

Development and Persistence of Local and Systemic Antibody Responses in Adults Given Live Attenuated or Inactivated Influenza A Virus Vaccine

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An enzyme-linked immunosorbent assay was used to measure nasal-wash and serum isotype-specific hemagglutinin antibody responses in 109 seronegative (hemagglutination-inhibiting titer $\leq 1:8$) adults vaccinated intranasally with live attenuated A/Washington/897/80 (H3N2) or A/California/10/78 (H1N1) cold-adapted (*ca*) virus or with licensed subvirion vaccine subcutaneously. Live and inactivated virus elicited serum immunoglobulin A (IgA) responses in 83 and 96% of vaccinees, respectively, and elicited serum IgG responses in 72 and 100% of vaccinees. Inactivated virus induced higher titers of serum antibodies than did live virus and stimulated a nasal-wash IgG response more often than did live virus (94 versus 59%, $P < 0.01$). In contrast, only 38% of inactivated virus vaccinees had local IgA responses compared with 83% of live virus vaccinees. Serum IgA and IgG and nasal IgG antibody titers remained elevated above prevaccination levels for at least 6 months in most of the live and inactivated vaccine responders, but the mean level of local IgA antibody induced by infection with live virus vaccine, in particular, decreased substantially. Considered in the context of previous work, the finding that live virus vaccine induced relatively long-lasting antibody in both local and serum compartments suggested that this vaccine may be a suitable alternative to inactivated vaccine for use in healthy persons.

Commercially available, inactivated influenza virus vaccine administered parenterally stimulates high levels of serum antibody and offers protection against influenza A disease, but the resistance induced is incomplete (18, 26). For this reason, there is interest in intranasally administered, live attenuated influenza A vaccines that stimulate local and systemic immunity and may provide longer-lasting, more complete protection. Live influenza A virus vaccines consisting of attenuated, cold-adapted (*ca*) reassortant viruses that possess the six internal RNA segments from the *ca* A/Ann Arbor/6/60 (H2N2) donor virus and both hemagglutinin (HA) and neuraminidase surface glycoproteins of epidemic wild-type virus are promising (2, 14, 20, 21, 24).

Data from a previous study indicated that intranasal vaccination of seronegative children with live attenuated *ca* reassortant H1N1 or H3N2 influenza A vaccine efficiently stimulated both systemic and local antibody responses (29). Local HA-specific antibody responses to live and inactivated influenza A virus vaccines have been incompletely studied in adults. The present study is the first to evaluate the magnitude and duration of the serum and nasal-wash immunoglobulin A (IgA), IgG, and IgM HA antibody responses evoked by vaccination with live or inactivated H1N1 or H3N2 influenza A virus in seronegative adult volunteers.

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MATERIALS AND METHODS

Vaccines. The influenza virus vaccines used in this study included two live attenuated, *ca* reassortant viruses and one commercial, trivalent, inactivated subvirion vaccine. The live attenuated A/Washington/897/80 (H3N2) (antigenically similar to A/Bangkok/1/79 virus) and the A/California/10/78 (H1N1) (antigenically similar to A/Brazil/11/78 virus) *ca* reassortant viruses each possessed the six internal RNA segments derived from the attenuated A/Ann Arbor/6/60 (H2N2) *ca* donor, whereas the two remaining genes (i.e., those that code for the HA and neuraminidase surface glycoproteins) were derived from their respective wild-type influenza A virus parents. The production and safety testing of these *ca* reassortant viruses and their evaluation in adults have been described (12, 13, 28). The commercial vaccine was ether extracted and contained 15 μ g each 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.).

