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Immune response of mice immunized with mouse hepatitis virus strain 3

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1. INTRODUCTION

Coronaviruses are widespread in nature and are associated with various diseases in infected hosts [1,2]. The coronavirus, mouse hepatitis virus strain 3 (MHV 3), infects many strains of mice causing fulminant hepatitis [1]. We have previously described the separation of the three main structural components of MHV 3 using sucrose density gradient centrifugation after nonidet P40 treatment [3]. The components were the surface projections containing polypeptides of M_r 170000 and 105000, a membrane fraction comprising polypeptides of M_r 22000 and 20000, and the RNP containing a 50000 M_r polypeptide and the RNA genome [4]. Polypeptides with similar M_r-values have been observed for MHV 3 and other MHV strains [5–8]. In this paper we have used an enzyme-linked immunosorbent assay (ELISA) to study the immune response in mice to immunization with MHV 3.

2. MATERIALS AND METHODS

MHV 3 was grown and purified as previously described [9] and preparations containing about 10^9 particles per ml by electron microscopy were used. Virus preparations were disrupted with nonidet P40 and surface projection, membrane and RNP fractions were separated on sucrose density gradients as described previously [3,10]. Subcomponent fractions were shown to be uncontaminated with the other subcomponents by labelling their polypeptides with ^{125}I and analysing them on polyacrylamide gels [3,10]. Sera were collected from mice immunized with MHV 3 as previously described [3] with simple modifications. Briefly, three groups of five C57 mice were immunized intraperitoneally at 2-day intervals with two 0.5 ml doses of purified, dialysed, formalin-inactivated MHV 3 emulsified in an equal volume of complete Freund's adjuvant. Mice in the three groups were inoculated with 1:50, 1:100 and 1:200 dilutions of denatured MHV 3 preparations, respectively. Three mice within each group were boosted 50 days after the initial immunization. Each animal was bled from the tail at 3, 6, 9, 12, 15, 20, 24, 32, 35 days after inoculation and then each 5th day up to 24 weeks. Sera were absorbed with equal volumes of foetal calf serum before use. The ELISA procedure used has been described previously [3]. The rabbit anti-mouse IgA and IgG antisera (Miles Laboratories) were used at dilutions of 1:400, and the IgM antiserum (Miles Laboratories) at a dilution of 1:800. Checkerboard titrations were done to obtain the optimum serum and antigen dilutions for the ELISA [11]. The antigen and serum dilutions selected are shown in the appropriate figure legends.

3. RESULTS

We have previously shown that mice from the specific pathogen-free unit of this Centre had no MHV 3 antibodies as determined by ELISA [3]. On immunization with denatured MHV 3 particles, IgG antibodies were detected against whole virus particles, and surface projection, membrane and RNP subcomponents. The quantity of antibody was measured by ELISA ratios, which are the ratio of postimmunization to preimmunization absorbance values at the same serum and antigen dilutions [11]. The highest immune response was obtained with mice inoculated with a 1:50 dilution of denatured MHV 3; results with this dilution are shown below.

The antibody responses to virus particles, and surface projection and membrane subcomponents in sequential sera from mice immunized with MHV 3 are shown in Figs. 1 and 2. The IgA, IgG and IgM responses for one mouse are shown in Fig. 1: similar responses for all three antibody classes were observed with other mice immunized in the same way. For example, Fig. 2 shows the IgG responses to virus particles, and surface projection and membrane subcomponents of a second mouse together with those of the first mouse.

IgG antibody to virus particles increased steadily from the 6th day after immunization, reaching a peak by the 32nd day (Figs. 1a and 2a), before declining steadily with antibody still detectable at the 110th day after immunization (Fig. 1a). However, the other antibody classes appeared immediately after immunization, reaching peaks at the 6th and 12th days after immunization for IgA and IgM, respectively, before steadily decreasing (Fig. 1a). The antibody responses to the surface projection (Fig. 1b) and membrane (Fig. 1c) components were similar to those to virus particles (Fig. 1a). The most marked differences were in the later IgA and IgM, and earlier IgG responses to surface projection components (Fig. 1b), and in the much lower antibody responses to membrane components (Fig. 1c). A very small antibody response was observed to the RNP component (data not shown). This relatively poor antibody response to membrane and RNP components has been observed in previous studies [3], in which the surface

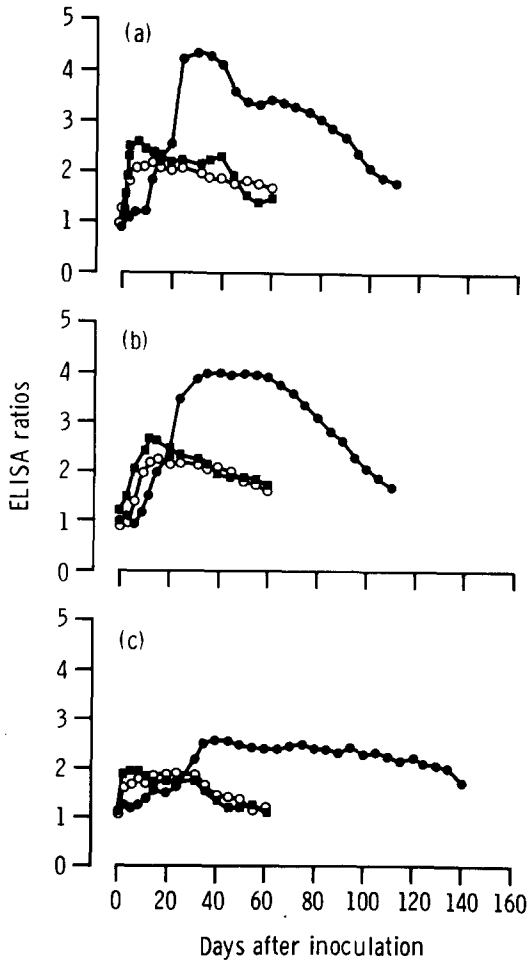

Fig. 1. ELISA ratios for MHV serum antibodies in sequential serum samples from mice immunized with MHV 3. (a) Antibodies to virus particles; antigen dilutions 1:100, serum dilutions 1:100. (b) Antibodies to surface projection components; antigen dilutions 1:50, serum dilutions 1:100. (c) Antibodies to membrane components; antigen dilutions 1:50, serum dilutions 1:50. All readings were taken at 405 nm after 30 min. ●——●, IgG antibodies; ○——○, IgM antibodies; ■——■, IgA antibodies.

