Anti-Ganglioside Antibody Induction by Swine (A/NJ/1976/H1N1) and Other Influenza Vaccines: Insights into Vaccine-Associated Guillain-Barré Syndrome

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Background. Receipt of an A/NJ/1976/H1N1 “swine flu” vaccine in 1976, unlike receipt of influenza vaccines used in subsequent years, was strongly associated with the development of the neurologic disorder Guillain-Barré syndrome (GBS). Anti-ganglioside antibodies (e.g., anti-GM1) are associated with the development of GBS, and we hypothesized that the swine flu vaccine contained contaminating moieties (such as Campylobacter jejuni antigens that mimic human gangliosides or other vaccine components) that elicited an anti-GM1 antibody response in susceptible recipients.

Methods. Surviving samples of monovalent and bivalent 1976 vaccine, comprising those from 3 manufacturers and 11 lot numbers, along with several contemporary vaccines were tested for hemagglutinin (HA) activity, the presence of Campylobacter DNA, and the ability to induce anti-Campylobacter and anti-GM1 antibodies after inoculation into C3H/HeN mice.

Results. We found that, although C. jejuni was not detected in 1976 swine flu vaccines, these vaccines induced anti-GM1 antibodies in mice, as did vaccines from 1991–1992 and 2004–2005. Preliminary studies suggest that the influenza HA induces anti-GM1 antibodies.

Conclusions. Influenza vaccines contain structures that can induce anti-GM1 antibodies after inoculation into mice. Further research into influenza vaccine components that elicit anti-ganglioside responses and the role played by these antibodies (if any) in vaccine-associated GBS is warranted.

Because of fears of an influenza pandemic, the 1976 National Influenza Immunization Program A/New Jersey “swine flu” influenza (A/NJ/1976/H1N1) vaccination campaign was designed to immunize almost the entire adult population in the United States as well as children at risk for serious influenza virus infection. The program was stopped after reports of vaccine-associated Guillain-Barré syndrome (GBS), an acute, postinfectious, immune-mediated attack on the nervous system characterized by rapidly evolving, bilateral, ascending motor neuron paralysis. The A/NJ/1976 vaccine was shown to be associated with development of GBS, with attributable risk estimates for GBS in the 6 weeks after vaccination ranging from 4.9 to 11.7 cases per million adult vaccinees [1–4]. Studies of subsequent influenza vaccines used after 1976 in general detected no significant increase in the overall risk for GBS in adult vaccinees, although a borderline statistically significant elevated risk of ~1 excess case per million adult vaccinees was reported during the 1992–1993 and 1993–1994 influenza seasons combined [5–9]. Questions about what caused the excess GBS cases after immunization with A/NJ/1976 vaccine—and why this strong association was restricted to that particular vaccine—remain unanswered.
Patients with GBS develop anti-ganglioside antibodies that are implicated in the pathogenesis of this disease. Antibodies to a number of different complex gangliosides, including GM1, GD1a, GD3, GT1b, and GQ1b, can be detected in patients with GBS [10]. Infections with several agents are associated with the development of GBS [11], and Campylobacter jejuni, a common cause of bacterial gastroenteritis, is one of the most frequently identified bacterial pathogens associated with the development of GBS [12]. It has been deduced that the development of GBS after C. jejuni infection is the result of molecular mimicry between the bacterial surface lipooligosaccharide (LOS) expressing ganglioside-like epitopes and relevant targets in peripheral nerves [10, 13]. Rabbits immunized with gangliosides or with ganglioside-like epitopes and relevant targets in peripheral nervous tissue [14, 15], and antibodies raised against C. jejuni ganglioside–mimicking LOS exhibit reactivity with nerve tissue identical to that seen with anti-ganglioside antibodies in patients with GBS [16].

Because C. jejuni is often present in poultry [17] and because influenza vaccines are made in chicken eggs [18], we hypothesized that contamination by C. jejuni of eggs used to produce the vaccine, contamination of the vaccine virus itself during the process, or vaccine components directly induced GBS in susceptible hosts by eliciting anti-ganglioside antibodies after influenza vaccination. We tested the hypothesis by obtaining surviving bottles of swine flu vaccine kept in storage since 1976–1977, immunizing mice with vaccine lots from different manufacturers, and measuring the anti-Campylobacter and anti-GM1 response to these vaccines. We also conducted similar experiments using influenza vaccine preparations from 2 more recent seasons, 1991–1992 and 2004–2005.