Clinical studies. Healthy volunteers, 18 to 35 years old, without a history of influenza vaccination and who were seronegative [i.e., having a serum hemagglutination-inhibiting antibody titer of 1:8 or less to either A/Washington/897/80 (H3N2) or A/California/10/78 (H1N1) virus] were recruited from college students in Maryland. Informed consent was obtained from the study participants. The study protocol was approved by the Clinical Research Subpanel of the National Institute of Allergy and Infectious Diseases and the Human Volunteer Research Committee at the University of Maryland. Volunteers who were H1N1-seronegative were randomly assigned to receive either two 0.5-ml doses of inactivated virus vaccine subcutaneously 1 month apart or a single dose ($10^{7.8}$ 50% tissue culture infective doses) of A/California/10/78 *ca* reassortant virus

TABLE 1. Serum HA-specific IgA, IgG, and IgM antibody responses of volunteers who received either live cold-adapted or inactivated virus vaccine

Vaccine group (no. in group)	Serum ELISA anti-HA titer (reciprocal mean ^a log ₂) on specified day after vaccination											
	IgA				IgG				IgM			
	Before vaccination	14 Days	28 Days	% with increase	Before vaccination	14 Days	28 Days	% with increase	Before vaccination	14 Days	28 Days	% with increase
Live H1N1 (23)	7.5	10.0	9.6	87	11.0	13.1	12.8	65	4.6	5.1	5.0	13
Inactivated H1N1 (20) ^b	7.0	10.6	9.9	90	11.3	14.8	14.4 ^c	100 ^d	4.6	8.5	8.6	65 ^d
Live H3N2 (31)	6.5	8.9	8.5	81	10.4	12.0	12.1	77	6.3	7.1	7.0	26
Inactivated H3N2 (35)	7.0	11.9	10.8 ^c	100	10.4	14.0	14.0 ^c	100	6.4	9.6	9.2	89 ^d

^a Standard deviations of the means were similar, ranging from 1.2 to 3.4 log₂.

^b Titers for volunteers vaccinated on days 0 and 28 were also measured on day 42 and were 10.1 and 15.0 in IgA and IgG isotypes, respectively. Increases for this group were cumulative for both vaccinations.

^c Statistically significant difference between the mean antibody increase of the live and inactivated vaccine groups, $P < 0.002$ by the paired t test.

^d Statistically significant difference between the percentages of increases in the live and inactivated vaccine groups, $P < .001$ by χ^2 test.

administered intranasally. Volunteers who were H3N2 seronegative were randomly assigned to receive one dose of inactivated virus vaccine (0.5 ml) subcutaneously or $10^{7.5}$ 50% tissue culture infective doses of A/Washington/897/80 *ca* reassortant virus intranasally.

Laboratory studies. Serum and nasal-wash specimens were collected before vaccination, and the immunological methods have been described (29, 30). For detection of IgA, IgG, and IgM HA antibodies, serum and nasal-wash specimens were tested by enzyme-linked immunosorbent assay (ELISA) with specific rabbit anti-human immunoglobulin, as previously described (29). Briefly, U-bottomed polystyrene microtiter plates (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.) were used with a ladder of reagents from the solid-to-liquid phase consisting of (i) purified HA, (ii) human serum or nasal wash, (iii) rabbit anti-human IgA, IgG, or IgM, (iv) goat anti-rabbit IgG serum conjugated with alkaline phosphatase, and (v) substrate. The A/California/10/78 (H1N1)- or the A/Bangkok/1/79 (H3N2)-purified HA was used as the homologous antigen in the ELISA. The ELISA titer was expressed as the highest dilution in which the optical density of the antigen-containing well was at least twice the optical density of the respective control well lacking antigen. The ELISA nasal-wash IgA, IgM, and IgG HA antibody titers were corrected to an IgA concentration of 10 mg/100 ml, as was done previously (30). A significant serum or nasal-wash ELISA response was defined as a fourfold or greater increase in antibody titers between prevaccination and postvaccination specimens. Antibody titers were expressed as reciprocal log₂ values.

The absence of a clinical history consistent with influenza illness and any significant increase in serum or nasal-wash antibody titers of these vaccinees within 8 to 28 weeks after vaccination suggested that natural influenza infection did not occur in the volunteers during the study period.

Analysis of data. Student's t test, the chi-square test with Yates' correction, and Fisher's exact test were performed where appropriate. For correlation of serum and nasal-wash antibody titers, the Spearman rank correlation test was used.

