SHORT COMMUNICATION

Cross-protection by MF59™-adjuvanted influenza vaccine: Neutralizing and haemagglutination-inhibiting antibody activity against A(H3N2) drifted influenza viruses

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Summary   Adjuvants enhance antibody response against vaccination. We compared the ability of MF59™-adjuvanted and non-adjuvanted subunit influenza vaccines, containing A/Wyoming/3/03(H3N2), to confer cross-protection against four consecutive drifted strains in the elderly. Neutralizing and haemagglutination-inhibiting antibody were measured. MF59™-adjuvanted vaccine induced a stronger booster response against A/Panama/2007/99(H3N2) than non-adjuvanted vaccine. A/Panama/2007/99(H3N2) circulated widely during the previous 5 years and was included in vaccines over four consecutive seasons. Broader serological protection against drifted strains that circulated 1 and 2 years after vaccination with A/Wyoming/3/03(H3N2) was observed with MF59™-adjuvanted vaccine. Thus, MF59™-adjuvanted vaccine confers greater immunogenicity than non-adjuvanted vaccines in vulnerable populations.
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Introduction

Influenza vaccines need to elicit an effective immune response against the virus strains included in the vaccine and against antigenically different virus strains, as drifted strains can appear following annual recommendation of vaccine composition. Such drifted strains can compromise vaccine-induced immunity, due to antigenic mismatch

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with the vaccine strain, and resulting seroprotection rates (assessed as serum haemagglutination-inhibiting [HI] assay titres ≥40 IU) can vary according to the antigenic distance between the vaccine strain and the circulating strain [1—4]. In elderly subjects, seroprotection rates can be as low as 20% against drifted strains, dropping from ≥70% in years where a good antigenic match is observed [1—4].

Although the HI assay is considered the ''gold standard'' for evaluation of vaccine-induced antibody response, there are some well-known limitations of this technique, in terms of sensitivity and specificity [5]. For example, during an outbreak caused by a drifted strain, protective HI titres against the strain were identified in 87% of vaccinated elderly nursing home residents diagnosed with influenza. These data suggest that such antibody levels might not be sufficient to neutralize viral infectivity [6]. In contrast, neutralization (NT) assays may be more sensitive than the HI test, both in detecting a higher rate of antibody increases and in detecting antibody levels in individuals who are seronegative according to the HI assay. Furthermore, they may provide a more functional measure of vaccine-induced immunity [2,7].

Several strategies have been proposed to address the need for vaccines that offer enhanced protection against drifted strains, including the use of adjuvanted vaccines, universal vaccines, and vaccines that exploit mechanisms of cross-protective immunity [8]. Studies have demonstrated that addition of the adjuvant MF59[TM] to subunit influenza vaccine can lead to higher seroprotection rates against drifted strains not included in the vaccine than are achieved with non-adjuvanted subunit vaccine [3,9]. These studies have predominantly used the HI assay for the estimation of antibody response, and there is currently a lack of data on the effect of drift on the neutralization ability of antibody elicited by influenza vaccines against heterovariant strains. To gain a more complete understanding of the effect of MF59[TM] on antibody response, we compared the ability of an MF59[TM]-adjuvanted vaccine and a non-adjuvanted subunit influenza vaccine to confer cross-protection against four consecutive drifted variants, measuring both the HI and NT activity of antibody. The study was conducted at a time when

a new A(H3N2) strain had been recommended for vaccine inclusion.

Methods

Study population

Prior to the 2004—2005 influenza season, healthy, elderly subjects ($n=50$; ≥65 years of age) were randomly assigned (1:1) to receive either a single dose of MF59[TM]-adjuvanted subunit influenza vaccine (FLUAD®; Novartis Vaccines, Siena, Italy) or a non-adjuvanted subunit influenza vaccine (Agrippal®; Novartis Vaccines, Siena, Italy). Sera were collected immediately before and 21 days after vaccination. Both vaccines contained 15 μg of each of the influenza strains recommended for the Northern hemisphere 2004—2005 influenza season (A/New Caledonia/20/99(H1N1); A/Wyoming/3/03(H3N2); B/Shanghai/361/02). All subjects provided written, informed consent prior to participation in the study, and institutional guidelines were followed.

