The Journal of Infectious Diseases

MAJOR ARTICLE

Intranasal M2SR (M2-Deficient Single Replication) H3N2 Influenza Vaccine Provides Enhanced Mucosal and Serum Antibodies in Adults

Joseph Eiden,[1] Carlos Fierro,[2] Howard Schwartz,[3] Mark Adams,[4] Kimberly J. Ellis,[5] Roger Aitchison,[6] Renee Herber,[1] Yasuko Hatta,[1] David Marshall,[1] Michael J. Moser,[1] Robert Belshe,[7] Harry Greenberg,[8] Kathleen Coelingh,[9] Yoshihiro Kawaoka,[10] Gabriele Neumann,[10] and Pamuk Bilsel[1]

[1]FluGen, Inc, Madison, Wisconsin, USA; [2]Johnson County Clin-Trials, Lenexa, Kansas, USA; [3]Research Centers of America, Hollywood, Florida, USA; [4]Alliance for Multispecialty Research, Lexington, Kentucky, USA; [5]Alliance for Multispecialty Research, Norfolk, Virginia, USA; [6]North Rim Consulting, Longmont, Colorado, USA; [7]Saint Louis University, St Louis, Missouri, USA; [8]Stanford University, Stanford, California, USA; [9]St Helena, California, USA; and [10]Influenza Research Institute, University of Wisconsin, Madison, Wisconsin, USA

Background. We previously demonstrated that an intranasal dose of 10^8 50% tissue culture infectious dose ($TCID_{50}$) M2-deficient single replication (M2SR) influenza vaccine protected against highly drifted H3N2 influenza challenge in a subset of subjects who demonstrated ≥2-fold increase in microneutralization (MN) antibodies to Belgium2015 (the challenge strain) after vaccination. Here, we describe a phase 1b, observer-blinded, dose-escalation study demonstrating an increased proportion of responders with this signal of immune protection.

Methods. Serosusceptible subjects aged 18–49 years were randomized to receive 2 doses (10^8–10^9 $TCID_{50}$) of M2SR or placebo administered 28 days apart. Clinical specimens were collected before and after each dose. The primary objective was to demonstrate safety of M2SR vaccines.

Results. The vaccine was well tolerated at all dose levels. Against Belgium2015, ≥ 2-fold increases in MN antibodies were noted among 40% (95% confidence interval [CI], 24.9%–56.7%) of subjects following a single 10^8 $TCID_{50}$ M2SR dose and among 80.6% (95% CI, 61.4%–92.3%) after 10^9 dose ($P < .001$). A single 10^9 $TCID_{50}$ dose of M2SR generated ≥4-fold hemagglutination inhibition antibody seroconversion against the vaccine strain in 71% (95% CI, 52.0%–85.8%) of recipients. Mucosal and cellular immune responses were also induced.

Conclusions. These results indicate that M2SR may provide substantial protection against infection with highly drifted strains of H3N2 influenza.

Clinical Trials Registration. *NCT03999554.*

Keywords. H3N2; drift; influenza; intranasal; live; mucosal; vaccine.

Lay Summary. In recent years, influenza A H3N2 viruses have evolved into multiple cocirculating clades, resulting in low vaccine efficacy and highlighting the need for more effective influenza vaccines. In a previous challenge study, a single intranasal dose of the investigational vaccine M2SR demonstrated protection against a highly drifted H3N2 influenza challenge virus in a subset of vaccine recipients with a signature immune response.

Increasing the dose of the M2SR vaccine in this phase1b study demonstrated a statistically significant increase in the proportion of subjects with the signature immune responses seen previously. The vaccine-induced antibodies were cross-reactive with a panel of drifted H3N2 viruses from 2007 to 2019.

Additionally, M2SR generated a rise in serum hemagglutination inhibition antibody titer in 71% of subjects. In contrast, the H3N2 seroresponse rate for the licensed intranasal vaccine FluMist is 10% in seronegative adults. Moreover, M2SR elicited mucosal and cell-mediated immune responses.

This study demonstrates that the intranasal M2SR generates a multifaceted immune response and has the potential to provide better efficacy against vaccine-matched strains and influenza drift variants reducing the need to update the vaccine on an annual basis. This is a noteworthy step in the development of a broadly protective influenza vaccine.

Received 13 September 2022; editorial decision 25 October 2022; accepted 02 November 2022; published online 9 November 2022

Correspondence: Pamuk Bilsel, PhD, FluGen, Inc, 597 Science Drive, Madison, WI 53711 (PBilsel@flugen.com).

The Journal of Infectious Diseases® 2023;227:103–12

https://doi.org/10.1093/infdis/jiac433

In recent years, influenza A/H3N2 viruses have evolved into multiple cocirculating clades, resulting in low vaccine efficacy and highlighting the need for broadly protective and effective influenza vaccines [1]. For the fifth consecutive year, the World Health Organization (25 February 2022) announced a strain change for the H3N2 component of the Northern Hemisphere 2022–2023 influenza vaccine [2]. Despite record low levels of influenza during the coronavirus disease 2019 (COVID-19) pandemic, multiple H3N2 subclades were detected and were poorly recognized by sera from humans who had received the licensed and

recommended 2021–2022 influenza vaccines, thus necessitating another vaccine strain change. Cocirculation of multiple H3N2 lineages presents formidable challenges for current strain-specific influenza vaccines that require nearly annual updates in attempts to maintain match with virus antigenic drift.

Despite updating of vaccine strains to account for antigenic drift, vaccine-induced protection against H3N2 remains low, especially in years when there is a mismatch between the vaccine and circulating strains. Vaccine effectiveness (VE) against H3N2 in the United States for the last 10 years has been below 40% and as low as 6% during mismatch seasons. During 2021–2022, the VE in the United States against H3N2 was 35% [3]. Thus, new influenza vaccines are needed to induce broader, cross-reactive immune responses that provide greater protection against infection and disease.

