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Prevaccination Antibody Confers Additional Immune Responses to Repeated Yearly Influenza Vaccination in an Elderly Population

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Abstract

Annual vaccination is necessary to maintain humoral immunity in the elderly population. However, the factors influencing the response to influenza vaccination have not been completely identified. The aim of this study was to explore the factors that influenced antibody responses to repeated vaccination using measures that were both objective and quantitative. A total of 111 volunteers aged > 61 years were vaccinated subcutaneously with one dose of influenza vaccine from the 2005-2006 season through the 2009-2010 season. The factors that influenced antibody responses after vaccination were evaluated. The seroprotection rates (PRs) were significantly higher in responders (subjects with a higher antibody titer in the 2005-2006 season) than in non-responders only in the 2006-2007 and 2007-2008 seasons. PRs after vaccination were significantly higher in seropositive individuals (subjects with a higher prevaccination antibody titer in the 2006-2007 season) than in seronegative individuals for all three virus strains in almost all of the 5 years. Age, gender, and vaccination in the 2004-2005 season did not influence the response. These results suggest that an immune response at a certain time point would predict immune responses only in the near future. However, prevaccination antibody titer in the following season is the ideal predictor for future responses that last over several influenza seasons.

Keywords

Influenza Vaccine, Elderly Persons, Repeated Vaccination, Seroprotection Rate

1. Introduction

Influenza infection is a severe public health problem especially for elderly indi-

viduals. Influenza and its complications are associated with increased morbidity and mortality in these individuals [1]. Influenza vaccination represents an important strategy to minimize the excess morbidity and mortality caused by influenza infection. A meta-analysis showed that influenza vaccination in elderly people reduces the rate of influenza-related illness by 50% [2].

Recent studies have suggested that repetitive vaccination against seasonal influenza influences the effectiveness and response to current vaccination strategies [3] [4] [5] [6] [7]. A Japanese research group had previously investigated antibody responses over a 5-year period and found that annual vaccination was essential to maintain humoral immunity in the elderly population. This group also revealed that annual seasonal vaccination was not associated with reduced vaccine effectiveness [8].

This is a post hoc analysis of a previously published study [8]. The new clinical questions in this study are below: 1) What is the covariate which affects vaccine response? 2) Are there any relationships between previous vaccine response/pre-existing antibody titer and vaccine responses? It is known that nonresponders are more susceptible to influenza infections than serological responders [9] [10]. Therefore, we investigated what kind of demographics can identify nonresponders. Sexual dimorphisms account for the differences in clinical manifestations or the incidence of infectious diseases between women and men [11]. One group has previously shown that people who have a low antibody titer before vaccination ("seronegative") do not acquire sufficient antibody levels with one dose of influenza vaccine through a single-year analysis [12]. However, they did not examine the factors influencing immune responses among those undergoing yearly vaccination in the elderly population. Therefore, to clarify the impact of vaccination in a quantitative and impartial manner, we evaluated the factors that influence antibody responses both before and after vaccination in a closed cohort over 5 consecutive years.

2. Methods

This prospective study was conducted in a rural community located in Kochi Prefecture, Japan. We enrolled 111 participants who were aged > 61 years at the start of the study. Each of these participants was vaccinated subcutaneously with one dose (0.5 ml) of inactivated influenza vaccine comprising the three virus strains, as recommended by the World Health Organization. The composition of the influenza vaccines administered over the 5-year period, from the 2005-2006 season through the 2009-2010 season, has been described previously [8]. Blood samples were collected twice annually from each participant, *i.e.*, once before the vaccination and at 4 weeks after the vaccination. Subsequently, the hemagglutinin inhibition (HI) antibody titers of the three influenza virus strains, A/H1N1, A/H3N2, and B, were evaluated.

