Protective immunization against Epstein–Barr virus-induced disease in cottontop tamarins using the virus envelope glycoprotein gp340 produced from a bovine papillomavirus expression vector


Departments of Pathology and Microbiology, and Anatomy, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD and Cancer Research Campaign Laboratories, Paterson Institute for Cancer Research, Christie CRC Research Centre, Wilmslow Road, Manchester M20 9BX, U.K.

Inoculation with Epstein–Barr virus (EBV) induces malignant lymphomas in the cottontop tamarin (Saguinus oedipus oedipus). This provides an experimental animal model for assessing the efficacy of candidate EBV vaccines which are intended to reduce the incidence of human tumours associated with EBV infection. Previous work has shown that experimental vaccines based on the major virus envelope glycoprotein gp340 prepared from the membranes of EBV-infected cells are effective in protecting cottontop tamarins against EBV-induced disease. However, not all purified gp340 preparations induce protective immunity against EBV lymphoma in the tamarin. In this work, cottontop tamarins were immunized with recombinant gp340, produced using a bovine papillomavirus (BPV) expression vector, and a threonyl muramyl dipeptide adjuvant formulation. Although the recombinant-derived gp340 lacked the membrane anchor sequence of authentic gp340 and was expressed in mouse cells, it was immunogenic and induced virus-neutralizing antibodies. Healthy vaccinated tamarins were protected against EBV-induced disease. The demonstration that a recombinant gp340 product is able to elicit protective immunity in the cottontop tamarin is a significant step in the development of an EBV vaccine because previously it had not been clear whether a recombinant product would have the exact tertiary structure, including the necessary carbohydrate components, to induce protective immunity. A recombinant gp340 vaccine offers various advantages over production of the authentic molecule by laborious biochemical separation, including lower cost and the absence of potentially oncogenic EBV DNA. Therefore, recombinant gp340 produced using the BPV expression vector is suitable for development as a candidate EBV vaccine for a human Phase I trial and beyond.

Epstein–Barr virus (EBV) infection is strongly associated with a number of human diseases, including the epithelial malignancy nasopharyngeal carcinoma, endemic Burkitt’s lymphoma and B cell lymphomas arising in immunosuppressed patients with AIDS or those undergoing transplant surgery. In addition to infectious mononucleosis, which is directly caused by delayed primary infection by the virus, severe complications can also arise during primary infection in individuals with various immunodeficiencies, and fatal cases of infectious mononucleosis are well documented in certain families with an X-linked lymphoproliferative syndrome (Purtilo, 1976). More recently, the link between Hodgkin’s lymphoma and EBV has been strengthened (Pallesen et al., 1991).

It has been a number of years since the development of an EBV vaccine was proposed (Epstein, 1976) with the aim of eliminating or at least reducing the incidence of EBV-related disease, and since then considerable progress has been made. A range of experimental EBV vaccines has been developed based on the major virus envelope glycoprotein gp340, against which neutralizing antibodies are directed (Morgan & Epstein, 1989).

The host range for EBV is limited to humans and certain subhuman primates whose B lymphocytes express CD21, the receptor for the C3d component of complement which also functions as the receptor for EBV (Fingeroth et al., 1984). The cottontop tamarin (Saguinus oedipus oedipus) is the only animal species which consistently succumbs to experimental EBV-induced disease; tamarins inoculated with a large dose of EBV develop multiple B cell lymphomas within 3 weeks.
(Cleary et al., 1985). These tumours have been well characterized and resemble the large cell lymphomas which arise in transplant recipients in terms of their histology, mono- or oligoclonality, normal karyotype and EBV gene expression (Cleary et al., 1985; Young et al., 1989). In addition to providing a model of EBV oncogenesis in vivo, the tamarin also provides an in vivo system for testing the efficacy of EBV vaccines, because the development of EBV-induced lymphoma can be easily monitored by external palpation and measurement of involved lymph nodes.

A variety of candidate EBV vaccines have been tested in the cottontop tamarin and have been found to elicit protective immunity. These vaccines have been based on the major virus envelope glycoprotein, gp340, prepared from the membranes of EBV-infected B95-8 cells and purified by fast protein liquid chromatography (FPLC) (David & Morgan, 1988). Two novel adjuvant systems have been used and found to be effective in the cottontop tamarin model. In the first case, FPLC-purified gp340 was incorporated into immune-stimulating complexes (Morgan et al., 1988) and, in the second, a synthetic muramyl dipeptide (MDP) adjuvant formulation was used with FPLC gp340 (Morgan et al., 1989). Thus, authentic gp340 used in conjunction with an adjuvant acceptable for human use (possibly MDP) appears to be suitable for a small-scale human trial to test for immunogenicity in man (Morgan et al., 1989). However, it would not be economically feasible to use this material beyond a Phase I trial.

