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Secretory Immunological Response After Intranasal Inactivated Influenza A Virus Vaccinations: Evidence for Immunoglobulin A Memory

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An intranasal, inactivated trivalent influenza A vaccine containing 7 μg of A/Bangkok/1/79 (H3N2) hemagglutinin was administered to 20 children aged 1 to 6 years to assess the local and systemic immune responses to antigen delivered to the respiratory tract. Six children without prior influenza virus infection exhibited no local immune response and manifested only a minimal systemic response to the intranasal vaccine. In contrast, five individuals who were previously infected with a live attenuated influenza A H3N2 virus vaccine, although having no residual secretory antibody at the time of challenge, promptly developed a local antibody response to intranasal, inactivated antigen. Therefore, the live influenza A virus vaccine had induced memory in the local immunoglobulin A (IgA) immune system. The third group of nine children had previously been infected with wild-type H3N2 influenza virus. A majority of these children had residual local and systemic antibody at the time of challenge but they demonstrated some boosting of local IgA antibody with administration of intranasal inactivated vaccine. The competence of the secretory IgA immune system in young children in mounting primary and secondary responses to influenza antigens has important implications for approaches to prevention of influenzal illness.

Intranasal administration of both live attenuated and inactivated vaccine has been advocated as a means of achieving effective local and systemic immunity against influenza (16, 17). Animal studies have suggested that local respiratory tract immunoglobulin A (IgA)-mediated immune response is the primary effector of resistance to influenza and other respiratory viruses (1). In humans, however, and particularly in young children, the role of local immunity in resistance to influenza A virus infection is less clearly characterized.

The development of sensitive immunoglobulin class-specific antibody assays which allow accurate quantitation of total IgA and influenza hemagglutinin-specific IgA antibody has facilitated studies on the local immune response to infections with wild-type and attenuated influenza viruses (9). By using an enzyme-linked immunosorbent assay (ELISA), it recently has been demonstrated that a short-lived, locally produced anti-influenza IgA response is seen after primary infection with an intranasally administered live attenuated vaccine (8). To date, the height and duration of the IgA response after other approaches to vaccination have not been clarified nor has the presence of influenza A virus-specific local memory been established. In the present experiment, we have addressed these questions by measuring the local and systemic immune responses of 20 children who were given intranasal inactivated influenza vaccine. The children were assigned to three groups based on their previous experience with influenza: (i) no prior influenza; (ii) prior live attenuated intranasal vaccine; and (iii) prior natural infection. The data indicate that an anamnestic recall of influenza-specific IgA antibody occurred in those children with prior infection with a wild-type or attenuated influenza A virus.

MATERIALS AND METHODS

The vaccinated children were enrolled in the Vanderbilt Vaccine Clinic where they had received comprehensive medical care since birth. Regularly collected serum samples and viral cultures obtained with each respiratory illness identified all prior infections with influenza virus. Six children whose average age at the time of immunization was 23 months had never had influenza. Five children had received a live attenuated

intranasally administered vaccine, A/Alaska/6/77 (H3N2) CR-29, 10 to 18 months before inactivated intranasal challenge (17). Their average age at intranasal challenge was 52 months. Nine children had experienced natural infection with H3N2 viruses similar to A/Bangkok/1/79 or A/Texas/1/77. Eight of these children had a primary infection with A/Bangkok in December of 1980, 3 to 6 months before vaccination. Their average age at time of vaccination was 27 months. The remaining child, age 51 months at the time of the intranasal challenge, had undergone natural A/Texas infection 3 years before vaccination with a boost in antibody 2 years before vaccination.

Inactivated influenza vaccine was administered intranasally as drops in a volume of 0.5 ml. The vaccine was a commercially formulated trivalent whole virus vaccine (lot no. 2760D) containing 7 μg each of A/Brazil/11/78 (H1N1), A/Bangkok/1/79 (H3N2), and B/Singapore/222/79 prepared by Merck Sharp & Dohme, West Point, Pa. Children were contacted 24 h after vaccination to assess any local or systemic reactions. Serum was obtained before and 2 and 6 weeks after vaccination. Nasal washes were obtained before and 1, 2, and 6 weeks after vaccination and concentrated from a volume of 10 to 15 ml to approximately 1 ml with Aquacide (Calbiochem-Behring, La Jolla, Calif.).

Influenza-specific antibody was determined by ELISA, using A/Bangkok/79 H3N2 hemagglutinin purified from the A/Bangkok/1/79 X-73 virus. The hemagglutinin was adsorbed to a 96-well flat-bottomed plate followed by the sequential addition of (i) serum or nasal wash specimen; (ii) rabbit anti-human IgA, IgG, or IgM; (iii) goat anti-rabbit serum conjugated with alkaline phosphatase; and (iv) substrate as previously described (9). The ELISA titers were expressed by the positive-over-negative method in which the endpoint was the highest dilution that gave an antigen-added (positive) well a twofold or greater optical density than an antigen-free (negative) well that received a comparable dilution of serum or nasal wash.

