



Inflammatory parameters associated with systemic reactogenicity following vaccination with adjuvanted hepatitis B vaccines in humans



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ABSTRACT

Background: Adjuvants like AS01_B increase the immunogenicity of vaccines and generally cause increased transient reactogenicity compared with Alum. A phase II randomized trial was conducted to characterize the response to AS01_B and Alum adjuvanted vaccines. A post-hoc analysis was performed to examine the associations between reactogenicity and innate immune parameters.

Methods: The trial involved 60 hepatitis B-naïve adults aged 18–45 years randomized 1:1 to receive either two doses of HBsAg-AS01_B on Day (D)0 and D30, or three doses of HBsAg-Alum on D0, D30, D180. Prior to vaccination, all subjects received placebo injection in order to differentiate the impact of injection process and the vaccination. Main outcomes included reactogenicity symptoms, vital signs, blood cytokines, biochemical and hematological parameters after vaccination. Associations were explored using linear regression.

Findings: The vaccine with AS01_B induced higher HBsAg-specific antibody levels than Alum. Local and systemic symptoms were more frequent in individuals who received HBsAg AS01_B/Alum vaccine or placebo, but were mild and short-lived. Blood levels of C-reactive protein (CRP), bilirubin, leukocyte, monocyte and neutrophil counts increased rapidly and transiently after AS01_B but not after Alum or placebo. Lymphocyte counts decreased in the AS01_B group and lactate dehydrogenase levels decreased after Alum. Modelling revealed associations between systemic symptoms and increased levels of CRP and IL-6 after the first HBsAg-AS01_B or HBsAg-Alum immunization. Following the second vaccine dose, CRP, IL-6, IP-10, IFN- γ , MIP-1 β and MCP-2 were identified as key parameters associated with systemic symptoms. These observations were confirmed using an independent data set extracted from a previous study of the immune response to HBsAg-adjuvanted vaccines (NCT00805389).

Conclusions: IL-6 and IFN- γ signals were associated with systemic reactogenicity following administration of AS01_B-adjuvanted vaccine. These signals were similar to those previously associated with antibody and T-cell responses induced by HBsAg-adjuvanted vaccines, suggesting that similar innate immune signals may underlie adjuvant reactogenicity and immunogenicity.

Trial registration: www.clinicaltrials.gov NCT0177295.

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Abbreviations: AE, adverse event; CI, confidence interval; DX(hY), day X at hour Y; CRP, C-reactive protein; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IP-10, inducible protein 10; LDH, lactate dehydrogenase; MIP-1 β , macrophage inflammatory protein-1 β ; MCP-2, monocyte chemotactic protein 2; MPL, 3-O-desacyl-4'-monophosphoryl lipid A; pIMD, piMD, potential immune-mediated disease; PC, principal component; PCA, principal component analysis; SAE, serious adverse event.

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1. Introduction

Adjuvants are immune stimulants included in vaccines to enhance their immunogenicity by increasing, broadening and/or prolonging the antigen-specific immune response [1,2]. Adjuvants work by rapidly activating the innate immune response, a key step to direct the magnitude and quality of the antigen-specific immune response [3]. The increased immunogenicity of adjuvanted vaccines is commonly associated with increased local and systemic

reactions, referred to as reactogenicity [3,4]. Vaccine reactogenicity is carefully assessed in several thousands of subjects during pre-licensure clinical trials and is well characterized by the time a vaccine is authorized for use in humans [5]. However, the underlying mechanisms that cause vaccine reactogenicity remain poorly understood and have not been systematically analyzed [5,6]. Understanding such mechanisms would further inform the design of adjuvanted vaccines with optimal immunogenicity and reactogenicity profiles.

Adjuvant System AS01 is a liposome-based adjuvant that contains the toll-like-receptor 4 agonist 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) and the saponin QS-21 (*Quillaja saponaria* Molina, fraction 21). AS01 potentiates antibody and CD4⁺ T-cell responses to vaccines against viruses and intracellular pathogens where classical approaches have proven less effective [7–9]. AS01_B is included in the herpes zoster vaccine (*Shingrix*, GSK), and in the candidate RTS,S/AS01 malaria vaccine, both of which have demonstrated efficacy in clinical trials [10–12]. The potent immunogenicity of AS01 adjuvanted vaccines was recently shown to be associated with the activation of specific innate immune responses including the IL-6 and IFN signals [13]. These studies did not investigate the immune signals associated with the adjuvanted vaccine's reactogenicity.

We hypothesized that the reactogenicity of AS01_B adjuvanted vaccine is associated with the activation of specific innate immune signals that could be distinct from those associated with the vaccine immunogenicity. To test this hypothesis, we performed a post-hoc analysis of a randomized phase II study of clinical and innate immune parameters following immunization with 20 µg hepatitis B (HBV) surface antigen (HBsAg) combined with either AS01_B or Alum in healthy adults. Clinical and immune parameters were also measured following the administration of a placebo to the same subjects 30 days prior to immunization as control, to evaluate the potential impact of injection, repeated venipuncture and circadian variations. Innate immune signals associated with reactogenicity were identified in this cohort, and were compared to signals identified in a larger cohort previously studied for correlates between innate and adaptive responses to adjuvanted vaccines [14].

2. Methods

2.1. Study design and participants

The single-center phase II, single-blind randomized study (www.clinicaltrials.gov NCT01777295) was performed in Belgium (Feb 2013–Sept 2016). The study protocol and complete statistical analysis plan are available in [Supplement 1](#). The protocol was approved by the institutional Ethics Committee and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from each participant before enrolment. A randomization list was used to number the vaccines and placebo, with a block size of 2. Sixty participants were randomized 1:1 by study staff to receive either two doses of HBsAg-AS01_B on Day (D)0 and D30, or three doses of HBsAg-Alum (*Engerix B*, GSK) on D0, D30 and D180. Vaccination was preceded by placebo injection in all subjects in order to investigate the actual impact of the vaccine. The primary study objective was to measure innate immune mediators in plasma following immunization in all participants. Secondary objectives reported here were to measure the antibody response to the vaccines, and to assess their safety. Participants were asked to follow specific dietary and lifestyle recommendations on the day before and the day of specific protocol-defined time points (see [Supplement 2 Methods](#)).

Blood was collected and reactogenicity parameters were measured before and following each injection ([Fig. 1](#)). A sub-cohort of 15 participants in each group stayed overnight at the study center for blood collection and reactogenicity assessment. Further detail on the study design and procedures are provided in [Supplement 2 Methods](#).

2.2. Evaluation of reactogenicity and safety

Reactogenicity was assessed by participants on diary cards as well as directly by the study staff ([Fig. 1](#)). For the first method, participants recorded local symptoms of pain, redness and swelling, and systemic symptoms of fatigue, fever (oral temperature ≥ 37.5 °C), gastrointestinal symptoms, headache, malaise, myalgia and shivering on diary cards for 7 days after each injection, (D0–D6), referred to here as 'standard procedures'. The intensity of symptoms was graded on a 3-point scale (mild-moderate-severe), where Grade 3 symptoms (severe) were defined as redness or swelling >100 mm in diameter, oral temperature >39.5 °C, and as preventing normal activity for all other symptoms. Unsolicited adverse events (AEs) were recorded for 28 days after each injection. Serious adverse events (SAEs), potential immune-mediated diseases (pIMDs, listed in the [Supplement 2](#)) and other events including pregnancy were recorded during the entire study period (until D210).

