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Analysis of Immunoglobulin G Antibody Responses after Administration of Live and Inactivated Influenza A Vaccine Indicates that Nasal Wash Immunoglobulin G Is a Transudate from Serum

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Following intranasal administration of live influenza A virus vaccine or parenteral inoculation of inactivated influenza virus vaccine, immunoglobulin antibody to the influenza virus hemagglutinin was detected in nasal wash specimens from adult volunteers. Several observations supported the suggestion that this immunoglobulin G hemagglutinin nasal wash antibody appeared to be mainly derived from the serum by a process of passive transudation.

The mucosal surfaces are an important host defense system against a variety of infectious agents (1). Although previous studies have shown that secretory antiviral antibodies are predominantly of the immunoglobulin A (IgA) isotype (8, 9, 13, 14, 17, 20), a few studies have shown the presence of IgG antiviral antibodies in nasal wash specimens (2–4, 7, 10, 11, 21). Whether the source of local IgG antibodies is a transudate from serum or arises from local mucosal production has been a subject of recent investigation. There is evidence from children infected with live attenuated influenza A vaccine for local production of IgG hemagglutinin (HA) antibody in a minority of cases (6). In addition, after administration of inactivated influenza A vaccine, children respond to live virus challenge with a greater increase in IgG HA antibody levels in nasal secretions than in serum (6). In adults the source of local IgG HA antibody has been less clearly defined. To further investigate the origin of nasal wash IgG HA antibody, we evaluated the IgG antibody responses to influenza A virus HA in serum and nasal wash specimens from adult volunteers vaccinated with either live attenuated or inactivated influenza A virus vaccine.

If nasal wash IgG antibody were derived mainly from plasma cells in the submucosa of the nasal turbinates, the concentration of IgG HA antibody achieved in the submucosal space of some individuals might exceed the background level of submucosal antibody produced by passive diffusion from serum. In such individuals, the specific activity of antibody (i.e., the ratio of antibody titer to IgG concentration) achieved in the nasal wash specimens should be higher than that in serum. Therefore, we compared the specific activities of serum and nasal wash IgG HA antibodies from the recipients of live- and inactivated-virus vaccines. Since it is presumably more likely for live virus administration rather than inactivated virus administration to stimulate local antibody responses, a higher specific activity of HA antibody might be observed in the nasal wash specimens from the recipients of live-virus vaccine. In addition, the titer of HA-specific IgG subclass antibody was measured to determine whether the relative amounts of IgG1, IgG2, IgG3, and IgG4 HA antibodies were similar in serum and nasal wash specimens. The different ratios of serum and nasal wash IgA1 and IgA2 concentrations have suggested different origins of IgA subclass antibody in these two compartments (5). Finally, we compared the levels of IgG HA antibody in postinfection sera and nasal washes and examined the slopes and intercepts of the regression lines defined by serum and nasal wash titers in both the live- and inactivated-virus vaccine recipients. A significant difference in the slope or intercept of the two regression lines would have suggested that the nasal wash IgG HA antibody had different origins in the two vaccine groups. The findings of these studies indicate that nasal wash IgG HA antibody is derived from serum as a transudate in both live- and inactivated-virus vaccine recipients, with little detectable contribution by mucosal plasma cells.

The subjects used in this study were selected from a larger group and were known to have had an increase in serum or nasal wash IgG antibody titer to purified A/Bangkok/79 H3 HA (4). These volunteers were vaccinated with a live attenuated cold-adapted influenzaA/Washington/897/80 (H3N2) virus or a commercial inactivated vaccine containing 15 μg of HA of A/Brazil/11/78 (H1N1), A/Bangkok/1/79 (H3N2), and B/Singapore/222/79 per 0.5-ml dose (Fluogen; Parke, Davis & Co., Morris Plains, N.J.). Serum and nasal wash samples were collected before vaccination and at 28 days postvaccination as previously described (4).

Murine monoclonal antibodies to human IgG subclasses (HP6001, αIgG1; HP6014, αIgG2; HP6048, αIgG3; HP6023, αIgG4) were produced and tested for specificity as previously described (16). The enzyme-linked immunosorbent assay (ELISA) for detection of subclass antibody responses was performed as previously described (18), except that the antigen used was purified A/Bangkok/79 H3 HA (12), which is closely related antigenically to the A/Washington/80 H3

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TABLE 1. IgG and IgG subclass responses to influenza A hemagglutinin in sera and nasal washes from subjects vaccinated with inactivated or live attenuated influenza A virus vaccine

Source of specimen	Type of vaccine administered	No. of subjects	IgG			IgG1		
			Reciprocal of $\log_2$ titer (mean ± SEM)		% With fourfold or greater increase	Reciprocal of $\log_2$ titer (mean ± SEM)		% With fourfold or greater increase
			Prevaccination	Postvaccination		Prevaccination	Postvaccination	
Serum	Inactivated	24	11.4 ± 0.4	15.8 ± 0.3	100	5.7 ± 0.4	10.4 ± 0.3	100
	Live	18	11.8 ± 0.4	13.6 ± 0.3	67	6.2 ± 0.3	8.1 ± 0.3	83
Nasal wash[a]	Inactivated	22	1.7 ± 0.4	6.2 ± 0.5	91	0.7 ± 0.2	2.2 ± 0.2	32
	Live	18	2.3 ± 0.3	5.3 ± 0.3	78	1.0 ± 0.1	1.1 ± 0.1	0

[a] Titers were corrected to 10 mg of IgA per dl.