**METHODS**

**Vaccines.** Monovalent and bivalent 1976 vaccines, comprising those from 3 manufacturers (manufacturer A, 7 monovalent lots; manufacturer B, 2 monovalent lots; and manufacturer C, 2 bivalent lots) and 11 lot numbers, as well as trivalent 1991–1992 vaccine (A/Taiwan/1/86, A/Beijing/353/89, and B/Panama/45/90) had been stored at 4°C at Baylor University before analysis in the present study. Vaccines prepared in 1976 contained the high-yielding influenza recombinant X-53. This recombinant contained 2 genes coding for the hemagglutinin (HA) and neuraminidase (NA) antigens derived from the swine influenza virus parent, A/NJ/1/76, and 6 genes acquired from the A/PR/8/34 parent, the laboratory strain that provided the high-yield characteristic [19]. Subsequent experiments documented that the X-53 recombinant produced for swine influenza for the National Influenza Immunization Program showed genetic dimorphism, X-53 and X-53a, 2 mutants that apparently are ubiquitous [20]. Additional trivalent vaccine preparations from the US 2004–2005 vaccination program (A/New Caledonia/20/99, A/Wyoming/03/2003, and B/Jiangsu/10/2003) were also tested (table 1).

**Viral antigens.** A/NJ/1976 was grown in eggs, and β-propiolactone−inactivated alantoic fluid was used as a source of viral antigen. Recombinant influenza HA from A/HK/156/97(H5N1) and A/Vietnam/1203/04(H5N1), prepared in a baculovirus expression system, were obtained from BEI Resources.

**HA activity and hemagglutination inhibition (HAI) titers.** HA activity of the vaccine preparations and HAI titers in mouse serum samples were assessed according to Centers for Disease Control and Prevention protocols [21]. Receptor-destroying enzyme serology was obtained from Denka Seiken (Accurate Chemical and Scientific). Ferret serum was used as a positive control in the HAI assays.

**Bacterial strains.** As a positive control for inducing anti-GM1 antibodies, we used C. jejuni HB93-13 (ATCC 700297), which expresses GM1, ganglioside mimicry, and an isogenic waaF knockout mutant, which lacks expression of GM1, mimicry [22], as controls for mouse immunization experiments [23].

**Campylobacter serology.** An ELISA was used to measure anti–C. jejuni antibodies in mouse serum samples by employing a modification of an assay for detecting human antibodies [24]. The assay was modified by using horseradish peroxidase−conjugated goat anti–mouse IgG or IgM antibodies (Cappel; MP Biomedicals). The assay was also modified to use purified C. jejuni LOS from strain HB93-13 as the antigen.

**Anti-ganglioiside antibodies.** Anti-GM1 antibodies were measured by use of an ELISA, as described elsewhere by Willison et al. [25]. Bovine brain GM1, ganglioside was obtained from EMD Biosciences. Mouse serum samples were tested at a 1:100 dilution. Polyclonal anti-GM1 and anti–asialo GM1 rabbit antisera were obtained from Matreya, LLC. Murine anti-GM1 monoclonal antibody was obtained from Associates of Cape Cod, Inc. (clone GM1-2b). An unrelated murine anti-phosphotyrosine monoclonal antibody was obtained from Rockland.

**Table 1. Vaccine samples used in the present study and their hemagglutinin (HA) activity.**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Lots tested, no.</th>
<th>Formulation*</th>
<th>HA titer range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>Monovalent</td>
<td>512–1024</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Monovalent</td>
<td>128–512</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>Bivalent</td>
<td>&gt;2048</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>Trivalent</td>
<td>&gt;2048</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>Trivalent</td>
<td>512</td>
</tr>
</tbody>
</table>

