Pre-existing citrulline specific CD4 T cells can be efficiently harnessed for tumour therapy

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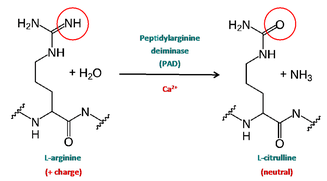
INTRODUCTION

- CD4 T cells are potent effectors but CD4 responses to self antigens are often attenuated.
- Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system [(2)]. One such modification is citrullination (cit).



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

- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated [(1)].
- Cancer cells citrullinate proteins [(3)]. Citrullinated proteins in cancer cells include ubiquitous cytoskeletal protein Vimentin and glycolytic enzyme α-Enolase.
- Stressful conditions in tumour microenvironment and inflammation leads to presentation of modified peptides on MHC class II which are a target for CD4 T cells. We have shown that these can be harnessed for tumour therapy [(4,5)].
- Citrulline specific CD4 responses have been previously detailed in HLA-DR4+ healthy donors although lower frequency than in rheumatoid arthritis patients[(6)]. We too have demonstrated that responses can be detected in healthy donors with a broader HLA range[(4,5,unpublished)].
- In this study we demonstrate the rapid detection of HLA-DR4 and HLA-DP4 restricted citrulline specific CD4 responses in mice which mediate regression of large established tumours.
- We also show evidence of oligoclonal CD4 responses to citrullinated peptides in healthy human donors that in some donors may also originate in the memory pool.

Citrullinated peptides stimulate Th1 responses restricted through HLA-DR4 and HLA-DP4 which mediate tumour therapy

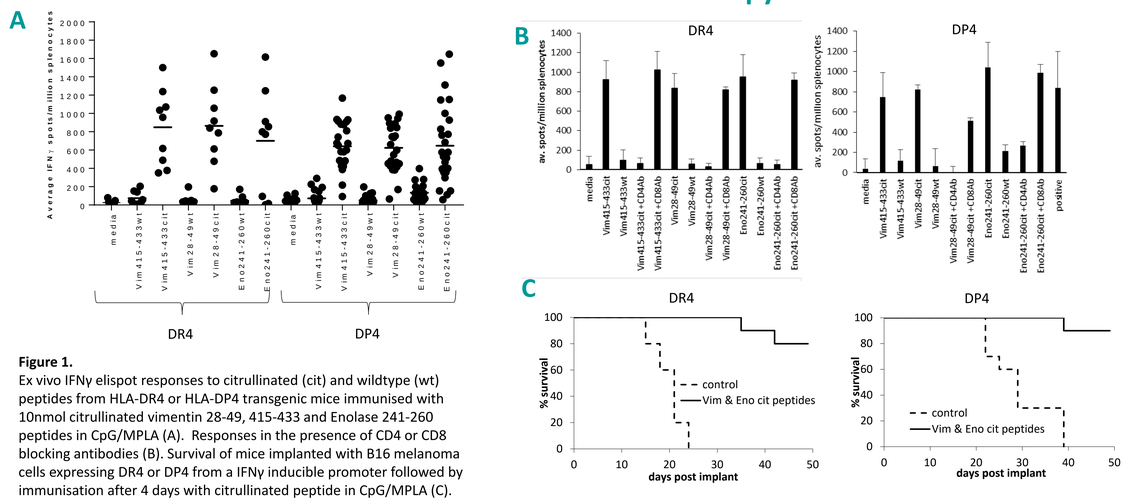


Figure 1.
Ex vivo IFNγ elispot responses to citrullinated (cit) and wildtype (wt) peptides from HLA-DR4 or HLA-DP4 transgenic mice immunised with 10nmol citrullinated vimentin 28-49, 415-433 and Enolase 241-260 peptides in CpG/MPLA (A). Responses in the presence of CD4 or CD8 blocking antibodies (B). Survival of mice implanted with B16 melanoma cells expressing DR4 or DP4 from a IFNγ inducible promoter followed by immunisation after 4 days with citrullinated peptide in CpG/MPLA (C).

Th1 responses are rapidly detected and promote fast regression of large tumours

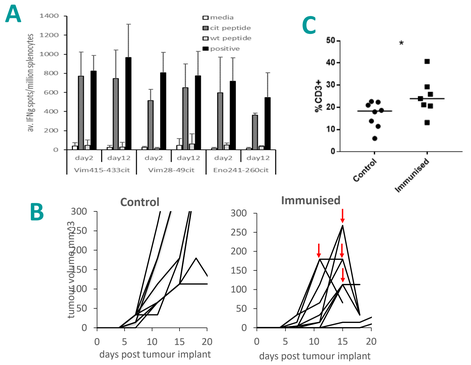


Figure 2. *Ex vivo* IFNγ ELISpot responses at day 2 or 12 post vaccination in HLA-DP4 transgenic mice immunised with 10nmol citrullinated peptides mixed with CpG/MPLA adjuvant. HLA-DP4 (B) transgenic mice were challenged with B16 tumour expressing HLA-DP4 under an IFNγ inducible promoter and immunised with 10nmol citrullinated peptides mixed with CpG/MPLA when tumours reached 5-9mm diameter. Tumour volume monitored for 4 days post vaccination and tumour assessed for T cell infiltrate by flow cytometry(C).