2.3. Analysis of peripheral blood parameters

Clinical laboratory hematological and biochemical parameters, anti-HBs IgG antibodies and a panel of 24 cytokines ([Supplement 2 STable1](#)) were measured in peripheral blood of all participants ([Fig. 1](#)). Anti-HBs IgG antibodies were measured using chemiluminescent immunoassay (CLIA, ADVIACentaur anti-HBs2, Siemens Healthcare): assay cut-off 6.2 mIU/mL, cut-off for seroprotection 10 mIU/mL. Cytokines were measured in serum in all participants using Myriad RBM's luminex xMAP technology, HMPC42 and HMPCORE1 multiplexes according to the manufacturer's instructions ([Supplement 2 STable1](#)).

2.4. Vaccines

Placebo consisted in 0.5 mL of saline solution (NaCl). HBsAg-Alum contained 20 µg HBsAg with 500 µg aluminium hydroxide (1 mL). HBsAg-AS01_B contained 20 µg HBsAg with AS01_B (50 µg MPL, 50 µg QS-21 [licensed by GSK from Antigenics-LLC, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation (NASDAQ: AGEN)] and liposomes, 0.5 mL). Vaccines were administered intramuscularly into the non-dominant deltoid. All vaccines were manufactured by GSK. The administered vaccines differed in appearance and volume. Therefore, the study was single-blind and unblinding occurred at D60.

2.5. Sample size

Different standard deviations were considered for the sample size calculation namely; 0.3, 0.5 and 0.8. With 27 subjects per group the study should achieve a 2- to 4-fold ratio between the upper and the lower limit of the 95% confidence interval (CI) for the geometric mean estimation at each time point (precision estimated using PASS 2005 software, CI of a mean, alpha = 5%). Allowing for 10% of non-evaluable subjects for immunogenicity evaluations, a total of 60 subjects were enrolled.

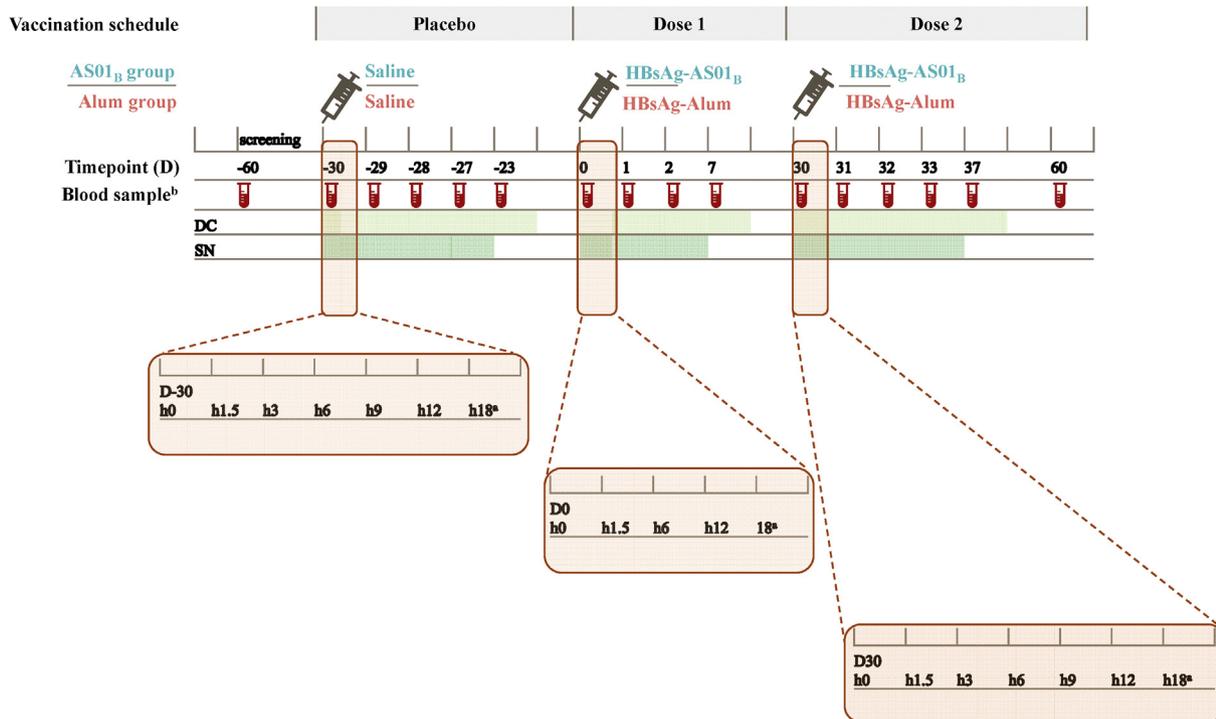


Fig. 1. Study design and procedures. ^aIn a subcohort of 15 participants out of 30 in each group. ^bAntibody measurement: D0(h0), D60. Hematology and biochemistry: D0(h0), D0(h6), D0(h12), D0(h18), D1, D7, D30(h0), D30(h6), D30(h12), D30(h18)^a, D31, D37, D60; Cytokines: D-30(h0), D-30(h1.5), D-30(h3), D-30(h6), D-30(h9), D-30(h12), D-30(h18)^a, D-29, D-28, D-27, D-23, D0(h0), D0(h1.5), D0(h6), D0(h12), D0(h18)^a, D1, D2, D7, D30(h0), D30(h1.5), D30(h3), D30(h6), D30(h9), D30(h12), D30(h18)^a, D31, D32, D33, D37. D = day; h = hour; DC = reactogenicity recorded daily by the participant on diary cards; SN = recording of temperature, heart rate and reactogenicity by the study nurse/investigator. AS01_B group received placebo and two doses of HBsAg-AS01_B. Alum group received placebo and two doses of HBsAg-Alum (and a third dose on D180 not shown here).

2.6. Statistical analysis

Descriptive analyses were performed on peripheral blood parameters and reactogenicity symptoms in the according-to-protocol cohort up to D60. Unsolicited AEs, SAEs and pIMDs were reported for the total vaccinated cohort. A post-hoc analysis was performed to evaluate the correlations between changes in peripheral blood parameters after each immunization and the reactogenicity measured the day after the second vaccination (D31), representing the peak of reactogenicity symptoms. A reduction of the number of parameters, both for the peripheral blood parameters and the reactogenicity measurement was first applied. Regarding peripheral blood, only parameters with evidence of a “treatment effect” were selected for inclusion in the model, i.e., when the levels of the parameter were above the limit of detection and the 95% CI measured for the parameter did not overlap between the AS01_B and Alum groups, at least for one post-vaccination time point. On that basis, five cytokines were selected: IFN- γ , IL-6, inducible protein 10 (IP-10), monocyte chemoattractant protein 2 (MCP-2) and macrophage inflammatory protein (MIP-1 β); and seven hematological/biochemical parameters: lymphocytes, leukocytes, lactate dehydrogenase (LDH), C-reactive protein (CRP), neutrophils, bilirubin and monocytes. Principal component analyses (PCA) (see Supplement 2 Methods) were performed with both the AS01_B and Alum groups in order to explain most of the variability obtained from the measure of peripheral blood parameters after the first dose (D0 to D7) or after the second dose (D30 to D31) of vaccine, reducing then the number of parameters to evaluate to 3 or 5 principal components (PCs). For the calculation of the reactogenicity scores, the solicited local AEs (pain, redness, swelling) and solicited systemic AEs (fatigue, fever [axillary temperature ≥ 37.5 °C], headache, malaise, myalgia) were considered separately. The individual score (per subject) was then calculated

by summing for either all local AEs, or all systemic AEs, the AE gradings recorded at Day 31 (based on the intensity grading described in Supplement 1).

Correlation analyses were then performed using the first PCs to identify peripheral blood signatures governing potential associations with local and systemic reactogenicity scores. A sensitivity analysis was performed on participants who received HBsAg-AS01_B. An analysis also considered individual reactogenicity parameters measured by the study staff, taking into consideration the maximum values of 18 h or one day after the second vaccination (D30 h18 or D31).