Currently available inactivated and recombinant influenza vaccines primarily depend on a close match between the vaccine immunogen and circulating viruses to be effective. Such vaccines are therefore relatively ineffective against newly emerging influenza viruses or viruses that have drifted away from the vaccine strain. In addition, mucosal and T-cell immune responses are generally not elicited following vaccination with conventional inactivated vaccines, even though these types of immune responses are associated with a reduction in influenza illness severity in subjects seronegative for influenza virus-specific antibody [4–8]. Although live influenza virus vaccines offer immunologically diverse responses, accumulating data with the licensed live attenuated influenza vaccine FluMist suggest that preexisting, cross-reactive immunity present in most adults limits vaccine virus replication, which in turn mitigates effective immune responses, thereby limiting its use to predominantly children [9–12].

We recently described an intranasal vaccine platform, M2SR (M2-deficient single replication influenza virus), that delivers negative-strand influenza RNA to the mucosa with subsequent production of influenza antigens that stimulate broad host immune responses [13–15]. M2SR mimics a single replication cycle of wild-type influenza virus, but no infectious virus is produced, resulting in broad-spectrum protection in animal models [16]. In a recent phase 2a challenge study with a highly drifted H3N2 influenza strain, a subset (27%) of serosusceptible adult subjects (aged 18–55 years) demonstrated a signature immune response, ≥ 2-fold increases in serum microneutralization titers (MNT), following a single intranasal administration of 10^8 50% tissue culture infectious dose ($TCID_{50}$) M2SR [17]. This subset of individuals had reduced rates of influenza infection after challenge (38% vs 71% of placebo subjects, $P = .0505$) and reduced illness [17]. We hypothesized that higher doses of M2SR would further increase the response rate to the single-cycle replication vaccine.

The phase 1b study described here subsequently explored the potential for higher doses of M2SR and a 2-dose regimen to increase the proportion of subjects with the protective signature immune response previously detected in the challenge study with a highly drifted influenza virus. The M2SR vaccine in this dose-response study was updated with the hemagglutinin (HA) and neuraminidase (NA) sequence from a strain similar to the H3N2 component of the 2018–2019 commercial seasonal influenza vaccine (A/Singapore/INFIMH-16-0019/2016).

METHODS

Study Design

This phase 1b, randomized, placebo-controlled, sequentially enrolled, dose-escalation study assessed safety and immunogenicity of M2SR when delivered as 2 administrations 28 days apart to healthy, influenza serosusceptible adults, 18–49 years of age (ClinicalTrials.gov NCT03999554). The study permitted exploration of safety and the potential for M2SR to induce among a higher proportion of adults the signature, protective immune response signal observed previously [17].

The study was conducted in accordance with the ethical principles of Good Clinical Practice as required by the major regulatory authorities based on the Declaration of Helsinki. All participants provided informed consent. The institutional review board (Western Institutional Review Board-Copernicus Group, WA) approved notices for recruitment of study subjects (print and radio advertisements, email blasts, and social media).

Subjects were prescreened for MNT against H3N2 A/Singapore/INFIMH-16-0019/2016 (Sing2016) within 90 days of enrolment and were eligible if Sing2016 MNT was ≤1:20. Major exclusion criteria were high serum antibody titers, positive test to recreational drugs, and significant history of lung disease.

Two investigational M2SR vaccines expressing HA and NA from A/Brisbane/10/2007 (Bris2007) or from Sing2016 (GISAID accession No. EPI1341068) were used. Vaccines were manufactured as previously described in a qualified complementing cell line [13] and provided frozen in single-use cryovials to the sites. Placebo was sterile normal saline.

A pharmacist or designee thawed the vial contents to room temperature just prior to dose administration and prepared final diluted product, which was drawn into 1-mL disposable polypropylene syringes fitted with a mucosal atomization device (MAD301; Teleflex) for intranasal delivery. Subjects were asked to record posttreatment symptoms for reactogenicity in a symptom memory aid for 7 days after each vaccination.

Randomization and Masking

Eligible subjects were screened and randomized at Johnson County Clin-Trials (Lenexa, KS), Research Centers of America (Hollywood, FL), Alliance for Multispecialty Research (Lexington, KY), and Alliance for Multispecialty Research (Norfolk, VA) to receive 2 administrations 28 days apart of Sing2016 M2SR at 1 of 3 dose levels

(10^8, $10^{8.5}$, 10^9 $TCID_{50}$), Bris2007 M2SR at 1 dose level (10^8 $TCID_{50}$), or placebo in a 1:1:1:1:1 ratio. The Bris2007 M2SR was used as a bridging group to the previous challenge study in which the protective immune response was observed in a subset of individuals [17].

Three sentinel groups were vaccinated first, consisting of 15 subjects each (9 subjects receiving escalating doses of Sing2016 M2SR, 3 subjects receiving Bris2007 M2SR, and 3 subjects receiving placebo), followed by the remainder of the subjects in an expansion group. Initial dosing was staggered to allow for review of adverse events (AEs) through day 3 postadministration of investigational product for each sentinel group prior to dose escalation. An independent safety review committee reviewed available safety data from all sentinel subjects after subjects completed 7 days postvaccination, prior to dosing of the expansion group.

Only the pharmacist or designee preparing the treatment, the unblinded monitor, and the unblinded statistician (R.A.) who prepared the randomization list were unblinded to treatment.

All investigative site staff and members of the study team and laboratory staff remained blinded to treatment assignments. All participants were blinded to the study treatment.

Procedures

AEs were monitored from first administration of vaccine or placebo until day 57 (28 days after dose 2). Serious AEs were recorded through day 209. Blood samples (serum and peripheral blood mononuclear cells [PBMC]) for antibody response and cell-mediated assessments were collected on days 1 (prevaccination), 29 (before second dose administration), and 57. Serum was collected from all subjects, PBMC from subjects at 2 of the 4 study sites (approximately 63% of subjects). Nasal swabs for mucosal antibody assessment were collected from all subjects on days 1 (prevaccination), 29 (before second dose administration), and 57. Nasal swabs for vaccine virus evaluation were collected on days 1 (prevaccination) and 7.

