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Response of Influenza Vaccines Against Heterovariant Influenza Virus Strains in Adults with Chronic Diseases

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The ability of influenza vaccination to provide cross-protection against heterovariant influenza strains was evaluated in a double-blind, randomized, trial in north-east Italy during the winter of 2005–2006. Of 238 adult subjects with underlying chronic diseases, 120 received MF59-adjuvanted subunit vaccine (Sub/MF59) and 118 received a conventional subunit vaccine (Subunit). Immunogenicity was measured for A/H3N2 and B influenza strains against both the homologous vaccine strains (A/New York/55/2004 and B/Jiangsu/10/2003), and the heterovariant strains recommended for the 2006–2007 season (A/Wisconsin/67/2005 and B/Malaysia/2506/2004). Although both vaccines conferred serological protection against the homologous vaccine strains and the 2006–2007 heterovariant A/H3N2 strain for a majority of subjects, the antibody response was highest in the Sub/MF59 vaccine group. For example, MF59-adjuvanted vaccination conferred significantly greater ($P = 0.002$) protection against the heterovariant A/H3N2 strain than the conventional subunit vaccine (79.2% *vs*. 61.0% of subjects, respectively). In conclusion, these results demonstrate that protection provided by influenza vaccination in adults affected by chronic diseases is lower against heterovariant strains than for homologous strains. However, addition of MF59 adjuvant to a subunit vaccine enhances immunogenicity against the A/H3N2 heterovariant strain, conferring broader protection than a conventional subunit vaccine in this population, who are at higher risk of influenza-related complications.

KEY WORDS: influenza A; influenza B; heterovariant; vaccine; MF59 adjuvant; high risk.

INTRODUCTION

Modifications in the genetic structure of influenza A and B viruses lead to the continuous appearance of new antigenic strains. Frequent mutations in the RNA encoding the hemagglutinin and neuraminidase viral surface glycoproteins, described as antigenic drift, are responsible for seasonal epidemic influenza infection (1, 2), with an attack rate ranging from 5 to 30% (3). Seasonal influenza epidemics impose weighty consequences on productivity and working activity, and increase utilization of health services (4, 5). In addition to antigenic drift, less-frequent genetic reassortment between different coinfecting influenza virus subtypes may create novel human variants with different surface antigen subtypes (antigenic shift) (1, 2). These novel virus subtypes have pandemic potential, as the population lacks immunity against the emerging virus (2); in the eventuality of a global pandemic, the attack rate for influenza infection could reach up to 50% (6, 7). A global pandemic is expected to cause a considerable health and economic burden, and as the timing, or the true burden, of the next pandemic is difficult to anticipate, an active and careful influenza surveillance and prevention programme during interpandemic periods is of primary importance (8, 9).

Influenza vaccination is considered to be the most appropriate means of preventing infection and its related complications. The efficacy of influenza vaccines, however, depends on the degree of similarity between the viral strains in the vaccine and those that are in circulation, and

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the age and immunocompetence of the vaccine recipient. Influenza vaccines are changed each year to ensure that the vaccine strains match the strains that are predicted to circulate in the population during the influenza season. Most often, the match between strains is satisfactory. However, occasionally, vaccine strains do not perfectly match the circulating strain leading to diminished vaccine effectiveness, as occurred during the 2003–2004 season (10, 11). When a good match is achieved, influenza vaccination prevents influenza illness among approximately 70–90% of healthy adults less than 65 years of age (12–14). However, in elderly people, and in adults with underlying chronic diseases, postvaccination antibody titres may be lower and the risk of influenza and its complications higher, compared with healthy young adults (15–18). A number of studies have shown that adjuvanted influenza vaccines can offer enhanced immunogenicity and favorable safety profiles in the elderly (19–22) and in adults with chronic diseases (23). Furthermore, the use of adjuvanted vaccines offers broader protection against heterovariant influenza virus strains in the elderly (24, 25) and against emerging strains with pandemic potential (26, 27). Few studies have assessed the potential of adjuvanted influenza vaccines against heterovariant strains in adults with chronic diseases.

Vaccination-induced antibodies against heterologous epidemic virus variants may better indicate the protection achieved by vaccination than antibodies to homologous strains (28). Therefore, as an extension to our previous study (23), we have evaluated whether inactivated influenza vaccines distributed during the 2005–2006 influenza season might be able to confer protection against heterovariant strains (A/Wisconsin/67/2005 and B/Malaysia/2506/2004) in adults with chronic disease. We have compared the ability of an MF59-adjuvanted subunit vaccine (Sub/MF59) to confer cross-protection with that of a conventional subunit vaccine against homologous vaccine strains.