A data set extracted from a previously published clinical study (NCT00805389) [13,14], was used to further validate the correlations observed in the current cohort. The study involved healthy HBV-naïve adults 18–45 years of age randomized (1:1:1:1) to receive two doses of HBsAg-combined with AS01_B, AS01_E, AS03, AS04 or three doses of HBsAg-Alum. Upon extracting data from participants vaccinated with HBsAg-AS01_B or with HBsAg-Alum, the same post-hoc statistical analysis was performed (considering time points D0 + 3 to 6 h post first vaccination, D1; D30 3 to 6 h post second vaccination, D31); fold changes of peripheral blood parameters measured in the study that are common in both studies (IFN- γ , IL-6, IP-10, lymphocytes, leukocytes, CRP, neutrophils and monocytes) were analyzed using a PCA, and an appropriate regression model (see Supplement 2 Methods) to evaluate the correlation of the first PCs with the scores of local or systemic reactogenicity measured at D31. Bilirubin, LDH, MIP-1 β , MCP-2 were not evaluated in this earlier study.

3. Results

Participants who received the AS01_B and Alum adjuvanted HBsAg vaccines were similar in terms of age, gender distribution

and geographic ancestry (Supplement 2 STable2). There were no withdrawals from the study due to AEs (Fig. 2).

3.1. AS01_B induces higher antibodies than Alum adjuvanted HBsAg vaccine

Anti-HBs antibody titers after two doses of HBsAg-AS01_B were higher than after three doses of HBsAg-Alum (Fig. 3), confirming previous results [13].

3.2. AS01_B induces transient increases in peripheral blood parameters

The administration of the placebo and of the Alum adjuvanted vaccine did not induce variations in serum levels of the measured cytokines (according-to-protocol cohort; Fig. 4).

Immunization with the AS01_B adjuvanted vaccine induced rapid and transient increases in serum levels of several cytokines, including IL-6, IFN- γ , IP-10, MCP-2 and MIP-1 β . A similar increase of IL-6 levels after the first and second dose of HBsAg-AS01_B was detected

whereas the magnitude of the increase was higher after the second dose for IFN- γ , IP-10, MIP-1 β and MCP-2. Levels of IL-6 peaked 12 h after vaccination whereas IFN- γ levels peaked after 18 h and levels of IP-10, MIP-1 β and MCP-2 peaked one day after vaccination. Levels of all cytokines decreased rapidly, and all returned to baseline by day 7 after each vaccination (Fig. 4). No changes in serum levels of the other measured cytokines were observed after vaccination (data not shown).

Serum levels of CRP and bilirubin increased after vaccination with HBsAg-AS01_B, but not after HBsAg-Alum (Fig. 5), peaking one day after vaccination and returning to baseline by 7 days after vaccination. Peak CRP and bilirubin levels were above normal values in 100% and 75% of HBsAg-AS01_B immunized subjects as per United States Food and Drug Administration Guidance for Industry, but these changes had no clinical relevance [15,16]. Serum LDH levels remained within normal values after vaccination in the AS01_B group but decreased 18 h post-vaccination in the Alum group.

Levels of leukocytes, including monocytes and neutrophils, increased rapidly (by H6) after each AS01_B dose and reduced to

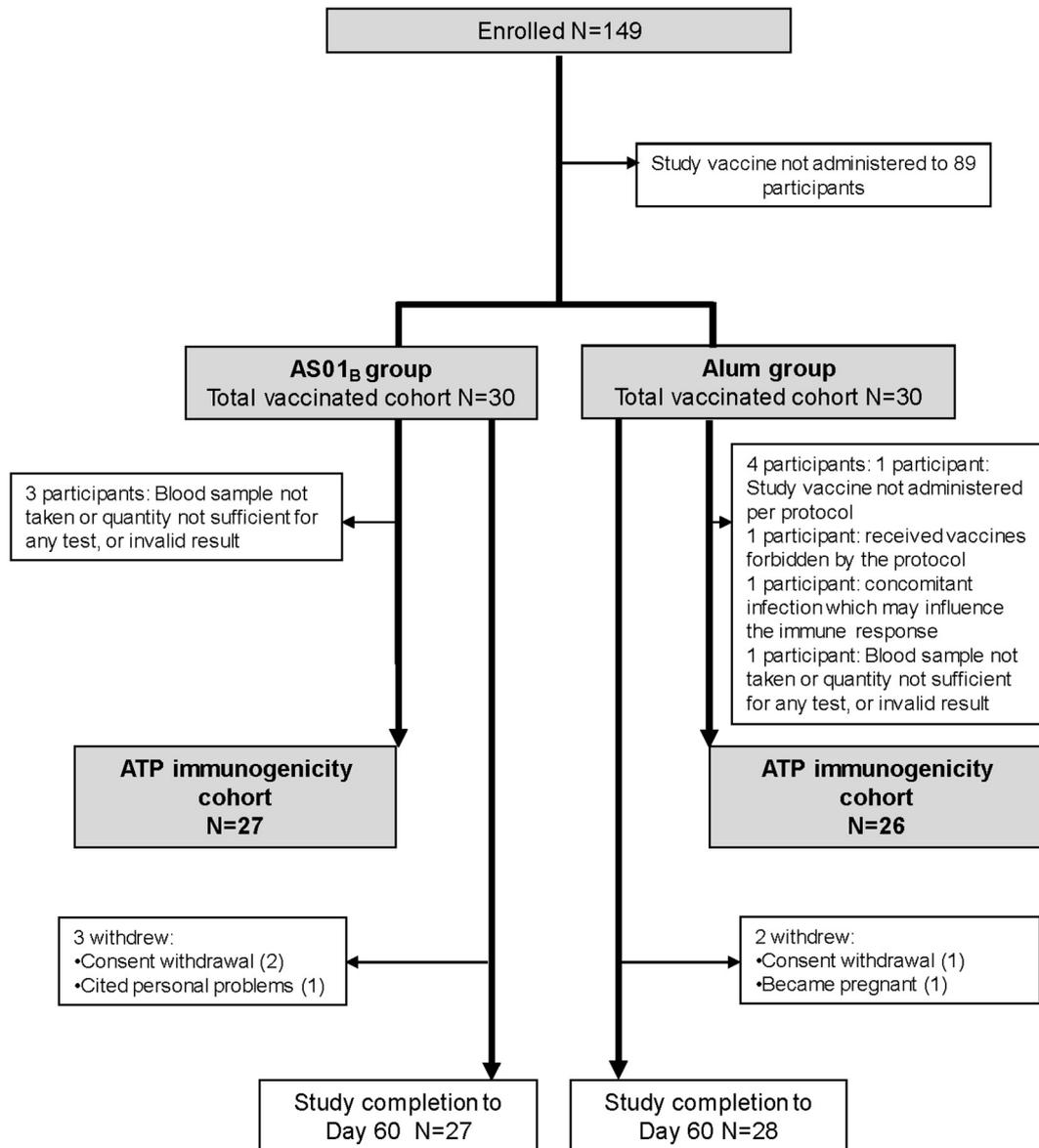


Fig. 2. Study flow. AS01_B group received placebo and two doses of HBsAg-AS01_B, Alum group received placebo and three doses of HBsAg-Alum, N = number of subjects in the indicated cohort, ATP = according to protocol.

