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Replication of avian influenza viruses in humans

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Summary. Volunteers inoculated with avian influenza viruses belonging to subtypes currently circulating in humans (H1N1 and H3N2) were largely refractory to infection. However 11 out of 40 volunteers inoculated with the avian subtypes, H4N8, H6N1, and H10N7, shed virus and had mild clinical symptoms: they did not produce a detectable antibody response. This was presumably because virus multiplication was limited and insufficient to stimulate a detectable primary immune response. Avian influenza viruses comprise hemagglutinin (HA) subtypes 1–14 and it is possible that HA genes not so far seen in humans could enter the human influenza virus gene pool through reassortment between avian and circulating human viruses.

Introduction

Avian influenza viruses, family *Orthomyxoviridae,* genus *Orthomyxovirus,* are widespread in aquatic birds, especially ducks and shorebirds [5, 7]. Of the 14 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes identified in birds, only the 3 HA-NA combinations, H1N1, H2N2, and H3N2, have infected humans in recent times. There is, however, an increasing body of evidence that the influenza viruses currently circulating in humans originated from avian reservoirs approximately 150 years ago [3]; and it is generally thought that the surface antigens of Asian-57 and Hong Kong-68 pandemic influenza viruses also arose from avian strains [8, 13, 16]. More recently it has been shown that the gene coding for the PB1 protein, likewise, had an avian origin [6]. In the present studies, avian influenza viruses with the human-like surface antigens, H1N1 and H3N2, and avian viruses antigenically dissimilar from human viruses, were given to human volunteers to compare their ability to infect and to cause clinical effects.

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Materials and methods

Viruses

Viruses used for infection are listed in Table 1 and were from the repository at St. Jude Children's Research Hospital. They were given under approved conditions at Salisbury and propagated in specific pathogen-free eggs.

Volunteers

Methods of housing and infecting volunteers have been described [2]. People aged 18–50 of either sex were inoculated with a minimum $10^{6.0}$ fifty percent egg infecting doses (EID_{50}). All had initially been screened for haemagglutination-inhibiting (HI) antibodies to the relevant test viruses and were allocated to the trials if reciprocal HI titres were 24 or less. Nasal washings were collected on 3 or 4 days post inoculation and were cultured in embryonated hens' eggs for virus recovery [1]. Virus isolations were confirmed by retesting of duplicate original nasal washings. Clinical reactions were recorded daily by methods described earlier: in brief, they were graded severe (influenza-like), moderate (respiratory and some constitutional symptoms), mild (local symptoms only), very mild (trivial discomfort), and nil [2].

Serology

Blood was collected before and 14–21 days after infection. Paired sera treated with receptor-destroying enzyme were tested simultaneously for HI antibodies to the trial viruses.

Results

As far as possible viruses used for inoculation were low passage material (Table 1). Those with H1N1 antigens related to human strains, duck/Alberta/35/76 (H1N1) and duck/Alberta/573/70 (H1N1), induced minimal clinical effects in 21 volunteers. It can, however, be assumed that many people had prior epidemiological experience of H1N1 viruses and had residual resistance. Two H3 viruses, duck/Ukraine/1/63 (H3N8) and duck/New York/6784/78 (H3N2), were given to 6 and 3 volunteers respectively: virus excretions were again undetectable but there were 2 clinical reactions and 4 HI antibody rises. Very likely priming by natural infection had boosted antibody responses and inhibited virus shedding.

Duck/Pennsylvania/486/69 (H6N1) has an avian HA and an NA subtype common to birds and humans. Anti-NA is less effective than anti-HA at preventing virus spread and, if present in the volunteers, would not prevent infection. Two out of 11 inoculated volunteers shed this virus. An attempt was made to enhance its human infectivity by passage of nasal washings to 5 more volunteers but this was unsuccessful (results not shown). No antibody responses were detected in the 2 infected volunteers presumably because the HA of duck/Penn/486/69 (H6N1) was novel and virus growth, although detectable, was insufficient to induce a primary immune response.

