**In Vivo** Assessment of Melanoma Peptide Vaccine Formulations

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**ABSTRACT SUMMARY**

The ability of melanoma peptide vaccine formulations to induce cytotoxic T-cell responses was investigated in an *in vivo* model. Coupling of two short Trp2 peptides and consequent immunization with various adjuvants and formulations enhanced immune responses but did not meet expectations for a successful peptide vaccine for the treatment of melanoma.

**INTRODUCTION**

Therapeutic peptide vaccines, which target cancer cells explicitly, are an appealing alternative to conventional cancer chemotherapy. In this approach, tumor-associated antigens (TAA) were used to prime the immune system to react to cancer cells and destroy them.

Commonly used TAAs consist of 6-8 amino acids and were shown to induce an immune response *in vivo* but have the disadvantage of inducing immunological tolerance due to exogenous loading of peptides onto MHC class I molecules. Longer peptides (22-45 amino acids) have to be processed prior to their presentation on MHC molecules and are therefore more likely to induce a potent immune response. Here we investigate the effect of using a long peptide (long Trp2–peptide), incorporating both a CD8 and CD4 tumor epitope, to induce a cytotoxic T-cell response *in vivo*. To increase uptake by antigen presenting cells (APCs) and APC activation, the long Trp2 peptide was formulated in chitosan nanoparticles (CNP), Montanide ISA 51® or aluminium hydroxide (alum). We further analyzed the effect of co-administration of interferon-α (IFN-α) with the peptide vaccine.

**EXPERIMENTAL METHODS**

**Formulations**

Six to eight week old male C57BL/6 mice were immunized (s.c.) with 20 nmol of the long Trp2-peptide formulated in CNP, alum or Montanide ISA 51® on day 0 and 14 and mice were sacrificed on day 18.

CNP were prepared by the ionotropic gelation method using sodium sulfate as the gelation agent. Average particle size was 783 ± 46 nm. Montanide ISA 51® was emulsified with saline (PBS) in a 1:1 ratio using a stop-cock connector. In some experiments IFN-α (5x10⁴ IU) was added to formulations prior to administration and also injected s.c. every second day for 6 days.

**CFSE labelling and transfer**

Lymph node and spleen cells were harvested from C57BL/6 mice, incubated with 2.5 μM or 0.5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) and either pulsed with 20 nmol Trp2 peptide (CFSE^high^) for 2h or left untreated (CFSE^low^). Cells were washed, resuspended in PBS and transferred to recipient mice on day 17.

**Flow cytometry analysis**

Lymph nodes and spleens were harvested from recipient mice on day 18, single cell suspensions prepared and cells stained with antibodies: CD8 APC-Cy7 and MHC Pentamer PE (H-2Kb SYDFFVWL) after blocking Fc-receptors with 2.4G2 antibody. Flow cytometry was performed on a FACs Canto II. Specific cell lysis of CFSE-labelled splenocytes was determined using the following equation:

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\% \text{ cytotoxicity} = 100 - \left[ \left( \frac{\text{peptide pulsed}_{\text{expt mice}}}{\text{peptide unpulsed}_{\text{expt mice}}} \right) / \left( \frac{\text{peptide pulsed}_{\text{naive mice}}}{\text{peptide unpulsed}_{\text{naive mice}}} \right) \times 100 \right]
\]

**RESULTS AND DISCUSSION**

To investigate the effect of a long peptide, comprised of a CD4 and CD8 epitope of Trp2, on the immune response *in vivo*, mice were immunized with the long peptide formulated in alum, montanide, CNP or with PBS. Cytotoxic T-cell responses in mice challenged with CFSE-labelled splenocytes pulsed with the long Trp2-peptide were surprisingly low and only resulted in 0-20% killing of the target-loaded cells (Figure 1). As a comparison, mice were immunized with the short CD4 (Trp288-102) and CD8 (Trp2180-188) peptides and cytotoxicity determined. The short peptides, when administered in montanide, led to a similar response, comparable to the long Trp2 peptide, with a slight increase in cytotoxicity.
To examine if the unexpectedly low response to immunization with the Trp2 peptides was caused by the lack of a strong adjuvant, we conducted another study and added IFN-α to the formulations. IFN-α has been reported to support cytotoxic T-cell responses when administered with peptide vaccines. However, we could not detect an increase in killing when IFN-α was co-administered with peptides (data not shown).

When cells were stained with a MHC pentamer that recognizes the T-cell receptor for Trp2<sub>180-188</sub> differences in cell populations were only statistically significant when the long Trp2-peptide was formulated in montanide (Figure 2). Addition of IFN-α failed to affect the proliferation of CD8<sup>+</sup> pentamer<sup>+</sup> populations.

**CONCLUSION**

Immunization with Trp2 peptides increased specific lysis of peptide pulsed cells and led to an increase of Trp2 specific CD8<sup>+</sup> T-cells. However, responses were minimal and it needs to be determined if cytotoxic immune responses to the combination of the two tumor epitopes can be further improved through the use of a more potent adjuvant or formulation.

**REFERENCES**


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