

and $P = 0.03$ (death). The corresponding values for all ethnic groups were $P = 0.03$ and $P = 0.02$.

23. To test for a significant additive effect (that is, for an advantage in having protective genotypes both at the *SDF1* locus and at one or both of the *CCR* loci), we performed a Cox model test with an interaction variable and a single covariable for *SDF1* or *CCR* protection. This test had significant log likelihood P values ($P < 0.01$) for AIDS-1987 and death for Caucasians in combined cohorts, with relative hazards of 0.31 (AIDS-1987) and 0.0 (death) (no deaths in doubly protected group), showing a significant advantage to having both protective genotypes. As an additional test of the additivity of the interaction between *SDF1* and *CCR*, a Cox model test was performed with separate variables for protection by *SDF1* and *CCR*, plus an interaction variable. The relative hazards of the interaction term were 0.55 (AIDS-1993), 0.31 (AIDS-1987), and 0.0 (death), with the P values falling short of significance ($P = 0.13$ to 0.31). These results indicate that the nonadditivity in the interaction between *SDF1* and *CCR* protective genotypes is not significant, but that the interaction tends toward being stronger than additive—that is, synergistic.

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25. The *SDF1* gene contains four exons over a 5.6-kb region of chromosome 10q11.1 (6). Two alternatively spliced transcripts that specify SDF-1 α and SDF-1 β are made from the gene; the isoforms differ by the addition of four COOH-terminal amino acids from the fourth exon in SDF-1 β (6). The two transcripts have completely different 3'UTRs, and the *SDF1*-3'A mutation is found in the SDF-1 β transcript within a sequence block that is conserved between mouse and human SDF-1 β UTR sequences. The possibility of linkage disequilibrium tracking of the *SDF1*-3'A mutation through linkage disequilibrium was investigated first by sequence determination of the four *SDF1* coding exons in eight *SDF1* 3'A/3'A homozygotes.

No additional polymorphisms were detected. Further sequence analysis of two *SDF1*-+/+ homozygotes and two *SDF1*-3'A/3'A homozygotes for 3253 nucleotides (out of 3524 in the entire transcript) identified two variants (positions 1912 and 2950) in single *SDF1*-+/+ individuals and revealed no additional mutations tracking with *SDF1*-3'A.

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Characterization of an Avian Influenza A (H5N1) Virus Isolated from a Child with a Fatal Respiratory Illness

Kanta Subbarao,* Alexander Klimov, Jacqueline Katz, Helen Regnery, Wilina Lim, Henrietta Hall, Michael Perdue, David Swayne, Catherine Bender, Jing Huang, Mark Hemphill, Thomas Rowe, Michael Shaw, Xiyan Xu, Keiji Fukuda, Nancy Cox

An avian H5N1 influenza A virus (A/Hong Kong/156/97) was isolated from a tracheal aspirate obtained from a 3-year-old child in Hong Kong with a fatal illness consistent with influenza. Serologic analysis indicated the presence of an H5 hemagglutinin. All eight RNA segments were derived from an avian influenza A virus. The hemagglutinin contained multiple basic amino acids adjacent to the cleavage site, a feature characteristic of highly pathogenic avian influenza A viruses. The virus caused 87.5 to 100 percent mortality in experimentally inoculated White Plymouth Rock and White Leghorn chickens. These results may have implications for global influenza surveillance and planning for pandemic influenza.

The introduction and subsequent spread in the human population of influenza A viruses with a novel hemagglutinin (HA) or a novel HA and neuraminidase (NA) subtype results from a sudden and major change in virus antigenicity, which is referred to as an antigenic shift. Lack of protective immunity in the human population against the new HA and NA proteins can result in rapid global spread of the virus, leading to widespread morbidity and mortality. Pandemic strains contain

new HA or NA genes derived from animal influenza A viruses. Influenza A viruses of 15 recognized HA subtypes and 9 NA subtypes are known to circulate in birds and other animals, creating a reservoir of influenza A virus genes available for genetic reassortment with circulating human strains of influenza virus. However, on the basis of seroarchaeology and virus isolation since 1933, only viruses of the H1, H2, and H3 subtypes are known to infect and cause disease in humans (1).

