Vaccination of Tamarin with Recombinant Vaccinia Virus Expressing Epstein Barr Virus (EBV) Latent Proteins

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ABSTRACT

The aim of this work was to see whether tamarin immunisation with recombinant vaccinia virus expressing Epstein Barr Virus latent proteins could prime T cells which were, on activation, able to inhibit the outgrowth of Epstein Barr virus transformed cells *in vitro*.

The vaccination appeared to be successful as all vaccinated tamarins developed vaccinia lesions. However, the vaccination protocol did not elicit a cell-mediated response capable of inhibiting the outgrowth of autologous Lymphoblastoid Cell Lines (LCLs) as seen in the tamarin infected with whole EBV, even though the recombinant vaccinia viruses used expressed the antigens commonly recognised by sero positive humans.

Keywords: Cytotoxic T cell response, Cottontop tamarin, Epstein Barr virus, Lymphoblastoid cell lines (LCLs)

INTRODUCTION

The Epstein Barr virus (EBV) is an important human pathogen which is associated with a range of diseases affecting both lymphoid and epithelial cells. To date the cottontop tamarin (Saguinus Oedipus Oedipus) model is much more developed than other animal models and has been used to evaluate potential EBV vaccines.[1,2]

A number of subunit EBV vaccines have been developed based on EBV glycoprotein 340 (gp340) from EBV – infected cells with different adjuvant formulations. In a recent work, a subunit vaccine composed of the EBV gp340 with aluminium hydroxide (alum) as the adjuvant elicited protective immunity against EBV-induced lymphoma in three out of five cottontop tamarins[3] but neutralizing antibody was only detected in one of the animals. These results indicated that cell-mediated responses were an important component of protective immunity against experimentally induced EBV lymphoma in the tamarins.

Advances in recombinant DNA technology, peptide synthesis, and hybridoma production are providing important tools for analyzing the protective immune response. Recent advance is focused on one new approach, namely the use of vaccinia virus as a general expression vector for stimulation of humoral and cell mediated immunity to specified proteins and for determination of target antigens of cytotoxic T lymphocytes (CTL).[4]

Vaccinia virus, the live virus vaccine which has previously been used to eradicate smallpox has been genetically engineered to express foreign proteins.[5] The protective effect of vaccination with live recombinant vaccinia viruses and their potential as vaccines for veterinary and medical purposes have been discussed in the past. However, recombinant virus vaccines are known to elicit more broad-ranging immune responses with effective immunological memory.[5]

The aim of these experiments was to see whether tamarin immunisation with recombinant vaccinia

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virus expressing EBV latent proteins could prime T cells which were, on activation, able to inhibit the outgrowth of EBV transformed cells *in vitro*. The cytotoxic T lymphocyte (CTL) response can be seen using a tissue culture, growth inhibition assay.[5,7] Compared to radioactive isotope techniques (^{3}H thymidine incorporation assay or a ^{51}Cr release assay), the growth inhibition assay is safer and economical (no redioactive isotopes are used, no additional reagents like scintillation fluid is required). Also the results obtained strongly correlate to those obtained with other techniques.

As any T cell response elicited by this immunization protocol would be directed against latent gene proteins and as Lymphoblastoid cell lines (LCLs) express all latent gene proteins they can be used as potential targets, it was therefore, planned to set up growth inhibition assay from tamarins vaccinated with a pool of vaccinia virus recombinants expressing a number of EBV latent proteins.

MATERIALS AND METHODS

(i) Six healthy adult cottontop tamarins (Saguinus Oedipus Oedipus) were obtained from the breeding colony in the University of Bristol.[8,9]

(ii) Tamarin EBV transformed LCLs.

(iii) Recombinant vaccinia viruses were provided by Dr. M. Mackett (Cancer Research Campaign, Paterson Institute for Cancer Research, Manchester). Five viruses were received, the parental virus WRSC8 and four recombinant viruses expressing each of Epstein Barr nuclear antigen 2A (EBNA2A), Epstein Barr nuclear antigen 3A (EBNA3A), Latent membrane protein 1 (LMP1) and Latent membrane protein 2 (LMP2).

Establishment of EBV Transformed Lymphoblastoid Cell Lines

Tamarin EBV-transformed LCL were established from peripheral blood lymphocytes as previously described.[10] Briefly, frozen virus stocks prepared from the B95-8 cell line and having a titre of 10^5 lymphocyte transforming units per ml were thawed quickly and diluted 1 in 5 in tissue culture medium and 0.5 ml was added to 2 x 10^6 tamarin lymphocytes. Cells were incubated in a CO_2 incubator at 37° C for 1 hour with regular shaking. Cells were then washed and resuspended at the required cell concentration in 10% FCS RPMI 1640 medium containing 100 IU/ml penicillin, 100 µg/ml streptomycin and 1% phytohaemaglutinin (PHA) (1% solution of a stock). Cells were plated out in 2ml wells and incubated in 5% CO_2 in a humidified incubator at 37ºC. After 2-3 days cells stimulated with PHA were clumped together and blast cells appeared. EBV transformed clumps were grown and cell numbers increased and then sub-cultured into fresh wells after about 2-3 weeks and then into 25 cm² plastic tissue culture flasks.

After establishment, LCLs were fed twice a week by doubling the volume or replacing half the culture with fresh medium.