Blood samples were stored at −20˚C, and the HI antibody titers were measured by the HI antibody test using the standard procedure [13]. Briefly, HI anti-

body titers were measured using both goose erythrocytes and influenza A/B test antigens of the vaccine strains used in the season. The initial serum dilution ratio was 1:10. A titer of <1:10 was arbitrarily assigned as 1:5 for calculation. The seroprotection rate (PR; the proportion of subjects with HI antibody titers of ^{3}1:40) was used to evaluate antibody responses. These values are presented as percentages. The cut-off age to define the younger elderly or the very elderly group was determined based on our previous report to compare our results with the previous results as a historical control [14].

The PR was compared using Fisher's exact probability test, and multiple regression analysis. All statistical analyses were conducted using SPSS version 19.0 (Statistical Package for Social Science, Inc., Chicago, IL, USA). The study protocol was approved by the ethics committee of our institution. Written informed consent regarding the use of data in this study was obtained from all study participants.

3. Results

Table 1 shows the serological parameters measured before vaccination and 4 weeks after vaccination in all 111 subjects. The PRs against all three virus strains were significantly higher at 4 weeks after vaccination than at a time point before vaccination in each of the five seasons ($p < 0.01$). The PRs decreased to the original prevaccination levels just before revaccination in the following year. The

Table 1. Prevaccination PR and postvaccine response from 2005-2006 season to 2009-2010 season in all subjects.

Strains	2005-2006 season		2006-2007 season		2007-2008 season		2008-2009 season		2009-2010 season	
	Before (95% CI)	4 weeks after vacc. (95% CI)	Before (95% CI)	4 weeks after vacc. (95% CI)	Before (95% CI)	4 weeks after vacc. (95% CI)	Before (95% CI)	4 weeks after vacc. (95% CI)	Before (95% CI)	4 weeks after vacc. (95% CI)
H1N1	33.3 (24.5 - 42.1)	77.5 (69.7 - 85.3)	26.1 (17.9 - 34.3)	46.8 (37.5 - 56.1)	6.31 (1.79 - 10.8)	60.4 (51.3 - 69.5)	27.9 (19.6 - 36.2)	49.5 (40.2 - 58.8)	19.8 (12.4 - 27.2)	45.9 (36.6 - 55.2)
p-value	<0.01		<0.01		<0.01		<0.01		<0.01	
H3N2	31.5 (22.9 - 40.1)	78.4 (70.7 - 86.1)	26.1 (17.9 - 34.3)	56.8 (47.6 - 66.0)	45.9 (36.6 - 55.2)	73.9 (65.7 - 82.1)	17.1 (10.1 - 24.1)	60.4 (51.3 - 69.5)	30.6 (22.0 - 39.2)	63.1 (54.1 - 72.1)
p-value	<0.01		<0.01		<0.01		<0.01		<0.01	
B	20.7 (13.2 - 28.2)	47.7 (38.4 - 57.0)	9.91 (4.35 - 15.5)	20.7 (13.2 - 28.2)	13.5 (7.14 - 19.9)	25.2 (17.1 - 33.3)	15.3 (8.60 - 22.0)	28.8 (20.4 - 37.2)	17.1 (10.1 - 24.1)	36.9 (27.9 - 45.9)
p-value	<0.01		<0.01		<0.01		<0.01		<0.01	

p-value; Fisher's exact probability test. Data in parentheses are 95% confidence intervals (CI).

PRs at 4 weeks after vaccination were similar against all influenza strains and during most of the five evaluated seasons.

Regarding the factors that influenced the response, we examined the effect of age, gender, and the effect of vaccination in the 2004-2005 season. Our results demonstrated that PRs at 4 weeks after vaccination against all strains were similar between the "younger elderly" who were aged 61 - 74 years and the "very elderly" who were aged 75 - 102 years. No gender-based differences were observed in the responses against all the strains. The PRs against all influenza strains after vaccination were similar between the vaccinated group and the unvaccinated group.

