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Multimodal nanoparticles that provide immunomodulation and intracellular drug delivery for infectious diseases

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Abstract

Infectious diseases are a worldwide health concern. For some infections, a common feature is the intracellular residence of the pathogen and evasion of the host immune response. In the case of tuberculosis (TB), *Mycobacterium tuberculosis* evades clearance within macrophages through suppression of intracellular reactive oxygen and nitrogen species (ROS/RNS) and pro-inflammatory cytokines. We propose new nanoparticle designs for infectious diseases, functionalized with ligands able to modulate the cellular immune response and concurrently deliver drug. We have designed 1,3-β-glucan functionalized chitosan shell, poly(lactide)co-glycolide core nanoparticles to stimulate ROS/RNS, pro-inflammatory cytokine secretion, and delivery of rifampicin inside human alveolar like macrophages (ALM). Nanoparticles significantly enhanced ALM secretion of IL-12p70 (2.9-fold), TNF-α (16-fold) and INF-γ (23-fold) compared to controls over 24 h, and doubled ROS/RNS generation over 6 h. Nanoparticles could deliver 4-fold greater rifampicin into ALM compared to rifampicin solution. These results provide proof-of-concept of multimodal nanoparticles and support their further development.

From the Clinical Editor: In this paper, a new nanoparticle design is proposed to address hard to treat infectious diseases such as TB, through the use of nanoparticles functionalized with ligands that are able to concurrently modulate the cellular immune response and deliver a drug. The authors have designed 1,3-β-glucan functionalized chitosan shell - poly(lactide)co-glycolide core nanoparticles to stimulate reactive oxygen and nitrogen species production, pro-inflammatory cytokine secretion, and delivery of rifampicin inside human alveolar-like macrophages.

Key words: Infectious diseases; Multimodal nanoparticles; 1,3-β-glucan; *Mycobacterium tuberculosis*; PLGA

Infectious diseases are responsible for considerable mortality globally, with developing countries disproportionately bearing the burden of these diseases. Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), Tuberculosis (TB) and malaria rank among the most deadly of infectious diseases. The World Health Organization reported over 1.7 million deaths from HIV in 2011, 1.4 million deaths from TB in 2012 and 660,000 deaths from malaria in 2010.[1–3] In the absence of effective vaccines against these diseases, drug therapy remains the only treatment option. The increasing prevalence of drug resistant pathogenic strains, including multi-drug resistant TB, and growing HIV and malaria resistance has become a global concern. The development of new therapies based on nanomedicines, to reduce drug doses and dose frequencies and to shorten treatment duration, with the goal of

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increasing patient compliance, improving treatment outcomes and reducing occurrence of drug resistance, is a major priority for these diseases.[4,5] One feature of some pathogens in infectious diseases is their residence in the intracellular space, where they are able to evade the immune system, persist and multiply to infect other cells.[4,6] In the case of HIV, TB and malaria, the pathogens are present in CD4+ T cells, macrophages and red blood cells, respectively, at some stage of the infection.[6] The pathogens have developed complex mechanisms to evade clearance by the host. For example, *Mycobacterium tuberculosis* (*M.tb*), the causative pathogen of TB, is able to persist within macrophages by suppressing the antimicrobial response of the host macrophage, through mechanisms which include suppression of intracellular generation of bactericidal reactive oxygen and nitrogen species (ROS/RNS), and secretion of pro-inflammatory cytokines such as Interleukin-12 (IL-12) and Interferon-gamma (IFN-γ).[7–9] Such pro-inflammatory cytokines are considered key components in intracellular eradication of mycobacterium through (i) the promotion of fusion of phagosomes which contain the bacterium to form the bactericidal lysosomes, (ii) the induction of intracellular ROS/RNS generation, and (iii) the priming of T lymphocytes.[9,10] With such knowledge of intracellular events which pathogens exploit for their survival, nanomedicines could be designed to impact the immunological aspects of the diseases in addition to delivering drug pay-load.[4] We view this as a new generation of "intelligent" nanomedicines for infectious diseases, functionalized through carefully selected pharmacologically active ligands carried on the surface of nanoparticles, to modulate immunology at the cellular level, and concurrently deliver therapeutic drug concentrations inside the cell. This represents a multimodal approach to eradication of pathogens as the induced pharmacological effect could act synergistically with the action of the drugs to eradicate the pathogen. Multifunctional nanoparticles are expected to play an important role in the future of nanomedicine.[4,11] In this report, we explore this concept, by designing 1,3-β-glucan functionalized chitosan (CS) shell, poly(lactide)co-glycolide (PLGA) core nanoparticles loaded with the anti-tubercular drug Rifampicin (RIF), and evaluate their impact on ROS/RNS generation and cytokine production, and intracellular concentrations of RIF in human alveolar like macrophages (ALM). The functionalizing ligand we have selected, i.e. 1,3-β-glucan is carried on the nanoparticle surface and can interact with Dectin-1 receptors on the surface of macrophages to induce various downstream signal transduction pathways which promote intracellular ROS/RNS generation as well as pro-inflammatory cytokine production.[12,13] Pro-inflammatory cytokines produced through Dectin-1 activation include IL-12.[12] Dectin-1 activation also enhances phagocytosis,[12] providing an opportunity for enhanced particle uptake and intracellular delivery of drugs.