To plot longitudinal values and determine antibody rises, ELISA end points were corrected to 1 μg of IgA and expressed as $\log_2$ values. Serum hemagglutinin-inhibiting (HAI) antibodies were determined by standard procedures, using A/Bangkok/1/79 reference antigen supplied by the Centers for Disease Control, Atlanta, Ga. Representative sera from all children were examined by HAI to determine from birth each child's prior exposure to influenza. Nasal wash neutralizing antibodies were determined by plaque reduction assay in MDCK, a continuous canine kidney line, using 50 PFU of A/Bangkok virus and a 50% reduction in plaque titer as an endpoint (6).

RESULTS

Trivalent whole virus vaccine with a total antigen content of 21 μg of hemagglutinin was administered intranasally and was well tolerated with no identifiable side effects in the 20 children. The serum and nasal wash antibody response to the H3 component of the vaccine (the only response analyzed in detail) clearly correlated with the extent and nature of prior contact with influenza H3N2 strains as described below.

No prior influenza. Two of six children with no prior influenza had a serum antibody response to a single dose of intranasal vaccine (Table 1). None had a detectable nasal wash antibody rise in the 6 weeks after vaccination (Fig. 1, Table 1). Thus, no primary local immune response was generated to inactivated influenza vaccine.

Prior intranasal live vaccine. Each of the five children who had received live CR-29 A/Alaska vaccine 10 to 18 months before the present study had prechallenge serum IgG H3 hemagglutinin ELISA antibody and four manifested a rise in either IgG or IgA serum antibody after inactivated intranasal vaccination (Table 1). However, prevaccination nasal wash ELISA antibody ti-

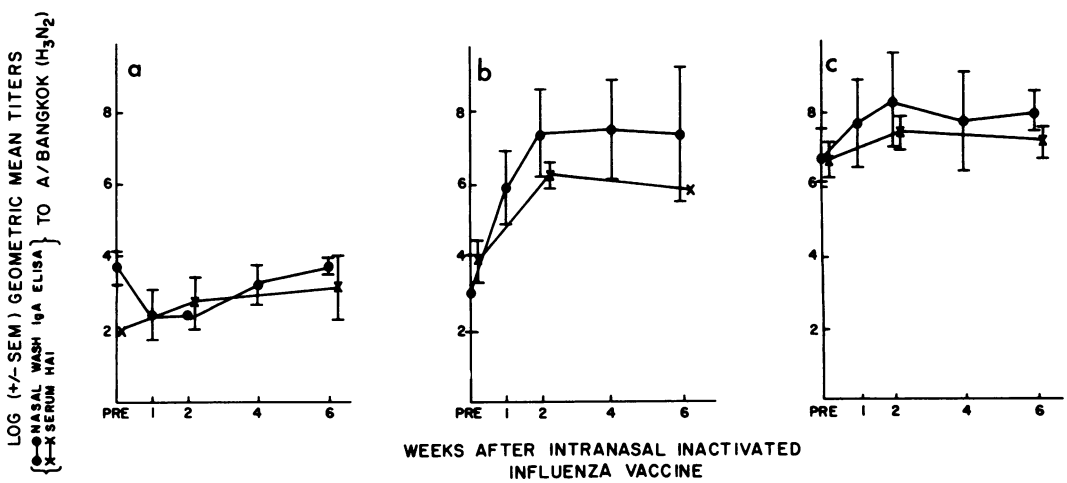

FIG. 1. Antibody response versus time for (a) children with no previous H3N2 influenza infection, (b) children who received live attenuated A/Alaska/6/77 (H3N2) CR-29, and (c) children with previous natural infection with A/Bangkok. Symbols: ●, ELISA nasal wash IgA; X, serum HAI.

TABLE 1. Children with greater than or equal to fourfold antibody responses after inactivated intranasal influenza A/Bangkok (H3N2) vaccine

Group	No.	No. with greater than or equal to fourfold antibody response			
		Serum			Nasal wash ELISA IgA
		ELISA		HAI	
		IgA	IgG		
No previous H3N2 influenza	6	0	1	2[a]	0
Previous intranasal CR-29 A/Alaska (H3N2)	5	1	4	3	5
Previous natural infection with A/Bangkok	9	3	4	2	5

[a] One child with an HAI rise without IgA or IgG ELISA rise was demonstrated to have an IgM response.

ters were similar to those with no prior influenza (Fig. 1). In contrast, nasal wash titers of the children who had previously received intranasal live vaccine promptly rose after inactivated vaccine, with four of five exhibiting a fourfold or greater rise within 1 week of vaccination and all five exhibiting such a rise in antibody levels by 2 weeks. The antibody levels achieved were comparable to those reached by children who previously had been infected by wild-type virus and were then given intranasal vaccine. The titers of IgA antibody achieved were significantly greater than those of the group with no prior influenza exposure ($P < 0.05$).

