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Review

MF59®-adjuvanted vaccines for seasonal and pandemic influenza prophylaxis

Angelika Banzhoff,[a] Michele Pellegrini,[b] Giuseppe Del Giudice,[b] Elena Fragapane,[b] Nicola Groth,[a] Audino Podda[b]

[a]Novartis Vaccines, Marburg, Germany. [b]Novartis Vaccines, Siena, Italy.
Correspondence: Angelika Banzhoff, Novartis Vaccines, Marburg, Germany. E-mail: angelika.banzhoff@novartis.com

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Abstract Influenza is a major cause of worldwide morbidity and mortality through frequent seasonal epidemics and infrequent pandemics. Morbidity and mortality rates from seasonal influenza are highest in the most frail, such as the elderly, those with underlying chronic conditions and very young children. Antigenic mismatch between strains recommended for vaccine formulation and circulating viruses can further reduce vaccine efficacy in these populations. Seasonal influenza vaccines with enhanced, cross-reactive immunogenicity are needed to address these problems and can confer a better immune protection, particularly in seasons were antigenic mismatch occurs. A related issue for vaccine development is the growing threat of pandemic influenza caused by H5N1 avian strains. Vaccines against strains with pandemic potential offer the best approach for reducing the potential impact of a pandemic. However, current non-adjuvanted pre-pandemic vaccines offer suboptimal immunogenicity against H5N1. For both seasonal and pre-pandemic vaccines, the addition of adjuvants may be the best approach for providing enhanced cross-reactive immunogenicity. MF59®, the first oil-in-water emulsion licensed as an adjuvant for human use, can enhance vaccine immune responses through multiple mechanisms. A trivalent MF59-adjuvanted seasonal influenza vaccine (Fluad®) has shown to induce significantly higher immune responses to influenza vaccination in the elderly, compared with non-adjuvanted vaccines, and to provide cross-reactive immunity against divergent influenza strains. Similar results have been generated with a MF59-adjuvanted H5N1 pre-pandemic vaccine, which showed higher and broader immunogenicity compared with non-adjuvanted pre-pandemic vaccines.

Keywords Adjuvants, antigenic mismatch, cross-reactivity, MF59®, pandemic influenza, seasonal influenza.

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Introduction

Influenza is a highly contagious disease associated with substantial morbidity and mortality in vulnerable populations, which include infants and young children, subjects with chronic underlying diseases and the elderly.[1,2] Morbidity and mortality rates are highest among infants and individuals over 65 years of age, which presents a major challenge to public health services.[3] The problem is compounded by the reduced efficacy of seasonal influenza vaccines in the elderly, with estimated vaccine efficacy at 17–53%, compared with 70–90% in young adults.[4] This is mainly due to immunosenescence that compromises the ability to mount protective immune responses to vaccine antigens.[3–5] In addition, antigenic drift during the influenza season further reduces the efficacy of seasonal influenza vaccination in the most vulnerable populations, such as the elderly.[6] The small changes in the haemagglutinin (HA) and neuraminidase (NA) genes that occur during antigenic drift are sufficient to hinder the match between the strains recommended by WHO for inclusion in the vaccine formulation and circulating viruses, which can, in turn, reduce the immune response to vaccination.[3–5] In elderly subjects, seroprotection rates as low as 20% against drifted viruses have been reported, often failing to meet Committee for Medicinal Products for Human Use (CHMP) criteria for seroprotection and seroconversion against drifted strains.[7–11] Consequently, influenza vaccines that confer cross-reactive immunogenicity are needed for seasonal use to address the problem of reduced efficacy in years where antigenic mismatch occurs.

