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VIROLOGY 131, 482-491 (1983)

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Analysis of Hantaan Virus RNA: Evidence for a New Genus of Bunyaviridae

CONNIE S. SCHMALJOHN[1] AND JOEL M. DALRYMPLE

United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

Received June 13, 1983; accepted September 9, 1983

Hantaan virus, the prototype virus of hemorrhagic fever with renal syndrome, was examined for nucleic acid characteristics which would support its previously proposed inclusion in the virus family Bunyaviridae. Nucleocapsid RNA from Hantaan virions and a control bunyavirus were examined for ribonuclease A (RNase A) sensitivity. Both viruses exhibited a similar accessibility of RNA within nucleocapsids to digestion by RNase A. Complete digestion of the RNA of both viruses was affected with high concentrations of ribonuclease. Evidence for negative strand RNA polarity was obtained by an *in vitro* transcriptase assay. RNA dependent RNA polymerase activity was associated with Hantaan virions. Polymerase activity required manganese and nucleoside triphosphates and was enhanced by magnesium, 2-mercaptoethanol, and sodium chloride. Oligonucleotide map analysis of the large (L), medium (M), and small (S) genome segments of Hantaan virus demonstrated that each RNA species was unique with respect to each other and was different from host cell ribosomal RNA. A common 3′ terminal sequence of the three genome segments was determined to be *3′AUCAUCAUCUG*. This sequence is different from those reported for viruses within the four recognized genera of the Bunyaviridae. Because all other data were consistent with nucleic acid characteristics of the Bunyaviridae, we propose a separate genus within the Bunyaviridae with Hantaan as its prototype virus.

INTRODUCTION

Hemorrhagic fever with renal syndrome (HFRS) is a collective name adopted for a variety of clinically similar diseases including Korean hemorrhagic fever (KHF), nephropathia epidemica (NE), and epidemic hemorrhagic fever (EHF) which are endemic throughout Korea, Scandinavia and Europe, and China, respectively (Gajdusek *et al.*, 1982; WHO, 1982). A viral agent (designated Hantaan virus) has been isolated from epizootic and epidemic regions of Korea and can be identified using convalescent sera from patients clinically diagnosed with Korean hemorrhagic fever (Lee *et al.*, 1978, 1982a, b). Because of the strong epidemiological correlation between characteristic human disease (HFRS) and subsequent development of anti-Hantaan antibody, Hantaan virus is believed to be the etiologic agent of Korean hemorrhagic fever or a close serological relative (Lee *et al.*, 1978).

Hantaan virus was originally isolated from the Korean striped field mouse (*Apodemus agrarius corea*) and has recently been adapted to and assayed in continuous cell lines (French *et al.*, 1981; McCormick *et al.*, 1982). Preliminary morphological characterization of Hantaan virus, strain 76-118, suggested that this virus may be similar to members of the Bunyaviridae family (McCormick *et al.*, 1982; White *et al.*, 1982). Stronger evidence for this classification, however, comes from the tripartite single-stranded RNA genome reported for Hantaan virus (Schmaljohn *et al.*, 1983). The only animal virus family known to possess a three segmented RNA genome is the Bunyaviridae.

The absence of documented serological

[1] To whom reprint requests should be addressed.

0042-6822/83 $3.00

relationships between Hantaan virus and members of recognized virus families has thus far precluded definitive classification. Consequently, the molecular description of this virus has become the primary available means for taxonomic identification. In the present study we have attempted to expand the molecular characterization of Hantaan virion RNA, to compare it to RNA of viruses within the Bunyaviridae and to determine whether, biochemically, Hantaan virus conforms to criteria established for the Bunyaviridae.

MATERIALS AND METHODS

Propagation and purification of Hantaan virus. Hantaan virus strain 76-118 was passaged in Vero E6 cells (American Type Culture Collection C1008) as previously described (Schmaljohn *et al.*, 1983). All experiments were performed using infectious inocula from the second Vero E6 passage. Cells were propagated and maintained in growth medium consisting of Eagle's minimal essential medium, Earles' salts (EMEM), supplemented with 5% heated fetal bovine serum, and penicillin and streptomycin (100 units and 100 μg/ml, respectively). Vero E6 cells were routinely infected at low multiplicities of infection (m.o.i. less than 0.1). Growth medium was removed 4 days postinfection and replaced with fresh growth medium. Infected cell culture supernatants were harvested 8 days postinfection and virus was concentrated by polyethylene glycol precipitation and purified by rate-zonal sedimentation in sucrose as previously described (Schmaljohn *et al.*, 1983).

