CD4 T cells targeting citrullinated vimentin reject advanced tumors

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USING IMMUNOLOGY TO FIGHT CANCER

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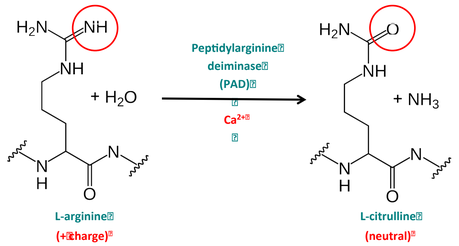
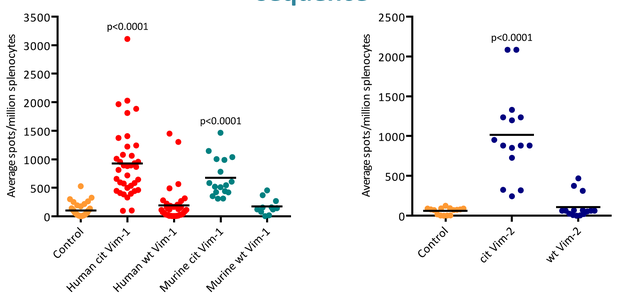
Introduction

- 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.
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated.
- T cells targeting modified self-antigens play a role in the pathophysiology of several autoimmune diseases.
- In this study the ability of these CD4 cells to target cancer has been explored.



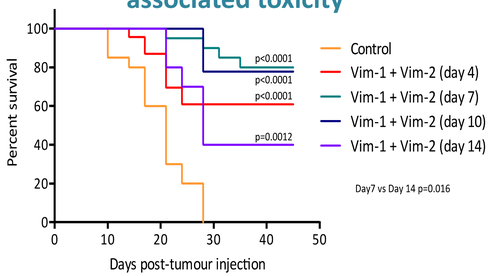
Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and converts arginine to citrulline by altering the positively charge aldimine group (=NH) group of arginine to the neutrally charged ketone group (=O) of citrulline.

Immunization with citrullinated vimentin peptides induced IFNγ responses to citrullinated self peptide epitopes which show minimal reactivity to unmodified sequence



HLA-DR4 transgenic mice were immunized with citvim-1 and citvim-2 peptides. Splenocytes were analyzed for peptide IFNγ specific responses to the cit or wild type peptides by Elispot assay.

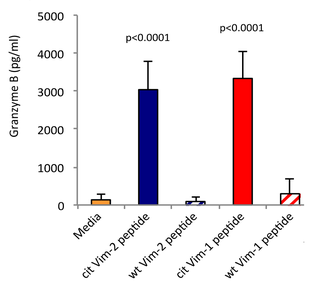
citVim-1 and citVim-2 stimulate potent responses against established tumors with no associated toxicity



Survival of HLA-DR4 transgenic mice challenged with B16DR4 tumor (Day 1) and Immunized with citVim-1 + citVim-2 administered on Day 4, Day 7, Day 10 or Day 14 (with CpG-MPLA adjuvant).

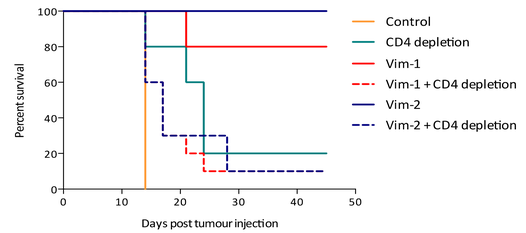
citVim-1 and citVim-2 stimulate potent anti-tumor responses

- Is this CD4 mediated? ✓
- Does this require recognition of MHC-II? ✓
- Is this dependent upon IFNγ? ✓
- Is it CD8 mediated ? X
- Is this dependent upon autophagy? ✓
- Is this dependent upon PAD enzymes? ✓
- Are cytotoxic CD4+ T cells involved? ✓



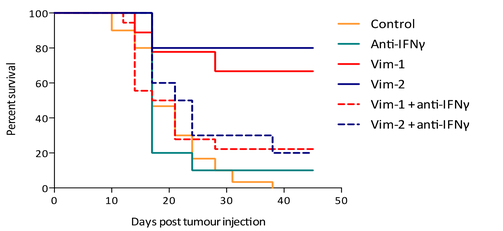
Splenocytes from citrullinated peptide immunized mice were tested for Granzyme B release by Elisa. Granzyme B was secreted in response to stimulation with citVim-1 or citVim-2, but not with wild-type peptides (hatched bars).