MATERIALS AND METHODS

Subjects

A double-blind, randomized study was conducted in Pianiga, Venice, north-east Italy during the influenza vaccination campaign for the 2005–2006 winter influenza season, as described previously (23). The study recruited adult subjects (18–60 years of age) with medical conditions such as chronic respiratory, cardiac, or renal disease, immunosuppression, diabetes, or cancer. Subjects were randomized (1:1) to receive one of two commercial trivalent inactivated influenza vaccines, a subunit vaccine adjuvanted with MF59 (FLUAD®, Novartis Vaccines [Sub/MF59]), or a conventional subunit vaccine (Influpozzi Subunità®, IVP [Subunit]). Both vaccines contained 15 μg of each of the three influenza viral antigens recommended by the World Health Organization (WHO) for the northern hemisphere during the 2005–2006 influenza season: A/New Caledonia/20/99 (H1N1); A/New York/55/2004 (H3N2) and B/Jiangsu/10/2003. Subjects were excluded if they experienced any acute disease at the time of vaccination, had a known allergy to any vaccine component, or had experienced any known or suspected neurological reactions following influenza vaccination.

Assessment of Immune Response

Blood samples were obtained from each subject before vaccination and at 4 weeks postvaccination. Following sera collection, hemagglutinin inhibition (HI) antibody titres were measured in all samples, as described elsewhere (29).

Immunogenicity was measured for the A/H3N2 and B influenza virus strains against both the homologous strains included in the 2005–2006 vaccine (A/New York/55/2004 (H3N2) and B/Jiangsu/10/2003) and the heterovariant strains recommended for the forthcoming 2006–2007 season (A/Wisconsin/67/2005 and B/Malaysia/2506/2004) (30). Purified antigens supplied by Novartis Vaccines were used.

Immunogenicity was determined using the following parameters: geometric mean titres (GMT) and the corresponding 95% confidence intervals (CI); meanfold increase in titres (MFI; ratio of post- to prevaccination titre); seroprotection rate, defined as the percentage of subjects achieving an HI titre $\geq$1:40; and the percentage of subjects achieving a significant increase in titre (defined as at least a 4-fold increase in HI titre from a nonnegative prevaccination titre [$\geq$1:10] or a rise from $<$1:10 to $\geq$1:40 in those who were serum-negative) (18, 31).

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences for Windows (SPSS 14.0; Chicago, IL, USA). The χ^2 test was used to analyze differences between proportions. Significance levels between pre- and postvaccination titres were assessed using the paired Student's *t*-test. Comparison of different vaccine groups was determined by Student's *t* test for unpaired data. In addition, HI titres were transformed into binary logarithms and corrected for prevaccination status as

Table I. Baseline Characteristics of the Study Population According to Vaccine Group

	Sub/MF59 ($n = 120$)	Subunit ($n = 118$)
Mean age, years ± SD	51.4 ± 12.1	50.7 ± 12.7
Male, *n* (%)	67 (55.8)	53 (44.9)
Previously vaccinated, *n* (%)	69 (57.5)	65 (55.1)
Underlying disease,[a] *n* (%)		
Cancer	17 (14.2)	17 (14.4)
Diabetes mellitus	22 (18.3)	28 (23.7)
Heart condition	57 (47.5)	51 (43.2)
Lung condition	41 (34.2)	33 (28)
Other	6 (5)	6 (5.1)

Note. SD, standard deviation; Sub/MF59, MF59-adjuvanted subunit influenza vaccine; Subunit, conventional subunit influenza vaccine.
[a]It was possible for each subject to have more than one risk factor.

described elsewhere (31). A P value < 0.05 was considered statistically significant.

RESULTS

Overall, the study population consisted of 238 adult subjects affected by chronic diseases and at higher risk for influenza complications. Age, gender, previous influenza vaccination status, and category of underlying diseases were equally distributed between the two vaccine groups (Table I).

Immune Response Against the A/H3N2 Viral Strain

For both vaccine groups, prevaccination GMT and seroprotection rates were higher for the homologous A/H3N2 2005–2006 virus strain (A/New York) than against the new heterologous 2006–2007 variant (A/Wisconsin) (Table II). However, similar values were recorded for both the vaccination groups (Table II).