Ribonuclease digestion of viral nucleocapsids. Hantaan virus infected Vero E6 cell cultures were radiolabeled with 20 μCi/ml [5,6-^{3}H]uridine (New England Nuclear) or 15 μCi/ml ^{3}H-labeled amino acids (Amersham) from 4 to 8 days postinfection. Vesicular stomatitis virus (VSV Indiana, Mudd Summers strain) and LaCrosse virus infected Vero cells were radiolabeled with 20 μCi/ml [5,6-^{3}H]uridine from 1 to 18 hr postinfection. Virion nucleocapsids were released by treatment of gradient purified viruses with 1% NP-40 in 0.01 *M* Tris-HCl, 1.0 *M* NaCl, and 0.001 *M* EDTA, pH 7.4, for 10 min at room temperature. Disrupted virus preparations were layered onto 10–30% linear cesium chloride gradients prepared in 0.01 *M* Tris-HCl, 0.1 *M* NaCl, 0.001 *M* EDTA, pH 7.4 (TNE), and centrifuged for 24 hr at 35,000 rpm in a Beckman SW-41 rotor. Gradient fractions containing nucleocapsids were identified by scintillation counting. Viral nucleocapsids were treated with 0, 10, 50, or 100 μg/ml RNase A (Sigma) in 1 ml of buffer containing 0.01 *M* Tris-HCl and 0.4 *M* NaCl, pH 7.4. Samples were incubated at 37° for 30 min. Ribonuclease sensitivity was assayed by precipitation of samples with 10% trichloroacetic acid, collection by filtration onto glass fiber filters (Whatman GF/A) and scintillation counting.

In vitro transcriptase assay. Hantaan virions were concentrated and subjected to rate zonal sedimentation as described above and the visible virus band was collected and layered onto a second linear 10–60% sucrose gradient prepared in TNE. Gradients were centrifuged for 15 hr at 35,000 rpm in a Beckman SW-41 rotor. The quantity of viral protein in the recovered gradient fractions was estimated using a Bio-Rad protein assay kit. Transcriptase reactions were carried out using conditions similar to those described for Uukuniemi virus (Ranki and Pettersson, 1975) or for Newcastle disease virus and vesicular stomatitis virus (Huang *et al.*, 1971) and are fully detailed in the appropriate figure legends.

Ribonuclease T1 digestion and two-dimensional electrophoresis. Infected Vero E6 cell cultures were radiolabeled from 4 to 8 days postinfection with 0.2 mCi/ml [^{32}P]orthophosphate (ICN). Cell culture supernatants were harvested and virus was concentrated and gradient purified by a single rate zonal sedimentation in sucrose as described above. Virions were dissolved in 1% sodium dodecyl sulfate (SDS) and RNA was extracted three times with phenol:cresol:8-hydroxy-quinoline (500 ml:70 ml:0.5 g). The aqueous phase containing the RNA was recovered by precipitation in 0.3 *M* sodium acetate with 3 volumes of cold ethanol. Individual RNA segments

were resolved by electrophoresis in 1.4% low melting point agarose (Marine Colloids) in 10 *mM* sodium phosphate buffer, pH 7.0, in a horizontal, submarine electrophoresis unit (Bio-Rad). The three RNA segments were located by exposure of the gel to XAR film (Kodak). Gel slices containing each RNA were excised and melted at 60° and transferred to 37°. RNA was digested by addition of 10 units of T1 ribonuclease (Sigma) in 10 m*M* Tris–HCl, 1 m*M* EDTA, pH 7.0. Following incubation at 37° for 1 hr, buffer containing 8 *M* urea, 0.1 *M* citric acid, and bromophenol blue and xylene cyanol dye markers was added and the samples were applied to the wells of the first dimension gel. The agarose was allowed to solidify in the wells prior to application of running buffer. Gel and electrophoresis conditions were as described by DeWachter and Fiers (1972) and modified by Clewley *et al.* (1977).

