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Antibody Response to Monovalent A/New Jersey/8/76 Influenza Vaccine in Pregnant Women

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The decision to implement a mass immunization program with A/New Jersey/8/76 (Hsw1N1) influenza vaccine provided a unique opportunity to evaluate immunological responses during pregnancy. Fifty-nine pregnant and 27 nonpregnant women participated in this study. Influenza virus hemagglutination-inhibition antibody titers were determined to A/New Jersey/8/76 (Hsw1N1), A/Japan/305/57 (H2N2), and A/Hong Kong/8/68 (H3N2) before and after a single dose of monovalent (200 chick cell agglutination units) influenza A/New Jersey/8/76 (Hsw1N1) vaccine. The difference in titers between pregnant and nonpregnant women was insignificant. Treatment of the sera with 2-mercaptoethanol disclosed a similar immunoglobulin M response to the vaccine in both groups. The mean fold rise in heterologous antibody titer was similar in pregnant and nonpregnant women. This study demonstrated that pregnant women were able to respond to an original myxovirus antigen, influenza A/New Jersey/8/76, in a manner equivalent to nonpregnant, age-matched controls.

Pregnant women are considered to be at risk of excess rates of mortality during influenza pandemics (3). Hardy et al. (8) during the Asian influenza pandemic of 1957 reported a high overall attack rate (85%) in pregnant women. During this pandemic, the outcome of pregnancy in terms of abortion, stillbirth, prematurity, neonatal morbidity, neonatal death, or congenital malformation could not be demonstrated to be different from experiences in previous and subsequent years (8) or from that of noninfected pregnant women (15).

There have been no adequately controlled studies of the protective effect of immunization during pregnancy in terms of either prevention of maternal disease or effect on pregnancy outcome (7). After the decision to implement a mass immunization program with A/New Jersey/8/76 (Hsw1N1) influenza vaccine, a study was designed to evaluate the effect of immunization for swine influenza vaccine on pregnant women. However, since the swine influenza vaccination program was terminated in February of 1977, the number of pregnant women enrolled in this study was too small to determine the effect of the vaccine on pregnancy outcome.

This report, however, summarizes the results of the antibody responses in pregnant women enrolled in the study and examines the immunogenicity of monovalent A/New Jersey/8/76 (Hsw1N1) influenza vaccine in pregnant women. The reactions and serological responses in normal adults and children and in high risk individuals have been summarized extensively in "Clinical Studies of Influenza Vaccines—1976" (J. Infect. Dis. **136**[Suppl.]:S341-S744, 1977).

MATERIALS AND METHODS

Subjects. Before the termination of the swine influenza vaccination program, 59 pregnant women, ages 18 to 34 (mean age, 24.3), in good general health and without recent or concurrent febrile illness, gave written informed consent to receive immunization with influenza A/New Jersey/8/76 (Hsw1N1) vaccine. Five were in the first trimester of pregnancy, 22 were in the second trimester, and 32 were in the third trimester. Twenty-seven nonpregnant women, ages 20 to 40 (mean age, 30.7), served as controls.

All persons received a single injection of 0.5 ml of monovalent (200 chick cell agglutination units) influenza A/New Jersey/8/76 (Hsw1N1) vaccine (Merrill National lot no. 1511 FK) intramuscularly. The vaccine virus was grown in eggs, inactivated in Formalin, purified by rate-zonal ultracentrifugation, and thus represented a "whole-virus" vaccine. Serum samples were obtained from all patients before immunization and at 4 to 6 weeks post-immunization.

Antibody determinations. Influenza antibody titers were measured by the standard hemagglutination-inhibition (HI) antibody test with influenza A/New Jersey/8/76 (Hsw1N1), influenza A/Japan/305/57 (H2N2), and influenza A/Hong Kong/8/68 (H3N2)

antigens. The procedures and reagents were kindly provided by the Center for Disease Control, Bureau of Laboratories, Atlanta, Ga.

The sera were also tested simultaneously with and without 2-mercaptoethanol treatment. The procedures as described by Cherry et al. (5) and Boyer et al. (2) were followed. Treatment of sera with 2-mercaptoethanol causes an effect of sulfhydryl reduction on the antibody, greatly reducing the amount of immunoglobulin M (IgM) antibody. A fourfold reduction in titer after 2-mercaptoethanol treatment was considered indicative of specific IgM antibody. A twofold reduction was considered suggestive of an IgM response.

