	100†	100†	100†	100†	10	10
SR-11	30	30	100†	100†	100†	100†	3	10
PH	1	1	1	1	1	1	30*	3
PA	3	1	1	1	1	1	10*	10*
Normal (control)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

ologic agents of KHF (*15*) and NE (*17*). Three isolates from U.S. rodents were also included as representatives of a geographic area with no known associated HFRS (*9–12*).

Antigenic relations among these viruses were examined by solid-phase radioimmunoassay (RIA) and plaque-reduction neutralization tests (PRNT's) with antisera produced by experimental infection of Wistar rats. Of three antigenic groups distinguishable by RIA, the greatest homology appeared among rat isolates that displayed two-way cross-reactivity, that is, antisera with equivalent homologous and heterologous titers (Table 2). One-way cross-reactivity was observed with prototype HTN virus and the LEE, urban rat (UR), Tchoupitoulas (TCH), and Girard Point (GP) isolates; RIA titers to HTN antigen were equivalent for serum against each virus, while antiserum directed against HTN could differentiate homologous and heterologous antigens. A similar one-way cross was observed between the two vole isolates, Prospect Hill (PH) and Puumala (PA). Antiserum to PA displayed the same RIA titer to both PA and PH antigen, but PH antiserum reacted preferentially with homologous antigen.

The more specific PRNT also suggested antigenic similarities among the rat isolates, but clearly differentiated the vole isolates (Table 3). HTN and LEE antisera had high neutralization titers to HTN and LEE viruses yet did not cross-react extensively with rat isolates. In contrast, antisera to rat viruses showed broad neutralizing reactivity among all rat isolates as well as to HTN. Although all the rat virus antisera had some neutralizing activity against PH virus, PH antiserum showed little neutralizing activity against any heterologous virus. Antiserum to PA virus did not neutralize any of the other viruses, nor was PA virus neutralized by any heterologous sera. Antisera to UR and LEE had higher neutralizing titers against heterologous than homologous viruses. While this could represent a genuine difference in accessibility of neutralizing epitopes on different viruses, it could also be attributed to a higher proportion of noninfectious viral antigens in the homologous virus inocula.

These findings show that each of the isolates examined was antigenically unique yet exhibited a pattern of cross-reactivity that appeared to be correlated with host factors rather than geographic location. For example, the rat isolate from Korea (UR) appeared to share a closer antigenic relation with rat viruses isolated from the United States and Japan than with viruses isolated from different hosts in the same endemic disease area of Korea. Although many of these rodent species are believed to be capable of transmitting HFRS, what role particular host influences might have on the potential for human pathogenicity is unknown. A more detailed examination of cross-reactive, neutralizing, and protective epitopes of several human isolates will be required to identify antigenic determinants relevant to HFRS-associated diseases.

Genetic studies may provide clues to the basis for the antigenic diversity and unusual biological properties of HTN-like viruses. The genome of HTN virions consists of three single-stranded RNA segments of negative polarity contained in three ribonuclease-sensitive nucleocapsids (*5, 6*). The only recognized family of animal viruses with these genome characteristics is the Bunyaviridae. Genetic investigations of many members of the Bunyaviridae have shown that mutational drift and genome reassortment appear to be responsible for the genetic diversity in the family (*18*). Oligonucleotide fingerprint analyses have been used extensively to investigate both of these evolutionary mechanisms and have proved valuable for differentiation of even very closely related viruses. Comparison of the genome oligonucleotide maps of viruses used in this study showed that each virus has a unique RNA fingerprint (Fig. 1). Even the serologically closely related U.S. rat isolates, GP and TCH, had readily distinguishable oligonucleotide maps. Furthermore, comparison of these T1 maps to those of host cell 28 and 18S ribosomal RNA demonstrated that none of the isolates

