Autoimmune Hemolytic Anemia Following MF59-Adjuvanted Influenza Vaccine Administration: A Report of Two Cases

Sabrina Montagnani, Marco Tuccori, Giuseppe Lombardo, Arianna Testi, Stefania Mantarro, Elisa Ruggiero, and Corrado Blandizzi

OBJECTIVE: To describe 2 cases of autoimmune hemolytic anemia (AIHA) following the administration of MF59-adjuvanted influenza vaccine.

CASE SUMMARY: An 83-year-old white woman developed persistent hyperpyrexia, polyarthralgia, and lower limb hypostenia about 2 days after receiving influenza vaccine. Clinical signs and laboratory evaluations suggested AIHA. The patient was treated with high-dose corticosteroids and immunoglobulins, and her clinical condition improved. A 74-year-old white woman developed severe abdominal pain and asthenia 3 days after the administration of influenza vaccine. Clinical signs and laboratory evaluations disclosed AIHA. She was treated with corticosteroids, rehydration, and blood transfusion; however, she died about 48 hours after hospitalization.

DISCUSSION: AIHA has been rarely described following influenza vaccine administration. In the cases described here, the causal relationship between influenza vaccination and the occurrence of AIHA, assessed by means of World Health Organization criteria, was scored as probable. It has been proposed that the mechanism whereby vaccines induce autoimmune responses can be molecular mimicry, although a possible role of other vaccine constituents, with particular regard for adjuvants, such as MF59, can not be excluded. Squalene, a constituent of MF59, has been suggested as a causative agent of autoimmune reactions. However, it is not clear how and under what conditions squalene can cause immune responses.

CONCLUSIONS: Influenza vaccination may rarely trigger severe AIHA, shortly after vaccine administration. A mechanism of molecular mimicry is probably involved in the development of these reactions, although the possible role of adjuvants can not be excluded. Patients should be instructed to report signs and symptoms of autoimmune disorders occurring in the first weeks after administration of influenza vaccine.

KEY WORDS: autoimmune hemolytic anemia, MF59-adjuvanted influenza vaccine.

Ann Pharmacother 2011;45:e8.

Published Online, 28 Dec 2010, *theannals.com*, DOI 10.1345/aph.1P480

MF59 adjuvant is an oil-in-water emulsion consisting of microvesicles made by a drop of squalene surrounded by a monolayer of nonionic detergents. It has been the first adjuvant developed for human use to be licensed since the introduction of aluminum as an adjuvant and, as a part of an enhanced influenza vaccine for the elderly, it is now commercially available in 23 countries worldwide, including Italy and other 11 European Union countries (not authorized in the US).[1]

Trivalent inactivated-subunit influenza vaccine adjuvanted with MF59 has shown higher immunogenicity compared with nonadjuvanted vaccines.[2,3] The most frequent adverse events associated with MF59-adjuvanted vaccine influenza include flu-like syndrome (ie, myalgia, respiratory symptoms, and fever) and injection site reactions, which are usually transient and mild-to-moderate in severity.[2] Cases of severe hematologic adverse reactions, including autoimmune hemolytic anemia (AIHA), following influenza vaccination have been rarely reported in the medical literature.[4,5] We describe 2 cases of AIHA in patients treated with intramuscular trivalent MF59-adjuvanted influenza vaccine, reported to the Italian Pharmacovigilance Network.

Author information provided at end of text.

