Vaccine 35 (2017) 3249–3255

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Carbohydrate fatty acid monosulphate esters are safe and effective adjuvants for humoral responses

Luuk A.Th. Hilgers [a,*], Peter Paul L.I. Platenburg [a], Jeffrey Bajramovic [b], Jennifer Veth [b], Robert Sauerwein [c], Will Roeffen [c], Marie Pohl [d], Geert van Amerongen [d], Koert J. Stittelaar [d], Johannes F. van den Bosch [a]

[a] LiteVax BV, Oss, The Netherlands
[b] Biomedical Primate Research Centre, Rijswijk, The Netherlands
[c] Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[d] Viroclinics Biosciences BV, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 14 April 2017
Accepted 19 April 2017
Available online 4 May 2017

Keywords:
Vaccine adjuvants
synthetic carbohydrate derivatives
influenza
ferrets
rabbits
TLR4

ABSTRACT

Carbohydrate fatty acid sulphate esters (CFASEs) formulated in a squalane-in-water emulsion are effective adjuvants for humoral responses to a wide range of antigens in various animal species but rise in body temperature and local reactions albeit mild or minimal hampers application in humans. In rabbits, body temperature increased 1 °C one day after intramuscular (IM) injection, which returned to normal during the next day. The effect increased with increasing dose of CFASE but not with the number of injections (up to 5). Antigen enhanced the rise in body temperature after booster immunization ($P < 0.01$) but not after priming. Synthetic CFASEs are mixtures of derivatives containing no sulphate, one or multiple sulphate groups and the monosulphate derivatives (CMS) were isolated, incorporated in a squalane in-water emulsion and investigated. In contrast to CFASE, CMS adjuvant did not generate rise in body temperature or local reactions in rabbits immunized with a purified, recombinant malaria chimeric antigen R0.10C. In comparison to alum, CMS adjuvant revealed approximately 30-fold higher antibody titres after the first and >100-fold after the second immunization. In ferrets immunized with 7.5 μg of inactivated influenza virus A/H7N9, CMS adjuvant gave 100-fold increase in HAI antibody titres after the first and 25-fold after the second immunisation, which were 10–20-fold higher than with the MF59-like AddaVax adjuvant. In both models, a single immunisation with CMS adjuvant revealed similar or higher titres than two immunisations with either benchmark, without detectable systemic and local adverse effects. Despite striking chemical similarities with monophospholipid A (MPL), CMS adjuvant did not activate human TLR4 expressed on HEK cells. We concluded that the synthetic CMS adjuvant is a promising candidate for poor immunogens and single-shot vaccines and that rise in body temperature, local reactions or activation of TLR4 is not a pre-requisite for high adjuvanticity.

© 2017 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Novel adjuvants are needed for the new generation of well-defined antigens to improve the level and duration of immunity and to reduce the number of non-responders. Of particular interest is their use in emergency vaccines thereby reducing the dose of antigen and the number of injections. Avoiding the need for a booster reduces costs, time-to-immunity and complexity of vaccination campaigns, which are critical factors in regions with poor infrastructure and in emergency situations. Furthermore, one instead of multiple vaccinations results in lower overall risks of adverse events associated with vaccination.

Synthetic carbohydrate fatty acid sulphate esters (CFASEs)-based adjuvants are known for several years to effectively enhance humoral responses to a wide range of antigens [1–8] in different animal species. Multiple cycles of lead-finding and optimisation focused on efficacy/toxicity (E/T)-ratio and structure-response-relationship in large, non-rodent species, resulted in a series of CFASE adjuvants. Incorporated into a squalane-in-water emulsion, CFASEs exerted strong synergy [1–4] and outperformed oily adjuvants with respect to efficacy, safety and quality [4,9–13], which is unprecedented for an aqueous adjuvant formulation.

The first generation of CFASE adjuvants consisted of polysaccharide derivatives and is exploited in single-shot vaccines for veteri-

* Corresponding author at: LiteVax BV, Akkersestraat 50, 4061 BJ Ophemert, The Netherlands.
E-mail address: luuk.hilgers@litevax.com (L.A.Th. Hilgers).

http://dx.doi.org/10.1016/j.vaccine.2017.04.055
0264-410X/© 2017 The Author(s). Published by Elsevier Ltd.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

nary purposes [3] and investigated in a cancer vaccine [14,15]. The second generation based on a disaccharide known as 'CoVaccine HT™ [4–6] reached phase IIa clinical trial with two therapeutic, peptide-protein vaccines [16,17]. Drawbacks of these CFASEs are rise in body temperature one day after injection and local reactions at the site of injection albeit mostly mild or minimal. To annihilate these adverse effects while retaining adjuvanticity, we examined several factors influencing E/T-ratio including dose of adjuvant and antigen, number of injections and chemical structure of the active molecule. Results on a third generation of carbohydrate derivatives are reported here.

2. Materials and methods

2.1. Antigens

Three different antigens were used to assess *in vivo* performance of the adjuvants. A peptide-protein conjugate (gonadotropin-releasing hormone peptide coupled to keyhole limpet haemocyanin; 'GnRH-KLH') prepared for nonclinical GLP toxicity studies as described by Turkstra et al. [13] was used at 400 and 800 μg of protein per dose. A chimeric recombinant malaria antigen R0.10C composed of a fragment of *Plasmodium falciparum* glutamate rich protein fused with a correctly folded fragment of Pfs48/45, produced in *Lactococcus lactis* and immune-purified ([18]; Radboud UMCN, Nijmegen, The Netherlands) was used at 10 μg protein per dose. Whole virion A/H5N1 and A/H7N9 grown on cell culture, inactivated and purified, were kindly provided by National Health Research Institute of Taiwan and Medigen Vaccine Biologicals Corp. (Taipei, Taiwan) and used at 7.5 μg HA per dose.

