

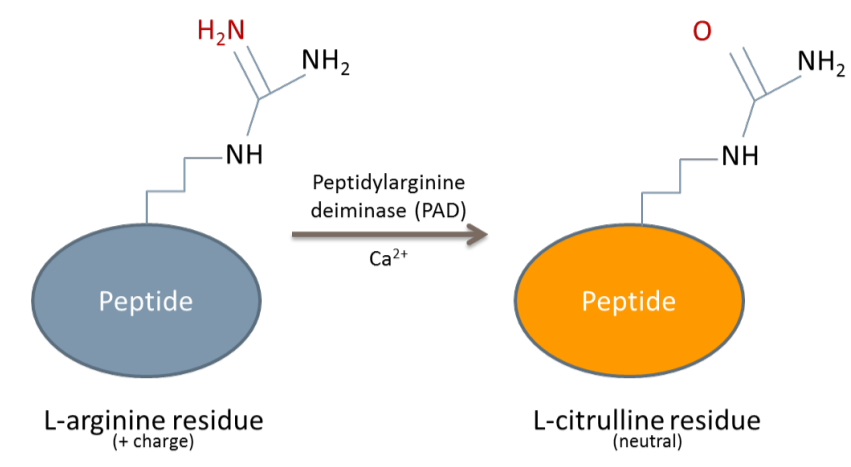
Katherine Cook¹, Peter Symonds¹, Victoria Brentville¹, Rachael Metherringham¹, Wei Xue¹ and Lindy Durrant (lindy.durrant@nottingham.ac.uk)^{1,2}

¹Scancell Ltd, Nottingham UK, ²University of Nottingham, Nottingham UK

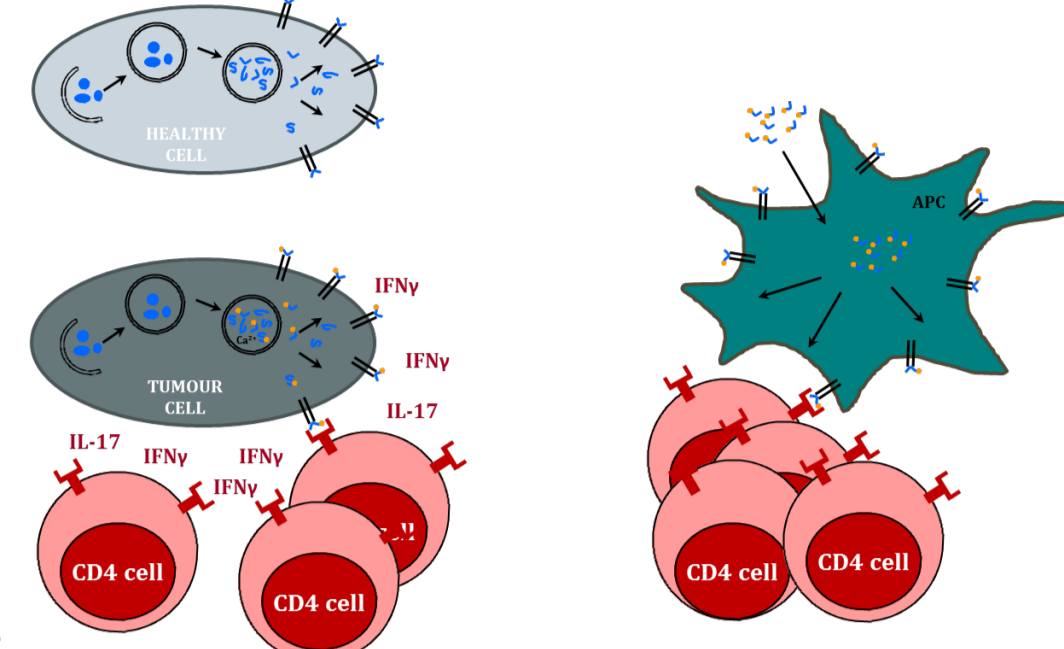
Introduction

Citrullination

Citrullination is a posttranslational modification which occurs as a result of cellular stress and leads to the generation of neoantigens. In rheumatoid arthritis this process generates potent immune responses.



