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Dose Response of A/Alaska/6/77 (H3N2) Cold-Adapted Reassortant Vaccine Virus in Adult Volunteers: Role of Local Antibody in Resistance to Infection with Vaccine Virus

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An attentuated influenza A candidate vaccine virus, derived from the A/Ann Arbor/6/60 (H2N2) cold-adapted (*ca*) donor virus and the A/Alaska/6/77 (H3N2) wild-type virus, was evaluated in adult seronegative volunteers (serum hemagglutination-inhibiting antibody titer, ≦1:8) for level of attenuation, infectivity, antigenicity, and genetic stability. Four groups with similar preinoculation mean titers of serum and nasal wash antibodies were inoculated intranasally with $10^{4.5}$, $10^{5.5}$, $10^{6.5}$, or $10^{7.5}$ 50% tissue culture infectious doses ($TCID_{50}$) of the *ca* reassortant virus, and eight other seronegative adult volunteers received the wild-type virus. Only 2 of 66 vaccinees developed fever or mild and brief systemic or upper respiratory tract illness or both. Both volunteers with vaccine-related reactions received the highest dose ($10^{7.5}$ $TCID_{50}$) of *ca* virus, which indicates that the vaccine retains some mild reactogenicity at a high dosage. In contrast, four of eight volunteers infected with the wild-type virus became ill. Each of the 54 isolates tested retained the temperature-sensitive phenotype of the vaccine virus. Thus, the *ca* reassortant was genetically stable and attenuated at $10^{4.5}$ to $10^{7.5}$ $TCID_{50}$ for seronegative adults. The 50% human infective dose of *ca* virus was approximately $10^{5.5}$ $TCID_{50}$. Ten and one hundred 50% human infectious doses infected 73 and 83% of vaccinees, respectively, and approximately 75% developed an immunological response at these doses. The failure of the vaccine virus to infect some volunteers was correlated with the presence of pre-inoculation nasal wash immunoglobulin A hemagglutinin antibody.

Commercially available inactivated influenza virus vaccines offer protection against influenza illness, but the protection is incomplete and transient (6, 15, 36, 38). Because of the uncertainty about the efficacy of these vaccines, more attention has been directed toward the development of a live attenuated vaccine that will efficiently stimulate local, as well as systemic, immunity and provide broad, long-lasting protection against influenza. At present, the most promising method of rapid attenuation of new influenza viruses involves the transfer of attenuating genes from the A/Ann Arbor/6/60 (H2N2) cold-adapted (*ca*) virus to a new variant of wild-type influenza A virus (11, 12, 14, 20, 25, 41). When gene reassortment is carried out at 25°C, and progeny are selected in the presence of antibodies to the A/Ann Arbor/6/60 parent, the reassortant virus usually receives six internal genes of the *ca* donor and the hemagglutinin and neuraminidase surface glycoproteins from the wild-type parent (4, 8). In general, tests in animals and humans have shown that *ca* reassortants possessing all six transferable genes from the A/Ann Arbor/6/60 *ca* donor virus exhibit satisfactory attenuation, antigenicity, nontransmissibility, and genetic stability after virus replication in vivo (5, 7, 10, 12–14, 17, 20, 22, 23, 25, 32, 40, 41). Recent studies in doubly seronegative children and in adults immunized with A/Alaska/6/77 (H3N2) or A/Hong Kong/123/77 (H1N1) *ca* reassortant indicated that the six transferable genes from the *ca* donor reproducibly attenuated both subtypes of influenza A virus and that these genetically stable reassortant vaccine viruses were safe, even in fully susceptible individuals (10, 23, 25, 41). These promising results prompted us to continue our evaluation of these influenza A reassortants as candidate vaccine strains.

Since most of the studies conducted with *ca* vaccine viruses have utilized $10^{6.0}$ to $10^{7.5}$ 50% tissue culture infectious doses ($TCID_{50}$), the relationship between the dose of *ca* vaccine

virus and its level of attenuation and antigenicity in seronegative individuals has not been clearly determined. Recently, the A/Alaska/6/77 *ca* reassortant virus was shown to be safe and antigenic in seronegative individuals at a dose of $10^{7.5}$ $TCID_{50}$/volunteer (20). The present study was conducted to further characterize the level of virus shedding and the immune response of seronegative adult volunteers to different quantities of the A/Alaska/6/77 (H3N2) *ca* reassortant virus. This study also provided us the opportunity to evaluate the relationships between preinoculation immunoglobulin A (IgA) and IgG anti-hemagglutinin (anti-HA) antibodies in nasal secretions or serum or both and resistance to infection with the *ca* vaccine virus.

