Med Microbiol Immunol (2006) 195: 45–47
DOI 10.1007/s00430-005-0008-4

EDITORIAL

H.W. Doerr · Domenica Varwig · Regina Allwinn
J. Cinatl

Will the next human influenza pandemic be caused by the virus of the avian flu A/H5N1? Arguments pro and counter

Published online: 13 January 2006

Abstract In 1997, the avian influenza A subtype H5N1 that caused big outbreaks of fowl pest in mass poultry farming had emerged in Hong Kong. Its spread throughout Eurasia had given rise to concerns in terms of the possible imminence of the next human influenza pandemic. In this article, epidemiological and virological arguments supporting or declining this fear are outlined and discussed with regard to viral infectivity and pathogenicity.

Keywords Influenza A · Avian flu H5N1 · Viral infectivity · Pathogenicity · Pandemic

Introduction

Avian influenza (avian flu) is an infectious disease affecting birds. Its symptoms resemble the human influenza in terms of respiratory disorder, but in contrast to the human virus it can also affect the gastrointestinal tract. In chickens, the classic fowl pest may cause huge losses, especially in mass poultry farms. The influenza virus type A, as the infectious agent of avian flu, especially classic fowl pest, belongs to the genus of the orthomyxoviridae family. Influenza viruses B, C and D represent other genera, which are respective types of this virus family. Apart from afflicting birds, type A is also endemic in humans and other animal species; types B and C are mainly detected in humans. Influenza D virus is endemic only in mice. Its taxonomic name is thogotovirus.

H.W. Doerr (✉) · D. Varwig · R. Allwinn · J. Cinatl
Institute for Medical Virology,
Johann Wolfgang Goethe-University,
Frankfurt on Main, Germany
E-mail: H.W.Doerr@em.uni-frankfurt.de

Natural biology of influenza virus infection

The group of influenza A viruses can be divided into many subtypes. Subtyping is based on two antigenic structures on the surface of the virus (envelope): (1) The haemagglutinin (H) shows a spike-like structure, which mediates the virus adsorption to the cell membrane, thus inducing the infection. In vitro assays demonstrated that this structure can also bind to erythrocytes causing a haemagglutination. (2) The neuraminidase (N) forms a second type of spikes on the virus envelope. It cleaves neuraminic acid containing mucus which is secreted by respiratory cells as a protective barrier. More importantly, it destroys the cell receptor and prevents readsorption of the viral progeny released by the infected cell. The influenza virus-specific cell receptor is comprised of sialic acid (SA), an *N*-acetylic neuraminic acid. In humans, this sialic acid contains galactose in the alpha(2,6)-binding configuration, while avian cells express alpha(2,3)-binding galactose in their SA receptors. Both receptors differ slightly in their steric configuration. Correspondingly, the anti-receptor structure on top of human H spikes differs from bird-adapted influenza virus; thus, the receptor as well as the viral structure are host specific.

The infolded, pocket-like antireceptor is non-immunogenic in vivo. Inhibition of H is immunologically mediated by antibody formation against H domains in the neighbourhood of the anti-receptor. While the anti-receptor is genetically relatively stable, the other domains of H undergo rapid mutations. Thus, the virus can partially escape the immune defence of the host. This antigenic drift can be ruled out in vitro by haemagglutination inhibition tests. A new H subtype is defined, if it does not cross react even in high avidity antibody tests. The intrasubtypic species-specific infectivity has to be investigated in animal experiments. This phenotyping can be exactly defined by sequencing of the H gene (genotyping). In a similar way, the N spike can also be sero-, pheno- and genotyped. The N antigenic

drift is the second (less important) parameter of influenzaviral infectivity.

Influenza A epidemiology in animals and humans: crossing the species barrier

Waterfowl (goose, duck, gull) are considered the main reservoir of influenza A viruses, because all known 16 H and 9 N subtypes are endemic in these birds. The influenza viral subtype (or phenotype) is defined as one of 16×9 H/N combinations. Since only a part has been identified, yet, new emerging influenza viruses may be expected, like influenza virus A/H5N1. The genome of influenza A (and B) viruses is segmented. H and N genes are located on two of eight ''chromosomes''(segments). By double infection of the same cell, the gene segments can be re-assorted. That way, antigenic drifting is supported when two strains of one subtype afflict the same host. If two different influenza A virus subtypes infect the same cell, H and N genes can produce a new combination, which causes a drastic antigenic shift. To date, only H1, H2 and H3, respectively, N1 and N2 serologic subtypes have been detected in humans: as phenosubtypes H1N1 (''Spanish'' influenza 1918), H2N2 (Asiatic influenza 1958) and H3N2 (Hong Kong influenza 1968). All three virus subtypes have analogues in birds and have undergone mutation in the H-located anti-receptor structure, which permits an easy interaction with human cell receptors. (The original avian anti-receptor needs high virus dose for binding.) Today, only H3N2 and H1N1 influenza viruses circulate in many drift variants, which have to be considered in the current vaccine production. H2N2 is less prevalent, since H1N1 has re-emerged in 1978. The human H1N1 influenza A virus subtype reveals an exchange of a single amino acid compared to the avian H1 at short distance from the anti-receptor, which seems to be indirectly affected. The human-adapted H structures have proven to be genetically stable. However, transmission to pigs is, in principle, possible, because their respiratory cells express two different cell receptors, one resembling the human configuration, the other resembling the avian SA configuration. Thus, pigs were regarded as 'mixing vessels' for porcine and human-adapted influenza A virus strains [1, 2].