In the current study, we demonstrate that these functionalized nanoparticles are able to stimulate enhanced ROS/RNS and pro-inflammatory cytokines in human macrophages, and concurrently increase intracellular concentrations of the anti-mycobacterial drug RIF. These nanoparticles therefore have the potential for eradication of intracellular pathogens including *M.tb* and HIV, which will be investigated in future studies.

Methods

Isolation of ALM: Blood donors were recruited at the University at Buffalo; consents were obtained consistent with the policies of the University at Buffalo Health Sciences Institutional Review Board and the National Institutes of Health. Peripheral blood samples from three healthy individuals were drawn into a syringe containing heparin (20 units/ml, Sigma-Aldrich, MO). Peripheral blood mononuclear cells (PBMC's) were separated by Ficoll-Paque (GE Health Care, Piscataway, NJ) gradient centrifugation. Monocytes were isolated from PBMC's by negative selection using Dynabeads® Untouched™ Human Monocytes (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Monocytes were cultured in complete medium [RPMI 1640, 10% fetal calf serum, 5% human AB serum, 10 mM/L HEPES, 1% Penicillin-Streptomycin, 20 ng/ml granulocyte-macrophage colony stimulating factor (Millipore)] for 10 days, for differentiation into ALM. To purify macrophages from monocytes, 5×10^5 cells/mL adhered to 6-well tissue culture plates for 2 h, non-adherent cells were then removed by washing. Purity was analyzed by CD11b FACS analysis[14] (Data not shown).

Immunofluorescence immunostaining for Dectin-1 on ALM

ALM (1×10^5cells/ml) cultured on Lab-Tek chambered coverglasses (Nalgene Nunc International, Rochester, NY) were fixed and permeabilized in cold 70% methanol for 30 min at 4 °C. Cells were washed in phosphate-buffered saline (PBS), incubated with Image-iT™ FX signal enhancer (Invitrogen) for 30 min at room temperature in a humid environment. Cells were washed in PBS then incubated with primary antibody (polyclonal goat anti-dectin-1, sc-26094) overnight at 4 °C. Cells were washed with PBS and incubated for 1 h at room temperature with a secondary antibody conjugated to ALEXA Fluor 647 (Alexa Fluor® 647 Donkey Anti-Goat IgG, Invitrogen). Cells were then counterstained with the nuclear stain 4′, 6-diamidino-2-phenylindole, dilactate (DAPI, Molecular Probes, Invitrogen) and imaged by confocal microscopy described below.

Functionalized nanoparticle synthesis and characterization

Glu-CS-PLGA loaded with RIF were synthesized using a modified emulsion-solvent evaporation technique.[15] Briefly a 20 mg/ml PLGA (lactide:glycolide: 50:50; Mw 30,000-60,000 Da) solution in ethyl acetate containing 2 mg/ml RIF was added drop-wise to a 10 mg/ml polyvinyl alcohol (Mowiol® 4–88) (Mw 31,000 Da) solution in de-ionised water, containing 3 mg/ml chitosan oligosaccharide lactate (CS); Mn 5, 000 Da), under stirring at 1000 rpm for 1 h, at room temperature. Ethyl acetate was subsequently removed by evaporation at 40 °C for 1 h and nanoparticles were collected through centrifugation at 10 000 × *g* for 5 min, followed by washing in de-ionized water. Nanoparticles (0.1% (v/v) in de-ionized water) were then incubated with a 0.025 μg/ml 1,3-β-glucan (1,3-β-glucan from *Euglena gracilis*) solution in water for 10 min, before washing to remove unattached 1,3-β-glucan. Previously the 1,3-β-glucan solution was subjected to ultrasonication (Qsonica, Newtown, CT) (to reduce the polymer chain length) for 30 min at an