HA inhibition (HAI), MNT, and NA inhibition (NAI) titers were determined at Viroclinics Biosciences (Rotterdam, the Netherlands) or Southern Research (Birmingham, AL) as described previously [13, 17]. Mucosal immunoglobulin A (IgA) or secretory IgA (sIgA) and serum enzyme-linked immunosorbent assay (ELISA) assays and interferon-γ (IFN-γ) ELISpot assays were conducted at FluGen or VisMederi (Siena, Italy) [13, 18]. Vaccine virus shedding evaluation by quantitative real-time polymerase chain reaction (qRT-PCR) was conducted at FluGen as described previously [13].

Outcomes

The primary end point for this study was safety and tolerability of M2SR vaccines delivered intranasally to healthy adult subjects. The secondary end points were influenza-specific immune responses at specified time points and vaccine virus shedding at day 7 postvaccine. Immunological end points included serum MNT and HAI, mucosal IgA and sIgA ELISA titers against vaccine-matched and drifted H3N2 influenza viruses and cellular immune responses evaluated by ELISpot. Baseline and postvaccination cell-mediated immune responses were assessed by an ex vivo IFN-γ ELISpot assay following stimulation with a peptide pool representing the influenza nucleoprotein.

Statistical Analyses

With 40 subjects per group, this study has at least 80% power to detect a 35% difference between groups for the proportion responding (defined as ≥2-fold increase in MNT) using Fisher exact 2-sided test. Significance level was .05 with no correction for multiple testing. Pearson correlation coefficients among the different antibody measurements are reported. Statistical tests were performed using GraphPad Prism 7 and SASV9.4.

RESULTS

Between September and November 2019, 206 subjects were enrolled and randomly assigned to 5 groups (Figure 1). All 206 randomized subjects received at least 1 dose of study M2SR or placebo and were included in the safety set. A total of 185/206 randomized subjects (90%) received both administrations of vaccine or placebo and were included in the evaluable set (Supplementary Table 1).

Demographic information is shown in Table 1. Baseline characteristics of participants were similar across treatment groups. Most subjects were white (81%) and approximately half (47%) were Hispanic. Seventy percent were female. The mean age was 39 years.

All immunized participants were included in the safety analyses. All doses of Sing2016 M2SR and Bris2007 M2SR vaccines were well tolerated through day 57 postvaccination. Most treatment-emergent AEs (TEAEs) were mild in severity across the treatment groups (34% to 52%) (Supplementary Table 2). The proportion of subjects with at least 1 TEAE in the each of the Sing2016 M2SR dose-escalation vaccine groups (62% to 75%) was consistent with the 10^8 $TCID_{50}$ Bris2007 M2SR group (67%) and slightly higher than placebo (59%) (Supplementary Table 3). Blinded study investigators (C.F., H.S., M.A., and K. J. E.) designated AE relatedness to investigational product, and the proportions of subjects with at least 1 related TEAE were also generally higher across all vaccine groups (49% to 62% in Sing2016 M2SR groups and 50% in Bris2007 M2SR group) compared with placebo (39%) (Supplementary Table 3). The most frequently reported TEAEs were nasal congestion, headache, fatigue, rhinorrhea, cough, and myalgia (Figure 2 and Supplementary Table 4). No general pattern of increasing frequency of TEAEs was noted with increasing Sing2016 M2SR doses, albeit nasal congestion,

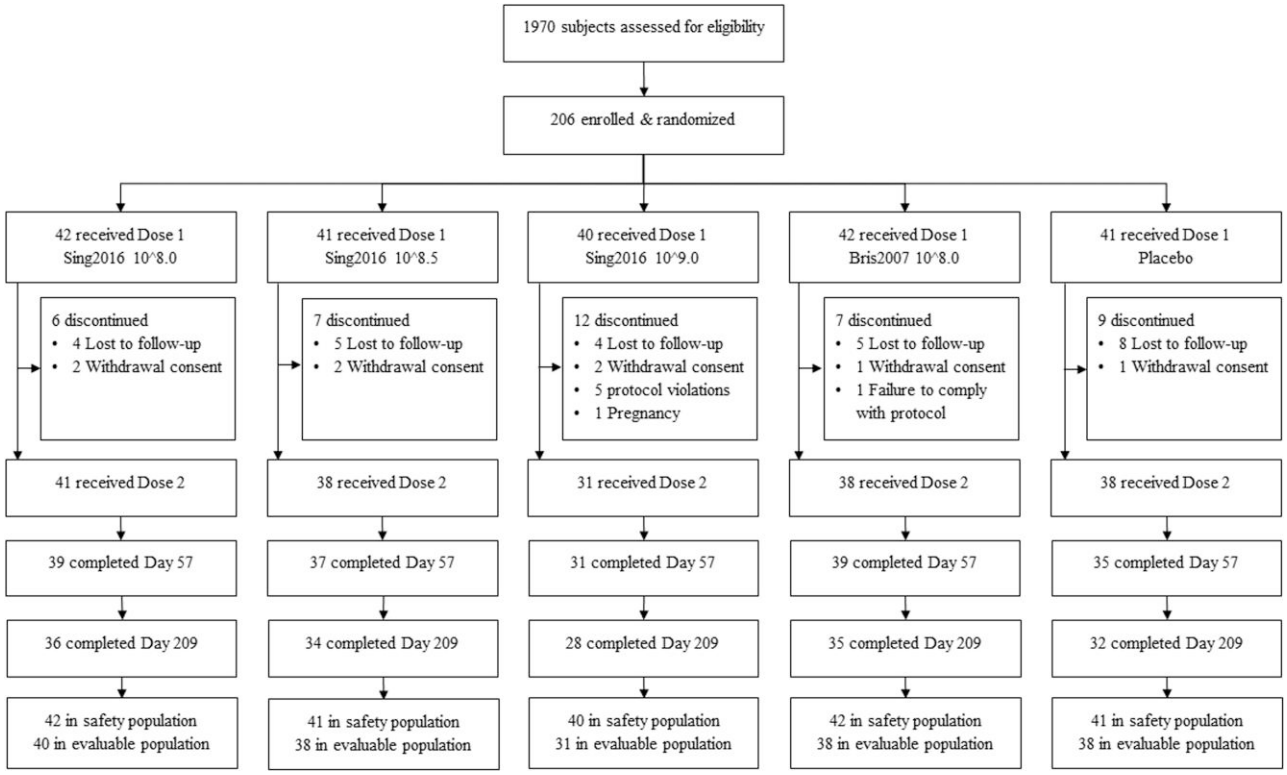

Figure 1. Trial profile. Abbreviations: Bris2007, H3N2 A/Brisbane/10/2007; Sing2016, H3N2 A/Singapore/INFIMH-16-0019/2016.