RESULTS

Serum antibody responses. Both the frequency and the magnitude of serum IgA, IgG, and IgM HA antibody increases of the two groups of volunteers who were vaccinated intranasally with the live virus vaccines of the H1N1 or H3N2 subtype were similar (Table 1). Likewise, the fre-

quency and magnitude of serum HA antibody responses in the groups given one or two doses of inactivated virus vaccine were comparable.

Almost all vaccine recipients manifested a significant increase in ELISA serum IgA, IgG, or IgM HA antibody titers after vaccination. However, inactivated virus vaccine was more effective than live virus vaccine in inducing serum antibody responses (Table 1). When data of the two groups of live virus vaccinees were combined and compared with those of the volunteers who were vaccinated with inactivated virus, inactivated virus vaccinees had significant increases in serum IgA and IgG HA antibodies more frequently ($P < 0.001$) than did live virus vaccinees (96 versus 83% and 100 versus 72%, respectively). Also, the magnitude of serum IgA, IgG, and IgM antibody responses was significantly greater ($P < 0.001$) in inactivated virus vaccinees than in live virus vaccinees.

Nasal-wash antibody responses. The frequency and magnitude of increases in nasal-wash IgA and IgG HA antibodies of the two groups given live H1N1 or H3N2 virus vaccine were comparable (Table 2). Also, the frequency and magnitude of nasal-wash IgA and IgG HA antibody responses of the inactivated virus vaccinees were similar whether they received one or two doses. Immunization with either live or inactivated H1N1 virus vaccine elicited local IgM antibody responses more frequently ($P < 0.001$) than did vaccination with either H3N2 virus vaccine. (Presumably, the majority of initially H1N1-seronegative volunteers had not been previously infected with an H1N1 virus, whereas the initially H3N2-seronegative volunteers had been previously infected with H3N2 viruses.)

Interestingly, inactivated virus vaccine was as effective as live virus vaccine in stimulating nasal-wash HA antibody responses in the volunteers (Table 2). Overall, significant increases in IgA, IgG, or IgM antibodies were detected in postvaccination nasal washes of all but one of those who received inactivated virus vaccine. Similarly, live H1N1 virus vaccination resulted in a significant increase in at least one of these immunoglobulin class-specific HA antibodies in postvaccination nasal secretions of 22 (96%) of 23 H1N1-seronegative volunteers and in 27 (87%) of 31 H3N2-seronegative volunteers. However, the predominant isotypes of the antibody elicited by live or inactivated virus vaccine differed. Comparison of the combined data of the two groups of live virus vaccinees with those of the inactivated virus vaccinees indicated that inactivated virus vaccination stimulated nasal-wash IgG HA antibody responses more frequently than did live virus vaccination (94 versus

TABLE 2. Nasal-wash HA-specific IgA, IgG, and IgM antibody responses of volunteers who received either live cold-adapted or inactivated virus vaccine

Vaccine group (no. in group)	Nasal-wash ELISA anti-HA titer (reciprocal mean ^a log ₂) on specified day after vaccination											
	IgA				IgG				IgM			
	Before vaccination	14 Days	28 Days	% with increase	Before vaccination	14 Days	28 Days	% with increase	Before vaccination	14 Days	28 Days	% with increase
Live H1N1 (23)	4.3	7.6	7.3	87	2.8	5.5	5.6	61	0.5	3.6	2.4	61
Inactivated H1N1 (20) ^b	4.0	5.4	5.0 ^c	30 ^d	2.0	6.4	5.9 ^c	95 ^d	-0.2	2.4	1.4	65
Live H3N2 (31)	4.6	7.9	7.6	81	2.8	4.7	5.1	58	-0.1	0.2	0.04	6
Inactivated H3N2 (35)	3.8	5.6	4.6 ^c	44 ^d	1.9	6.1	5.7 ^c	94 ^d	-0.1	0.8	0.4	17

^a Standard deviations of the means were similar, ranging from 0.4 to 2.8 log₂.

^b Titers for volunteers vaccinated on days 0 and 28 were also measured on day 42 and were 5.0 and 6.5 in IgA and IgG isotypes, respectively. Increases for this group were cumulative for both vaccinations.