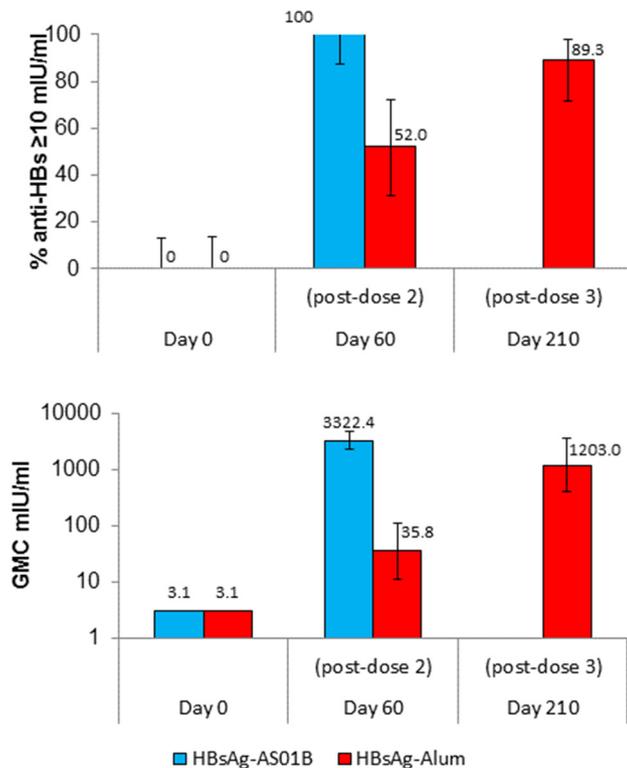


Fig. 3. Anti-HBs antibody titers induced by AS01_B or Alum adjuvanted vaccine. Geometric mean of anti-HBs antibody concentrations (GMCs) (as represented by the numbers in the graphs) with 95% confidence intervals in HBsAg-AS01_B and -Alum groups (according-to-protocol for adaptive immunogenicity up to Day 60, booster total vaccinated cohort for Day 210).

baseline levels by day 7 post-vaccination (Fig. 5). Peak leukocyte counts were above normal values in 57% of subjects but these changes were mild [16]. Such changes were not observed in the Alum group. Levels of lymphocytes increased from 12 h in the Alum group but not in the AS01_B group, returning to baseline by D1. Hematological and biochemical parameters that did not change after vaccination are listed in SFig 1 from Supplement 2.

Principal component analysis (PCA) of the blood dataset was conducted to identify the parameters most affected by the adjuvanted vaccines (Fig. 6). After the first dose of vaccine, the first principal component (PC1) captured 40% of the variability in the dataset, with CRP and IL-6 levels determining most of the variability, whereas the PC2 and PC3 jointly captured 22% of the variability (Fig. 6A and B). For the PC2, the IFN-dependent cytokines IP-10 and MCP-2 were the predominant parameters, while variability in the levels of IP-10 and neutrophils accounted for most of the variability captured by the PC3.

After the second vaccination, the PC1 explained 54% of the variability, which was mostly determined by IFN- γ , IP-10 and CRP (Fig. 6C and D). The variability captured by the PC2 and PC3 (collectively 17%) was mostly influenced by CRP, IP-10, MCP-2 and MIP-1 β .

3.3. AS01_B adjuvanted vaccine induces higher reactogenicity than Alum adjuvanted vaccine or placebo

The profiles of solicited symptoms were similar following the administration of Alum adjuvanted vaccine and placebo (Figs. 7 and 8). Local and systemic symptoms were more commonly observed after the administration of the AS01_B adjuvanted vaccine. These symptoms were transient, peaking one day after vaccination

and resolving by the second to third day (Fig. 8). Local symptoms, especially pain, were reported by more participants after injections with HBsAg-AS01_B than after HBsAg-Alum, and after injections with HBsAg-Alum than with placebo. Fatigue, malaise, increase in body temperature and shivering were more common after the second dose as compared to the first dose of AS01_B adjuvanted vaccine. Unsolicited symptoms are summarized in the Supplement 2 results. No pIMDs or related SAEs were reported during the study. Regular temperature measurement by study staff (Fig. 8) detected an increase in body temperature in HBsAg-AS01_B recipients at 12 and 18 h post-dose 2 (29.6% with fever after the second dose), and to a lesser extent post-dose 1, with a peak at 18 h (during the night when a nadir in body temperature is expected) [17]. Heart rate was maximal at 18 h post-dose 2 in the AS01_B group (Supplement 2 SFig 2), coinciding with the peak in body temperature (Fig. 8). Median heart rate remained within the normal range [16] at all time points (Supplement 2 SFig 2). There was little change in respiratory rate after vaccination (data not shown).

3.4. Changes in IL-6, CRP, IFN- γ , and IP-10 are associated with reactogenicity

Regression analysis was conducted to identify blood parameters associated with local or systemic reactogenicity measured one day after the administration of the second vaccine dose (day 31). For this purpose, a global score of solicited systemic and local symptoms was calculated (Supplement 2 SFig 3). Analyses included the principal components of blood parameters identified after the first and second dose of vaccine (Fig. 6).

A significant association was observed between blood parameters measured after the first dose of vaccine and systemic reactogenicity at D31 (Table 1). Among the five components of the PCA, the first PC (PC1) was the only individual component significantly associated with the systemic reactogenicity. After the second dose of vaccine, significant associations were detected between systemic reactogenicity and blood parameters measured summarized by PC1 and PC3 (Table 2). Collectively, these results suggest that PC1 (post-one and post-two vaccine doses) captured the most relevant blood parameters associated with systemic reactogenicity at D31 induced by adjuvanted vaccines. Similar results were obtained in a sensitivity analysis including only the AS01_B group, indicating that the overall association was not the result of a treatment effect (data not shown).

In contrast, no significant association was detected between local reactogenicity at D31 and blood parameters measured after the first vaccine dose, and only a weak association involving PC3 was detected after the second vaccine dose (Tables 1 and 2). Non-standard parameters of reactogenicity measured by study staff did not improve the performance of the models (data not shown). No regression analysis including other AEs was performed.

To validate the results in an independent cohort, data from a previously published study of healthy adults immunized with HBsAg combined with Alum or AS01_B were analyzed (N = 43 and 52, respectively) [13,14]. In this study, peripheral blood parameters induced one day after immunization were identified that correlated with the immunogenicity of adjuvanted vaccines. Changes in peripheral blood parameters and reactogenicity were similar to the present study (Supplement 2 SFig 4) [13]. Peripheral blood parameters common to both studies were included in this secondary analysis. No significant association was observed between systemic reactogenicity at D31 and peripheral blood parameters measured after the first vaccine dose, even if a trend for discrimination between subjects was observed with PC1 and PC3 (explaining together about 61% of the variability in peripheral blood parameters, and mostly influenced by IL-6, CRP and IP-10, Supplement 2 SFig 4A). In contrast, a strong association was observed

