Synthetic Small Molecule Nucleic acid Hybrid compounds stimulate TLR-7 in Macrophages and Dendritic cells infected with BCG vaccine and enhance efficacy of the BCG vaccine through an Adjuvant action against Tuberculosis

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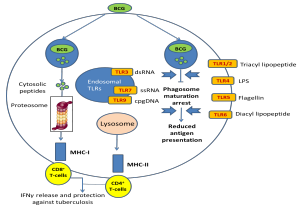
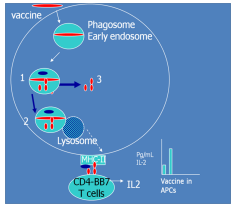
Summary

BACKGROUND: BCG vaccine, which is widely used against human tuberculosis, and the vaccine, as well as, the pathogen Mycobacterium tuberculosis (Mtb) infect macrophages and avoid lysosomal fusion. Both BCG and Mtb sequester in immature phagosomes thereby helping Mtb survival and immune evasion. TLR activation during mycobacterial infection may enhance innate responses although certain mycobacterial lipids are known to suppress immune responses of APCs including MHC-II expression. Although TLR7 has an endosomal localization, we found that BCG vaccine did not co-localize with fluorescent ligands of TLR7 within macrophages suggesting decreased TLR7 activation. We therefore evaluated TLR7 activators to modulate immune responses to BCG vaccine. METHODS & RESULTS: Adjuvant action on APC function: To determine whether TLR7 activation could enhance efficacy of BCG vaccine, APCs were incubated with several TLR7-activating synthetic small nucleic acid hybrid analogs (SMNHs), followed by BCG addition. The cells were overlaid with a T cell hybridoma specific for the immunodominant antigen-85B, which secreted IL2 upon recognition of an epitope from Ag85B from BCG infected APCs. SMNH activation of APCs markedly enhanced in vitro antigen presentation. Of the 14 synthetic analogs of SMNHs analyzed, four analogs had marked adjuvant activity. All four SMNHs also enhanced the levels of Th1 cytokines (IL12, IL1β and TNFα) from BCG-treated APCs, suggesting adjuvant activity. Adjuvant efficacy of SMNH analog in mice: One lead candidate SMNH adjuvant (SB 9922) was then combined with BCG vaccine and used for immunization of mice which were then challenged with virulent Mtb. Four weeks after challenge, lung and spleen counts of Mtb were significantly ($p < 0.009$ vs. BCG) reduced by SMNH-BCG combination, compared to BCG vaccinated mice and naive but challenged mice. SMNH-BCG also induced a stronger expansion of antigen-specific CD8 T cells, and enhanced the levels of both effector and memory precursor CD8 T cells correlating with protection. Thus, SMNH mediated TLR7-activation enhanced innate responses of BCG infected APCs, markedly amplified adaptive immune responses against tuberculosis in mice, culminating in increased vaccine-induced memory. SMNHs activating TLR7 are therefore novel adjuvants for BCG vaccine. *Supported by AI78420, NIAID, NIH.*

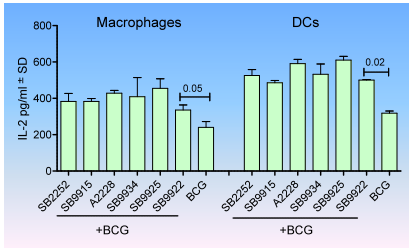
Methods



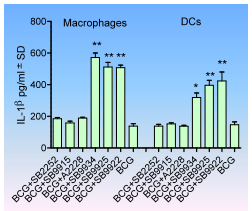


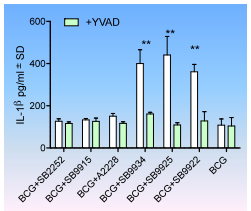
MHC class II presentation begins with the phagocytosis of a pathogen followed by the step wise maturation of the 'phagosome' into a phagolysosome. Lysosomal degradation produces peptides that are loaded onto MHC-II in endosomal compartments for export to plasma membrane and subsequent presentation to CD4+ T cells. Since phagosome maturation arrest is observed in both Mtb and BCG, we tested the MHC-II-dependent peptide presentation in the presence or absence of TLR-7-activating SMNH adjuvants (Singh et al, J. Immunol, 2006). We then tested the ability of SMNH compounds to induce Th1 cytokines and boost vaccine activity in mice.