2.2. Adjuvants

CFASE was prepared as described by Blom and Hilgers [4]. Briefly, maltose dissolved in pyridine plus N,N-dimethylformamide was contacted with one equivalent of SO_3.pyridine and 8 equivalents of decanoylchloride. Monosulphate derivatives (CMS) and polysulphate derivatives (CPS) were isolated from the CFASE by preparative liquid chromatography on silica with 12 v/v% triethylamine and 12 v/v% 2-isopropanol in n-heptane (all from Sigma-Aldrich, St. Louis, MO) as eluent. Fractions containing either CMS or CPS as determined by thin layer chromatography were pooled and dried and purity was determined by LC-MS.

Adjuvant formulations were prepared by passing mixtures of 40 g of CFASE or CMS or CPS, 80 g of squalane (Bayer, Germany), 40 g of Polysorbate 80 (Montannox 80 PPI; Seppic, Paris, France) and 840 g phosphate buffered saline (PBS; Fisher Scientific) three times through a high-pressure emulsifier (Microfluidizer Y110, Microfluidics, Newton, USA). The submicron emulsions obtained were passed through a 0.2 μm filter (PES Supor EKV, Pall Life Sciences, Portsmouth, UK), stored in sterile containers at 4 °C and tested as described below.

Alhydrogel (Brenntag Biosector, Frederikssund, Denmark) and the MF59-like adjuvant AddaVax (InvivoGen, Toulouse, France) were used as benchmarks.

Vaccines were prepared by mixing antigen, adjuvant and PBS at appropriate volume ratio one day before administration. Prefilled 1-mL syringes with 0.5 mL (plus 0.1–0.2 mL excess) of either vaccine, equipped with 25G needles, labelled with animal ID, study ID and date were supplied to the animal facility.

The dose of adjuvant was 22 mg of CFASE or 8 mg of CMS or 8 mg of CPS (22 mg CFASE contains approximately 8 mg CMS and 8 mg of CPS), 0.2 w/v% Alhydrogel or 50 v/v% of AddaVax.

2.3. Animals

SPF Female rabbits (New Zealand White, Harlan Laboratories, UK), 3–6 months of age were housed individually with supply of tap water *ad libitum* and 100 g food per animal per day.

Male ferrets (undisclosed provider in Denmark), 6–12 months of age and negative for relevant (circulating and vaccine) influenza strains (A/H7N9, A/H1N1, A/H3N2 and B) and Aleutian Disease virus, were housed in standard group cages with *ad libitum* supply of tap water and food (Hope Farms, Ferret super).

The animals were randomly assigned to treatment groups using a generalized randomized block design on the base of body weight. Animals were checked daily for overt signs of disease and treatment-related adverse events.

2.4. Ethical statement

The use of animals was performed in accordance with the regulations set forth by the relevant national and/or local Ethical Committee for Animal Experimentation and in accordance with European Community Directive 86/609. All the techniques and procedures were refined to provide for minimal discomfort and stress to the animals.

2.5. Immunization and blood collection

Test items in 0.5 mL were injected IM two times alternating in the left and right thigh muscle at 3-week interval, unless stated otherwise. At different time intervals before and after each immunization blood samples of 1–5 mL were collected and rectal body temperature was measured.

2.6. Antibody titres to malaria in rabbits

Antibody titres in serum samples were measured by Radboud UMCN (Nijmegen, the Netherlands) by ELISAs with purified R0.10C or gametocyte extract as coating antigens. Microtiter plates (Sterilin® ELISA plates, Netherlands) were coated overnight, blocked with 5% milk in PBS, incubated with serial dilutions of serum samples in PBS-0.05% Tween 20 (PBST) for 4 h at room temperature, incubated with anti-rabbit IgG-HRP (H + L) (DAKO, Denmark) and developed with tetramethyl benzidine (TMB) substrate solution for 20 min at room temperature. The colour reaction was stopped with 0.2 N H_2SO_4 and the optical density was read at 450 nm in a Microplate Reader (Labtec BV, Germany). After each incubation step plates were washed extensively with PBST – 0.5 M NaCl. A positive and negative control serum were included in each test to confirm validity of the test. The antibody concentration was expressed as the EC50-value calculated by using GraphPad Prism (GraphPad Software, USA).

2.7. Antibody titres to H7N9 in ferrets

Antibody responses to H7N9 in ferrets were determined by Viroclinics Biosciences BV (Rotterdam, the Netherlands) as described elsewhere [19]. Briefly, serum samples were pre-treated with cholera filtrate resulting in a pre-dilution of 1:6 and serially two-fold dilutions were tested in the HAI test using 2:6 reassortant (A/Anhui/1/2013 H7N9 and A/PR/8/34) as described elsewhere [20].

2.8. Safety assessment in rabbits

Rectal body temperature of rabbits was measured 6 h before and 3 or 6, 24, 48 and 96 h after each immunization. Local reactions were determined by palpation and visual inspection during

Download English Version:

https://daneshyari.com/en/article/5536514

Download Persian Version:

https://daneshyari.com/article/5536514

Daneshyari.com