Animals. C3H/HeN mice, 8–10 weeks of age, were purchased from Jackson Laboratories and acclimatized for 2 weeks before immunizations. Each of the 11 lots of anti-influenza A/NJ/1976 vaccine, as well as 2 additional vaccine formulations from the 1991–1992 and 2004–2005 immunization programs, was administered to a group of 4 animals. Other mouse groups (n = 4) were immunized with C. jejuni HB93-13 (positive control) or the waaF knockout mutant (negative control) bacterial strain, as described elsewhere [23]. Unless otherwise stated, animals immunized with influenza vaccines were injected with the recommended adult human dose (0.5 mL) of vaccine on days 0 and 21. Each dose was split by injecting 0.125 mL intramuscularly into each front and hind leg. Serum samples were obtained weekly (via tail vein) until day 35, when the animals were killed. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

Detection of bacterial DNA. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was used to detect the presence of bacterial DNA in vaccine samples [26] with the following universal primers: 27F, 5’-AGAGTTTGATCCTTGGCTCAG-3’; 355F, 5’-ACTCCTACGGGAGGCAGC-3’; and 556R, 5’-CTTTACGCCACARTRAWTCCG-3’. The 355F and 556R primers were used to amplify a 200-bp fragment, and 27F and 556R were used to amplify a 500-bp fragment. DNA was extracted from 0.5 mL of each vaccine preparation by phenol-chloroform extraction. We used Escherichia coli DNA as a positive control. Any amplification products were subjected to DNA sequencing.

Detection of glycolipids and ganglioside mimicry. After being dissolved in a chloroform-methanol solution (1:1), vaccine samples, egg-grown A/NJ/1976 virus inactivated with propiolactone, and uninfected chick alantoic fluid were analyzed for the occurrence of glycolipids by thin-layer chromatography (TLC) with diphenylamine or resorcinol staining, as described elsewhere [27, 28]. To detect ganglioside mimicry, samples were analyzed on days 21 (P = .007) and 35 (P = .009), with median titers of 50 and 280 (range, 128 to >2048) on days 21 and 35, respectively. Control titers of antibody to HA from animals immunized with egg-grown inactivated A/NJ/1976 (n = 4) showed a median of 200 (range, 80–320). Mice immunized with C. jejuni HB93-13, which exhibits GM1 mimicry (n = 24), and those immunized with a C. jejuni waaF knockout mutant lacking GM1 mimicry (n = 8) did not develop antibodies to HA (data not shown).

RESULTS

Unopened vaccine samples from 11 vaccine lots containing A/NJ/1976 antigen produced by 3 different manufacturers were first tested for their ability to hemagglutinate chicken red blood cells, to determine whether these influenza vaccines retained HA activity. All of the vaccine preparations had HA activity, with HA titers ranging from 1:128 to >1:2048 (hereafter, titers are reported as reciprocal dilutions), thus demonstrating the retention of HA activity during storage (table 1). All of the vaccine preparations induced antibodies to HA in immunized mice, as measured by HAI assay (figure 1). Anti-HA antibodies were not detected on day 0, and significantly increased titers were observed on days 21 (P = .007) and 35 (P = .009), with median titers of 50 and 280 (range, 128 to >2048) on days 21 and 35, respectively. Control titers of antibody to HA from animals immunized with egg-grown inactivated A/NJ/1976 (n = 4) showed a median of 200 (range, 80–320). Mice immunized with C. jejuni HB93-13, which exhibits GM1 mimicry (n = 24), and those immunized with a C. jejuni waaF knockout mutant lacking GM1 mimicry (n = 8) did not develop antibodies to HA (data not shown).

We next immunized groups of mice with the 11 lots of influenza vaccines containing A/NJ/1976 antigen. To test the hypothesis that the 1976 anti-influenza vaccines induced GBS because of contamination by C. jejuni derived from vaccine production in eggs, we tested all serum samples from vaccine-immunized mice for antibodies to C. jejuni, using a pooled surface-antigen preparation that has been employed to examine the human immune response to C. jejuni infection [31]. Antibodies to C. jejuni...
were not observed in immunized mice, suggesting that Campylobacter antigens were not present in the vaccine formulations (data not shown). Furthermore, we used PCR with universal (eubacterial) primers to amplify the 16S rRNA gene from vaccine preparations, but the gene was not detected in any of the samples (data not shown).

