Prevention of Epstein–Barr (EB) Virus-induced Lymphoma in Cottontop Tamarins by Vaccination with the EB Virus Envelope Glycoprotein gp340 Incorporated into Immune-stimulating Complexes

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SUMMARY

Experimental induction of malignant lymphomas can be achieved in the cottontop tamarin by inoculation with Epstein–Barr (EB) virus. This system provides an animal model for assessing the efficacy of vaccine protection against the virus which is intended to reduce the incidence of human tumours associated with EB virus infection, namely endemic Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma. Cottontop tamarins have been vaccinated with the major envelope glycoprotein of EB virus, gp340, incorporated into immune-stimulating complexes (iscoms) and were thereby protected against a 100% lymphomagenic dose of virus. The gp340 iscoms are highly immunogenic, requiring only a few micrograms of immunogen to induce protective immunity and thus would be a strong candidate for further development as an EB virus vaccine for use in man.

Epstein–Barr (EB) virus is strongly implicated as one of the causative agents in the chain of events that leads to the development of two important human malignancies, undifferentiated nasopharyngeal carcinoma (Shanmugaratnam, 1971) and endemic Burkitt's lymphoma (Burkitt, 1963). The former represents a major world health problem, being the most common cancer in men of southern Chinese origin. The need for a vaccine to prevent or reduce the incidence of these diseases has been recognized for some time (Epstein, 1976) and in the ensuing period efforts have been focused on the major EB virus envelope glycoprotein, gp340, as a potential subunit vaccine (Epstein & Morgan, 1987). An animal model for EB virus-induced malignant disease has been developed using the cottontop tamarin (Saguinus oedipus oedipus) where inoculation with a large dose of EB virus invariably leads to the development of multiple tumours within 2 to 3 weeks. Histological and molecular biological criteria have been used to demonstrate that these tumours are induced by EB virus, that they are monoclonal or oligoclonal (Cleary et al., 1985) and are large cell malignant lymphomas consisting of immunoblastic and follicular centre cell types (Dorfman et al., 1982).

It has already been demonstrated that appropriately purified gp340 can induce protective immunity in tamarins when incorporated into artificial liposomes (Epstein et al., 1985). More recently protective immunity was obtained in tamarins after immunization with a vaccinia virus recombinant (WR strain) expressing the gp340 gene, by what appears to be a cell-mediated mechanism (Morgan et al., 1988). These results provide further evidence that the gp340 molecule is able to prevent EB virus-induced lymphoma in tamarins when suitably presented to the immune system. However, the use of live virus vectors for vaccination in man remains controversial and alternative types of vaccine for immunization studies with gp340 have therefore been sought.
Immune-stimulating complexes (iscoms) are recently developed structures, based on the glycoside Quil A (Dalsgaard, 1978) for the immunogenic presentation of membrane proteins. They combine the characteristics of multimeric presentation of antigen with an inbuilt adjuvant (Morein et al., 1987; Sundquist et al., 1988) and vaccines utilizing these properties are now being developed, including a hepatitis B virus vaccine (Howard et al., 1987). Protective immunity has been obtained against feline leukaemia virus (FeLV) in cats following vaccination with iscoms carrying the gp70/85 component of FeLV (Osterhaus et al., 1985). Since experiments with vaccinia virus recombinants expressing gp340 indicated that cell-mediated immunity is an important protective mechanism, at least in the tamarin model (Morgan et al., 1988), and that iscom preparations induce cell-mediated as well as humoral immune responses (Wahren et al., 1987) we have constructed iscoms carrying EB virus gp340 for vaccination experiments in tamarins.

Gp340 was prepared from B958 cells (Miller et al., 1972) using a simple procedure consisting of anion-exchange chromatography and gel filtration giving rise to a pure product as judged in SDS-PAGE (David & Morgan, 1988). The iscoms were prepared by a dialysis method as previously described (Lovgren et al., 1987). Briefly, 200 μg of gp340 in 1 ml phosphate-buffered saline (PBS) was mixed with Quil A to give a final concentration of 0.1% (w/v) and a mixture of 5 μg cholesterol and 5 μg phosphatidylcholine and dialysed extensively against PBS, first (4 to 6 h) at room temperature, then at 4 °C. Free Quil A was removed by centrifugation of the iscoms through a cushion of 10% sucrose. The pellet was resuspended in 500 μl PBS and stored at 4 °C until use. These preparations were characterized by electron microscopy and exhibited the characteristic cage-like morphology of iscoms (Morein et al., 1987) (Fig. 1).