In addition to antigenic drift, completely new variants emerge periodically through antigenic shift.[12–14] The highly pathogenic avian influenza A/H5N1 virus, first reported in China in 1996, has been responsible for severe avian influenza outbreaks.[15–17] The disease is now widespread among poultry and migratory birds in many parts of the world and, significantly, more than 380 humans have been infected, with approximately 240 (63%) deaths.[18] Based on

the number and severity of human infections, an A/H5N1-influenza virus is considered by most experts to be the most likely candidate to cause the next pandemic,[19] which is expected to spread quickly and to cause substantial global morbidity and mortality.[20,21] Pre-pandemic vaccination against H5N1 and other strains with pandemic potential could therefore form the first line of defence against pandemic influenza. However, since neither the timing nor the causative agent of future pandemics can be predicted with complete accuracy, it is important that pre-pandemic vaccines induce long-lasting immunological memory and cross-reactivity to other H5 strains.[22]

The reported immunogenicity of several conventional non-adjuvanted H5N1 vaccines is not encouraging. One study showed that two vaccinations with 90 μg HA of a non-adjuvanted vaccine induced an antibody response at protective levels in only half of an immunologically naive population.[23] Another study found that two 30 μg doses of an aluminium-adjuvanted split-virion H5N1 vaccine were needed to induce an immune response that met two of three criteria required for European Union licensure.[24] As the amount of antigen tested in both these studies is substantially more than is needed for protection against seasonal influenza strains, and given current limits on worldwide vaccine production capacity, measures to increase the immune response and reduce the antigen content are essential. This is particularly important as clinical trials have shown that two doses of adjuvanted H5N1 vaccine are necessary to satisfy regulatory criteria for immunogenicity.[24–26] Use of improved adjuvants may provide the best approach for cross-reactive immune responses for both seasonal and pre-pandemic vaccination.

Mechanism of action of MF59

MF59® (Novartis Vaccines and Diagnostics Inc., MA, USA) is the first oil-in-water emulsion licensed as a vaccine adjuvant for human use [27] and triggers a cascade of immunostimulatory events.[28,29] Several studies have established that MF59 generates a local immunostimulatory environment at the injection site, activating local immune cells.[30] In mice, MF59 specifically enhances maturation of monocytes into dendritic cells, recruitment of antigen-presenting cells and uptake of antigen.[30] In addition, MF59 strongly induces the homing receptor CCR7 on maturing dendritic cells, facilitating an adaptive immune response.[30] In mice, MF59 stimulates the secretion of cytokines such as CCL2, CCL4 and CXCL8, which are important for the recruitment of immune cells to the injection site. MF59 also facilitates the cellular immune response by enhancing surface expression of MHC class II and increasing endocytosis of antigens by monocytes. Therefore, MF59 appears to enhance the immune response to antigens at a number of specific points. Many of these effects have not been reported for other approaches that have been developed for improving vaccine immunogenicity, including alternative adjuvants, virosomal antigen presentation[31] and intradermal vaccination.[32]

Immunogenicity and protection in animals

Preclinical studies established that MF59 enhances antibody responses to influenza vaccination in mice.[33] Subsequent studies in mice focused on whether MF59-adjuvantation of the influenza vaccine could protect against lethal intranasal challenge with influenza viruses and whether protection could be conferred if the antigenic content of the vaccine was reduced.[34] The addition of MF59 significantly increased the antibody response to the vaccine antigens over a wide dose range; equivalent antibody titres were achieved using a 50- to 200-fold reduction in antigen content. Furthermore, the humoral immune response was sustained for at least 6 months following immunization. MF59-adjuvanted trivalent influenza vaccine provided improved protection against lethal intranasal challenge with influenza viruses and the rate of survival of the mice was significantly increased compared with non-adjuvanted vaccine.[34] The use of a 65- to 80-fold reduced antigen content still induced full protection from a lethal intranasal challenge for up to 6 months.[34] MF59 enhanced the protective efficacy of the vaccine, both in terms of the percentage of survivors and the reduction of influenza viral titre in the lungs of mice. As a major target population for influenza vaccines is the elderly, the mouse model was used to determine whether the addition of MF59 could enhance the immunogenicity of influenza vaccines in elderly mice.[35] Addition of MF59 to the influenza vaccine induced an antibody response in previously infected elderly mice similar to that normally achieved in young mice.[35]