In general, avian influenza A viruses, including those that are highly pathogenic in birds, do not appear to replicate efficiently or cause disease in humans. The only reported natural infections of humans by avian viruses are two cases of conjunctivitis associated with avian H7 viruses, one of which was an infection with a seal virus of avian origin (2). Serosurveys of farm work-

ers in southern China by single radial hemolysis revealed a seroprevalence ranging from 1 to 38% for avian viruses of the H4 through H13 subtypes, including 7% seroprevalence for H5 viruses (3). In contrast, there were no documented infections in U.S. poultry workers exposed to strains of avian (H5) influenza A viruses that were highly pathogenic in poultry (4).

On 9 May 1997, a previously healthy 3-year-old boy, who was a resident of Hong Kong, developed a sore throat, dry cough, and fever. He was diagnosed with pharyngitis and prescribed antibiotics and aspirin. The child continued to be symptomatic and febrile and was hospitalized on 15 May. Upon admission, he was noted to be febrile (axillary temperature above 39°C) and irritable. His laboratory tests were most remarkable for leukopenia (2000 white blood cells per cubic millimeter). His chest x-ray was within normal limits. The next day, he was transferred to another hospital, where he developed progressive respiratory distress associated with hypoxemia, consistent with acute respiratory distress syndrome. He also became increasingly unresponsive. Computerized tomography of the head was unremarkable, and examination of his cerebrospinal fluid was not suggestive of an inflammatory process. Despite mechanical ventilation and broad antibiotic coverage, the child died on 21 May with several complications, including respiratory failure, renal failure, and disseminated intravascular coagulopathy. Postmortem liver and kidney biopsies showed evidence of microvascular fatty infiltration consistent with Reye's syndrome, which is a recognized complication of influenza.

A tracheal aspirate specimen was obtained on day 10 of illness and was cultured for respiratory viruses. A cytopathic effect was noted in mammalian Madin Darby canine kidney (MDCK) cells and rhesus monkey kidney (LLC-MK2) cells 2

K. Subbarao, A. Klimov, J. Katz, H. Regnery, H. Hall, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Fukuda, N. Cox, Influenza Branch, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA. W. Lim, Government Virus Unit, Queen Mary Hospital, Hong Kong, China.

M. Perdue and D. Swayne, Southeast Poultry Research Laboratory, Agriculture Research Service, U.S. Department of Agriculture, Athens, GA 30605, USA.

*To whom correspondence should be addressed.

and 3 days after inoculation, respectively. The isolate, designated A/Hong Kong/156/97, was typed as an influenza A virus by means of monoclonal antibodies to viral nucleoprotein in an immunofluorescence test, and it did not stain with monoclonal antibodies to respiratory syncytial virus and parainfluenza viruses 1, 2, and 3 (5). Additionally, bacterial and fungal cultures of the tracheal secretions did not yield pathogenic organisms. The influenza A virus isolate was not inhibited by sheep hyperimmune antisera to H1 and H3 influenza viruses included in the World Health Organization's Influenza Reagent Kit. Confirmation of the initial identification of the isolate as influenza A was obtained by immunoperoxidase staining of infected MDCK monolayers with influenza virus-specific monoclonal antibodies (5, 6). A hemagglutination-inhibition (HI) test with a panel of antisera to different subtypes of influenza A virus gave a reaction (HI titer 1280) only with the A/Tern/South Africa/61 (H5N3) antiserum, providing an identification of the HA as H5 (7).

is and treatment of influenza A virus infections, it was of interest to establish the antiviral sensitivity of this virus. Results of an in vitro assay revealed that the virus was sensitive to amantadine (5).

were conserved in the A/Hong Kong/156/97 virus. The residues of the H5 receptor binding site described by Garcia *et al.* are conserved in the A/Hong Kong/156/97 virus as well (12).

Nucleotide sequence and phylogenetic analysis determined that genes encoding all internal and nonstructural proteins were most closely related to known genes of avian origin (Table 1). The similarity of the A/Hong Kong/156/97 PB1 gene to the corresponding gene of human A/Singapore/1/57 (H2N2) virus can be explained by the suggestion that, in 1957, this gene was transmitted to human influenza viruses from avian species (15). The highest percent identities

Fig. 1. Alignment and comparison of complete HA coding sequence for the A/Hong Kong/156/97 virus isolate. Dashes under the sequence adjacent to the cleavage site indicate the positions of the insertions relative to the reference strain A/Turkey/England/91. The boxed amino acids indicate potential glycosylation sites. Underlined, bold peptides describe the location of the receptor binding site (12). The nucleotide sequence has been deposited in GenBank (accession number AF036356).

