Murine gamma-herpesvirus 68 glycoprotein 150 protects against virus-induced mononucleosis: A model system for gamma-herpesvirus vaccination

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Abstract

Murine gamma-herpesvirus 68 (MHV-68) is a model for the study of the pathogenesis of gamma-herpesviruses. Epstein–Barr virus (EBV) is a highly related gamma-herpesvirus that causes significant disease in humans. The major membrane antigen gp350 of EBV is a candidate vaccine antigen for protection against EBV-related disease. An MHV-68 glycoprotein, gp150, has significant homology to EBV gp350. We have therefore used the MHV-68 gp150 to model the potential efficacy of EBV gp350 in protecting from virus-associated disease. A recombinant vaccinia virus expressing MHV-68 gp150 was constructed. This recombinant vaccinia virus was used to infect mice via the subcutaneous route. This vaccination resulted in production of MHV-68-neutralising antibodies. Mice were then challenged intranasally with MHV-68. MHV-68-associated mononucleosis was virtually abrogated in immunised mice. However, mice did establish MHV-68 latency. The results suggest that gp350 may be effective as an immunogen to prevent EBV-associated infectious mononucleosis in humans that are EBV-seronegative. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The Epstein–Barr virus (EBV) is a member of the gamma-herpesvirus sub-family that is associated with a number of human diseases including infectious mononucleosis (Glandular fever), Burkitt’s lymphoma, Hodgkin’s lymphoma, post-transplant lymphoma and nasopharyngeal carcinoma. Vaccination against EBV might therefore be a valid way to protect against these diseases. No EBV vaccine is currently available, however, it has been proposed that the EBV gp350/220 protein may be a good immunogen for protection against EBV-associated disease [1]. This rationale is based upon the facts that first, gp350 is responsible for the virus binding to its cellular receptor on B lymphocytes and secondly, antibodies to gp350 are virus-neutralising [2,3]. Animal model studies with gp350 have showed that immunisation was efficacious in protecting cotton-top tamarins from the development of EBV-associated lymphomas [4,5]. However, the route of infection with EBV (intra-muscular and intra-peritoneal) and the disease course in the tamarin are far removed from those encountered in humans.

Murine gamma-herpesvirus (MHV-68) will infect inbred strains of mice via the respiratory route (intra-nasally), which represents an authentic route for gamma-herpesviruses [6]. MHV-68 replicates initially in alveolar epithelial cells and mononuclear cells in the lung causing a clinically silent peri-bronchiolar and interstitial pneumonia [7]. The virus becomes latent in B lymphocytes resulting in an acute splenomegaly [8]. Splenomegaly is the result of expansion of all splenocyte subsets, is dependent upon the presence of both CD4 T cells and virus-infected B cells and is in all respects analogous to EBV-associated mononucleosis [9–11]. Long-term virus latency in B cells follows resolution of splenomegaly [7–9] and the
control of infection appears to involve a critical role for CD8⁺ T cells [12]. Thus, MHV-68 parallels what is known about the biology of EBV and other gammaherpesviruses.

The MHV-68 homologue of EBV gp350 called gp150 was recently characterised [13]. Like EBV gp350, MHV-68 gp150 is expressed as a transmembrane glycoprotein that is a component of the infected cell membrane and virus particle and is a target for virus-neutralising antibodies. MHV-68 gp150 was therefore used as an immunogen in the MHV-68 system to model the effects of using EBV gp350 as a vaccine antigen. The work in this paper describes the efficacy of vaccination with gp150 delivered via a recombinant vaccinia virus.

2. Materials and methods

2.1. Virus and cells

Working stocks of MHV-68 clone g2.4 were grown and titred by plaque assay in BHK-21 cells as described by Sunil-Chandra et al. [7]. Vaccinia virus strain WR was used to generate recombinants and stocks were grown and titred on CV-1 cells.