Parameters of influenza pathogenicity

The course of influenza virus infection may not be apparent or may not lead to a severe and lethal disease. This depends on the interference of host resistance and virulence factors of the infectious agent. In this context, the action of haemagglutinin has been studied intensely. The adsorption of the virus induces a receptor-mediated endocytosis. The second step of infection consists of a cleavage in the mean part of the haemagglutinin by a cellular protease. At this position, the H scaffold is opened and bound to the vacuole-like internalized cell membrane. Simultaneously protons from the vacuole enter the virus by special ion transporter proteins inserted in the matrix underlying the envelope. Haemagglutinin cleavage and proton influx cause a fusion between viral envelope and internalized cell membrane, thus releasing the viral ribonucleoprotein into the cytoplasma to be transported to the cell nucleus, where the genome transcription takes place. It is obvious, that the virus is more or less infectious, depending on the number of its haemagglutinin cleavage sites and thus the amount of cell proteases available. Highly pathogenic avian influenza (HPAI) virus strains reveal a lot of cleavage sites for different cell proteases, especially for the ubiquitous furin. Thus, they spread and can rapidly be replicated in any organ (systemic infection). In particular, if cells of the capillary system are affected, a highly dangerous haemorrhagic infection can be induced. Low PAI strains express H molecules on the envelope, which can be cleaved only by trypsin-like proteases secreted by respiratory or gastrointestinal cells. Influenza pathogenicity not only depends on H and N activity, but also on many other viral enzymes and proteins, which interfere with host-specific cell regulation in different ways. Thus, the viral polymerase complex has been studied intensely with regard to mutations increasing or decreasing the speed of viral replication. Sequence analyses of the corresponding genes located on three different genomic segments have revealed, that HPA influenza A/H5N1 strains have undergone point mutations similar to the original H1N1 virus of the Spanish influenza pandemic 1918 [3, 4]. These strains of the influenza A/H1N1 are not circulating anymore. No information is available on the relation of N1 in H5N1 and human H1N1 in terms of antigen drift. The H3N2 pandemic was not as dangerous as H2N2, because H3N2 was re-assorted out of an avian H3 strain and human H2N2. Although H5N1 virus is highly pathogenic for humans too, the crucial mutation of H (anti-receptor), that would facilitate an easy infection, is still missing.

Emergence and spread of influenza virus A/H5N1

Influenza virus A subtype H5N1 emerged in 1997 in a mass poultry farm in Hong Kong [1]. In this environment, huge numbers of viruses could be produced, which led to the infection of a few humans who fell ill and died. This outbreak proved false the notion that avian influenza viruses need pigs (or other animals) as vehicles to be transmitted to humans. In the following years, more and more outbreaks in mass poultry farms were registered in several countries in East and Southeast Asia causing the death of thousands of chickens. To date, about 140 humans have been infected and fallen ill. In nearly every case, a close contact with the highly infectious avian material could be detected. Apart from typical respiratory signs, many patients also presented with gastroenteritis similar to the

infected chickens. The lethality rate mounts to more than 50%. In parallel, isolated H5N1 strains revealed a high grade of mutagenic activity concerning amplified cleavage sites in the H5 spike [5]. Obviously, it is the mass holding of poultry, which favours the spread of HPAI strains. Under other circumstances, the chain of infection would disrupt because of rapid host elimination. Unfortunately, killing the poultry did not stop the viral transmission, because HPAI strains of chicken are not necessarily pathogenic for other birds like ducks or geese. These can carry the virus to the next poultry farm, without developing symptoms. At the moment, we are confronted with the spread of the virus throughout Eurasia along bird migration routes. To date, there is no explanation why those migratory flocks of birds do not fall ill and die.

Does avian H5N1 epidemic cause a human pandemic?

Serious concerns have risen that HPAI H5N1 would be the origin of the next human influenza pandemic [6]. The following arguments support or decline this fear:

Pro

- Birds are the reservoir of all influenza A viruses. Each human influenza A virus subtype derives from an avian influenza through direct transmission and adaptation or indirect transmission and genetic reassortment.
- Mass animal farming facilitates mutation and selection of highly pathogenic and rapidly replicating virus strains, like the avian influenza virus A/H5N1.
- In principle, this virus can be transmitted to humans, if the dose is sufficiently high.
- The genome of avian flu H5N1 shows considerable similarities in terms of genetic virulence factors compared to influenza virus H1N1, which has caused the greatest human influenza pandemic to date.

Counter

Fowl pest outbreaks of previously detected avian flu subtypes have not been known to be responsible for a human epidemic. The former human influenza pandemics could not be traced to animal epidemics, but to inapparent zoonoses similar to SARS corona virus.

- High lethality rates limit the spread of infection.
- After 8 years and nearly 140 documented avian influenza virus A/H5N1 transmissions to humans, a virus strain variant expressing an H molecule adapted to human cell receptor has not been detected yet.
- In contrast to influenza A/H1N1 (original strain 1918), there has occurred no mutation in the H5N1 neuraminidase, which would promote proteolytic cleavage of H5.
- For more than 100 years, only three of 16 H influenza virus subtypes (H1, H2, H3) have been detected, circulating and re-circulating in humans. Thus, there are epidemiologists, who consider that H2 will cause the next human epidemic.

Conclusions

Molecular investigations on avian and human cases of H5N1 influenza gives evidence why at present this virus is highly pathogenic, but less infectious to human beings. Because of epidemiological issues, it is probable that an increase in infectivity of humans will lead to a decrease in pathogenicity and lethality.

The H1N1 subtype developed such a high pathogenicity and infectivity possibly because it spread among soldiers along the western front of the First World War. Thus, another risk factor promoting dangerous strains is the close social interaction and living together (“mass housing of men”).

Everything depends on measures to slow down the speed of the virus circulation, which can be best achieved by observing strict classical hygiene.

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