Table 1. Demographic Characteristics of Trial Participants

Characteristic	Sing2016 M2SR $10^{8.0}$ n = 42	Sing2016 M2SR $10^{8.5}$ n = 41	Sing2016 M2SR $10^{9.0}$ n = 40	Bris2007 M2SR $10^{8.0}$ n = 42	Placebo n = 41
Sex, n (%)					
Female	25 (60)	26 (63)	29 (72)	35 (83)	31 (76)
Male	17 (40)	15 (37)	11 (28)	7 (17)	10 (24)
Race, n (%)					
American Indian/Alaska Native	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Black/African American	4 (10)	7 (17)	6 (15)	7 (17)	11 (27)
Indian	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Mexican American	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Multiracial	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Native Hawaiian/Other Pacific Islander	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
White	36 (86)	34 (83)	34 (85)	33 (79)	29 (71)
American Indian/Alaska Native	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Ethnicity, n (%)					
Hispanic	24 (57)	16 (39)	18 (45)	20 (48)	18 (44)
Not Hispanic	18 (43)	25 (61)	22 (55)	22 (52)	23 (56)
Age, y					
Mean	39.4	39.9	39.2	39.1	38.1
SD	7.03	8.60	7.48	6.94	7.17
BMI, kg/m^2, n (%)					
<30	19 (45)	28 (68)	27 (68)	24 (57)	25 (61)
≥30	23 (55)	13 (32)	13 (32)	18 (43)	16 (39)

Abbreviations: BMI, body mass index; Bris2007, H3N2 A/Brisbane/10/2007; M2SR, M2-deficient single replication; Sing2016, H3N2 A/Singapore/INFIMH-16-0019/2016.

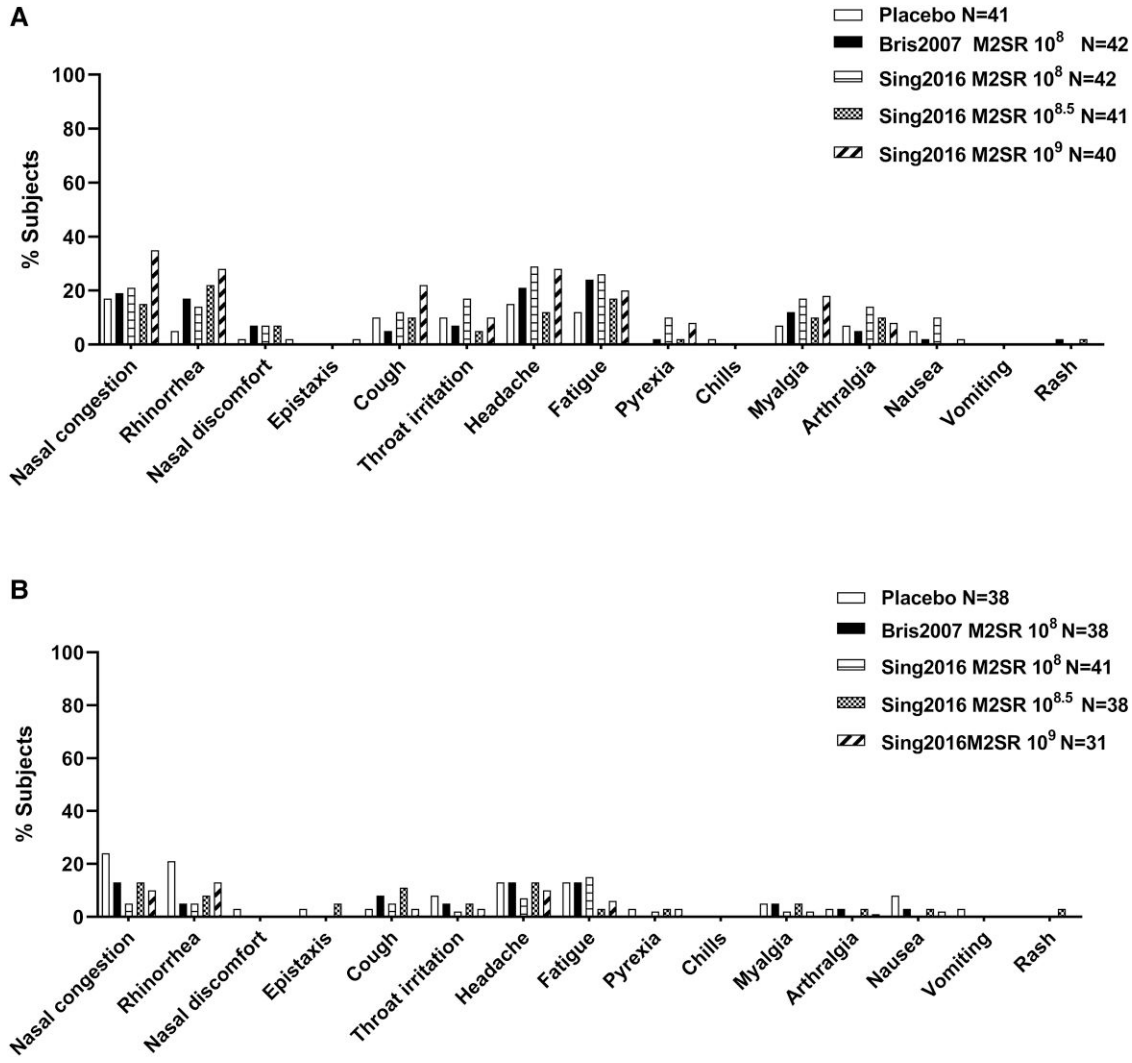

Figure 2. Treatment-related adverse events. *A*, Proportion of subjects with treatment-emergent adverse events (TEAE) 8 days after the first dose. *B*, Proportion of subjects with TEAE 8 days after the second dose.

rhinorrhea, and cough were higher with the 10^9 dose (Figure 2). Frequency of total subjects with any TEAE were lower after the second dose compared with the first dose for the vaccine groups (Figure 2). There were no deaths, serious AEs, or TEAEs that led to study withdrawal. No halting rules defined in the study protocol were met. Vaccine virus shedding was not detected in subjects receiving 10^9 dose (Supplementary Table 5), as observed previously with lower doses [13].

