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Comparison by Studies in Squirrel Monkeys, Chimpanzees, and Adult Humans of Avian-Human Influenza A Virus Reassortants Derived from Different Avian Influenza Virus Donors

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We evaluated the abilities of three different avian influenza A viruses to attenuate the wild-type human influenza A/Korea/1/82 (H3N2) virus in squirrel monkeys, chimpanzees, and adult seronegative human volunteers. Two of these, avian influenza A/Mallard/NY/78 and A/Mallard/Alberta/76 viruses, appeared to be satisfactory donors of attenuating genes for the production of live influenza A reassortant virus vaccines for human use because the reassortants exhibited an acceptable balance between attenuation and immunogenicity.

Previously we observed that a group of 10 avian influenza A viruses varied in their level of replication in the lower respiratory tracts of squirrel monkeys (8). To determine whether the degree of restriction of replication of an avian influenza A virus in the lower respiratory tracts of squirrel monkeys was predictive of the degrees of restriction of replication of avian-human influenza A reassortant viruses in humans, we compared the abilities of avian influenza viruses representative of the entire spectrum of replication in monkeys to attenuate the wild-type human influenza A/Korea/1/82(H3N2) virus. Avian influenza A/Mallard/NY/6750/78(H2N2), A/Pintail/Alberta/119/79, and A/Mallard/Alberta/88/76(H3N8) viruses, which replicated to low, intermediate, or moderately high levels, respectively, in the lower respiratory tract of squirrel monkeys, were used as donor strains. Avian-human influenza A reassortant viruses derived from mating each of the three avian strains with the wild-type human influenza A/Korea/82 virus were evaluated first in monkeys and subsequently in seronegative volunteers for their safety, levels of replication, and immunogenicity.

The avian-human influenza reassortant viruses were selected and plaque purified as previously described (4, 8, 11). Each contained RNA segments coding for the hemagglutinin and neuraminidase surface glycoproteins from the wild-type human virus parent, whereas the internal genes of each reassortant virus were derived from its respective avian influenza virus parent. The reassortant viruses and their avian influenza virus parents produced plaques efficiently at temperatures (41 to 42°C) restrictive for wild-type human influenza A viruses. The final viral suspensions administered to humans were grown in the allantoic cavities of specific-pathogen-free eggs and tested for adventitious agents as previously described (6).

An inoculum of 10^7 50% tissue culture infective doses ($TCID_{50}$) of wild-type or avian-human influenza A reassortant virus was administered intratracheally to squirrel monkeys as previously described (8). The three influenza A reassortant viruses exhibited degrees of restriction of replication in the upper respiratory tracts of squirrel monkeys similar to that of wild-type human influenza A virus (Table 1). However, in lower respiratory tracts, the level of replication of the wild-type human influenza A/Korea/82 virus was lower than we had previously observed for H3N2 subtype viruses (2), and this precluded determination of the relative degrees of restriction of replication of the avian-human reassortant viruses in lower respiratory tracts.

Because the avian influenza A/Mallard/Alberta/76 virus was more virulent in squirrel monkeys than either previously evaluated avian donor virus and because the poor replication of the wild-type human influenza A virus in the tracheas of squirrel monkeys prevented full assessment of the potential virulence of the current reassortant viruses, we evaluated the wild-type virus and reassortant viruses derived from the most and least virulent avian virus donors (influenza A/Mallard/Alberta/76 and A/Mallard/NY/78 viruses, respectively) in chimpanzees as previously described (M. H. Snyder, W. T. London, E. L. Tierney, H. F. Maassab, and B. R. Murphy, J. Infect. Dis., in press). The avian-human reassortant viruses infected each animal but were significantly restricted in replication in both upper and lower respiratory tracts compared with the wild-type human influenza A virus. Replication of the avian-human influenza A reassortant viruses was not detected in the lower respiratory tracts of chimpanzees despite intratracheal administration of virus. In contrast, a high level of viral replication was exhibited in the lower respiratory tracts of chimpanzees infected with the homologous wild-type virus under similar conditions, as reported previously (Snyder et al., in press).

