Polyarginine-Containing Peptide Nanofibers Promote Nucleotide-Adjuvant-Based DC Activation In Vitro

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Background

Sublingual (under the tongue) vaccines hold promise to eliminate the need for vaccine delivery via injections. Previously we showed that self-assembling peptide nanofibers enable effective sublingual immunization against specific peptide epitopes when conjugated with polyethylene glycol (PEG). These nanofibers combine the effects of the self-assembling Q11 peptide with the mucous penetrating PEG polymer for delivery.



Qingguo Xu, Laura M. Ensign, Nicholas J. Boylan, Arne Schön, Xiaoqun Gong, Jeh-Chang Yang, Nicholas W. Lamb, Shutian Cai, Tao Yu, Ernesto Freire, and Justin Hanes ACS Nano 2015 9 (9), 9217-9227

Motivation

 However, past work in the lab demonstrates for optimal performance, the addition of adjuvants such as cholera-toxin are necessary. STING (stimulator of IFN genes) pathway agonists such as cyclic dinucleotides (CDNs) have shown preclinical potential as mucosal adjuvants. Here, we demonstrate the in vitro potential of incorporating CDN adjuvants into a supramolecular nanofiber vaccine platform by leveraging their strong interactions with polyarginine sequences.



Co-delivery of GMP Adjuvant Promoted by Electrostatics and Hydrogen Bonding





Titrating R9 Content in Peptide Increases Nanofiber Zeta Potential



- Q11-assembly system allows for increased incorporation of R9, suggesting that nona-arginine can be dosed into the nanofiber platform. Incorporation of Nona-arginine into Cyclic-di-AMP or Cyclic-di-GMP Adjuvanted Nanofibers Leads to Dose-dependent Increase in Dendritic Cell Activation.



Depicts that DC 2.4 activation increases alongside the nonaarginine concentration with respect to CD80 and CD86 markers in the presence of (A/B) Cyclic – di-GMP and (C/D) Cyclic di-AMP. ****p*<.0.005 by linear regression of fold change in MFI and R9 content in nanofibers.

Nona-arginine Incorporation Specifically Improves Dendritic Cell Activation with Cyclic-di-Nucleotides



Cells were incubated for 24 hours and treated with 0.1 mM peptide solution and 20 µg of GMP per well. Dinucleotide adjuvants improved DC 2.4 activation with respect to the (A) CD80 and (B) CD86 markers. There was no significant affect of the presence of dinucleotide adjuvants on DC activation for the (C) MHCII marker. The presence of CpG did not affect DC activation in all markers.

Nona-arginine Promotes DC 2.4 Uptake and Presentation Independent of CDN Adjuvants



(A)The incorporation of R9 in the peptide platform increased tamra-labeled nanofiber uptake in DC 2.4 cells in short time intervals. (B) Treatment of 50% R9 content in conjugated peptides to DC cells significantly promoted presentation to DOBW cells overnight.

Concluding Points

- 1. Incorporation of nona-arginine into cyclic-di-AMP or cyclic-di-GMP adjuvanted nanofibers leads to dose-dependent increase in dendritic cell DC 2.4 activation.
- 2. Presentation of the pOVA epitope in MHC class II molecules to T cells is promoted by nona-arginine independent of CDN adjuvants.