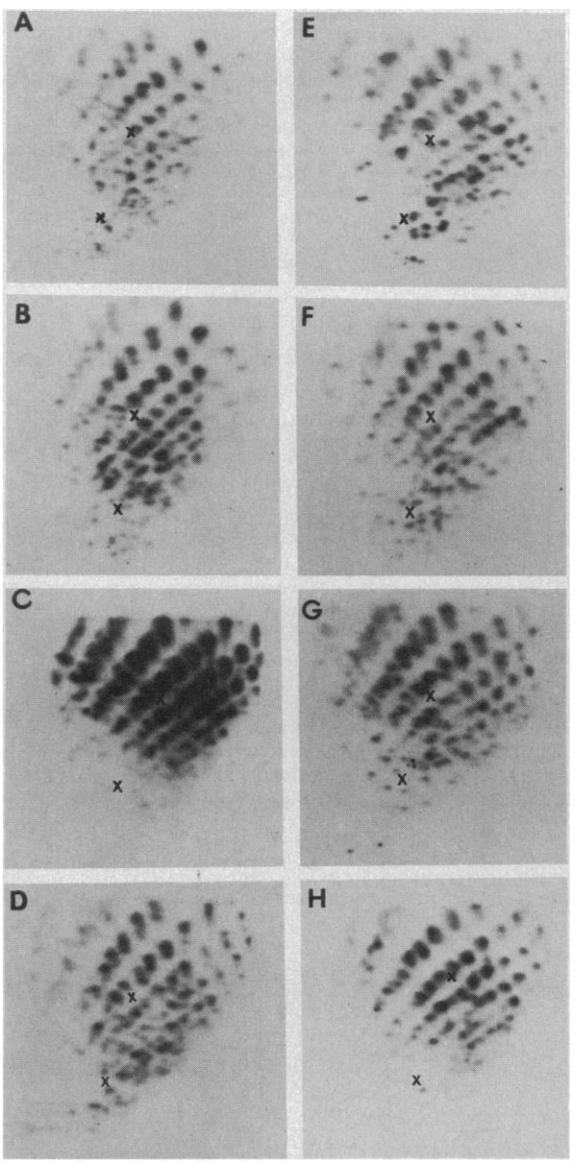

Fig. 1. (A to H) Oligonucleotide maps of HFRS viruses. Methods for virus propagation, concentration, and purification have been described elsewhere (*5, 6*). The RNA's of HTN, SR-11, GP, TCH, and PH were labeled by incorporation of [^{32}P]orthophosphate into cell culture media (330 μCi/ml) during the final 4 days of infection. Because UR, LEE, and PA viruses replicated slowly and to low titers (less than 10^6 PFU/ml) and consequently less RNA could be obtained (typically, 1 to 5 μg compared to 10 to 20 μg), the T1 resistant oligonucleotides of these viruses were labeled at the 5′ termini with [γ-^{32}P]ATP and polynucleotide kinase (*25*). Similar end-labeling of 5 μg of HTN oligonucleotides yielded a T1 map indistinguishable from that obtained with metabolically labeled RNA, indicating that these techniques were interchangeable. Two-dimensional polyacrylamide gel electrophoresis was performed as described elsewhere (*26, 27*). (A) HTN, (B) LEE, (C) UR, (D) SR-11, (E) TCH, (F) GP, (G) PH, and (H) PA; x's indicate the positions of xylene cyanol and bromophenol blue reference dye markers.

contained detectable host RNA. Previous comparison of fingerprints of the individual large, medium, and small RNA species of HTN and LEE demonstrated that each corresponding genome segment, as well as composite maps of all three segments, displayed considerable diversity and indicated that genetic changes are not limited to any one RNA segment (*19*).

Reassortment studies with various Bunyaviridae have shown that only closely related viruses are capable of exchanging genome segments and that reassortment does occur in vivo as well as in cell culture (*18*). While no attempts have yet been made to examine genome reassortment with HFRS-related viruses, the persistent noncytopathic nature of these viruses would appear to allow ample opportunity both for genetic drift and for coinfection of rodents with two or more viruses and subsequent genome reassortment.

Serologically related Bunyaviridae have a conserved nucleotide sequence at the 3′ termini of each of their three virion RNA segments. This characteristic 3′ terminal nucleotide sequence is common to all viruses examined in a genus, but is different from those of other genera (*20*). Terminal 3′ sequence analysis of the large, medium, and small genome segments of HTN revealed a conserved sequence 3′AUCAUCAUCUG on each segment (*6*). This sequence is unique with respect to the consensus sequences reported for other genera of Bunyaviridae. We similarly analyzed the 3′ termini of the seven HTN-related isolates and determined that, in accordance with other Bunyaviridae genera, all these presumptive members of the proposed *Hantavirus* genus have a consensus sequence, identical to that of HTN, which is conserved on each of their three RNA segments (Table 4).

Prototype HTN meets all criteria established for inclusion in the Bunyaviridae (*18*). Among these are the following:

1) Virion particles appear to be spherical (diameter, about 95 nm) and have an envelope (*7, 8*).

2) Viruses have single-stranded, three-segmented, negative-sense RNA genomes with a total molecular weight of about 4.5×10^6 (*5, 6*).

3) Three viral nucleocapsids composed of a nucleocapsid protein and a single RNA species have been described (*6*).

4) Two virus-specified glycoproteins associated with virion membrane components have been identified (*19, 21*).

5) Virus replication occurs in the cytoplasm of infected cells (*22*), and morphogenesis involves budding, primarily into the Golgi cisternae (*22*).