MATERIALS AND METHODS

Viruses. The production of the *ca* reassortant virus, CR29 clone 2, from the A/Ann Arbor/6/60 (H2N2) *ca* donor and cloned A/Alaska/6/77 (H3N2) wild-type virus has been described elsewhere (4). The characterization, preparation, and safety testing of the cloned wild-type and *ca* reassortant A/Alaska/6/77 viruses administered to volunteers were performed as described previously (9, 18, 20–22). Data obtained from volunteers who received the wild-type virus or the *ca* reassortant virus at a dose of $10^{7.5}$ $TCID_{50}$ were reported previously and are included here for the purpose of comparison (20, 24, 26). The same lot of vaccine was used in the current and previous studies to insure the comparability of the different groups of vaccines. The previous study was extended here by characterizing the serum and nasal-wash enzyme-linked immunosorbent assay (ELISA) IgA and IgG antibody responses.

Clinical studies. Adult volunteers (mean age, 24.0 ± 0.3 years) who had a serum hemagglutination-inhibiting (HAI) antibody titer of 1:8 or less were recruited with informed consent from the community and from students and employees at the University of Maryland in Baltimore. The wild-type virus ($10^{4.2}$ $TCID_{50}$) and the reassortant *ca* virus ($10^{7.5}$ $TCID_{50}$) were evaluated in isolation facilities at the Center for Vaccine Development of the University of Maryland School of Medicine, Baltimore, as previously reported (20). After the *ca* reassortant virus was shown to be safe at a dose of $10^{7.5}$ $TCID_{50}$, 42 seronegative volunteers were randomly assigned to receive $10^{6.5}$, $10^{5.5}$, or $10^{4.5}$ $TCID_{50}$ of the attenuated virus intranasally as outpatients. Four additional volunteers received $10^{3.5}$ $TCID_{50}$ of the vaccine virus; none became infected. The data collected from this group will not be discussed further since the number of volunteers receiving this dose was too small to permit us to draw any meaningful conclusions.

The methods for the intranasal administration of virus, observation of clinical response, collection of pre- and post-inoculation serum and nasal-wash specimens, quantitation of virus, determination of the temperature-sensitive phenotype of virus isolates, measurement of serum HAI and neuraminidase-inhibiting (NI) antibodies, and detection of serum and nasal-wash IgA and IgG anti-HA antibodies by ELISA have been described elsewhere (20, 21, 23, 24, 37). Paired sera from volunteers who did not develop a serum HAI, serum NI, or nasal-wash ELISA IgA anti-HA antibody response have been tested by ELISA for IgG antibodies against inactivated whole influenza A/Texas/X-47/77 (H3N2) vaccine virus (26). A volunteer was considered infected if viral shedding or a rise in serum or nasal-wash antibodies or both were detected.

All procedures and observations in the outpatient study were performed as described previously for the inpatient studies (20), except for the following modifications: volunteers recorded their own temperature and pulse rate at home four times a day for 4 days; and volunteers were examined, and nasal-wash specimens

TABLE 1. Response of seronegative volunteers to A/Alaska/6/77 (H3N2) *ca* reassortant (CR29) or wild-type virus

A/Alaska/6/77 virus administered	Dose ($TCID_{50}$)	No. of volunteers	% Infected[a]	Virus shedding			% with indicated illness[d]			
				% Shedding	Avg duration (days ± SE)[b]	Peak mean log_{10} titer (± SE)[b,c]	Febrile (37.8°C) or systemic or both	Upper respiratory tract	Lower respiratory tract	Any illness
Wild type CR29	$10^{4.2}$	8	100	100	4.9 ± 0.9	4.5 ± 0.6	38	50	25	50
	$10^{7.5}$	24	83	46	0.9 ± 0.2	1.0 ± 0.2	10	5	0	10
	$10^{6.5}$	15	73	53	2.1 ± 0.6	2.2 ± 0.5	0	0	0	0
	$10^{5.5}$	15	53	20	1.1 ± 0.6	1.3 ± 0.5	0	0	0	0
	$10^{4.5}$	12	25	17	2.0 ± 1.0	2.5 ± 1.1	0	0	0	0

[a] Evidence of virus shedding or rise in antibody titer or both.

