Comparative preclinical evaluation of AS01 versus other Adjuvant Systems in a candidate herpes zoster glycoprotein E subunit vaccine

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ABSTRACT
The candidate vaccine HZ/su is being developed to prevent herpes-zoster disease (HZ). HZ occurs at a rate of 30% in adults aged ≥ 60 years, with an incidence that increases with age, with immunosuppressive treatments or in immunocompromised individuals. The occurrence of HZ has been attributed to a decline in T-cell mediated immunity to VZV. A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). In mice, MPL and QS-21, in combination, synergistically induce gE-specific CD4+ T-cell responses to vaccination. Therefore the objective of this study was to compare CD4+ T-cell responses to gE vaccines adjuvanted with AS01B or AS01E, with those to gE vaccines adjuvanted with AS03 or AS04, in mice primed with live-attenuated VZV. In clinical trials, 2 other Adjuvant Systems, AS03 and AS04, have been shown to enhance antigen-specific CD4+ T-cell responses to influenza and human papillomavirus HPV vaccines, respectively, but have not been evaluated with the gE antigen. Therefore the objective of this study was to compare CD4+ T-cell responses to gE vaccines adjuvanted with AS01B or AS01E, with those to gE vaccines adjuvanted with AS03 or AS04, in mice primed with live-attenuated VZV. Antibody responses to vaccination were also evaluated. Two independent experiments were performed in which C57Bl/6 mice (Harlan Horst, Netherlands) were primed with one sub-cutaneous dose of a live-attenuated varicella vaccine (full-human dose of Varilrix, 10^4 pfu). Five weeks after priming on Days 0 and 28, mice were administered intramuscular (tibialis) injections of a gE vaccine or saline (0.9% NaCl; control group). One gE-vaccine dose contained 5 μg gE and an

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Herpes zoster (HZ) or shingles is a disease with symptoms including skin rash and postherpetic neuralgia, and is caused by the reactivation of dormant varicella zoster virus (VZV). The lifetime risk of having HZ has been estimated at around 30%, and the incidence of disease increases with age, with immunosuppressive treatments or with immunocompromised conditions. The occurrence of HZ has been attributed to a decline in T-cell mediated immunity to VZV. A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). A live-attenuated vaccine is licensed (Zostavax, Merck). In mice, MPL and QS-21, in combination, synergistically induce gE-specific CD4+ T-cell responses to vaccination. Therefore the objective of this study was to compare CD4+ T-cell responses to gE vaccines adjuvanted with AS01B or AS01E, with those to gE vaccines adjuvanted with AS03 or AS04, in mice primed with live-attenuated VZV. Antibody responses to vaccination were also evaluated. Two independent experiments were performed in which C57Bl/6 mice (Harlan Horst, Netherlands) were primed with one sub-cutaneous dose of a live-attenuated varicella vaccine (full-human dose of Varilrix, 10^4 pfu). Five weeks after priming on Days 0 and 28, mice were administered intramuscular (tibialis) injections of a gE vaccine or saline (0.9% NaCl; control group). One gE-vaccine dose contained 5 μg gE and an

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tems AS01B, AS01E, AS03, or AS04 are defined by the quantities of the following components in a full-human dose: AS01B contains 50 μg MPL and 25 μg QS-21; AS01E contains 11.86 mg α-tocopherol and squalene in an oil-in-water emulsion, and AS04 contains 50 μg MPL adsorbed on 500 μg aluminum salt. For the purpose of this article, gE/AS01B, gE/AS01E, gE/AS03, and gE/AS04 refer to the mouse vaccines in which the Adjuvant System contains one tenth of the respective quantities used in a full-human dose.

Antigen-specific CD4+ T cells expressing at least one of the 2 cytokines IFN-γ and IL-2, were detected in all vaccine groups at 30 d after dosing. In Experiment 1, the geometric mean frequency (GMF) of gE-specific CD4+ T cells was 6.2% in the gE/AS01B group; whereas it was 3.5% in the gE/AS04 group, 2.2% in the gE/AS01E group and 1.3% in the gE/AS03 group (Fig. 1A). In Experiment 2, the GMF of gE-specific CD4+ T cells was 9.1% in the gE/AS01B group; whereas it was 5.8% in the gE/AS01E group, 1.9% in the gE/AS04 group, and 1.5% in the gE/AS03 group (Fig. 1A). In Experiments 1 and 2, the frequencies of gE-specific CD4+ T cells in the NaCl group were either close to or below the cut-off for the assay (GMFs were 0.3% and 0.05%, respectively). The frequencies of gE-specific CD8+ T cells in any of the adjuvanted-vaccine groups were not significantly higher than the baseline frequencies observed in the NaCl group (not shown).

In both experiments, the differences between the vaccine groups were mostly associated with gE-specific CD4+ T cells that were IFN-γ positive (Fig. 1A). Moreover, the magnitude of IFN-γ production in IFN-γ+ CD4+ T cells relative to IFN-γ− CD4+ T cells appeared higher in the gE/AS01B group than in the other groups (measured by fluorescent-staining intensities; not shown). Overall, gE-specific CD4+ T cells were 5.4, 2.8 and 2.2-fold more frequent in response to gE/AS01B than in response to gE/AS03 gE/AS04 and gE/AS01E (p < 0.001), respectively; and were 2.5-fold more frequent in response to gE/AS01E than in response to gE/AS03 (p < 0.001; Fig. 1B).

