Original Articles

Neuraminidase Antibody Response to Inactivated Influenza Virus Vaccine Following Intranasal and Intramuscular Vaccination

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Abstract

Background: The evaluation of influenza vaccine activity and potency are based on the immune response to hemagglutinin, and protection is indicated when the titer of hemagglutination inhibition serum antibody is $\geq$ 1:40. Neuraminidase, the second surface glycoprotein, may also have a role in protection, but little information is available on the immunologic response to this component.

Objectives: To determine whether any response to neuraminidase is evoked by intranasal immunization with a novel, whole, inactivated anti-influenza vaccine.

Methods: This study was part of a more comprehensive study of mucosal and serum immune response to this vaccine. Fifty-four young adults were immunized intranasally, 9 intramuscularly and 18 received a placebo. Twenty-three elderly people were immunized intramuscularly, and 21 elderly and 17 children were immunized intranasally. Serum and nasal antibodies to antigens N1 and N2 were determined by the lectin neuraminidase test.

Results: Serum response following intranasal vaccination was lower than after intramuscular vaccination, and ranged from 21.4 to 35.3% and 33.3 to 64.7% following intranasal vaccination and from 52.2 to 77.8% and 47.8 to 88.9% after intramuscular vaccination, to N1 and N2 respectively. Nasal antibody response was low and was found only after intranasal vaccination, and response to N2 was better than to the N1 antigen.

Conclusions: It may be beneficial if future vaccines would include competent hemagglutinin and neuraminidase, which would afford a higher level of protection.

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Influenza, a major winter respiratory infection, ranges from a mild to severe form and can result in complications and mortality, particularly in high risk groups. While the old and the very young are particularly vulnerable to influenza, the general population is sensitive as well, imposing a heavy economic burden and considerable pressure on public health services. The preferred strategy for controlling the disease is annual vaccination.

Routinely used vaccines include inactivated vaccines (whole virions, split or purified viral subunit proteins) designated for intramuscular administration. Mostly sera antibody directed against the major viral envelope protein, hemagglutinin, is produced. Recently,

vaccines for intranasal administration (inactive and attenuated) were developed [1,2]. The principal advantage of mucosal immunization is that an enhanced mucosal as well as systemic immunity is created, constituting a double barrier against the virus [3]. Also, SIgA antibodies are considered to have a broader spectrum of activity than the serum immunoglobulin G antibodies [4]. One of the most acute problems related to influenza vaccines is the narrow range of their specificity and the restricted strain-specific activity. The rapid changes that occur in the viral surface antigen, hemagglutinin, limit the efficacy of the currently available vaccines, which are based on standardization of hemagglutinin content (15 µg hemagglutinin of each strain/dose). Also, evaluation of vaccine activity and potency are based on the immune response to hemagglutinin, and protection is indicated when the hemagglutination inhibition serum antibody titer is $\geq$ 1:40.

The second viral surface glycoprotein, neuraminidase, is less variable and its antigenic evolution occurs at a slower rate than that of hemagglutinin [5-7] and can, therefore, provide cross-protection not only against homologous virus but also drift viruses [6]. Anti-neuraminidase antibodies may play a role in protection. Although infection is not inhibited, there is a decrease in virus replication and disease manifestations [8-10]. The importance of neuraminidase in influenza infection is also indicated by the beneficial effect of the recently developed anti-neuraminidase drugs in prevention and cure [11,12]. However, neuraminidase concentration in the vaccines and its antigenic potency following vaccination has not been sufficiently investigated and only limited data have accumulated [13-15]. In the present preliminary study, we sought to determine whether any response to the neuraminidase was evoked following intranasal immunization with a novel, whole, inactivated anti-influenza vaccine.