The open reading frame encoding gp340 in the EBV genome is known (Biggin et al., 1984), so it has been possible to produce recombinant gp340 in a number of eukaryotic expression systems, but only at relatively low levels or in unstable cell lines (Whang et al., 1987; Emini et al., 1989; Motz et al., 1987). Consequently, a bovine papillomavirus (BPV) vector expressing gp340 has also been developed (Conway et al., 1987). Since gp340 has been shown to be toxic for eukaryotic cells, this approach has been developed further (Motz et al., 1987; Whang et al., 1987) and a recombinant BPV vector has been constructed containing a gp340 gene lacking the sequence encoding the membrane anchor region of the protein, resulting in its secretion into the cell culture medium (M. Madej et al., unpublished results). Purification of secreted gp340 is much more straightforward than isolation of the authentic protein from cell membrane fractions. It is this secreted recombinant gp340 which was used in the present work to immunize cottontop tamarins. The biochemical characterization and purification of this recombinant product will be described elsewhere (M. Madej et al., unpublished results).

The aim of the work described here was to demonstrate that recombinant-derived gp340 prepared using the BPV expression system could induce protective immunity against lymphomagenic EBV challenge in the cottontop tamarin. Although this has been done using the authentic molecule and a vaccinia virus recombinant expressing gp340, it is very important to determine whether the recombinant subunit product is more or less protective in the tamarin model. The sources of gp340 used in the past, even when considered to be identical, have had profoundly different results in protection experiments. Authentic gp340 purified by monoclonal antibody affinity chromatography did not induce protective immunity in the cottontop tamarin, whereas SDS–PAGE-purified material did (Morgan et al., 1989; Epstein et al., 1986). The relative contribution of cell-mediated and humoral immune responses to protective immunity in the tamarin following vaccination with various gp340 preparations is not known. The absence of the membrane anchor sequence and the variation in carbohydrate composition depending on the host cell used (Whang et al., 1987) for recombinant gp340 production might have a profound effect on the induction of protective immunity against EBV.

In the absence of carbohydrate on bacterially expressed gp340 fragments (Pitser et al., 1991) and in the presence of different carbohydrates attached to gp340 expressed in yeast (Schultz et al., 1987), the antibody responses in rabbits are very different. Neither of these products induces virus-neutralizing antibodies, whereas when gp340 is expressed in several mammalian cells, virus-neutralizing antibodies can bind to it (Emini et al., 1988). It is for these reasons that it was essential to establish whether gp340 expressed in the BPV expression system could induce protective immunity in the tamarin against a lymphomagenic challenge with EBV.

Four cottontop tamarins were immunized with 50 μg of BPV-derived gp340 using the Syntex threonyl muramyl dipeptide formulation as an adjuvant as described (Allison & Byars, 1986; Morgan et al., 1989). Four intramuscular injections were given into the thigh at 10-day intervals. Tamarins were challenged as described earlier (Cleary et al., 1985) with a 100% tumorogenic dose of EBV from a pretested batch of virus 10 days after the fourth immunization; two non-immunized tamarins were challenged at the same time as controls. The batch of EBV used for this challenge had been previously inoculated into two healthy tamarins seronegative for EBV and both had developed extensive EBV-related disease of the peripheral and abdominal lymph nodes. Around 4 weeks after EBV challenge, tumour indices of 17 and 18 were present (see below and Fig. 1 for definition), which indicated a considerable tumour burden in each animal. Blood samples were taken at the beginning of the experiment (pre-bleed), following each immunization and immediately before the EBV chal-
Fig. 1. Tumour development in immunized and non-immunized cottontop tamarins following challenge with a tumorigenic dose of EBV. The tumour index is defined as the increase in the sum of the radii (in mm) of palpable lymph nodes following EBV challenge. Immunized animals: (○) R94, (●) R98 and (■) B143; non-immunized animals: (□) R141 and (▲) R157.

Challenge to assess the antibody response to gp340 by ELISA (Randle & Epstein, 1984) and the ability of plasma to neutralize EBV in vitro, as measured by inhibition of EBV-induced transformation of cord blood lymphocytes (De Schryver et al., 1974). Following EBV challenge, all six tamarins were regularly examined by external palpation and measurements were taken of enlarged lymph nodes and induced tumours.

Prior to immunization, the plasma of each tamarin was negative for antibodies to gp340 as determined by ELISA and negative for antibodies to the viral capsid antigen as judged by immunofluorescence. The antibody responses obtained in the four immunized tamarins immediately prior to EBV challenge are shown in Table 1. The levels of virus-neutralizing activity are seen to parallel the anti-gp340 titres, so that plasma from the two animals with higher titres showed strong virus neutralization. These were able to reduce the titre of EBV in vitro around 10-fold, in contrast to the plasma from the low responder animal, R94, which showed no virus-neutralizing activity because the plasma did not reduce the EBV titre in vitro at all. However, this low responder animal had developed idiopathic colitis during the immunization schedule and received treatment. This underlying disease may have been a factor in the poor response to immunization observed in this animal. The anti-gp340 antibody titres in the three remaining animals were of the same order as those reported previously following four 50 μg doses of authentic gp340 purified from the membranes of EBV-infected cells and given with the same MDP adjuvant (Morgan et al., 1989). All four immunized tamarins showed some minor local lymphadenopathy during the course of the immunizations. This was detected in the superficial inguinal lymph nodes draining the inoculation sites in the thigh.

