Molecular aspects of the epidemiology of virus disease

by C. Scholtissek

Institut für Virologie, Justus-Liebig-Universität Giessen, Frankfurter Strasse 107, D-6300 Giessen (Federal Republic of Germany)

Summary. With regard to molecular epidemiology, influenza A viruses belong to the best-studied virus systems. At least two large reservoirs of influenza A viruses have been built up in nature, one in humans and another one in water fowls. The latter one is very heterogenous, consisting of viruses belonging to 13 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes in almost all possible combinations. The segmented structure of the influenza virus genome allows the creation of new influenza strains by reassortment. By replacement of the HA gene of human strains new pandemic viruses can be generated (antigenic shift). The particular structure of the HA enables the human influenza A-viruses to create variants which can escape the immune response of the host (antigenic drift). The nucleoprotein is responsible for keeping those two large reservoirs apart. Mixing of genes of viruses from these two reservoirs seems to happen predominantly by double infection of pigs, which apparently are tolerant for infection by either human or avian influenza viruses. The molecular mechanisms described for influenza viruses can be explained by the particular structure of their genome and their components and cannot be generalized. Each virus has developed its own strategy to multiply and to spread.

Key words. Epidemiology of influenza; antigenic drift; antigenic shift; virus reservoirs; reassortment.

1. Introduction

Viruses which are unable to be transmitted from one host to the next are bound to die out. Transmission of viruses can occur by several means: by aerosols, in food or drinking water, by casual or intimate contact, by vectors such as insects, etc. Humans and most animals try to prevent transmission by eliminating the infecting virus mainly by the host immune defense mechanisms. Therefore, viruses have developed means to escape these defences in different ways: 1) Viruses whose surface antigens are genetically relatively stable, because mutations in the corresponding genes are functionally not well tolerated, will infect only individuals who have never been in contact with the virus before. Polio or measles viruses, which infect mainly children, are examples. 2) Another class of viruses may infect an organism latently and cause symptoms only occasionally in spite of the presence of antibodies. An example of this is the Herpes simplex virus. 3) A third class of viruses might exhibit a great variation in their surface components, brought about by mutations, so that variants can always escape the immune response. The influenza A viruses are an example. For these viruses the hemagglutinin gene carries sequences which are not necessary for its function but form the various epitopes against which neutralizing antibodies are directed. Thus, the hemagglutinin consists of highly variable parts, consisting of the epitopes where mutations are tolerated, and highly conserved parts, which are indispensable for its function[17]. Such viruses can cause epidemics by infecting individuals of all age groups, because the virus infecting a particular individual the next time is antigenically altered, although it might be genetically highly related to the foregoing strain (antigenic drift). Because of the segmented nature of their genome, influenza A viruses have developed an additional mechanism to escape the immune status of a population. New viruses can be constructed by replacement of the genes coding for the surface antigen(s) hemagglutinin (and neuraminidase) (antigenic shift).

Since influenza A viruses belong to the best-studied virus group, with respect to molecular epidemiology, they will be discussed here in more detail.

2. Structure and properties of the influenza A viruses

The genome of influenza viruses consists of single-stranded RNA of negative polarity. The virion RNA can be separated by polyacrylamide gel electrophoresis into 8 segments (fig. 1, left), each segment consisting of one gene. The assignment of the RNA segments to the various viral gene products has been achieved (fig. 1, right)[10, 14, 21]. For our considerations the most important viral components are the hemagglutinin (HA), against which the infected organism induces neutralizing antibodies and by which the virus binds to the cell

