Vaccine 28 (2010) 6159–6163

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immune response of single dose vaccination against 2009 pandemic influenza A (H1N1) in the Taiwanese elderly

Tsui Mai Kao[a], Szu Min Hsieh[a,*], Hsiang Chi Kung[a], Yi Chien Lee[a], Kuo Chin Huang[b], Li Min Huang[c], Feng Yee Chang[d], Ning Chi Wang[d], Yung Ching Liu[e], Wen Sen Lee[f], Hsingjin Eugene Liu[f], Chin I. Chen[f], Chien-Hui Chen[g]

[a] Department of Internal Medicine, National Taiwan University Hospital, Medical College of National Taiwan University, Taipei, Taiwan
[b] Department of Family Medicine, National Taiwan University Hospital, Medical College of National Taiwan University, Taipei, Taiwan
[c] Department of Pediatrics, National Taiwan University Hospital, Medical College of National Taiwan University, Taipei, Taiwan
[d] Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan
[e] Department of Internal Medicine, Taipei Medical University – Shuang Ho Hospital, Taipei, Taiwan
[f] Department of Internal Medicine, Wang Fang Hospital, Taipei, Taiwan
[g] Taiwan Product Quality Research Institute, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 5 February 2010
Received in revised form 27 June 2010
Accepted 12 July 2010
Available online 24 July 2010

Keywords:
2009 Pandemic influenza A (H1N1)
Vaccination
Immune response

ABSTRACT

We conducted a multi-center, randomized and laboratory-blinded clinical trial with subgroup analyses, involving adults aged greater than 60 years old (range 61–86 years old), to investigate the immunogenicity and the potential factors affecting the immune response of a monovalent, unadjuvanted, inactivated, split-virus vaccine. A total of 107 subjects were randomized to receive 15 and 30 μg of hemagglutinin antigen in a 1:1 ratio. The immunogenicity was detected through hemagglutination inhibition (HAI) test of serum obtained before and 3 weeks after vaccination. By 3 weeks after vaccination, HAI titer ≧1:40 was observed in 75.5% and 81.1% of participants receiving 15 and 30 μg of hemagglutinin antigen, respectively. Positive seroconversion was observed in 71.7% and 81.1% of recipients of the 15 and the 30 μg, respectively. The GMTs increased by a factor of 10.7 and 17.4 in the groups of 15 and 30 μg, respectively. This study indicated that one dose of 15 μg hemagglutinin antigen without adjuvant induced protective immune response in the majority of elderly. Multivariate logistic regression analyses showed that gender, age and diabetes were statistically significant factors affecting the seroprotection rate ($p = 0.04$, 0.01 and 0.01, respectively) and seroconversion rate ($p = 0.01$, 0.01 and 0.01, respectively).

1. Introduction

In the spring of 2009, the pandemic influenza A (H1N1) virus was first identified in Mexico and the United States [1,2]. The rapidly global spread of 2009 pandemic influenza A (H1N1) virus prompted the World Health Organization (WHO), on 11 June 2009, to declare the influenza pandemic [3]. A previous study found that vaccination with recent seasonal nonadjuvant or adjuvant influenza vaccines provided little or no cross-reactive antibody protection against 2009 pandemic influenza A (H1N1) in any age group [4]. In the Northern Hemisphere, the incidence of 2009 pandemic influenza A (H1N1) was expected to increase substantially in the approaching influenza season. Therefore, a safe and effective vaccine against 2009 pandemic influenza A (H1N1) is urgently needed. The previous preliminary report showed that antibody titers ≧1:40 were observed in 96.7% of subjects, aged 18–64 years, 3 weeks after receiving the 15 μg of monovalent, unadjuvanted, inactivated, split-virus vaccine [5].

However, the quality of the immune response to influenza vaccination in the elderly is still debated [6–8]. The objective of this study was to investigate the immunogenicity and the potential factors affecting the immune response of single dose of vaccine against 2009 pandemic influenza A (H1N1) in the elderly.

2. Materials and methods

2.1. Study design

This was a multi-center, randomized and laboratory-blinded clinical trial with the subgroup analysis focusing on the elderly greater than 60 years old. The study was conducted and the data analysed by the nonindustry investigators. All the authors had full access to all study data, and vouch for the accuracy and completeness of the analysis and the data. The study protocol, amendments

* Corresponding author. Tel.: +886 2 2312 3456x7831; fax: +886 2 2370 7772.
E-mail address: hsmaids@hotmail.com (S.M. Hsieh).

