A materials-based approach for immunotherapeutics design: Mechanisms of thiol-associated complement activation of dendritic cells

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Statement of Purpose: Accumulating evidence suggests that complement, which is better known for its role in innate immunity, is also an important regulator of adaptive immunity. However, the molecular mechanisms by which complement differentially modulates immunity remain unclear. In complement's alternative pathway, tickover of complement component C3 leads to C3a release and generation of C3b with an exposed thioester bond. This thioester can then react with nearby nucleophiles such as terminal hydroxyl groups or thiols to form a covalent bond. In this way, bacterial surfaces in the blood become covered with C3b which triggers internalization of C3b-opsonized pathogens by phagocytic cells. Our group has recently reported that lymph node-targeting, complement-activating nanoparticles may be used as an effective vaccine platform via direct maturation and activation of dendritic cells[1]. Here we explore mechanisms of nanoparticle "synthetic pathogen" activation of C3 via free surface thiols and the subsequent immunological response induced to explore their potential use in material-based vaccines.

Methods: We used nanoparticles (NPs) composed of the polymer polypropylene sulfide (PPS) to form the hydrophobic core, surface stabilized with Pluronic (a block copolymer of polyethylene glycol and polypropylene glycol terminated by α and ω hydroxyl groups)[2]. The hydrophobic core is stabilized by disulfide crosslinking of the linear PPS chains but not all the PPS chains become crosslinked, leaving free sulfhydryl groups on the NP surface. These free surface thiols can be irreversibly blocked by covalent attachment of the alkylating sulfhydryl reagent, iodoacetamide. The high density of hydroxyl groups from Pluronic on the nanoparticle surface mimics the carbohydrate residues that typically coat bacterial cell walls, while the presence of free thiols from PPS chains mimics bacterial outer membrane proteins which possess free cysteines residues. Thus with our model synthetic pathogens we assessed the role of free thiols in the initiation and propagation of the complement cascade and subsequent immunological response relative to lipopolysaccharide (LPS), a potent activator of the immune (and complement) systems.

Results: OH-NP strongly induced complement activation as measured by C3a release after exposure to human serum (Figure 1A), similar to levels seen with a high concentration of LPS (0.22 mg/ml). However OH-SH-NP, NPs containing both hydroxyl and thiol nucleophiles, activated complement to significantly higher levels and it required an extremely high concentration of LPS (0.70 mg/ml) to reach similar complement activity (Figure 1A). Furthermore, OH-SH-NP matured dendritic cells, when pre-incubated with serum to levels similar to lipopolysaccharide (LPS) while no maturation was seen with exposure to phosphate buffered saline (PBS), serum alone, OH-SH-NP alone or OH-NP pre-incubated with serum (Figure 1B).

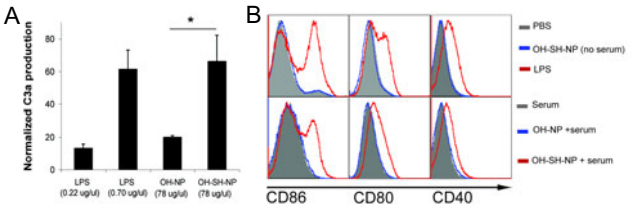

Figure 1. A, C3a release of OH-NP, OH-SH-NP and LPS following exposure to human serum as measured by ELISA. **B,** Flow cytometry histograms showing maturation profiles of murine bone marrow derived dendritic cells (BMDCs), as measured by expression of co-stimulatory molecules CD86, CD80, CD40, after a 24h incubation with controls, OH-SH-NP and OH-NP.

DCs express a significant repertoire of complement receptors on their surface which have been implicated in the LPS triggering of the CD14/TLR4-MD2 signaling complex[3-5]. Hence, we sought to determine if the DC maturation observed from thiol-associated complement activation was being mediated by a TLR4-dependent pathway. *In vivo* assessment of the activation mechanisms by OH-SH-NP revealed that maturation of lymph node DCs occurred in wildtype (WT) but not in TLR4-deficient (TLR4d) mice. OVA conjugated OH-SH-NPs also induced high levels of anti-OVA IgG titers in WT mice while no positive titers were observed in TLR4d mice. Thus it appears as though the antigen-specific adaptive immune responses induced by thiol-associated activation of complement is also TLR4 dependent, suggesting that it is likely a direct result of the lack of DC maturation.

Conclusions: We found that the presence of nucleophiles on the NP, in particular free surface thiols and/or hydroxyls, affects the quantity of complement activation after NP exposure to serum. In particular, SH-OH-NPs but not OH-NPs are able to induce *in vitro* maturation of BMDCs. Furthermore, we found that activation of lymph node DCs as well as subsequent DC-induced antigen-specific humoral immunity takes place via a TLR4-dependent mechanism. These findings motivate the further investigation of complement-activating biomaterials-based schemes for immunotherapeutic design.

References: [1]Reddy ST. Nat Biotech. 2007;25: 1159-64.
[2]Rehor A. Langmuir 2005; 21, 411-417
[3]Ingalls RR. J Exp Med 1995; 181, 1473-9
[4]Ingalls RR. 1998; J Immunol 161, 5413-20
[5]Triantafilou K 2001; Nat Immunol 2, 338-45