SMNHs that activate TLR-7 enhance antigen presentation, & Th1 function in macrophages and DCs

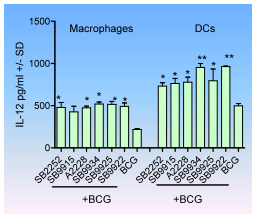


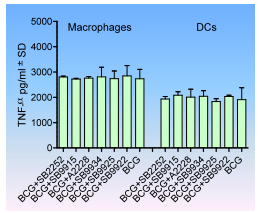
Macrophages and DCs (referred to as APCs) were treated with Small Molecule Nucleic acid Hybrids (SMNHs) which activate TLR-7 and infected with BCG vaccine. Four hrs later, they were washed and overlaid with BB7 CD4 T cells specific for Ag85B. Several SMNHs were found to enhance antigen presentation in APCs. [P values determined by t test].





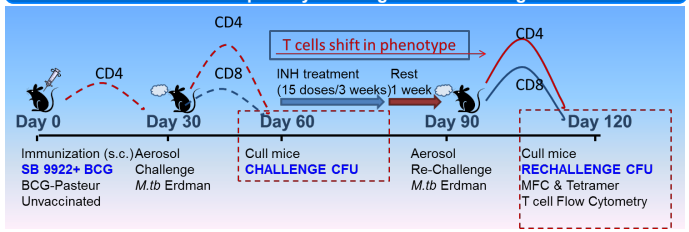
SMNHs that activate TLR-7 enhance IL-1β in BCG infected macrophages. Macrophages were treated with SMNH compounds and infected with BCG. Two analogs of SB 9922 enhanced IL-1β which was inhibited by prior incubation with the Caspase-inhibitor Y-VAD.

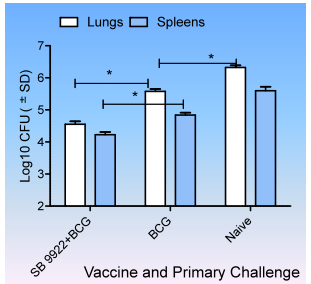




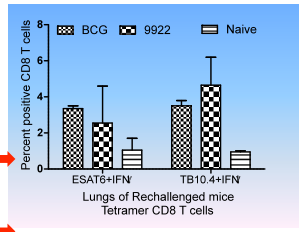
SMNH analogs related to SB 9922 enhance IL-12 in BCG infected macrophages. SB 9922 and related analogs enhanced BCG-induced Th1 cytokine IL-12 in APCs maintaining high levels of TNFα. Based on above activity data SB 9922 was selected as a lead adjuvant for further evaluation in animal models.

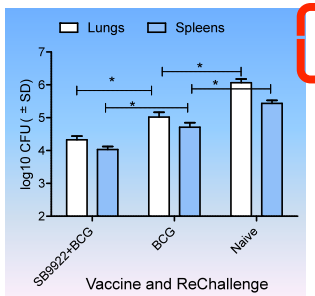
SB 9922 enhances efficacy of BCG vaccine against tuberculosis in mice both after primary challenge and rechallenge

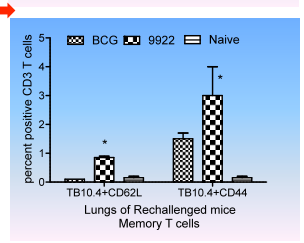




C57Bl/6 mice were immunized with BCG alone or BCG with SB 9922 (25 µg/dose) given once s.c to mice, which were then challenged with virulent MTB Erdaman as shown. Organ CFUs of MTB were analyzed on time points and expressed as log10 per organ (SD) (5 mice per group) (p value using 2-way ANOVA * < 0.009. SB 9922 enhances BCG efficacy.







To determine increased protection against rechallenge, T cells of the lungs were analyzed for antigen specificity and immune memory using flow cytometry. Data show that SB 9922 in combination with BCG increases the proportion of memory T cells that express CD62L and CD44 compared to BCG vaccine alone with a marginal increase in CD8 T cells positive for tetramer TB10.4. **Conclusion**: These data suggest that SB 9922 is an efficient adjuvant, which can increase immune memory to BCG vaccine.