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.07.026

as well as informed consent form were reviewed and approved by the Institutional Review Board (IRB) of each participating medical centers. In Taiwan, the study was conducted in accordance with the ethical principles. All volunteers were randomized to receive 15 or 30 μg of hemagglutinin antigen in a 1:1 ratio. Injections were given intramuscularly (0.5 mL vs. 1.0 mL) in the deltoid muscle. The individual treatment for each subject was determined by a randomization scheme which was established by biostatistician and operated by a computer software program incorporating a standard procedure to generate random numbers. The study group was assigned using the randomization code once a subject was eligible for this study. The investigators were blinded to the assignment group for each subject and all serum samples sent to the analytical laboratory were also under the blinded matter. There was no information, regarding dose group and subject background data, marked on the serum samples for antibody titration. For each subject, two randomization numbers were allocated, one for the pre-vaccination serum sample and the other for the post-vaccination one (week 3). Immunogenicity was detected through serum hemagglutination inhibition (HAI) tests before and 3 weeks after vaccination.

2.2. Subjects

All volunteers were recruited from National Taiwan University Hospital (NTUH), Tri-Service General Hospital (TSGH) and Wan Fang Hospital (WFH). The inclusion criteria were adults greater than 60 years old, willing and being able to adhere to visiting schedules as well as all study requirements, being in good physical health on the basis of medical history and physical examination, and agreeing and signing the informed consent. The exclusion criteria were previous influenza vaccination within 6 months; history of hypersensitivity to eggs/egg protein; personal or family history of Guillain–Barré syndrome; an acute febrile illness within the last 72 h prior to vaccination; bleeding or any coagulation disorder, and thus posing a contraindication for intramuscular injection; presenting of influenza-like illness defined by fever (temperature ≧38.5 °C) and at least two of the following four symptoms: headache, muscle/joint arches and pains (e.g. myalgia/arthralgia), sore throat and cough; treatment with an investigational drug or device, or participation in a clinical study within 3 months before consent; immunodeficiency, immunosuppressive or household contact with immunosuppression; history of wheezing or having been using bronchodilators within 3 months prior to the study; receipt of any inactivated vaccine within 2 weeks prior to study or expected receipt of vaccination within 3 weeks after the immunogenicity evaluation period; receipt of live virus vaccine within 1 month prior to study vaccination or expected receipt within 2 months after study vaccination; receipt of any blood products, including immunoglobulin in the prior 3 months; underlying condition that may be inappropriate for vaccination in the investigator's opinion.

2.3. Vaccine

This 2009 pandemic influenza A (H1N1) monovalent, split-virus vaccine was developed by Adimmune corporation, and the seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College, New York), one of the candidate reassortant vaccine viruses recommended by the WHO [9]. The vaccine virus was propagated in chicken embryos according to the same standard techniques that are used for the production of seasonal trivalent inactivated vaccine. The virus-containing fluids are harvested, and the virus is inactivated within formaldehyde and purified by zonal centrifugation. The vaccine contains 30 μg hemagglutinin antigen per mL, thimerosal 0.1 mg/mL as a preservative, formalin 0.1 μL/mL and polysorbate 80 0.1 μL/mL as a stabilizer.

2.4. Immunogenicity

Serum samples were obtained before and 3 weeks after vaccination. They were tested for anti-hemagglutinin antibodies by hemagglutination inhibition assay (HAI). The three immunogenicity end points after vaccination were chosen based on international guidelines used to evaluate influenza vaccines [10,11]. The immunogenicity profiles including the seroprotection rate (the proportion of subjects with antibody level ≧1:40 on HAI assay), seroconversion rate (the proportion of subjects with a pre-vaccination HAI antibody titer <1:10 and a post-vaccination titer ≧1:40, or a pre-vaccination titer ≧1:10 and an increase in the titer by a factor of four or more) and geometric mean fold rises of HAI antibody titer were analysed. Subjects were considered to be seronegative if serum HAI titer was less than 1:10. Reference antiserum to A/California/7/2009 was obtained from National Institute for Biological Standards and Control (NIBSC). Serum samples were treated with receptor-neutralizing enzymes to eliminate non-specific hemagglutination inhibitors. Duplicate HAI assays were performed in each sample for validation. Pre- and post-vaccination sera were titrated simultaneously and tested using 2-fold serial dilution starting with 1:10 of the tested serum.