Prior wild-type influenza. Each of the nine naturally infected children had serum and nasal wash antibodies before intranasal vaccination. Rises determined by ELISA were found for either IgA or IgG class-specific serum antibody in six of nine children, although the HAI titer showed a significant (greater than or equal to fourfold) rise in only two of nine (Table 1). Nasal wash antibodies showed a greater than or equal to fourfold rise in five of nine subjects.

Correlation of nasal wash ELISA and neutralizing antibody. The local antibody responses of the three groups of children after administration of intranasal inactivated vaccine were also determined by using a conventional plaque neutralization assay. None of the 27 specimens obtained either before or after vaccination from children with no prior influenza experience manifested a neutralization titer (uncorrected to total IgA level) of greater than 8. In contrast, 14 of 25 specimens from children who had previously received live attenuated vaccine contained measurable antibody by neutralization after inactivated intranasal vaccine administration, and 29 of 41 specimens from children with prior natural infection had measurable antibody. Thus, two independent assays, IgA ELISA and neutralization, clearly demonstrated a local antibody response to inactivated intranasal vaccine in children with prior influenza, but no response in children without such experience.

Response to other vaccine components. The serum antibody responses to the H1N1 and influenza B components of the inactivated vaccine were measured only by HAI. Antibody responses to these two components of the trivalent vaccine were minimal after a single dose of inactivated vaccine by the intranasal route. Three of 20 children (1 of 12 initially seronegative, 2 of 8 initially seropositive) had an HAI response to the A/Brazil (H1N1) antigen. One of two initially seropositive children had a response to B/Singapore, whereas none of 18 initially seronegative children responded to the influenza B component.

DISCUSSION

The importance of secretory immunity in the defense against respiratory viruses has been suggested by work of Smith et al., which shows that the level of neutralizing antibody to parainfluenza type 1 in secretions is the major determinant in resistance to experimental infection of adult volunteers with wild-type virus (15). Influenza antibody can be produced in previously primed adults by administration of inactivated vaccine into the respiratory tract (J. M. Zahradnik, J. A. Kasel, R. R. Martin, H. R. Six, R. B. Couch, and T. R. Cate, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 664, 1980) and this can be protective on subsequent challenge (16). In adults without serum antibody, local IgA antibody is a major determinant in resistance to experimental challenge with wild-type influenza (7).

In children, three to five doses of parenterally administered inactivated polio vaccines did not stimulate local antibody, nor did a single parenteral dose of live attenuated rubella vaccine (10, 12). In contrast, natural infection with rubella or polio and live, intranasally administered polio and rubella vaccines regularly stimulate local respiratory tract IgA antibody detectable by radioimmunodiffusion and autoradiography (10, 12). Large doses of inactivated intranasal polio vaccine will also stimulate local secretory IgA immune response (11).

Local antibody to respiratory syncytial virus and parainfluenza viruses after natural infection in children has been detected by indirect immunofluorescence, but not by neutralizing antibody assays (18). These observations suggest a dissociation between functional antibody measured in

the plaque neutralization test and antibody detected by assays for antigen-antibody binding. In our studies, a correlation of neutralizing and ELISA antibody to influenza in secretions is reassuring that the IgA antibody measured has a functional role (8).

Further, the present study extends our understanding of the secretory immune system against influenza in several important respects. First, the local antibody response to natural infection is of considerable duration with antibody persisting in eight of eight children for at least 3 to 6 months after primary infection. Second, live attenuated vaccine administered 10 to 18 months before inactivated vaccine challenge clearly primed the secretory immune system for local memory and an anamnestic response on reexposure. Local systemic responses are not seen before 2 weeks on primary exposure (9), whereas a local response was demonstrated at 1 week on reexposure. Third, inactivated antigen given intranasally in a dose that might be expected to elicit a response if given parenterally did not elicit a strong primary serum or nasal wash response in children seronegative to the strains contained in the vaccine (3).

Studies in animals and humans certainly suggest that the local immune system is optimally primed by the local administration of antigen (2, 5). Furthermore, studies with a variety of antigens, including polio and shigella, have suggested that a live replicating antigen is superior to an inactivated preparation (4, 11). However, inactivated vaccines and various toxin preparations can still elicit local immunity (2, 5, 11, 14). Several recent studies in animals have demonstrated that a memory system exists which produces a rapid and enhanced immune response on reexposure to an antigen (4, 5, 13). Initial priming of one area of the mucosal-associated immune system can prime distal sites in the mucosal immune system (4, 14), although some dissociation may exist between the enteric and respiratory system (14).