[b] Data from each infected volunteer were used for the calculations. Each vaccinee was tested for 4 to 7 days, and volunteers who received the wild-type virus were tested for 10 days.

[c] The amount of virus in the nasal-wash specimen from each volunteer was determined, and the peak titers from the volunteers who shed virus were averaged.

[d] Volunteers who became ill had evidence of infection. Systemic illness was defined as the occurrence of myalgias or chills and sweats or both. Upper respiratory tract illness was defined as an illness observed on 2 consecutive days that consisted of one or both of the following: (i) rhinitis—the development of rhinorrhea or (ii) pharyngitis—the occurrence of pharyngeal erythema and discomfort. The presence of a constant, dry, hacking cough on 2 consecutive days signified a lower respiratory tract illness.

TABLE 2. Antibody response of seronegative volunteers to A/Alaska/6/77 (H3N2) *ca* reassortant (CR29) or wild-type virus

A/Alaska/6/77 virus administered	Dose ($TCID_{50}$)	No. of volunteers	% Infected[a]	Serum antibody response[b]		
				HAI antibody		
				Reciprocal of mean $\log_2$ titer (±SE)[c]		% with fourfold or greater rise in antibody
				Pre-inoculation	Post-inoculation	
Wild type	$10^{4.2}$	8	100	1.5 ± 0.3	3.8 ± 0.7	75
CR29	$10^{7.5}$	24	83	1.5 ± 0.1	2.9 ± 0.3	50
	$10^{6.5}$	15	73	1.8 ± 0.1	3.1 ± 0.4	40
	$10^{5.5}$	15	53	1.5 ± 0.2	2.1 ± 0.3	20
	$10^{4.5}$	12	25	1.3 ± 0.1	1.8 ± 0.3	8

[a] Evidence of virus shedding or rise in antibody titers or both.

[b] All serum pairs were tested for HAI antibody. Serum pairs from individuals without an HAI rise were tested in ELISA with goat anti-human IgG antiserum. Serum HAI and NI antibodies were tested with ^{H}A/Texas/77 ^{N}Equi or ^{H}Equi-1 ^{N}A/Texas/77 reassortant virus, respectively, as antigens. Nasal-wash ELISA IgA HA antibodies were tested with purified HA antigen of A/Alaska/77 virus.

[c] Data from each volunteer were used for the calculations.

[d] Insufficient quantities of nasal-wash specimens were available to test. NT, Not tested.

[e] Nasal-wash ELISA IgA HA antibodies were tested in paired specimens from 22 of 24 volunteers.

for virus isolation were obtained daily for 4 successive days (instead of 7 to 10) after inoculation.

Fever was defined as an oral temperature greater than or equal to 37.8°C. A volunteer was considered ill if he or she developed fever or had symptoms and physical findings consistent with an influenzal illness on 2 consecutive days after inoculation. Influenza-like illnesses were categorized by the following criteria: systemic illness—the occurrence of myalgias or chills and sweats or both; upper respiratory tract illness—the development of coryza (rhinorrhea) or pharyngitis (pharyngeal erythema and discomfort) or both; and lower respiratory tract illness—the presence of a persistent, dry, hacking cough.

RESULTS

Response of volunteers to A/Alaska/6/77 *ca* reassortant or wild-type virus. The clinical, virological, and immunological responses of adult volunteers to A/Alaska/6/77 *ca* reassortant or wild-type viruses are shown in Tables 1 and 2. Eight volunteers received $10^{4.2}$ $TCID_{50}$ of the cloned wild-type virus, and 66 individuals received one of four doses ($10^{7.5}$, $10^{6.5}$, $10^{5.5}$, or $10^{4.5}$ $TCID_{50}$) of the A/Alaska/77 *ca* virus. Each of the five groups of volunteers had a comparable mean pre-inoculation serum HAI antibody titer, whereas the mean pre-inoculation serum NI antibody titer of the volunteers who received the wild-type virus was higher than that of the vaccinees. Each group of vaccinees had similar serum HAI, NI, and nasal-wash ELISA IgA anti-HA antibody titers before inoculation.