2.5. Statistical analyses

Instead of power calculations, the sample size in this study was determined through meeting the requirements of 50 subjects per dosing group by European guidelines for yearly influenza trials [10]. To compare the baseline characteristics and medical conditions between the two dosing groups, the student's t test was applied for continuous variables and the χ^2 test was used for discrete variables. The protection and conversion rates were determined by exact 95% confidence intervals. The two-sided Fisher's exact test was applied to compare proportions of the groups. Ninety-five

Table 1
Demographic characteristics and medical conditions of study subjects.

	15 μg $N=54$	30 μg $N=53$	p-value
Gender-no. (%)			0.07
Male	25 (46.3%)	15 (28.3%)	
Female	29 (53.7%)	38 (71.7%)	
Age–year old			0.68
Mean ± SD	69 ± 6.3	68.5 ± 5.5	
Median	67	68	
Range	61–86	61–3	
Weight–kg			0.82
Mean ± SD	63.1 ± 10.4	62.8 ± 12.9	
Median	63	62	
Range	44–97	38–108	
Body mass index–kg/m^2			0.33
Mean ± SD	24.8 ± 3.1	25.6 ± 4.9	
Median	25	25	
Range	18–32	18–41	
Pre-vaccination antibody titer-no. (%)			0.54
Seronegative	34 (63.0%)	37 (69.8%)	
Seropositive	20 (37.0%)	16 (30.2%)	
Medical conditions-no. (%)			
Hypertension	25 (47.2%)	26 (49.1%)	0.84
Hyperlipidemia	13 (24.5%)	15 (28.%)	0.66
Diabetes mellitus	9 (17%)	10 (18.9%)	0.80
Joint disorders	8 (15.1%)	4 (7.5%)	0.35
Coronary artery disorders	5 (9.4%)	5 (9.4%)	1.00

Table 2
Seroprotection rate (HAI titer ≧1:40), seroconversion rate and geometric mean titers of subjects receiving single dose of study vaccine.

Seroprotection	15 μg	30 μg	Difference (%) (95% CI)	*p*-value
Pre-vaccination (n/N)	2/54	3/53	−2.0%	0.67
%	3.7%	5.7%	(−10.0%, 6.1%)	
95% CI	(0.5%, 12.7%)	(1.2%, 15.7%)		
3 weeks (n/N)	40/53	43/53	5.7%	0.63
%	75.5%	81.1%	(−23.3%, 10.0%)	
95% CI	(61.7%, 86.2%)	(68.0%, 90.6%)		

Seroconversion	15 μg	30 μg	Difference (%) (95% CI)	*p*-value
3 weeks (n/N)	38/53	43/53	−9.4%	0.36
%	71.7%	81.1%	(−25.5%, 6.6%)	
95% CI	(57.7%, 83.2%)	(68.0%, 90.6%)		

Geometric mean titers (GMT)	15 μg	30 μg	Group ratio (95% CI)	*p*-value
Pre-vaccination (N)	54	53	1.03	0.78
Mean ± SD	7.3 ± 1.79	7.1 ± 1.91	(0.82, 1.31)	
95% CI	(6.3, 8.6)	(6.0, 8.5)		
3 weeks (N)	53	53	0.62	0.09
Mean ± SD	79.5 ± 4.37	124.0 ± 5.10	(0.35, 1.10)	
95% CI	(52.9, 119.3)	(79.1, 194.2)		
Mean fold rise				
Mean ± SD	10.7 ± 4.62	17.4 ± 4.11		
95% CI	(7.0, 16.4)	(11.8, 25.7)		

percent confidence intervals of the geometric mean titers were obtained by transforming the mean of the log titer with antilog, which were compared by means of one-way analysis of co-variance (ANCOVA) on the log transformed titers with pre-vaccination level of titer as the covariate. To identify independent factors associated with increasing antibody titers of vaccination, multivariate analyses were conducted and independent variables were selected based on the results of simple logistic regression (p-value < 0.1). Statistical significance for all comparisons was determined at $p < 0.05$. All statistical analyses were performed by SAS software (version 9.2).