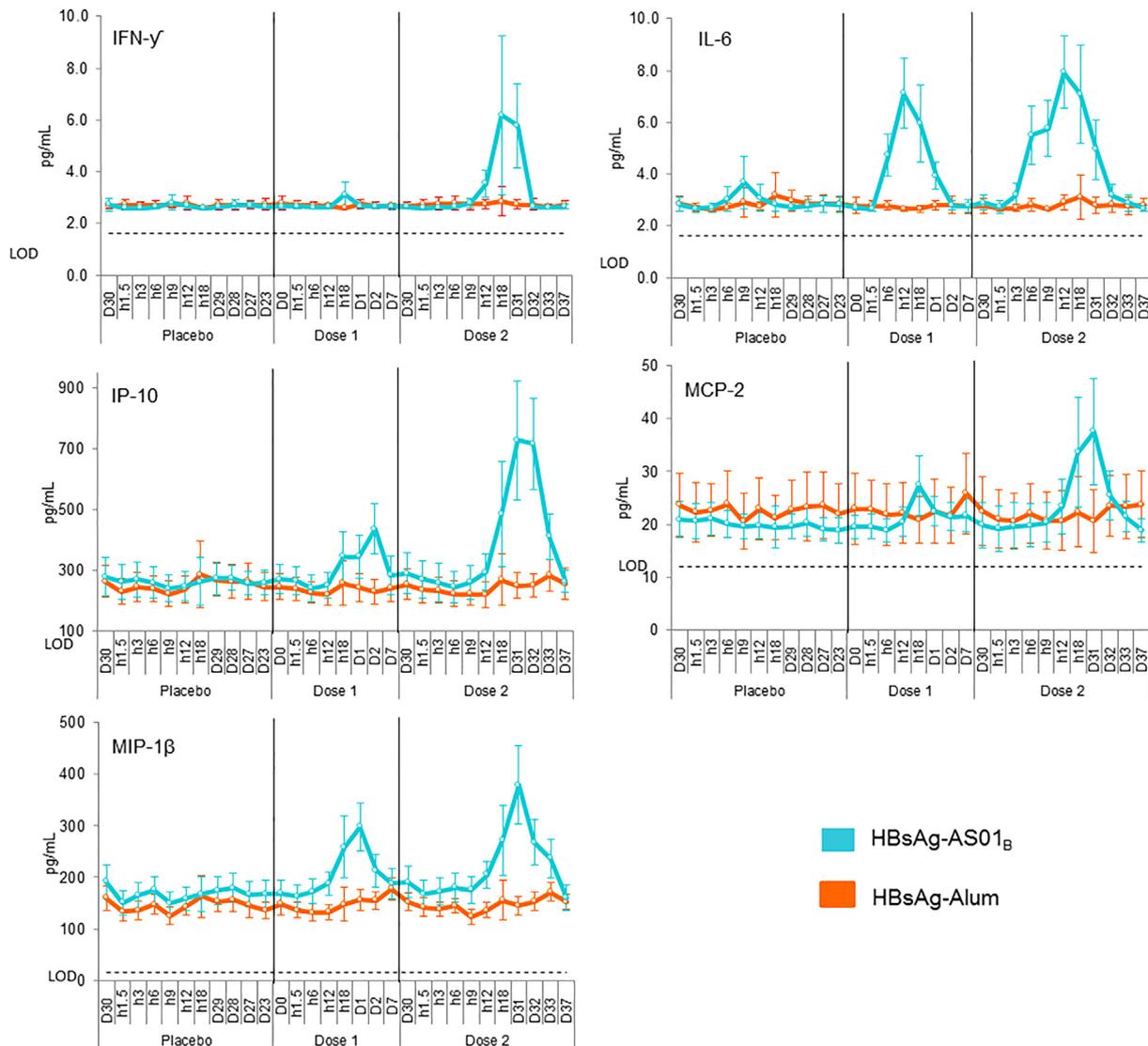


Fig. 4. Inflammatory cytokines in serum induced by AS01_B or Alum adjuvanted vaccine. Kinetics of 5 out of 24 cytokines over placebo, dose 1 and dose 2 periods, geometric mean and 95% confidence interval (according-to-protocol immunogenicity for innate immunity up to Day 60).

between systemic reactivity at D31 and peripheral blood parameters measured after the second vaccine dose, essentially due to PC1, explaining approximately 50% of the variability of peripheral blood parameters, and mostly influenced by IL-6, CRP, IFN- γ , IP-10 (Supplement 2 SFig 4B). As observed in the primary cohort, no association was observed between peripheral blood parameters and local reactivity (Supplement 2 SFig 4). Together, these results of this validation cohort confirm the association between reactivity and peripheral blood parameters identified in the present study.

4. Discussion

Intensive monitoring of reactivity and indicators of inflammation in peripheral blood were performed to provide in depth characterization of the response to AS01_B and Alum adjuvanted HBsAg vaccines. A post-hoc analysis explored associations between those parameters. Inclusion of a placebo phase controlled for any potential impact of injection, repeated venipuncture, and diurnal variation within the same individuals who later received vaccination. By providing a contextual analysis of symptoms reported after administration of adjuvanted vaccine, we observed that injection

of placebo had no impact on the production of cytokines, but was followed by local and systemic symptoms in a percentage of individuals.

Local and systemic symptoms were more common after HBsAg-AS01_B than HBsAg-Alum, with an increase in systemic symptoms after the second dose of HBsAg-AS01_B as compared to the first dose, as observed in another clinical study [13]. In line with other vaccines using AS01_B [11,12], the majority of signs and symptoms were short-lived and were mild-to moderate in intensity.

Analysis of multiple time points after vaccination, including a time point not previously investigated, i.e., 18 h, allowed a detailed analysis of the kinetics of modifications in blood parameters. Modulation of some blood parameters began as early as 3 h after administration of the AS01_B adjuvanted vaccine. The several waves of blood parameter changes detected during the first 48 h post-vaccination suggest sequential responses induced by intermediate mediators. IL-6 levels peaked 12 h after vaccination, followed by increased levels of the acute phase reactant CRP. IFN- γ levels peaked at 18 h and was followed by the production of three IFN-dependent cytokines, IP-10, MIP-1 β and MCP-2 [18–20]. The highest response of several parameters, including IFN- γ , IP-10, MIP-1 β and MCP-2, after the second dose of vaccine, suggests the

