Enhancing the efficacy of innate immune agonists: could nanolipoprotein particles hold the key?

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Innate immune modulation for infectious disease

Immune modulation strategies have been applied extensively in cancer therapeutics for several decades with varied success. Applying similar strategies to prevent or treat infectious disease has transformative potential and remains poorly explored. Modulation of the innate immune system for infectious disease has the potential for truly broad-spectrum efficacy due to the highly conserved nature of the system and its co-evolution with pathogens over millennia [1], such that disparate pathogens utilize similar host innate immune pathways during the infectious process. Therefore, targeting of these pathways offers an attractive, alternative broad-spectrum approach for infectious disease therapeutics. Along these lines, several studies have elegantly demonstrated that prophylactic and postexposure methodologies involving innate immune stimulation prevent or ameliorate infections in animal models [2–9]. The concept of immune modulation as a strategy for infectious diseases relevant for public health and biosecurity has been reviewed over the last several years by a number of groups, which noted that a countermeasure strategy targeting the host and not the pathogen directly might greatly reduce the emergence of antibiotic resistance and may prove to be efficacious in special populations for which antibiotic and vaccination therapies are not optimal [10,11].

Our knowledge of how innate immune responses are initiated, driven and maintained during infection, coupled with our

knowledge of those key innate immune cells, receptors and signaling pathways involved, has opened the possibility for the rational design of targeted host-based therapeutics. Toll-like receptors (TLRs), a key class of pattern recognition receptors, are integral in initiating the immune response to infection [1,12]. Furthermore, robust TLR activation is a key driver in sustaining an adaptive immune response, as well as eventual pathogen clearance [1,12]. When coupled with their well-documented association with myriad infectious diseases, the importance of TLRs as therapeutic targets is widely accepted. While TLR agonists have been extensively explored as vaccine adjuvants, their use in TLR monotherapy to induce innate immune activation has been less thoroughly explored to enhance host resistance to infection by a variety of pathogens [2–9] and represents a potential paradigm shift in our approach to combat infectious diseases.

Drawbacks of current approaches

To date, the clinical potential of TLR agonists has not been fully realized despite these compounds being rigorously evaluated in numerous clinical trials for a variety of indications. While more progress has been made toward the clinical translation of TLR agonists as vaccine adjuvants rather than as standalone therapeutics, significant limitations exist for both applications [12,13]. With respect to TLR monotherapy, significant obstacles for the widespread application of this approach need to be addressed in order to facilitate clinical translatability. First, studies

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using TLR agonists to prevent infection demonstrate only marginal and short-lived protection, severely limiting their utility in both pre- and post-exposure scenarios. Second, high doses are often required to afford protection *in vivo* raising substantial toxicity concerns. Finally, TLR agonists tend to exhibit poor biodistribution and pharmacokinetic profiles and poor cellular uptake *in vivo*, thereby limiting their bioavailability and efficacy [12,13]. Hence, innovative strategies to improve the therapeutic index of TLR agonists *in vivo* are urgently needed in order to fully realize and maximize their potential for infectious disease treatment and/or prevention for both adjuvants and monotherapy applications.

Nanoparticle delivery of innate immune agonists

Nanoparticle platforms have recently been developed as tools to more efficiently deliver a variety of therapeutics, including drugs, vaccines and imaging agents [14]. It has been widely demonstrated that delivery of therapeutics via a nanoparticle platform can overcome many of the limitations of conventional administration, including nonspecific biodistribution and targeting, poor aqueous solubility, limited oral bioavailability and low therapeutic indices [15]. Based on these previous findings, we believe that successful treatment of infectious diseases through immune modulation strategies targeting TLRs may benefit significantly from the use of nanoparticle platforms for the delivery of TLR agonists.

One particular platform that is well suited for such an application is nanolipoprotein particles (NLPs; also known as reconstituted high density lipoproteins and nanodiscs). These biomimetic nanoparticles, which are comprised of apolipoproteins and lipids, have been successfully used to address both fundamental science, as well as clinically driven applications. NLPs assume a loosely discoidal morphology, nominally featuring a lipid bilayer stabilizing the apolipoprotein. While these types of nanoparticles are primarily used for solubilizing and stabilizing membrane proteins in discrete, native lipid environments for biophysical and structural characterization [16], their utility for *in vivo* applications has not been neglected. Of particular note are the uses of these biomimetic nanoparticles in the delivery of therapeutic drugs, enhancing a drug's therapeutic index, diagnostic imaging and vaccine applications [17–20].