Anti-tumor responses are mediated by CD4 cells



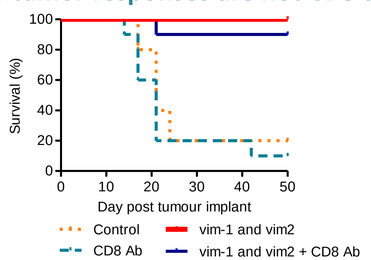
Survival of HLA-DR4 transgenic mice challenged with B16DR4 tumor (Day 1) and immunized (Day4) with either citVim-1 or citVim-2 (with CpG-MPLA adjuvant) in the presence or absence of a anti-CD4 depleting monoclonal antibody. CD4 depletion *in vivo* abrogates anti-tumor response

Anti-tumor responses are mediated by IFNγ



B16DR4 tumor was established in transgenic HLA-DR4 mice (Day 1). The citVim-1 or citVim-2 peptides were administered on Day 4 (with CpG-MPLA adjuvant) in the presence or absence of a IFNγ neutralizing monoclonal antibody. Blockade of IFNγ *in vivo* abrogates anti-tumor responses.

Anti-tumor responses are not CD8 dependent

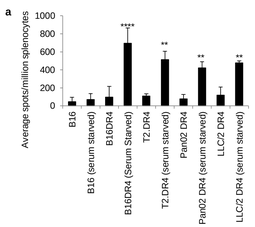


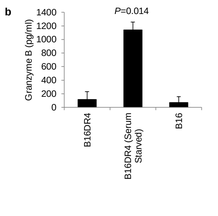
Survival of HLA-DR4 mice challenged with B16DR4 tumor (Day 1). Immunized with citVim-1 or citVim-2 administered on Day 4 (with CpG-MPLA adjuvant) in the presence or absence of a CD8 depleting monoclonal antibody. Removal of CD8 cells has no influence on the anti-tumor responses

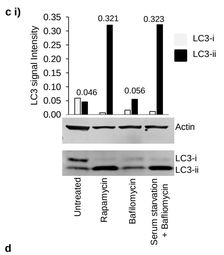
Conclusion

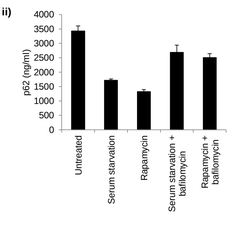
These results show that CD4 T cells can mediate potent anti-tumor responses against modified self–epitopes presented on tumor cells.

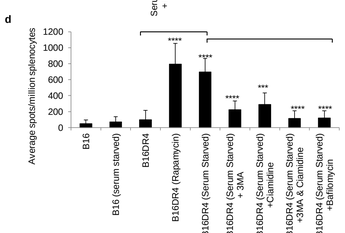
Tumor recognition depends upon citrullination and autophagy











Splenocytes from mice immunized with both citrullinated peptides were assessed for the ability to recognize tumor cells in IFNγ Elispot assay (a) or by granzyme B Elisa (b). (c) (i) Western blot of B16DR4 tumor cell lysates probed for the LC3 autophagy marker with associated histogram summarizing the densitometric analysis of the LC3-I and LC3-II bands normalized to β actin control and (ii) p62 Elisa showing induction of autophagy when treated with rapamycin, bafilomycin and serum starvation. (d) Recognition of nutrient-starved tumor cells and inhibition in the presence of autophagy and PAD inhibitors.. ***$P < 0.001$, ****$P < 0.0001$.