Nucleotide sequence of the L genome segment of Hantaan virus

AD-A233 151

C.S.Schmaljohn

Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011, USA

Hantaan virus, strain 76-118, is the prototype virus of the *Hantavirus* genus of the Bunyaviridae family and is the etiologic agent of Korean hemorrhagic fever. Hantaan virus has a tripartite, single-stranded, negative sense RNA genome with segments designated as large (L), medium (M), and small (S). Molecular cloning, sequence analysis, and expression studies revealed that the S and M segments of Hantaan virus, respectively, encode the nucleocapsid and envelope glycoproteins (1, 2, 3, 4, 5). The L genome segment is presumed to encode the virion-associated transcriptase. To complete the characterization of the Hantaan virus genome, the nucleotide sequence of the L segment of Hantaan virus was determined.

For molecular cloning, first-strand synthesis was performed essentially as previously described for the S segment (1). A specific oligonucleotide primer, complementary to the 3′ terminal nucleotide sequence of Hantaan L (6), was used to prime cDNA synthesis on virion RNA templates. RNAse H digestion and cDNA synthesis with Klenow were used for second-strand synthesis (Pharmacia). Double-stranded cDNA was cloned into Bluescript vectors (Strategene), either by the addition of EcoRI linkers or by blunt end ligation. The authenticity of cDNA clones was confirmed by Northern blot hybridization of cDNA to Hantaan virion L RNA. Sequences were determined for both cDNA strands by dideoxy chain termination reactions, and those at the 5′ terminus of virion RNA were confirmed by dideoxy chain termination reactions with virion RNA templates as previously described (7).

The L genome segment of Hantaan virus was found to consist of 6,530 nucleotides. A hard copy of the sequence can be obtained from the author with requests for reprints. As reported for the M and S segments of Hantaan virus (1, 2), the L segment displayed a consensus sequence 3′ terminal nucleotide sequence of AUCAUCAUC and had complementary 5′ terminal oligonucleotides. A long, open reading frame identified in the virus-complementary sense RNA could potentially encode 2,150 amino acids or a polypeptide of 246,5000 daltons (Figure 1, reading frame 1, nucleotides 38-6490). The L protein of

Hantaan virus was previously estimated to be 200,000 daltons, based upon electrophoretic migration (8).

Recently, the nucleotide sequence of the L genome segment of one other virus in the Bunyaviridae family was reported: Bunyamwera virus, a member of the *Bunyavirus* genus (9). No significant nucleotide or amino acid homology could be detected between the Hantaan L gene or gene product and those of Bunyamwera virus (not shown). As reported for the L segment of Bunyamwera virus, the L segment of Hantaan virus has an open reading frame (ORF) of unknown significance in the virus-sense RNA which overlaps that encoding the putative L protein (Figure 1), reading frame -3, nucleotides 6302-5637). This ORF could encode a polypeptide consisting of 221 amino acids or approximately 24,000 daltons. No homology was detected between this potential polypeptide and that encoded by the analogous ORF of Bunyamwera virus.

ACKNOWLEDGEMENT

REFERENCES

1. Schmaljohn,C.S., Jennings,G.B., Hay,J. and Dalrymple,J.M. (1986) *Virology* **155**, 633-643.
2. Schmaljohn,C.S., Schmaljohn,A.L. and Dalrymple,J.M. (1987) *Virology* **157**, 31-39.
3. Schmaljohn,C.S., Sugiyama,K., Schmaljohn,A.L. and Bishop,D.H.L. (1988) *J. Gen. Virol.* **69**, 777-786.
4. Pensiero,M.N., Jennings,G.B., Schmaljohn,C.S. and Hay,J. (1988) *J. Virol.* **62**, 696-702.
5. Schmaljohn,C.S., Chu,Y.K., Schmaljohn,A.L. and Dalrymple,J.M. (1990) *J. Virol.* **64**, 3162-3170.
6. Schmaljohn,C.S., Hasty,S.E., Dalrymple,J.M., LeDuc,J.W., Lee,H.W., von Bonsdorff,C.-H., Brummer-Korvenkontio,M., Vaheri,A., Tsai,T.F., Regnery,H.L., Goldgaber,D. and Lee,P.W. (1985) *Science* **227**, 1041-1044.
7. Schmaljohn,C.S., Arikawa,J., Hasty,S.E., Rasmussen,L., Lee,H.W., Lee,P.W. and Dalrymple,J.M. (1988) *J. Gen. Virol.* **69**, 1949-1955.
8. Elliot,L.H., Kiley,M.P. and mcCormick,J.B. (1984) *J. Gen. Virol.* **65**, 1285-1293.
9. Elliot,R.M. (1989) *Virology* **173**, 426-436.
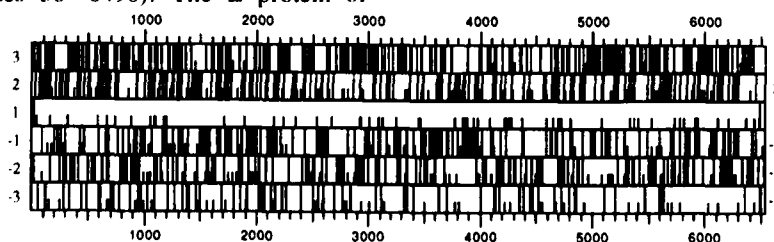10. Marck,C. (1988) *Nucleic Acids Res.* **16**, 1829-1836.

Figure 1. Potential open reading frames encoded by the L segment of Hantaan virus were identified by using the DNA Strider Program (10). Half bars represent translation initiation codons and full bars represent termination codons.