HAI antibody titers measured in serum samples on days 1 (prevaccination) and 29 demonstrated a dose-related response against matched and drifted H3N2 influenza viruses (Figure 3). A single intranasal dose of 10^9 $TCID_{50}$ Sing2016 M2SR generated a $\geq$ 4-fold rise in HAI titer in 71% of subjects against the vaccine antigen, Singapore 2016 (Figure 3*A*). Moreover, Sing2016 M2SR at this dose elicited HAI titers against 4 drifted H3N2 vaccine strains from multiple clades ranging from 2007 to 2019, demonstrating induction of broadly reactive protective antibodies. A second intranasal dose of M2SR further increased seroconversion rates against all the H3N2 strains on day 57 (Figure 3*B*). The Sing2016 M2SR 10^9 dose induced a geometric mean fold rise (GMFR) of 5.29 against matched Sing2016 ($P < .009$ compared to all the other cohorts) and GMFRs ranging from 1.68 to 4.97 for the drifted H3N2 strains ($P < .05$) after the first dose (Supplementary Table 6). By design, none of the subjects had seroprotective HAI titers (HAI $\geq$ 40) against the vaccine antigen Sing2016 at baseline (Supplementary Table 7). The Sing2016 M2SR 10^9 dose induced seroprotective HAI titers in 58.1% (95% CI, 39.1%–75.5%) after the first dose and in 77.4% (95% CI, 58.9%–90.4%) after the second dose compared to 0% (95% CI, .0%–9.3%) and 2.9% (95% CI, .1%–14.9%) for placebo (Supplementary Table 7).