Enhancing and broadening seasonal vaccine immune responses in the elderly

In order to assess the safety and immunogenicity of an MF59-adjuvanted trivalent subunit influenza vaccine (Fluad®, Novartis Vaccines and Diagnostics Inc., MA, USA) in elderly subjects, a number of comparative studies against non-adjuvanted conventional influenza vaccines have been undertaken using haemagglutination inhibition assays to measure immunogenicity. One of the first trials performed evaluated primarily the safety and tolerability of the MF59-adjuvanted vaccine in the elderly over three consecutive seasons, showing no reports of any vaccine-related serious adverse events or of safety concerns associated with the vaccine after the first, second or third vaccination (Table 1).[36] The adjuvanted vaccine did induce more local reactions than the conventional vaccine, but the reactions were typically mild and limited to the first 2–3 days after

Table 1. Incidence of reported adverse events following influenza immunization from September 1997 to August 2006

Adverse events	Reported cases (*n*)	Number of cases assessed as possibly related	Reporting rate per 100 000 doses
All reported events*	387	249	1·4
Serious cases	107	34	0·39
Fatal cases	13	0	0·05
Vaccine failures	4	4	0·01
Allergic reactions	39	34	0·14
Neurological disorders	51	21	0·18
ADEM, encephalitis, myelitis	8	2	0·02
GBS	9	7	0·03
Parsonage–Turner syndrome	3	2	0·01
Blood and vascular disorders	9	2	0·03

Reproduced with permission from Schultze *et al.* [41]
ADEM, acute disseminated encephalomyelitis; GBS, Guillain–Barré syndrome.
*Sold doses of Fluad or Fluad-like vaccine 27 374 412.

vaccine injection. Although the study was not statistically powered to test for cross-reactive immunogenicity, antibody responses in the adjuvanted group were higher both against the vaccine antigens and mismatched strains. Similar safety and immunogenicity data were reported in another study performed in the elderly population, across three consecutive influenza seasons. In particular, Hemagglutination inhibition (HI) antibody titres induced by Fluad ($n = 94$) resulted consistently higher when compared with a non-adjuvanted subunit vaccine ($n = 98$), especially in the elderly with low baseline HI titres.[37]

The higher immunogenicity of Fluad was confirmed in a larger trial (Fluad, $n = 204$; non-adjuvanted subunit comparator, $n = 104$), which also showed a clinical tolerability of the adjuvanted vaccine comparable to that of the conventional vaccine.[38]

For seasonal influenza vaccines, valuable cross-reactive antibodies versus drifted strains are relevant to potentially cover antigenic mismatch.

A very recent analysis including both neutralization and haemagglutination inhibition assays evaluated cross-reactive immunity against A/H3N2-drifted influenza viruses, comparing sera taken from the elderly vaccinated with either Fluad ($n = 25$) or a non-adjuvanted subunit vaccine ($n = 25$).[8] Broader immune responses were observed with Fluad against four consecutive drifted A(H3N2) variants, A/Panama/2007/99 (circulated over 5 years prior to this study), A/Wyoming/3/03 (included in the vaccine formulation), A/California/7/04 and A/Wisconsin/67/05, representing A/H3N2 vaccine changes over a decade. For the drifted strains only the MF59-adjuvanted vaccine induced a substantial immune response, meeting all CHMP requirements against A/Panama/2007/99, A/California/7/04 and A/Wisconsin/67/05.

Against A/California/7/04 and A/Wisconsin/67/05, Fluad induced significantly higher HI Geometric Mean Titer (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 A/Panama/2007/99 ($P < 0·01$) and A/California/7/04 ($P < 0·01$).[8]

These data confirmed previous observations showing broader immunogenicity of the 2003/2004 A/Panama/1999 (H3N2)-like strain against the mismatched strain A/Fujian/411/2002, if included in the MF59 adjuvanted vaccine, compared with conventional subunit and split formulations.[10]

Finally, a meta-analysis of 20 clinical trials involving the use of MF59-adjuvanted vaccine in more than 10 000 elderly subjects confirmed that greater immunogenicity conferred by Fluad in the elderly (Figure 1).[39] The greatest adjuvant effect was shown in subjects with low pre-immunization titres and in those affected by chronic underlying diseases including cardiovascular or respiratory diseases or diabetes.

