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Impact of adjuvants on CD4$^+$ T cell and B cell responses to a protein antigen vaccine: Results from a phase II, randomized, multicenter trial

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ABSTRACT

Immunogenicity and safety of different adjuvants combined with a model antigen (HBsAg) were compared. Healthy HBV-naïve adults were randomized to receive HBs adjuvanted with alum or Adjuvant Systems AS01B, AS01E, AS03A or AS04 at Days 0 and 30. Different frequencies of HBs-specific CD4+ T cells 14 days post dose 2 but similar polyfunctionality profiles were induced by the different adjuvants with frequencies significantly higher in the AS01B and AS01E groups than in the other groups. Antibody concentrations 30 days post-dose 2 were significantly higher in AS01B, AS01E and AS03A than in other groups. Limited correlations were observed between HBs-specific CD4+ T cell and antibody responses. Injection site pain was the most common solicited local symptom and was more frequent in AS groups than in alum group. Different adjuvants formulated with the same antigen induced different adaptive immune responses and reactogenicity patterns in healthy naïve adults.

The results summary for this study (GSK study number 112115 – NCT# NCT00805389) is available on the GSK Clinical Study Register and can be accessed at www.gsk-clinicalstudyregister.com.

1. Introduction

Adjuvants are included in vaccines with the aim of accelerating, prolonging or enhancing the intrinsic immunogenicity of antigens [1]. Aluminum salts were first used as adjuvants in the 1920′s [2] and are still widely utilized in human vaccines. However, aluminum salts predominantly promote antibody responses [3,4] that reach protective levels only after multiple vaccine doses. In addition, aluminum-adjuvanted vaccines are of more limited use when strong T cell responses are required to protect against complex pathogens, chronic infections, or in populations such as the elderly or immunocompromised [5–8]. These limitations have led to the development of variety of new adjuvants based on oil-in-water (o/w) emulsions, saponins and Toll-like receptor agonists. These substances, used alone or in combination, are essential

Abbreviations: AE, adverse event; AS, adjuvant System; ATP, according-to-protocol; CLIA, chemiluminometric immunoassay; ELISPOT, enzyme-linked immunosorbent spot; GMC, geometric mean concentration; GMF, geometric mean frequency; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HPV, human papillomavirus vaccine; IgG, immunoglobulin G; mIU, milli-international units; MPL, 3-*O*-desacyl-4′-monophosphoryl lipid A; PBMC, peripheral blood mononuclear cell; pIMD, potential immune-mediated disease; QS-21, *Quillaja saponaria* Molina, fraction 21; R, Pearson's correlation coefficient; SAE, serious adverse event; SD, standard deviation.

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components of currently licensed vaccines and candidate vaccines under development [9].

AS01 is an Adjuvant System family containing the TLR4 agonist 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) and QS-21 (*Quillaja saponaria* Molina, fraction 21) formulated with liposomes. It has been developed to potentiate T cell responses against challenging pathogens where classical approaches have proven less effective [10,11]. This is exemplified by the RTS,S/AS01 candidate vaccine which targets *Plasmodium falciparum* and has been shown to elicit 31% and 50% protective efficacy against clinical malaria in infants and children, respectively [12,13], and by the candidate subunit glycoprotein E varicella zoster HZ/su vaccine inducing >95% protection in older adults [14]. $AS03_A$, an Adjuvant System containing α-Tocopherol and squalene in an o/w emulsion promotes the rapid production of cross-reactive antibodies and allows for antigen-sparing as demonstrated with a pre-pandemic H5N1 candidate vaccine [15,16] and with the licensed H1N1 influenza vaccine [17]. AS04, an Adjuvant System containing MPL adsorbed on Al salt enhances antibody and T cell responses and is included in the licensed human papillomavirus vaccine HPV-16/18 for prevention of cervical cancer [18,19] and in a hepatitis B virus (HBV) vaccine for use in patients with renal insufficiency [20].

These Adjuvant Systems have been shown to induce enhanced antibody and T cell responses in numerous clinical studies targeting a variety of pathogens and some have been compared in clinical trials (e.g., in combination with the candidate malaria RTS, S antigen and the candidate Herpes Zoster antigen), providing valuable information on differential responses in the respective settings [21–23]. Additionally, as a precursor to the current study, Adjuvant Systems containing MPL and QS-21 were compared in a clinical trial using a well-characterized model antigen (recombinant hepatitis B virus surface antigen [HBsAg]) [24,25]. However, AS03 and AS04, which are already used in licensed products, have not previously been compared with AS01. To gain further insight into the induced immune profile and to assist in their rational inclusion in future vaccines, a study has been set up to compare the immunogenicity and safety of AS01, AS03, AS04 and alum in a head-to-head clinical trial. These adjuvants have been combined with HBsAg, and were evaluated in healthy, young HBV-naïve adults to minimize confounding factors.

