ARE DIFFERENCES IN BIOLOGICAL PROPERTIES OF MHV ASSOCIATED WITH DIFFERENCES IN SPECIFIC REGIONS OF THEIR NUCLEOCAPSID mRNA?

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The purpose of this study is to compare specific regions of the nucleocapsid mRNA for murine coronaviruses and to determine if any differences are associated with differences in pathogenicity. Murine hepatitis viruses (MHV) differ in the types of diseases they produce in rodents (see review[1]). In particular, MHV-JHM, causes demyelinating lesions in the central nervous system of many strains of rats while MHV-3 does not. MHV-A59 and MHV-S causes chronic demyelinating disease in mice. MHV-1 causes hepatitis in weanling VS mice.

Experiments with JHM monoclonal antibodies have indicated that both the nucleocapsid protein and E2 glycoprotein may play a critical role in determining the ability of a MHV strain to produce a demyelinating disease[2,3]. Furthermore, it has been shown that different regions of the nucleocapsid gene of MHV-A59 and JHM do not share uniform homology[4,5]. We therefore sought to determine if differences in any region of the nucleocapsid gene could be correlated with differences in the biological properties of these viruses. In order to determine the relative homology various MHV strains share at specific regions of the nucleocapsid gene, three synthetic oligonucleotides were prepared. These oligonucleotides (CKP-1, CKP-2 and CKP-3) were used as primers to bind to purified JHM nucleocapsid mRNA and to make cDNAs, which were used in hybridization experiments (Fig. 1).

Our data indicated that all regions of the nucleocapsid gene of MHV-3 and JHM consistently shared greater homology than was seen between A59 and JHM. Since sequence data has previously shown that A59 and JHM nucleocapsid sequences share an overall homology of 93%[4,5], the biological differences observed between JHM and MHV-3 are only associated with a small overall difference ($<7\%$) in their nucleocapsid coding sequences. In addition, because of the redundancy of the genetic code, the amino acid divergence is likely even less. Therefore, other viral genes may be associated with differences in the ability of JHM and MHV-3 to produce demyelinating disease in rats. This idea is supported by our observation that an altered E2 mRNA is present in the CNS tissue of Wistar Furth rats with a JHM induced demyelinating disease; in addition, the level of E2 antigen detected in these infected rats is significantly reduced[6]. Our data also indicated that the biological differences between the other tested murine hepatitis viruses are not associated with major differences in the coding regions of their nucleocapsid genes.

This work is supported by the MS Society of Canada and the Medical Research Council of Canada.

M. M. C. Lai et al. (eds.), *Coronaviruses*

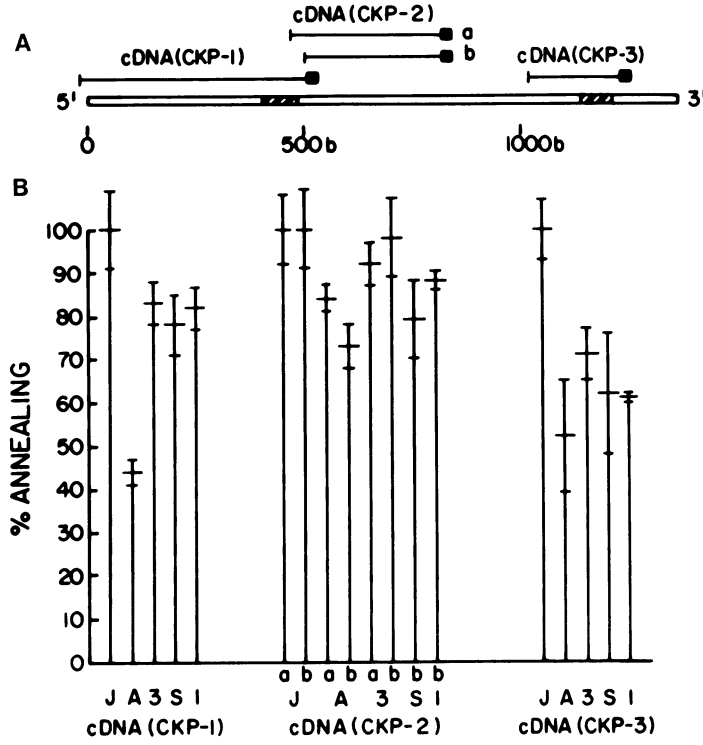

Fig. 1. Panel A

 indicates the coding region for the JHM nucleocapsid gene.

 indicates the binding sites for the synthetic oligonucleotides.

 indicates portion of the JHM nucleocapsid gene homologous to each cDNA.

 indicates areas of reduced homology between JHM and A59. The homology falls to 65% for the region toward the 5' side and 68% for the 3' region.

Panel B. The average normalized hybridization value between the oligonucleotide primed cDNAs and MHV infected L cell RNAs is shown. MHV strains are indicated as follows: J is JHM; A is A59; 3 is MHV-3; S is MHV-S; 1 is MHV-1. The two cDNA (CKP-2) probes that were prepared are indicated by "a" and "b". The error bars indicate the standard deviation.

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