2.2. Recombinant vaccinia virus construction

All standard molecular cloning techniques were performed as described by Sambrook et al. [14]. The complete gp150 coding sequence as a cDNA [13] was excised from pSP72 (Promega) using the restriction enzymes Hin dIII and Eco RI (both Gibco) and inserted between the Hin dIII and Eco RI sites of the vaccinia plasmid insertion vector p1108 [15]. This vector placed the membrane antigen cDNA into the vaccinia virus thymidine kinase locus under the control of the vaccinia 7.5 K early/late promoter and also provided the E. coli gpt gene as a dominant selectable marker. Purified plasmid DNAs from either p1108 containing the gp150 cDNA or p1108 alone were used to make recombinant vaccinia viruses as described previously [15]. Briefly, CV-1 cells were infected with vaccinia virus at a multiplicity of infection of 0.01. After 1 hour, plasmid DNA (10 μg) was transfected into the same cells by using Lipofectin (Gibco). After growth for 3 days, the cells were harvested and recombinant vaccinia viruses selected by three rounds of plaque purification in CV-1 cells in the presence of 25 μg/ml mycophenolic acid (Sigma), 250 μg/ml xanthine (Sigma) and 15 μg/ml hypoxanthine (Sigma) as selective agents. Individual plaques were then grown to high titre in CV-1 cells and tested for expression of recombinant gp150.

2.3. Radio-immunoprecipitation

For MHV-68, cells were infected at 10 pfu/cell and infection was allowed to proceed for 18 h prior to labelling. For vaccinia, the cells were infected at 30 pfu/cell and labelled 3 h post-infection. Cells were then incubated for 30 min in methionine free minimum essential medium (Gibco) before being metabolically labelled by the addition of 500 μCi [35S] methionine (Amersham) contained in 3 ml medium. After incubation for 4 h cells were solubilised in 2 ml RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.5% deoxycholate, 1% NP40, 0.1% SDS). A monospecific rabbit antiserum to gp150 was then used to immunoprecipitate gp150. This antiserum had been previously using a protein consisting of gp150 fused to glutathione-S-transferase [13]. Antibody (2.5 μl) was added to 500 μl solubilised protein and incubated for 30 min at room temperature. A volume (50 μl) of protein A-sepharose beads (Pharmacia) was then added and incubated for a further 30 min at room temperature. Immunoprecipitated proteins were then collected by centrifugation, washed five times with 1 ml RIPA buffer and collected finally in 50 μl SDS-PAGE sample buffer. Samples were then analysed by electrophoresis through 5.5% SDS–PAGE gels along with [14C]-methylated proteins (Amersham) as molecular weight markers. Gels were impregnated with Enhance (DuPont), dried and exposed to autoradiographic film (X-Omat, Kodak).

2.4. Immunofluorescence

Cell smears were fixed in acetone for 5 min. Primary antibody, either rabbit anti-gp150 or pre-immune control, diluted 1 in 100 in PBS was added and incubated for 1 h at 37°C. Slides were then washed 3 times with PBS and FITC-conjugated swine anti-rabbit antibodies (Dakopatts) diluted 1 in 30 in PBS was added and incubated for 1 h at 37°C. Slides were then washed three times in PBS and mounted in a 50:50 solution of PBS: glycerol before visualisation with a UV microscope.

2.5. Vaccination and virus challenge protocol

Three-week old female mice, strain Balb/C (Bantin and Kingman) were vaccinated at four weeks of age. Mice in two groups of were inoculated with either RVVgp150 or with RVV1108 under halothane anaesthetic. Inoculations were done with a dose of 1 × 10⁷ pfu of recombinant vaccinia virus administered subcutaneously in a volume of 100 μl into the scruff of the neck. After 28 d the inoculations were repeated exactly as before. After a further 28 d, mice from both groups were challenged via the intra-nasal route with 4 × 10⁵
pfu MHV-68. Serum was separated from blood on the
day of challenge. At 10, 14, 21 and 28 d post-chal-
lenge, four mice from each group were killed hu-
amely, blood was taken for serum and the spleen
was removed. The spleen was analysed by conven-
tional plaque assay for the presence of infectious virus
after homogenisation of the organ. Latent virus was
detected via an infective centre assay [7] as follows.
Splenocytes were added to confluent monolayers of
BHK-21 cells and co-cultured for 5 d. The number of
plaques in the monolayer was then determined. The
amount of infectious virus present in the same sample
was determined by plaque assay after one cycle of
freezing and thawing and subtracted from the number
of plaques seen in the co-cultivation to give the num-
ber of infective centres. These infective centres are a
biological representation of virus reactivating from
latency.