We examined each group of immunized mice for the development of anti-GM1 antibodies by ELISA. All mice developed IgM and IgG antibody responses to GM1 ganglioside (figure 2, top). Significant increases in both IgM and IgG antibodies to GM1 ganglioside were observed starting on day 7 after initial immunization ($P = .0007$ and $P = .0017$, respectively) and generally increased over time through day 35 ($P = .0007$ and $P = .0008$, respectively). The antibody responses to each of the 11 swine flu vaccine lots are shown in figure 3. As a positive control, C. jejuni HB93-13, which expresses GM1, was used to immunize mice, and animals developed anti-GM1 IgG and IgM antibodies (figure 2, bottom). A negative control of an isogenic C. jejuni waaF knockout mutant did not induce antibodies to GM1, as has been shown previously [23], owing to the structurally confirmed absence of GM1-mimicking core oligosaccharide of LOS from this mutant [22].

Subsequently, 2 additional influenza vaccines not associated with an increased risk of GBS in vaccine recipients (trivalent formulations from the 1991–1992 and 2004–2005 influenza seasons) were tested for their ability to induce anti-GM1 antibodies (figure 4). Mice immunized with these vaccines developed HAI responses on day 35. None of the mice developed anti-C. jejuni antibodies (data not shown), but they did develop anti-GM1 IgM and IgG antibody responses after vaccination.

We asked whether the influenza vaccines exhibited ganglioside mimicry or contained ganglioside-like epitopes. Initial testing of vaccines using a cholera toxin dot blot assay [32] showed that all 1976 vaccines bound cholera toxin, suggesting the presence of GM1-like structures in the vaccine (data not shown). The 1976 vaccine lots and the more contemporary influenza vaccines, egg-grown A/NJ/1976 virus, and uninfected chick alantoic fluid were analyzed by TLC with chemical staining. All vaccine samples were shown to contain glycolipid-like molecules ($R_f = 0.27$ and $R_f = 0.43$), as indicated by diphenylamine staining. However, 1 vaccine sample (2004–2005) had an additional glycolipid band ($R_f = 0.57$) that stained with resorcinol, indicative of the occurrence of sialic acid, which is found in certain gangliosides, including GM1. Subsequently, for detection of ganglioside mimicry, samples were subjected to TLC with immunostaining using individual polyclonal anti-GM1, anti–asialo GM1, anti-GM2, anti–asialo GM2, and anti-GD3 rabbit antisera as probes at a 1:100 dilution. Again, the vaccine lot (2004–2005) showed reactivity with anti-GM1 (moderate reaction), anti–asialo GM1 (strong reaction), and anti-GM2 (weak reaction) antibodies, according to a standardized semiquantitative measure [27], indicating the presence in the sample of ganglioside epitopes that are related to a GM1-like mimic. Moreover, A/NJ/1976 virus and chick alantoic fluid reacted weakly with the anti-GM1 antibody preparation. Authentic gangliosides (GM1, asialo GM1, GM2, asialo GM2, and GD3) in the assay performed as expected for controls. Other assays, including dot blotting, Western blotting, and ELISA, confirmed these results (data not shown).
shown), showing that the assay format did not influence the reactions observed.

Finally, we asked whether HA was involved in inducing anti-GM$_1$ antibodies observed in vaccine-immunized mice. In preliminary studies, anti-GM$_1$ antibody preparations from 2 commercial sources were tested for HAI activity to determine their reaction with HA (table 2). Monoclonal and polyclonal anti-GM$_1$ antibodies exhibited HAI titers of 10 and 20, respectively, whereas an anti–asialo GM$_1$ polyclonal antibody preparation did not. Likewise, an irrelevant anti-phosphotyrosine monoclonal antibody preparation showed no HAI activity. Ferret antiserum and mouse polyclonal antiserum to A/NJ/1976 were positive for HAI activity, as expected. Furthermore, 2 recombinant HA proteins derived from the H5N1 viruses A/HK/156/97 and A/Vietnam/1203/04 elicited a significant anti-GM$_1$ antibody response after a single immunization (figure 5).

**DISCUSSION**

We found that commercial influenza vaccines containing A/NJ/1976 induced IgG and IgM antibodies to GM$_1$, but not to *C. jejuni*, after immunization in mice. Unexpectedly, this was not restricted to the 1976 vaccines but was also observed in both the 1991–1992 and 2004–2005 commercial vaccines. All tested 1976 vaccine lots retained HA activity and induced antibodies to HA in mice after the rather lengthy storage period. There was, however, variability in the HA titers among 1976 vaccine lots, which may represent some, but not complete, degradation of the product during storage.