Case Reports

CASE 1

In November 2006, an 83-year-old white woman developed persistent hyperpyrexia, polyarthralgia, and lower limb hypostenia about 2 days after receiving intramuscular

influenza vaccine.[a] No concomitant treatments with other medications or parapharmaceutical products were reported. Her clinical history did not include allergy or immune diseases. Laboratory parameters, evaluated at admission, are listed in Table 1. Virologic assessments were negative for hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, Epstein-Barr virus (EBV) and cytomegalovirus. Cerebrospinal fluid analysis showed an increase in albumin level (7.3 g/dL), which was interpreted by physicians as indication that the blood-brain barrier had been compromised. Chest X-ray, electrocardiogram, and computed tomography scan did not detect any relevant findings. Magnetic resonance imaging suggested myelitis. Based on clinical signs and laboratory evaluations (ie, decrease in red blood cells, hemoglobin and hematocrit, increase in reticulocytes and total bilirubin, and positive direct Coombs test), AIHA was diagnosed. The patient was treated intravenously with high-dose corticosteroids and immunoglobulins (2 g/kg), and her clinical condition improved. Complete recovery was achieved in about 10 days.

CASE 2

A 74-year-old white woman had a history of aortic valvulopathy treated chronically with low-dose aspirin (100 mg/day orally), atenolol (100 mg/day orally), and ramipril (5 mg/day orally). In October 2009, she presented to the emergency department with severe abdominal pain and asthenia, in the absence of hyperpyrexia, 3 days after intramuscular administration of influenza vaccine.[b] The clinical signs and laboratory evaluations, indicating marked reductions of red blood cells, hemoglobin, haptoglobin, and hematocrit, together with increments of total bilirubin and positive direct Coombs test, supported the diagnosis of AIHA (Table 1), and the patient was hospitalized. At that time, when the patient's blood type was determined, the presence of autoantibodies was detected. A few hours after presentation, the patient developed jaundice; her renal function was normal. Virologic assessments excluded HBV, HCV, HIV, EBV, and cytomegalovirus. Her clinical history did not disclose allergy or immune diseases. She was treated with corticosteroids, rehydration, and blood transfusion, with a modest improvement of hemoglobin (5.5 g/dL) 24 hours after the first assessment. Nevertheless, her clinical condition remained critical and, about 48 hours after hospitalization, despite a temporary improvement of laboratory parameters, the patient died.

Discussion

A causal relationship between influenza vaccination and the occurrence of AIHA, assessed by means of World Health Organization criteria,[6] was scored as probable in both cases.

Adverse reactions associated with adjuvanted vaccines can be classified as short-term (up to a 1-day risk period), such as allergic reactions; medium-term (1-week risk period), such as local and systemic reactions, including rash, seizures, and unspecified adverse events; and long-term (1 week to 1 month and more than 1-month risk window), such as possible delayed reactions to immunization that might occur via immune-mediated mechanisms.[7]

To our knowledge, only 2 cases[4,5] of AIHA associated with influenza vaccine have been reported in the medical literature, both as a part of a clinical picture of thrombotic microangiopathy; the reactions occurred 5 and 14 days after vaccine administration. Of note, there were no signs of thrombotic microangiopathy in our 2 patients.

Table 1. Laboratory Parameters at the Time of Admission

Parameter (reference range)	Case 1	Case 2
WBCs, /mm^3 (5,000-10,000)	13,900	9,000
RBCs, $\times 10^6$/mm^3 (4.4-5.6)	3.36	0.45
Platelets, /mm^3 (150,000-400,000)	291,000	216,000
Neutrophils, % (40-75)	79.3	
Lymphocytes, % (20-40)	12.3	
Hemoglobin, g/dL (12-16)	8.3	4.6
Hematocrit, % (37-46)	28.8	4.2
Reticulocytes, % (0.5-1.5)	3.6	5.4
ESR, mm/h (10-20)	120	
CRP, mg/L (<10)	141.8	
Serum iron, µg/dL (49-151)	19	
Haptoglobin, mg/dL (50-300)	27.9	< 7
Total bilirubin, mg/dL (0.1-1.2)	8.60	9.41
Direct bilirubin, mg/dL (0.1-0.25)	1.40	1.61
DCT	positive	positive
DCT IgG		positive
DCT IgA		positive
DCT IgM		positive
DCT C3c		positive
DCT C3d		positive
ICT	positive	positive

CRP = C-reactive protein; DCT = direct Coombs test; ESR = erythrocyte sedimentation rate; ICT = indirect Coombs test; RBCs = red blood cells; WBCs = white blood cells.