^c Statistically significant difference between the mean antibody increase of the live and inactivated virus vaccine groups, $P < 0.003$ by the paired t test.

^d Statistically significant difference between the percentage of increases in the live and inactivated virus vaccine groups, $p < 0.03$ by χ^2 test.

59%, respectively, $P < 0.001$). In contrast, only 21 (38%) of 55 inactivated virus vaccine recipients developed nasal-wash IgA HA antibody increases after vaccination compared with 45 (83%) of 54 live virus vaccinees ($P < 0.001$).

Relationship of serum and nasal-wash antibodies. Serum and nasal-wash HA antibody titers of 53 inactivated vaccine recipients and 54 live virus vaccinees on day 28 after vaccination were compared to determine if there was a correlation between the magnitude of these antibody populations. There was a significant positive relationship between the levels of serum and nasal-wash IgA and IgG antibodies induced by each type of vaccine (Fig. 1).

Duration of serum and nasal-wash antibodies. To study the duration of the immune response induced by live or inactivated virus vaccine, serial serum and nasal-wash specimens from vaccinees who had manifested an increase in systemic or local IgA or IgG HA antibody after vaccination were

tested. Only the data of those vaccine responders whose specimens were collected serially for at least 16 weeks after vaccination were analyzed; these data were obtained from at least 80% of the live virus vaccine responders and from 83% of the inactivated vaccine responders.

The pattern of serum and nasal-wash IgA and IgG HA mean antibody responses in the H1N1 vaccinees is shown (Fig. 2). Live or inactivated virus vaccine induced similar levels of serum IgA and IgG antibodies and nasal IgG antibody. These antibody levels peaked at 2 weeks after vaccination with live virus and 4 weeks after the second vaccination with inactivated virus. In contrast, the live virus vaccine stimulated higher levels of nasal IgA antibody than did inactivated virus vaccine; this antibody peaked at 2 weeks after vaccination. The serum IgG and IgA and nasal-wash IgG HA antibodies remained significantly elevated (with a fourfold or greater titer) above prevaccination levels for at least 28 weeks in most of the vaccinees in both immunization groups, whereas the nasal-wash IgA antibody titers remained elevated in about one-half of these vaccinees (Table 3). The levels of serum and nasal-wash IgG antibody remained stable between 2 and 28 weeks after vaccination in both vaccine groups. However, during this interval there was a statistically significant decrease in the levels of both serum and nasal-wash IgA antibodies induced by live H1N1 virus vaccination ($P < 0.0001$) and in the level of serum (but not nasal-wash) IgA antibody induced by inactivated virus vaccination ($P < 0.002$). This decrease in local IgA antibody titers was not statistically significant because of the small number of specimens tested.

The pattern of serum and nasal-wash antibody responses to live or inactivated virus vaccine in the H3N2 vaccinees is illustrated in Fig. 3. The inactivated virus vaccine induced higher levels of serum IgG and IgA antibodies and of nasal IgG antibody than did the live virus vaccine. As before, the level of nasal IgA antibody achieved by vaccination with live virus was higher than that achieved by inactivated virus vaccine. The antibody levels generally peaked at 2 weeks after vaccination. The IgG and IgA serum and nasal-wash IgG (but not IgA) antibodies remained significantly elevated above prevaccination levels in most of the vaccinees in both immunization groups for 28 weeks (Table 3). The levels of serum and nasal-wash IgG antibodies remained stable between 2 and 28 weeks postvaccination. In contrast, a significant decline in levels of both serum and nasal-wash IgA antibodies occurred between 2 and 28 weeks after vaccination with live or inactivated H3N2 vaccine ($P < 0.003$).