The wild-type virus infected each recipient. Four of these eight volunteers (50%) developed coryza or pharyngitis which lasted for 2 to 6 days; three of these individuals had accompanying fever or systemic illness or both. In contrast, the *ca* reassortant caused illness in only 2 of 42 infected vaccinees (5%); both illnesses occurred in the group of 24 volunteers who received the highest dose ($10^{7.5}$ $TCID_{50}$) of vaccine virus. One vaccinee who shed virus and had an HAI seroresponse had coryza and myalgias lasting for 2 days. The other ill vaccinee experienced a febrile (37.9°C) systemic illness that began 4 days after inoculation and lasted only 1 day. He developed a 16-fold rise in nasal-wash ELISA IgA anti-HA antibodies but failed to shed virus or manifest a rise in serum HAI, NI, or ELISA IgG antibodies to inactivated whole virus vaccine.

Vaccinees shed significantly less virus over a shorter interval than did the recipients of the wild-type virus ($P < 0.01$ and $P < 0.05$, respectively, Student's t test). Significantly, each of the 54 isolates recovered from the vaccinees and tested for plaque formation at 34 and 39°C on Madin-Darby canine kidney cell cultures retained the temperature-sensitive phenotype. In addition, each of 16 isolates tested retained the *ca* phenotype (20). These results indicate that this *ca* virus was genetically stable in adult volunteers.

The *ca* reassortant virus was less antigenic for seronegative adults than the wild-type virus (Table 2). The magnitude of serum HAI and NI antibody responses of vaccinees given the highest dose of *ca* virus was about 60% of that of individuals who received the wild-type virus.

The dose-dependent response to the A/Alaska/6/77 reassortant virus is shown in Fig. 1. In general, an increase in the dose of *ca* vaccine

TABLE 2—*Continued*

Serum antibody response[b]				Nasal-wash ELISA IgA anti-HA antibody response[b]			
NI antibody							
Reciprocal of mean $\log_2$ titer (±SE)[c]		% with threefold or greater rise in antibody	% with serum HAI, NI, or ELISA antibody rise	Reciprocal of mean $\log_2$ titer (±SE)[c]		% with fourfold or greater rise in antibody	% with any antibody response
Pre-inoculation	Post-inoculation			Pre-inoculation	Post-inoculation		
1.5 ± 0.5	3.6 ± 0.4	75	87	NT[d]	NT	NT	87
1.0 ± 0.4	2.3 ± 0.4	42	67	2.0 ± 0.5[e]	4.3 ± 0.6	55	75
0.3 ± 0.4	1.5 ± 0.6	40	73	2.0 ± 0.7	5.5 ± 0.8	60	73
0.7 ± 0.4	1.4 ± 0.4	20	40	2.3 ± 0.8	3.9 ± 0.7	33	47
1.1 ± 0.5	1.6 ± 0.5	17	25	2.8 ± 0.5	3.7 ± 0.7	8	25

virus in 10-fold increments from $10^{4.5}$ to $10^{6.5}$ $TCID_{50}$ resulted in a graded increase in virus infectivity. Most infections with the *ca* reassortant virus were detected by two or more of the assays depicted in Fig. 1. One infection, however, was detected only by a rise in serum ELISA IgG antibodies to inactivated whole virus vaccine. The 50% human infectious dose (HID_{50}) was approximately $10^{5.5}$ $TCID_{50}$ for the *ca* reassortant virus, whereas 73 and 83% infectivity was achieved by 10- and 100-fold-higher doses, respectively. The two highest doses of *ca* virus evoked the highest mean titers of serum HAI, NI and nasal-wash IgA anti-HA antibodies.