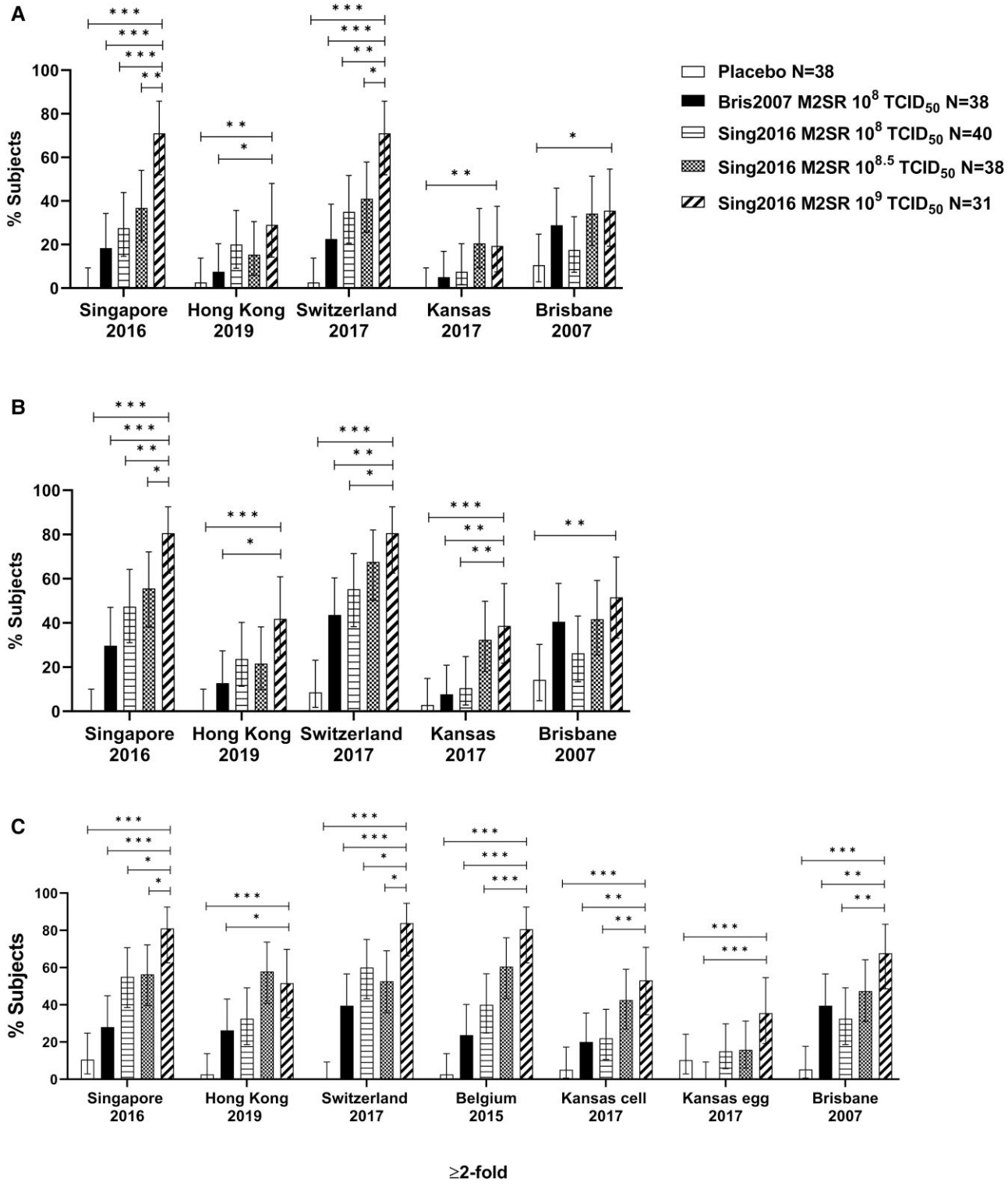

Figure 3. Serum HAI and neutralizing antibody responses after intranasal vaccination. *A*, Proportion of subjects with ≥4-fold rise in HAI titer 28 days after the first dose against matched and drifted H3N2 influenza virus strains. *B*, Proportion of subjects with ≥4-fold rise in HAI titer 28 days after the second dose against matched and drifted H3N2 influenza virus strains. Error bars are 95% CI. *C*, Proportion of subjects with ≥2-fold rise in MNT 28 days after the first dose against matched and drifted H3N2 influenza virus strains. Error bars are 95% CI. H3N2 test strains are: Singapore 2016, A/Singapore/INFIMH-16-0019/2016; Hong Kong 2019, A/Hong Kong/2671/2019; Switzerland 2017, A/Switzerland/8060/2017; Belgium 2015, A/Belgium/4217/2015; Kansas 2017, A/Kansas/14/2017; Kansas 2017 cell, A/Kansas/14/2017 cell-based; Kansas 2017 egg, A/Kansas/14/2017 egg-based; Brisbane 2007, A/Brisbane/10/2007. ***$P < .001$, **$P < .01$, *$P < .05$. Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition; MNT, microneutralization titer; $TCID_{50}$, tissue culture infectious dose.

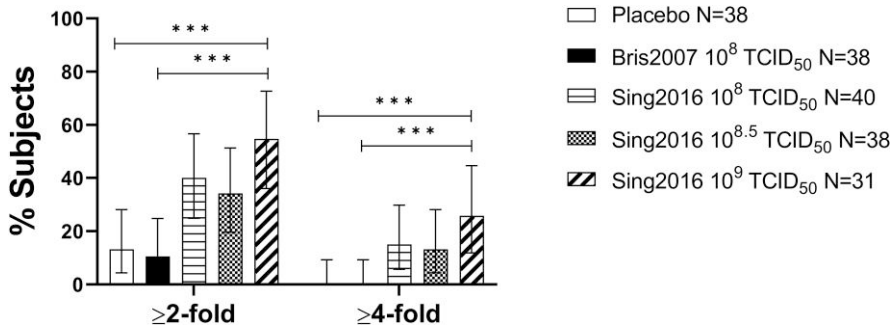

Figure 4. Serum neuraminidase inhibition antibody titers after intranasal vaccination. Shown are proportion of subjects with ≥2-fold and ≥4-fold rise in neuraminidase inhibition 28 days after the first dose against H6N2 reassortant influenza virus encoding the neuraminidase from A/Switzerland/9715293/2013. Error bars are 95% confidence interval. ***$P < .001$, **$P < .01$, *$P < .05$. Abbreviations: Bris2007, H3N2 A/Brisbane/10/2007; Sing2016, H3N2 A/Singapore/INFIMH-16-0019/2016; $TCID_{50}$, tissue culture infectious dose.

MNT against vaccine-matched and drifted H3N2 strains were also evaluated. A dose-related response trend similar to serum HAI was seen against the vaccine strain, Sing2016 (clade 3c.2a1), and the H3N2 influenza drift strains ranging from Bris2007 (clade 1) to Hong Kong2019 (clade 3C.2a1b.1b) (Figure 3*C*). The 10^9 dose of Sing2016 M2SR stimulated ≥2-fold (Figure 3*C*) and ≥4-fold (Supplementary Figure 1) increases from baseline in a significantly greater proportion of subjects against the matched and 5 drifted H3N2 viruses including Belgium2015 (the challenge virus from the previous phase 2a study [17]). A single 10^8 $TCID_{50}$ dose of Bris2007 M2SR generated ≥2-fold serum MNT responses against Belgium2015 among a similar proportion of study subjects (24%) as observed in the previous challenge study. Response frequencies for the 10^8 $TCID_{50}$ dose of Sing2016 M2SR (40% after first dose) were slightly higher compared to Bris2007. The response frequencies for the 10^9 dose of Sing2016 M2SR was 81% after the first dose. Sing2016 M2SR 10^9 dose induced ≥2-fold MNT increases in a greater proportion of subjects against the cell-produced Kansas2017 virus (epidemic virus like) than against the egg-produced Kansas2017 virus (53.1% vs 35.5%) (Figure 3*C*). The GMFR from baseline was 4.42 for matched Sing2016, and ranged from 2.17 to 7.71 for the drifted H3N2 strains after first dose and up to 13.97 after the second dose for the Sing2016 M2SR 10^9 dose (Supplementary Table 8).

Serum antibodies against the influenza NA were also elicited by Sing2016 M2SR in a dose-response manner with 54.8% and 25.8% of subjects in the high dose of Sing2016 M2SR demonstrating a ≥ 2-fold and ≥4-fold rise from baseline, respectively, compared with 13.2% and 0% for placebo subjects after first dose administration (Figure 4). At baseline, 8/31 (25.6%) of subjects in the high-dose Sing2016 M2SR cohort had NAI titers of 40 and above; by day 29 the proportion had increased to 15/31 (48.3%). After the second dose, 74.2% and 32.3% of subjects in the high dose of Sing2016 M2SR demonstrated a ≥ 2-fold and ≥4-fold rise from baseline, respectively, compared with 11.4% and 2.9% of placebo subjects (Supplemental Figure 2).

Dose-related increases in mucosal secretory IgA (sIgA) directed against vaccine-specific HA were observed after both first and second doses of Sing2016 M2SR vaccination (Figure 5*A*). The proportion of high-dose Sing2016 M2SR subjects who demonstrated a ≥ 2-fold rise from baseline in sIgA titers was 45% after the first dose compared with 0% in placebo subjects, and 76% and 3% after the second dose for M2SR and placebo subjects, respectively (Figure 5*B*). The rise against HA-specific mucosal IgA against the vaccine strain Sing2016 and drift strain Bris2007 were similar after the first and second doses, demonstrating broad mucosal antibody responses (Supplementary Figure 3). The Sing2016 M2SR 10^9 dose elicited ≥2-fold rise from baseline sIgA antibodies against multiple recent H3N2 strains including Cambodia2020 and Darwin2021, the vaccine strains for the 2021–2022 and 2022–2023 northern hemisphere influenza vaccines, respectively (Supplementary Figure 4).