Our research suggests that the citrullination of peptides in rapidly growing tumour cells may be exploited to generate specific CD4 responses which lead to tumour rejection. This requires immunisation with peptides and an appropriate adjuvant.



Mod-1 vaccine

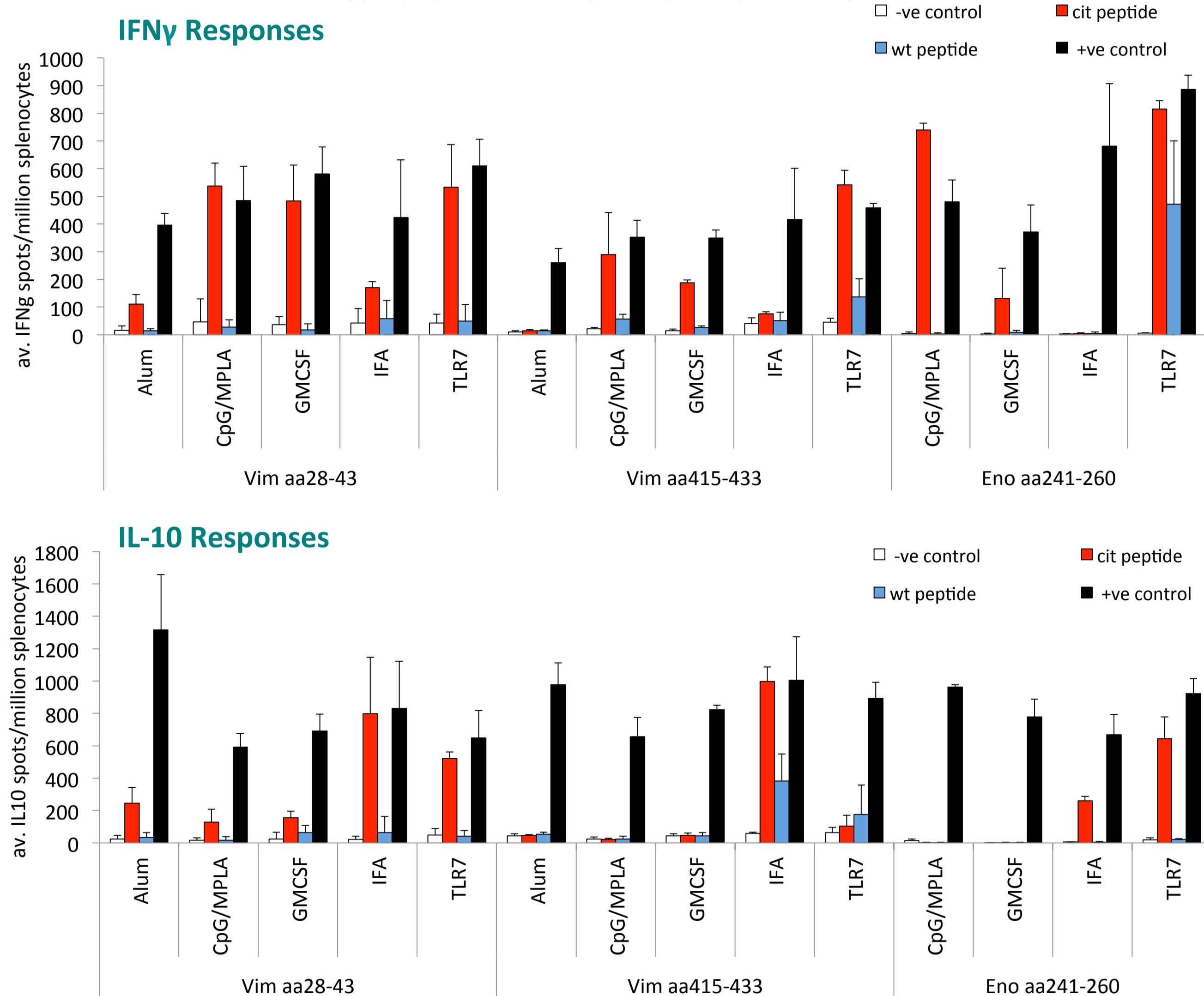
Mod1 is a combination of three citrullinated peptides; two Vimentin (aa28-43cit and 415-433cit) and one alpha-enolase (aa241-260cit). Enolase is a glycolytic enzyme which is upregulated in many cancers. Vimentin is a cytoskeletal protein of the type III IF protein family and a marker for and regulator of epithelial to mesenchymal transition (EMT). Both proteins are over-expressed in various cancers and are known to undergo citrullination.