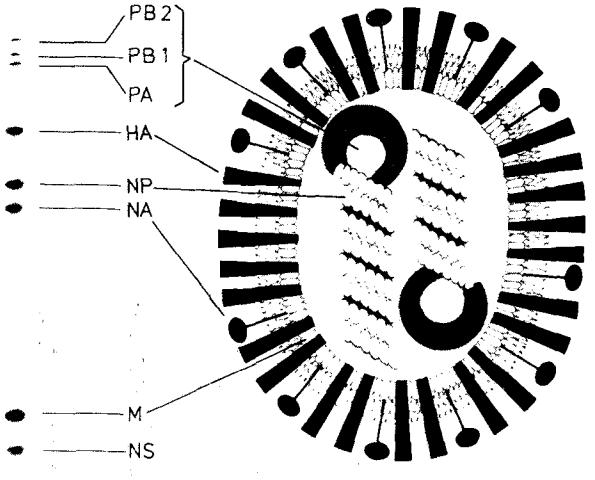

Figure 1. Structure and structural elements of an influenza A virus (right) and assignment of the gene products (middle) to the viral RNA segments (genes) separated by polyacrylamide gel electrophoresis (left). The polymerase complex is located in the interior of the particle. It consists of the three P proteins PB2, PB1 and PA and the nucleoprotein NP. The lipid bilayer is covered on the inside by the membrane or M protein. The two glycoproteins hemagglutinin (HA) and neuraminidase (NA) are located on the exterior surface. The smallest RNA segment contains the information for two nonstructural proteins (NS).

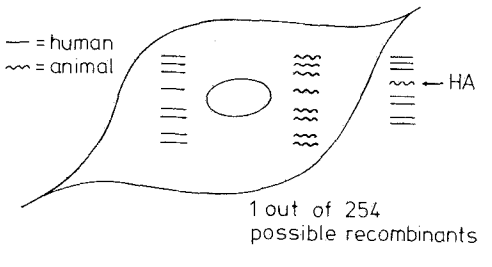

Figure 2. Scheme to explain the creation of a new pandemic influenza A strain (antigenic shift). A cell becomes doubly infected by an animal virus and by the prevailing human influenza A strain. One of the 254 possible new reassortants has the gene constellation shown on the right. Only the HA gene is derived from the animal strain, while all the other genes are derived from the human virus.

Table 1. Multiplication of reassortants between fowl plague virus (F) and A/chicken/Germany 'N'/49 (N) in lungs and brains after intranasal inoculation of 2-day-old mice. With exception of the first virus (virus N) the genes PB2, HA, NA and M of the reassortants are derived from fowl plague virus. If the PB1- or/and PA-gene is derived from virus N, the reassortants are neurotropic for mice. If in addition the NS-gene is derived from virus N, the virus titer found in the brain increases even further. However, replacement of the NP-gene causes loss or decrease of neurotropic properties. All animals with detectable virus in their brains die. However, the mean death time correlates inversely with the virus titer in their brains[1].

Origin of the genes				Virus titer 4 days after infection	
PB1	PA	NP	NS	Lung (plaque-forming suspension)	Brain units/ml
N	N	N	N	10^4	$< 10^1$
F	F	F	F	10^1	$< 10^1$
N	F	F	F	10^6	10^3
N	F	F	N	10^6	10^5
F	N	F	F	10^6	10^4
N	N	F	F	10^7	10^5
N	N	F	N	10^7	10^6
F	F	N	F	< 101	$< 10^1$
F	N	N	F	$< 10^1$	$< 10^1$
N	N	N	F	10^6	10^4

receptors, and the nucleoprotein (NP), which is the major component of the nucleocapsid. The latter complex exhibits RNA polymerase activity, synthesizing viral mRNA in vitro as well as in vivo.

The influenza A viruses can be subdivided with regard to the surface glycoproteins into 13 different HA (H) and 9 different NA (N) subtypes which have been found in nature in almost all possible combinations.

Influenza viruses, like all viruses with a segmented genome, exhibit the phenomenon of reassortment in that, after double infection of a cell or an organism with two different influenza A strains, the 16 RNA segments behave like chromosomes and reassort freely (fig. 2) giving rise to maximally 254 different reassortants with new properties. Not all of these theoretically possible reassortants are compatible for growth in a certain host cell. For example, some might be able to multiply in chick embryo cells (CEC) but not in dog kidney (MDCK) cells, some other reassortants might grow in MDCK cells but not in CEC. This is the background for understanding how we can change the host range and organ tropism by reassortment and how in nature new strains with new properties can be created.

