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Short communication

AS03-adjuvanted H7N1 detergent-split virion vaccine is highly immunogenic in unprimed mice and induces cross-reactive antibodies to emerged H7N9 and additional H7 subtypes

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ABSTRACT

Avian H7 is one of several influenza A virus subtypes that have the potential to cause pandemics. Herein we describe preclinical results following administration of an investigational H7N1 inactivated detergent-split virion vaccine adjuvanted with the AS03 Adjuvant System. The adjuvanted H7N1 vaccine was highly immunogenic compared to the non-adjuvanted H7N1 vaccine in unprimed mice with less than 100 ng of hemagglutinin antigen per dose. In addition, compared to the non-adjuvanted vaccine, the AS03-adjuvanted H7N1 vaccine also induced robust HI and VN antibody responses that cross-reacted with other H7 subtypes, including recently emerged H7N9 virus. These H7 data from the preclinical mouse model add to the existing H5 data to suggest that AS03 adjuvant technology may be generally effective for formulating antigen-sparing detergent-split virion vaccines against intrinsically sub-immunogenic avian influenza A virus subtypes.

Low pathogenic H7 influenza viruses circulate in wild birds throughout the world, sporadically are introduced into domestic poultry populations, and typically cause sub-clinical infections that can be difficult to detect and monitor [1–3]. Prior to 2013, both low pathogenic and highly pathogenic avian H7 influenza viruses caused sporadic human infections in Europe and the Americas, primarily in persons exposed to poultry [4–9]. In 2003, a large outbreak of human disease caused by highly pathogenic H7N7 virus occurred in the Netherlands [5,6]. Although most infections were mild, there was a single fatality. Sustained human-to-human transmission of H7 subtype viruses has not been reported, but some H7 viruses exhibit enhanced transmissibility in mammalian models [10]. More recently, in February 2013, human influenza cases caused by a novel low pathogenic H7N9 virus were reported in the Anhui and Shanghai regions of eastern China [11,12]. By mid-May 2015, the total number of H7N9 cases has exceeded 650 and in addition to the cases in China, isolated cases have been identified in Taiwan, Malaysia, and Canada. Consistent with the epidemiology in humans where sustained human-to-human transmission has not been reported [13], H7N9 viruses have demonstrated a limited ability to transmit through respiratory droplets in the ferret transmission model [14,15] suggesting additional genetic changes are needed for the virus to fully adapt to humans. However, since H7N9 viruses continue to circulate in domestic poultry in China, and additional human cases are likely to occur, the pandemic risk posed by H7N9 viruses has heightened the need for highly effective H7 subtype vaccines.

Previous attempts to develop H7 subtype vaccines as part of pandemic preparedness programs have met with mixed success. Adjuvanted and non-adjuvanted inactivated H7 vaccines were poorly immunogenic in Phase I testing even with relatively high quantities of HA antigen [16,17]. More recently, live attenuated H7 subtype vaccines [18–20] as well as adjuvanted VLP and inactivated

Abbreviations: HA, hemagglutinin; NA, neuraminidase; HI, hemagglutination inhibition; VN, virus neutralization; GMT, geometric mean titers; AS03, Adjuvant System 03; SRID, single radial immunodiffusion; NIBSC, National Institute for Biological Standards and Control.

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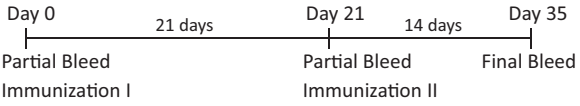

Fig. 1. *Study treatment schedule.* On study day 0, mice were bled (pre-immunization baseline) and then immunized with either the H7N1 or the H5N1 vaccine (immunization I). On study day 21, mice were bled (post-I immunization) and then immunized with the respective vaccine a second time (immunization II). On study day 35, mice were bled (post-II immunization) a final time. Serum HI antibody titers to the homologous H7N1 or H5N1 vaccine antigens were measured post-I and post-II immunization to determine the boosting effect of the respective vaccine.

H7 subtype vaccines were generally well-tolerated in early clinical testing [21,22], and the adjuvanted inactivated vaccine achieved higher immunogenicity with relatively lower quantities of HA than the previous H7 subtype inactivated vaccines.

We have undertaken early development of an investigational H7N1 inactivated detergent-split virion vaccine adjuvanted with the oil-in-water emulsion AS03 Adjuvant System (hereafter referred to as AS03) that can serve as a model for H7 subtype, including H7N9, vaccine development. In the current studies in mice, we demonstrated that the AS03-adjuvanted H7N1 vaccine was highly immunogenic with an antigen-sparing effect. This H7N1 vaccine performed similarly to a licensed AS03-adjuvanted H5N1 inactivated detergent-split virion vaccine which was included as a comparator in the current studies. In addition, the AS03-adjuvanted H7N1 vaccine induced robust serum antibody responses against other H7 viruses from both the Eurasian and North American lineages, including the recently emerged H7N9 virus as well as highly pathogenic H7N7 and H7N3 viruses. These H7 data from the preclinical mouse model add to the existing H5 data to suggest that AS03 adjuvant technology may be generally effective for formulating antigen-sparing detergent-split virion vaccines against intrinsically sub-immunogenic avian influenza A virus subtypes that have historically presented several challenges to vaccine development.