Role of IgA anti-HA antibodies in resistance to infection with vaccine virus. Some vaccinees were infected with 10 ($10^{6.5}$ $TCID_{50}$) or 100 ($10^{7.5}$ $TCID_{50}$) HID_{50}. Since nasal-wash antibodies have been correlated with resistance to infection with other viruses that infect the respiratory tract, we sought to examine their possible role in preventing infection with the attenuated vaccine virus. This was examined by an analysis of the immunological status of the volunteers before inoculation with 10 or 100 HID_{50} of *ca* vaccine virus. The proportion of volunteers with detectable pre-inoculation nasal-wash IgA anti-HA antibodies who resisted infection with *ca* virus

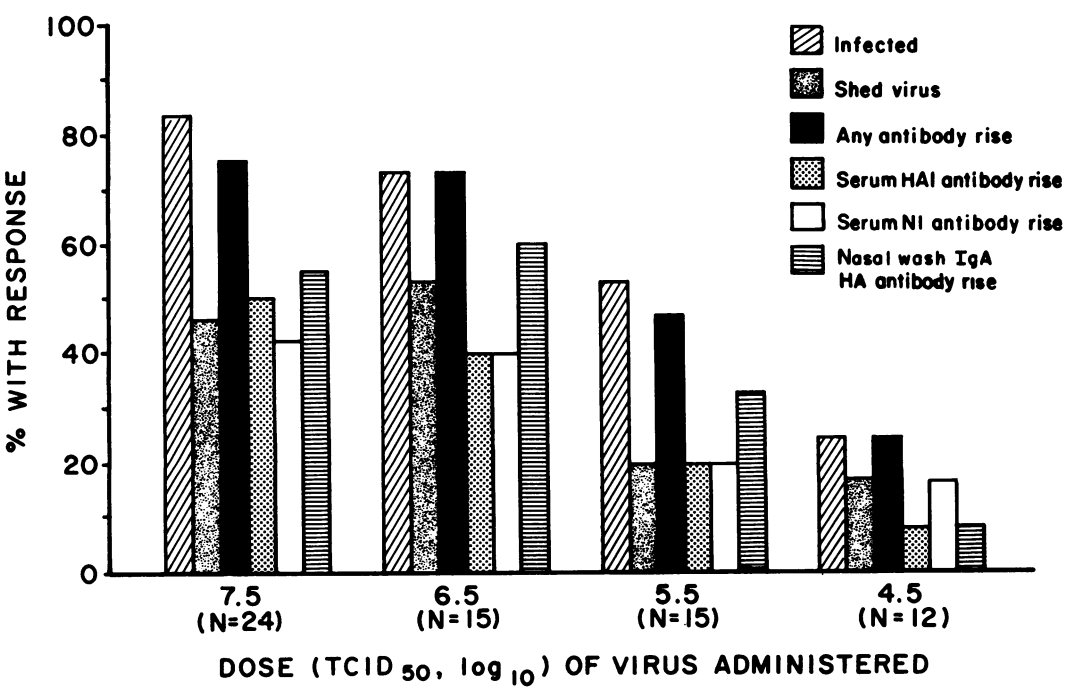

FIG. 1. Dose response to influenza A/Alaska/6/77 *ca* reassortant vaccine virus. Seronegative adult volunteers were inoculated intranasally with $10^{4.5}$ to $10^{7.5}$ $TCID_{50}$ of *ca* vaccine.

was significantly greater than that of those who lacked nasal-wash IgA anti-HA antibodies (Table 3). Similarly, the geometric mean titer of local IgA antibodies before inoculation was significantly higher in those who resisted infection than in those who became infected with *ca* virus (Table 4). In contrast, neither the presence nor the magnitude of pre-inoculation IgA or IgG anti-HA antibodies in serum was correlated with resistance to infection with *ca* virus (Tables 3 and 4).

DISCUSSION

The present study characterized the response of seronegative adults to different doses of a *ca* reassortant influenza vaccine virus that received its attenuating genes from the A/Ann Arbor/6/60 *ca* donor virus. In the dosage range of $10^{4.5}$ to $10^{6.5}$ $TCID_{50}$, the A/Alaska/6/77 (H3N2) *ca* reassortant was fully attenuated in 42 adults, 22 of whom were infected. A few vaccine-related reactions, however, were observed in the group of adults who received $10^{7.5}$ $TCID_{50}$ of the vaccine virus. At this dose, 10% of 20 infected vaccinees had brief, mild febrile or systemic reactions, and one of the ill vaccinees had rhinorrhea. Nevertheless, these reactions were less severe and occurred less often with the vaccine virus than during infection with the A/Alaska/77 wild-type virus (20). The wild-type virus induced a febrile or systemic reaction in three of eight (38%) volunteers and upper respiratory symptoms in four (50%).