Terminal 3′ sequence analysis of Hantaan virion RNA segments. The RNA of purified Hantaan virions was extracted and recovered as above. Following centrifugation for 10 min in a microcentrifuge (Eppendorf) the RNA pellet was rinsed with cold ethanol, lyophylized to dryness, and resuspended in sterile distilled water. Terminal 3′ end labeling was accomplished by incubation of 50 μg of virion RNA with 50 μCi of cytidine 3′,5′-bis [^{32}P]phosphate (ICN, 2000 Ci/mmol) and 30 units of T4 RNA ligase (P-L Biochemicals, 10,000 units/ml) in a 15 μl reaction mixture containing 50 m*M* HEPES, 3 m*M* dithiothreitol, 10 m*M* $MgCl_2$, and 5 μ*M* ATP (Sigma). Following incubation on ice for at least 18 hr, RNA was recovered by addition of 5 μg of carrier RNA, 0.3 *M* sodium acetate, and 3 volumes of cold ethanol. Precipitates were dried and redissolved in buffer containing 15 m*M* sodium sulfate, 1 m*M* EDTA, 50 m*M* boric acid, 5 m*M* sodium borate, pH 8.19 with 10 m*M* methyl mercury hydroxide (Alfa) and loaded onto a 1% low melting point agarose gel prepared in the same buffer. Electrophoresis was at 100 V for approximately 2 hr or until the bromophenol blue dye marker had reached the bottom of the 12 cm gel. RNA bands were located by exposure of the gel to XAR-5 film (Kodak). Gel slices containing individual RNA segments were excised, melted at 60° in at least 3 volumes of TNE containing 0.5% SDS and the RNA extracted twice with the phenol mixture as above. Following ethanol precipitation, the RNA was subjected to direct chemical cleavage as described by Peattie (1979). RNA fragments were resolved on 20% acrylamide gels containing 7 *M* urea, 50 m*M* Tris-borate pH 8.3, and 1 m*M* EDTA. Electrophoresis was at 900–1200 V until the bromophenol blue dye marker had migrated 15 cm. Gels were frozen at −70° and exposed to XAR-5 film (Kodak) with lightning plus screens (Dupont).

RESULTS

Ribonuclease Sensitivity of Hantaan Virus Nucleocapsids

We previously demonstrated that Hantaan virions disrupted with nonionic detergents released three distinct nucleocapsid species similar in size to nucleocapsids of members of the family Bunyaviridae (Schmaljohn *et al.*, 1983). Ribonuclease (RNase) sensitivity of virion nucleocapsids has been reported for members of the Bunyaviridae (Pettersson *et al.*, 1971; Obijeski *et al.*, 1976). This characteristic distinguishes bunyaviruses from rhabdoviruses which contain nucleocapsids resistant to digestion by RNase A (Soria *et al.*, 1974). We compared the ribonuclease sensitivity of Hantaan virus nucleocapsids to that of nucleocapsids from the rhabdovirus, vesicular stomatitis virus (VSV), and the bunyavirus LaCrosse (LAC). Virions radiolabeled with [5,6-^{3}H]uridine or with ^{3}H-mixed amino acids were purified by density sedimentation in sucrose and nucleocapsids were released by treatment with the nonionic detergent NP-40 in 1 *M* sodium chloride. Nucleocapsids were sedimented to density in cesium chloride gradients, recovered, and treated with various concentrations of RNase A. Following a 30-min incubation at 37°, acid-precipitable counts were measured. The nucleic acid contained within Hantaan virus nucleocapsids was sensitive to degradation by RNase A at 10 μg/ml. LaCrosse virus RNA was also sen-

sitive to degradation at low concentrations of RNase A, however VSV RNA appeared resistant to RNase A concentrations in excess of 50 μg/ml. Hantaan virus nucleocapsids which were radiolabeled with ^{3}H-labeled amino acids exhibited no difference in the acid-precipitable counts after RNase A treatment and served to document the absence of any contaminating proteases which could have contributed to the accessibility of RNA to RNase A digestion by removal of normally protective proteins. We conclude that Hantaan virus nucleocapsid RNA, like RNA of Bunyaviridae nucleocapsids, was not protected from ribonuclease digestion by interaction with nucleoprotein.