The observations reported here in young children undergoing primary wild-type or vaccine infection with influenza with subsequent challenge with inactivated intranasal vaccine extend the domain of secretory antibody memory to the respiratory tract of humans. The stimulation of a persistent local antibody response, one with demonstrated recall, or both have been major objectives in efforts to develop live attenuated vaccines for viruses that infect mucosal surfaces. The present studies clearly advance this goal. It is possible that individuals who lack measurable influenza IgA antibody but have a primed IgA memory will respond differently to an influenza virus challenge than will virgin individuals.

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LITERATURE CITED

1. **Barber, W. H., and P. A. Small, Jr.** 1978. Local and systemic immunity to influenza infections in ferrets. Infect. Immun. **21**:221–228.
2. **Fuhrman, J. A., and J. J. Cebra.** 1981. Special features of the priming process for a secretory IgA response. J. Exp. Med. **153**:534–544.
3. **Glezen, W. P., J. A. Kasel, R. G. Webster, and L. M. Tabor.** 1977. Alternative approaches to immunization of children with inactivated influenza virus vaccines. J. Infect. Dis. **136**:S677–S682.
4. **Keren, D. F., S. E. Kern, D. H. Bauer, P. J. Scott, and P. Porter.** 1982. Direct demonstration in intestinal secretions of an IgA memory response to orally administered *Shigella flexneri* antigens. J. Immunol. **128**:475–479.
5. **Klipstein, F. A., and R. F. Engert.** 1980. Influence of route of administration on immediate and extended protection in rats immunized with *Escherichia coli* heat-labile enterotoxin. Infect. Immun. **27**:81–86.
6. **Meguro, H., J. D. Bryant, A. E. Torrence, P. F. Wright.** 1979. Canine kidney cell line for isolation of respiratory viruses. J. Clin. Microbiol. **9**:175–179.
7. **Murphy, B. R., E. G. Chalhub, S. R. Nusinoff, J. Kasel, and R. M. Chanock.** 1973. Temperature-sensitive mutants of influenza virus. III. Further characterization of the *ts*-1 (E) influenza A recombinant (H3N2) virus in man. J. Infect. Dis. **128**:479–487.
8. **Murphy, B. R., D. L. Nelson, P. F. Wright, E. L. Tierney, M. A. Phelan, and R. M. Chanock.** 1982. Secretory and systemic immunological response in children infected with live attenuated influenza A virus vaccines. Infect. Immun. **36**:1102–1108.
9. **Murphy, B. B., M. A. Phelan, D. L. Nelson, R. Yarchoan, E. L. Tierney, D. W. Alling, and R. M. Chanock.** 1981. Hemagglutinin-specific enzyme-linked immunosorbent assay for antibodies to influenza A and B viruses. J. Clin. Microbiol. **13**:554–560.
10. **Ogra, P. L., D. T. Karzon, F. Righthand, and M. MacGillvray.** 1968. Immunoglobulin response in serum and secretions after immunization with live and inactivated polio vaccine and natural infection N. Engl. J. Med. **279**:893–899.
11. **Ogra, P. L., and D. T. Karzon.** 1969. Poliovirus antibody response in serum and nasal secretions following intranasal inoculation with inactivated poliovaccine. J. Immunol. **102**:15–23.
12. **Ogra, P. L., D. Kerr-Grank, G. Umana, J. Dzierba, and D. Weintraub.** 1971. Antibody response in serum and nasopharynx after naturally acquired and vaccine induced infection with rubella virus. N. Engl. J. Med. **285**:1333–1339.
13. **Pierce, N. F.** 1978. The role of antigen form and function in the primary and secondary intestinal immune responses to cholera toxin and toxoid in rats. J. Exp. Med. **148**:195–206.
14. **Pierce, N. F., and W. C. Cray, Jr.** 1981. Cellular dissemination of priming for a mucosal immune response to cholera toxin in rats. J. Immunol. **127**:2461–2464.
15. **Smith, C. B., R. H. Purcell, J. A. Bellanti, and R. M. Chanock.** 1966. Protective effect of antibody to parainfluenza type 1 virus. N. Engl. J. Med. **275**:1145–1152.
16. **Waldman, R. H., J. J. Mann, and P. A. Small, Jr.** 1969. Immunization against influenza. J. Am. Med. Assoc. **207**:520–524.
17. **Wright, P. F., N. Okabe, K. T. McKee, Jr., H. F. Maassab, and D. T. Karzon.** 1982. Cold-adapted recombinant influenza virus vaccines in seronegative young children. J. Infect. Dis. **146**:71–79.
18. **Yanagihara, R., and K. McIntosh.** 1980. Secretory immunological response in infants and children to parainfluenza viruses types 1 and 2. Infect. Immun. **30**:23–28.