

Introduction:

➤ CD4 cells are potent effectors but CD4 responses to self antigens are attenuated.

➤ Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system ⁽²⁾.

➤ In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated ⁽¹⁾.

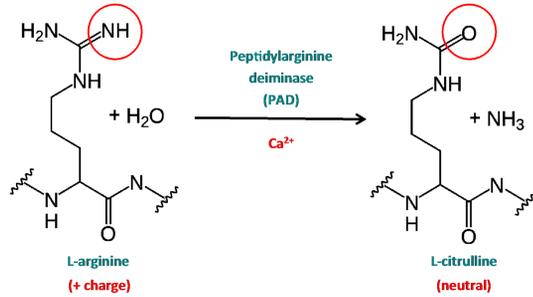
➤ T cells targeting modified self-antigens play a role in the pathophysiology of several autoimmune diseases.

➤ Cancer cells citrullinate proteins ⁽³⁾ and present modified peptides as targets for CD4 T cells.

➤ Responses can be induced by peptide vaccination.

➤ Selection of an appropriate adjuvant is necessary in order to induce immunity with peptide vaccines ⁽⁴⁾.

➤ In this study we investigate the effect of adjuvant on the induction and cytokine profile of self antigen specific CD4 responses.



Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and convert arginine to citrulline by altering the positively charged aldimine group (=NH) of arginine to the neutrally charged ketone group (=O) of citrulline.

Methods:

➤ HLA-DR4 or C57Bl/6 mice were immunised with a single dose of 25µg citrullinated vimentin (Vim), enolase (Eno), myelin oligodendrocyte glycoprotein (MOG) or ovalbumin (Ova) peptide in combination with adjuvant.

➤ Splenocytes were monitored at indicated time points by cytokine elispot assays or ELISA for peptide specific immune responses.

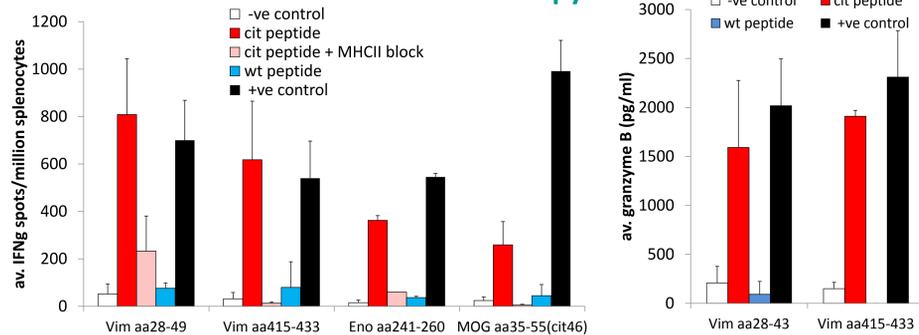
➤ HLA-DR4 transgenic mice were challenged with B16F1 tumour expressing HLA-DR4 either prior to or post vaccination with citrullinated peptide in adjuvant.

References:

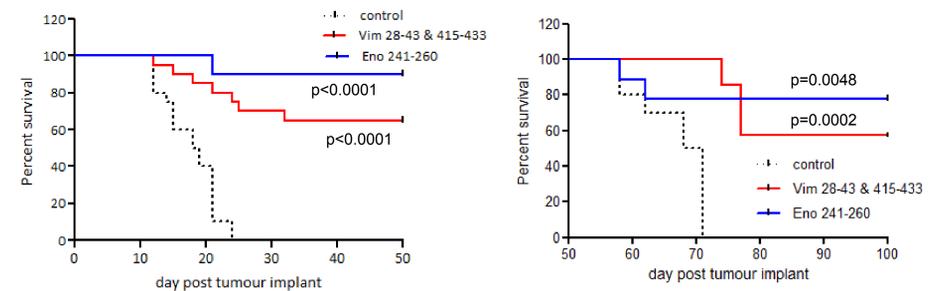
1. Feitsma AL *et al.* Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. *Arthritis Rheum.* 2010 Jan;62(1):117-25
2. Ireland JM, Unanue ER. Autophagy in antigen presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J Exp Med.* 2011 Dec 19;208(13):2625-32.
3. Jiang Z *et al.* Investigating citrullinated proteins in tumour cell lines. *World J Surg Oncol.* 2013 Oct 7;11:260.
4. Hailemichel Y & Overwijk W. Peptide-based anticancer vaccines: The making and unmaking of a T-cell graveyard. *Oncoimmunology* 2013. 2(7):e24743.