2.6. Virus neutralisation assay

Sera were heat-treated at 56°C for 30 min to destroy
complement. Serum concentrations varying between 1
in 10 and 1 in 80 were added to 200 pfu MHV-68 for
1 h at 37°C. The virus/serum combination was then
used to infect sub-confluent BHK-21 cells. After 4 d
the monolayers were fixed and plaques counted. Each
serum concentration was assayed in triplicate. The
neutralisation titre was expressed as the reciprocal of
the serum dilution required to cause a 50% reduction
in plaque number.

3. Results

3.1. Expression of gp150 via a recombinant vaccinia
virus

The complete gp150 coding sequence, as a
cDNA [13] was inserted into the vaccinia transfer vec-
tor p1108. This approach was used because it had pro-
ven useful in expressing recombinant proteins that
were effective in generating both antibody and T cell
responses [17]. Recombinant vaccinia viruses were
made with either p1108 alone (termed RVV1108) or
p1108 containing the gp150 cDNA (termed RVVgp150) as
described in Section 2.

The expression of recombinant gp150 by
RVVgp150-infected cells was analysed by radio-immu-
noprecipitation. Cells were radiolabelled with [35S]-
metionine and proteins precipitated with monospecific
rabbit anti-gp150 antiserum, the results of which are
shown in Fig. 1. No proteins were immuno-preci-
pitated from wild-type vaccinia virus-infected cells (panel

![Diagram](image-url)

Fig. 1. Immunoprecipitation analysis of gp150 expression by recombinant vaccinia virus. Cells (BHK) were either mock-infected or infected with
virus for either 3 h (vaccinia) or 18 h (MHV-68) prior to radiolabelling with [35S]-methionine. Cells were then solubilised and immunoprecipitated
with anti-gp150 antiserum as described in Section 2. Proteins were then electrophoresed through 5.5% SDS-PAGE gels, the gels dried and
exposed to autoradiographic film. Panel A shows extracts derived from either wild-type vaccinia virus-infected or RVVgp130-infected cells. Panel
B shows extracts from either MHV-68-infected or mock-infected cells. The position of molecular weight standards (×10^3) is shown to the left
of panel A and to the right of panel B.
A) or mock-infected cells (panel B) with anti-gp150 antiserum. A similar result was obtained when a pre-
immune serum from the same rabbit was used with extracts from RVVgp150-infected or MHV-68-infected
cells (results not shown). The predominant proteins immuno-precipitated from RVVgp150-infected cells
were gp150 and its precursor gp130 (panel A). The
smaller p110, which is derived from the use of an
alternative translational initiation site [13] was also
seen. Two additional proteins of Mr 220,000 and
190,000 not previously observed were precipitated
from RVVgp150-infected cells. Panel B shows that
similar patterns of proteins were precipitated from
MHV-68-infected cells including the novel higher mo-
olecular-weight forms. These minor, higher molecular
weight proteins were most likely alternatively-glycosyl-
lated forms of gp150 and were called gp190 and gp220
accordingly.

Expression of gp150 on the surface of RVVgp150-
infected cells was tested by indirect immunofluores-
cence on live cells and found, like MHV-68-infected
cells, to be positive (data not shown). Thus, RVVgp150-infected cells expressed gp150 in an auth-
etic fashion.

3.2. Serological response to vaccination with RVVgp150

Two groups of mice were vaccinated twice, 28 d
apart, with either RVVgp150 or the control vaccinia
RVV1108 as described. 28 d after the second vacci-
nation the mice were challenged with MHV-68 via the
intra-nasal route.

Sera obtained from blood taken from mice prior to
challenge with MHV-68 as well as sera obtained fourteen and 21 d post-challenge were tested for their abili-
ty to neutralise MHV-68 infection. The results are shown in Fig. 2. Sera obtained prior to challenge with
MHV-68 (day 0) from mice vaccinated with the con-
tral RVV1108 (open circles) contained no virus-neutralis-
ing activity. In contrast, at the same time-point, sera
from mice vaccinated with RVVgp150 (closed circles)
had a virus-neutralising titre of approximately 20. At
subsequent time-points, there was evidence of neu-
tralising antibody in both groups of mice. The levels in
the RVVgp150-vaccinated group showed a significant
increase in titre but were not significantly different
from the titres of the RVV1108-vaccinated group.
Thus, mice inoculated with RVVgp150 showed a sig-
ificant serological response to vaccination that was
virus-neutralising.