[a]Fluad, Novartis Vaccine and Diagnostic, Siena, Italy. Surface antigens (hemagglutinin and neuraminidase of influenza virus containing the following strains: A/New Caledonia/20/99 [H1N1], equivalent strain [A/New Caledonia/20/99 IVR-116] 15 µg hemagglutinin; A/Wisconsin/67/2005 [H3N2], equivalent strain [A/Hiroshima/52/2005 IVR 142] 15 µg hemagglutinin; B/Malaysia/2506/2004, equivalent strain [B/Malaysia/2506/2004] 15 µg hemagglutinin; cultivated in eggs and adjuvanted with MF59).

[b]Fluad, Novartis Vaccine and Diagnostic, Siena, Italy. Surface antigens (hemagglutinin and neuraminidase of influenza virus containing the following strains: A/Brisbane/59/2007 [H1N1], equivalent strain [A/Brisbane/59/2007, IVR-148], 15 µg hemagglutinin; A/Brisbane/10/2007 [H3N2], equivalent strain [A/Uruguay/716/2007, NYMC X-175C] 15 µg hemagglutinin; B/Brisbane/60/2008, equivalent strain [B/Brisbane/60/2008], 15 µg hemagglutinin; cultivated in eggs and adjuvanted with MF59).

In both cases, the short time lag between vaccination and onset of symptoms (2 and 3 days) is strongly suggestive of a causal relationship. Furthermore, major alternative explanations for the occurrence of AIHA are unlikely, since several viral infections and malignancies were ruled out by blood analysis and imaging evaluations. However, the available data do not allow exclusion of the possible contribution of other infectious diseases, particularly in patient 1, who, together with the picture of AIHA, displayed clinical and laboratory signs consistent with an inflammatory activity of possible infectious origin (ie, hyperpyrexia and elevated white blood cell count, erythrocyte sedimentation rate, and C-reactive protein). It is noteworthy that, among the concomitant medications reported in patient 2, hemolytic anemia has been described in patients treated with aspirin,[8] and it is labeled also in the adverse reaction section of ramipril summary of product characteristics.[9] However, since the woman had been receiving chronic treatment with aspirin and ramipril at the time of AIHA onset, their causative role in the occurrence of this adverse event appears to be unlikely.

A probable mechanism whereby vaccines may induce autoimmune responses is molecular mimicry, which is defined as the possibility that amino acid sequence similarities between foreign and self peptides are sufficient to elicit cross activation of autoreactive T or B cells by pathogen-derived peptides. Despite the indiscriminate nature of several peptide sequences, which can be both foreign and self, a single antibody clone or T cell receptor can be activated by even a few crucial amino acid residues, a circumstance that highlights the importance of structural homology in the theory of molecular mimicry. Upon activation of B or T cells, it is believed that these "peptide mimic" specific immune cells can cross react with self-epitopes, thus leading to pathologic events (autoimmunity).[10] Molecular mimicry usually requires several days following the first exposure to an antigen.[11] However, as prior influenza infections or vaccinations can not be excluded, a second exposure to the same antigen or a similar one might elicit an adverse response within a shorter period. When considering other mechanisms accounting for autoimmune reactions, it is worthy to note that the increased risk of autoimmunity among recipients of vaccines may stem not solely from antigenic-mediated responses, but also from other constituents of the vaccine, such as yeasts, adjuvants, and preservatives.[11] Adjuvants are known to rarely induce autoimmune reactions, which have been designated as "adjuvant disease." However, in most cases, the rarity of post-influenza vaccination autoimmunity makes it difficult to establish a clear causal relationship.