Similarly, HA-specific IgA responses in serum were seen in a dose-dependent manner after Sing2016 M2SR immunization with the highest increase (2.4-fold) seen with the 10^9 $TCID_{50}$ dose (Figure 5*C*). A ≥ 2-fold rise from baseline in serum IgA titers was seen in 54.8% of high-dose Sing2016 M2SR subjects compared with 0% in placebo subjects (Figure 5*D*). Serum IgA titer correlated positively with the other serum antibody measurements for high-dose M2SR (MNT, $r = 0.4148$, $P < .0203$ and HAI, $r = 0.4076$, $P < .0228$) and with mucosal sIgA ($r = 0.6215$, $P < .0003$). Postvaccination cell-mediated immune responses assessed by IFN-γ ELISpot demonstrated a dose-dependent increase from baseline in influenza nucleoprotein reactive cells (Figure 6).

DISCUSSION

Previous clinical trials and investigations in animal models have demonstrated that intranasal administration of M2SR stimulates a broad range of adaptive immune responses [13–17]. The results of the current phase 1b study confirm the range and

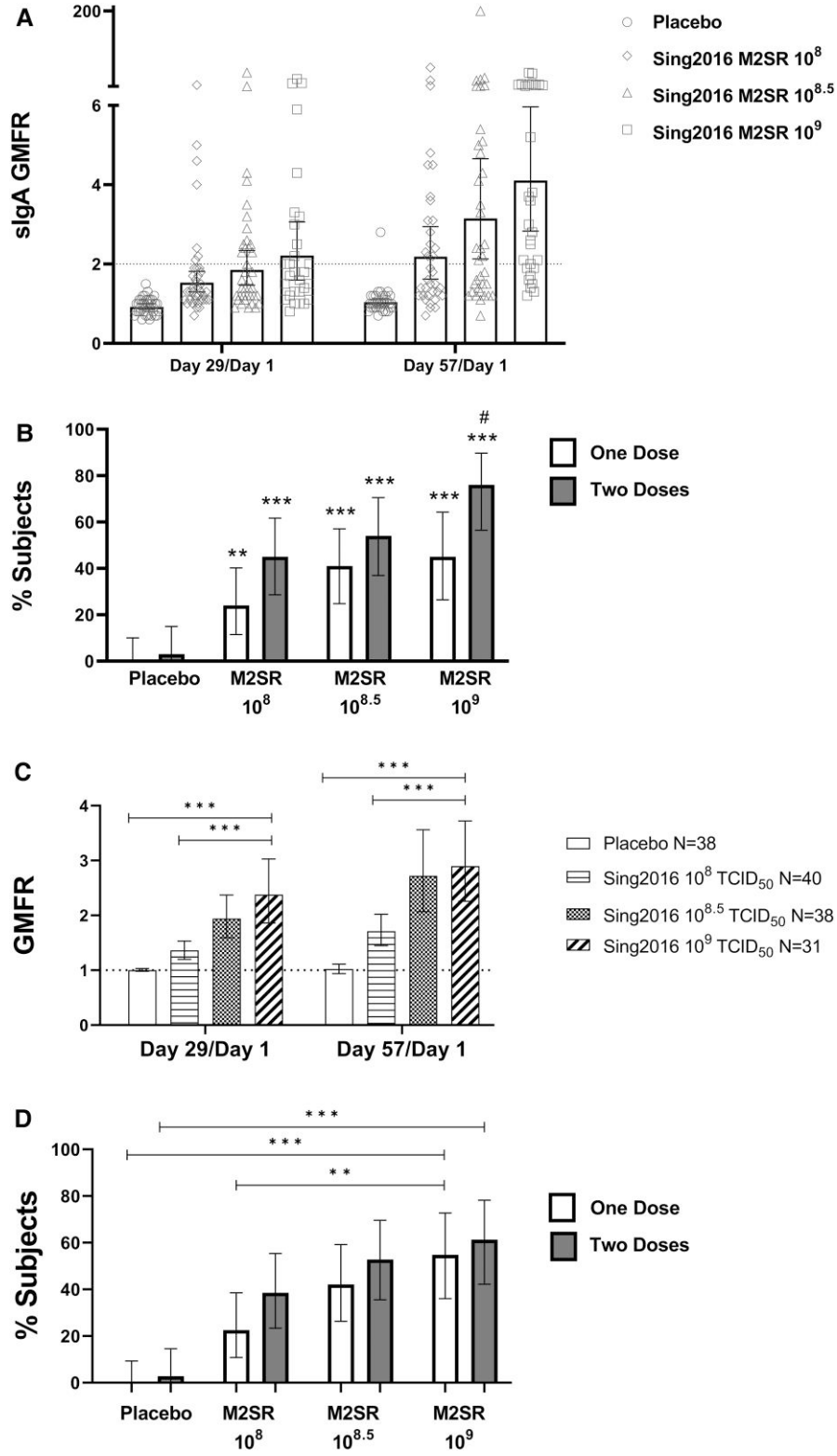

Figure 5. Mucosal sIgA and serum IgA antibody ELISA titers in nasal swabs after intranasal vaccination. *A*, GMFR from baseline in sIgA titer 28 days after first dose and 28 days after second dose. Anti-Sing2016 HA ELISA titers were normalized to total sIgA in the nasal specimens. *B*, Proportion of subjects demonstrating ≥2-fold rise in sIgA titer 28 days after first dose and 28 days after second dose. ***$P < .001$; **$P < .01$, M2SR dose levels versus placebo. #$P < .05$, 10^9 M2SR versus 10^8 M2SR. *C*, GMFR from baseline in anti-Sing2016 HA serum IgA ELISA titer 28 days after first dose and 28 days after second dose. *D*, Proportion of subjects demonstrating ≥2-fold rise in serum IgA titer 28 days after first dose and 28 days after second dose. ***$P < .001$, **$P < .01$, *$P < .05$. Abbreviations: ELISA, enzyme-linked immunosorbent assay; GMFR, geometric mean fold rise; HA, hemagglutinin; M2SR, M2-deficient single replication; sIgA, secretory immunoglobulin A; Sing2016, H3N2 A/Singapore/INFIMH-16-0019/2016; $TCID_{50}$, tissue culture infectious dose.