Each of the virus isolates examined in this study exhibited a specific antigenic reaction and a characteristic RNA oligonucleotide pattern sufficient to identify it as a separate virus. However, the serologic cross-reactivity and conserved 3′ terminal RNA sequence of these viruses suggest their grouping within a genus. The absence of a detectable serologic relation with any virus in the four established genera of Bunyaviridae, a differ-

Table 3. Antigenic relations of HFRS-related viruses: 80 percent PRNT titers. PRNT's were performed on Vero E6 cell monolayers grown in Eagle's minimal essential medium (EMEM) with rat antisera (as described in the legend to Table 2) and cell culture–adapted viruses. Approximately 100 PFU of virus were incubated with twofold dilutions of sera for 1 hour at room temperature before inoculation of 25-cm^2 flasks. Inoculated cells were further incubated for 1 hour at 37°C before addition of an overlay of EMEM containing 0.6 percent agarose (Seakem ME), 10 percent heated fetal bovine serum (FBS), 2 m*M* L-glutamine (Gibco), penicillin (100 U/ml), streptomycin (100 μg/ml), and Fungizone (0.5 μg/ml; Gibco). Following incubation for 7 to 14 days after infection a second overlay identical to the first except for a reduced FBS concentration (2 percent) and inclusion of neutral red (final concentration, 0.167 mg/ml) was applied. Plaques were counted as they became visible 1 to 5 days after addition of the second overlay. Titers were expressed as the reciprocal of the highest dilution of antibody, resulting in greater than 80 percent reduction of approximately 100 plaques. Asterisks indicate homologous titers.

Serum pools	Virus							
	HTN	LEE	UR	TCH	GP	SR-11	PH	PA
HTN	4,000*	4,000	0	160	80	40	0	0
LEE	4,000	2,000*	20	160	160	200	0	0
UR	2,000	80	4,000*	16,000	16,000	8,000	160	0
TCH	2,000	200	4,000	32,000*	8,000	8,000	200	0
GP	200	80	2,000	8,000	8,000*	8,000	200	0
SR-11	2,000	20	2,000	2,000	2,000	2,000*	200	0
PH	0	0	0	0	20	20	4,000*	0
PA	0	0	0	0	0	0	0	1,280*

Table 4. Terminal 3′ nucleotide sequences of genome segments from HFRS-related viruses. Virions were concentrated and purified from cell culture supernatants harvested 8 to 22 days after infection (*5*). Virion RNA (1 to 10 μg) was extracted and end-labeled with [5′-^{32}P]3′,5′-cytidine bisphosphate and T4 RNA ligase (*6*). Labeled genome segments were resolved by electrophoresis in 1 percent low-gelling-temperature agarose (Sigma) containing 10 m*M* methylmercury (Alfa Biochemicals), located by autoradiography, purified free of agarose by phenol extraction, and subjected to chemical sequencing (*24*). RNA sequences were determined by electrophoresis of fragments on acrylamide:bisacrylamide gels (20:0.7 percent) (*6*). Homology is indicated by dots and undetermined sequences by X's.

Virus	Sequence
	Large RNA
HTN	AUCAUCAUCUGAGGGAUUUAUUGAU
LEE	·····CU·CAUG·UUG
UR	·····CC··CUC··U·
TCH	·····CU·C·C·GUUA
GP	·····CU·CAUG·UGG
PH	·····XCUC·AUXXXXX
PA	·····CU·C·C·GUU·
SR-11	·····CC··CUC·CG·
	Medium RNA
HTN	AUCAUCAUCUGAGGCGUUUUCUUUG
LEE	·····A···C
UR	·····C·U·G·C
TCH	·····C·U·G·C
GP	·····C
PH	·····A··CU··CX
PA	·····C·UC··C
SR-11	·····C·U·G·C
	Small RNA
HTN	AUCAUCAUCUGAGGGAUUUCUCGAU
LEE	·····
UR	·····
TCH	·····
GP	·····U··X
PH	·····A·C·····
PA	·····A·C·····GAGA
SR-11	·····

ent mode of transmission, and a unique terminal genome sequence define a new and separate genus of Bunyaviridae. We propose the name *Hantavirus* for this genus.

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Gene for α-Chain of Human T-Cell Receptor: Location on Chromosome 14 Region Involved in T-Cell Neoplasms

Abstract. *A human complementary DNA clone specific for the α-chain of the T-cell receptor and a panel of rodent × human somatic cell hybrids were used to map the α-chain gene to human chromosome 14 in a region proximal to the immunoglobulin heavy chain locus. Analysis by means of in situ hybridization of human metaphase chromosomes served to further localize the α-chain gene to region 14q11–q12, which is consistently involved in translocations and inversions detectable in human T-cell leukemias and lymphomas. Thus, the locus for the α-chain T-cell receptor may participate in oncogene activation in T-cell tumors.*