This study aimed to determine the best adjuvant to use in conjunction with these peptides to obtain the best product for clinical trial

Adjuvant effects the type of response

Does the type of adjuvant effect the cytokine profile of the immune responses?

HLA-DR4 transgenic mice were immunised with a single dose of citrullinated peptides in various adjuvants. Splenocytes were analysed for peptide specific IFN γ or IL-10 responses to the citrullinated or wild type peptides at day 12 by ELISpot assay.



Peptides induce a predominantly IL-10 or IFN γ response depending on adjuvant

Does adjuvant type alter the efficacy of a Mod1 vaccine against tumour challenge?

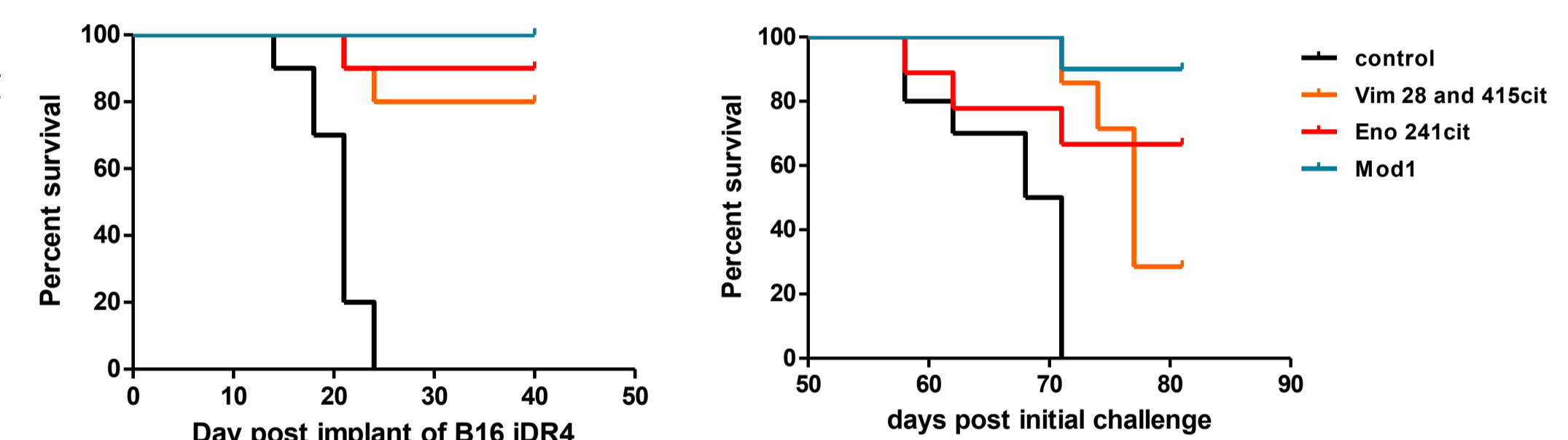
To determine the effect of adjuvant, HLA-DR4 mice were immunised with a single dose of Vim aa28-49 & aa415-433 citrullinated peptides in various adjuvants on day 1. On day 12 mice were challenged subcutaneously with B16 tumour expressing HLA-DR4 and survival was monitored.