Materials and Methods

Specimens and study group

Sera and nasal washing specimens used for the detection of anti-neuraminidase antibodies were part of the samples collected in a more comprehensive study of the mucosal and serum immune response to our inactivated trivalent anti-influenza vaccine [1,3,16-18]. Peripheral blood and nasal swabs were collected before vaccination and 21 days after, and sera and secretions were

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Table 1. Experimental groups

Route	Group	Age	Year	Vaccinees no
Intranasally	A-1	Young adults	1996/7	26
	A-3	Young adults	1997/8	28
	A-4	Placebo	1997/8	18
	B-5	Elderly	1998/9	21
	C-7	Children 12 yrs old	1998/9	17
Intramuscularly	A-2	Young adults	1996/7	9
	B-6	Elderly	1998/9	23

Individuals were vaccinated once, intranasally or intramuscularly

frozen at -20°C until needed for analysis. Anti-neuraminidase response to the B strain in the vaccine was not studied. Samples were obtained from groups of vaccinees as described in Table 1. All elderly participants were previously submitted to annual intramuscular vaccination. For the groups of young adults and children this was the first influenza vaccination. The study was approved by the Israel Ministry of Health and the Helsinki Committee of the Hadassah University Hospital. Informed consent was obtained from each vaccinee.

Antigens comprising the vaccine were according to the recommendation for each season: 1996/97 included A/Texas/36/91, H1N1 and A/Nanchang/933/95 (H3N2); 1997/98 included A/Nanchang/933/95 and A/Johannesburg/82/96 H1N1); and 1998/99 included A/Beijing/262/95 (H1N1), and A/Sydney/5/97 (H3N2).

Antigens
Recombinant viruses with hemagglutinin from equine origin were used:

N2: Wic-250 (H7N2) A/Equi/Prague/1/56 (H7) – A/Gd/25/93 (N2)
N1: Wic-121 (H7N1) A/Equi/Prague/1/56 (H7) – A/Singapore/6/86 (N1)

Antibodies to neuraminidase: lectin neuraminidase test [19]
Twofold serial dilutions of inactivated sera were mixed with 4 neuraminidase units of virus, for 1 hour at room temperature; 3% of washed human O+ red blood cells were added for 3 hours at 37°C. At the end of this period the reaction mixture was shaken well and lectin was added for 1 hour at room temperature. Inhibition of hemagglutination caused by lectin indicated the presence of

Table 2. Serum Antibody Geometric Mean Titer and Antibody Response to neuraminidase and to hemagglutinin following intranasal or intramuscular vaccination

Route of admininstration	Season	Group	N1[a] GMT[b] Pre-	Post-	x-fold	Antibody response[c] No.	%	H1[a] GMT[b] Pre-	Post-	x-fold	Antibody response[d] No.	%
Antigen N1												
Intranasally	1996/97	A-1	2.8	3.8*	1.4	8/26**	30.8	23.5	77.9*	3.3	15/26	57.6
	1997/98	A-3	3.4	3.9	1.1	6/28	21.4	100.0	128.1	1.3	5/28	17.9
	1998/99	B-5	2.9	3.2	1.1	5/21	23.8	10.0	14.4*	1.4	4/21	19.0
		C-7	3.8	4.5	1.2	6/17	35.3	22.3	90.4*	4.0	12/17	70.0
Intramuscularly	1996/7	A-2	2.3	5.0*	2.2.	7/9**	77.8	25.2	160.0*	6.3	8/9	88.8
	1998/9	B-6	1.9	2.6	1.4	12/23	52.2	12.7	66.8*	5.2	16/22[e]	72.7

Route of admininstration	Season	Group	N2[a] GMT[b] Pre-	Post-	x-fold	Antibody response[c] No.	%	H3[a] GMT[b] Pre-	Post-	x-fold	Antibody response[d] No.	%
Antigen N2												
Intranasally	1996/97	A-1	3.4	5.2*	1.5	14/26	53.8	17.5	37.0*	2.1	10/26	38.5
	1997/98	A-3	7.6	10.8*	1.4	13/28	46.4	28.3	37.2	1.3	6/28	21.4
	1998/99	B-5	14.0	18.3*	1.3	7/21	33.3	11.8	23.6	1.2	5/21	23.8
		C-7	15.4	25.1*	1.6	11/17	64.7	30.1	153.6*	5.1	9/17	52.9
Intramuscularly	1996/7	A-2	3.4	10.9*	3.2	8/9	88.9	21.6	137.2*	6.3	7/9	77.8
	1998/9	B-6	4.8	10.8*	2.3	11/23	47.8	17.2	55.7*	3.2	12/22[e]	61.5

Antibody titers to hemagglutinin and neuraminidase were evaluated 3 weeks after vaccination.