We examined the relationship between antibody responses in the 2005-2006 season and those in the following seasons. We divided all participants into two groups depending on the postvaccination antibody titer in the 2005-2006 season as follows: "responder" for subjects with a high antibody titer (³40) and "nonresponder" for subjects with a low antibody titer (<40). The numbers of responders were 86 (77.5%) for H1N1, 87 (78.4%) for H3N2, and 87 (78.4%) for B strains. The numbers of nonresponders were 25 (22.5%) for H1N1, 24 (21.6%) for H3N2, and 24 (21.6%) for B strains. As shown in **Table 2**, the PRs after vaccination were significantly higher in responders than in nonresponders for both A/H3N2 and B strains in all the five seasons. For A/H1N1 strain, the PRs after vaccination were higher only in the 2006-2007 and 2007-2008 seasons.

Finally, we compared the relationship between prevaccination antibody titers and PRs after vaccination. We divided all participants into two groups according to the prevaccination antibody titer in the 2006-2007 season as follows: "seronegative" for subjects with a low antibody titer (<10) and "seropositive" for subjects with a high antibody titer (³10). The numbers of seronegative subjects were 32 (28.8%) for H1N1, 28 (25.2%) for H3N2, and 28 (25.2%) for B strains. The numbers of seropositive subjects were 79 (71.2%) for H1N1, 83 (74.8%) for H3N2, and 83 (74.8%) for B strains. The PRs after vaccination were significantly higher in seropositive subjects than in seronegative subjects for all three virus strains in all of the 5 years, except for A/H1N1 strain in the 2008-2009 season (**Table 3**).

4. Discussion

The PR (the proportion of individuals with HI antibody titers ³1:40) at 4 weeks after vaccination is a good surrogate parameter for the protection provided by a given vaccine and is relevant to public health [15]. The antibody responses to an inactivated influenza vaccine depend on several factors, including age, sex, history of vaccination, antibody response to a certain vaccination, prevaccination antibody titer, underlying medical conditions, functional disability, previous exposure to influenza viruses, quality and quantity of vaccine products, and vaccine dose regimen. Our aim in this study was to identify any factors that distinguish responders from nonresponders.

Table 2. Influence of response in 2005-2006 season in H1N1, H3N2, and B strains.

strains		2005-2006 season		2006-2007 season		2007-2008 season		2008-2009 season		2009-2010 season	
		before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)
H1N1	nonresponder (n = 25)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	12.0 (−0.74 - 24.7)	0.00 (0.00 - 0.00)	36.0 (17.2 - 54.8)	16.0 (1.67 - 30.4)	36.0 (17.2 - 54.8)	8.00 (−2.63 - 18.6)	28.0 (10.4 - 45.6)
	responder (n = 86)	43.0 (32.5 - 53.5)	100.0 (100.0 - 100.0)	33.7 (23.7 - 43.7)	57.0 (46.5 - 67.5)	8.14 (2.36 - 13.9)	67.4 (57.5 - 77.3)	31.4 (21.6 - 41.2)	53.5 (43.0 - 64.0)	23.3 (14.4 - 32.2)	51.2 (40.6 - 61.8)
	p-value	0.000	-	0.000	0.000	0.346	0.010	0.204	0.173	0.152	0.067
H3N2	nonresponder (n = 24)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	29.2 (11.0 - 47.4)	12.5 (−0.73 - 25.7)	41.7 (22.0 - 61.4)	0.00 (0.00 - 0.00)	37.5 (18.1 - 56.9)	4.17 (−3.83 - 12.2)	37.5 (18.1 - 56.9)
	responder (n = 87)	40.2 (29.9 - 50.5)	100.0 (100.0 - 100.0)	33.3 (23.4 - 43.2)	64.4 (54.3 - 74.5)	55.2 (44.8 - 65.6)	82.8 (74.9 - 90.7)	21.8 (13.1 - 30.5)	66.7 (56.8 - 76.6)	37.9 (27.7 - 48.1)	70.1 (60.5 - 79.7)
	p-value	0.000	-	0.000	0.003	0.000	0.000	0.011	0.017	0.001	0.008
B	nonresponder (n = 24)	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	1.72 (−3.48 - 6.92)	10.3 (−1.86 - 22.5)	3.45 (−3.85 - 10.8)	10.3 (−1.86 - 22.5)	0.00 (0.00 - 0.00)	6.90 (−3.24 - 17.0)	8.62 (−2.61 - 19.8)	17.2 (2.10 - 32.3)
	responder (n = 87)	43.4 (33.0 - 53.8)	100.0 (100.0 - 100.0)	18.9 (10.7 - 27.1)	32.1 (22.3 - 41.9)	24.5 (15.5 - 33.5)	41.5 (31.1 - 51.9)	32.1 (22.3 - 41.9)	52.8 (42.3 - 63.3)	26.4 (17.1 - 35.7)	58.5 (48.1 - 68.9)
	p-value	0.000	-	0.003	0.009	0.002	0.000	0.011	0.000	0.022	0.000