projection components were found to be much more immunogenic than the membrane and RNP components.

The IgG antibody responses to virus particles, surface projection and membrane components, after boosting mice with MHV 3, 50 days after the first immunization, are shown in Figs. 2a, b and c,

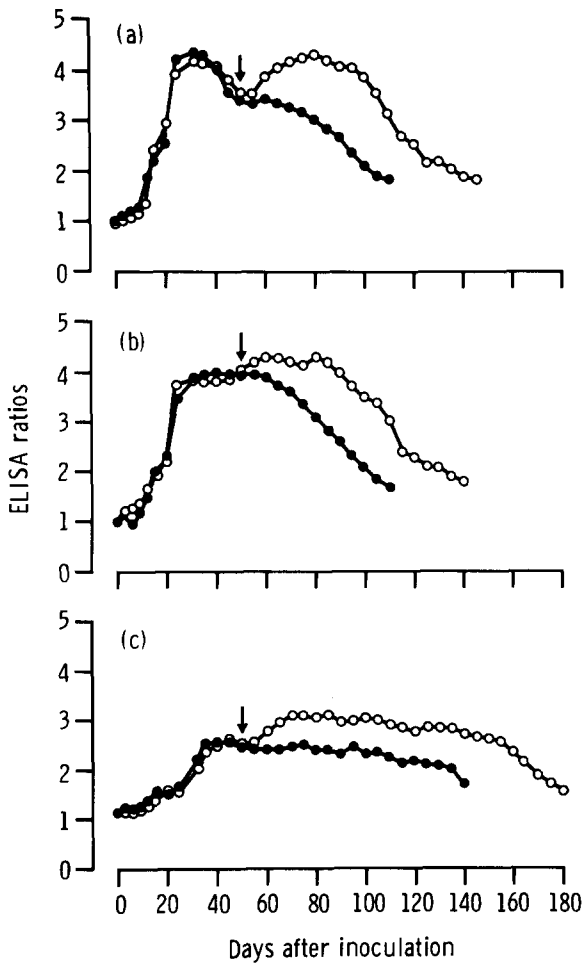

Fig. 2. ELISA ratios for MHV serum antibodies in sequential serum samples from mice immunized with MHV 3. (a) IgG antibodies to virus particles; antigen dilutions 1 : 100, serum dilutions 1 : 100. (b) Antibodies to surface projection components; antigen dilutions 1 : 50, serum dilutions 1 : 100. (c) Antibodies to membrane components; antigen dilutions 1 : 50, serum dilutions 1 : 50. All readings were taken at 405 nm after 30 min. ●———● and ○———○ represent IgG serum antibodies from two mice, one of which, ○———○, was boosted with MHV 3, 50 days after the first immunization (see arrows).

respectively. Increased antibody responses to all antigens were seen in these mice, compared with mice that were not given an MHV 3 boost. However less IgG antibody was made after the second immunization compared to the antibody produced after the first immunization. The levels of IgA and IgM antibodies were not significantly increased in the sera from the boosted mice (data not shown).

4. DISCUSSION

In this paper, we have confirmed and extended previous results [3] by showing that the surface projection components were the most immunogenic of the MHV 3 subcomponents: IgA and IgM, as well as IgG [3] were produced to all the MHV 3 components except the RNP. The almost complete absence of any antibody directed against RNP may be due to the use of denatured antigen preparations in the immunization procedure. Denaturation may lead to a loss in the antigenicity of the RNP, as untreated RNP preparations produced an antibody response in mice [3]. It is difficult to correlate the antibody responses produced by this immunization procedure with those produced by natural infections, as studies with other viruses have shown that greater antibody responses are produced during natural virus infections than on vaccination with attenuated viruses [12,13]. In addition, the low IgA and IgM response may be due to the use of inactivated antigens as immunogens, although production of IgA and IgM increased on using multi-dose immunization procedures. Similar results have been obtained with the coronavirus transmissible gastroenteritis virus (TGEV). Administration of inactivated TGEV particles [14–17] or surface projections [15] to pigs stimulated circulating IgG antibodies, while very little serum IgA and IgM was produced in pigs inoculated with inactivated whole virus or surface projections [15].

The antibody response to inoculation of mice with MHV 3 may be a useful model for human coronavirus (HCV) infections, although unlike MHV 3 infections in mice, HCV infections are common in man and the majority of the population have HCV antibodies [18,19]. Nevertheless, there is at present no clear role for these HCV antibodies in protection against infection as most individuals infected with HCVs already had HCV serum antibodies [2]. Our results show that IgG membrane antibodies remain much longer than IgG surface projection antibodies in the serum of

immunized mice: these membrane antibodies are not protective against infection [3]. A similar situation may occur after HCV infections, explaining at least partially the apparent non-protective nature of HCV antibodies. Further studies are required to examine the antibody response to infection and immunization with other coronaviruses, in particular HCVs, to determine the requirements for vaccination against these infections.

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