[a] Neuraminidase inhibition antibody assays were performed with the lectin neuraminidase test (see Methods). Hemagglutination inhibition assays were performed with the standard micro-titer method.
[b] Results are given as geometric mean titer of antibody of the tested group.
[c] Serum increase in neuraminidase inhibition antibody levels was twofold compared to baseline.
[d] Antibody titer increase was at least fourfold as compared to baseline, or attaining hemagglutination inhibition antibody titer ≥ 1:40.
[e] Data are missing for one vaccinee.
* $P = 0.01–0.03$, comparing increased GMT in serum between pre- and post-vaccination (unpaired Student's t-test).
** $P < 0.05$, comparing response between intranasal and intramuscular groups (A-1 vs. A-2) (Fisher's exact test).

anti-neuraminidase antibodies in the serum as well as in mucosal samples. Antibody titer is the last dilution with no hemagglutination. A twofold increase in antibody titer (in serum and nasal secretions) following immunization was considered positive. Pre-vaccination and post-vaccination antibody titer from each vaccinee in serum and nasal washing were measured simultaneously.

Antibody response to the hemagglutinin

Serum antibody levels were determined by the standard hemagglutination inhibition microtiter assay as described previously [3]. A fourfold increase or more in antibody titer or ≥ 1:40 hemagglutination inhibition titer following immunization was considered positive. Mucosal antibody levels were determined by the enzyme-linked immunosorbent assay method [3]. Mucosal immune response was defined as a ≥ 1.4 increase in nasal antibody levels compared to baseline.

Statistics

The geometric mean titer pre-vaccination was compared to that of post-vaccination using unpaired Student's t-test. Total responses in the intranasal and intramuscular groups were compared by Fisher's exact test. The Microsoft Excel and SigmaStat 2.03 programs were used.

Results

Serum antibody response

Serum neuraminidase inhibition antibody response to intranasal or intramuscular vaccination is presented in Table 2. There were no significant differences in the serum GMT to N1 and N2 before vaccination in the intramuscular and intranasal compatible groups; a higher titer to N2 component of the A/Sydney/5/97 was found in the intranasal vaccinated elderly (B-5) and children (C-7). Pre-vaccination hemagglutination inhibition antibody titers were similar in all the groups except for the higher titer to H1 (group A-3). Pre-vaccination titers to the hemagglutinins were higher than to the neuraminidases.

The serum antibody response to neuraminidases among intramuscular vaccine recipients (groups A-2 and B-6) was 7/9 (77.8%), 12/23 (52.2%), 8/9 (88.9%) and 11/23 (47.8%) to N1 and N2 antigens, respectively, in seasons 1996/97 and 1998/99. Serum antibody response following intranasal vaccination was lower than that after intramuscular vaccination, but was significantly lower only in 1996/97 for N1 in group A-1 compared to group A-2 ($P < 0.05$). The antibody response was 8/26 (30.8%), 5/21 (23.8%), 14/26 (53.8%) and 7/21 (33.3%) to N1 and N2, respectively. Serum antibody response in the intranasal vaccine recipients in 1997/98 was comparable to the response following intranasal vaccination in the other two seasons [Table 2], 6/28 (21.4%) and 13/28 (46.4%) for N1 and N2, respectively. A similar antibody response following intramuscular vaccination was found to H1 and H3 (88.8%, 72.7%, 77.8% and 61.5%) respectively. The latter response was similar to the response to N1 and N2 [Table 2].