3. Results

3.1. Baseline characteristics and medical conditions of subjects

A total of 107 subjects (range from 61 to 86 years old) were enrolled. One subject in 15 μg group declined follow-up at week 3. The mean age and body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) of study subjects were approximately 69 years old and 25 kg/m^2, respectively. There were no significant differences of age, BMI, and medical conditions between the two dosing groups. The proportion of female subjects in 30 μg group (71.7%) was higher than those in 15 μg group, but the difference between the two dosing groups was not statistically significant ($p = 0.07$). Pre-vaccination antibodies was detected, which was identified by seropositive HAI assay (titer ≧1:10), in 33.6% of total subjects without significant difference between the groups. The demographic characteristics and medical conditions of the study subjects were summarized in Table 1.

3.2. Pre- and post-vaccination seroprotection, seroconversion and geometric mean titer (GMT)

Prior to the vaccination, 5 (4.7%) of 107 subjects had antibody titers ≧1:40, without significant differences between the two groups ($p = 0.67$). Post-vaccination serum HAI titer ≧1:40 was observed in 75.5% of recipients with the 15 μg dose and in 81.1% of recipients receiving the 30 μg dose. There was no significant difference in the seroprotection rate between the 15 and 30 μg dosing group ($p = 0.63$) (Table 2). Positive seroconversion was observed in 71.7% and 81.1% of recipients with the 15 and the 30 μg, respec-

Table 3
Factors associated with seroprotection and seroconversion rate at 3 weeks after vaccination.

		Seroprotection			Seroconversion		
Variable	Comparison	OR	(95% CI)	*p*-value[a]	OR	95% CI	*p*-value[a]
Dose	30 μg vs. 15 μg	1.397	(0.551, 3.542)	0.48	1.697	(0.682, 4.222)	0.25
BMI	BMI ≧ 27 vs. BMI < 27	0.824	(0.299, 2.270)	0.70	0.959	(0.352, 2.608)	0.93
Gender	Female vs. male	2.696	(1.049, 6.928)	0.03	3.360	(1.327, 8.505)	0.01
Age	<70 years vs. ≧70 years	2.531	(0.986, 6.499)	0.053	2.573	(1.027, 6.445)	0.04
Medical condition							
Hypertension	With vs. without	0.813	(0.322, 2.048)	0.65	1.006	(0.410, 2.468)	0.98
Hyperlipidemia	With vs. without	0.774	(0.280, 2.140)	0.62	0.900	(0.330, 2.458)	0.83
Diabetes mellitus	With vs. without	6.092	(0.768, 48.320)	0.08	6.857	(0.867, 54.217)	0.06
Joint disorders	With vs. without	0.507	(0.138, 1.862)	0.30	0.575	(0.158, 2.100)	0.40
Coronary artery disorders	With vs. without	1.120	(0.221, 5.678)	0.89	0.694	(0.165, 2.909)	0.61

[a] Result was analysed by simple logistic regression. BMI: body mass index.

tively. The 30 μg recipients had an approximate 10% higher rate of seroconversion compared to the 15 μg group, but the difference was not statistically significant ($p = 0.36$) (Table 2). The GMTs were 7.3 (ranging from <10.0 to 40) and 7.1 (ranging from < 10.0 to 80) in the 15 and the 30 μg groups prior to the vaccination; afterward, they increased by a factor of 10.7 and 17.4, respectively, 3 weeks after vaccination. Furthermore, it was higher in the 30 μg group; however, the difference was also statistically insignificant ($p = 0.09$) (Table 2).

The serological results indicated that single dose of 15 μg hemagglutinin antigen without adjuvant induced protective immune response in the majority of elderly.

3.3. Factors affecting the immune response at 3 weeks

Simple logistic regression analyses showed that gender, age and diabetes significantly affected seroprotection and seroconversion rates ($p < 0.1$) (Table 3). Multivariate analyses of the seroprotection rate showed that age was a statistically independent factor. On the other hand, multivariate analyses of the seroconversion rate showed that gender, age and diabetes were significantly factors. After adjusting other confounders, such as BMI (BMI ≥ 27 vs. BMI < 27), hypertension, hyperlipidemia, joint disorders, coronary artery disorders, we found that gender, age and diabetes significantly influenced both the seroprotection rate ($p = 0.04$, 0.01 and 0.01, respectively) and seroconversion rate ($p = 0.01$, 0.01 and 0.01, respectively).

Table 4.

