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Evaluation of novel synthetic TLR7/8 agonists as vaccine adjuvants

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ABSTRACT

Small-molecule adjuvants that boost and direct adaptive immunity provide a powerful means to increase the effectiveness of vaccines. Through rational design several novel imidazoquinoline and oxoadenine TLR7/8 agonists, each with unique molecular modifications, were synthesized and assessed for their ability to augment adaptive immunity. All agonists bound human TLR7 and TLR8 and induced maturation of both human mDCs and pDCs. All agonists prompted production of type I interferon and/or proinflammatory cytokines, albeit with varying potencies. In most *in vitro* assays, the oxoadenine class of agonists proved more potent than the imidazoquinolines. Therefore, an optimized oxoadenine TLR7/8 agonist that demonstrated maximal activity in the *in vitro* assays was further assessed in a vaccine study with the CRM197 antigen in a porcine model. Antigen-specific antibody production was greatly enhanced in a dose dependent manner, with antibody titers increased 800-fold compared to titers from pigs vaccinated with the non-adjuvanted vaccine. Moreover, pigs vaccinated with antigen containing the highest dose of adjuvant promoted a 13-fold increase in the percentage of antigen-specific $CD3^+/CD8^+$ T cells over pigs vaccinated with antigen alone. Together this work demonstrates the promise of these novel TLR7/8 agonists as effective human vaccine adjuvants.

1. Introduction

Vaccines are remarkably successful at preventing diseases. However, development efforts for many disease targets have failed to yield effective vaccines. One limitation of development has been the inability of conventional vaccines to stimulate an effective antigen-specific $CD8^+$ T cell response, which is critical for protection against many viral diseases and cancers [1]. Since replicating viruses induce cytotoxic T cells (CTL), attenuated viruses are being developed as vaccines or vectors to deliver recombinant antigens. However, the fear that an attenuated virus vaccine will revert to its pathogenic state, as has occurred with the Sabin polio vaccine [2], remains concerning. Another potentially safer approach is to utilize recombinant antigens formulated with vaccine adjuvants. Approved adjuvants already widely used in the US and/or Europe include alum, oil in water emulsions and monophosphoryl lipid A (MPL) [3]. These adjuvants are safe and enhance immune responses to antigens [4,5] however, they are not effective at promoting $CD8^+$ T cells. Adjuvants that target the same pathogen-associated molecular pattern (PAMP) receptors engaged by viruses may induce the signaling pathways necessary to induce a $CD8^+$ T cell response. An important set of these receptors are the Toll-like family of receptors.

The transmembrane Toll-like receptors (TLR) have binding domains specific for different microbial and viral components [6]. TLR7/8 recognize single-stranded viral RNA [7,8]. Upon ligation of the receptor-ligand pair, intracellular signaling is initiated and downstream transcription factors activate genes of proinflammatory cytokines (TNF, IL-1), type I interferons (IFNα) and co-stimulatory molecules (CD80, CD86) [9–11]. The activation of

Abbreviations: CTL, cytotoxic $CD8^+$ T cell; DC, dendritic cell; EC50, half-maximal effective concentration; IM, intramuscular; IP-10, interferon γ-induced protein 10; IRF, interferon regulator factor; mDC, myeloid dendritic cell; MPL, monophosphoryl lipid A; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; TIR, toll/interleukin-1 domain; TLR, Toll-like receptor; HBV, hepatitis B virus; HCV, hepatitis C virus; APC, antigen presenting cell.

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these genes promotes maturation of dendritic cells (DCs), facilitating the presentation of antigen and stimulation of the ensuing adaptive immune response. The exact immune response generated depends upon the precise stimuli and resultant transcriptional and cellular changes. Cell-mediated immunity executed by $CD8^+$ T lymphocytes is enabled through accessory T_h1 cells and the cytokines IFNα, IL-2 and IL-12 [12]. However, if innate, proinflammatory cytokines such as IL-1 and TNFα are produced in excess both local and systemic inflammation results. Accordingly, to create a safe adjuvant, that augments the immune response to a given antigen, the proper combination of these signals must be generated.

Several TLR7/8 agonists, including R848 and R837, have been investigated as potential vaccine adjuvants. Some of these agonists have found success as topically administered agents, but demonstrate dose limiting toxicity when given orally or intravenously before ever reaching efficacious concentrations. This profile renders the current TLR7/8 agonists ineffective as systemic vaccine adjuvants [13,14]. Therefore in this study the immune-stimulating ability of a set of novel, structurally distinct TLR7/8 agonists (Figs. 1 and 5, [15]) was assessed, initially, *in vitro*. These agents were designed to be more potent than R848 or R837 (data not shown) and induce lower inflammatory cytokine levels, thereby overcoming their issues as systemic adjuvants. The most potent oxoadenine compound was evaluated *in vivo* and demonstrated strong enhancement of humoral and cell-mediated immune responses to the CRM197 antigen. Collectively, this data establishes the utility of these novel TLR7/8 agonistic compounds to enhance immune response to a cognate vaccine antigen.