Both vaccines induced a significant rise ($P < 0.001$) in GMT against the homologous and the heterologous vaccine strains at 4 weeks postvaccination (Fig. 1). For both strains, significantly higher GMTs ($P = 0.001$) were recorded in the Sub/MF59 group compared with the Subunit group (Fig. 1). Furthermore, postvaccination seroprotection rates were significantly ($P = 0.002$) higher for A/New York and A/Wisconsin in the Sub/MF59 vaccine group than the Subunit group. In fact, the highest seroprotection rate (79.2%) was achieved for the heterovariant virus in the Sub/MF59 vaccination group. Correction of the data for prevaccination titres did not alter these findings (data not shown).

Although both vaccines induced a substantial immune response to both the homologous and the heterologous virus strains, the highest MFI (8.0) was observed for the Sub/MF59 group against the A/Wisconsin variant (Table II). In addition, the percentage of subjects with at least a 4-fold increase in postvaccination GMT was significantly higher for both the A/New York ($P = 0.02$) and A/Wisconsin ($P = 0.008$) viruses in the Sub/MF59 group, compared with the Subunit group. Interestingly, when the results were compared by virus strain, the MFI and the percentage of subjects with at least a 4-fold increase in postvaccination GMT were higher against the A/Wisconsin heterovariant strain, compared with the homologous virus for both vaccine groups (Table II).

Immune Response Against the B Viral Strain

Figure 2 and Table III detail the antibody response with regard to the influenza B virus strains. For both vaccine groups, prevaccination GMT were higher (approximately 1.8-fold) against the heterovariant B/Malaysia than the B/Jiangsu vaccine strain (Fig. 2). Despite this difference, the prevaccination seroprotection rate was comparable between both strains for the two vaccine groups.

Vaccination induced an increase in GMT for both strains and both vaccine groups, with higher titres being reported against B/Jiangsu (Fig. 2). Postvaccination

Table II. Antibody Response, as Determined by the Hemagglutinin Inhibition Test, Pre- and Postvaccination for the Sub/MF59 and Subunit Vaccine Groups, According to Viral Strain (A/H3N2 Viral Strain)

	Sub/MF59 ($n = 120$)		Subunit ($n = 118$)	
	A/NewYork/55/2004	A/Wisconsin/67/2005	A/NewYork/55/2004	A/Wisconsin/67/2005
Seroprotection rate (% of subjects with titre ≥1:40)				
Prevaccination	15.8	10.0	14.4	7.6
Postvaccination	75.0[a]	79.2[a]	57.6	61.0
MFI	4.8	8.0	3.2	4.7
4-fold titre increase (% of subjects)	52.5[b]	67.5[c]	33.1	50.8

[a]$P = 0.002$.
[b]$P = 0.02$.
[c]$P = 0.008$ versus Subunit data.

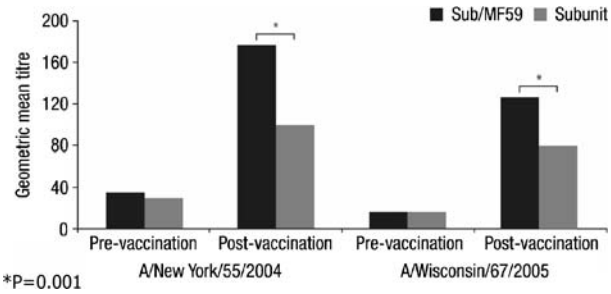

Fig. 1. Geometric mean titre against the homologous vaccine strain (A/New York/55/2004) and the heterovariant vaccine strain (A/Wisconsin/67/2005) in subjects prevaccination and at 4 weeks post-vaccination. *Note*. For both vaccines and both strains, post-vaccination titres were significantly ($P < 0.001$) higher than pre-vaccination titres.

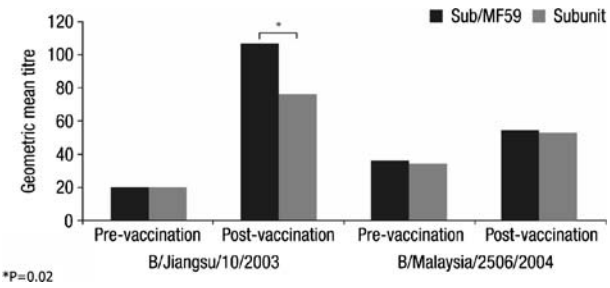

Fig. 2. Geometric mean titre against the homologous vaccine strain (B/Jiangsu/10/2003) and the heterovariant vaccine strain (B/Malaysia/2506/2004) in subjects prevaccination and at 4 weeks postvaccination.