In general, the etiopathogenesis of autoimmune disorders remains unknown. However, in the past decades, vaccines were suggested as possible causative agents for autoimmune diseases.[12] These autoimmune events often develop within a few days after vaccine administration and usually have a favorable outcome. Among these adverse events, Guillain-Barré syndrome remains the most frequently reported autoimmune reaction following influenza vaccination.[13] However, other manifestations of autoimmune responses, including vasculitis and hematologic events, such as thrombocytopenia and AIHA, have also been rarely reported.[14-16]

The safety of vaccine formulations represents one of the major limiting factors in the introduction of new vaccine adjuvants. MF59 adjuvant has been developed to enhance the immunogenicity of recombinant and subunit antigens. Extensive clinical immunogenicity and safety information on various MF59-adjuvanted vaccine antigens has been shown in clinical trials during the last 15 years. Current data show that MF59-adjuvanted antigens can elicit a marked antibody response, allowing the amount of antigen in the vaccine to be reduced, while remaining safe and generally well tolerated.[17] MF59 adjuvant contains squalene, a natural component of cell membranes involved in the biosynthesis of cholesterol.[18] Some studies have evaluated the possible immunotoxicologic effects of squalene-based adjuvants and have described the presence of antibodies to squalene among individuals who had received anthrax vaccine, although these findings remain controversial.[19] The possibility of autoimmunity induction by squalene is of particular interest, since it is an endogenous lipidic compound that is abundant in serum and it acts as a normal precursor of cholesterol and steroid hormones.[20] How and under what conditions such a compound of endogenous origin can cause immune reactions remain unclear. However, several studies have established that MF59 can stimulate the formation of a local immunostimulant environment at the injection site, thus promoting localized activation of immune cells.[21]

Available evidence suggests a lack of significant difference in the risk of autoimmune adverse events between MF59-adjuvanted influenza vaccine [MF59(+)] and influenza vaccine not adjuvanted with MF59 [MF59(–)]. Indeed, a low number of adverse events of potential autoimmune origin was reported following the analysis of 64 trials, where a total of 20,447 subjects had received MF59(+) and 7526 MF59(–) influenza vaccines. Five cases (2 cases of Crohn's disease and single cases of type 1 diabetes mellitus, rheumatoid arthritis, and multiple sclerosis) were found in the MF59(+) group; 2 adverse events (rheumatoid arthritis and temporal arteritis) were recorded in the MF59(–) group.[22]

In conclusion, influenza vaccination may rarely trigger severe AIHA shortly after vaccine administration and, in very rare cases, may result in mortality. The etiopathogenesis of autoimmune disorders induced by influenza vaccination remains unclear. However, a mechanism of molecular mimicry is probably involved in the development of these

reactions, although the possible role of adjuvants cannot be excluded. Further investigations are needed to assess possible risk factors. Patients should be instructed to report signs and symptoms of autoimmune disorders occurring in the first weeks after administration of influenza vaccine.

Sabrina Montagnani PharmD, Pharmacovigilance Fellow, Tuscan Regional Centre of Pharmacovigilance, Interdepartmental Centre for Research in Clinical Pharmacology and Experimental Therapeutics, University of Pisa, School of Medicine and Surgery, Pisa, Italy

Marco Tuccori PharmD, Pharmacology Specialist, Tuscan Regional Centre of Pharmacovigilance, Unit of Pharmacology, University Hospital of Pisa

Giuseppe Lombardo MD, Clinical Specialist, Intensive Care Unit, Hospital of Empoli, Florence, Italy

Arianna Testi PharmD, Pharmacology Specialist, Tuscan Regional Centre of Pharmacovigilance, Pharmaceutical Unit, Health District of Pisa

Stefania Mantarro PharmD, Pharmacology Specialist, Interdepartmental Centre for Research in Clinical Pharmacology and Experimental Therapeutics, University of Pisa

Elisa Ruggiero PharmD, Pharmacovigilance Fellow, Tuscan Regional Centre of Pharmacovigilance, Pharmaceutical Unit, Regional Health System, University Hospital of Pisa

Corrado Blandizzi MD, Professor of Pharmacology, Tuscan Regional Centre of Pharmacovigilance, Division of Pharmacology and Chemotherapy, Department of Internal Medicine, University of Pisa