Table 1. Molecular analysis of the gene segments of the A/Hong Kong/156/97 virus. Nucleotide sequences were compared, and identity was determined by FASTA (Wisconsin Package, version 9.0) searches of GenBank and European Molecular Biology Laboratory (EMBL) databases as of August and June 1997, respectively. The nucleotide sequences have been deposited in GenBank (accession numbers AF036356 through AF036363).

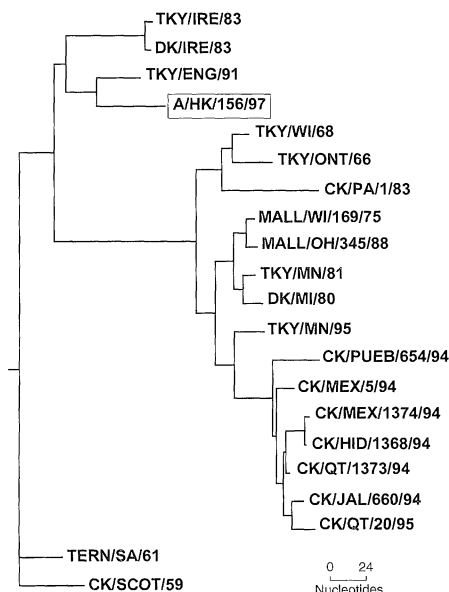


Fig. 2. Phylogenetic relationship of the HA1 domain of the hemagglutinin gene of representative avian H5 viruses and the H5 virus isolated from a human (A/Hong Kong/156/97, boxed). The tree is rooted in A/Chick/Scotland/59 (H5N1) as an outgroup. This is a neighbor-joining analysis determined by PHYLIP.

of the PB2, PA, NP, M, and NS genes of A/Hong Kong/156/97 to human influenza virus genes were 83.3, 83.6, 85.4, 91.2, and 89.3%, respectively, 3.1 to 9.9% lower than the percent identity with avian viruses listed in Table 1. Therefore, all gene segments of the A/Hong Kong/156/97 virus appeared to be avian in origin with no evidence of genetic reassortment with recent human influenza A virus genes.

To determine whether the A/Hong Kong/156/97 virus isolated in MDCK cells retained pathogenicity for avian species, we inoculated the virus by intravenous and intranasal routes into 16 3-week-old White Plymouth Rock chickens (*Gallus domesticus*) and by combined intranasal and intratracheal routes into six adult female White Leghorn chickens. The virus killed all eight chickens inoculated intravenously within 3 days; all of the chickens inoculated intranasally or by combined routes died within 9 days, except one intranasally inoculated White Plymouth Rock chicken, which survived. The chickens that died had lesions consistent with highly pathogenic avian influenza (fowl plague) and included necrosis of the combs and subcutaneous hemorrhages of the legs, thoracic wall, fat pads, proventriculus, and serosa of the heart. Most chickens had moderate to severe diffuse interstitial pneumonia with demonstration of influenza viral antigen in blood vessel and air capillary endothelium and macrophages. The virus also replicated in other tissues, as indicated by the presence of viral antigen in neurons, cardiac myocytes, and blood capillary endothelial cells. The A/Hong Kong/156/97 virus remained highly pathogenic for birds after

replication in mammalian cells.

To rule out laboratory contamination as a source of the isolate, we confirmed that the H5N1 virus could be identified directly in the tracheal aspirate obtained from the patient. The MDCK cells and embryonated eggs re-inoculated with the original clinical material yielded only an H5 virus. In addition, successful PCR amplification of the HA gene from RNA extracted from the clinical material was achieved with only H5 HA-specific primers and not with H1, H3, or B HA-specific primers (Fig. 3). The nucleotide sequences of the coding regions of the HA and NA genes amplified from RNA extracted from the clinical material were identical to the sequences determined for the virus isolated in MDCK cells (5). Cells from the clinical material were stained by immunofluorescence with monoclonal antibodies against the H5 HA and influenza A NP but not with monoclonal antibody against the H3 HA and a pool of influenza B-specific monoclonal antibodies (Fig. 4). Paired sera from the patient were not available; however, a single serum specimen collected on day 10 of illness did not react with the isolate in an HI test.

Although a clear epidemiologic link was not established between the infected child and infected poultry, there were three outbreaks of influenza in poultry on farms in Hong Kong between late March and early May 1997. Two viruses from one of these outbreaks were H5N1 viruses. Additionally, there were a few sick chickens at the child's preschool, but there is no evidence that the chickens were infected with avian influenza or that the child was in close contact with them.