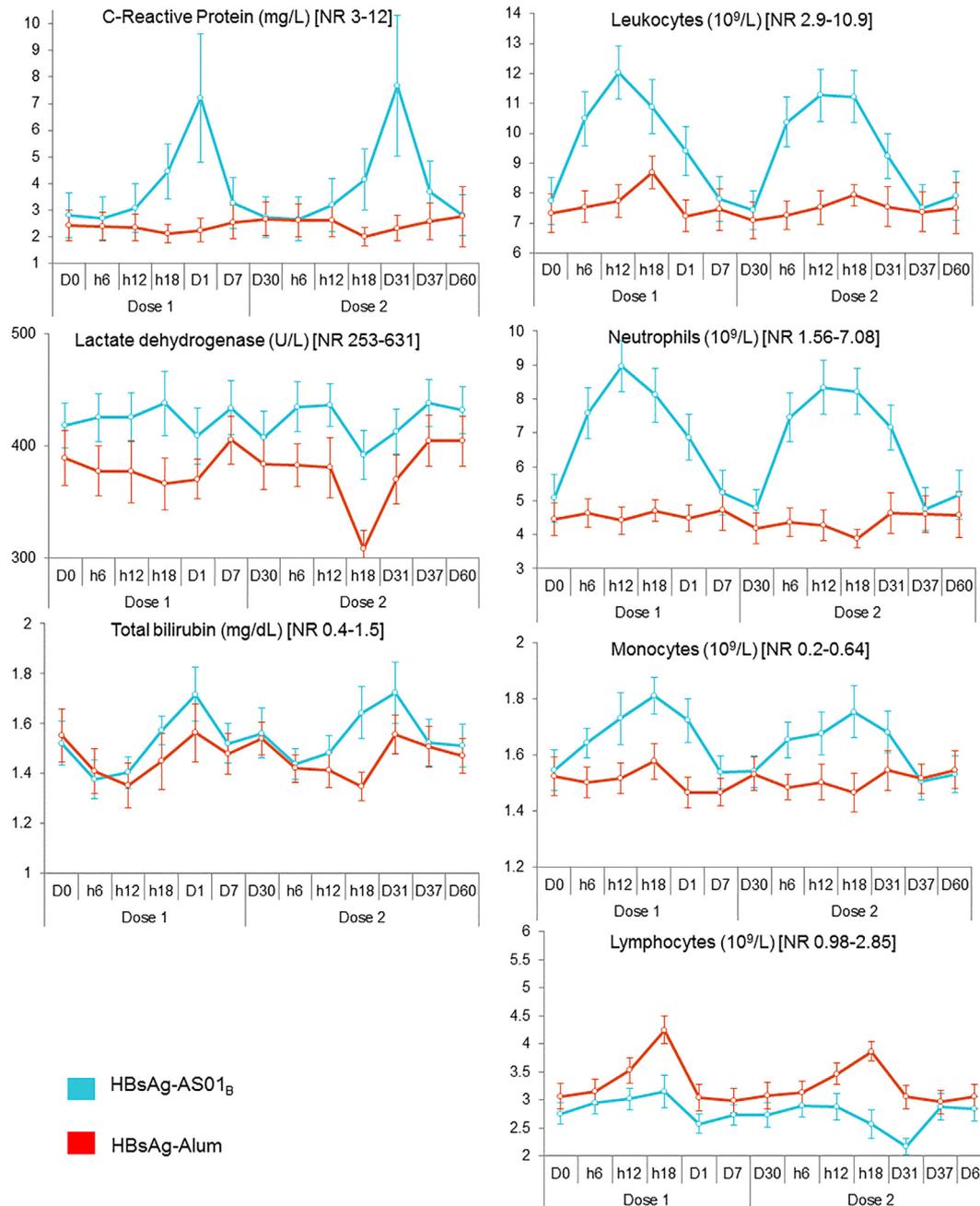


Fig. 5. Hematological and biochemical parameters induced by ASO1_B or Alum adjuvanted vaccine. Kinetics of 7 hematological or biochemical parameters showing change over time after vaccination over dose 1 and dose 2 periods, geometric mean and 95% confidence interval (Total vaccinated cohort, up to Day 60). NR, normal range; U/L, units per litre.

involvement of the adaptive immune response induced by the vaccine after the first dose. Alternatively, this phenomenon may involve the induction of immunological memory at the level of innate cells, a phenomenon called trained immunity [21]. Peak cytokine responses at 18 h coincided with peaks in body temperature and heart rate, although both remained within normal physiological ranges [21]. The kinetics of response observed in this clinical study is analogous to the responses observed in preclinical models with, for example, an early detection of IL-6 in the muscle and draining lymph nodes of mice, followed by a more prolonged IP-10 response [22,23], and of CRP in the serum of rabbits after administration of ASO1_B-adjuvanted vaccines [24,25]. The common induction of increased levels of CRP by ASO1_B-adjuvanted vaccine suggests that this marker could be used in the monitoring of the innate immune

response induced by adjuvanted vaccines. It also indicates that the possibility of a vaccine response should be considered in the clinical evaluation of patients showing a mild increase in CRP levels in the first days after administration of an adjuvanted vaccine.

While the intensity of systemic symptoms was correlated with levels of specific markers (IL-6, CRP, IFN- γ , IP-10, and MCP-2), only weak associations were observed between local reactogenicity and various modifications in blood parameters following vaccination. This suggests that systemic and local reactions after vaccination may involve different innate immune response parameters, or a different kinetic of the same parameters at the local level. Importantly, the associations were reproduced in an independent cohort of healthy volunteers vaccinated with ASO1_B and Alum adjuvanted vaccines, emphasizing the robustness of the results.

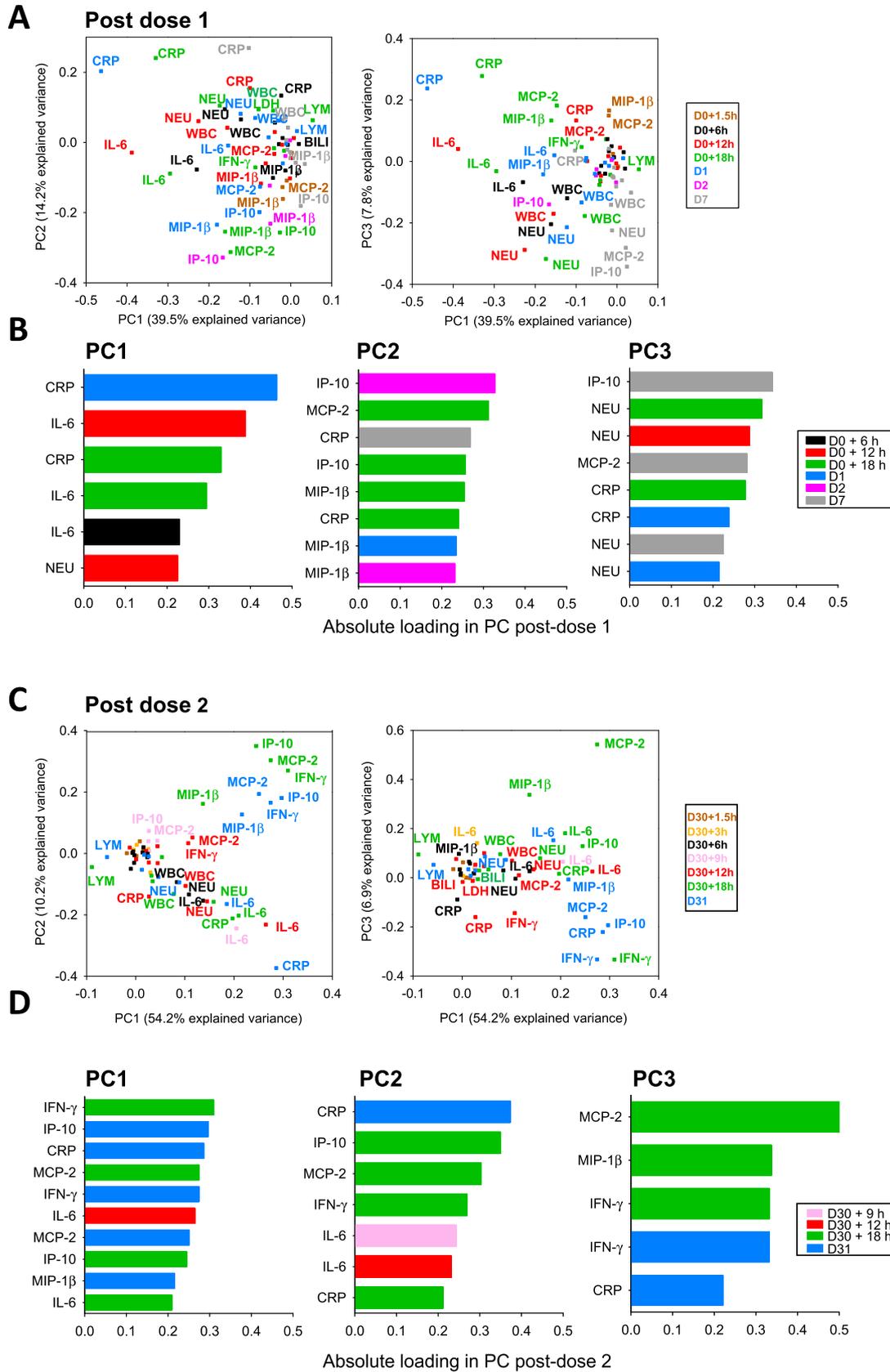


Fig. 6. Principal component analysis of the blood parameters that were most affected by the adjuvanted vaccines. The innate response data-sets comprising selected blood parameters were summarized by principal component (PC) analyses performed after the first dose (A and B) and the second dose (C and D) of the AS01_B- or Alum-adjuvanted vaccines. The first three PCs (PC1–3) are presented. A/C: The variables represented by the PC1–3, measured either post-dose 1 (A) or post-dose 2 (C) are shown for the post-vaccination time-points that are indicated by the color coding in the corner of the plots. LYM: lymphocytes. NEU: neutrophils. WBC: White blood cells. BILI: bilirubin. B/D: Bar graphs representing the absolute loading (using an arbitrary cutoff of $> |0.2|$) of the blood parameters in the PC1, PC2 and PC3 (left, middle and right plots respectively) are shown for the time-points post-dose 1 (B) or post-dose 2 (D).