Virion-Associated Transcriptase

Bunyaviridae possess tripartite, single-stranded RNA genomes which are negative sense with respect to messenger activity. Consequently, members of this virus family require RNA transcription prior to viral protein synthesis. Like other negative-stranded viruses, an RNA polymerase is associated with the virions and is presumably coded for by virus RNA (Bishop and Shope, 1979). Conditions for *in vitro* assay of virion transcriptase activity have been described for two members of the Bunyaviridae (Ranki and Pettersson, 1975; Bouloy and Hannoun, 1976). To determine whether Hantaan virions possessed an endogenous polymerase capability, we assayed detergent disrupted virions for transcriptase activity under conditions optimized for other negative-stranded viruses.

Standard transcriptase assays used were similar to those described for the bunyavirus Uukuniemi (Ranki and Pettersson, 1975) or for the rhabdovirus VSV (Huang *et al.*, 1971). Unlike rhabdoviruses, Bunyaviridae transcriptase activity has been reported to be dependent upon the presence of manganese divalent cations while the requirement for magnesium cations appears to be less strict (Ranki and Pettersson, 1975; Bouloy and Hannoun, 1976). Like viruses within the Bunyaviridae, purified Hantaan virions appeared to contain transcriptase activity which was absolutely dependent upon the presence of manganese cations (Table 1). Detergent disruption of virions and the presence of nucleoside triphosphates were also required for transcription. Magnesium, 2-mercaptoethanol, and sodium chloride also increased transcriptase activity but the requirement was not absolute. Transcriptase activity appeared sensitive to treatment with RNase A but not to DNase I. Incorporation of the radiolabeled nucleoside-monophosphate was specific and could be competitively reduced by inclusion of nonradiolabeled CTP in the reaction mixtures. Longer incubation times (100 and 200 min) did not result in increased radiolabel incorporation (data not shown). Under conditions suitable for transcription of VSV (Huang *et al.*, 1971), Hantaan virus transcriptase was only minimally active, even when 30 μg of virus protein was added (Table 2). These data indicate that Hantaan virions contain an RNA-dependent RNA polymerase which functions well under *in*

TABLE 1

TRANSCRIPTASE ACTIVITY ASSOCIATED WITH HANTAAN VIRIONS

Reaction conditions	^{3}H cpm incorporated
Complete[a]	15,151
−Virus	535
−NP-40	787
−Mg^{2+}	2,380
−Mn^{2+}	532
−GTP	655
−UTP	805
−2-ME	1,161
−NaCl	3,341
+RNase A	3,669
+DNase	11,605
+1 μ*M* CTP	5,864
+5 μ*M* CTP	2,735
+10 μ*M* CTP	963

[a] Complete reaction mixtures contained 20 μg of virion protein, 50 m*M* Tris–HCl pH 8.0, 25 m*M* NaCl, 5 m*M* $MnCl_2$, 1.5 m*M* $MgCl_2$, 10 m*M* 2-mercaptoethanol (2-ME), 1 m*M* each ATP, GTP, and UTP, 10 μCi [^{3}H]CTP (24.6 Ci/mmol), and 0.2% NP-40. Samples were incubated at 37° for 1 hr and incorporation of radiolabel determined by acid precipitation of samples.

TABLE 2

In Vitro TRANSCRIPTASE ASSAY OF VESICULAR STOMATITIS VIRUS AND HANTAAN VIRUS

Reaction conditions	^{3}H cpm incorporated
Vesicular stomatitis virus (VSV)	
Complete (20 μg)[a]	21,434
−VSV	252
−Triton N-101	1,879
−ATP	624
Hantaan virus	
Complete (10 μg)	433
(20 μg)	737
(30 μg)	1,085
−Hantaan	581

[a] Reaction mixtures contained the indicated amount of Hantaan or VSV virion protein, 50 m*M* Tris-HCl pH 7.3, 100 m*M* NaCl, 4 m*M* $MgCl_2$, 3 m*M* dithiothreitol, 0.7 m*M* each ATP, GTP, and UTP, 10 μCi [5-^{3}H]CTP and 80 μg Triton N-101. Incubation was at 32° for 1 hr.

vitro assay conditions suitable for Bunyaviridae transcriptase but functions poorly under conditions which are optimal for rhabdoviruses.