GMT against the homologous vaccine strain were significantly higher for the Sub/MF59 group than the Subunit group (106.8 *vs*. 76.6; $P = 0.02$). No significant differences were observed between vaccine groups for the post-vaccination seroprotection rate, MFI, or the percentage of subjects with at least a 4-fold increase in postvaccination GMT (Table III); however, comparison of the results by viral strain revealed that these values were higher for the homologous vaccine strain than the heterovariant strain.

DISCUSSION

As an extension to our previous study in subjects with chronic diseases vaccinated during the 2005–2006 winter season (23), we investigated the ability of two influenza vaccines to provide protection against the heterovariant strains, A/Wisconsin/67/2005 and B/Malaysia/2506/2004 included into the vaccine recommended for the forthcoming 2006–2007 season (30).

The ability of a vaccine to evoke an immune response against viruses different from the vaccine strains (i.e., drift variants) has a clinical and epidemiological significance, since the seasonal influenza epidemics are frequently caused by circulating viruses that are antigenically distinct from those in the vaccine formulation. In addition, the antibodies to heterologous strains induced by vaccination can provide a better indication of the degree of protection conferred by different vaccines (28).

In this study, both inactivated vaccines were able to confer serological protection against the new 2006–2007 antigenic variant A/Wisconsin/67/2005 in a majority of adult subjects with underlying chronic disease. The postvaccination rise in antibody titre and the rate of protection reached after vaccination were, however, higher in subjects vaccinated with the MF59-adjuvanted vaccine than the conventional vaccine (e.g., 79.2% *vs*. 61% of subjects achieved ≥1:40 protective titres against A/Wisconsin/67/2005, respectively [$P = 0.002$]).

Our data are consistent with findings reported in a recent study performed in elderly subjects (25), and demonstrate that addition of the MF59 adjuvant to a subunit influenza vaccine enhances serological protection against the heterovariant A/H3N2 viral strains. Thus, in vulnerable populations, the MF59-adjuvanted vaccine is protective even when there is no close genetic homology between the vaccine influenza strains and the circulating influenza strains. The benefits of higher immunogenicity and broader protection to emerging new influenza variants conferred by the MF59-adjuvanted vaccine, compared with a conventional nonadjuvanted vaccine, assume particular importance in vulnerable individuals,

Table III. Antibody Response, as Determined by the Hemagglutinin Inhibition Test, Pre- and Postvaccination for the Sub/MF59 and Subunit Vaccine Groups, According to Viral Strain (B Viral Strain)

	Sub/MF59 ($n = 120$)		Subunit ($n = 118$)	
	B/Jiangsu/10/2003	B/Malaysia/2506/2004	B/Jiangsu/10/2003	B/Malaysia/2506/2004
Seroprotection rate (% of subjects with titre ≥1:40)				
Prevaccination	17.5	18.3	16.9	22.0
Postvaccination	69.2	39.2	61.0	42.4
MFI	5.4	1.5	4.0	1.5
4-fold titre increase (% of subjects)	46.7	10.0	36.4	9.3

such as the elderly and subjects with chronic disease who are susceptible to influenza and its severe associated complications.

In contrast, in this study, the enhanced immunogenicity observed with the MF59-adjuvanted vaccine for the A/H3N2 strain was not seen against the type B heterologous vaccine strain (B/Malaysia); both vaccines induced a comparable increase in geometric mean titres against the heterovariant B strain. The influenza B virus belongs to two major phylogenetic lineages that cocirculate in the population: the B/Victoria/2/87 or the B/Yamagata/16/88 lineage (32). Reassortment among strains from different lineages plays a role in generating the genetic diversity of influenza B viruses (32, 33). Influenza B viruses of both lineages circulated during the 2005–2006 influenza season; however, the B/Victoria/2/87 lineage was predicted to dominate during the 2006–2007 season (30, 34). Thus, genetic differences in the B strain from one year to the next are not as subtle as for the type A strains, resulting in a greater risk of strain mismatch.

In conclusion, these results demonstrate that protection provided by influenza vaccination in adults affected by chronic diseases is lower against heterovariant strains than for homologous strains. However, addition of MF59 adjuvant to subunit vaccine enhances immunogenicity against the A/H3N2 heterovariant strain, conferring broader protection than a conventional subunit vaccine in this population, who are at higher risk of influenza-related complications. Thus, high-risk adults should be encouraged to receive influenza vaccine annually to protect against seasonal influenza epidemics, as a suitable vaccine is available.

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