Ganglioside GM$_1$ is a monosialylated glycosphingolipid, which is one of several gangliosides considered a target antigen in the pathogenesis of GBS [10, 33]. One of the best-studied examples of a precedent infection associated with GBS development is *C. jejuni*–mediated gastroenteritis. *C. jejuni* expresses ganglioside-like mimicry in the core region of its bacterial surface LOS, and these structures can induce anti-ganglioside antibodies, such as anti-GM$_1$ [16, 27], and reproduce the disease in rabbits [14]. It is currently postulated that loss of tolerance to naturally occurring gangliosides in susceptible hosts is a mechanism for how these antibodies arise after *C. jejuni* infection and trigger autoimmune-mediated disease [13, 33, 34]. Activation of autoreactive T cells to self-glycolipids after infection may also play a role in the development and progression of disease [35]. Nonetheless, most patients infected with *C. jejuni* strains expressing ganglioside-like mimicry do not develop GBS [32]; thus, host susceptibility factors may contribute to GBS development [36].

The vaccines produced in 1976, as well as the more contemporary formulations examined here, were produced in eggs and purified by physical and chemical procedures. These vaccine preparations contain not only HA but also other viral components—such as NA, nucleoprotein, and other viral structural proteins [37]—as well as egg proteins and other excipient remnants from production [18]. One hypothesis for why the 1976 vaccines elicited GBS was that bacterial antigens, such as those from *C. jejuni*, contaminated the eggs and/or were introduced during processing of the vaccine and thus elicited anti–*C. jejuni*

**Table 2. Hemagglutination inhibition (HAI) activity of different antibody preparations, including anti–GM$_1$, ganglioside, anti–influenza virus, anti–*Campylobacter jejuni*, and anti–influenza vaccine antibodies.**

<table>
<thead>
<tr>
<th>Antibody preparation</th>
<th>HAI titer$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti–GM$_1$, mouse monoclonal IgG</td>
<td>10</td>
</tr>
<tr>
<td>Anti–GM$_1$, rabbit polyclonal antibody</td>
<td>20</td>
</tr>
<tr>
<td>Anti–asialo GM$_1$, polyclonal antibody</td>
<td>0</td>
</tr>
<tr>
<td>Anti–phosphotyrosine monoclonal antibody</td>
<td>0</td>
</tr>
<tr>
<td>Anti–swine influenza virus ferret antiserum</td>
<td>320</td>
</tr>
<tr>
<td>Anti–<em>C. jejuni</em> HB93-13 mouse antiserum</td>
<td>0</td>
</tr>
<tr>
<td>Anti–monovalent A/NJ/1976 vaccine mouse antiserum$^b$</td>
<td>80</td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Titers are expressed as the reciprocal of the highest serial dilution that inhibited hemagglutination of the preparation.

$^b$ Serum sample from one of the mouse vaccine groups immunized with monovalent vaccine.
immune responses in susceptible vaccinated individuals, leading to the development of GBS. However, this is an unlikely scenario, because *C. jejuni* is not transmitted vertically from hen to egg [38]. Alternatively, external egg contamination with *C. jejuni* through residual fecal material on the exterior of the eggs may have increased during the massive ramping up of swine flu vaccine production. We provide evidence, however, that *C. jejuni* contamination is probably not the cause of the anti-GM₁ antibody production in animals immunized with A/NJ/1976 and other influenza vaccines. Importantly, antibodies to *C. jejuni* antigens were not detected in mice immunized with the various vaccine preparations, nor did any of the vaccines contain bacterial DNA.

On the other hand, we present preliminary evidence that influenza HA may be involved in eliciting anti-GM₁ antibodies in mice, on the basis of 2 experiments. First, commercial monoclonal and polyclonal antibodies to GM₁ had low but detectable antibody activity against HA, and this was not detected with irrelevant monoclonal antibody or with mouse anti-*C. jejuni* polyclonal antibody. The specificity of the reaction was further underscored by the absence of HAI activity in an anti-asialo GM₁ (the related, nonsialylated form of GM₁) polyclonal antibody preparation. Serum samples from mice immunized with a *C. jejuni* isolate expressing GM₁ ganglioside mimicry, which contained anti-GM₁ antibodies (figure 2), did not have HAI activity. This is likely explained by different fine specificities of the anti-GM₁ response in animals immunized with ganglioside-like structures borne on different chemical entities [13]. Second, experiments with recombinant HA of the H5 subtype also showed that immunized mice developed low but significant responses to GM₁ ganglioside (figure 5).