Four cottontop tamarins were immunized twice, subcutaneously with an interval of 2 weeks with 2 to 5 μg each of gp340 complexed as iscoms followed by a third vaccination after a further 2 weeks with less than 2 μg gp340 iscoms for each animal. Serum antibodies to gp340 in the vaccinated animals were measured by an ELISA (Randle & Epstein, 1984). At the time of virus challenge serum antibody titres to gp340 were in the range 1/400 to 1/800. Sera from these animals were able to neutralize EB virus in an in vitro assay based on the inhibition of EB virus-induced transformation of cord blood lymphocytes (De Schryver et al., 1974).

Two weeks after the third injection the four tamarins received a challenge dose of EB virus (Cleary et al., 1985). The development of tumours in the animals was assessed by physical examination. The results obtained are shown in Fig. 2 and are compared with those obtained in four unvaccinated control animals that had received the same dose of EB virus from the same batch as that used to challenge the vaccinated animals. Tumour development occurred in the control animals in the characteristic way (Cleary et al., 1985) with multiple lesions appearing during the 3rd week following challenge. All four vaccinated animals remained free of significant palpable lesions although some transient lymph node enlargement was detected. In order to check that the transient enlargement was not tumour, a biopsy was taken from the tamarin displaying the enlarged lymph node (Y20) at approximately 3 weeks post-challenge. Histological examination showed the transient swelling to be due to inflammation and consisted of a reactive lymphoid infiltrate with no evidence of the presence of lymphomatous tissue. In contrast to the monomorphic appearance of lymphomas induced by EB virus this infiltrate was polymorphous and showed a spectrum of lymphoid differentiation. There appeared to be an admixture of small and large lymphoid cells including transformed cells i.e. immunoblasts in addition to plasma cells. The lesion was reminiscent of the so-called polymorphous B cell hyperplasia found in renal transplant recipients (Frizzera et al., 1981).

These data indicate that microgram doses of gp340 incorporated into iscoms can protect tamarins against a 100% lymphomagenic challenge of EB virus, although the possibility that iscoms without gp340 may alter the disease process following virus challenge has not been excluded. Since such small amounts of protein are required to establish protective immunity it would appear that the preparation of sufficient gp340 for use as a subunit vaccine of defined composition in human trials is a practical possibility. No side effects have ever been observed in a variety of species at the dosage level of iscoms required for effective vaccination (Morein et al., 1987). A recent formal toxicological study of local reactions to Quil A and to an iscom measles...
Fig. 1. Electron micrograph of gp340 iscoms. Bar marker represents 100 nm.

Fig. 2. Tumour development in cottontop tamarins following challenge with $10^{5.3}$ transforming units of EB virus (Cleary et al., 1985) (a) in control animals B41 (○), B57 (▲), B24 (●) and B36 (△) and (b) in animals R69 (▲), Y20 (●), Y9 (★) and R70 (△) vaccinated with gp340 iscoms. The tumour index is defined as the sum of the radii of palpable tumours in mm in an animal at the time indicated following virus challenge. Before use all animals were determined to be seronegative with respect to EB virus since antibodies to the virus capsid antigen (Henle & Henle, 1966) could not be detected nor could antibodies to gp340 be detected using a sensitive ELISA (Randle & Epstein, 1984).

vaccine following intramuscular injection into rats has confirmed these observations (Speijers et al., 1988). Iscom vaccines avoid the complications associated with live virus vectors and they also appear to be of low toxicity in animals, although this aspect will require further systematic testing. The cottontop tamarin is not a perfect model for the prevention of EB virus infection in man since it appears that persistent infection is not maintained in this animal (Finerty et al., 1988) nor is it possible to predict the effectiveness of EB virus vaccines in preventing infection of epithelial cells in the nasopharynx and subsequent associated malignancies. Nevertheless, the cottontop tamarin provides the only suitable animal model for EB virus-induced malignancy and the demonstration that tamarins can be protected against EB virus-induced lymphoma by immunization with gp340 iscoms strongly argues for their further development for possible use in man as an EB virus vaccine.
Short communication

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REFERENCES


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