Assessment of immune response and statistical analysis

HI and NT antibodies were titred, as previously described [5,10], against four consecutive drifted A(H3N2) variants: A/Panama/2007/99 (Pan/99); A/Wyoming/3/03 (Wyo/03); A/California/7/04 (Cal/04); and A/Wisconsin/67/05 (Wisc/05), representing vaccine composition changes for A(H3N2) during the last decade. The A(H3N2) subtype was chosen because it is most associated with disease burden in the elderly. The antigenic and molecular distances between the vaccine strain (Wyo/03) and Pan/99, Cal/04, and Wisc/05 are shown in Fig. 1A. Pan/99, Cal/04, and Wisc/05 presented 16, 10, 11 amino acid changes with respect to Wyo/03 on the globular head of haemagglutinin, respectively. Purified strains were kindly supplied by Alan Hay, World Health Organization Influenza Centre, London, United Kingdom.
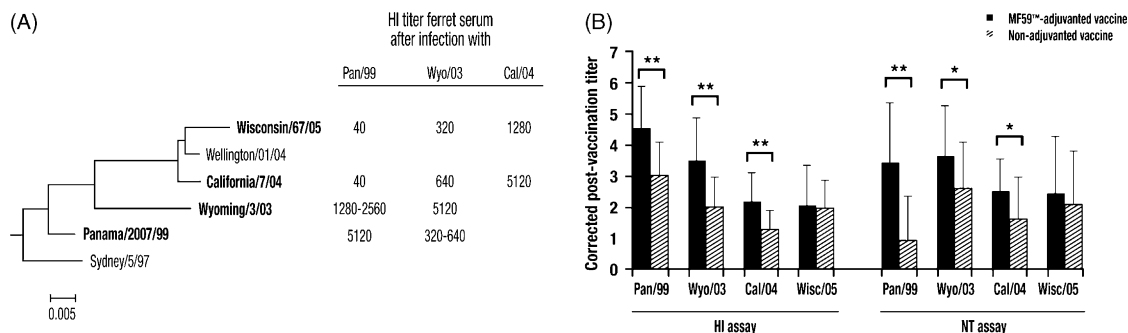
Figure 1 (A) Antigenic and molecular distances between the vaccine strain (A/Wyoming/3/03), A/Panama/2007/99, A/California/7/04, and Wisconsin/67/05. The phylogenetic tree was based on sequence analysis of the region codifying for the globular head region of haemagglutinin and including the vaccine strains in the last decade. The strains used in this study are in bold and (B) post-vaccination haemagglutination-inhibiting (HI) and neutralization (NT) titres, corrected for pre-vaccination status, in subjects vaccinated with an MF59[TM]-adjuvanted vaccine or a non-adjuvanted vaccine, each containing A/Wyoming/3/03 antigen, according to viral strain. Data expressed as mean titres. Error bars denote standard deviation. **P < 0.01, *P < 0.05, MF59[TM]-adjuvanted vs. non-adjuvanted vaccine.

Table 1 Antibody response determined using HI and NT assays after vaccination with an MF59™-adjuvanted vaccine and non-adjuvanted vaccine, according to viral strain

Test and parameters	MF59™-adjuvanted vaccine ($n = 25$)				Non-adjuvanted vaccine ($n = 25$)			
	Pan/99	Wyo/03	Cal/04	Wisc/05	Pan/99	Wyo/03	Cal/04	Wisc/05
HI test								
Pre-vaccination GMT	99.0	75.7	18.4	13.0	110.0	64.0	15.3	11.3
Post-vaccination GMT	422.3[a]	347.7[a,b]	50.5[a,b]	38.3[a,b]	205.3[a]	129.9[a]	21.9[a]	23.2[a]
MFI	4.26	4.59	2.74	2.95	1.86	2.02	1.43	2.05
Seroprotection rate (%)	100	100	80[b]	64[b]	100	96	28	20
Seroconversion rate (%)	60[b]	68[b]	48[b]	44	24	28	8	20
NT test								
Pre-vaccination GMT	117.9	28.6	13.2	16.0	131.8	20.6	15.2	9.7
Post-vaccination GMT	588.0[a]	194.2[a,b]	47.2[a]	80.0[a]	357.5[a]	62.3[a]	36.8[a]	45.9[a]
MFI	4.99	6.79	3.57	5.00	2.71	3.02	2.42	4.73

HI, hemagglutination-inhibiting; GMT, geometric mean titer; MFI, mean fold increase; NT, neutralization.
[a] $P < 0.05$, compared with pre-vaccination GMT.
[b] $P < 0.05$, compared with non-adjuvanted vaccine.