Volunteer studies, virus isolation, and antibody assays were performed as previously described (1–3, 5, 7, 9–11). Each of the avian-human influenza A/Mallard/Alberta/76, A/Mallard/NY/78, and A/Pintail/Alberta/79 reassortant viruses was restricted in replication and produced less illness in seronegative (hemagglutination inhibition [HAI] $\leq$ 1:8) adult volunteers compared with the homologous wild-type virus (Table 2).

None of four volunteers who received placebos and were housed with vaccinees infected with influenza A/Mal-

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TABLE 1. Responses of squirrel monkeys and chimpanzees to avian-human influenza A reassortant or wild-type human influenza A/Korea/1/82 (H3N2) virus[a]

Test animal and avian-human influenza A reassortant or wild-type human influenza A virus administered	No. of animals	No. infected[b]	Virus shedding					
			Nasal swab			Tracheal lavage		
			No. shedding	Mean duration (days ± SE)[c]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SE)[c]	No. shedding	Mean duration (days ± SE)[c]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SE)[c]
Squirrel monkey								
A/Pintail/Alberta/79 × A/Korea	4	3	2	2.7 ± 1.8	1.6 ± 0.8	1	0.6 ± 0.5	0.8 ± 0.3
A/Mallard/NY/78 × A/Korea	4	4	1	0.3 ± 0.2	0.6 ± 0.1	4	2.0 ± 0.0	1.5 ± 0.0
A/Mallard/Alberta/76 × A/Korea[d]	4	4	2	2.3 ± 1.4	1.7 ± 0.8	4	2.0 ± 0.0	1.8 ± 0.3
A/Korea/82 wild type	12	12	12	5.0 ± 0.7	3.2 ± 0.4	11	3.6 ± 0.5	2.0 ± 0.2
Chimpanzee								
A/Mallard/NY/78 × A/Korea	2	2	1	2.0 ± 1.4	0.8 ± 0.2	0	0.0 ± 0.0	≤0.5 ± 0.0
A/Mallard/Alberta/76 × A/Korea	2	2	2	3.5 ± 1.8	1.1 ± 0.3	0	0.0 ± 0.0	≤0.5 ± 0.0
A/Korea/82 wild type[e]	2	2	2	10.0 ± 0.0	4.5 ± 0.0	2	6.0 ± 0.0	4.0 ± 0.4

[a] Squirrel monkeys and chimpanzees received 10^7 $TCID_{50}$ of virus intratracheally in a 0.5-ml inoculum. Nasopharyngeal washes were performed daily on postvaccination days 1 to 10. Tracheal lavage was performed on days 2, 4, and 6.
[b] Virus isolation, fourfold rise in HAI antibody, or both signified infection.
[c] Data from infected monkeys were used for calculations. The lowest detectable quantity of virus shedding was $10^{0.75}$ $TCID_{50}$/ml. S.E., Standard error.
[d] Shown to be safe in squirrel monkeys before use in humans as previously described (2, 4).
[e] Studies with this virus were previously reported (Snyder et al., in press).

lard/Alberta/76 reassortant virus showed evidence of influenza virus infection. Virus was not recovered from the blood or stools of the 30 vaccinees who received the avian-human influenza A/Mallard/Alberta/79 reassortant virus (three volunteers received $10^{6.0}$ $TCID_{50}$ of this virus [data not shown]). Each of 13 nasal wash isolates of the influenza A/Mallard/Alberta/76 reassortant virus and each of three nasal wash isolates of avian-human influenza A/Pintail/Alberta/79 virus retained the ability to replicate efficiently at temperatures restrictive for wild-type human influenza A virus.

The proportion of volunteers who developed local immune responses or serum HAI antibody responses after receiving either the avian-human influenza A/Mallard/Alberta/76 or A/Mallard/NY/78 reassortant virus was similar to that of volunteers who received the wild-type human influenza A virus. The systemic immune responses of volunteers who received the influenza A/Pintail/Alberta/79 × A/Korea/82 reassortant virus were significantly diminished ($P < 0.05$; Fisher exact test, two tailed) compared with those of volunteers who received the other avian-human reassortant viruses as indicated by a lower percentage of serum HAI or immunoglobulin G enzyme-linked immunosorbent assay antibody responses in infected volunteers. The local immune responses were not significantly different in the three groups of vaccinees.

