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NOTES

Identification of Hemagglutinin and Neuraminidase Genes of Influenza B Virus

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The genome of influenza B viruses was shown by electrophoresis to consist of eight RNA segments. The fifth largest segment coded for hemagglutinin and the sixth coded for neuraminidase.

By polyacrylamide gel electrophoresis with high concentrations of urea, the RNA of influenza A viruses was resolved into eight distinct bands. Each RNA segment was assigned to one of the virus-specific polypeptides (3, 5). Influenza B viruses were also shown to contain eight RNA segments (4). Recently, the fifth largest RNA segment was reported to code for hemagglutinin (HA) (V. R. Racaniello and P. Palese, *in* R. D. Barry and B. W. J. Mahy, ed., *Negative Strand Viruses and the Host Cell,* in press.

The study presented in this communication has confirmed the above finding and has shown, in addition, that the sixth segment codes for neuraminidase (NA).

Propagation of MDCK cells and virus growth have been reported elsewhere (6). Influenza B virus strains B/Lee/40 (Lee), B/Massachusetts/1/71 (Mass), and two reciprocal recombinants derived from the cross between them, clone 12 ($HA^{Mass}NA^{Lee}$) and clone 28 ($HA^{Lee}NA^{Mass}$), have been described in detail (7). For the preparation of ^{32}P-labeled virus, cells were infected at a multiplicity of 10 PFU/cell and overlaid with maintenance medium lacking phosphate. A 60-μCi amount of carrier-free $^{32}PO_4^{3-}$ per ml was added immediately after infection. RNA was extracted and electrophoresed as described in the legend to Fig. 1. The eight discrete bands were observed in all four influenza B virus strains. A comparison of the RNA patterns of the two parent viruses, Lee and Mass, shows that no RNA segment of Lee virus migrated to the same position as that of the Mass RNA segments. RNA bands of recombinant viruses were located at the same migration distance as that of corresponding segments of either Lee or Mass. Therefore, the origin of all RNA segments of recombinants could be traced to either of the two parent viruses. Clone 12 and 28 virus genomes shared six out of eight RNA bands, bands 1, 3, and 7 being derived from Lee and bands 2, 4, and 8 from Mass, thus leaving the derivation of only bands 5 and 6 to be attributed to a different genotype. Serological characterization had shown that clone 12 inherited the HA from Lee, and vice versa for clone 28. The electropherogram shows that clone 12 obtained band 5 from Mass and band 6 from Lee virus, and vice versa for clone 28. Therefore, there is no ambiguity in concluding that band 5 of both Lee and Mass codes for HA and band 6 codes for NA.

It is noteworthy that B/Lee and B/Mass, despite the absence of antigenic shift in influenza B viruses, showed recognizable differences in all RNA segments.

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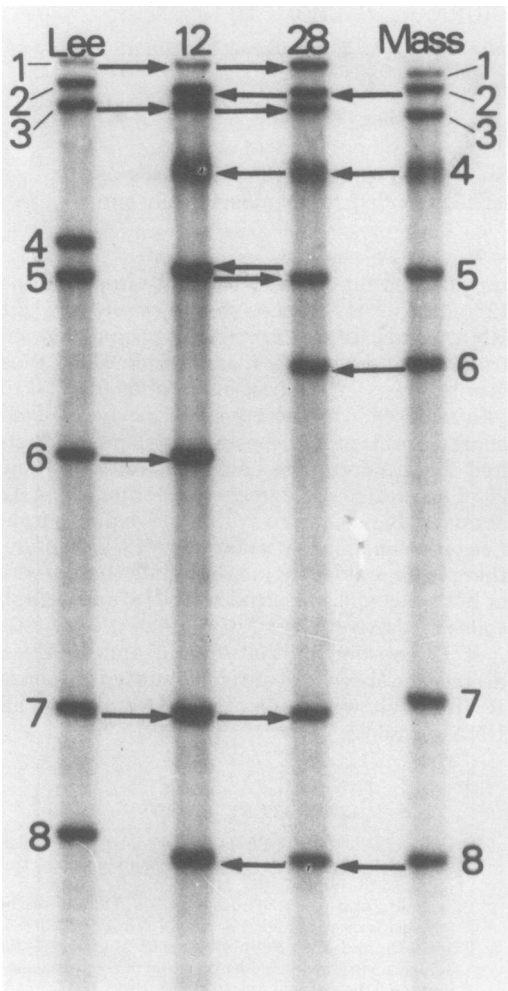

FIG. 1. *Polyacrylamide gel electrophoresis of RNAs of influenza virus strains Lee and Mass, and the recombinant viruses clone 12(HA^{Mass}NA^{Lee}) and clone 28(HA^{Lee}NA^{Mass}). RNA was extracted from ^{32}P-labeled virus. Methods of virus purification and RNA extraction have been described (2). RNA was dissolved in Tris-acetate buffer (40 mM Tris–20 mM sodium acetate–2 mM EDTA [pH 7.8]) (1) containing 0.05% sodium dodecyl sulfate, boiled for 30 s, and quenched in ice water. Before electrophoresis, an equal volume of Tris-acetate buffer containing 9 M urea, 0.003% bromophenol blue, and 30% glycerol was added. The slab gel (dimensions, 14 cm wide by 28 cm long by 1.5 mm thick) consisted of 2.8% polyacrylamide, 6 M urea, and Tris-acetate buffer. The electrode tank contained Tris-acetate buffer. Electrophoresis was done at 120 V for 38 h at room temperature. Fuji 400 X-ray film was exposed to the dried gel. The RNA segments are numbered 1 through 8. The arrows next to the RNA segments indicate their derivation from either Lee (→) or Mass (←).*