Toll-like receptor 8 agonist nanoparticles mimic immunomodulating effects of the live BCG vaccine and enhance neonatal innate and adaptive immune responses

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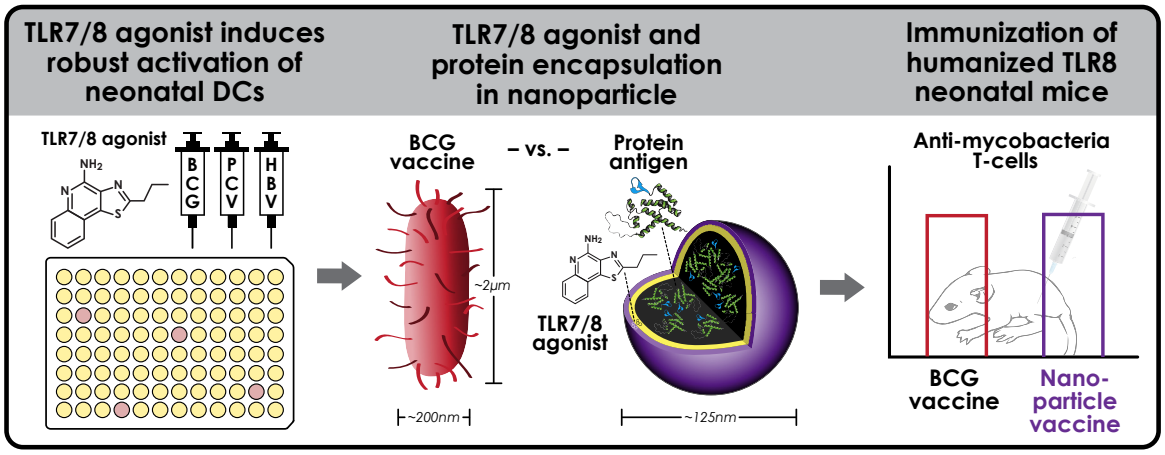
GRAPHICAL ABSTRACT



Background: Newborns display distinct immune responses, leaving them vulnerable to infections and impairing immunization. Targeting newborn dendritic cells (DCs), which integrate vaccine signals into adaptive immune responses, might enable development of age-specific vaccine formulations to overcome suboptimal immunization.

Objective: Small-molecule imidazoquinoline Toll-like receptor (TLR) 8 agonists robustly activate newborn DCs but can result in reactogenicity when delivered in soluble form. We used rational engineering and age- and species-specific modeling to construct and characterize polymer nanocarriers encapsulating a TLR8 agonist, allowing direct intracellular release after selective uptake by DCs.

Methods: Chemically similar but morphologically distinct nanocarriers comprised of amphiphilic block copolymers were engineered for targeted uptake by murine DCs *in vivo*, and a range of TLR8 agonist–encapsulating polymersome formulations were then synthesized. Novel 96-well *in vitro* assays using neonatal human monocyte-derived DCs and humanized TLR8 mouse bone marrow–derived DCs enabled benchmarking of the TLR8 agonist–encapsulating polymersome formulations against conventional adjuvants and licensed vaccines, including live attenuated BCG vaccine. Immunogenicity of the TLR8 agonist adjuvanted antigen 85B (Ag85B)/peptide 25–loaded BCG-mimicking nanoparticle formulation was evaluated *in vivo* by using humanized TLR8 neonatal mice.

Results: Although alum-adjuvanted vaccines induced modest costimulatory molecule expression, limited T_H-polarizing cytokine production, and significant cell death, BCG induced a robust adult-like maturation profile of neonatal DCs. Remarkably, TLR8 agonist polymersomes induced not only newborn DC maturation profiles similar to those induced by BCG but also stronger IL-12p70 production. On subcutaneous injection to neonatal mice, the TLR8 agonist–adjuvanted Ag85B peptide 25 formulation was comparable with BCG in inducing Ag85B-specific $CD4^+$ T-cell numbers.

Conclusion: TLR8 agonist–encapsulating polymersomes hold substantial potential for early-life immunization against

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intracellular pathogens. Overall, our study represents a novel approach for rational design of early-life vaccines. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Newborn, dendritic cells, Toll-like receptor 8, polymersome, nanoparticle, BCG, vaccine*

Human newborns and infants have a high frequency of infection compared with older children and adults,[1] in part because of distinct early-life immunity with impaired host defense against intracellular pathogens.[2] On challenge with many stimuli, including bacterial components, children less than 2 months of age express a strong innate T_H2 and T_H17 cell polarization and impaired T_H1 cell and innate antiviral type 1 interferon responses.[3] Relatively low innate T_H1 cell support at birth appears to gradually increase over the first years of life.[2] Distinct newborn T_H17- and infant T_H2-polarized responses potentially limit the efficacy of early-life immune response against certain pathogens[4,5] and vaccines.[6,7] Because birth is the most reliable point of health care contact worldwide, neonatal vaccines, such as the live attenuated BCG vaccine, achieve high global population penetration. Therefore developing early-life immunization strategies, including those directed at tuberculosis, might be highly advantageous.[8]

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play a vital role in shaping adaptive immunity. DC maturation begins when endogenous or exogenous danger molecules are recognized by pattern recognition receptors (eg, Toll-like receptors [TLRs]), triggering upregulation of costimulatory molecules and production of immune-polarizing cytokines.[9] Of note, human newborn DCs demonstrate impaired T_H1 responses and particularly low production of TNF and IL-12p70, which are important for vaccine-induced protection against intracellular pathogens.[2] Accordingly, development of novel adjuvanted vaccine formulations that enhance the maturation and functionality of human neonatal DCs might enable a new generation of early-life vaccines.[10,11] Unlike agonists of most TLRs that elicit reduced T_H1 cytokine production by newborn leukocytes, agonists of the endosomal TLR8,[12] such as the synthetic imidazoquinoline (IMQ) CL075 (TLR8/7 agonist), induce robust T_H1-polarizing responses from both neonatal and adult DCs.[13,14]

Abbreviations used

Ag85B:	Antigen 85B
APC:	Antigen-presenting cell
BMDC:	Bone marrow–derived dendritic cell
DC:	Dendritic cell
FITC:	Fluorescein isothiocyanate
HBV:	Hepatitis B vaccine
IMQ:	Imidazoquinoline
LDH:	Lactate dehydrogenase
MHCII:	MHC class II
MoDC:	Monocyte-derived dendritic cell
PCV:	Pneumococcal conjugate vaccine
pDC:	Plasmacytoid dendritic cell
PE:	Phycoerythrin
PEG-*bl*-PPS:	Poly(ethylene glycol)-bl-poly(propylene sulfide)
PGE_2:	Prostaglandin E_2
p25:	Peptide 25
Tet^+:	Tetramer positive
TLR:	Toll-like receptor
WT:	Wild-type

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