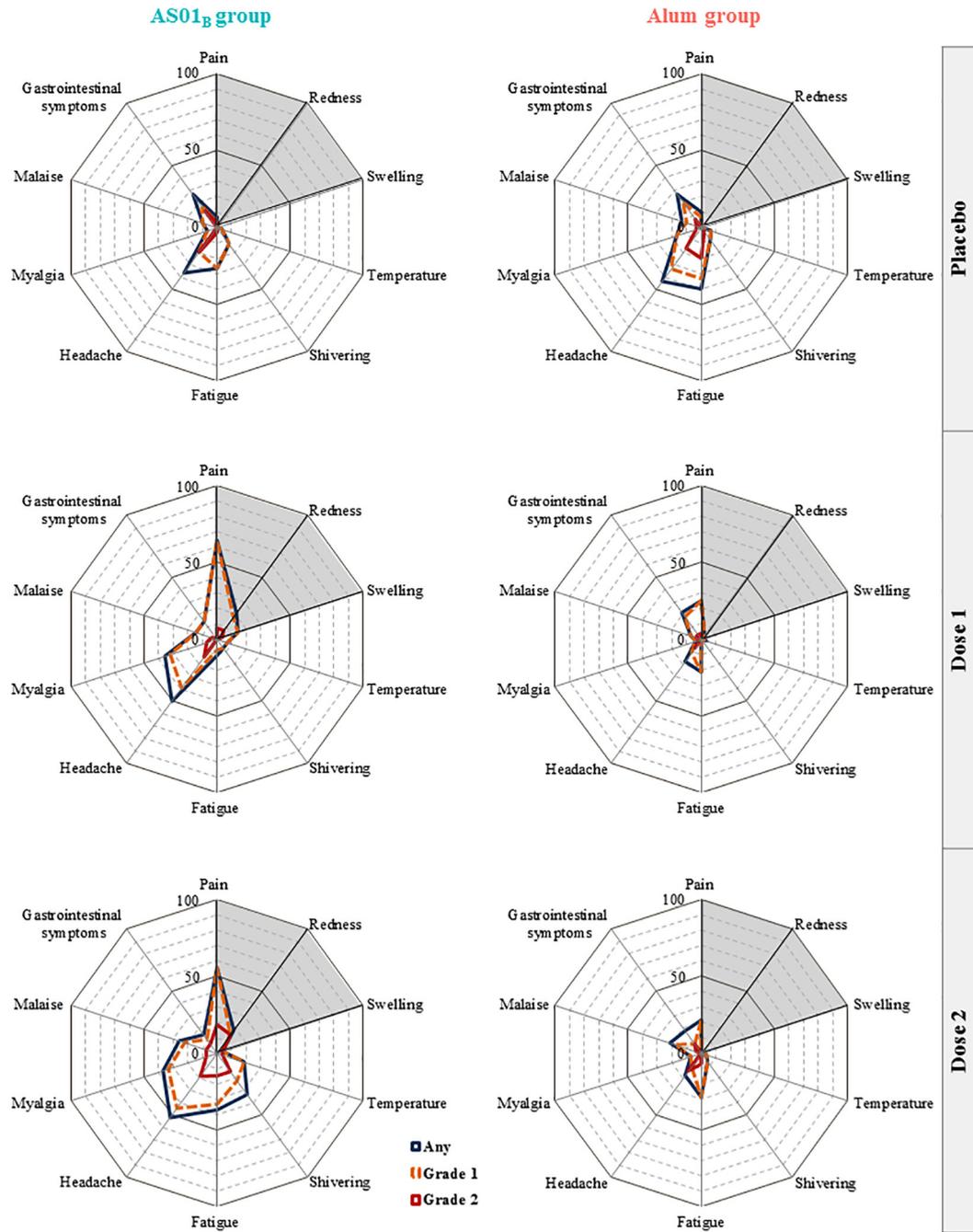


Fig. 7. Star graph of the prevalence of each solicited symptoms reported during the 7-day (Days 0–6) post-placebo/vaccine period after each dose (Total vaccinated cohort). Shaded = local symptoms. Pain: Grade 1 mild: Any pain neither interfering with nor preventing normal every day activities. Grade 2 moderate: Painful when limb is moved and interferes with every day activities. Severe: Significant pain at rest, preventing normal every day activities. Redness, Swelling: Grade 1 mild: >20 – ≤50 mm, Grade 2 moderate: >50 – ≤100 mm, Grade 3 severe > 100 mm. Systemic symptoms. Temperature: Any: ≥37.5 °C, Grade 1 mild: ≥37.5 °C – <38.6 °C, Grade 2 moderate: ≥38.6 °C – <39.5 °C, Grade 3 severe: ≥39.5 °C. Fatigue, Headache, Gastrointestinal symptoms, Myalgia, Malaise, Shivering: Grade 1 mild: Symptom that is easily tolerated, Grade 2 moderate: symptom that interferes with normal activity, Grade 3 severe: prevents normal activity. Concentric grid represents 0–100% of participants reporting the symptom. Grade 3 is not shown as the number of cases is too small to be clearly visible. AS01_B group received placebo and two doses of HBsAg-AS01_B. Alum group received placebo and three doses of HBsAg-Alum.

Previous analyses of the cohort used to confirm our observations indicated an association between the IL-6 and IFN signals and the adaptive immune response induced by AS01_B adjuvanted HBsAg vaccine [14]. It appears therefore that the same early cytokine responses are associated with both systemic reactivity and adaptive immune response induced by an AS01_B adjuvanted vaccine. Causal relationship between innate and adaptive parameters was demonstrated in animal studies whereby the beneficial effect of AS01_B on the adaptive immune response required the

stimulation of innate cells and signals, including dendritic cells, natural killer cells, macrophages, TLR4, caspase-1 and Myd88 signals [22,23,26]. Although our study has not established causal relationships, it suggests that the reactogenicity associated with the addition of adjuvants to vaccine formulations involves innate immune response parameters that are also associated with their adjuvant effect on the antigen-specific immune response. This may complicate the development of future adjuvants with potent immunogenicity and minimal reactogenicity profiles. However,

