Abstract

Listeria monocytogenes has been previously used as a vector in cancer immunotherapy to only transmit a full length protein into a dendritic cell *in vitro*. Our objective was to instead target dendritic cells *in vivo*.

We hypothesized that by using minigenic constructs in a plasmid vector that codes for minimal tumor/dengue peptide it would get presented by dendritic cells to CD8+ T cells. The T cells then mount an effector response by secreting IFNγ.

2 mice were immunized for each of the 4 peptides with Listeria bearing the construct ΔActA-Ub-peptide. Ub is ubiquitin which helped release the peptide directly after being hydrolyzed in cytoplasm. Using a minimal peptide also ensured that we bypassed proteasomal activity.

Intracellular staining was done to measure IFNγ production and data was analyzed by flow cytometry. 3 out of 4 peptides elicited a response from CD8+ T cells with the response especially high for the dengue peptide.

Thus, we were able to confirm that tumor cells can indeed be targeted *in vivo* and future studies should reveal the immunization strategies that produce the best response.

Background and Introduction

Cancer immunotherapy:
- Problem: Tumor cells are not very immunogenic.
- Solution: Dendritic cells
  - Are professional antigen presenting cells.
  - Tumor-associated antigens (TAAs) are targeted to CD8+ (cytotoxic) T cells through dendritic cells.
  - T cells response triggered against all cells expressing the TAAs i.e. the cancer cells.

New problem:
- Dendritic cells need to be removed from the patient, pulsed with TAAs and then placed back in patient’s body.
- Cumbersome and expensive.
- Solution: How do we circumvent this problem?

Methods

- Overnight cultures were set up using Act-A deficient Listeria strain DP-L4029 as vector.

The Listeria expressed 4 different peptides:
- ΔAct A-Ub-mGARC-177-185 (AALLOWLY
- ΔAct A-Ub-mTRP2-180-188 (SYVDFVWL
- ΔAct A-Ub-mPhA2-682-689 (VSVKHMK
- ΔAct A-Ub-D2 NS4a (YSQVNPTTL)

Glioma associated peptides

- After 1 week, spleens were harvested and incubated with Listeria expressing stimulating peptide or control peptide SIINFEKL
- Cells were fixed and stained for flow cytometry with anti-CD8, anti-IFNγ and anti-Fcγ receptor

Results

- This was a pilot study to verify that our dendritic cell vaccine approach is feasible. The response by CD8+ T cells showed that we can elicit an immune response using this method.
- This indicates that it will be possible to target tumor cells by using dendritic cells infected with *Listeria in vivo*.
- While, the response to the glial peptide mPhA2 remained inconclusive, the response to the other two glial peptides mGARC and mTRP2 was significant.
- In the future, multiple constructs involving the minimal glial peptides can be used to see if we can measure a measurable response.
- We could also provide a booster dose of stimulating peptide to further prime the immune response.

Conclusion

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