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Antigenicity of Mouse Hepatitis Virus Strain 3 Subcomponents in C57 Strain Mice

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With 2 Figures

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Summary

C57 strain mice were inoculated intraperitoneally with denatured mouse hepatitis virus strain 3 particles and virus surface projection, membrane and ribonucleoprotein subcomponents, obtained from detergent treated purified virus preparations. All immunised animals developed high levels of serum antibody directed against the respective antigens, detectable by enzyme-linked immunosorbent assay. Mice that had been immunised with denatured virus particles or surface projections were protected against infection with mouse hepatitis virus strain 3, whereas immunisation with virus membrane or ribonucleoprotein subcomponents failed to protect mice against virus challenge.

Introduction

Mouse hepatitis virus (MHV) is a member of the Coronaviridae group of viruses which are all lipid-containing, enveloped, positive-stranded viruses that bud from endoplasmic reticular membranes (17, 23). The structural polypeptides of a number of MHV strains, including strains A59 (2, 21), JHM (1, 2, 25) and 3 (1, 12) have been described, and consist of 4 to 6 polypeptides of similar size and composition. These polypeptides are of 3 main types, with up to 3 high mol. wt. glycopolypeptides comprising the surface projections, up to 2 low mol. wt. polypeptides forming membrane proteins, and a single polypeptide of about 50,000 mol. wt. comprising the ribonucleoprotein (RNP) (12, 21, 25). Several reports have described the separation of some or all of the subviral components of MHV A59 (22) and other coronaviruses (4, 6, 14, 18) by disruption of virus particles with Nonidet P40 or Triton X100.

Most strains of mice can be infected with MHV 3 with the development of fulminant hepatitis, although numerous other organs are also infected (17). The

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susceptibility of the C57 mouse strain to MHV 3 has been studied previously in this (16) and other laboratories (26), with the first deaths occurring within 5 days after infection.

In this report we describe the isolation and purification of MHV 3 subviral components, and the immune response of susceptible C57 mice inoculated with inactivated MHV 3 particles and subviral components.

Materials and Methods

Virus Growth

MHV3 was grown in confluent secondary mouse embryonic fibroblasts. Monolayers were infected at an input multiplicity of 0.1 infectious particles per cell and following an adsorption period of 1.5 hours at 37° C, were incubated for 72 hours at 37° C in Eagle's MEM with 2 per cent foetal calf serum (13). Aliquots of this virus suspension were stored at −70° C and used for the preparation of purified virus particles and subcomponents.

Preparation of Purified Virus

Virus was purified at 0° to 4° C as described previously (13). The virus was pelleted at 75,000 × *g* for 1 hour and then resuspended in 1 ml Dulbecco's phosphate buffered saline "A" (PBSA). The resuspended virus was overlaid on to a linear 25 to 55 per cent (w/w) sucrose gradient in PBSA and centrifuged for 16 hours at 90,000 × *g*. The virus peak at 1.18 g/ml was collected.

Iodination Procedure

Preparations of purified virus particles were dialysed against PBSA for 16 hours at 4° C, and then disrupted with 1 per cent Nonidet P40 in PBSA at 21° C in order that all virus components were available for iodination. The iodination procedure used was based on that described by GREENWOOD *et al.* (7).

50 µl of 0.5 M sodium ^{125}I iodide in 0.5 M NaH_2PO_4 2 H_2O and 0.5 M Na_2HPO_4 buffer (phosphate buffer, pH 7.5) was added to 1 ml samples of dialysed, Nonidet P40 treated virus. 40 µl of chloramine T (1 mg/ml) in phosphate buffer was added for 30 to 60 seconds at 21° C. 40 µl sodium metabisulphite (1 mg/ml) in phosphate buffer was then added to stop the reaction.

Isolation of Purified Virus Subcomponents

Sucrose-gradient-purified virus particles were disrupted at 21° C with 1 per cent Nonidet P40 in PBSA and layered on to either 10 to 55 per cent (w/w) or 25 to 65 per cent (w/w) sucrose gradients in PBSA and centrifuged for 16 hours at 90,000 × *g* at 4° C. Subcomponent peaks were located at 1.13 g/ml in 10 to 55 per cent (w/w) sucrose gradients and at 1.23 and 1.27 g/ml in 25 to 65 per cent (w/w) sucrose gradients as described previously for HCV 229E (14).

Polyacrylamide Gel Electrophoresis

Iodinated virus and virus subcomponent fractions were treated with 5 per cent sodium dodecyl sulphate, 2 per cent 2-mercaptoethanol at 100° C for 1.5 minutes. A trace of bromophenyl blue was added to the reduced preparations, and the polypeptides were electrophoresed through 7.5 per cent polyacrylamide gels as described previously (15). After electrophoresis the gels were extruded and sliced into 1 mm discs and their radioactivity determined.

Immunisation Procedure

4—6 weeks old C57 BL/10 strain mice were obtained from the specific-pathogen-free (SPF) unit of this Centre. Groups of 10 mice were immunised with dilutions of purified denatured MHV3 particle preparations of titres about 10^7 ID_{50}, virus sub-