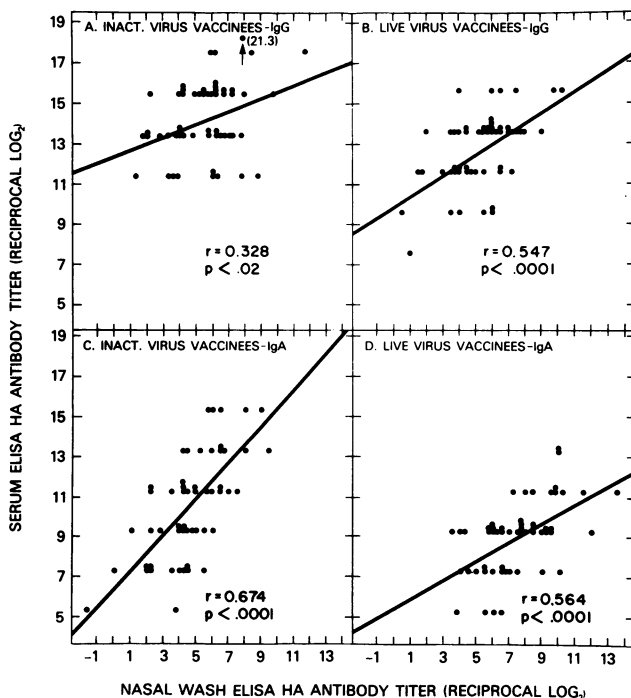


FIG. 1. Relationship between serum and nasal-wash HA antibody titers on day 28 postvaccination. Correlations were calculated by a nonparametric method (Spearman rank correlation [r]).

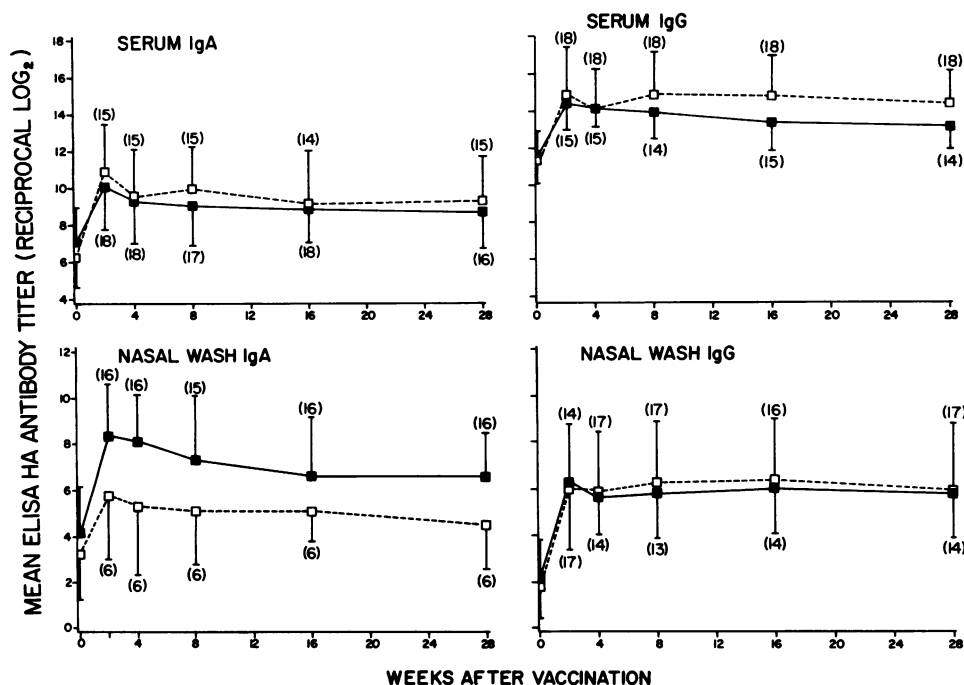


FIG. 2. Serum and nasal-wash IgA and IgG HA mean antibody responses in initially H1N1-seronegative adults after immunization with live virus (■) and inactivated virus (□) vaccines. Each square represents the mean value and each vertical bar represents one standard deviation. The values in parentheses are numbers of volunteers.