Here we first report on the antibody, T and B cell responses to HBsAg as well as the reactogenicity and safety profiles of the different formulations up to Day 60. Additional analyses deciphering innate and adaptive immune responses will be the subject of future reports.

2. Material and methods

2.1. Study design and participants

This was an observer-blind, randomized, controlled trial (ClinicalTrials.gov identifier: NCT00805389) conducted at 14 study centers (4 in Belgium and 10 in Germany) from December 2008 to July 2011. The protocol was approved by all institutional Ethics Committees and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from each participant before trial participation. Eligible participants were healthy men and women aged 18–45 years. Exclusion criteria were: previous vaccination against HBV; positive for anti-HBs antibodies, *anti*-HBc antibodies, HBsAg, antibodies against hepatitis C virus and/or HIV; previous administration of MPL or QS-21 (*Q. saponaria* Molina, fraction 21) (Licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation); administration of any other investigational or non-registered product (drug or vaccine) within the last 30 days or planned use during the study period; administration or planned administration of a vaccine not foreseen by the study protocol within the last 30 days with the exception of the influenza vaccine which could be administered >21 days preceding or following each primary vaccine dose and >7 days preceding or following the booster dose; chronic administration of immunosuppressants or other immune-modifying drugs within the last six months; administration of immunoglobulins and/or any blood products within the last three months, any confirmed or suspected immunosuppressive or immunodeficient condition, history of allergic disease or reactions likely to be exacerbated by vaccine components; other conditions that the investigator judged may interfere with study findings.

A target of 710 eligible participants (142 per group) were to be randomized (1:1:1:1:1) to receive 20 μg HBsAg adjuvanted with alum or one of four GSK proprietary Adjuvants Systems ($AS01_B$, $AS01_E$ [containing half the quantity of MPL and QS-21 as in $AS01_B$], $AS03_A$ or AS04) at Days 0 and 30. The vaccine formulations are shown in Fig. 1. Vaccine doses were administered by intramuscular injection into the deltoid muscle of the non-dominant arm. The primary endpoint (HBs-specific T cells) was assessed for all participants. Secondary and exploratory endpoints were evaluated in a sub-cohort of participants (375 planned; 75 per group) (Supplementary Fig. 1). Allocation of participants to the sub-cohort was based on HLA type, determined at screening, in order to allow analyses of HBsAg-derived peptide specific T cell responses (see Supplementary Material).

2.2. Treatment allocation and blinding

Participants were allocated a unique treatment number using a centralized randomization system on internet. The randomization algorithm used a minimization procedure accounting for country of recruitment, pre-selected HLA type and gender. When 375 participants had been allocated to the sub-cohort, HLA typing was stopped and the remaining participants were then randomized only according to gender and country. The study was conducted in an observer-blinded manner. Full blinding could not be done due to the different appearance and preparation of the vaccines. Vaccine preparation and administration were performed by authorized medical personnel who did not participate in any of the clinical evaluations. Study participants and those responsible for the evaluation of study endpoints were unaware of group allocation.

2.3. Immunological evaluation

For analyses described here, blood samples were collected on Days 0, 14, 30, 37, 44 and 60 (Fig. 1). All assays were done at central laboratories (ImmuneHealth, Gosselies, Belgium for cell-mediated immunity assays; GSK Vaccines, Rixensart, Belgium for measurement of anti-HBs antibodies) as described below.

2.3.1. HBs-specific $CD4^+$ and $CD8^+$ T cells

HBs-specific $CD4^+$ and $CD8^+$ T cells were measured using frozen peripheral blood mononuclear cells (PBMCs) by the intracellular cytokine staining assay using adaptations of previously described methods [24,26,27]. Briefly, PBMCs were stimulated in vitro with a pool of peptides (15-mers overlapping by 11 amino acids and covering the entire HBsAg sequence; at 0.5 μg/mL/peptide; Eurogentec S.A.) and medium (negative control) for 2 h in the presence of *anti*-CD28 (CD28.2) and anti-CD49d (9F10) antibodies (from BD Biosciences). Cytokine secretion inhibitor (Golgi Plug, BD Pharmingen containing Brefeldin A) was added 2 h after start of culture (stimulation with peptides) and the culture was further incubated overnight. After *in vitro* stimulation, PBMC were stained with extracellular markers, CD4 V450 (SK3) and CD8 APC Cy7 (SK1) (BD Biosciences) and permeabilized in Cytofix/Cytoperm solution (BD Pharmingen). The cells were then stained with the following antibodies: CD40L PE (TRAP1), IL-2 FITC (MQ1-17H12), TNF-α PE-Cy7 (Mab11), IFN-γ Alexa 700 (4S·B3) all from BD Biosciences; IL-13 APC (JES10-5A2) from Biolegend, IL-17 PerCp Cy 5.5 (eBio64DEC17) from eBiosciences Inc. and CD3 Pacific Orange (UCHT1) from Caltag Medsystems Ltd. Finally, cells were acquired on a LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo version 9 software (Tree Star Inc., Ashland, OR, USA).