Targeting Cancer Cells by Antigen Presentation of Tumor Peptides to Cytotoxic T Cells

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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 minigene 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

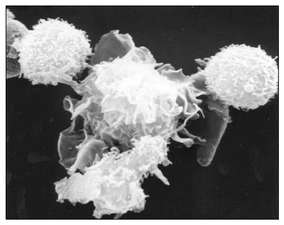
Cancer immunotherapy:-
Problem: Tumor cells are not very immunogenic.
Solution:
Dendritic cells -

- **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?



Dendritic cell interacting with lymphocytes
www1.imperial.ac.uk/medicine/research/researchthemes/inflamandimmun/immuno/northwick/

Methods

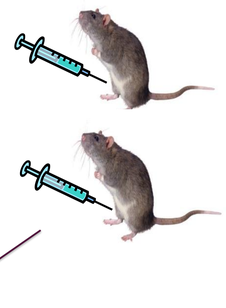
• Overnight cultures were set up using Act-A deficient Listeria strain DP-L4029 as vector.

The Listeria expressed 4 different peptides:

ΔAct A-Ub-mGARC$_{177-185}$ (AALLNKLY
ΔAct A-Ub-mTRP2$_{180-188}$ (SVYDFFVWL
ΔAct A-Ub-mEphA2$_{682-689}$ (VVSKYKPM } **Glioma associated peptides**

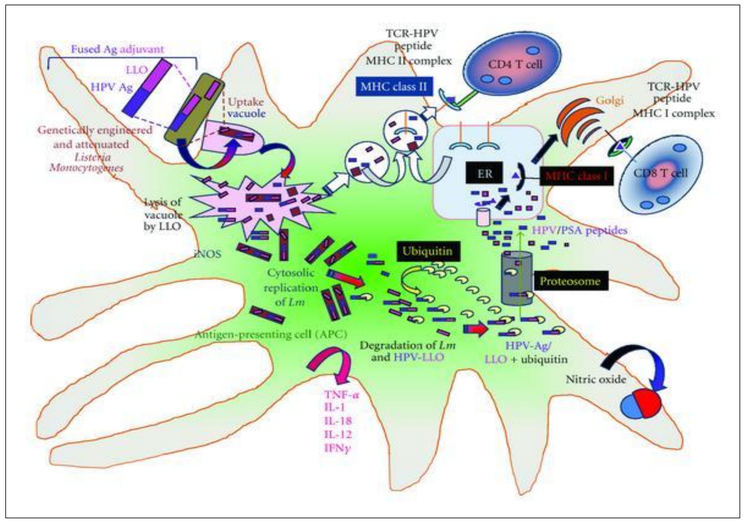
ΔAct A-Ub-D2 NS4a (YSQVNPTTL) } **Dengue peptide**

Each peptide expressing Listeria



• 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



Antigen Presentation pathways in cells
http://www.hindawi.com/journals/jo/2012/542851/fig1/

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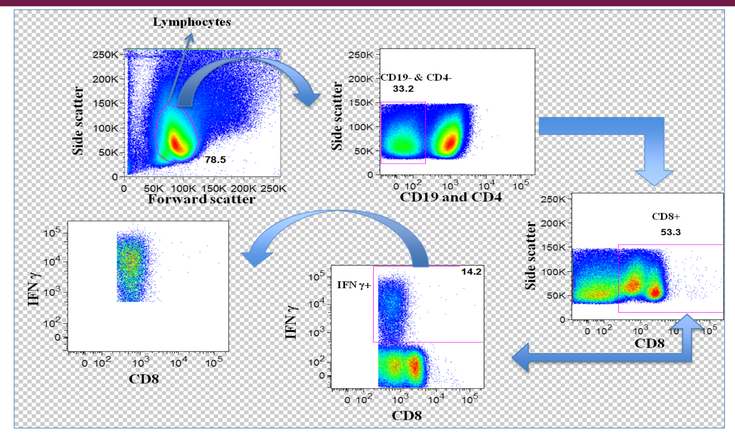
Results



Gating strategy used for C57BL/6 mouse 1 immunized with Listeria expressing dengue peptide D2 NS4a. 1st graph – live gating of lymphocytes; 2nd – gating of CD19- and CD4- lymphocytes; 3rd – gating of CD8+ from CD19- & CD4- cells; 4th – gating of IFNγ secreting CD8+ T cells

• The immunization with the dengue virus peptide had the greatest response (IFNγ secretion by T cells) equivalent to the entire CD8+ T cell response to Listeria. Two of the glioma peptides, mGARC and mTRP2, also gave measurable responses but the mEphA2 peptide did not.

Peptide		% IFNγ+ CD8+ T cells/ Total CD8+ T cells
Dengue virus peptide D2 NS4a	Mouse 1	**13.8 %**
	Mouse 2	**6.5 %**
Glioma peptide mEphA2$_{682-689}$	Mouse 1	0.12 %
	Mouse 2	-0.03 %
Glioma peptide mGARC$_{177-185}$	Mouse 1	0.37 %
	Mouse 2	0.16 %
Glioma peptide mTRP2$_{180-188}$	Mouse 1	0.15 %
	Mouse 2	0.18 %

Conclusion

•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 mEphA2 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 cause a measurable response.
• We could also provide a booster dose of stimulating peptide to further prime the immune response.