DISCUSSION

The relative role of specific antibodies in protection against influenza has not been established. However, evidence from studies in animals and humans suggests that both secretory and serum antibodies may be mediators of immunity to influenza infection, disease, or both (1, 4, 15, 16, 23, 32, 34). Thus, antibodies in both compartments should be measured when the immunogenicity of a new vaccine candidate is assessed. The present study has characterized local and systemic HA-specific antibody responses in seronegative adults immunized with live attenuated and inactivated influenza A vaccines. The immune responses to intranasally administered, live virus vaccination differed in two aspects from those observed with parenteral, inactivated virus vaccination. (i) Inactivated virus vaccine induced greater serum IgA, IgG, and IgM HA antibody responses than did live virus vaccine. (ii) Although vaccination with both types resulted in significantly increased levels of nasal-wash HA

antibodies, there was a striking difference in the predominant isotype of local antibody induced by each type of vaccine. Recipients of live virus vaccine developed IgA antibody more frequently, whereas recipients of inactivated influenza vaccine developed IgG antibody more frequently. Interestingly, these respective local antibodies remained elevated above prevaccination levels for at least 6 months (28 weeks) in more than one-half of the vaccinees who had a nasal IgG antibody response and in at least one-third of those who had a nasal IgA antibody response.

The finding that parenteral vaccination with inactivated influenza vaccine stimulates systemic antibodies in ferrets and in humans more efficiently than does intranasal vaccination with live attenuated virus has been reported previously (10, 11, 13, 16, 25, 37). In general, intranasal vaccination with attenuated *ca* influenza A vaccine virus does not produce as extensive a systemic antibody response as does intranasal inoculation of the homologous wild-type virus (14, 31). However, some live attenuated influenza viruses administered at high doses can induce levels of serum antibodies of the same magnitude as those induced by the homologous wild-type virus. For example, the A/Washington/897/80 (H3N2) and A/California/10/78 (H1N1) *ca* vaccine viruses, when administered intranasally at doses comparable to those in the present study, stimulated serum antibody levels as high as those induced by experimental infection with wild-type virus (12, 28).

Previous studies in primed and unprimed children and adults have demonstrated that the levels of antibody in serum decline significantly within 6 to 8 months after parenteral vaccination with subvirion vaccine (9, 11, 22) but not after intranasal vaccination with live attenuated virus or after natural infection (11, 18a, 36). In the current study, we found that the serum IgA and IgG HA antibody levels in

TABLE 3. Persistence of serum and nasal-wash HA antibodies induced by live cold-adapted or inactivated virus vaccines

Vaccine administered	Proportion (%) of vaccine responders with persistently elevated antibody titers ^a at 28 weeks after vaccination			
	Serum		Nasal wash	
	IgG	IgA	IgG	IgA
Live H3N2	16/22 (73)	22/24 (92)	11/18 (61)	13/23 (56)
Inactivated H3N2	27/30 (90)	28/31 (90)	20/28 (71)	6/13 (46)
Live H1N1	11/14 (79)	12/16 (75)	12/14 (86)	6/16 (38)
Inactivated H1N1	18/18 (100)	15/15 (100)	16/17 (94)	2/6 (33)

^a Persistently elevated titers were defined as a fourfold or greater increase between prevaccination and 28-week postvaccination titers.