4. Discussion

The immunogenicity results showed that the effectiveness of vaccine against 2009 pandemic influenza A (H1N1) fulfilled the requirements of the international guidelines evaluating influenza vaccines [10,11] and a single 15 μg dose of hemagglutinin antigen without adjuvant can generate the protective immune response in the majority of the Taiwanese elderly 3 weeks after vaccination. In our study, although there is ∼1:1 gender representation in 15 μg group and ∼2.5:1 female:male ratio in the 30 μg group, after adjusting the gender imbalance between the two dosing groups, the difference in seroprotection and seroconversion rate was still not statistically significant after adjusting gender ($p = 0.75$ and 0.504, respectively). It was surprising, contrary to the previous studies [4,5,12], that only 4.7% of study subjects had pre-existing HAI titer ≧1:40. This result may imply different infection stages of the ongoing 2009 pandemic influenza among different countries. It also clearly indicated that a different vaccination strategy may be required to achieve the most effective influenza prevention and control because the epidemiology of the pandemic may be different from countries to countries.

The quality of the immune response to seasonal influenza vaccine in the elderly is, however, still equivocal [6–8]. Goodwin et al. performed a quantitative review of 31 antibody response to influenza vaccination studies conducted from 1986 to 2002 and compared antibody responses in elderly and younger adults [8]. They concluded that the antibody response in the elderly (17–53%) was considerably lower than that in younger adults (70–90%). Similar to the recent study in 2009 pandemic influenza A (H1N1) vaccine [13], our study also found that subjects greater than 60 years old had less increment of GMTs than those who were younger. The inverse correlation between immunogenicity and age was disclosed in all measures of immunogenicity. These results may highlight the need of more immunogenic vaccine formulations of 2009 pandemic influenza A (H1N1) for the elderly.

Table 4
Multivariate analyses of seroprotection and seroconversion rate at 3 weeks after vaccination.

Variable		Seroprotection OR	(95% CI)	p^a	Seroprotection Adjusted OR	(95% CI)	p^b	Seroconversion OR	(95% CI)	p^a	Seroconversion Adjusted OR	(95% CI)	p^b
Gender	Female vs. male	2.43	(0.895, 6.456)	0.08	2.895	(1.002, 8.362)	0.04[c]	3.084	(1.160, 8.198)	0.02[c]	3.594	(1.264, 10.219)	0.01[c]
Age	<70 years vs. ≧70 yrs	3.006	(1.096, 8.244)	0.03[c]	3.928	(1.253, 12.315)	0.01[c]	3.124	(1.146, 8.515)	0.02[c]	3.919	(1.271, 12.082)	0.01[c]
Diabetes mellitus	With vs. without	8.237	(0.975, 69.593)	0.052	20.285	(1.660,247.813)	0.01[c]	9.696	(1.132,83.030)	0.03[c]	28.224	(2.057, 387.233)	0.01[c]

[a] Independent variables were selected according to the results of simple logistic regression (p-value < 0.1).
[b] Independent variables were selected according to the results of simple logistic regression (p-value < 0.1); adjusted for other confounders (i.e. BMI, hypertension, hyperlipidemia, joint disorders, coronary artery disorders).
[c] $p < 0.05$.

In addition to age, several factors contributing to poor or suboptimal vaccine effectiveness in seasonal influenza vaccination of elder adults included immunogenetics, immunosenescence, nutritional status, co-morbid conditions and frailty [14]. Our study showed that gender was significantly associated with the seroprotection rate and seroconversion rate of vaccination of 2009 pandemic influenza A (H1N1) in the Taiwanese elderly. The previous randomized, single-blinded placebo-controlled study also reported that gender was one of the significant predictors for the seroconversion rate of influenza A (H3N2) vaccine in the community-dwelling Chinese elderly persons ($p = 0.01$) [15]. As immunity has been observed to be sexually dimorphic, it might be expected that gender discrepancy exists in immune response with vaccination [16,17]. The comprehensive search of the literature retrieved seven studies of influenza vaccine, found sex-difference in the clinical efficacy of influenza vaccines [18]. The actual mechanism of the difference has not yet been defined.

Some investigators reported that diabetic patients had poor immune responses to influenza vaccine despite of widespread agreement that diabetic patients should be routinely vaccinated against influenza [19]. However, some clinical trials reported that the diabetic patients had a comparable immune response with the healthy controls [20]. Our subjects, ambulatory diabetic elderly (mean, HbA1C 6.59), appeared to have better immune response to vaccination. This might be due to the small sample size, among which diabetic subjects only accounted for 17.9%. Moreover, 94.7% of diabetic subjects had immune responses to the study vaccine.