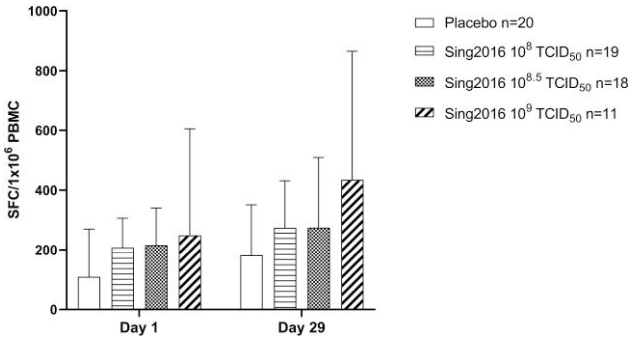

Figure 6. Cell-mediated immune responses after intranasal M2SR vaccination. Geometric mean ELISpot responses (SFC per 10^6 PBMC) on day 1 (baseline) and day 28 after vaccination. PBMC were stimulated with a nucleoprotein peptide pool. Error bars are 95% confidence interval. Abbreviations: M2SR, M2-deficient single replication; PBMC, peripheral blood mononuclear cell; Sing2016, H3N2 A/Singapore/INFIMH-16-0019/2016; SFC, spot forming cell; $TCID_{50}$, 50% tissue culture infectious dose.

breadth of those responses after 1 or 2 vaccinations administered 4 weeks apart. These findings indicate the potential for high-dose M2SR for primary immunization and suggest the potential for boosting of influenza-specific immunity with administration of M2SR at increased intervals between doses such as a midwinter booster vaccine. M2SR was well tolerated at the dose levels tested after both the first and second intranasal administrations and no difference in reactogenicity to intranasal saline was seen.

A single 10^9 $TCID_{50}$ dose of Sing2016 M2SR vaccine induced serum HAI and MNT responses in a significantly greater proportion of adult subjects than a single 10^8 dose of either Bris2007 M2SR or Sing2016 M2SR. The data in the current phase 1b study show that a 10-fold higher dose of M2SR than that employed in our human challenge study is both well tolerated and significantly increases the proportion of serosusceptible adults who respond to M2SR with ≥2-fold increases of serum MNT. Using the challenge strain Belgium2015 as the example, where the 10^8 M2SR dose gave 40% responder frequency, a 10^9 dose provided a similar response in 81% of recipients after a single dose. These results suggest that a 10^9 dose of M2SR has the potential to provide substantially greater protection against both matched and drifted influenza strains than indicated in the prior challenge study.

Serum HAI titers of ≥1:40 are accepted as the correlate of protection against influenza [19]. However, for the intranasal M2SR vaccine, the signature immune response identified in the previous challenge study (≥2-fold increase in serum MNT) is unlikely to be the only immune response that contributed to protection by M2SR. As shown in that study, as well as in this phase 1b clinical trial, M2SR induces not only serum antibody responses against HA and NA but also stimulates broad mucosal and cell-mediated responses against influenza. Therefore, the serum MNT increase following intranasal M2SR is likely a marker for the much broader immune response generated by M2SR, and the stimulation of each of these types of immunity probably are engaged in the protection provided by this investigational intranasal vaccine.

Of note, the high proportion of serosusceptible adults with ≥4-fold increases in HAI titers after intranasal M2SR is in contrast to the much lower proportions reported for serosusceptible adults of similar ages following administration of FluMist vaccine. For example, the H3N2 seroresponse rate for FluMist is reported to be 10% (95% CI, 8%–11%) in seronegative adults (n = 1906) [20]. The high proportion of responders to 10^9 $TCID_{50}$ Sing2016 M2SR is more like the serum responses to FluMist reported for young children, an age group for which FluMist generally provides a much greater level of protection against influenza [21]. Because the protection provided by FluMist is believed to be dependent upon vaccine virus replication after inoculation, the relatively limited immune response by serosusceptible adults may result from greater blunting of FluMist replication by older individuals who have experienced repeated exposures to wild-type influenza and vaccines [12, 22]. The immune responses after a second dose of M2SR suggest that the single-replication vaccine is not as susceptible to blunting by preexisting immunity as compared to the currently licensed live attenuated influenza vaccine FluMist. A single 10^9 dose of M2SR appears to overcome baseline immunity in serosusceptible adults and permits enhanced engagement of the immune response, which may predict increased protection against influenza infection and illness.

Moreover, M2SR not only elicited HAI titers against previous and subsequent H3N2 vaccine strains, demonstrating induction of broadly reactive protective antibodies, but also mucosal antibodies that were also broadly reactive against drift H3N2 strains. M2SR additionally elicits cell-mediated immune responses against highly conserved influenza antigens in humans. The multifaceted immune response of M2SR suggests the potential for M2SR to protect against influenza drift variants and reduce the need to update the vaccine on an annual basis.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgment. We thank Kasi Quale, Katie Radspinner, and Shari Seymour for technical assistance.

Disclaimer. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

Financial support. This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Peer Reviewed Medical Research Program (award No. W81XWH-17-1-0430). The US Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office.

Potential conflicts of interest. J. E. is a consultant to FluGen. R. B., H. G., and K. C. serve on FluGen's Clinical Advisory Board. Y. K. and G. N. are founders of FluGen. R. H., Y. H., D. M., M. M., and P. B. are employees of FluGen. All other authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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