p-value; Fisher's exact probability test. Data in parentheses are 95% confidence intervals (CI).

Table 3. Influence of prevaccination antibody titer of 2006-2007 season in H1N1, H3N2, and B strains.

strains		2005-2006 season		2006-2007 season		2007-2008 season		2008-2009 season		2009-2010 season	
		before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)	before (95% CI)	4 weeks after vacc. (95% CI)
H1N1	seronegative[a] (n = 32)	3.13 (−2.90 - 9.16)	40.6 (23.6 - 57.6)	0.00 (0.00 - 0.00)	9.38 (−0.72 - 19.5)	3.13 (−2.90 - 9.16)	37.5 (20.7 - 54.3)	12.5 (1.04 - 24.0)	34.4 (17.9 - 50.9)	6.25 (−2.14 - 14.6)	25.0 (10.0 - 40.0)

Continued

	seropositive[b] (n = 79)	45.6 (34.6 - 56.6)	92.4 (86.6 - 98.2)	36.7 (26.1 - 47.3)	62.0 (51.3 - 72.7)	7.59 (1.75 - 13.4)	69.6 (59.5 - 79.7)	34.2 (23.7 - 44.7)	55.7 (44.7 - 66.7)	25.3 (15.7 - 34.9)	54.4 (43.4 - 65.4)
	p-value	0.000	0.000	-	0.000	0.671	0.003	0.021	0.059	0.033	0.006
	seronegative[a] (n = 28)	0.00 (0.00 - 0.00)	32.1 (14.8 - 49.4)	0.00 (0.00 - 0.00)	21.4 (6.21 - 36.6)	7.14 (−2.40 - 16.7)	32.1 (14.8 - 49.4)	3.57 (−3.30 - 10.4)	32.1 (14.8 - 49.4)	7.14 (−2.40 - 16.7)	28.6 (11.9 - 45.3)
H3N2	seropositive[b] (n = 83)	42.2 (31.6 - 52.8)	94.0 (88.9 - 99.1)	34.9 (24.6 - 45.2)	68.7 (58.7 - 78.7)	59.0 (48.4 - 69.6)	88.0 (81.0 - 95.0)	21.7 (12.8 - 30.6)	68.7 (58.7 - 78.7)	38.6 (28.1 - 49.1)	74.7 (65.3 - 84.1)
	p-value	0.010	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.001
	seronegative[a] (n = 28)	4.84 (−3.11 - 12.8)	33.9 (16.4 - 51.4)	0.00 (0.00 - 0.00)	6.45 (−2.65 - 15.5)	0.00 (0.00 - 0.00)	9.68 (−1.27 - 20.6)	3.23 (−3.32 - 9.78)	12.9 (0.48 - 25.3)	1.61 (−3.05 - 6.27)	17.7 (3.56 - 31.8)
B	seropositive[b] (n = 83)	40.8 (30.2 - 51.4)	65.3 (55.1 - 75.5)	22.4 (13.4 - 31.4)	38.8 (28.3 - 49.3)	30.6 (20.7 - 40.5)	44.9 (34.2 - 55.6)	30.6 (20.7 - 40.5)	49.0 (38.2 - 59.8)	36.7 (26.3 - 47.1)	61.2 (50.7 - 71.7)
	p-value	0.001	0.003	-	0.000	0.000	0.000	0.000	0.002	0.000	0.000

a: Subjects with hemagglutination inhibition antibody titer of <1: 10 before vaccination; b: Subjects with hemagglutination inhibition antibody titer of ≥1: 10 before vaccination. p-value; Fisher's exact probability test. Data in parentheses are 95% confidence intervals (CI).