The GMT of antibody increased significantly following intra-

GMT = geometric mean titer

muscular and intranasal vaccination as compared to pre-vaccination GMT for N2 except for group B-5. A significant increase in antibody titer to N1 was less frequent. Increase in antibody titer was higher to H1 and H3 than to N1 and N2 following intranasal or intramuscular vaccination [Table 2]. There was no difference in the response following intramuscular vaccination between N1 and N2 in the same calendar year, although response was higher in 1996/97. On the other hand, following intranasal vaccination, the serum response was higher to N2 than to N1 ($P = 0.025$–0.05) in the A-1 and C groups but not in the elderly (B-5). In children (group C), following intranasal vaccination, antibody response in the serum to N2 was higher than in the elderly, 64.7% vs. 33.3% ($P = 0.1$)

Nasal antibody response

Nasal antibody response, or increase in GMT to neuraminidase or hemagglutinin, was only negligible following intramuscular vaccination. Following intranasal immunization, neuraminidase inhibition antibody response was rather poor. A significant increase in GMT was found only for N2 in 1997/98 in group A-3 and in 1998/99 in the children (group C) (increase of 1.0 to 1.6 and 1.7 to 2.8, respectively). Antibody response to N2 antigen was only found in 4/26 (15.4%), 10/28 (35.7%), 5/20 (25%) and 6/15 (40%) of the vaccinees (groups A-1, A-3, B-5 and C, respectively). Antibody response to N1 antigen was only found in 2/20 (10.0%) and 4/28 (14.3%) of the vaccinees' antigen (B-5 and A-3 groups, respectively). Mucosal response to the H1 and H3 antigens was higher and ranged between 25% and 60.0%. The specificity of the response was demonstrated by the complete lack of serum or mucosal responses following intranasal administration of placebo.

Discussion

The immunogenicity of currently available influenza vaccines (the inactivated and the live attenuated) is mostly measured by the level of antibodies raised against hemagglutinin. Fewer data are available concerning the response to the second surface glycoprotein, neuraminidase, despite the known role of antibodies to neuraminidase in preventing infection, decreasing level of viral progeny, duration in viral shedding, and reducing the intensity of disease symptoms [8-10,20]. In this report we present the response to neuraminidase following intramuscular and intranasal vaccination, and compare it to the response to hemagglutinin. The trend of the immune response found to neuraminidase is comparable to that of hemagglutinin [1,3,16-18]. Serum antibody response to hemagglutinin and neuraminidase was higher following intramuscular than following intranasal vaccination. Mucosal response was absent or negligible following intramuscular vaccination but was evident following intranasal vaccination. However, response to neuraminidase was higher in the serum than in the mucosa, even following intranasal vaccination, while mucosal response to hemagglutinin was higher than serum response or similar (not shown). The higher increase in antibody titers to hemagglutinin, found also by others [21], may be due to a low concentration of neuraminidase in the vaccine because of the relatively greater molar amounts of hemaglutinin than

neuraminidase on the virion surface (concentration in the tested vaccine was not determined) [22], loss of antigenicity during vaccine production [21,23], or as a result of antigenic competition between hemagglutinin and neuraminidase [24]. This may explain the low responses to N1 and H1 in group A-3 where pre-vaccination hemagglutination inhibition antibody titer was high (1:100). We did not compare, statistically, the response to the two antigens since different methods with different sensitivity were used: for the hemagglutinin a fourfold increase or attaining 1:40 titer was considered positive, compared to a twofold increase for neuraminidase.

The lower response in the elderly group (1998/99) may be attributed to frequent, previous exposure to antigens by either infection or vaccination [25], or to age (65–85 years old). The higher response in the 1996/97 season may be due to the difference in the strains included in the vaccine. There is some indication that the mucosal response to N2 was higher than to N1. The lower response to N1 in the serum and mucosa is acceptable as antigenicity of N1 is considered to be inferior.

Conclusion

Immune response to neuraminidase was detected in the serum and in the mucosa following intramuscular or intranasal vaccination. Because of the slower rate of antigenic variation in neuraminidase and its role in protection, it is important that vaccine developers consider standardization and procedures that would maintain its antigenicity. Competent hemagglutinin and neuraminidase in vaccines may afford a high level of protection.

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