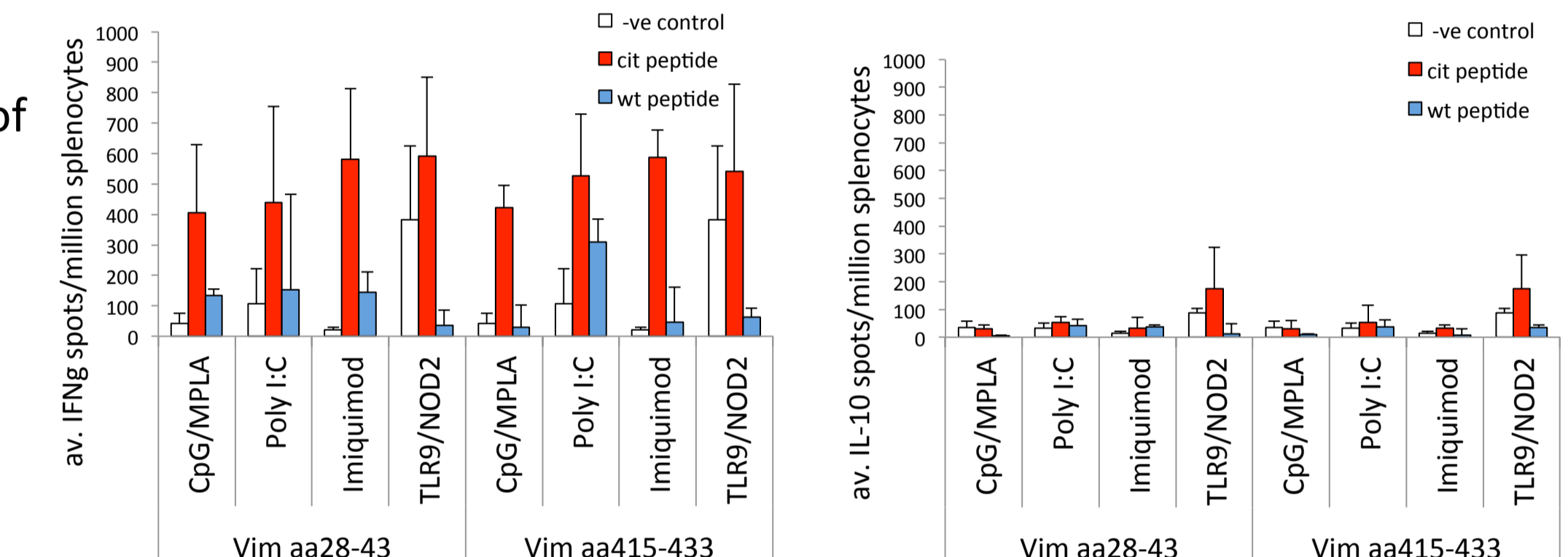
Efficacy of Mod1 as an anti-tumour vaccine is dependent on adjuvant used

Do Mod-1 peptides induce a therapeutic anti-tumour response?

HLA-DR4 mice challenged with a B16 tumour expressing IFN γ inducible DR4 were immunised with citrullinated peptides in CpG and MPLA on day 4. Survival after initial challenge and a rechallenge on day 42 are shown.



HLA-DR4 mice were immunised with doses of citrullinated Vimentin peptides in different adjuvants. Splenocytes analysed by ex vivo ELISpot for IFN γ or IL10 responses



A number of adjuvants induce similar predominantly IFN γ responses to the Vimentin peptides

Adjuvant effects the dose required

Can the dose required be reduced by using adjuvants conjugated directly to peptides?

