Replication of avian influenza viruses in humans

A. S. Beare1,* and R. G. Webster2

1 Clinical Research Centre, Harvard Hospital, Salisbury, Wiltshire, U.K.
2 St. Jude Children's Research Hospital, Department of Virology and Molecular Biology, Memphis, Tennessee, U.S.A.

Accepted December 12, 1990

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.

* Present address: St. Albans, Hertfordshire, U.K.
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 $10^6$ 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 (H3NS) 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