3.3. Effect of vaccination on splenomegaly and virus
latency in the spleen

The effect of MHV-68 on the lymphoid system was
assessed by counting the numbers of cells in the spleen
and assessing the number of infective centres in the
spleen at 10, 14, 21 and 28 d post-challenge. The for-
mer gives a measure of splenomegaly and the latter a
measure of the number of latently-infected cells in the
spleen. The experiment was performed twice with com-
parable results and the results of one experiment are
shown in Figs. 3 and 4 respectively.

A sharp rise in the number of spleen cells was
observed in mice vaccinated with the control RVV1108
(Fig. 3; open circles). Cell numbers peaked at day 14
to a level of approximately 2.5 times normal and had
dropped back to a normal level by day 28. This pat-
tern is typical of that seen after MHV-68 infection [7].
Mice vaccinated with RVVgp150 (closed circles)

Fig. 2. Detection of MHV-68-neutralising antibody. MHV-68 (200
pfu) was incubated with serum at a range of dilutions for 1 h at
37 C. BHK cells were then infected using the virus-antibody mixture
and the number of plaques counted after 4 d. The data is presented
as the mean (plus or minus the standard deviations) of the reciprocal
antibody dilution required to cause 50% of the maximum inhibition
of plaque formation. Open circles represent mice vaccinated with
RVV1108 and closed circles represent those vaccinated with RVVgp150.

Fig. 3. Spleen cell number in infected mice. Mean cell numbers per
spleen ± standard deviation for four mice per group for each given
time post-challenge with MHV-68. Numbers from mice vaccinated
with RVVgp150 are shown by closed circles and those from mice
vaccinated with RVV1108 by open circles.
showed a slight increase in spleen cell number, which again had resolved by day 28.

No infectious virus was seen in any spleen samples. A typical, sharp rise in infective centres (latent virus) was observed in mice vaccinated with control RVV1108 (Fig. 4; open circles). This peaked at day 14 post-infection and resolved to a basal level (mean 230 infective centres per spleen) by day 28 post-infection. Mice vaccinated with RVVgp150 (closed circles) showed a rise in infective centres which again peaked at day 14 and had returned to a basal level (mean 150 infective centres) by day 28. However, the peak in this case was significantly lower than that seen in the control group. Thus, both splenomegaly and the amount of latent virus in the spleen were greatly reduced in mice vaccinated with RVVgp150 as compared with control RVV1108-vaccinated mice.

4. Discussion

The experiments described in this paper form a study on the efficacy of vaccination against MHV-68 using the virion membrane glycoprotein, gp150, as vaccine antigen to model the potential of EBV gp350 to protect against EBV. The immunogen was delivered via a recombinant vaccinia virus (RVVgp150) which expressed gp150 in an authentic fashion and stimulated the production of virus-neutralising antibodies. After challenge with MHV-68, splenomegaly was markedly reduced in immune mice and only low numbers of latently-infected cells were detected in the spleen following the acute infection.

A recombinant vaccinia virus expressing gp150 was used since this approach had been successful in expressing and delivering other herpesvirus glycoproteins as vaccine antigens [5,18–21]. Also, recombinant vaccinia viruses have the ability to stimulate not only antibody but also cell-mediated immune responses. In our experiments, mice were inoculated twice with RVVgp150 and elicited a neutralising antibody response, however, this was relatively low in titre. Future attempts to improve the efficacy of vaccination with this antigen may therefore employ purified recombinant gp150 in combination with adjuvant in an attempt to generate higher neutralising titres.

Vaccination against a pathogen seldom results in protection from infection or sterile immunity but does have the ability to protect from disease. This appears to be the case in these experiments. Thus, whilst there was little splenomegaly and lower levels of latent virus during splenomegaly, RVVgp150-vaccinated mice were infected and did establish long-term latency in the spleen. The changes seen in the lymphoid compartment during acute infection with MHV-68 (i.e. splenomegaly) are highly reminiscent of EBV-associated mononucleosis. This work in the MHV-68 model system therefore suggests that vaccination against EBV with a gp350-based vaccine may be a useful approach in protecting against infectious mononucleosis. However, if one wished to prevent or reduce the establishment of long-term latency and hence reduce the incidence of tumours, our experiments suggest that modification of the approach might be required, perhaps to include additional (maybe latency-associated) antigens. Testing the MHV-68 model in this respect awaits the elucidation of antigens associated with MHV-68 latency.

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References