CD25 depletion uncovers responses to citrullinated peptides in healthy donors

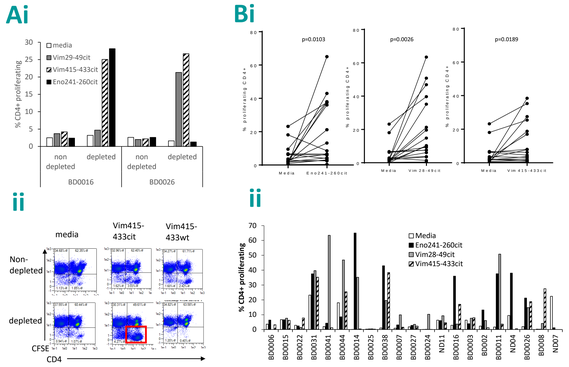


Figure 3. Responses to citrullinated peptides in healthy donors. A, CD25 depleted or undepleted PBMCs from healthy donors were labelled with CFSE and stimulated with 10μg/ml citrullinated or wt peptides. Proliferation by loss of CFSE was analysed at day 11 by flow cytometry in combination with CD4 staining. Results shown as percentage CD4+ cells proliferating is shown for each peptide (i) and an example set of flow cytometry dotplots (ii). B, Collated data for responses in CD25 depleted PBMCs from healthy donors showing responses to each peptide (i) and responses within individual donors (ii).

Responses in healthy donors show an oligoclonal response to citrullinated peptides with a Tem and Temra phenotype

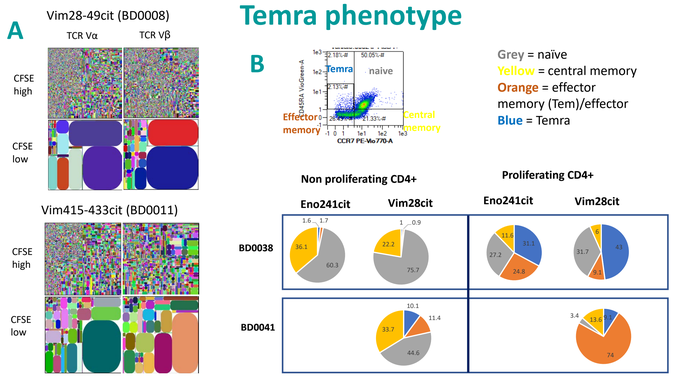


Figure 4. Characterisation of responses to citrullinated peptides. CD25 depleted PBMCs from healthy donors were labelled with CFSE and stimulated with 10μg/ml citrullinated peptides. A, Low frequency proliferating (CFSE low) CD4+ cells were sorted on MoFlow sorter (Beckman Coulter) at day 10 from two responding donors and sent for TCR repertoire analysis by iRepertoire Inc. Repertoire data for TCR Vα and Vβ is shown as Tree plots where each spot denotes a specific V-J CDR3 and the spot size denotes frequency. B, Proliferating CD4+ cells at day 10 from two donors were analysed for expression of memory markers (CD45RA and CCR7) by flow cytometry staining and percentage of the proliferating CD4+ population expressed as pie charts for responses.

Healthy donors can show both memory and naïve responses to citrullinated peptides

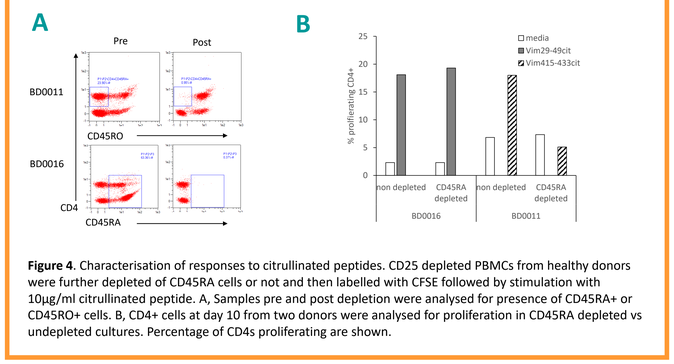


Figure 4. Characterisation of responses to citrullinated peptides. CD25 depleted PBMCs from healthy donors were further depleted of CD45RA cells or not and then labelled with CFSE followed by stimulation with 10μg/ml citrullinated peptide. A, Samples pre and post depletion were analysed for presence of CD45RA+ or CD45RO+ cells. B, CD4+ cells at day 10 from two donors were analysed for proliferation in CD45RA depleted vs undepleted cultures. Percentage of CD4s proliferating are shown.

CONCLUSIONS

- **Th1 responses to citrullinated peptides can be stimulated in HLA-DP4 and HLA-DR4 transgenic mice**
- **Responses are rapidly detected post vaccination suggesting they are pre-existing and cause swift regression of large tumours within 4 days**
- **Healthy donors show CD4 repertoires that respond to citrullinated peptides and these can be uncovered/enhanced by CD25 depletion suggesting regulatory mechanisms keep these responses in check.**
- **Responses in humans appear oligoclonal and show enhanced effector memory and Temra phenotypes.**
- **Responses in healthy humans can exist in both memory and naïve T cell pools**

References:

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