Safety of MF59

Preclinical toxicology studies of MF59 showed no genotoxicity, teratogenicity or sensitization, and treatment-related

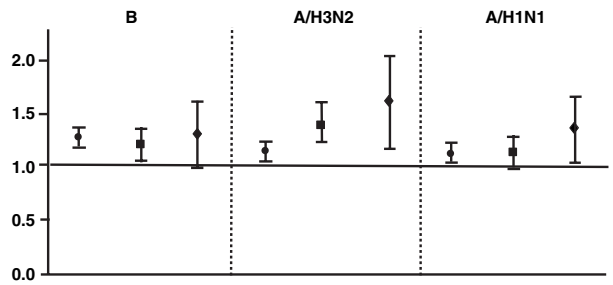

Figure 1. Fluad to comparator post-immunization GMT ratios and 95% confidence intervals for the B, A/H3N2 and A/H1N1 antigens after the first immunization ●, second immunization ■ and the third immunization ◆. These data are from a meta-analysis that included all Fluad recipients with a low re-immunization titre. The first immunization data are from 13 clinical trials (2102 Fluad recipients and 1498 comparator recipients), the second immunization data are from five clinical trials (463 Fluad recipients and 307 comparator recipients) and two clinical trials (149 Fluad recipients and 83 comparator recipients). Adapted from Podda A, 2001.[39]

safety issues were generally limited to inflammatory responses at the site of injection.[28] Early clinical studies also showed no increase in anti-squalene IgG or IgM antibody titres following immunization with an MF59-adjuvanted vaccine.[40] Clinical trials and post-marketing pharmacovigilance data from subjects who received the Fluad vaccine have provided extensive MF59 safety data base, covering more than 10 years of use, with more than 40 million doses distributed worldwide.[27,28,39,41]

A meta-analysis including safety data from over 2000 elderly subjects who received one or more Fluad vaccinations showed no immediate, allergic-type reactions after immunization.[39] The most common reactions after first, second and third Fluad vaccine injection was pain, which was experienced by 32%, 27% and 28% of patients respectively, compared with 14%, 21% and 16% of the elderly who received non-adjuvanted comparators. Erythema and induration were also experienced in >10% of the subjects, but most local reactions were rated as mild and were of short duration. Importantly, this meta-analysis showed no increased reactogenicity or significant change in the safety profile of Fluad between first and subsequent vaccine doses, showing long-term good tolerability of MF59 across multiple seasonal vaccinations.[39] Extensive Fluad pharmacovigilance data are also available, which further confirm the low frequency of adverse reactions associated with MF59.[41] This database contains all reports of adverse drug reactions following vaccination with Fluad or Fluad-like vaccines, and contains only 387 case reports from over 27 million doses, of which 107 cases fulfilled at least one seriousness criterion regardless of the severity and causality. Nine cases of Guillain–Barré syndrome were reported, irrespective of causality, giving an overall incidence of 0·03 cases per 100 000 doses. Importantly, there were no deaths considered possibly related to Fluad vaccination.[41] This incidence is similar to that observed after vaccination with conventional, non-adjuvanted vaccines.[42]

Although the majority of MF59 safety data are from patients who received Fluad, the adjuvant has been studied in a number of vaccines, including pandemic influenza, HIV-1, cytomegalovirus, herpes simplex virus and hepatitis B. These studies have further reinforced the very good safety profile of MF59 in subjects across a wide age range, from newborns to the elderly.[16,43–48]

Confronting pandemic influenza

The high pathogenicity of A/H5N1 influenza viruses and their capacity for transmission from birds to humans, coupled with a mortality rate of approximately 60%, has raised concerns about an impending worldwide pandemic similar to the H1N1 pandemic of 1918.[49] However, currently, transmission of circulating avian H5N1 influenza viruses to humans is inefficient and human-to-human transmission will require further adaptation of the virus. Therefore, it is not possible to predict precisely what a future pandemic strain will be. Present strategies are to develop vaccines that induce long-lasting immunological memory and cross-reactivity against strains with pandemic potential, in particular H5N1. This approach could represent the first line of defence against a pandemic, by providing at least partial protection before or during the early stages of an H5N1 pandemic until an optimally matched vaccine is produced.[22,50]