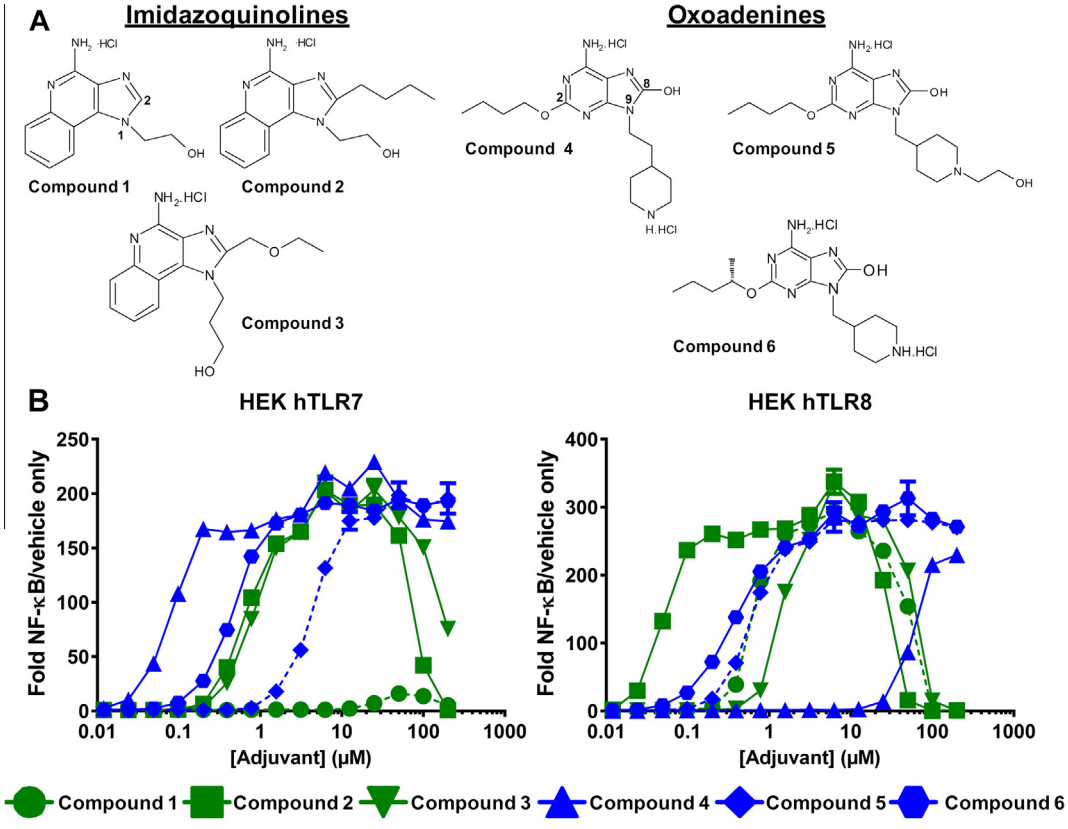
2. Materials and methods

2.1. TLR agonists and adjuvants

Compounds were synthesized following established procedures [15,16] and formulated in 2% glycerol in water. AS01 is a GSK proprietary Adjuvant System comprising liposomes, MPL and QS-21 (a triterpene glycoside purified from *Quillaja saponaria*, *fraction 21*; licensed by GSK from Antigenics Inc) [17].

2.2. HEK293 assay

HEK293 cells expressing human TLR7 or TLR8 with an NF-κB-responsive SEAP reporter gene were obtained from Invivogen (San Diego, CA). Cells were maintained in DMEM with 10% HI-FBS and selection antibiotics. Cells were plated at 5×10^5 cells/96-well and stimulated for 24 h. Supernatants were harvested



Cmpd	hTLR7 EC50 (μM)	hTLR8 EC50 (μM)	TLR7/TLR8 Ratio
1	24.66 (13.92/35.4)	2.68 (1.58/3.78)	9.2
2	0.78 (0.18/1.37)	0.26 (0.11/0.40)	3.0
3	0.92 (0.39/1.44)	6.12 (4.20/8.04)	0.15

Cmpd	hTLR7 EC50 (μM)	hTLR8 EC50 (μM)	TLR7/TLR8 Ratio
4	0.09 (0.06/0.12)	223 (219/228)	0.0004
5	4.76 (4.37/5.16)	2.62 (2.41/2.84)	1.8
6	0.49 (0.46/0.53)	2.18 (1.60/2.76)	0.22

Fig. 1. General structures and potencies of imidazoquinoline and oxoadenine TLR7/8 agonists. (A) Structures of each agonist. (B) Relative TLR7- or TLR8-induced NF-κB activity, determined in recombinant reporter HEK293 cells, versus control. Half maximal effective concentrations (EC50) for each compound was determined by non-linear curve fitting, numbers in parenthesis represent the 95% upper and lower confidence intervals.

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