Immunogenicity was determined by: geometric mean titre (GMT); mean-fold increase (MFI; ratio of post- to pre-vaccination titre); seroprotection rate (the percentage of subjects achieving an HI titre $\geq$40 IU); and seroconversion rate (the percentage of subjects with at least a 4-fold increase in HI titre from a non-negative pre-vaccination titre or a rise from <10 to $\geq$40 IU in those who were seronegative). The results were evaluated against the Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines in the elderly, which require that at least one of the following criteria be met: MFI > 2; seroprotection rate >60%, or seroconversion rate >30%.

HI and NT titres were also transformed into binary logarithms, corrected for pre-vaccination status, as described by Beyer et al. [11] and were expressed as mean titres, with the corresponding standard deviation. The observed distributions were confirmed to be normally distributed by the one-sample, Kolmogorov–Smirnov goodness-of-fit test procedure.

Comparisons between the MF59™-adjuvanted and the non-adjuvanted subunit influenza vaccines were analyzed by Student's *t* test for paired and unpaired data (titres) and by chi-square test (seroprotected subject proportions).

Results

Pre- and post-vaccination HI and NT GMTs, seroprotection rates, and seroconversion rates for both vaccines are shown in Table 1, according to viral strain. Pre-vaccination titres were not significantly different between vaccine groups, for all four strains (Table 1).

Both vaccines met CHMP requirements for MFI (>2) and seroprotection rate (>60%) against the vaccine strain (Wyo/03); however, the requirement for seroconversion rate (>30%) was only met for the MF59™-adjuvanted vaccine group. Subjects vaccinated with the MF59™-adjuvanted vaccine showed significantly higher post-vaccination HI and NT GMTs $P = 0.01$ and $P = 0.03$, respectively) and a significantly ($P < 0.01$) higher seroconversion rate against the vaccine strain (Wyo/03) than those in the non-adjuvanted vaccine

group. Seroprotection rates were high ($\geq$96%) for both vaccine groups.

For the drifted strains, only the MF59™-adjuvanted vaccine induced a substantial immune response, meeting all CHMP requirements against Pan/99, Cal/04, and Wisc/05. Against Cal/04 and Wisc/05, the MF59™-adjuvanted vaccine induced significantly higher HI GMTs ($P < 0.01$ and $P = 0.05$, respectively) and seroprotection rates ($P < 0.01$ and $P < 0.01$, respectively), compared with the non-adjuvanted vaccine. The MF59™-adjuvanted vaccine also induced significantly higher seroconversion rates against Pan/99 ($P < 0.01$) and Cal/04 ($P < 0.01$), compared with the non-adjuvanted vaccine (Table 1).

Following correction for pre-vaccination status, both HI and NT titres were significantly ($P < 0.05$) higher for the MF59™-adjuvanted vaccine, when evaluated against Pan/99, Wyo/03, and Cal/04, compared with the non-adjuvanted vaccine (Fig. 1B).

Discussion

The ability of both of the subunit influenza vaccines used in this study to confer seroprotection against a homologous strain, and of MF59™ to enhance the immune response, is consistent with other findings reported during the last decade [3,12,13]. Together, these data confirm that, against homologous strains, MF59™-adjuvanted vaccine elicits a stronger immune response than non-adjuvanted vaccine.

When the immune response was evaluated against drifted strains, however, the immunogenicity profile of the two vaccines differed markedly. In subjects vaccinated with the MF59™-adjuvanted vaccine, CHMP requirements for seroprotection and seroconversion rates and MFI were reached for Pan/99, Cal/04, and Wisc/05, while the non-adjuvanted vaccine failed to achieve the CHMP seroprotection rate criterion against Cal/04 and Wisc/05 or the CHMP seroconversion rate criterion against all of the drifted strains. Thus, the MF59™-adjuvanted vaccine offered broad serological protection against drifted strains that circulated 1 and 2 years after vaccination with Wyo/03. The adju-

Table 2 Influenza vaccine strains and circulating strains, 1997—2007 [19]

Season	Northern hemisphere vaccine strains			Circulating strains in Europe and the US		
	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B
1997–98	Bayern/95	Wuhan/95	Beijing/93	Bayern/95+ Beijing/95	Wuhan/95+ Sydney/97	Beijing/93+ Harbin/94
1998–99	Beijing/95	Sydney/97	Beijing/93	Bayern/95+Beijing/95	Sydney/97	Beijing/93
1999–00	Beijing/95	Sydney/97	Beijing/93	NewCal/99	Sydney/97	Beijing/93
2000–01	NewCal/99	Panama/99	Yamanashi/98	Bayern/95+ NewCal/99	Sydney/97	Sichuan/99
2001–02	NewCal/99	Panama/99	Sichuan/99	NewCal/99	Sydney/97	Sichuan/99+ HK/01
2002–03	NewCal/99	Panama/99	Hong Kong/01	NewCal/99	Fujian/02 (Panama/99)	Sichuan/99+ HK/01
2003–04	NewCal/99	Panama/99	Hong Kong/01	NewCal/99	Fujian/02	Shanghai/02
2004–05	NewCal/99	Wyoming/03	Jiangsu/03	NewCal/99	Cal/04	Shanghai/02+ HK/01
2005–06	NewCal/99	California/04	Jiangsu/03	NewCal/99	Cal/04+ Wisc/05	Mal/04+Florida/04 +Egypt/05
2006–07	NewCal/99	Wisc/05	Mal/04	NewCal/99+Solomon Islands/06	Wisc/05	Mal/04+Florida/04 +Egypt/05