Three other avian influenza viruses were given to other volunteers: turkey/Wisconsin/1/66 (H9N2) to 6 people, duck/Alberta/288/78 (H4N8) to 14, and turkey/Minnesota/3/79 (H10N7) to 15. The first of these that shares the N2

Table 1. Responses of human volunteers to infection with avian influenza viruses

Virus	Surface antigens	Passage history	Virus dose (EID_{50})	Virus excretions	Clinical reactions					HI antibody rises[a]
					severe	moderate	mild	very mild	nil	
Dk/Alberta/35/76	H1N1	E2	7.5	0/10	0	1	0	3	6	0/10
Dk/Alberta/35/76	H1N1	E3[b]	7.7	0/5	0	0	0	0	5	1/5
Dk/Alberta/573/78	H1N1	E2 SW1 E2	7.5	0/6	0	0	0	0	6	0/6
Dk/Ukraine/1/63	H3N8	>E10	7.7	0/6	0	0	0	1	5	3/6
Dk/NY/6874/78	H3N2	E2	8.0	0/3	0	0	1	0	2	1/3
Dk/Alberta/33/78 (triple cloned)	H6N2	E5	9.2	0/5	0	0	0	1	4	0/5
Dk/Penn/486/69 (triple cloned)	H6N1	E7	9.0	2/11	0	0	1	2	8	0/11
Turkey/WI/1/66	H9N2	E5	8.2	0/6	0	0	0	1	6	1/6
Dk/Alberta/288/78	H4N8	E3	7.5	3/14	0	1	2	3	8	0/14
Turkey/MN/3/79	H10N7	E2	6.8	6/15	0	0	2	6	7	0/15

Clinical reactions were based on pyrexia, coryza, handkerchief count and subjective symptoms

E Egg passages; *SW* passages in pigs

EID_{50} Fifty percent egg-infecting doses

HI Hemagglutination inhibition; numerators are numbers of positive specimens, denominators are numbers of specimens tested

[a] 4-fold or greater increase of antibody titer

[b] Terminal dilution passage of E2 virus

neuraminidase with human strains was unremarkable, but the second was excreted by 3 people and the third by 6. There were again no HI rises to HA antigens wholly alien to human beings. In this connection, it may be noted that antibodies induced by primary infection of ducks and turkeys with these viruses are also of low titre or are undetectable.

Discussion

The receptor specificity of avian influenza viruses differs from that of human influenza viruses: they preferentially bind SA2,3 Gal linkages on cell surface sialyloligosaccharides [12] whereas human strains bind SA2,6 Gal linkages. The optimal temperature for replication of avian viruses is 41 °C [9] and for human viruses 37 °C. Because of this, one would not expect avian strains to multiply readily in humans. Nevertheless, Hinshaw et al. [4] have reported that avian strains do infect pigs and Scholtissek and Naylor [14] suggest that pigs might serve as a mixing vessel and as the source of at least some pandemic strains.

Our studies establish that humans can support limited replication of avian influenza viruses unrelated to human strains when inoculated with high doses. The fact that they can be infected at all is noteworthy, for it raises 2 concerns, firstly that the receptor specificity and lower optimal replication temperatures are not complete barriers to host range spread, and secondly, that there is a possibility of reassortment of an avian virus with a current human strain and of the introduction of genes from the H4–H14 subtypes into the human population.

Avian influenza viruses have been studied in squirrel monkeys and avian-human reassortants have been used as experimental live human vaccines [9, 10]: the replication of both the avian parents and of the reassortants was found to be highly restricted in mammals. Our present studies also indicate very limited replication of avian influenza viruses in humans. The very high doses used in our trials suggests that they are not readily transmitted to humans; furthermore, person-to-person transmission could not be achieved by direct inoculation of infected nasal washings.

In nature, there is no virological evidence of human infection with avian influenza viruses even among those who handle wild ducks when they are shedding high concentrations of virus. Virological and serological studies of Canadian wildlife personnel in this category have been consistently negative and in keeping with the idea that avian to human transmission is a rare event. On the other hand, antibodies to avian influenza viruses have been reported in humans in Southern China [15]; these antibodies were detected by single radial hemolysis, an assay that is insensitive to inhibitors. The highest seropositive reactions were for the H11, H6, and H4 subtypes with 15%, 12%, and 11% respectively. In contrast, serological studies of humans in Italy showed no evidence of infection with avian influenza viruses [11]. Shortridge [15] raises the possibility of frequent exposure of humans to avian influenza viruses in

Southern China as an explanation for the presence of antibodies. Confirmation of these observations by the isolation of viruses remains to be done. The present experiments are limited but establish that restricted replication of avian influenza viruses can take place in humans without the induction of detectable HI antibodies.

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