Results:

Modified peptides induce strong Th1 responses that mediate tumour therapy



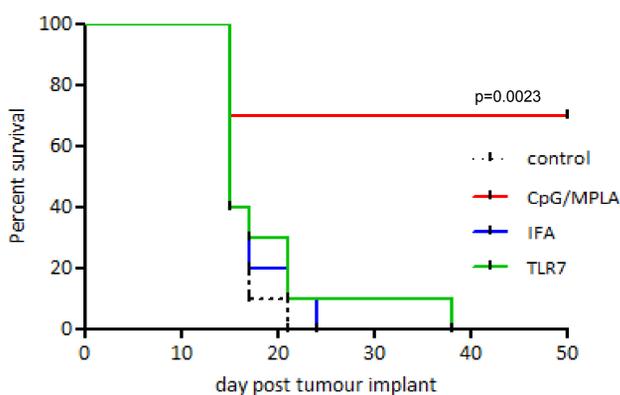
HLA-DR4 transgenic or C57Bl/6 mice were immunized with a single dose of citrullinated peptides in CpG and MPLA. Splenocytes were analyzed at day 12 for peptide specific IFN γ responses to the citrullinated or wild type peptides by Elispot assay. MHCII blocking antibody was used to prove CD4 nature of the response. Supernatant from elispot plates was tested for granzyme B by ELISA.



B16F1 tumour expressing IFN γ inducible HLA-DR4 was established subcutaneously in transgenic HLA-DR4 mice (Day 1). The citrullinated peptides were administered on Day 4 (with CpG-MPLA adjuvant) and survival monitored.

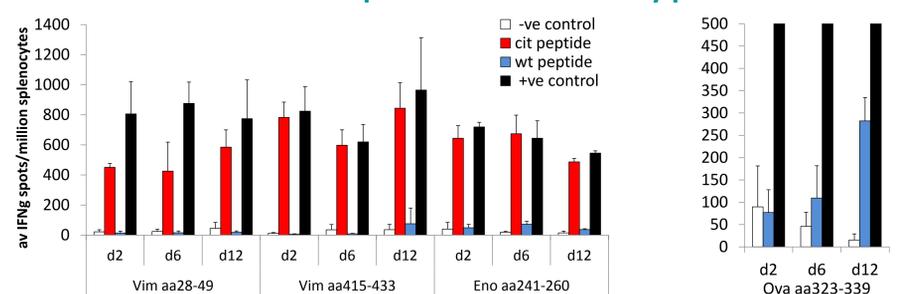
Surviving mice were rechallenged with tumour at day 50 to assess memory responses and survival monitored

Th1 response phenotype efficiently eradicates tumour whereas regulatory phenotype does not