The HA is the main determinant of the

host range of influenza A viruses, a phenotype conferred by its specificity for receptor recognition and binding. All key amino acid residues believed to be associated with the specificity of receptor binding of H5 HA (12) were conserved in the A/Hong Kong/156/97 virus. Identification of the specific linkages ($\alpha 2,6$ or $\alpha 2,3$ Gal) that can be bound by the A/Hong Kong/156/97 virus and knowledge of the distribution of receptors bearing these linkages in the human respiratory tract may help explain why this avian H5 virus infected this child.

In addition to its role as a determinant of host range, the HA gene is important in determining the virulence of avian influenza A viruses for poultry (16). All human influenza virus HA genes characterized to date, including the 1918 viruses (17), have a single, basic Arg residue at the cleavage site between HA1 and HA2. An insertion similar to the basic amino acid insertion adjacent to the cleavage site in the HA gene of the A/Hong Kong/156/97 virus has been found in several highly pathogenic avian H5 viruses. Basic amino acids adjacent to the cleavage site allow proteases other than trypsin-like proteases to cleave the HA into HA1 and HA2 domains. This event would enable the virus to spread systemically by altering the tissue range of these viruses from the respiratory and alimentary tract to other sites, such as the brain, heart, and blood vessels (18). It has been suggested that the acquisition of a ubiquitously cleavable avian

Fig. 3. Amplification of influenza HA gene from RNA extracted directly from the tracheal aspirate obtained from the patient. Lanes 1 and 14 contain a DNA ladder, with fragment sizes indicated along the left edge of the gel. RNA was extracted by means of the Qiagen (Valencia, California) RNeasy kit from the clinical material (lanes labeled HK/156/97: 4, 7, 10, 13), from known H1 (lane 2), H3 (lane 5), and H5 (lane 8) influenza A viruses, influenza B virus (lane 11), and water controls (lanes labeled —: 3, 6, 9, 12); reverse transcriptase reactions were carried out using 5 μ l of RNA and 25 units of avian myeloblastosis virus reverse transcriptase in a 25- μ l reaction. PCR was carried out using H1 (lanes 2 to 4), H3 (lanes 5 to 7), H5 (lanes 8 to 10), and B (lanes 11 to 13) HA-specific primers (PP, primer pairs); the expected product sizes are 1104, 785, 1110, and 1104 base pairs, respectively. PCR cycling conditions included denaturation at 94°C for 7 min, annealing for 2 min at 50°C, and extension for 3 min at 72°C, followed by 29 cycles of denaturation at 94°C for 1 min, annealing for 2 min at 50°C, extension for 3 min at 72°C, and an additional extension of 7 min at 72°C.

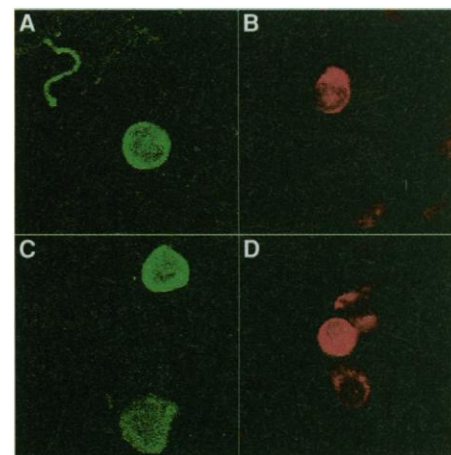
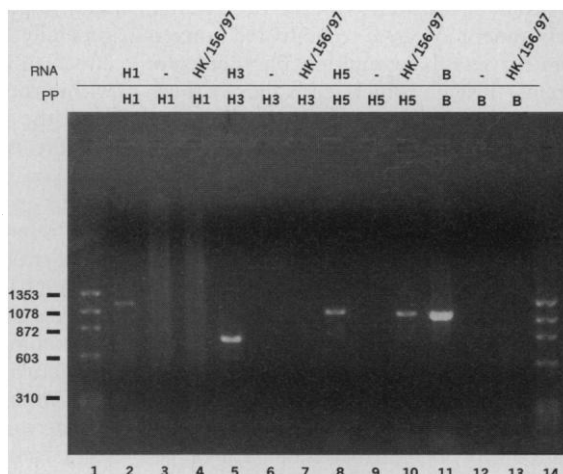


Fig. 4. Immunofluorescence staining of cells from the tracheal aspirate obtained from the patient. Acetone-fixed cells were incubated with influenza virus-specific monoclonal antibodies followed by fluorescein isothiocyanate-conjugated goat antibodies to mouse immunoglobulin G. The influenza virus-specific monoclonal antibodies used were (A) a pool of two antibodies raised against the H5 HA of A/chicken/Pennsylvania/1370/83, (B) an antibody that is broadly cross-reactive with the HA of human H3N2 viruses, (C) a pool of two antibodies specific for the NP of influenza A viruses, and (D) antibody specific for influenza B viruses.