HA. The ELISA for measuring total IgG antibody response to purified influenza A/Bangkok/79 H3 HA was performed as previously described (11). The dilution of rabbit anti-human IgG and the substrate incubation time were identical for the determinations of serum and nasal wash HA antibody titers. All nasal wash ELISA titers were corrected to an IgA concentration of 10 mg/dl. The total IgG concentrations in serum and nasal wash samples were determined by using a standard radioimmunoassay (19). The nasal wash specimens were concentrated approximately 10-fold by dialysis against aquacide (Calbiochem-Behring, La Jolla, Calif.).

The total IgG and IgG subclass responses to HA in sera and nasal washes are shown in Table 1. Each inactivated-virus vaccine recipient had a serum IgG HA antibody response, and 91% of these volunteers developed a nasal wash IgG HA titer increase. Among the live-virus vaccine recipients, the total IgG HA responses in sera and nasal washes were 67 and 78%, respectively. The specific activity of a serum or nasal wash sample was calculated by determining the value of the postvaccination IgG HA antibody titer over the total IgG concentration. The total IgG concentrations were 6,443 ± 426 μg/ml (mean ± standard deviation) for sera and 35.6 ± 3.4 μg/ml for nasal washes for the inactivated-virus vaccine recipients and 5,339 ± 432 μg/ml for sera and 30.6 ± 3.1 μg/ml for nasal washes for the live-virus vaccine recipients, indicating that the two groups of vaccine recipients had comparable IgG concentrations in their serum and nasal wash specimens. Next, for each vaccine recipient the ratio of the nasal wash specific activity to the serum specific activity was determined, and means for the live- and inactivated-virus vaccine recipients were calculated. The mean specific activity ratio for inactivated-virus vaccine recipients was 0.7 ± 0.3, and that for the live-virus vaccine recipients was 1.1 ± 0.4, values which are not significantly different from each other. This ratio of almost 1 suggests that the nasal wash IgG is derived by a process of passive transudation since the specific activities of HA antibody are similar in serum and nasal secretions. It was previously demonstrated that the ratios of nasal wash specific activity to serum specific activity for IgA and IgM HA antibodies were greater than 4.0 in 8 of 11 and 6 of 7 children, respectively, who received live influenza A virus vaccine (11). However, only two of nine children had specific activity ratios for IgG HA antibody that were greater than 4.0. In the present study, the number of adult live-virus vaccine recipients with specific activity ratios for IgG HA antibody that were greater than 4.0 was 1 of 18, and the number of inactivated-virus vaccine recipients was 1 of 22, indicating that, like the means, the frequencies of specific activity ratios greater than 4.0 were comparable in the two groups of vaccine recipients.

The IgG subclass responses to HA in sera and nasal washes are also shown in Table 1. The ranking of serum IgG subclass HA antibody titers among inactivated-virus vaccine recipients revealed that the order of the mean $\log_2$ postvaccination titers was as follows: IgG1, IgG3, IgG2, IgG4. A similar ordering of the magnitude of IgG subclass HA responses (IgG1, IgG3, IgG2, IgG4) was seen in the nasal washes of these volunteers. Live-virus vaccine recipients had the same ordering of the serum IgG subclass postvaccination HA antibody titers (IgG1, IgG3, IgG2, IgG4). However, the nasal wash IgG HA subclass antibody responses of these volunteers were too infrequent to detect a pattern of response. It has been demonstrated previously that there is a significant correlation between serum and nasal wash total IgG HA antibody titers (4), and a similar analysis was done for HA-specific IgG1 antibody, the IgG antibody subclass with the highest frequency and magnitude of response. Postvaccination titers in the sera and nasal washes were ranked, and the Spearman rank correlation coefficients were calculated. For the inactivated-virus vaccine group, the correlation coefficient was 0.436 ($P < 0.05$). A similar analysis for the correlation of the live-virus vaccine group was not performed because of the low frequency of nasal wash IgG responses.