Antigen-specific antibodies were detected in all vaccine groups at 14 and 28 d after dosing but were not detected in the NaCl group (concentrations were below the cut-off of the assay; i.e. < 500 EU/ml). For both experiments and in any given vaccine

Figure 1. Geometric mean frequencies (GMFs) of (A) gE-specific CD4+ T cells and (B) ratios of GMFs from different adjuvanted-vaccine groups. Spleens (Experiment 1, N=8 and Experiment 2, N =11; spleens pooled from 2 mice) were sampled at 30 d after the second vaccine dose (30dPII). The frequency of gE-specific CD4+ T cells was calculated as a percentage of cytokine-positive CD4+ T cells divided by all CD4+ T cells. Error bars represent 95% confidence intervals. In Experiments 1 and 2, the frequencies of antigen-specific CD4+ T cells in the NaCl group were either close to or below the cut-off for the assay (GMFs were 0.3% and 0.05%, respectively). In (B), horizontal gray reference line indicates a ratio = 1, and asterisks indicate significant differences from 1 (** p < 0.01; *** p < 0.001). Antigen-specific T cells were evaluated in splenocyte-restimulation cultures as described previously, with some modifications. Briefly, splenocyte cultures (110^6 cells per well of 96-well plate) were prepared from spleens of 2 mice and were incubated for 2 hours in the presence of gE peptides spanning the complete gE protein (6315-mer peptides, 11 amino-acid overlap) and then incubated 18 hours in the presence of brefeldin A. Subsequently, the cells were stained with fluorescent-monoclonal antibodies specific for CD4 and after permeabilization, for intracellular-cytokines IL-2 and IFN-γ. All antibodies were obtained from BD Biosciences, Belgium. Flow cytometry was performed using LSR II Facs (BD Biosciences, Belgium) and analyzed using FlowJo software (FlowJo, LLC, OR, USA). Statistical calculations were based on an analysis of variance with 2 factors (vaccine group, experiment) on log_{10} values using a heterogeneous variance model (i.e., identical variances were not assumed for the different levels of the factor). Estimates of the geometric mean ratios between groups and their 95% confidence intervals (CI) were obtained using back-transformation of log_{10} values. Adjustments for multiple testing were performed using Tukey’s method. All analyses were performed using SAS software (Version 9.2, SAS Institute Inc, NC, USA).
group, the magnitude of geometric mean concentrations (GMCs) of gE-specific antibodies appeared similar at 14 d compared with 28 d (Fig. 2A). Some significant differences were observed in the ratios of antibody concentrations between vaccine groups, although, no differences were more than 2-fold (Fig. 2B). In the gE/AS01B group at 14 and 28 days, GMCs were 1.6-fold \((p < 0.001)\) and 1.7-fold higher \((p < 0.001)\) than in the gE/AS03 group, respectively; 1.4-fold \((p < 0.001)\) and 1.7-fold \((p < 0.001)\) higher than in the gE/AS04 group, respectively; and 1.5-fold \((p < 0.001)\) and 1.4-fold \((p < 0.05)\) higher than in the gE/AS01E group, respectively \((p < 0.001)\).

Overall, the AS01B-based vaccine formulation induced the highest frequency of gE-specific CD4\(^+\) T cells compared with the AS03 and AS04 vaccine formulations, primarily reflecting differences in the frequencies of those T cells that were IFN-\(\gamma\) positive. The AS01E formulation also induced a higher frequency of gE-specific CD4\(^+\) T cells than AS03. The potential that these comparative differences are relevant in humans is suggested from the observation that gE-specific CD4\(^+\) T cell responses were higher to the AS01B formulation than to the AS01E formulation in VZV-primed mice (consistent with a previous study) as well as in the clinical setting.\(^{13,15}\)

Although VZV antibodies are not considered essential to confer protection against HZ,\(^2\) the AS01B-based vaccine formulation induced marginally higher gE-specific antibody concentrations compared with the other formulations (and our unpublished observations suggest that these antibody concentrations correlate with VZV-neutralizing activity). Hence, the differences between AS01B-based vaccine formulation and the AS03- and AS04-based vaccine formulations were primarily reflected in differences in CD4\(^+\) T cell frequencies and in line with nonclinical and clinical experience of other vaccines.\(^{17}\)

In humans, VZV-specific cell-mediated immunity appears to play an essential role in protection against both the occurrence and morbidity of HZ, although a clearly defined correlate of protection against HZ remains to be identified.\(^{1,2,4,5}\) CD4\(^+\) T cells expressing IFN-\(\gamma\) appear to predominate in the responses to VZV antigens in general or to gE alone,\(^{5,20}\) thus supporting the monitoring of CD4\(^+\) T cells in HZ-vaccine evaluations.\(^{13}\) Hence the present study and a previous preclinical study add further support for the use of AS01B rather than AS03, AS04, AS01E or aluminum salt in the candidate HZ vaccine formulation.

**Abbreviations**

- CI: confidence interval
- gE: glycoprotein E
- HZ: herpes zoster
- GMC: geometric mean concentration
- GMF: geometric mean frequency
- MPL: 3-O-desacyl-4’-monophosphoryl lipid A
- QS-21: Quillaja saponaria Molina, fraction 21
- VZV: varicella zoster virus

**Disclosure of potential conflicts of interest**

All authors were involved in the conception and design of the studies. ND, MB, MF acquired the data. All authors analyzed and interpreted the
results. All authors were involved in drafting the manuscript or revising it critically for important intellectual content. All authors had full access to the data and approved the manuscript before it was submitted by the corresponding author.

All authors have declared the following interests: all authors are employees of the GSK group of companies. MB and ND own GSK stocks.

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