Virus-induced Diabetes Mellitus. XXV. Difference in the RNA Fingerprints of Diabetogenic and Non-diabetogenic Variants of Encephalomyocarditis Virus


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SUMMARY

The genomes of diabetogenic and non-diabetogenic variants of encephalomyocarditis virus were analysed by nucleic acid hybridization and RNA fingerprinting. Hybridization and thermal elution profiles failed to show any difference between the RNAs of the two variants, whereas fingerprinting of the T1-digested RNAs revealed at least one oligonucleotide, 20 to 25 nucleotides long, missing in the non-diabetogenic variant.

Encephalomyocarditis (EMC) virus produces diabetes in mice by infecting and destroying pancreatic beta cells (Notkins, 1977). Recently, we showed that our virus pool contained two antigenically similar variants which could not be distinguished by hyperimmune sera (Yoon et al., 1980). One of the variants, designated D, produced diabetes, whereas the other variant, designated B, failed to produce diabetes. The present study was initiated to analyse the genomes of these two variants by nucleic acid hybridization and RNA fingerprinting. We found that both viral cDNA probes hybridized equally well to homologous and heterologous RNAs, but that the RNAs differed by at least one spot in the unique sequence region of their oligonucleotide fingerprints.

Viruses were purified from infected L929 cells by sucrose and CsCl gradient centrifugation (McClintock et al., 1980). RNA was obtained from purified virions after phenol–cresol–chloroform extraction and ethanol precipitation (Kacian & Myers, 1976). The viral RNA was treated with methylmercury hydroxide to enhance transcription according to the method of Payvar & Schimke (1979). Then, labelled cDNA was synthesized using [32P]dCTP and avian myeloblastosis virus reverse transcriptase. Oligo(dT) and oligo(dG) were used as primers and the cDNA was purified by Sephadex G-75 and DEAE column chromatography. Foldback sequences were removed by hydroxylapatite column chromatography as previously outlined by Aulakh & Gallo (1977). The single-stranded cDNA probes of EMC-D and EMC-B were shown to be representative of the complete viral genomes by their ability to protect the labelled viral RNAs from digestion with ribonucleases A and T1. The respective homologous cDNAs protected greater than 94% of EMC-D- and 95% of EMC-B-labelled RNAs.

Hybridizations were performed using 20 μg/ml viral RNA and approximately 0.05 μg/ml cDNA probe (50000 ct/min) in 0.48 M-sodium phosphate buffer pH 6.8, 3 mM-EDTA, and 0.4% SDS. After denaturing at 105 °C for 5 min, the hybrids were allowed to anneal at 65 °C for 10 h. Hybrids were separated from unhybridized strands by hydroxylapatite chromatography. When EMC-D cDNA was hybridized with EMC-D and EMC-B RNA, 90% and 91%, respectively, of the cDNA formed hybrids. Similarly, when EMC-B cDNA was hybridized with homologous and heterologous viral RNA, 90% and 89% of the cDNA formed hybrids. The hybrids were also analysed for thermal stability to evaluate mismatching in base pairing.
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Thermal elution midpoints ($t_{50}$) were determined by loading hydroxylapatite columns with the hybrids in 0.12 M-phosphate buffer pH 6.8 containing 0.2% SDS at 60 °C and subsequent elution with the same buffer at 4 °C increments up to 100 °C (Aulakh & Gallo, 1977). As shown in Fig. 1 (a), the $t_{50}$ values with EMC-D cDNA probe for homologous and heterologous hybrids were identical. Similarly, both EMC-B cDNA homologous and heterologous hybrids had identical $t_{50}$ values (Fig. 1 b).

The failure to detect any difference in the RNAs of EMC-B and EMC-D by hybridization techniques led us to look for differences in RNA fingerprints. RNA extracted from gradient-purified virus was further purified by sucrose density gradient centrifugation, digested with RNase T1, terminally labelled with [$\gamma$-32P]ATP and then fingerprinted on two-dimensional polyacrylamide gels (deWachter & Fiers, 1972; Pederson & Haseltine, 1980). Fig. 2 shows the large oligonucleotides of both EMC-B and EMC-D RNA, together with schematic diagrams. At least one oligonucleotide (no. 8a), about 20 to 25 nucleotides long, is missing from EMC-B RNA, but is present in the fingerprint of EMC-D RNA. The entire experiment, starting with freshly prepared virus or viral RNA, was repeated at least four times. Except for differences in the intensities of a few spots in some fingerprints (e.g. no. 3, 6, 12 and 14 in Fig. 2), or occasional minor spots which arose due to partial digestion, the oligonucleotide patterns of the experiments were similar to those shown in the schematic diagram of Fig. 2. To see if we could detect minor differences in migration of the unique spots (e.g. caused by point mutations), T1 digests of the two variants were mixed and run on the same gel. No increase in the complexity of the pattern was observed as compared to that seen with the individual variants. It should be remembered that the unique regions of any RNA fingerprint represent only 10 to 15% of its genome.

We also looked at other properties of the two variants. Four of the structural proteins (α, β, γ and δ) of the two variants migrated identically on SDS-PAGE, with a pattern similar to that already known for EMC virus capsid proteins (Rueckert, 1976). Moreover, we could not find any differences in the sensitivity to different pH values, salt concentrations, u.v. irradiation and temperature as measured by infectivity (data not shown).

Although the diabetogenic and non-diabetogenic variants of EMC virus could not be distinguished by hyperimmune sera (Yoon et al., 1980) or by nucleic acid hybridization, RNA fingerprints showed a difference in at least one oligonucleotide. Similarly, studies with other
Fig. 2. Fingerprints of EMC RNA. Unlabelled RNA (2 to 3 μg) was digested with ribonuclease T1 and calf intestine alkaline phosphatase for 45 min at 37 °C. The digested RNA was precipitated with ethanol after extraction of the reaction mixture with phenol–chloroform. The fragmented RNA was heated at 70 °C for 3 min and then terminally labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase. The labelled oligonucleotides were separated by two-dimensional gel electrophoresis. Top panels are actual fingerprints of RNA from EMC-D (D) and EMC-B (B). The bottom panels are schematic diagrams of the RNA fingerprints from EMC-D (D) and EMC-B (B).
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Picornaviruses, foot-and-mouth disease virus and poliovirus, have revealed oligonucleotide differences among very closely related subtypes within a serological group (Robson et al., 1980; Minor et al., 1982). The oligonucleotide missing from the non-diabetogenic B variant has not yet been mapped to a specific region of the viral genome nor has its function been determined. It is possible, however, that this oligonucleotide is part of a gene that controls certain biological properties of the virus. In cell culture or in the islets of Langerhans the D variant yields 10 to 50 times more infectious virus than the B variant. This, in part, may be due to the greater interferon-inducing capacity of the B variant (Yoon et al., 1980). There may also be differences in the receptor-binding capacity of the B and D variants for certain cell types (e.g. beta cells), analogous to the differences observed between the antigenically similar 2T variant of Mengo virus and the D variant of EMC virus for cultured neurons (Morishima et al., 1982). These factors, alone or in combination, might be responsible for the different capacity of the two variants to infect and destroy pancreatic beta cells and, in turn, produce diabetes in mice.

REFERENCES


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