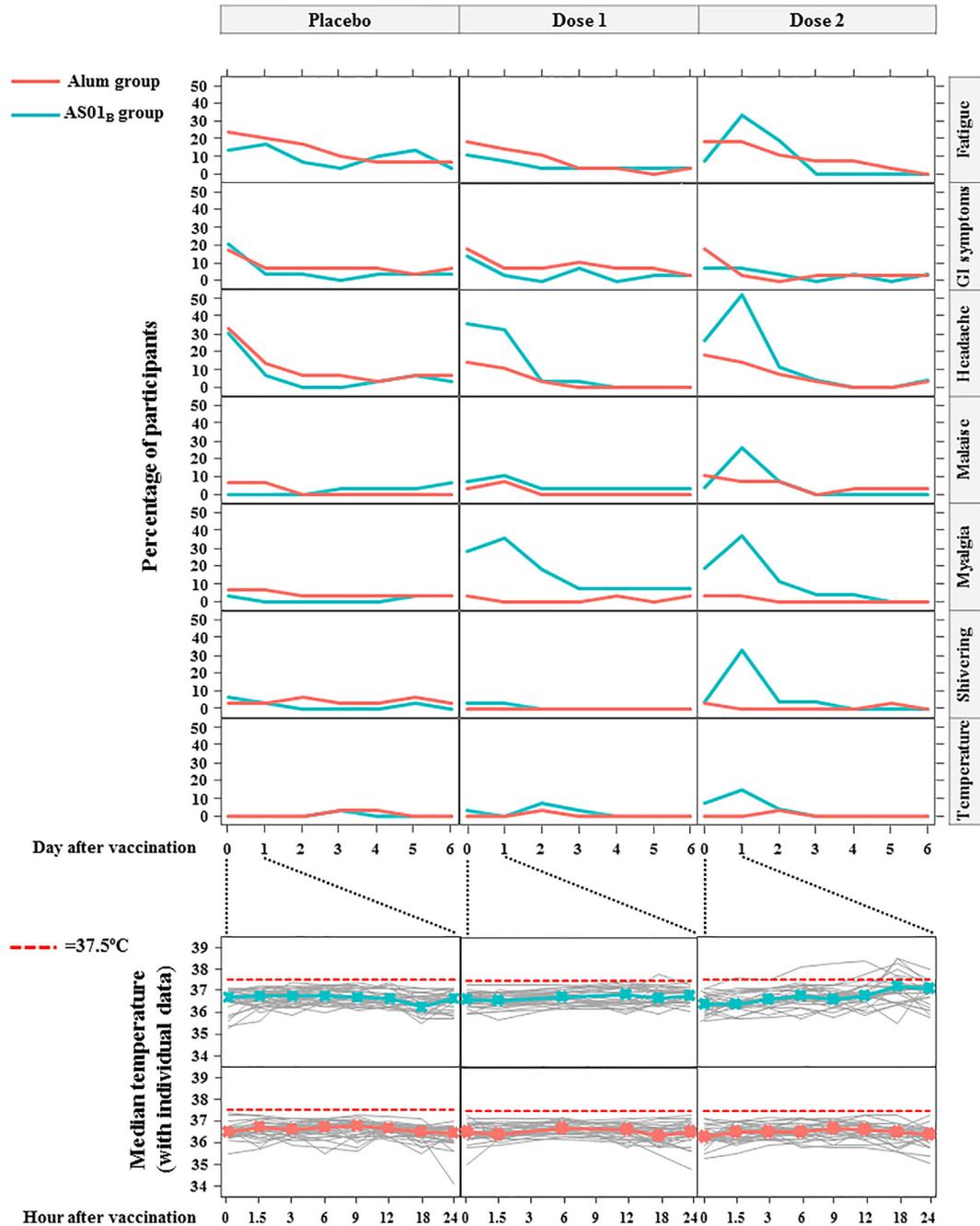


Fig. 8. Kinetics of the prevalence of each solicited general symptom (any severity and fever $\geq 37.5^\circ\text{C}$) reported during the 7-day (Days 0–6) post-placebo/vaccine administration (Total vaccinated cohort). AS01_B group received placebo and two doses of HBsAg-AS01_B. Alum group received placebo and two doses of HBsAg-Alum. GI = gastrointestinal. Data show symptoms as reported daily by participants on diary cards. The lower panels show body temperature as recorded by study staff at numerous defined time points after vaccination (median with individual data [grey lines]).

we cannot exclude that other parameters, not tested in our study, are specifically associated with the reactogenicity or the immunogenicity of adjuvanted vaccines. Indeed, in a recent study on yellow fever vaccination, innate immune signals specifically associated with reactogenicity but not immunogenicity were identified [27]. Identifying key innate immune parameters differentially involved in immunogenicity and reactogenicity will help in the design of future adjuvants targeting specifically cellular and molecular pathways underlying immunogenicity. This will require specifically designed and sufficiently powered systems vaccinology studies.

Several limitations to this study may be considered. Our discovery cohort included a limited number of subjects, nevertheless clear associations were found and the results were confirmed with an independent and larger cohort. Vaccine response parameters were analyzed in peripheral blood and may not reflect inflammatory signals induced at the site of vaccine injection. Finally, the study was conducted in healthy adults using HBsAg as antigen, and the applicability of findings to other age groups, populations and to other vaccines is not known.

In conclusion, the systemic reactogenicity of an AS01_B adjuvanted vaccine is associated with innate immune response elements

Table 1
Associations between systemic and local reactogenicity measured at Day 31 and the innate response post-dose 1 (N = 51).

β parameter	Systemic Reactogenicity D31				Local Reactogenicity D31			
	Est.	SE	P (> Z)	P (>F)	Est.	SE	P (> Z)	P (>F)
Intercept	−0.055	0.348	0.874		−2.201	0.766	0.004	
AS01B	0.281	0.512	0.583		2.016	0.953	0.034	
PC1	−1.304	0.311	<0.001		−0.564	0.505	0.264	
PC2	−0.212	0.295	0.474		0.003	0.509	0.996	
PC3	0.060	0.393	0.880		0.506	0.593	0.393	
PC4	0.535	0.469	0.254		1.098	0.640	0.086	
PC5	−0.072	0.601	0.905		0.969	0.987	0.326	
–				<0.001				0.332

Multi-parametric analyses of solicited reactogenicity symptoms recorded at 1 day post-dose 2 (day 31) performed for each coefficient (β) of the listed model input parameters are presented in terms of the estimate ('Est.') of the effect size, standard error (SE) and p-value (derived using a Z-test [P(>|Z|)] or partial Fisher test [P (>F)]). Input parameters included the innate responses post-dose 1, as represented by their first 5 principal components (PC1-5). PCs that were associated (p < 0.05) with reactogenicity are indicated by bold font.

Table 2
Associations between systemic and local reactogenicity measured at Day 31 and the innate response post-dose 2 (N = 51).

β parameter	Systemic Reactogenicity D31				Local Reactogenicity D31			
	Est.	SE	P (> Z)	P (>F)	Est.	SE	P (> Z)	P (>F)
Intercept	−0.169	0.334	0.612		−2.332	0.745	0.002	
AS01B	0.422	0.464	0.363		2.063	0.865	0.017	
PC1	0.727	0.166	<0.001		0.564	0.307	0.067	
PC2	−0.618	0.321	0.054		0.171	0.425	0.688	
PC3	−1.367	0.331	<0.001		1.717	0.675	0.011	
–				<0.001				0.024

Multi-parametric analyses of solicited reactogenicity symptoms recorded at 1 day post-dose 2 (day 31) performed for each coefficient (β) of the listed model input parameters are presented in terms of the estimate ('Est.') of the effect size, standard error (SE) and p-value (derived using a Z-test [P(>|Z|)] or partial Fisher test [P (>F)]). Input parameters included the innate responses post-dose 2, as represented by their first 3 principal components (PC1-3). PCs that were associated (p < 0.05) with reactogenicity are indicated by bold font.

that were also found to be associated with the adaptive immune response to the vaccine. This observation underscores the key role of the innate immune signals in driving the response to adjuvanted vaccines.

5. Trademarks

Shingrix and Engerix are trademarks owned or licensed to the GSK group of companies.

The GSK proprietary AS01 Adjuvant System contains QS-21 adjuvant licensed from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation (NASDAQ: AGEN), MPL and liposomes.

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Conflict of interest

AC, AD, CH, FTdS, JY, LF, MC, RvdM, and WB are employees of the GSK group of companies. AC, AD, FTdS, JY, RvdM and WB hold shares in the GSK group of companies. AD and RvdM own patents related to AS01. AM is research director at the Fonds de la Recherche Scientifique, F.R.S.-FNRS, Belgium, Belgium.

Author contributions

AD, AM, CH, FTdS, FW, LF, RvdM and WB participated to the conception, planning and/or design of the study. AM, CH, FT, FW, LG, and WB participated in the data generation. AC, AD, AM, CH, CL, CN, FTdS, FW, JY, LF, MC, RvdM and WB performed or supervised the analysis of data and interpretation of the results. AD, AM, FW, LG and WB participated in the performance of the study. AC, CN, CL, LF, MC provided with statistical expertise for the modelling and/or analysis and interpretation of the results. WB led the development of the outline. All authors participated in the development of this manuscript. All authors had full access to the data, gave final approval before submission and agree to be accountable for all aspects of the work. The corresponding author was responsible for submission of the publication.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2019.02.015>.

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