Correspondence: Dr Tuccori, m.tuccori@ao-pisa.toscana.it

Reprints/Online Access: www.theannals.com/cgi/reprint/aph.1P480

Conflict of interest: Authors reported none

References

1. Podda A, Del Giudice G. MF59-adjuvanted vaccine: increased immunogenicity with an optimal safety profile. Exp Rev Vacc 2003;2:197-203.
2. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006;354:1343-51.
3. Podda A. The adjuvanted influenza vaccines with novel adjuvants: experience with the MF59-adjuvanted vaccine. Vaccine 2001;19:2673-80.
4. Dias PJ, Gopal S. Refractory thrombotic thrombocytopenic purpura following influenza vaccination. Anaesthesia 2009;64:444-6.
5. Brown RC, Blecher TE, French EA, Toghill PJ. Thrombotic thrombocytopenic purpura after influenza vaccination (letter). Br Med J 1973; 861:303.
6. World Health Organization (WHO). International drug monitoring: the role of national centres. Report of a WHO meeting (498). Geneva: World Health Organization, 1972.
7. Schultze V, D'Agosto V, Wack A, Novichi D, Zorn J, Hennig R. Safety of MF59 adjuvant. Vaccine 2008;26:3209-22.
8. Sanford-Driscoll M, Knodel LC. Induction of hemolytic anemia by nonsteroidal antiinflammatory drugs. Drug Intell Clin Pharm 1986;20:925-34.
9. Product information. Altace tablets (ramipril). http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022021s006lbl.pdf (accessed 2010 Sept 1).
10. Kohm AP, Fuller KG, Miller SD. Mimicking the way to autoimmunity: an evolving theory of sequence and structural homology. Trends Microbiol 2003;11:101-5.
11. Agmon-Levin N, Kivity S, Shoenfeld Y. Influenza vaccine and autoimmunity. Isr Med Assoc J 2009;11:183-5.
12. Molina V, Shoenfeld Y. Infection, vaccines and other environmental triggers of autoimmunity. Autoimmunity 2005;38:235-45.
13. Haber P, DeStefano F, Angulo FJ, et al. Guillain-Barrè syndrome following influenza vaccination. JAMA 2004;24:2478-81.
14. Blunberg S, Biefang D, Kantrowitz FG. A possible association between influenza vaccination and small-vessel vasculitis. Arch Intern Med 1980; 140:847-8.
15. Vial T, Laine V, Delcombel M, et al. Vasculitis after anti-influenza vaccination. Report of 5 cases. Therapie 1990;45:509-12.
16. Schattner A. Consequence or coincidence? The occurrence, pathogenesis and significance of autoimmune manifestations after viral vaccines. Vaccine 2005;23:3876-86.
17. Podda A, Del Giudice G. MF59 adjuvant emulsion. New generation vaccines. 3rd ed. New York: Marcel Dekker, 2004:225-36.
18. Ott G, Radhakrishnan R, Fang JH, Hora M. The adjuvant MF59: a 10-year perspective. In: O'Hagan D, ed. Vaccine adjuvants: preparation methods and research protocols. Totowa, NJ: Humana Press, 2000:211-28.
19. Asa PB, Wilson RB, Garry RF. Antibodies to squalene in recipients of anthrax vaccine. Exp Mol Pathol 2002;320:1363-7.
20. Allison AC. Squalene and squalene emulsions as adjuvants. Methods 1999;19:87-93.
21. Mosca F, Tritto E, Muzzi A, et al. Molecular and cellular signatures of human vaccine adjuvants. Proc Natl Acad Sci U S A 2008;105:10501-6.
22. Pellegrini M, Nicolay U, Lindert K, Groth N, Della Cioppa G. MF59-adjuvanted versus non-adjuvanted influenza vaccines: integrated analysis from a large safety database. Vaccine 2009;27:6959-65.