The factors influencing the response to influenza vaccination have not been completely identified. We believed that those who responded to the vaccination in the 2005-2006 season would continue to exhibit better responses in the following 2006-2007, 2007-2008, 2008-2009, and 2009-2010 seasons. We divided all subjects into the following two groups depending on the postvaccination antibody titer in the 2005-2006 season: "responder" for subjects with a high antibody titer (³40) and "nonresponder" for subjects with a low antibody titer (<40). We found that the PRs after vaccination were significantly higher in responders than in nonresponders for all strains in both the 2006-2007 and 2007-2008 seasons. However, the differences were lost for A/H1N1 strain in both the 2008-2009 and 2009-2010 seasons. These findings suggest that an immune response at a certain time point would predict immune responses only in the near future. Therefore, antibody responses may not be the ideal predictor for future responses.

Since analysis of the factors affecting the antibody response showed that those who have a low antibody titer before vaccination ("seronegative") do not acquire

enough antibody with one dose of influenza vaccine, we divided all participants into two seronegative and seropositive groups depending on the prevaccination antibody titer in the 2006-2007 season [12]. The PRs after vaccination were found to be significantly higher in the seropositive group than in seronegative group for all three virus strains in all the 5 years, except for A/H1N1 strain in the 2008-2009 season. These findings suggest that seropositive individuals produce sufficient antibody levels in response to vaccination and this better response continues over several years. Therefore, prevaccination antibody titer in the following season is the ideal predictor for future responses that last over several influenza seasons. The results of our study show that humoral antibody responses may be enhanced by preexisting antibodies, probably through an anamnestic response to vaccination [16]. Preexisting immune cells are triggered to produce new antibodies, thus enhancing the protective efficacy. Therefore, a significant booster effect of vaccination would be observed in seropositive individuals. In contrast, the protection against influenza infection may not be sufficient in seronegative individuals, particularly influenza epidemic outbreaks. In general, it is believed that elderly individuals are well immunized by one dose of influenza vaccination [17] [18]. However, our results indicate that seronegative people would not acquire sufficient antibody levels to achieve protection against influenza infection after only one dose of influenza vaccination.

Nonresponders who do not respond to a certain vaccination are a serious problem in vaccination. To maximize the vaccine effects for nonresponders, we should try several strategies. One strategy might be to immunize elderly people with a booster dose of influenza vaccine and not with only one dose [14]. High-dose influenza vaccine could be another strategy since it was shown to be more immunogenic, and more efficacious in preventing influenza infections than the standard-dose vaccine [19]. Further studies are needed to clarify this issue.

There were several limitations in this study. First, it was conducted in a rural community. Considering that influenza is transmitted from one person to another, infection is more common in urban areas than in rural communities. Therefore, the results may not be generalizable to people living in urban areas. Second, our study cohort included only those aged > 61 years. Because immune responses are different among different age groups, it is not possible to generalize our study results [20] [21]. Third, life expectancy varies among countries. The average life expectancy in Japan is relatively high, and our study participants were healthy elderly people who are expected to live longer than the global average life expectancy. Therefore, their immune response to the vaccination might have been different from the global standard. Finally, vaccine efficacy, which is a comparison of infection rates between a vaccinated and unvaccinated cohort, was not included in our evaluation. Although the HI antibody titer is a meaningful parameter that adequately describes the immune response to vaccination, vaccine effectiveness is more clinically important [21].

5. Conclusion

These results suggest that an immune response at a certain time point would predict immune responses only in the near future. However, prevaccination antibody titer in the following season is the ideal predictor for future responses that last over several influenza seasons.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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