Chapter 3 (AD6) Methods

1. Experimental rationale

The discovery of AD-6 response within HCMV gB vaccine recipients outlined a potential mechanism with which the gB vaccine can induce protection, however, the exact function of AD-6 remained a mystery.

Conservation etc

In order to determine whether the target of native AD-6 peptide is viral or cellular, I developed a binding assay using a modified protocol for the purification of His-tagged proteins on a Ni column. The virus was run through the column against a pre-bound AD-6 peptide and a no-peptide control and then tested for both the amount of infectious particles and viral DNA.

1. *In silico* work
   1. Molecular frustration

Eee

* 1. Sequence analysis

Tools to align ? But that’s in generic ?

Maybe just the sequences

* + 1. Within HCMV

f

* + 1. Within CMV of different species

F

* + 1. Within HHV

What seqences used and how obtained

* 1. Structure conservation analysis

AD-6a identification!

In HHV and CMVs

1. *In vitro* work
   1. VZV production and harvest

VZV was amplified from a vial gifted to us by colleagues in Prof Judith Breuer group (UCL) in epithelial cells. Upon removing the media from the T175 flask, the virus vial was made up to 2mL with warm media containing 2% FBS and introduced onto the cells. The flask was transferred into a 37°C incubator for 2 hours, and then into a 34°C incubator for further culture. The flask was then monitored for CPE and plaque formation and the virus was harvested when about 60% of the cell monolayer was comprised of plaques.

Low titres were first amplified in a T75 flask until 60% CPE. After removing the media from the flask, the cells were scraped with a sterile scraper and resuspended in 2mL of 2% FBS media. The suspension was introduced onto a T175 flask with nearly confluent epithelial cells. Similarly to the previously described process, the new production flask was first kept in a 37°C incubator for 2 hours before being moved into a 34°C incubator for further culture.

Since VZV is highly cell-associated, I was not able to obtain the virus simply by harvesting the media above the infected monolayer, like with HCMV or HSV-1. To do that I introduced 5mL of mitomycin-C (manufacturer) made up to 0.05mg/mL in FBS-free media to the flask, before incubating it at 37°C for 3 hours. After the incubation, the cells were washed with PBS and trypsinised similarly to a regular cell splitting protocol. After 5 minutes, trypsin was deactivated with excess media and then removed by centrifuging for 5 minutes at 500g. The cell pellet was resuspended with 2mL of freezing media (45% FBS, 45% complete media, 10% DMSO), aliquoted, and kept at -80°C for further use.

* 1. Monoclonal antibody production and harvesting

Two hybridomas were available for murine AD-6 monoclonal antibody production, 12B2 and 147F1, both produced by (ref to marco’s paper if its published) . The cells were transferred into RPMI supplemented with IgG-depleted FBS (Capricorn manufacturer) and monitored for cell death for several days. Once more than 50% of cells became permissive to Trypan blue (manufacturer) all liquid in the flask was harvested. Cells and cell debris were removed by centrifuging at 500g for 5 minutes. Preliminary analysis to confirm presence of antibodies specific to HCMV AD-6 was done on unadulterated supernatant. For all other analyses the supernatant containing the monoclonal antibodies was stored at 4°C before antibody purification through a proteinG column (manufacturer).

* 1. Polyclonal antibody production

Custom rabbit polyclonal antibody against HCMV AD-6 peptide was purchased from Genscript UK. The amino acid sequence of the peptide immunogen used for rabbit immunisation can be found in the [Peptide table].

* 1. Spread assays

Fhfhf

* 1. Neutralisation assays

Eeejejjee

* + 1. Antibody neutralisation (include pab and mab)

Dksjdksjdksjd

* + 1. Peptide neutralisation

To assess

* 1. ELISA (include pab and mab and different proteins?)

The protocol described in [Generic Methods ELISA] was followed with the parameters specified below.

* + 1. Polyclonal AD-6 antibody

The plate was coated with HCMV AD-6 peptide, AD-6 analogue peptides from HHV, and AD-6 analogue peptides from animal CMV, made up to 1µg/mL in coating buffer. AD-6 polyclonal antibody was used as a primary antibody, and an anti-rabbit HRP something as a secondary antibody (manufacturer).

While the plate was coated in identical manner as in [2.6.1], the murine AD-6 monolonal antibody produced from B-cell hybridoma cultures was used as a primary antibody. An anti-mouse HRP something as a secondary antibody (manufacturer).

* 1. Ni column

List p much entire protocol here cause it’s a new one

Desalting

Epithelial and hffs were used

Optimisation included increasing the quantity of peptide and reducing the amount of virus introduced onto the column.