Tamarin B157 died suddenly of causes apparently unrelated to EBV 3 days after EBV challenge. No obvious cause of death was found at postmortem, although 4 weeks previously there was evidence of mild inflammatory bowel disease diagnosed following proctoscopy. The animal was only 2 years old so did not die of old age. Such unexplained deaths are not unusual in captive members of this species, which can appear healthy up to the time of death. Toxicity related to gp340 or the muramyl dipeptide adjuvant is not suspected because no tamarin suffered any side-effects during the immunization schedule, and both gp340 in various forms and this adjuvant have been used in previous experiments with no evidence of toxicity whatsoever. A possible cause of death was endotoxaemia secondary to the underlying inflammatory bowel disease. This possibility is under investigation.

The remaining three immunized tamarins, along with the two non-immunized controls, were regularly examined for EBV-induced tumour development; the results are illustrated in Fig. 1. The two non-immunized tamarins showed progressive disease exactly as described in earlier work (Cleary et al., 1985) and as seen previously in the two tamarins used to pretest this virus pool, including increasing involvement of the mesenteric lymph nodes with time. Both control tamarins R141 and R157 were put down owing to the size of their tumour burdens at days 52 and 57 after EBV challenge respectively. Histology samples of grossly enlarged lymph nodes taken at this time showed the standard features of neoplastic disease, including the loss of lymph node architecture, frequent mitoses and necrosis. In contrast to this progressive disease the two immunized tamarins R98 and B143 showed only slight

Table 1. Antibody responses to gp340 in immunized cottontop tamarins immediately prior to EBV challenge

<table>
<thead>
<tr>
<th>Tamarin</th>
<th>Anti-gp340 antibody titre</th>
<th>Virus-neutralizing activity in vitro (log_{10} reduction in virus titre)</th>
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<tr>
<td></td>
<td>Pre-immunization</td>
<td>Post-immunization</td>
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<tr>
<td>R94</td>
<td>0</td>
<td>1/50</td>
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<tr>
<td>R98</td>
<td>0</td>
<td>1/200</td>
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<tr>
<td>B143</td>
<td>0</td>
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<td>B157</td>
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peripheral lymph node enlargement, predominantly of the inguinal lymph nodes, with no abdominal involvement. This relatively minor lymph node enlargement has been reported previously in similar experiments using authentic gp340, in which histological examination revealed a reactive infiltrate (Morgan et al., 1989). The third immunized tamarin, R94, which developed colitis and responded only poorly to immunization with a low anti-gp340 antibody titre, did develop a progressive EBV-induced disease similar to that seen in the two non-immunized tamarins. Although R94 was not protected from EBV-induced lymphoma and had to be put down 37 days after challenge, the course of the disease was altered in this animal because there was involvement of the peripheral lymph nodes only, in contrast to the two non-immunized tamarins.

The results show that immunization with BPV-expressed gp340 elicits protective immunity against experimentally EBV-induced lymphoma in healthy cottontop tamarins. However, the anti-gp340 titres obtained after four immunizations ranged from 1/50 to 1/700, which is at the lower end of the range (1/340 to 1/1500) obtained previously using authentic gp340 as the immunogen under the same experimental conditions. This could simply reflect the range of immune responses to be expected in an outbred species or may be a consequence of the lower purity of BPV-expressed gp340, which had a lower specific activity than that obtained previously for authentic gp340 (North et al., 1982), or it may reflect unidentified antigenic differences between authentic gp340 and gp340 produced in the BPV expression system. This work demonstrates for the first time that a recombinant-derived gp340 subunit vaccine can elicit protective immunity.

Gp340 is a large and complex molecule with a large carbohydrate component so it was far from certain that a recombinant product would have the same protective properties as the authentic molecule, especially as the membrane anchor sequence was deleted in the product under study. In this context it should be noted that authentic gp340 isolated by monoclonal antibody affinity chromatography did not induce protective immunity against EBV lymphoma in the cottontop tamarin (Epstein et al., 1986). Indeed, the tertiary structure of the glycoprotein appears to be very important for the formation of B cell virus-neutralization epitopes (R. J. Pither et al., unpublished results), so any slight differences in the tertiary structure of the recombinant product could have influenced its ability to elicit protective immunity. Therefore, the present results are a very significant step towards the development of an EBV vaccine for use in man. The preparation of authentic gp340 by laborious biochemical separation techniques is not suitable for large-scale vaccine production, but the recombinant product can be produced in quantity at a relatively low cost and in the absence of potentially oncogenic EBV DNA. Further development will follow to meet the requirements of regulatory bodies prior to a human Phase I trial.

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References


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