HA by a human influenza A virus could make a virus lethal (16). It remains to be shown whether gene segments other than the HA may also have contributed to the ability of the virus to infect a human.

A pandemic of influenza could begin with isolated cases, in which avian or swine influenza viruses adapt to human hosts or, over time, genetically reassort with circulating human influenza A viruses, or it could begin as a rapid and explosive spread of a pandemic virus derived from a reassortment event in an intermediate host. Increased surveillance efforts have been initiated to identify other cases of illness associated with influenza A (H5N1) viruses. In addition, serosurveys are under way in an effort to identify asymptomatic or mild clinical infections in the region and substantiate the previous report of seroprevalence to H5 viruses (3). These studies may determine whether H5 viruses similar to A/Hong Kong/156/97 are circulating in the human population and if the isolation of the A/Hong Kong/156/97 virus is the first step in the recognition of an influenza A virus with pandemic potential or whether this case is simply an isolated event.

Note added in proof: Since the submission of this report, there have been 12 additional confirmed human cases of influenza A (H5N1) infections in Hong Kong, including three fatalities. Sequence analysis of the genes of six of the isolates revealed that all of the genes are of avian origin and are closely related to each other. The HA gene codes for a multiple basic amino acid insertion upstream of the cleavage site, associated with highly pathogenic avian influenza viruses and identical to that seen in the A/Hong Kong/156/97 virus.

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Extraocular Circadian Phototransduction in Humans

Scott S. Campbell* and Patricia J. Murphy

Physiological and behavioral rhythms are governed by an endogenous circadian clock. The response of the human circadian clock to extraocular light exposure was monitored by measurement of body temperature and melatonin concentrations throughout the circadian cycle before and after light pulses presented to the popliteal region (behind the knee). A systematic relation was found between the timing of the light pulse and the magnitude and direction of phase shifts, resulting in the generation of a phase response curve. These findings challenge the belief that mammals are incapable of extraretinal circadian phototransduction and have implications for the development of more effective treatments for sleep and circadian rhythm disorders.

Circadian rhythms are endogenously generated oscillations of about 24 hours that provide temporal structure to a wide range of behavioral and physiological functions. Because the endogenous clock tends to run at a period close to but not exactly 24 hours, a daily adjustment, usually by the natural light-dark cycle, is required to synchronize or entrain circadian rhythms to the external environment. Many vertebrate and nonvertebrate species have multiple photoreceptor systems through which circadian entrainment may be achieved (1-3). In the house sparrow, for example, three discrete input pathways for light to act on the circadian system have been identified (4). Similarly, a number of fish, amphibian, and reptile species have extraocular and extrapineal pathways for circadian light transduction (5).

The photoreceptors responsible for entraining the mammalian biological clock may not be the same cells that mediate vision (6). Mice homozygous for the autosomal recessive allele *rd* (retinally degenerate), which have no electrophysiological or behavioral visual responses to light, can be

entrained to a light-dark cycle (7). Likewise, bright light suppresses melatonin output in some totally blind humans, despite the fact that they have no conscious light perception and no pupillary light reflex (8). Such findings support the hypothesis that all vertebrates, including mammals, have specialized nonvisual photoreceptors that mediate circadian responses to the light-dark cycle. It is generally assumed, however, that nonvisual circadian photoreceptors in mammals reside within the retina, and that mammals do not have the capacity for extraocular circadian photoreception (1, 2, 9). This conclusion is based on studies showing a failure of several rodent species to entrain to a light-dark cycle or to respond to pulses of light with shifts in circadian phase after complete optic enucleation (10). In addition, Czeisler and co-workers found an absence of light-induced melatonin suppression during ocular shielding in two individuals who did show suppression when light fell on their eyes (8). A decade earlier, Wehr and co-workers reported a lack of clinical response in seasonal affective disorder when patients' skin (face, neck, arms, legs) was exposed to a bright light stimulus while their eyes were shielded (11). However, in that study, no physiological measures of light response, such as melatonin secretion or temperature phase re-

Laboratory of Human Chronobiology, Department of Psychiatry, Cornell University Medical College, 21 Bloomingdale Road, White Plains, NY 10605, USA.

*To whom correspondence should be addressed.