Vaccination

Four young adult cottontop tamarins (R145, R170, B175, B195) were vaccinated with a pool of the four recombinant viruses expressing EBNA2A, EBNA3A), LMP1 and LMP2. Each animal received two 50 µl intradermal injections of pooled virus, a total dose of 10^7 pfu (i.e. 2.5 x 10^6 pfu of each virus). One control animal (R149) received 10^7 pfu of the parental vaccinia virus (WRSC8) without any EBV genes inserted and another control (B152) remained unvaccinated. Blood samples were taken from the tamarins throughout the course of the experiment, a pre-bleed before vaccination and then afterwards at weeks 2, 4, 5, and weekly to week 14. Peripheral blood lymphocytes (PBLs) were prepared and their ability to inhibit the outgrowth of autologous EBV-transformed LCLs was investigated using the tissue culture growth inhibition assay.

Growth Inhibition Assay

The ability to inhibit the outgrowth of autologous EBV-transformed LCL in vitro as a measure of immune competence was carried out as previously described.[10] Briefly, for each animal, six replicate serial dilutions of autologous LCL from 2.5x 10^4/well to 1.7x 10^2/well were placed in 96- well plates. A constant number of 2 x10^4 peripheral blood lymphocytes was added to each well and the cultures were incubated at 37°C for 4 weeks and observed for the outgrowth of LCL. Control wells without the addition of effector cells (PBLs) were set up at the same time. All control wells showed out growth of LCL at 4 weeks. The results of the inhibition were expressed as the highest ratio of peripheral blood

lymphocytes to LCL that inhibited LCL growth in 50% of wells.

RESULTS

All tamarins vaccinated with the pool of the four recombinant viruses expressing EBNA2A, EBNA3A, LMP1 and LMP2 developed large lesions. However, two immunized animals (R170, B175) and one control animal (R149) died of causes apparently related to vaccinia virus 2 weeks after vaccinia virus injection.

Blood samples were taken as described from the remaining two immunized tamarins and unvaccinated control and their response to the autologous LCL investigated but no cell-mediated response was observed using the growth inhibition assay up to 14 weeks after recombinant virus injection (Table 1). In addition no serum antibody response to any of the EBNAs was detected in sera from the surviving tamarins taken 4 weeks after vaccination by Western blot (Personal communication).

DISCUSSION

Cell-mediated immunity to EBV in humans involves the recognition of epitopes in a number of latent proteins. Among EBV latent proteins, the EBNA3 family (3a, 3b, and 3c), LMP2 and EBNA2 appear to be targets but EBNA1 does not appear to have target epitopes for HLA class I restricted CTL responses.[2,6,7] EBV-induced tamarin lymphomas differ from Burkitt's Lymphoma (BL) and resemble organ transplant lymphomas on the criteria of EBV gene expression.[10-12]

Table 1. Ability of PBLs taken from tamarins immunized with a pool of recombinant vaccinia viruses expressing 4 EBV latent proteins (EENA 2A, EENA 3A, LMP1, LMP2) to inhibit outgrowth of autologous LCLs. Two immunized animals (R170, E175) and also one control animal (R149) died of causes related to vaccinia virus 2 weeks after injection.

Week	Animals	Ability of PBLs to inhibit outgrowth of autologous LCL
-1 (pre–bleed)	R145, R170, B195, B175 , B152, and R149 (controls)	No inhibition
0 (Vaccinia injection)	-	-
2	R145, B170, B195, B175 B152, R149 (controls)	No inhibition
3	- -	-
4	R145, B195 B152, (control)	No inhibition
5	R145, B195 B152, (control)	//
6	R145, B195 B152 (control)	//
7	R145, B195 B152, (control)	//
8	R145, B195 B152, (control)	//
9	R145, B195 B152, (control)	//
10	R145, B195 B152, (control)	//
11	R145, B195 B152, (control)	//
12	R145, B195 B152, (control)	//
13	R145, B195 B152, (control)	//
14	R145, B195 B152, (control)	//

EBV gene expression is highly restricted in BL as only EBNA-1 is expressed.[13] In contrast, tamarin lymphomas express a range of EBV latent proteins. Young *et al*,[11] were unable to detect the EBNA-3 family by immunoblotting in biopsy samples but these antigens were readily detectable in the derived cell lines. However, another investigation was able to detect the EBNA-3 family in a tumour biopsy.[14] This may be explained by the use of different antisera in the two studies or there may be variable expression of these high molecular weight EBNAs in different biopsy samples. However, EBNA-1, EBNA-2, EBNA-LP, and LMP1 are clearly expressed in the tumour biopsies.

In the present work, no cell-mediated response was observed in cottontop tamarins using a growth inhibition assay up to 14 weeks after injection with a pool of recombinant vaccinia viruses expressing a range of EBV latent proteins. In addition, no serum antibody response to any of the EBNAs was detected in sera from the surviving tamarins taken 4 weeks after vaccination by Western blot (Personal communication).

However, this is not unusual because in previous work no serum antibody response to EBV gp340 was found in any sera taken from animals that had received vaccinia expressing gp340 but the animals were protected from EBV-induced lymphoma.[1, 15] In this study, two immunized animals (R170, B175) and one control animal (R149) died of causes apparently related to vaccinia virus 2 weeks after vaccinia virus injection. Unfortunately, we could not find why the animals died.

The vaccination appeared to be successful as all vaccinated tamarins developed vaccinia lesions. However, the vaccination protocol did not elicit a cell-mediated response capable of inhibiting the outgrowth of autologous LCLs as seen in the tamarin infected with whole EBV, even though the recombinant vaccinia viruses used expressed the antigens commonly recognised by sero positive humans. This may indicate that the growth inhibition response seen in sero positive tamarins may be induced by different EBV antigens to those employed in the present experiment but this explanation seems unlikely. Alternatively, presentation of latent proteins to the tamarin immune system as individual proteins expressed in vaccinia virus may not simulate that of natural EBV infection.

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