Mice were serially immunized with the investigational H7N1 vaccine or the comparator H5N1 vaccine and bled according to the schedule described in Fig. 1. Serum antibody responses were measured by hemagglutination inhibition and viral neutralization assays to the vaccine strain as well as additional H7 strains. The vaccines, viruses, animal procedures, immuno assays, and statistical analysis are described in detail in the Supplementary Methods.

Mice were dosed with the investigational H7N1 vaccine based on estimated HA content using an HA:total protein ratio of 1:3 which translated to a bulk vaccine concentration of 100.4 μg HA/mL. Estimating the HA dose was necessary since the SRID antigen reference reagent calibration from NIBSC was unavailable at the time of study initiation. After the reagent calibration was issued, the actual HA content of the vaccine lot used in this study was determined to be 129.0 μg HA/mL after 30 months of storage at 2–8 °C, which is 29% higher than the estimated HA content. The underestimation of the HA content in the investigational H7N1 vaccine resulted in exceeding the targeted HA dosage used in the current study. However, the impact of this was not considered to be technically or biologically significant as the difference between the estimated and actual HA content was within the experimental variability of the SRID assay and below the sensitivity of the unprimed mouse immunogenicity model to detect when using AS03 as an adjuvant (unpublished observations).

The reciprocal geometric mean HI titers obtained post-I and post-II immunization (Fig. 1) with the H7N1 and H5N1 vaccines with and without AS03 are presented in Fig. 2. AS03 has previously been shown to adjuvant monovalent H3N2 and H5N1 detergent-split virion vaccine antigens effectively in different preclinical models including BALB/c mice [23], ferrets [24], and pigs [25], and these models have predicted enhanced immunogenicity

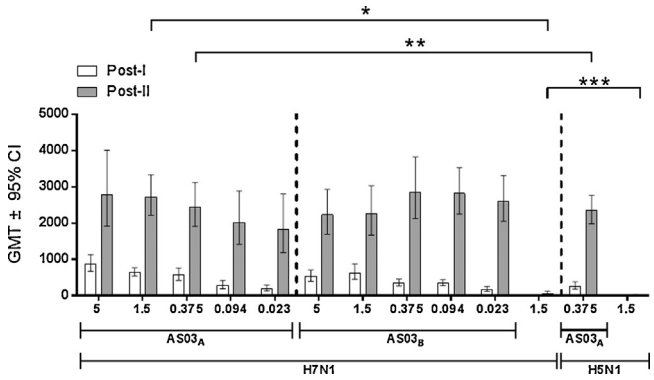

Fig. 2. *Serum hemagglutination inhibition (HI) antibody titers in mice immunized with the H7N1 vaccine (plus/minus indicated adjuvant) or the H5N1 vaccine (plus/minus indicated adjuvant).* HI titers for each treatment group are expressed as reciprocal geometric mean titers ± 95% confidence intervals post-I immunization (study day 21) and post-II immunization (study day 35). HI assays were performed as described in the Supplementary Methods using H7N1 or H5N1 detergent-split vaccine antigen, 0.5% horse erythrocytes, with 2 hrs of incubation of virus/erythrocyte mixtures. Numbers on X-axis indicate micrograms of estimated HA (H7N1 vaccine) or actual HA (H5N1 vaccine). No antibody titers (HI titers < 20) were detected in the PBS control mice (data not shown). * $P < 0.0001$ [H7N1 (1.5 μg estimated HA) with $AS03_A$ vs. H7N1 (1.5 μg estimated HA) without $AS03_A$]. ** $P > 0.05$ [H7N1 (0.375 μg estimated HA) with $AS03_A$ vs. H5N1 (0.375 μg actual HA) with $AS03_A$]. *** $P > 0.05$ [H7N1 (1.5 μg estimated HA) without $AS03_A$ vs. H5N1 (1.5 μg actual HA) without $AS03_A$].