An early clinical study on 451 healthy adults, examined a non-adjuvanted inactivated subvirion H5N1 vaccine in the USA, showed relatively poor immunogenicity and required two 90 μg doses, administered 4 weeks apart, to elicit neutralizing antibodies in 54% of vaccinees.[23] A second phase I, non-controlled clinical study in 300 adults compared non-adjuvanted split H5N1 vaccine with the same vaccine combined with an aluminium adjuvant and demonstrated that only adjuvanted vaccine produced an immune response consistent with European regulatory requirements, using two 30 μg doses, administered 3 weeks apart.[24] Clinical studies with MF59 as vaccine adjuvant for A/H5N1 vaccine antigen (n = 486 adults and elderly) found that even a dose of only 7·5 μg, administered twice, 3 weeks apart, was able to meet all three CHMP criteria for licensure of pandemic vaccines in the European Union.[51]

In animal models, heterosubtypic cross-protection against challenge with highly pathogenic H5N1 virus has been observed with influenza vaccines using mucosal adjuvants or immunostimulating complexes.[21,52] These data support the view that induction of cross-reactive antibodies in humans is clinically relevant. Furthermore, a clinical study demonstrated that an MF59-adjuvanted H5N3 (A/Duck/Singapore/97) vaccine stimulated cross-reactive neutralizing antibodies against highly pathogenic heterovariant H5N1 strains isolated from humans between 1997 and 2004 (Figure 2). By contrast, sera obtained from recipients of the same vaccine without MF59 adjuvantation showed limited or no cross-reactivity.[50]

More recently, an MF59-adjuvanted H5N1 (A/Vietnam/1194/2005) clade 1 subunit vaccine induced cross-reactive antibodies against an H5N1 A/Turkey/Turkey/05 (clade 2) influenza strain, indicating broad seroprotection against diverse H5N1 strains in adults (n = 313) and elderly (n = 173).[53] A booster given after 6 months induced higher antibody levels, indicating that initial vaccination had induced a strong and persistent immunological memory that was boosted upon re-vaccination.[53]

The durability of immune responses to MF59-adjuvanted H5N1 vaccination was demonstrated in a study where subjects, who had been vaccinated 6 years earlier with an MF59-adjuvanted (n = 12) or non-adjuvanted H5N3