Oligonucleotide Maps of Virion RNA

The three RNA species contained within Hantaan virions, designated large (L), medium (M), and small (S), have molecular weights of approximately 2.7, 1.2, and 0.6 $\times 10^6$, respectively (Schmaljohn *et al.*, 1983). Oligonucleotide map analysis of each of the three RNA species was performed to determine whether these RNA species were unique with respect to each other as well as to host ribosomal RNAs. Hantaan virus was radiolabeled with [^{32}P]orthophosphate, and virions were purified by rate zonal centrifugation. The L, M, and S RNA species, which were completely resolved by agarose gel electrophoresis, were recovered from the gels, digested with T1 ribonuclease and subjected to two-dimensional polyacrylamide gel electrophoresis (Fig. 1). It is apparent that the fingerprint patterns of the L, M, and S RNA species are unique. Direct comparison of the autoradiographs demonstrated no oligonucleotides indicative of cross-contamination of genome segments. The schematic diagram illustrates the probable segment identification of individual oligonucleotide spots in the composite map. A few of the largest oligonucleotides in the fingerprints of individual RNA segments migrated anomalously compared to the composite fingerprint, presumably due to retardation of larger oligonucleotides by the agarose applied to the first dimension wells of the individual maps but not the composite map. No similarity between these oligonucleotide maps and the maps of host cell 28 S and 18 S ribosomal RNAs could be detected (data not shown). The three RNA segments of Hantaan virions, like Bunyaviridae RNA, appear to be unique species, free of host cell ribosomal RNA.

Terminal 3′ Sequence of Hantaan Virion L, M, and S RNAs

The 3′ terminal RNA sequence of viruses representing all four currently recognized genera within the Bunyaviridae have been reported (Clerx-van Haaster *et al.*, 1982; Parker and Hewlett, 1981; Obijeski *et al.*, 1980). Viruses within each genus have very similar, if not identical 3′ end sequences. The 3′ termini of the L, M, and S RNA species of individual viruses appear to be highly conserved and, for the two viruses examined, complementary to several bases on the 5′ ends (Obijeski *et al.*, 1980). All serologically classified members of each genus within the family Bunyaviridae which have also been examined by terminal sequence analysis, can be assigned to that genus by their common 3′ terminal RNA sequence. Although the tripartite RNA genome of Hantaan virus strongly suggested classification of this virus in the family Bunyaviridae, no conclusive serological relationship to other members has yet been reported. We therefore examined the 3′ terminal RNA sequence of Hantaan virus to determine if it displayed characteristics common to Bunyaviridae such as conserved 3′ termini on the L, M, and S segments, and if so, whether the sequence was the same as that reported for viruses within existing genera.

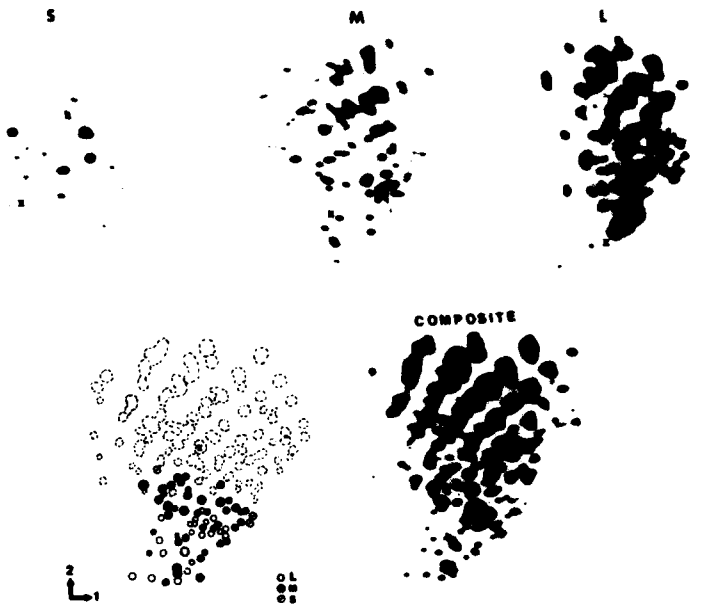

FIG. 1. Oligonucleotide maps of Hantaan virion RNA. Individual L, M, and S RNA segments (resolved by agarose gel electrophoresis) and total virion RNA (extracted from gradient purified virus) were digested with T1 ribonuclease and subjected to two-dimensional electrophoresis. The schematic diagram illustrates assignments of individual oligonucleotides in the composite map. Bromophenol blue and xylene cyanol dye markers are indicated with "X." Arrows on the schematic diagram show the direction of first and second dimension electrophoresis.

