Statement of Purpose: This study was aimed at testing the hypothesis that MHC class II molecules are suitable targets for ligand-facilitated nucleic acids entry into professional antigen-presenting cells.

Methods: Nanoparticles fabricated from poly (d, l-lactic-co-glycolic acid) (PLGA) or carboxylate polystyrene [1] with the cationic peptide O10H6 (O=ornithine, H=histidine) [2] were used as platforms for testing the targeting principle. Particles were anchored with VHA [3], a ligand binds to the murine class II molecule I-A^d expressed in BALB/c mice. The particles were loaded with 12 ng/ml of fluorescein isocyanate (FITC)-labeled oligodeoxynucleotides (ODN) encompassing nuclear factor-kappa B (NF-κB) binding sequence (5'-AGGGACTTTCCGCTGGGGACTTTCC-3').

Physical characteristics of the particles were determined based on size and zeta potential analyses, transmission electron microscopy, and infrared spectroscopy. Dendritic cells (DC) were generated from bone marrow progenitors of BALB/c (H-2^d) or C57BL6 (H-2^b) mice differentiated in the presence of 5 ng/ml of GM-CSF and IL-4 [4]. DC maturation and phenotypes are regulated by the NF-κB pathway [5]. DNA uptake was quantified and confirmed using flow cytometry and laser confocal microscopy. NF-κB blockade was evaluated based on expression of the costimulatory molecule CD86 in respond to 10 μg/ml of lipopolysaccharides (LPS) stimulation.

Results: VHA-anchored PLGA and polystyrene particles carrying O10H6-condensed ODN were found to be spherical in shape and nanometer in size (hydrodynamic diameter: 100-400 nm). VHA on the particles was evident by CH2 symmetric and asymmetric stretches in the infrared spectrum. MHC halotype-specific ODN uptake was demonstrated based on 2-fold (n =3, $p = 0.04$) higher fluorescent intensity detected in BALB/c (I-A^d+) than C57BL/6(I-A^b-) DC. The uptake was not a function of non-specific interaction between the particles and cell membrane for both PLGA and polystyrene particles possess negative zeta potential (-15 to -25 mV). Internalization of the DNA was confirmed in confocal imaging. Escape of the DNA from the lysosomal pathway was evident in Lysotracker-stained cells. LPS-dependent CD86-expression was suppressed ($n = 3$, $p < 0.05$) in BALB/c but not C57BL/6 DC with VHA-guided particles carrying NF-κB decoys.

Conclusions: These data demonstrate that a MHC class II ligand can function as the targeting moiety for nucleic acid delivery into DC. This strategy may be applied to attain allograft-selective immune modulation in organ transplant settings.

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