in humans. The post-I immunization HI titers were collected for informational purposes, whereas the post-II immunization HI titers were considered the definitive antibody endpoint, as the investigational H7N1 vaccine was anticipated to be a two-dose vaccine regimen in humans that will most likely be immunologically naïve to H7 viruses. The H7N1 vaccine was highly immunogenic across a broad range of antigen doses tested (5 to 0.023 μg estimated HA) when adjuvanted with $AS03_A$ or $AS03_B$ after both the first and second immunization. However, the magnitude of the HI titers increased substantially following the second immunization, thus demonstrating a strong boosting effect. Comparable immunogenicity has been achieved in unprimed mice with AS03-adjuvanted monovalent detergent-split virion vaccines produced from other avian subtypes, e.g., H7N9, down to a vaccine antigen level of 0.0015 μg HA (manuscript in preparation). Thus, $AS03_A$ and $AS03_B$ are able to adjuvant immune responses down to very low levels of vaccine antigen, thereby demonstrating antigen dose-sparing properties that could be critical in a pandemic setting where global vaccine demand could exceed global vaccine supply. Post-I and post-II immunization HI titers elicited by the $AS03_A$-adjuvanted H7N1 vaccine were very close to the titers elicited by the $AS03_A$-adjuvanted H5N1 vaccine when both vaccines were administered at comparable dosages ($P > 0.05$). A vaccine dose–response trend was observed post-I immunization when the H7N1 vaccine was adjuvanted with $AS03_A$ or $AS03_B$, whereas a more shallow vaccine dose–response trend was observed post-II immunization when the H7N1 vaccine was adjuvanted with $AS03_A$ only. Interestingly, 100% of mice were responders (HI titer > 40) in all adjuvanted ($AS03_A$ and $AS03_B$) vaccine groups post-I and post-II immunization when immunized with H7N1 vaccine levels as low as 0.023 μg estimated HA. Conversely, the non-adjuvanted H7N1 vaccine was poorly immunogenic post-I and post-II immunization compared to the $AS03_A$-adjuvanted H7N1 vaccine when tested at the same dosage (1.5 μg estimated HA), thus indicating a strong adjuvant effect ($P < 0.0001$). Indeed, 79% and 43% of the mice were non-responders (HI titer < 40) post-I immunization and post-II immunization, respectively, following immunization with the non-adjuvanted H7N1 vaccine, and HI titers post-I immunization (HI GMT = 16) and post-II immunization (HI GMT = 56) were low. The

Table 1
Anti-H7 hemagglutination inhibition (HI) and viral neutralization (VN) antibody titers in mouse sera following immunization with the H7N1/AS03$_A$ vaccine[a].

Virus Strain	Anti-H7N1/AS03$_A$ high dose pool		Anti-H7N1/AS03$_A$ medium dose pool		Anti-H7N1/AS03$_A$ low dose pool		PBS placebo pool	
	HI	VN	HI	VN	HI	VN	HI	VN
H7N9[b]	320	320	320	640	160	320	<10	10
H7N7[c]	160	ND[e]	160	ND	80	ND	<10	ND
H7N3 (EA)[d]	320	2560	320	2560	160	1280	<10	<10
H7N3 (NA1)[e]	320	ND	320	ND	160	ND	<10	ND
H7N3 (NA2)[f]	320	ND	320	ND	160	ND	<10	ND

[a] Serum pools were prepared from post-II immunization (study day 35) sera and HI and VN assays were performed as described in the Supplementary Methods (HI was performed using the respective wild-type virus, 1% horse erythrocytes, with 1 hr of incubation of virus/erythrocyte mixtures). Each HI and VN titer reported is the reciprocal titer and is the average of two replicates.
[b] H7N9: A/Anhui/1/2013 (Eurasian lineage/low pathogenic).
[c] H7N7: A/Netherlands/219/2003 (Eurasian lineage/highly pathogenic).
[d] H7N3 (EA): A/mallard/Netherlands/12/2000 (Eurasian lineage/low pathogenic).
[e] H7N3 (NA1): A/chicken/British Columbia/CN-6/2004 (North American lineage/low pathogenic).
[f] H7N3 (NA2): A/Mexico/InDRE7218/2012 (North American lineage/highly pathogenic).
[e] ND: not determined.

non-adjuvanted H7N1 vaccine at a level of 1.5 μg estimated HA induced lower HI titers compared to 0.023 μg estimated HA of the H7N1 vaccine adjuvanted with AS03$_A$ or AS03$_B$. However, these results are based on a single non-adjuvanted H7N1 vaccine antigen dosage of 1.5 μg estimated HA, and it is possible that a higher dosage would have induced a stronger HI antibody response. It is worth noting the magnitude of the HI response ($P > 0.05$) as well as the percentage of non-responders (HI titer < 40) following immunization with the non-adjuvanted H5N1 vaccine (100% of mice post-I immunization and 86% of mice post-II immunization) were similar to the results for the non-adjuvanted H7N1 vaccine when administered at a comparable vaccine antigen dosage, thus confirming a strong adjuvant effect for the H5N1 vaccine.