Previous studies have demonstrated that A/Victoria/3/75 and A/Queensland/6/72 H3N2 *ca* reassortants are attenuated for seronegative adults at a dose of less than $10^{7.5}$ $TCID_{50}$ (5, 7, 12, 17, 22). However, vaccine-related reactogenicity comparable to that associated with the *ca* A/Alaska/77 reassortant in this study was observed in 0 to 13% of adults infected with $10^{7.5}$ to $10^{7.7}$ $TCID_{50}$ of an A/Victoria/75 *ca* reassortant or another A/Alaska/6/77 *ca* reassortant (17, 20, 22). A *ca* A/Scotland/840/74 (H3N2) reassortant also caused febrile or systemic reactions in 10% of vaccinees who received $10^{7.5}$ $TCID_{50}$, and a 10-fold-higher dose was associated with such reactions in 33% of the recipients (22). Several studies have shown that *ca* vaccine viruses of the H1N1 subtype are clearly attenuated for seronegative adults at doses ranging from $10^{5.0}$ to $10^{7.4}$ $TCID_{50}$ and also for doubly seronegative children at $10^{6.4}$ $TCID_{50}$ (25, 40, 41). There is also some evidence that the H1N1 *ca* viruses may retain some reactogenicity at higher doses (25, 32). Three of 24 (13%) seronegative adults who received $10^{7.5}$ $TCID_{50}$ of A/Hong Kong/123/77 (H1N1) experienced mild upper respiratory symptoms (25). Also, in a recent study by Reeve et al., mild systemic reactions (headache, fatigue, muscular pains, or weakness) occurred in 8 of 25 (32%) adults who received $10^{7.4}$ 50% egg infective doses of an A/USSR/92/77 (H1N1) *ca* virus; 8 of 14 (57%) adults administered $10^{8.6}$ 50% egg infective doses of this virus became ill (32). In both instances, the frequency of reactions was greater than in volunteers administered a placebo. The factors responsible for the residual low-level reactogenicity of the *ca* viruses at high doses are not well understood. Reactogenicity appears to be a function of the dose, but not the genotype, since similar reactogenicity has been observed with H1N1 and H3N2 *ca* reassortants that possessed five or six internal genes derived from the A/Ann Arbor/60 *ca* parent (17, 20, 22, 25, 32).

The A/Alaska/6/77 (H3N2) *ca* reassortant virus exhibited a pattern of viral replication in adults characteristic of other attenuated *ca* reassortant viruses, i.e., a small amount of virus shed for a short period (17, 20, 22, 25, 32, 40). The proportion of volunteers who shed vaccine virus appeared to be dose related: approximately half of the vaccinees administered A/Hong Kong/77, A/Victoria/75, or A/Alaska/77 *ca* virus at a dose between $10^{6.5}$ and $10^{7.5}$ $TCID_{50}$ shed virus, whereas fewer of the vaccinees administered a lower dose shed virus (17, 20, 22, 25). In this study, as well as in most of the previous

TABLE 3. Relation between pre-inoculation nasal-wash or serum ELISA anti-HA antibody titer and resistance to infection with influenza A/Alaska/6/77 *ca* vaccine virus

Response to vaccine[a]	No. of volunteers tested	No. with pre-inoculation titer (reciprocal)[b]					
		Serum				Nasal wash IgA	
		IgG		IgA			
		≤7.2	>7.2	≤5.2	>5.2	≤1.5	>1.5
Not infected	8	1	7	1	7	0	8[c]
Infected	29	7	22	5	24	17	12[c]

[a] Virus recovery or antibody response or both signified infection.
[b] Log_2 titer.
[c] $P < 0.01$ by the Fisher exact test (two tail).

TABLE 4. Relation between pre-inoculation nasal-wash or serum ELISA anti-HA antibody titer and resistance to infection with influenza A/Alaska/6/77 *ca* vaccine virus

Response to vaccine[a]	No. of volunteers tested	Mean pre-inoculation antibody titer (reciprocal)[b]		
		Serum		Nasal wash IgA
		IgG	IgA	
Not infected	8	8.7 ± 0.6	6.4 ± 0.6	4.0 ± 0.5[c]
Infected	29	8.1 ± 0.3	6.6 ± 0.3	1.5 ± 0.5[c]

[a] Virus recovery or antibody response or both signified infection.

[b] Log_2 titer plus or minus the standard error.