All serological titer results were expressed as reciprocals of serum dilutions and compared as geometric mean titers. Student's t test was used to evaluate significance.

RESULTS

There were no significant side effects after immunization in any of the women. For age-matched comparison, 26 pregnant women, age 24 to 34 (mean age, 28.5), were compared with 18 nonpregnant, age-matched (mean age, 28.7) controls. All vaccinees in this group had pre-immunization HI titers <10 to influenza A/New Jersey/8/76.

In Table 1, the post-immunization geometric mean titer of the controls (nonpregnant) was 29.4. The pregnant women had a post-immunization geometric mean titer of 20.0. The difference between the two groups was not significant. There was also no significant difference in comparing the women in different trimesters with controls.

Four pregnant women (all trimesters) and one nonpregnant woman failed to respond to the single dose of influenza A/New Jersey/8/76 antigen (Table 2). Sixty-five percent of the pregnant women responded with an HI titer >20, and 35% had HI titers >40. The response to influenza A/New Jersey/8/76 (Hsw1N1) antigen at HI >20 was fairly uniform regardless of trimester of pregnancy.

TABLE 1. *Serum HI antibody responses to influenza A/New Jersey/8/76 vaccine in pregnant and nonpregnant women (24 to 34 age group)*

Group	No. tested	Reciprocal GMT[a]	
		Before	After
Nonpregnant	18	<10	29.4[b]
Pregnant	26	<10	20.0
1st trimester	3	<10	15.9
2nd trimester	9	<10	20.0
3rd trimester	14	<10	21.0

[a] GMT, Geometric mean titer before and after monovalent influenza A/New Jersey/8/76 vaccine. For calculation of geometric mean titer, titers <10 were assigned a value of 5.

[b] Geometric mean titers were compared by Student's t test for various groups and found not significant.

TABLE 2. *Serum HI antibody responses of pregnant and nonpregnant women 24 to 34 years old to monovalent influenza A/New Jersey/8/76 vaccine*[a]

Reciprocal HI titer	Nonpregnant[b]	Pregnant[b]		
		All trimesters	1st and 2nd trimester	3rd trimester
<10	1	4	1	3
10	4	5	3	2
20	5	8	5	3
40	4	7	2	5
80	1	1	1	0
160	2	0	0	0
320	1	1	0	1

[a] All pre-immunization titers were <10.

[b] Number of subjects who responded with antibody titers at levels indicated.

Figure 1 indicates the result of treatment of the sera with 2-mercaptoethanol. Only sera with post-immunization HI titers ≥10 were treated with 2-mercaptoethanol. The mean fold reduction in titer was fairly uniform and not statistically different in comparing various trimesters or in pregnant and nonpregnant groups.

When the heterologous antibody responses to influenza A/Japan/305/57 (H2N2) and influenza A/Hong Kong/8/68 (H3N2) were examined after immunization with influenza A/New Jersey/8/76 (Hsw1N1) vaccine, there was, as one would expect, a much higher mean fold rise to influenza A/New Jersey/8/76 than to the other antigens. Both groups, pregnant and nonpregnant, exhibited previous exposure to both H2N2 and H3N2 antigens. No statistical significant increase in geometric mean titer for the two heterologous antibodies was demonstrated; however, eight patients, four pregnant and four nonpregnant, out of 86 total vaccinees responded with a fourfold or greater rise in antibody to influenza A/Japan/305/57 (H2N2) after the single injection of influenza A/New Jersey/8/76 (Hsw1N1) vaccine.

DISCUSSION

Immunizations during pregnancy have always been somewhat controversial. Live attenuated virus vaccines, such as rubella, are contraindicated in pregnancy because of unknown or potential teratogenic properties (1). Inactivated viral vaccines, such as influenza, carry no known teratogenic potential and, at least in nonpreg-

