Synthetic Self-Assembled Nanorod Vaccine Confers Protection Against Influenza A Virus

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Statement of Purpose: Proteinaceous nanoparticles have recently emerged as a promising strategy to develop safe and efficient subunit vaccines. The ability of synthetic β-sheet self-assembling peptides to stabilize B- and/or T-cell epitopes and to potentiate the epitope-specific immune responses have highlighted their potential as an immunostimulating platform for antigen delivery [1, 2]. Nonetheless, the intrinsic polymorphism of the resulting cross-β fibrils, their length in the microscale and their structural similarity with pathological amyloids limit their usage in vaccinology. Herein, we harnessed electrostatic capping motifs to control the self-assembly of a chimeric peptide comprising a β self-assembling sequence [3] and a highly conserved epitope derived from the influenza virus (M2e). Self-assembly led to the formation of 100 to 200 nm long uniform nanorods (NRs) displaying the M2e epitope on their surface.

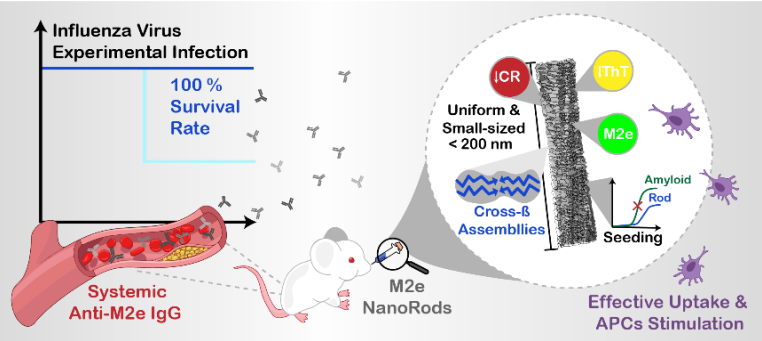

Figure 1. M2e-NRs present structural features differing from prototypical amyloids, resulting in an improved safety profile, and induce an enhanced immune response *via* efficient APC uptake and stimulation

Methods: A double-Lys (KK) positive capping unit was added at the N-terminus of a M2e-conjugated 10-mer peptide (I10; SNNFGAILSS), derived from the islet amyloid polypeptide (IAPP) to guide the morphology of cross-β assemblies into highly uniform M2e-functionalized nanorods (NRs). Peptides were synthesized on a Rink amide solid support using Fmoc chemistry and assembled into NRs by rotary agitation. Morphology of these assemblies was characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM) and dynamic light scattering (DLS). The supramolecular structure was determined by powder X-ray diffraction (PXRD), far-UV circular dichroism (CD) and binding by amyloid-specific fluorescent probes thioflavin T (ThT) and Congo Red (CR). Cytocompatibility of these assemblies was assessed using Live/Dead and Resazurin assays on macrophages (J774.A1) and dendritic-like cells (DC2.4). Uptake of FITC-labeled NRs by these antigen-presenting cells (APCs) was characterized by FACS and confocal microscopy, and DC activation was determined by MHC II and CD86 upregulation using FACS. To characterize the *in vivo* response, Balb/C mice were vaccinated intranasally at weeks 0, 2 and 4. Serum antibody titers at 2, 4 and 6 weeks were measured by enzyme-linked immunosorbent assay (ELISA). Mice were experimentally challenged at week 6 with 5 x LD50 of the H1N1 influenza strain A/PR8/1934 to evaluate survival and clinical signs

Results: Self-assembly led to the formation of 100 to 200 nm long uniform nanorods (NRs) displaying the M2e epitope on their surface. These cross-β assemblies differed from prototypical amyloids owing to low polydispersity, non-binding to ThT and CR dyes, and incapacity to seed homologous amyloid assembly. M2e-NRs were efficiently uptaken by APCs and the cross-β quaternary architecture activated the Toll-like receptor 2 (TLR2) and stimulated dendritic cells. Mice subcutaneous immunization revealed a robust M2e-specific IgG response, which was dependent on self-assembly into NRs and did not necessitate the addition of an adjuvant. Upon intranasal immunization in combination with montanide gel, M2e-NRs conferred complete protection with absence of clinical signs against a lethal infection with the H1N1 influenza A virus.

Conclusions: This study reveals that the electrostatic capping strategy to control the growth and morphology of cross-β-sheet fibrils can be implemented for the development of fully synthetic self-adjuvanted nanovaccines. These vaccines show an improved safety profile due to their non-amyloid properties and inability to seed amyloid assembly. These findings also indicate that, by acting as an immunostimulator of APCs and a delivery system, synthetic peptide-based NRs constitute a promising self-adjuvanted platform of subunit vaccines.

References:

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