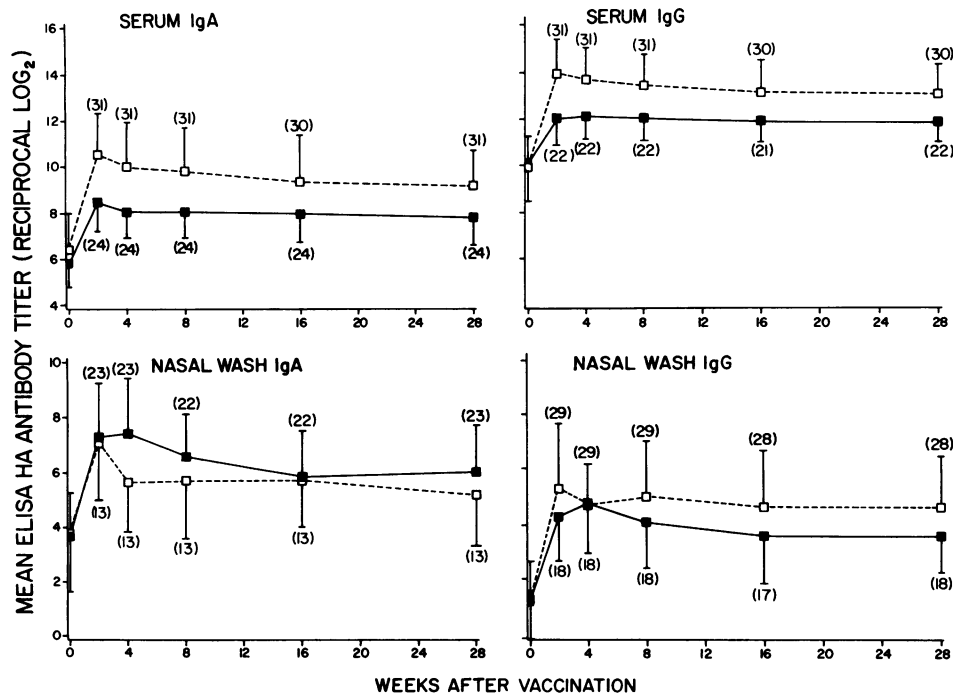


FIG. 3. Serum and nasal-wash IgA and IgG HA mean antibody responses in initially H3N2-seronegative adults after immunization with live virus (■) and inactivated virus (□) vaccines. Each square represents the mean value and each bar represents one standard deviation. The values in parentheses are numbers of volunteers.

most vaccinees remained elevated above prevaccination levels for at least 6 months after vaccination with either inactivated or live virus. The reason for the persistence of elevated serum antibody levels after inactivated virus vaccination in our study is not clear, but differences in various studies in antigenicity of the vaccines, immunological status of the vaccinees, and sensitivity of the assays used to detect antibodies may have been responsible.

Live influenza vaccines administered intranasally, like oral poliovirus vaccines, regularly elicit higher levels of local secretory IgA or neutralizing antibody than inactivated virus vaccines administered parenterally (15, 16, 18a, 25, 32, 37). In the current study, 83% of live virus recipients developed nasal IgA HA antibody responses compared with only 38% of inactivated vaccine recipients. It is interesting that parenterally administered, nonreplicating (i.e., inactivated) viruses also stimulate local antibody responses. More than 40 years ago, Francis (17) observed that subcutaneous injection of adults with influenza vaccine enhanced the inactivating capacity of their nasal secretions. Others confirmed this finding and identified this substance as neutralizing antibody, mainly polymeric IgA antibody containing secretory component (6, 19, 27, 32, 35). More recently, IgG and IgM HA-specific antibody responses to live and inactivated influenza vaccines have been measured in nasal-wash specimens (18a, 29, 37). In studies with seronegative adults, Zahradnik and co-workers (37) detected nasal IgG HA antibody increases by radioimmunoprecipitation assay in one-third of inactivated virus recipients but in only 6% of live virus vaccinees. Johnson and his colleagues (18a) also found nasal IgG (measured by ELISA to surface glycoproteins) in one-third of children who received inactivated influenza vaccine and in 7 of 10 children vaccinated with live virus vaccine. In contrast, in our study nearly all (94%)

inactivated virus vaccinees and 59% of live virus vaccinees developed nasal IgG HA antibody responses (measured by ELISA) after vaccination. These discrepancies in local IgG responses can be explained in part by the different antibody assays, dose of virus administered, and prevaccination immunological status of the vaccinees.

Our findings of a gradual decline in secretory IgA HA-specific antibodies induced by live virus vaccination over 6 months agree with previous studies which showed that local IgA antibody resulting from vaccination is relatively short-lived (8, 19). Our study did not address the persistence of local antibodies beyond 6 months after vaccination. However, data from studies in seronegative children have indicated that nasal IgA HA antibody could be detected for 1 or more years in 47% of naturally infected subjects and in 53% of live virus vaccinees, but in only 5% of inactivated vaccine recipients (18a). Nasal IgG HA antibody persisted in 77% of naturally infected children, 53% of live virus vaccinees, and 45% of inactivated virus vaccinees (18a).