We believe this platform, in particular, is advantageous for the delivery of immune agonists because there are several features intrinsic to the NLP that are of importance to any *in vivo* delivery platform. First, since these particles are naturally present in the human body,

the NLP platform is less likely to result in issues facing other nanoparticle systems that are currently used for the delivery of therapeutics, such as unwanted off-target effects, stability in complex biological fluids and toxicity. Second, the fundamental versatility in NLP self-assembly accommodates a plethora of starting reagents (i.e., apolipoproteins and lipidic molecules), whereby the NLP size can be tailored by both pairing of the constituents, as well as their molar ratios. Third, NLP-like particles provide multiple means of cargo conjugation or incorporation, spanning a large range of cargo molecules of disparate physicochemical characteristics. This last point cannot be overemphasized, as hydrophobic moieties can be contained within the hydrophobic core of the NLP lipid bilayer, amphipathic moieties can be anchored to the lipid bilayer, and bioconjugation of soluble molecules can be achieved by incorporating functionalized lipids bearing reactive polar headgroups. As such, the transformative potential of this class of nanoparticle as a universal platform for delivering and enhancing innate immune agonists is appealing.

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As a proof of concept, we recently examined the effect of TLR agonist delivery via the NLP platform relative to administration of the TLR agonist alone [2]. We demonstrated that conjugation of two disparate (in terms of physical and chemical characteristics) TLR agonists, MPLA and CpG, was readily achieved. Subsequent *in vitro* and *in vivo* assessment of immunostimulatory properties clearly demonstrated that conjugation of TLR agonists to NLPs improved cellular uptake and activation of key innate immune effector cells relative to administration of the TLR agonist alone. In addition, when administered to mice prior to a lethal influenza virus challenge, complete protection from weight loss-induced death was observed only when the TLR agonist was conjugated to the NLP. Similar activation profiles were also observed in primary human innate immune cells, a positive indicator for the translatability of TLR agonist monotherapy to humans. Taken together, these combined findings provide strong evidence for the potential of utilizing nanoparticle technologies in combination with TLR agonists for the treatment of infectious diseases.

Future prospects

In a natural infection, many distinct innate immune receptors contribute in concert to initiate the innate

immune response, and current data indicate that stimulation of multiple TLRs leads to synergistic cytokine production *in vivo*. This variety in signaling might suggest that multiagonist formulations activating a cluster of different TLRs, may lead to highly effective protection by more closely resembling the innate immune responses observed during natural infections. The versatility in accommodating chemically distinct and structurally diverse molecules during NLP production allows for the co-localization of multiple, different TLR agonists on a single NLP, thereby allowing rapid testing of multiple TLR agonist combinations. This multiagonist approach will likely ensure robust stimulation of key innate immune cells and may also facilitate the investigation of other pattern recognition receptors demonstrated to be important in a variety of diseases.

“Combining enhanced TLR therapy with traditional therapeutic modalities, such as antibiotics, is also a very promising consideration. Such a strategy may be especially useful in limiting the duration of antibiotic therapies...”

Identification and validation of enhanced innate immune modulation using NLP-based formulations would necessarily be followed by numerous important studies. Rigorous efforts to refine and optimize such formulations to minimize the potential for overstimulation and deleterious side effects is paramount, as such adverse effects would be difficult to predict *a priori*. Evaluating their efficacy in multiple disease models is also extremely important, given the documented dif-

ferences in sensitivities that exist between the immune response to TLR agonists among different species. Evaluation of dose and route of administration, as well as dosing regimen, are important parameters that need to be assessed for each infectious disease model. Combining enhanced TLR therapy with traditional therapeutic modalities, such as antibiotics, is also a very promising consideration. Such a strategy may be especially useful in limiting the duration of antibiotic therapies, which for many infectious agents relevant for both public health and biosecurity, is extremely prolonged. The prospect of a broad-spectrum therapy for infectious disease using innate immune modulation is extremely attractive, and platform strategies such as the NLP may hold the key by both enhancing and tailoring the innate immune response using available agonists to realize this goal.

Financial & competing interests disclosure

Two patents were filed on utilizing the nanolipoprotein particle platform for vaccine applications: ‘Nanolipoprotein particles and related compositions, methods and systems’ (application number: 12469533) and ‘Immunostimulatory nanoparticles and related compositions, methods and systems’ (application number: 12604362). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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