Influenza viruses enter the host cell by binding to sialic acid receptors on cell surfaces; this is mediated by HA, a viral surface glycoprotein with a receptor-binding pocket that can bind to specific sialylglycoproteins and cellular gangliosides [39–41] to facilitate host cell entry by influenza viruses. After intracellular viral replication, influenza viruses bud from the cell membrane but may become bound to the cell membrane through sialic acid receptors (e.g., a sialylated ganglioside [41]). Viral NA mediates virus release by removing sialic acid. It is unclear whether sialic acid is completely removed from the viral HA on virus release, and it is possible that sufficient sialic acid–associated HA occurs to mimic a GM₁ epitope. This is supported by our ability to detect GM₁ epitope mimicry in the egg-grown virus preparation as well as in commercial vaccine preparations.

It had been reported elsewhere that influenza viruses and the commercial influenza vaccines derived from them contained varying amounts of viral NA and the A/NJ/1976 vaccine contained little to no detectable NA activity [37, 42], compared with other vaccine formulations. This leads us to speculate that the low levels of viral NA in the 1976 vaccine may have allowed for sufficient sialic acid to remain bound to viral HA, forming a sialic acid–HA complex that mimics GM₁ ganglioside. Higher levels of viral NA in other vaccines could be sufficient to reduce the amount of sialic acid–HA complex so that it is less immunogenic and therefore did not trigger GBS, as observed for the 1976 vaccine. Thus, given that the immunogenicity of HA differs among influenza virus strains [43], one possibility is that A/NJ/1976 HA had different immunogenic properties than other influenza vaccine HA, resulting in a more potent anti-GM₁ (or other ganglioside) antibody response and GBS in susceptible hosts.

Several studies have examined the risk of GBS after various influenza immunization programs [1, 9, 44–47]. Compared with the risk associated with the A/NJ/1976 vaccines used in the 1976 vaccine program, the risk of GBS after immunization with other influenza vaccines, if there is one, is much smaller, perhaps contributing 1 excess GBS case per million individuals vaccinated. Thus, it has been difficult to establish a link between GBS and influenza vaccine–related GBS in recent years.

Previous studies of the immunogenicity of influenza vaccines in animals or humans have not examined anti-ganglioside antibody responses and should be considered in future vaccine studi-
ies. The present study is the first to do so and may lay the found-
dation for a more complete immunogenicity profile for these
vaccines. However, a limitation of the present study is that mice
were used to detect whether anti-GM₁ antibodies arose after im-
munization with the various influenza vaccines, and it remains
unknown whether this finding correlates with the anti-
ganglioside antibody response in humans and the development
of GBS after vaccination. We are unaware of any human serum
collections from the 1976 vaccination program, and testing of
any such collection for anti-ganglioside antibodies would be
helpful in corroborating the results of our animal studies. In
addition, whether the antibodies detected in mice are pathogenic
and capable of blocking nerve conduction or inducing other
pathological damage, as observed in GBS, cannot be determined
from the present study. Moreover, we used vaccine doses that
were relatively high in proportion to animal body weight, com-
pared with the proportion of dose to body weight for human
vaccine immunization. A further limitation of this study is that
we did not include other vaccines or viruses that do not bind to
sialic acid receptors, so we had no negative influenza virus con-
trol vaccine that failed to induce anti-GM₁ antibodies. Whether
influenza vaccines prepared from tissue culture–grown virus
elicit anti-GM₁ responses similar to those observed in our study
is another question for future investigation. Nevertheless, the
present study provides some intriguing evidence that some com-
ponent or moieties in influenza vaccines can induce anti-GM₁
antibodies after vaccination of mice; hence, further studies of the
nature of these entities and their impact on the development of
GBS in humans are clearly warranted.

Acknowledgments

We gratefully thank Robert Couch for providing the vaccine samples used
in this study; Robert Tauxe, Paul Glezen, Patricia Fields, and Bala Swami-
nathan for helpful discussions about the study; and Saien Lai for technical
assistance.

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