Purified Hantaan virions were radiolabeled with [^{32}P]cytidine-bis-phosphate (pCp) using T4 RNA ligase. The three RNA species were resolved by electrophoresis in 1% low melting point agarose gels containing methyl-mercury hydroxide. RNA bands were located, and extracted from agarose gels as described under Materials and Methods. Sequences of the RNAs were determined by the chemical cleavage method of Peattie (1979). Autoradiographs of the 20% acrylamide sequencing gels are shown in Fig. 2. The L, M, and S RNAs of Hantaan virus each possessed a conserved 3′ terminal sequence of 3′ *AUCAUCAUCUG*. Shadow bands, such as those observed at the "A greater than G" residues between positions 4–5 and 7–8 from the 3′ terminus, were not consistent from one sequencing gel to another and were not considered as true chemical cuts.

The 3′ sequence observed for Hantaan virus is different from those reported for viruses in the four genera of the Bunyaviridae (Table 3). Hantaan virus appears sufficiently distinct from other genera to merit the proposal of a new genus in the virus family Bunyaviridae.

DISCUSSION

Bunyaviridae is the largest and most recently described family of arthropod borne viruses. Only in the last few years has the family been subdivided into four genera (*Bunyavirus*, *Phlebovirus*, *Uukuvirus*, and *Nairovirus*) which are composed of numerous interrelated serogroups (Bishop *et al.*, 1980). All recognized members of the Bunyaviridae which have been examined possess morphologically similar spherical, virion particles, 90–110 nm in diameter, which are a composite of three distinct helical nucleocapsids surrounded by a lipid containing envelope. Two glycoproteins appear to be associated with the envelope,

FIG. 2. Terminal 3′ sequences of Hantaan virion L, M, and S RNA segments. Electrophoretic comparison of M and S segment sequences was performed on the same polyacrylamide gel and L segment sequences on a separate gel. Terminal sequences are read from bottom to top.

while a single polypeptide species forms the nucleocapsid structure. A fourth high-molecular-weight protein, which may be the RNA polymerase, has also been observed with some members of the Bunyaviridae (Bishop and Shope, 1979).

The preliminary physical description of Hantaan virus, including electron microscopic measurements of virion particles (White *et al.*, 1982; McCormick *et al.*, 1982) and sedimentation coefficients of Hantaan virions and nucleocapsids (Schmaljohn *et al.*, 1983), suggested that Hantaan virus satisfied the physical criteria for membership in the Bunyaviridae.

Although analysis of virion proteins is still incomplete, a single polypeptide with a molecular weight of about 50,000 was found to be associated with all three Hantaan virus nucleocapsids, and two other viral proteins (MW approx. 55,000 and 65,000) were released from intact virions by nonionic detergent treatment (Schmaljohn *et al.*, 1983 and unpublished obser-

TABLE 3

3′ TERMINAL NUCLEOTIDE SEQUENCES OF VIRUSES WITHIN EACH OF THE BUNYAVIRIDAE GENERA[a]

Virus	Genus	3′ Terminus
Lacrosse	*Bunyavirus*	UCAUCACAUGA
Hazara	*Nairovirus*	AGAGAUUCUUU
Sandfly fever (sicilian)	*Phlebovirus*	UGUGUUUCG
Uukuniemi	*Uukuvirus*	UGUGUUUCU
Hantaan	—[b]	AUCAUCAUCUG

[a] The 3′ terminal sequence of selected members of each genus are as reported by Clerx-van Haaster *et al.* (1982).