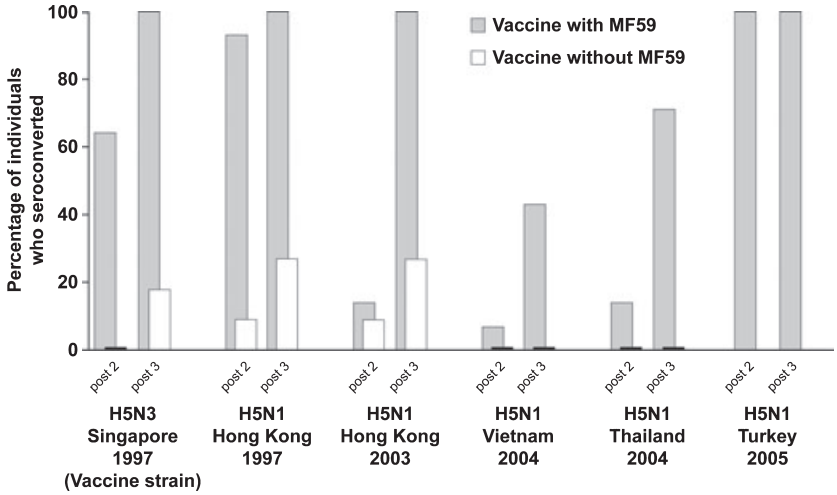

Figure 2. Broad cross-reactive immunity against heterologous H5N1 isolates. The figure shows the percentage of individuals who seroconverted following vaccination with an MF59-adjuvanted (grey bars) or non-adjuvanted (white bars) H5N3 vaccine. Participants received two doses of vaccine 21 days apart (post 2) and a booster vaccination 16 months later (post 3). Seroconversion was defined as a ≥ fourfold rise in pre-vaccination antibody titre and was measured for the homologous (H5N3 Singapore strain) and mismatched (H5N1 Hong Kong 1997; H5N1 Hong Kong 2003; H5N1 Vietnam 2004; H5N1 Thailand 2004) strains. Additionally, some subjects who had received two doses of MF59-adjuvanted H5N3 Singapore vaccine 6 years previously were re-vaccinated with two doses 21 days apart of an MF59-adjuvanted vaccine containing antigen derived from the H5N1 Vietnam strain. Seroconversion to the mismatched H5N1 Turkey strain was measured in these subjects after the first dose of H5N1 Vietnam (post 2) and after the second dose (post 3). Adapted from Stephenson I, *et al.* 2005, 2008.[50,54]

(n = 12) (A/Duck/Singapore/97) vaccine, were re-vaccinated with an MF59-adjuvanted H5N1 vaccine (Figure 2).[54] The MF59-adjuvanted H5N1 vaccine rapidly induced (within 7 days after one dose) cross-reactive antibody responses against diverse influenza H5N1 viruses, including clades 1, 2·1, 2·2 and 2·3. Cross-reactive immune responses were substantially higher among subjects who initially received the MF59-adjuvanted H5N3 vaccine, compared with the non-adjuvanted vaccine. Thus, priming with MF59-adjuvanted H5 antigen induces immune memory that can be rapidly mobilized by the single administration of a distinct H5 vaccine to provide broad heterologous cross-protection. Consistent with these findings, MF59-adjuvanted H5N1 vaccination has been shown to induce a large and a stable pool of H5N1-specific memory B cells that can be boosted with antigen to rapidly expand and differentiate into plasma cells.[55] This vaccine has also been shown to induce an immune response involving H5-specific $CD4^+$ T cells with a Th1 effector/memory phenotype ($IL\text{-}13^-$, $IL\text{-}2^+$, $IFN\text{-}\gamma^+$, $TNF\text{-}\alpha^+$) that can be boosted with a single dose of antigen.[56] This long-lasting cellular immunity and pool of specific memory B cells associated with MF59 adjuvantation are critical attributes for pre-pandemic vaccines.

Conclusions

Seasonal influenza epidemics necessitate annual influenza vaccination programmes and are associated with high morbidity and mortality rates in the most frail populations, particularly in the elderly. In addition, the threat of an H5N1 pandemic has heightened the awareness of some of the shortcomings of vaccines, particularly due to low immunogenicity in humans of the H5N1 subtype and the unpredictable antigenic variation of influenza strains. The administration of more immunogenic and cross-reactive influenza vaccines is therefore considered the best option for control of both seasonal and pandemic influenza for all risk groups.

Studies with MF59-adjuvanted inactivated influenza subunit vaccines, in comparison with non-adjuvanted vaccines, have shown the importance of MF59 adjuvantation for enhancing immunogenicity against both seasonal and pandemic influenza virus strains. In particular, MF59-adjuvanted vaccines have been shown to confer long-lasting cross-reactive immune responses not reported with non-adjuvanted vaccines. These responses are linked to the mechanism of action of MF59, which includes a multiplicity of immunostimulatory effects involving both humoral and cell-mediated immunity.

Thus, MF59-adjuvanted influenza vaccines offer higher and broader antibody responses to drifted viruses making them a strong candidate for seasonal influenza vaccination programmes in vulnerable populations. In addition, MF59 can stimulate H5N1 cross-clade antibody and cell-mediated immune responses that can be boosted at least 6 years following priming for potential use in an H5N1 pandemic. MF59 adjuvantation provides cross-reactive immune

responses with both seasonal and pre-pandemic vaccines, which is likely to be a necessary attribute for vaccines that address the critical issues of antigenic drift and pre-pandemic vaccine effectiveness.

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