Note: Cal/04, California/04; HK/01, Hong Kong/01; Mal/04, Malaysia/04; NewCal, New Caledonia/99; Wisc/05, Wisconsin/05. (▪) mismatch; (▨) partial mismatch; (□) match.

vanted vaccine was also able to induce a stronger booster effect against Pan/99, a strain that widely circulated in the previous 5 years and was in the vaccine composition for four consecutive seasons, than the non-adjuvanted vaccine, as demonstrated by higher post-vaccination GMTs and a higher seroconversion rate. Although these results are consistent with those of previous studies using MF59[TM]-adjuvanted vaccine [3,14,15], they are not completely in agreement with the findings of other studies using non-adjuvanted vaccine [3,9]. Both a non-adjuvanted subunit influenza vaccine and a non-adjuvanted split-virus influenza vaccine containing Pan/99 were able to confer good serological protection against the heterovariant strain Wyo/03 in the vast majority (75.9 and 80%, respectively) of elderly vaccinated subjects [3], and the seroprotection rate and MFI of antibody response induced by a non-adjuvanted subunit vaccine against the drifted variant Wisc/05 were higher than those against the vaccine strain A/New York/55/2004, a Cal/04-like virus [9]. The reason for the discrepancy between the findings of Baldo et al. and Del Giudice et al. and our own results could be the distance between vaccine and heterovariant strains: the antigenic distance between the vaccine strains Wyo/03 and Cal/04, measured by HI test using ferret antisera (Fig. 1A), is higher than that between Pan/99 and Wyo/03 or between Cal/04 and Wisc/05. Thus, HI titre against Cal/04 in ferrets infected with Wyo/03 was 8-fold lower than that against homologous strains, while a 2- to 4-fold decrease in HI titre against the drifted strains Wyo/03 and Wisc/05 was observed after Pan/99 and Cal/04 infections, respectively [16] (Fig. 1A). This point is supported by epidemiological and virological surveillance data:

the drift variant Cal/04 that appeared during the 2004—2005 influenza season exerted a heavy burden on the Italian population, with a high disease incidence reported in the elderly, even among vaccinated subjects [17].

Finally, NT titre evaluation confirmed the increased antibody response in subjects vaccinated against Pan/99 and Cal/04 with MF59[TM]-adjuvanted vaccine compared with those vaccinated with non-adjuvanted vaccine, suggesting the protective role of neutralizing antibodies that can cross-react with antigenically different strains. The broader and neutralizing serological response elicited by MF59[TM]-adjuvanted vaccine against A(H3N2) viruses, and the strong booster effect, is consistent with the high cross-reactivity to different A(H5N1) strains after primary vaccination with two doses of MF59[TM]-adjuvanted vaccine containing A/Dk/Sing/97(H5N3) and a booster dose after 16 months [18].

In conclusion, the appearance of drifted strains with an antigenic pattern highly different from the vaccine strain, such as Cal/04 and Wisc/05, highlights a limitation of the ability of non-adjuvanted vaccine to elicit an effective immune response. There is, therefore, a great need to develop drift-resistant vaccines, such as adjuvanted vaccines. As antigenic mismatch and/or partial mismatch occurs frequently (Table 2) [19], and the annual impact of antigenic drift on vaccine effectiveness is difficult to predict, it is necessary to continuously monitor the impact in order to develop a more comprehensive understanding of the benefit of vaccination. For the strains examined during this study, and for mismatched or partially mismatched strains during different seasons [3,14,15], addition of MF59[TM] to subunit

influenza vaccine allows for a broader serological response than is achieved by non-adjuvanted subunit vaccine in the elderly, who are at high risk of influenza-related complications. This is of particular importance during periods of antigenic drift, when vaccine efficacy may be compromised due to antigenic mismatch.

Acknowledgements

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