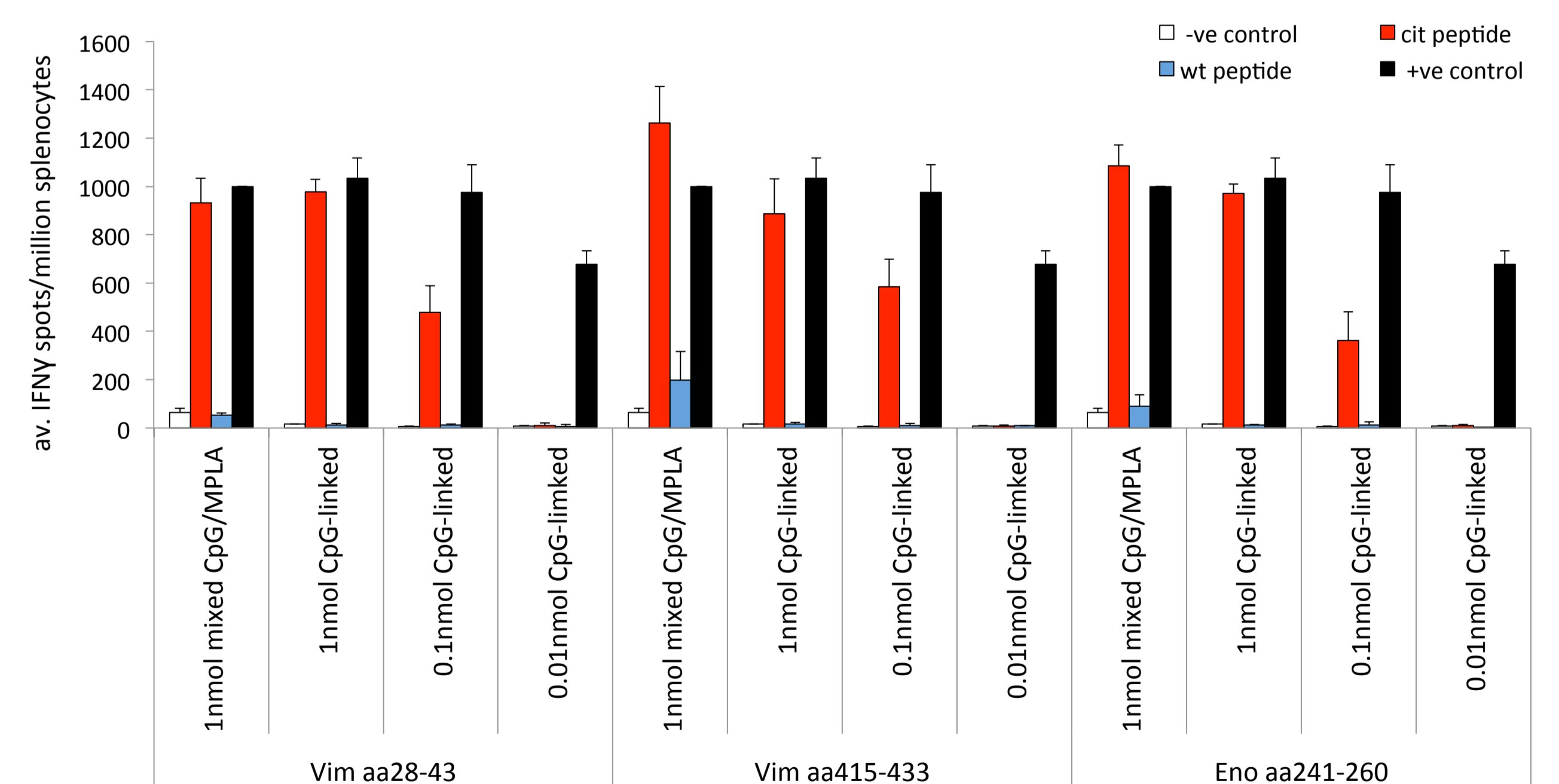
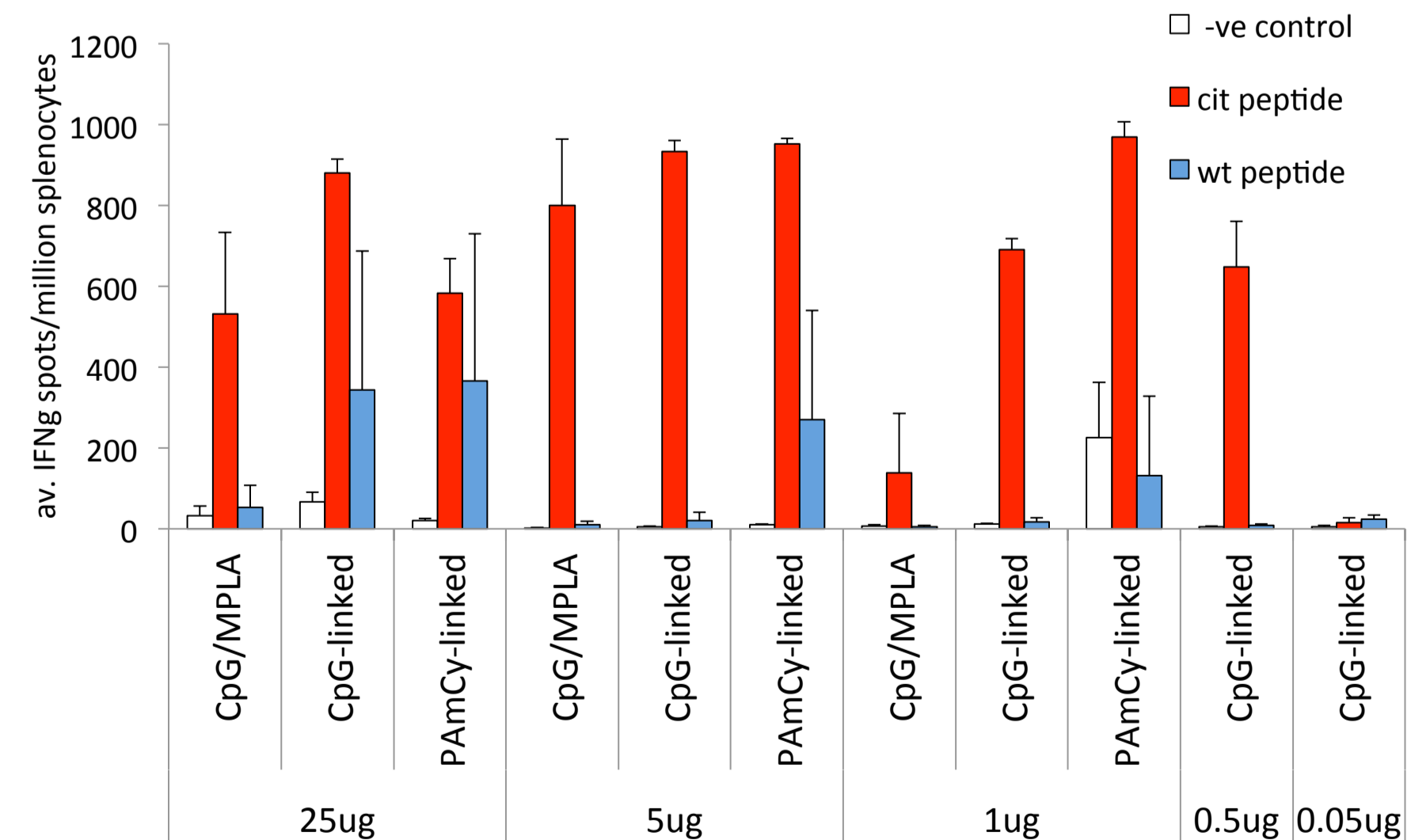
Two linked adjuvants were tested – CpG and Pam2Cys

Responses were first tested with the Vim28-49cit peptide and then confirmed with the combination of all three peptides

Mice were immunised with three doses of Vimentin 28-49 cit, Vim415-433cit and Eno241-260cit each linked to either CpG or Pam2Cys adjuvants at different concentrations

IFN γ Responses to adjuvant linked Vimentin 28cit peptide

Mice were immunised with three doses of citrullinated Vimentin 28-49 peptide linked to either CpG or Pam2Cys adjuvants at different concentrations



Linking peptides to adjuvants reduces the dose required for a detectable immune response

Conclusions

- Transgenic mice expressing human HLA DR4 show a strong response after immunisation with the Mod1 vaccine (Vimentin 415cit, Vimentin 28cit and Enolase 241cit peptides)
- The cytokines profile of the response is determined by the adjuvant used for immunisation
- The immune response profile induced by different adjuvants is reflected in the anti-tumour response with only those inducing Th1 type responses efficiently targeting tumour
- A number of adjuvants are capable of inducing pro-inflammatory Th1 type responses to the citrullinated peptides
- Linking the peptides directly to the adjuvants allows lower doses to be administered in order to induce detectable immune responses

These results suggest that adjuvant choice has a profound effect on the dose required and the response profile following immunisation with a self CD4 epitope peptide