[c] $P < 0.005$ by the Wilcoxon rank sum test (two tail).

studies, the *ca* reassortant viruses recovered from adults have retained their temperature-sensitive and *ca* phenotypes (5, 17, 20, 22, 25, 32, 40). Although there was evidence of the genetic instability of the *ca* phenotype in some of the isolates recovered from vaccinees infected with the A/Scotland/74 *ca* reassortant that possessed five genes derived from the *ca* parent, thus far, the reassortant viruses, with six internal genes from the *ca* parent, appear to be genetically stable after replication in adults (5, 17, 20, 22, 25, 32, 40).

The HID_{50} for the A/Alaska/77 *ca* virus in seronegative adults was about $10^{5.5}$ $TCID_{50}$. This *ca* virus was only slightly less infectious for seronegative adults than was the A/Hong Kong/77 (H1N1) *ca* virus that had a HID_{50} of $10^{5.0}$ $TCID_{50}$ in vaccinees who had not been previously infected with an H1N1 virus (25). At $10^{7.5}$ $TCID_{50}$, the A/Hong Kong/77 (H1N1) *ca* virus induced a seroresponse in all volunteers, whereas a comparable dose of the A/Alaska/77 (H3N2) *ca* reassortant infected 83%, and 75% developed an immunological response (25). As discussed below, some of the seronegative volunteers vaccinated with the H3N2 *ca* virus in the present study failed to become infected with vaccine virus, presumably because they had immunity from previous natural infection with a related H3N2 virus.

The relative roles of serum antibodies and local antibodies in protection against influenza A infection and illness in humans have not been clearly defined. The presence of specific secretory IgA antibodies or local neutralizing antibodies has been correlated with resistance to certain viral infections, including influenza (16, 19, 28, 29, 33–35, 39). In ferrets and mice, several investigators have found that local antibodies, not serum HAI antibodies, prevented influenza infection, whereas serum antibodies prevented viral pneumonia and appeared to enhance recovery from infection (1, 31, 33, 34). Observations made during volunteer studies indicated that nasal-wash neutralizing antibodies, presumably of the IgA class, in the absence of detectable serum HAI or NI antibodies, were associated with resistance to influenza A wild-type virus infection and illness (19). In the present study, we found that the presence of nasal-wash IgA antibodies correlated with resistance to infection with a *ca* vaccine virus. This relationship supports the concept that secretory IgA antibodies are an important mediator of immunity to influenza virus infection in humans. Our findings differ from those of Couch et al., who observed that the presence of serum antibodies most consistently correlated with resistance to infection with influenza A virus (3). The reasons for this difference are unclear. Individuals who lacked serum antibodies but who possessed local antibodies were underrepresented in their study. It was previously found that such a group of individuals were as resistant to challenge with wild-type virus as were individuals who possessed antibodies in both the local and systemic compartments (19). Evidence that serum IgG antibodies probably also contribute to resistance to influenza illness in humans has recently been obtained (30). A correlation of resistance to influenzal illness with the level of transplacentally acquired antibodies in neonates suggested that serum antibodies alone may confer protection against illness caused by influenza virus (30).

Since both local and serum antibodies appear to be mediators of immunity to influenza infection or disease or both, it is important to develop vaccines that will induce antibodies in both compartments (2). Recent evidence has shown that the intranasal vaccination of immunologically inexperienced children with live, attenuated influenza A *ca* viruses stimulated both local and systemic antibody responses (23). In the present dose-response study, A/Alaska/77 (H3N2) *ca* vaccine at a dose of $10^{6.5}$ to $10^{7.5}$ $TCID_{50}$ induced a high titer of nasal-wash and serum antibodies in adults, each of whom had some prior experience with H3N2 antigens. It is not possible to determine from these limited studies the dose required to protect against influenza illness. A dose greater than $10^{6.5}$ $TCID_{50}$ may be necessary in adults to stimulate satisfactory local and circulating antibody responses; however, the advantage of greater antigenicity must be weighed against the possibility of greater reactogenicity of the vaccine at a dose greater than $10^{6.5}$ $TCID_{50}$. Studies are under way to determine the optimal *ca* vaccine dose for the protection of adults against wild-type influenza A virus and to further elucidate the role that local and systemic antibodies induced by these

ca vaccines play in resistance to wild-type influenza virus.

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