In this study the avian influenza A/Mallard/NY/78 and A/Mallard/Alberta/76 viruses appeared to be acceptable donor strains for live influenza A vaccines for human use. In contrast, the avian-human influenza A/Pintail/79 reassortant virus appeared less infectious for volunteers in this study, although in previous studies the 50% human infectious dose of an avian-human influenza A/Pintail/79 × A/Washington/897/80 (H3N2) reassortant virus was only slightly less than that of an avian-human influenza A/Mallard/NY/78 × A/Washington/80 reassortant virus (2, 7).

Differences in levels of replication manifested by the three avian influenza A virus parents in the upper and lower respiratory tracts of squirrel monkeys were not observed

TABLE 2. Responses of seronegative (HAI ≤ 1:8) volunteers to avian-human influenza A reassortant or wild-type human influenza A/Korea/1/82 (H3N2) virus

Avian-human influenza A reassortant or wild-type human influenza A virus administered	Virus dose ($TCID_{50}$)	No. of volunteers	% Infected[a]	Virus shedding (nasal wash)			% with antibody response				% with illness[b]
				% Shedding	Mean duration (days ± SE)[c]	Mean peak titer (log_{10} $TCID_{50}$/ml ± SE)[c]	HAI	Serum IgG HA ELISA[d]	Nasal wash IgA HA ELISA	Any antibody response	
A/Pintail/Alberta/79 × A/Korea (lot E-227)	$10^{7.0}$	15	53	20	0.4 ± 0.2[e]	0.6 ± 0.0[e]	7	20	47	53	0
A/Mallard/NY/78 × A/Korea (11)	$10^{7.5}$	31	87	32	0.7 ± 0.2[e]	0.8 ± 0.1[e]	68	77	55	87	3
A/Mallard/Alberta/76 × A/Korea (lot E-235)	$10^{7.0}$	27	81	41	0.9 ± 0.2[e]	0.9 ± 0.1[e]	59	74	56	81	0
A/Korea/82 wild type (11)	$10^{6.2}$	14	100	100	5.5 ± 0.5	4.9 ± 0.4	71	93	57	100	50

[a] Virus isolation, antibody response, or both signified infection.
[b] Volunteers were considered ill if they developed any of the following signs or symptoms: fever (≥37.8°C); systemic illness—occurrence of myalgia, chills, and sweats; upper respiratory tract illness—rhinitis, pharyngitis, or both observed on two consecutive days; or lower respiratory tract illness—a persistent cough lasting for at least 2 days. Only volunteers with evidence of infection were considered to have influenza-related illness.
[c] Data from infected volunteers were used for calculations. The lowest detectable quantity of virus shedding was $10^{0.75}$ $TCID_{50}$/ml. S.E., Standard error.
[d] IgG, Immunoglobulin G, HA, hemagglutinin; ELISA, enzyme-linked immunosorbent assay.
[e] $P < 0.001$ (two-tailed Student t test) compared with the control group receiving wild-type virus.

when avian-human reassortant viruses were studied in humans. We were not able to demonstrate concordance between degrees of restriction of avian-human influenza A reassortant viruses in squirrel monkeys and humans.

This study provided confirmation of the safety of vaccination of seronegative adult volunteers with live avian-human influenza A reassortant viruses found previously (2, 7, 11). Despite gastrointestinal replication of parental avian influenza A viruses in birds undergoing natural infection, we found no evidence of viral replication outside the respiratory tract in 99 volunteers who received avian-human reassortant virus vaccines. Transmission of avian-human reassortant virus from infected vaccinees to contacts also has not been observed. The results of our study of chimpanzees complement clinical studies indicating the safety of these vaccines. If replication of avian-human influenza A reassortant viruses is as restricted in the lower respiratory tracts of humans as in chimpanzees, the chance of humans developing lower respiratory tract illness caused by these virus vaccines is small. Studies to evaluate the safety and immunogenicity of avian-human influenza A reassortant virus vaccines in young children are planned.

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