We next sought to examine in more detail the relationship between the serum and nasal wash total IgG ELISA antibody titers in the live- and inactivated-virus vaccine recipients. In this analysis the serum and nasal wash ELISAs were performed in an identical manner, whereas in a previously published comparison the ELISA titers were determined by using different assay conditions for measuring serum and nasal wash titers (4). Regression analysis of the relationship between serum (x axis) and nasal wash (y axis) postvaccination IgG HA titers indicated that the regression equation for the inactivated-virus vaccine group was $y = -3.44 + 0.61x$ and the equation for the live-virus vaccine group was $y = -3.04 + 0.62x$. If the live-virus vaccine recipients indeed produced significantly more local IgG ELISA HA antibody than the inactivated-virus vaccine recipients, one would expect that, for any given concentration of serum IgG antibody in the live-virus vaccine recipients, a greater quantity of nasal wash antibody would be present. This would be reflected by a significant difference in the x intercept as defined by the regression equation. The almost identical regression lines for the live- and inactivated-virus vaccine recipients suggests that the mechanisms of nasal wash IgG HA antibody production were similar in the two groups. The live-virus vaccine recipients had significantly greater local IgA antibody responses (4), supporting the suggestion of a greater mucosal antigenic stimulation in these vaccine recipients. Despite this greater mucosal antigenic stimulation,

TABLE 1—*Continued*

IgG2			IgG3			IgG4		
Reciprocal of $\log_2$ titer (mean ± SEM)		% With fourfold or greater increase	Reciprocal of $\log_2$ titer (mean ± SEM)		% With fourfold or greater increase	Reciprocal of $\log_2$ titer (mean ± SEM)		% With fourfold or greater increase
Prevaccination	Postvaccination		Prevaccination	Postvaccination		Prevaccination	Postvaccination	
3.8 ± 0.2	5.3 ± 0.5	46	4.3 ± 0.3	7.8 ± 0.5	88	3.3 ± 0	3.7 ± 0.2	12
3.8 ± 0.2	3.9 ± 0.3	11	3.3 ± 0	4.3 ± 0.3	39	3.3 ± 0	3.3 ± 0	0
0.7 ± 0.2	1.3 ± 0.2	14	0.7 ± 0.2	1.5 ± 0.2	4	0.7 ± 0.2	1.2 ± 0.2	4
0.9 ± 0.1	1.1 ± 0.2	6	0.9 ± 0.1	1.0 ± 0.1	0	0.9 ± 0.1	1.1 ± 0.1	0

similar patterns of local IgG antibody response were observed in the two groups.

If nasal wash IgG antibody is indeed a transudate from serum, it should be possible to estimate the gradient across which the antibody diffuses. A regression analysis including prevaccination titers as well as postvaccination titers was chosen so the analysis would include the full spectrum of ELISA titers. The regression equation for the combined live- and inactivated-virus groups was $y = 6.64 + 0.79x$. Solving this regression equation for the y intercept indicates that a serum reciprocal $\log_2$ IgG HA titer of 8.4 (approximately 1:350) is required before nasal wash IgG HA antibody becomes detectable.

Both IgA and IgM are known to be actively secreted from mucosal surfaces (1), but local secretion has not been shown consistently for IgG (B. R. Murphy, M. L. Clements, P. R. Johnson, and P. F. Wright, *in* W. Strober, ed., *Mucosal Immunity and Infection at Mucosal Surfaces*, in press). Whereas some studies have demonstrated mucosal production of IgG (3, 11), others have suggested a transudate from serum down a concentration gradient (4). The use of inactivated influenza virus vaccine administered parenterally and live attenuated influenza virus vaccine administered intranasally afforded the possibility of investigating again the mechanism(s) of local IgG production by the two methods of immunization. Several observations in this study support a serum transudate as the source of nasal wash IgG HA antibody. First, the ratio of nasal wash specific activity to serum specific activity was similar (approximately 1 for each group) in both inactivated-virus and live-virus vaccine recipients, which is evidence against a significant local mucosal IgG HA antibody contribution to the nasal wash IgG HA antibody. Second, the orders of magnitude of anti-HA titers of the IgG antibody subclasses in the inactivated-virus vaccine group were similar in sera and nasal washes, suggesting a common source for the serum and nasal wash IgG subclass HA antibodies. In addition, there was a significant correlation between the titers of serum and nasal wash IgG1 HA antibodies. The almost identical regression lines of the postvaccination IgG serum and nasal wash HA antibodies for the live- and inactivated-virus vaccine recipients suggests that similar mechanisms for the generation of nasal wash IgG HA antibody are operative in the two groups (i.e., passive diffusion from serum-derived antibody down a concentration gradient).

In this study, we showed that the concentration gradient of influenza HA antibody from sera to nasal washes was similar in our two vaccine groups and that a $\log_2$ titer of 8.4 (approximately 1:350) was required before HA-specific IgG was detected in nasal washes. Interestingly, it is approximately this level of serum neutralizing antibody that is associated with resistance in the lungs to another respiratory virus, respiratory syncytial virus (15). Thus, identification of the magnitude of this gradient might have general implications for the protection of mucosal surfaces by antibody present in serum.

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