Following the outbreak of H7N9 cases during 2013, there was interest in testing the antisera from the current study for broadly reactive HI and VN antibodies that could potentially cross-react against the emerged H7N9 virus as well as additional H7 subtypes such as highly pathogenic H7N7 and H7N3 viruses. Based on the range of the adjuvanted H7N1 vaccine antigen dosages that were administered, three post-II immunization antisera pools (high, medium, and low) were selected for these experiments to minimize variability in the antibody responses in the individual serum samples. As can be seen in Table 1, robust broadly-reactive antibody titers were detected against H7 viruses from both the Eurasian and North American lineages, including the recently emerged H7N9 virus (Eurasian lineage) as well as highly pathogenic H7N7 (Eurasian lineage) and H7N3 (North American lineage) viruses. HI titers to H7N9, H7N7, and two North American H7N3 viruses were very comparable (within 2-fold) to the titers measured against the Eurasian H7N3 virus which has an identical HA to the H7N1 vaccine virus (i.e., HA gene in H7N1 vaccine virus is from A/mallard/Netherlands/12/2000; see Supplementary Methods). A recently published phylogenetic tree of H7 viruses demonstrates the percentage of similarity between the viruses used in this study representing the two lineages [26]. Linear sequence alignment demonstrated that there is 97.14% identity at the amino acid level between the HA of the H7N3 virus of the Eurasian lineage (A/mallard/Netherlands/12/2000) and the H7N9 virus also of the Eurasian lineage (A/Anhui/1/2013), which provides some rationale for the observed cross-reactivity (sequence alignment data not shown). Of the sixteen amino acid differences in the HA of the two viruses, only seven are solvent exposed (as defined by >30% solvent accessibility) using solvent accessibility analysis based on the three-dimensional structure, and therefore are likely to be involved in antigen/antibody interaction (unpublished observations). On the other hand, the VN titers to the H7N3 virus were substantially higher (4- to 8-fold) than the titers to the H7N9 virus, which may, in part, be explained by the fact that the VN assay may detect a broader range of antibodies that target the HA compared with the HI assay. However, VN titers of 320 to 640 to the H7N9 virus were still considered to be robust. Interestingly, there was little to no difference in the antibody titers between the serum pools regardless of the H7 virus or the antibody assay utilized, even though the serum pools represented a 250-fold vaccine antigen dose range between the high dose pool and the low dose pool. The demonstration that the adjuvanted H7N1 vaccine was able to elicit serum antibody responses that cross-reacted with the H7N9 virus and other H7 viruses is consistent with recent preclinical and clinical data that H7N3 and H7N7 live attenuated vaccines elicited HI and VN antibody responses against emerged H7N9 [19,20,27]. Our data is also consistent with data of Krammer and colleagues [26] that have shown at both the preclinical and clinical levels that divergent H7 vaccines elicited cross-reactive serum antibody responses to H7N9 viruses, and these antibody responses cross-protected against H7N9 viral challenge at the preclinical level.

Conclusion

The investigational H7N1 vaccine assessed in the current preclinical study in a mouse model was highly immunogenic (i.e., robust HI titers in a dose–responsive manner down to nanogram levels of HA antigen) when formulated with AS03$_A$ or AS03$_B$. Conversely, the non-adjuvanted H7N1 vaccine was poorly immunogenic, which demonstrated the requirement of AS03 to induce robust HI titers. This immunogenicity profile highlighted by the adjuvant requirement was similar to that of a licensed H5N1 vaccine in the unprimed mouse model. Finally, the AS03$_A$-adjuvanted H7N1 vaccine also elicited antibody responses that cross-reacted with a diverse panel of H7 viruses from both the Eurasian and North American lineages, including the recently emerged H7N9 virus as well as highly pathogenic H7N7 and H7N3 viruses. These H7 data add to the existing H5 data to suggest that AS03 adjuvant technology may be generally effective for formulating antigen-sparing detergent-split virion vaccines against intrinsically sub-immunogenic avian influenza A virus subtypes, and clinical trials are currently underway to assess the immunogenicity of the AS03-adjuvanted H7N1 vaccine in humans.

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Contributions

CPM, EB, BB, MZL, JMK, BLI, and SLG were involved in the conception and design of the experiments described in this publication. EB, MHJ, XL, and FL acquired the data. CPM, EB, BB, MZL, JMK, BLI, and SLG analyzed and interpreted the results. All authors were involved in drafting the manuscript or revising it for critically important intellectual content. All authors had access to the data and approved the manuscript before it was submitted by the corresponding author.

Conflict of interest statement

All authors have declared that the following interests are relevant to the submitted work. CPM, EB, MHJ, BB, BLI, and SLG are employees of the GSK group of companies. CPM, EB, MHJ, BLI, and SLG own stock in GSK. CPM, BB, BLI, and SLG are listed as an inventor on patents owned by GSK. JMK has received funds from GSK for research not related to the present study. XL, FL, and MZL report no financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.06.053

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