The origin of nasal-wash HA antibodies induced by live and inactivated virus vaccination is not completely known. There are at least three possibilities: (i) local production of a J chain containing IgA dimers (or IgM pentamers) which are actively transported externally by nasal epithelium possessing secretory component to which polymeric IgA and IgM bind (3, 5); (ii) local synthesis of non-J chain-containing immunoglobulin by plasma cells in the nasal mucosa followed by its passive transport toward this lumen; and (iii) passive transudation of serum-derived antibodies. In the case of intranasal vaccination with live attenuated virus, there is considerable evidence that secretory IgA and to some extent IgM and IgG are synthesized by nasal epithelial cells and actively secreted locally (3-5, 7, 8, 29). In earlier studies (7, 29) a positive correlation between the levels of

nasal and serum IgA HA antibodies in live virus vaccinees was observed, suggesting that these antibodies might be derived from the same source. Brown and colleagues (6) found that IgA HA-specific antibody in nasal-wash specimens after infection with wild-type virus was composed almost entirely of polymeric (11S) secretory IgA. Furthermore, the serum IgA HA antibody from these volunteers contained a higher proportion of J-chain-containing polymeric IgA with respect to monomeric IgA than total serum IgA, suggesting that serum IgA HA-specific antibody induced by live virus vaccination was of mucosal origin. The findings in the present study are consistent with the concept of a mucosally derived serum and with nasal-wash IgA antibodies in live virus vaccinees, i.e., the magnitude of their serum and nasal-wash IgA HA antibodies were related and there was a concomitant decrease in these antibody populations over 6 months.

The origin of nasal antibodies, particularly IgG HA antibody, induced by subcutaneously administered inactivated virus vaccine, is intriguing but cannot be unequivocally identified by our findings. The positive correlation coefficient between postvaccination levels of nasal and serum IgG HA antibodies in our inactivated virus vaccinees suggests that these populations of antibody are derived from the same source. The observation that levels of both serum and nasal-wash IgG HA antibodies remained stable up to 6 months after vaccination while nasal-wash IgA levels declined supports the concept that nasal IgG HA antibody is derived from serum antibody by passive transudation. This possibility requires further study.

Serum IgG influenza antibody has been shown to prevent lethal pneumonia in infected animals and to correlate with resistance to influenza illness in neonates (33, 34); however, the role of nasal-wash IgG antibody in protection against influenza has not been defined. A previous study demonstrated a relationship between resistance to influenza infection and specific IgG in nasal secretions (15). Clearly, the function of inactivated influenza vaccine-induced IgG in nasal secretions should be investigated further.

Results of earlier studies have demonstrated that, in the absence of serum antibodies, local IgA antibody is a major determinant of resistance to infection and illness in volunteers challenged with wild-type virus (13). Liew and co-workers (23) recently reported data suggesting that specific IgA in the respiratory tract induced by infection of mice with live influenza virus may be the most important immunological mediator of protection against wild-type infection. In an earlier volunteer challenge study, we showed that immunity conferred by infection with live *ca* virus vaccine was more effective against wild-type virus infection than was inactivated virus vaccine (13). Because live virus vaccine stimulated nasal IgA more efficiently than did the inactivated vaccine, it is likely that nasal IgA HA antibody plays an important role in preventing experimentally induced influenza. The gradual attrition of nasal and serum IgA antibody induced by live virus vaccination, however, may represent a waning in immunity and may result in an increased susceptibility to influenza infection and illness with time.

In summary, both live and inactivated influenza vaccines induced antibodies in the serum and respiratory tract. Although the relative protective role of systemic and local IgA and IgG antibodies has not been clearly defined, the presence of antibodies in both compartments may be important in the prevention of infection or disease. The finding that live, attenuated, cold-adapted reassortant influenza A vaccine induces relatively long-lasting local and systemic anti-

bodies suggests that this vaccine may be a suitable alternative to the current licensed inactivated vaccine for use in healthy children and adults.

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