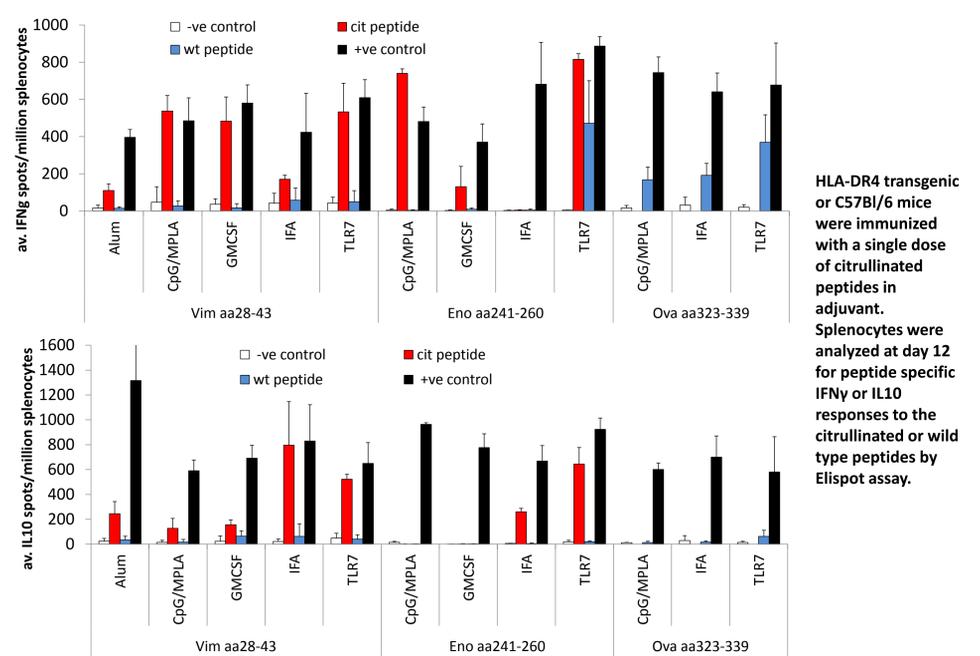
HLA-DR4 transgenic mice were immunised with a single dose of Vim aa28-49 & aa415-433 citrullinated peptide in various adjuvants on day 1. On day 12 mice were challenged subcutaneously with B16 DR4 tumour and survival was monitored.

Rapid induction of responses suggest vimentin and enolase specific T cells are present in the memory pool



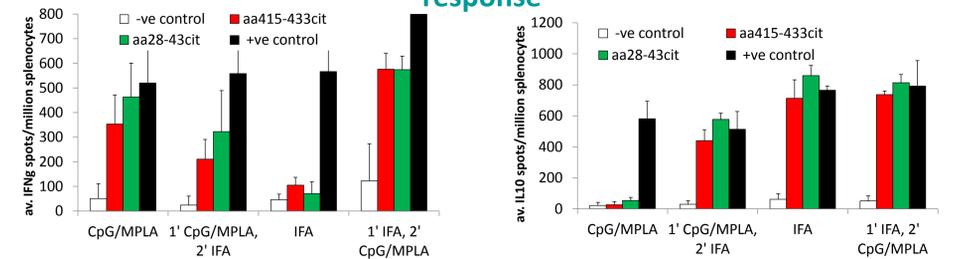
HLA-DR4 transgenic or C57Bl/6 mice were immunized with a single dose of citrullinated peptides in CpG and MPLA. Splenocytes were analyzed at day 2, 6 or 12 for peptide specific IFN γ responses to the citrullinated or wild type peptides by Elispot assay.

Adjuvant choice polarises the cytokine response of modified epitopes



HLA-DR4 transgenic or C57Bl/6 mice were immunized with a single dose of citrullinated peptides in adjuvant. Splenocytes were analyzed at day 12 for peptide specific IFN γ or IL10 responses to the citrullinated or wild type peptides by Elispot assay.

Adjuvant combination prime boost promotes a mixed cytokine response



HLA-DR4 transgenic mice were immunized with prime boost regime of citrullinated Vim aa28-49 and 415-433 peptides in adjuvant. At day 1 and 21. Splenocytes were analyzed at day 27 for peptide specific IFN γ or IL10 responses to the citrullinated or wild type peptides by Elispot assay.

Conclusion

These results demonstrate the stimulation of memory responses to self antigens that recognise and target tumour cells using modified peptide epitopes. Furthermore the cytokine phenotype of these self antigen specific responses can be dramatically polarised when combined with different adjuvant combinations and costimulation resulting in a change in tumour targeting ability.