There were some limitations in our study. First, the sample size was small, and our study is not placebo-controlled trial. Moreover, our study subjects focused on the ambulatory elderly with good cognitive and function status, so the result could not be expanded to the other populations, such as those living in long-term care facilities or with impaired immunity. Second, our study evaluated the immunogenicity 3 weeks after vaccination, so the persistence of the protective immune response of vaccination in the elderly in the following period is still inconclusive. Previous studies found that antibody induced by influenza vaccine declined more rapidly in the elderly, which may be below seroprotection level within 4 months [21,22]. However, Skowronski et al. conducted a literature review including 14 studies and they concluded that no compelling evidence to support more rapid decline of the influenza vaccine-induced antibody responses in the elderly compared with young adults [23]. It needs further exploration.

Acknowledgments

The study was sponsored by Adimmune corporation. The authors thank to Mr. Murphy Chen for the assistance in statistical analyses (ClinicalTrials.gov number, NCT01003145).

References

[1] Update: swine influenza A/H1N1 infections – California and Texas, April 2009. MMWR Morb Mortal Wkly Rep 2009;58:435–7.
[2] Swine influenza A/H1N1 infection in two children – Southern California, March – April 2009. MMWR Morb Mortal Wkly Rep 2009;58:400–2.
[3] New influenza A/H1N1 virus: global epidemiological situation, June 2009. Wkly Epidemiol Rec 2009;84:249–57.
[4] Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med 2009;361:1945–52.
[5] Greeberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009;361:2405–13.
[6] Brydak LB, Machała M, Myśliwska J, Myśliwski A, Trzonkowski P. Immune response to influenza vaccination in an elderly population. J Clin Immunol 2003;23:214–22.
[7] McElhaney JE, Dutz JP. Better influenza vaccines for older people: what will it take? J Infect Dis 2008;198:632–4.
[8] Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 2006;24:1159–69.
[9] World Health Organization. Availability of a candidate reassortant vaccine virus for the novel influenza A (H1N1) vaccine development X-179A. (Accessed Jan. 19, 2010, at http://www.who.int/csr/resources/publications/swineflu/candidatesX-179a/en/).
[10] European Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). London: The European Agency for the Evaluation of Medicinal Products; 1997.
[11] Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. Bethesda, MD: Food and Drug Administration; 2007.
[12] Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. MMWR Morb Mortal Wkly Rep 2009;58:521–4.
[13] Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, et al. A novel influenza A (H1N1) vaccine in various age groups. N Engl J Med 2009;361:2414–23.
[14] Sambhara S, McElhaney JE. Immunosenescence and influenza vaccine efficacy. Curr Top Microbiol Immunol 2009;333:413–29.
[15] Hui SL, Chu LW, Peiris JS, Chan KH, Chu D, Tsui W, et al. Immune response to influenza vaccination in community-dwelling Chinese elderly persons. Vaccine 2006;24:5371–80.
[16] Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. Science 1999;283:1277–8.
[17] Rider V, Abdou NI. In: Legato MJ, editor. Sexual dimorphism and the immune system in principles of gender-specific medicine. London: Elsevier Academic Press; 2004. p. 1071–81.
[18] Cook IF. Sexual dimorphism of humoral immunity with human vaccines. Vaccine 2008;26:3551–5.
[19] Bouter KP, Diepersloot RJ, Wismans PJ, Gmelig Meyling FH, Hoekstra JB, Heijtink RA, et al. Humoral immune response to a yeast-derived hepatitis B vaccine in patients with type 1 diabetes mellitus. Diabet Med 1992;9:66–9.
[20] Marseglia G, Alibrandi A, d'Annunzio G, Gulminetti R, Avanzini MA, Marconi M, et al. Long term persistence of anti-HBs protective levels in young patients with type 1 diabetes after recombinant hepatitis B vaccine. Vaccine 2001;19:680–3.
[21] Prevention and control of influenza: recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR Recomm Rep 1990;39(RR-7):1–15.
[22] Smith NM, Bresee JS, Shay DK, Uyeki TM, Cox NJ, Strikas RA, et al. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2006;55(RR-10):1–42.
[23] Skowronski DM, Tweed SA, De Serres G. Rapid decline of influenza vaccine-induced antibody in the elderly: is it real, or is it relevant? J Infect Dis 2008;197:490–502.