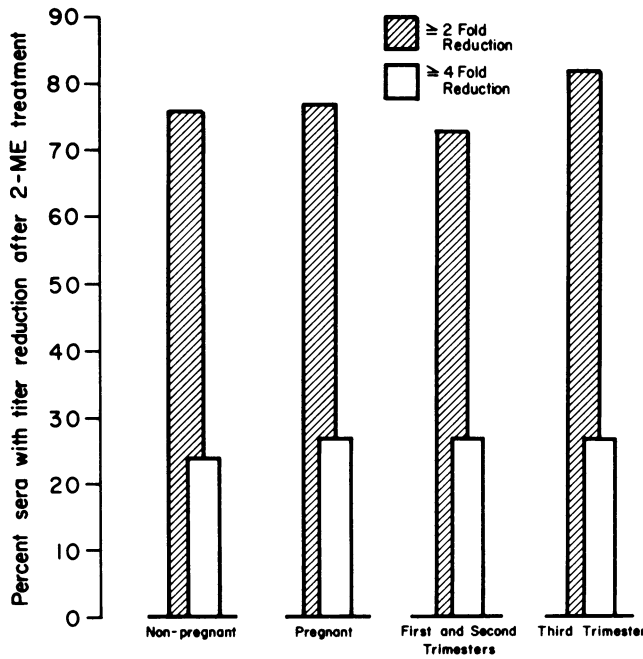

FIG. 1. *Reduction of influenza A/New Jersey/8/76 HI antibody titers of 4- to 6-week post-immunization serum from pregnant and nonpregnant women (24 to 34 age group) after 2-mercaptoethanol (2-ME) treatment.*

nant patients, play a role in modifying clinical disease. The hazard of clinical influenza during pregnancy, particularly during pandemics, has been documented (14). High mortality in puerperal women during the 1918 to 1920 pandemic was well recognized (3, 8). Transplacental transfer of influenza virus during infection in the mother has occurred (16), and during pandemics a high incidence of abortions has been recorded. The need for a study to determine the effectiveness of influenza vaccines for the protection of susceptible pregnant women is evident.

Immunological responses during pregnancy are poorly defined. One study showed a slight but gradual decline in blood levels of IgG toward the end of pregnancy and also postpartum, but this was considered to represent increased placental transfer of antibody to the fetus rather than decreased maternal antibody production (10). Immunization of pregnant women to a variety of antigens has shown no consistent weakening of the immune response (13). In general, the capacity to synthesize antibody during pregnancy has been thought to be at least equivalent to that of nonpregnant individuals.

In this study, we found no significant difference, in age-matched (age 24–34) comparison, between pregnant and nonpregnant vaccinees in response to a single dose of monovalent (200 chick cell agglutination units) influenza A/New Jersey/8/76 vaccine. The percentage of patients able to attain an HI titer ≥20 was similar to those of Cate et al. (4): 65% pregnant and 72% nonpregnant versus 81% in Cate's study (ages 22 to 43, males only). The percentages were somewhat less than the 87% observed by Dolin et al. (ages 24 to 59, males and females) (6).

When the total patient population immunized was used, regardless of age, a significantly lower geometric mean titer ($P < 0.002$) was found in the pregnant vaccinees compared to the nonpregnant vaccinees (data not presented in results). This disparity in antibody response may be explained by the fact that the pregnant vaccinees were younger (mean age, 24.3) than nonpregnant vaccinees (mean age, 30.7) and less responsive to this new influenza virus hemagglutinin as has been shown with other adult populations (12).

Specific antibody response after natural viral infection and immunization may be divided into IgM and IgG classes (9). A fourfold reduction in titer after 2-mercaptoethanol treatment is indicative of a specific IgM response. When comparing groups, however, a twofold reduction is also statistically valid (2). Our results of 77 and 76% (pregnant and nonpregnant) with a twofold or greater reduction and 27 and 24% (pregnant and nonpregnant) with a fourfold or greater reduction are similar to those of Boyer et al. (2) and indicated a primary response in vaccinees after immunization with influenza A/New Jersey/8/

76 vaccine. Most of our patients demonstrated high prevaccination antibody titers to H2N2 influenza viruses. The number of patients who exhibited a fourfold or greater rise in influenza A/Japan/305/57 (H2N2) antibody after influenza A/New Jersey/8/76 (Hsw1N1) vaccine was similar to that of Noble et al. (11). This probably represents cross-reactive determinants in the original primary hemagglutinin and the secondary stimulating hemagglutinin.

The immunological response of pregnant and nonpregnant vaccinees to an original myxovirus antigen, influenza A/New Jersey/8/76, was antigen specific and represented IgM as well as IgG antibody. This study indicates that the B-lymphocyte (including the modulating effect of suppressor and helper T-lymphocytes) response of pregnant women is intact.

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