The T-cell antigen receptor is a 80,000 to 90,000 dalton heterodimeric glycoprotein which, in reducing conditions, yields two subunits of 40 to 50 kD each (*1*). Clones of complementary DNA (cDNA) encoding the β-chain of the T-cell receptor have been isolated from murine (*2*) and human (*3*) T lymphocyte cDNA libraries. More recently, cDNA clones encoding what is believed to be the α-

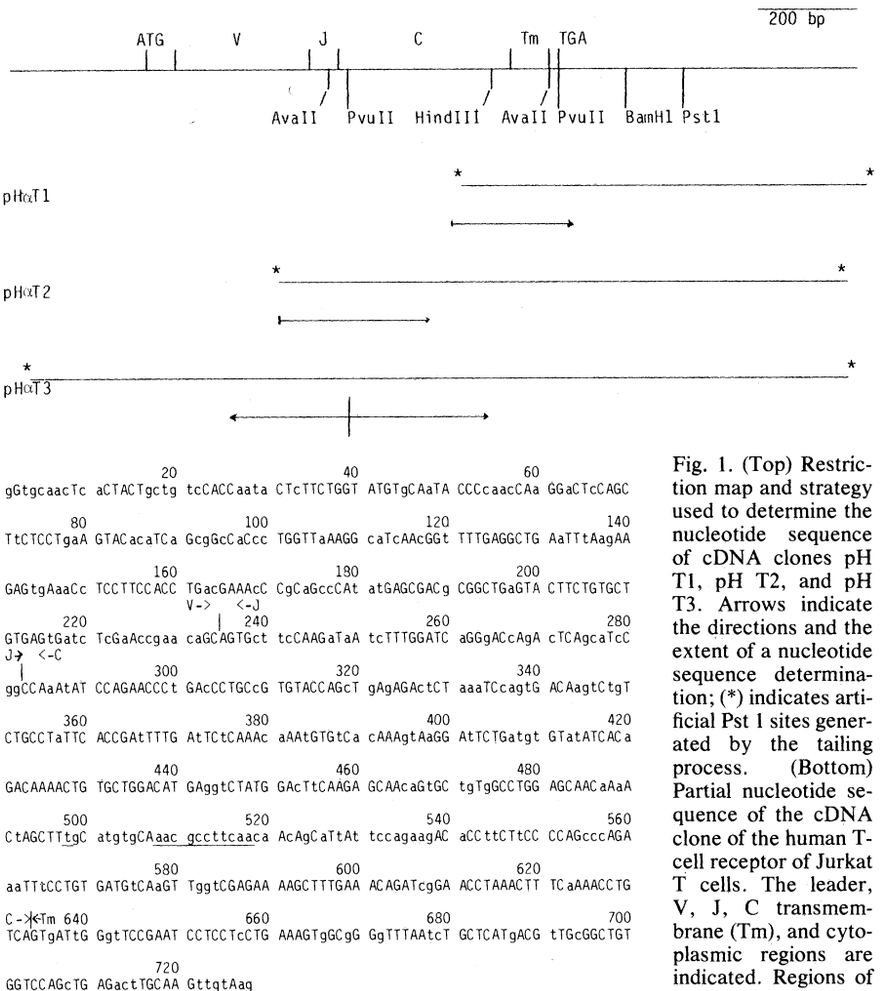

Fig. 1. (Top) Restriction map and strategy used to determine the nucleotide sequence of cDNA clones pH T1, pH T2, and pH T3. Arrows indicate the directions and the extent of a nucleotide sequence determination; (*) indicates artificial Pst I sites generated by the tailing process. (Bottom) Partial nucleotide sequence of the cDNA clone of the human T-cell receptor of Jurkat T cells. The leader, V, J, C transmembrane (Tm), and cytoplasmic regions are indicated. Regions of homology to the mouse cDNA α-chain clones described by Chien *et al.* (*4*) and by Saito *et al.* (*5*) are also indicated by capital characters. A cDNA library was prepared from 0.5 μg of polyadenylate [poly(A)]-containing RNA isolated from Jurkat (clone E) human T cells (*9*) by the method of Gubler and Hoffman (*20*) with minor modifications. The C-tailed double-stranded cDNA was annealed to the G-tailed Pst I site of PUC 8 (*21*). JM109 cells were transformed by the method of Hanahan (*22*) and approximately 700,000 colonies were collected. The cDNA library was screened by the method of Grunstein and Hofner (*23*) using the mouse α-chain-specific cDNA clone TT11. Thirty-six positive clones were isolated. Three clones (pHαT1, pHαT2, and pHαT3) were analyzed in detail and shown to be 900, 1200, and 1600 bp in length. Restriction map analysis and partial overlapping sequences of the three clones indicated that they were homologous. Sequencing was carried out in M13, mp18, and mp19 by the Sanger dideoxy method (*24*).