[b] Proposed fifth genus of the Bunyaviridae which would include Hantaan virus as its prototype member.

vations). A nucleocapsid protein similar in size to this has been reported for members of the *Nairovirus* genus, but viruses in the other four genera have smaller nucleocapsid proteins (Bishop *et al.*, 1980). In general, Bunyaviridae have either one large and one small envelope protein, such as the G1 (85–120,000) and G2 (29–41,000) glycoproteins of the *Bunyavirus* and *Nairovirus* genera, or two intermediately sized envelope proteins such as the G1 (55–75,000) and G2 (50–65,000) glycoproteins of the *Phlebovirus* and *Uukuvirus* genera (Bishop and Shope, 1979). If the two polypeptides separable from Hantaan nucleocapsids by detergent treatment of virions do prove to be viral envelope proteins, then none of the protein profiles of the four Bunyaviridae genera will be consistent with that of Hantaan virus.

While information on Hantaan virion proteins remains incomplete, and inconclusive, the virion RNA description reported here clearly indicates that Hantaan virus is a bunya-like virus. The Hantaan virus genome is composed of three unique, single-stranded, RNA species with a combined molecular weight of about 4.5×10^6. Virion polymerase activity, a feature of negative stranded viruses, is suggestive that Hantaan virions have an anti-message sense genome. We have been unable to program reticulocyte lysate *in vitro* translation systems with Hantaan virion RNA, although control messenger RNA activity could be observed. This observation provides additional supportive evidence that Hantaan virus is a negative-stranded virus, however, conclusive evidence must come from hybridization of polysome associated RNAs to the virion genome.

In the absence of documented serological relationships with other members of the Bunyaviridae, Hantaan virus could not be placed into any of the four Bunyaviridae genera. Intrageneric Bunyaviridae have recently been demonstrated to be readily discernible from intergeneric viruses based on molecular as well as serological characteristics (Obijeski *et al.*, 1980; Parker and Hewlett, 1981; Clerx-van Haaster *et al.*, 1982). The 3′ terminal sequence of the three RNA species has been shown to be highly conserved within a genus, but quite dissimilar among members of different genera. Furthermore, the 3′ terminal sequence of individual viruses appears to be identical or nearly identical on each of the three RNA segments. In the present study Hantaan virus has been demonstrated to be comparable to Bunyaviridae in that the 3′ terminal sequences of the L, M, and S RNA segments were conserved; however, the Hantaan virus 3′ termini were different from consensus sequences of each of the four Bunyaviridae genera. Consequently, we propose that Hantaan virus be considered as the prototype member of a new genus in the family Bunyaviridae, and that viruses serologically related to Hantaan virus be included in this genus. In support of this proposal, recent studies in our laboratory have resulted in the preliminary observation that two other viruses which are serologically related to, but distinguishable from, Hantaan virus have 3′ terminal sequences of at least 12 residues which are identical to those at the 3′ termini of corresponding Hantaan virion genome segments.

Numerous virus isolates, serologically related to Hantaan virus, have recently been obtained from several rodent species in widely diverse geographical regions (Kitamura *et al.*, 1983; Lee *et al.*, 1982; Tsai *et al.*, 1982; LeDuc *et al.*, 1982). Adaptation of these isolates to cell culture and subsequent virus characterization should con-

tribute significantly to the present data base and allow determination of the scope of the newly proposed genus.

An important consideration in placing Hantaan and related viruses in the Bunyaviridae recalls that this family contains many arthropod-borne viruses, but, as yet, no known arthropod vector has been reported for any of the Hantaan-like viruses. Intensive entomological studies have not been performed, however, and it is possible that arthropods may yet be implicated in the transmission of these viruses.

In summary, we have described molecular characteristics of Hantaan virus—a virus believed to be the etiologic agent of, or closely related to the agent of Korean hemorrhagic fever (Lee *et al.*, 1982). All virion RNA characteristics observed were consistent with classification of Hantaan virus in the Bunyaviridae family. Terminal sequence analysis of the RNAs, however, did not allow inclusion of Hantaan into any of the four existing genera. We have proposed, therefore, that Hantaan and antigenically related viruses comprise a fifth genus of the Bunyaviridae. Molecular examination of serological relatives of Hantaan virus will be essential for confirmation of the requirement for a new genus within the Bunyaviridae.

ACKNOWLEDGMENTS

We wish to thank S. Hasty for providing excellent technical assistance and D. H. L. Bishop for advice and interpretation of RNA sequence data.

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