AD5 Methods

1. Rationale

Vaccine effect thoguh neutralising and non-neutralising, Having had a chance to analysie novel AD6 response and historically well known neutralising response of AD2, we came back to the question remained on Which gB Epitopes Might Be Significant for the Neutralising Activity Observed in some gB/MF59 Vaccine Recipients?

* Arianes cold-neutralisation paper could divide the population into neutralisirers and non-neutralisers
* AD6 paper has provided us with detailed peptide screening data. Cobine the two together.

Having combined this knowledge BLAH

1. In silico work

1.1. AD5N identification

Quote matts analysis & the undergrad diss

Following Gomes et al. (87) discovering detectable pre-transplant neutralising antibodies using their modified 4ºC neutralisation assay, questions arose about the composition of this antibody pool, as it could potentially reveal mechanistic correlates of protection. To address these questions, the same research group combined data from their modified 4ºC assay study and their 2023 study where they identified AD6 (80,87). This data, although not published, was provided to me by my supervisor, who was involved in both studies from which the data was extracted. Using data from their 4ºC modified assay, vaccine recipients were characterised into neutralisers (those with post-vaccination neutralising antibodies detected in the 4ºC assay) and non-neutralisers. Upon characterisation of neutralisers and non-neutralisers, the data collected in their 2023 AD6 study, which involved antibody binding to a series of overlapping 15-mer peptides spanning the entire gB protein, was analysed. A MannWhitney test, based on non-parametric relationships, was then conducted to assess differences in responses of neutralisers and non-neutralisers to the various antigenic domains. As seen in Figure 7, the only two statistically significant differences (<0.05) (greater in neutralisers) were in the binding to AD1 and AD5, with responses to AD5 robustly more significant. Therefore, antibody binding to peptides within the AD5 region was further analysed. Peptides 24-81 of gB mapped onto the AD5 region, leading to analysis of antibody binding to these peptides in neutralisers and non-neutralisers. As illustrated in Figure 8, disparities in binding were particularly evident in peptides 31-45 and 76-90, with the former exhibiting significantly higher binding in neutralisers. This region was termed AD5N, representing the region of the AD5 domain closer to the N terminus. Subsequent analysis involved sequencing AD5N and AD5C (region of AD5 closer to the C terminus), achieving the sequences illustrated in Figure 9.

Thus, AD5N was identified as a 70 amino acid domain located largely at the N terminus of AD-5. The sequence is quoted in results.

1.2. Structure analysis

Pre-fusion and post-fusion structures of glycoprotein B (pdb refs) and Chimera. In detail is in [General Methods]

1.3. Sequence conservation analysis

1. In vitro work

Using the dividing into neutraslisers and not

2.1

2.2 Peptide pre-adsorption and cold neutralisation

* Pre-adsorption assays + cold neutralisation