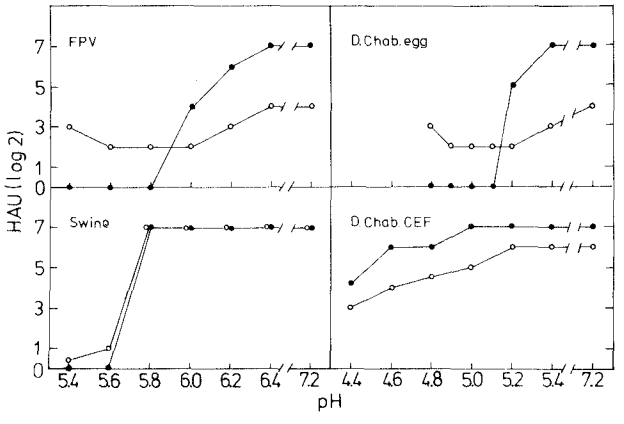

Figure 3. pH inactivation of various influenza A strains. Infectious allantoic fluids of A/FPV/Rostock/34, A/swine/1976/31, or A/duck/Chabarovsk/1610/72 were adjusted to the pH indicated on the abscissa and were incubated for 1h at 20 °C, before chick embryo cells were infected. The virus yield was determined by the HA test 7 h after infection (●). Open circles represent the HA titers of the infecting virus after pH treatment. Duck/Chabarovsk virus propagated on chick embryo cells having non-cleaved hemagglutinin (lower right) was also investigated. For replication (●) 10 μg/ml trypsin was present in the medium.

3. Creation of influenza A virus strains with new properties by reassortment

As outlined in figure 2, new pandemic influenza virus strains can be 'synthesized' by reassortment (antigenic shift). Thus, when we analyzed the Hong Kong (H3N2) strain (which caused the pandemic in 1968) by the hybridization technique we found that the new strain inherited 7 RNA segments from

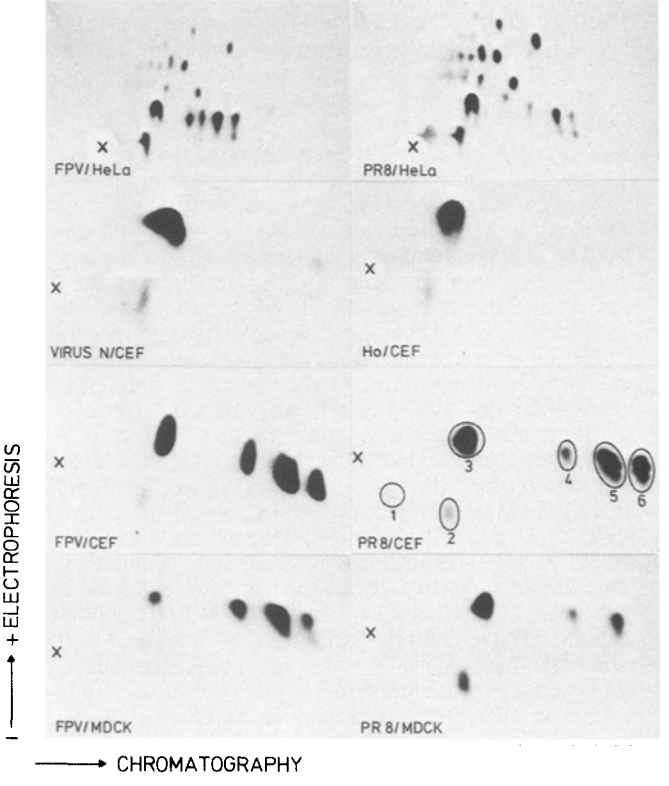

Figure 4. Phosphopeptide fingerprints of the nucleoproteins of different influenza A strains, which were labeled with (^{32}P)orthophosphate in different cells. The following viruses were investigated: fowl plague virus (A/FPV/Rostock/34), virus N (A/chicken/Germany 'N'/49), Ho (A/Hong Kong/1/68), and PR8 (A/PR/8/34). The following host cells were investigated: CEF, primary chick embryo fibroblast; MDCK, immortal dog kidney cells; HeLa = immortal human carcinoma cells. The virus yields in MDCK cells is on average about 10% of that in CEF; the yield in HeLa cells is about 0.1% of that in CEF. During growth in HeLa cells, the nucleoprotein (NP) is overphosphorylated. During growth in MDCK cells, the PR8 NP is missing phosphopeptide 6. In FPV-infected MDCK cells, the major phosphopeptide 3 is only weakly labeled[7].

the foregoing H2N2 virus, and that the only new gene, the HA, was highly related to the HA gene of a duck influenza virus isolated previously in 1963 in the Ukraine[15]. Thus, this new human strain was able to start a pandemic, because at this time no neutralizing antibodies against the H3 hemagglutinin were present in the human population. Since the host range and symptoms were not influenced by this reassortment, we have to assume that these properties are conferred by one or several of the other genes taken over from the original H2N2 virus.

During the antigenic shift 'wolves in sheep's clothing' are produced under natural conditions. We have succeeded in

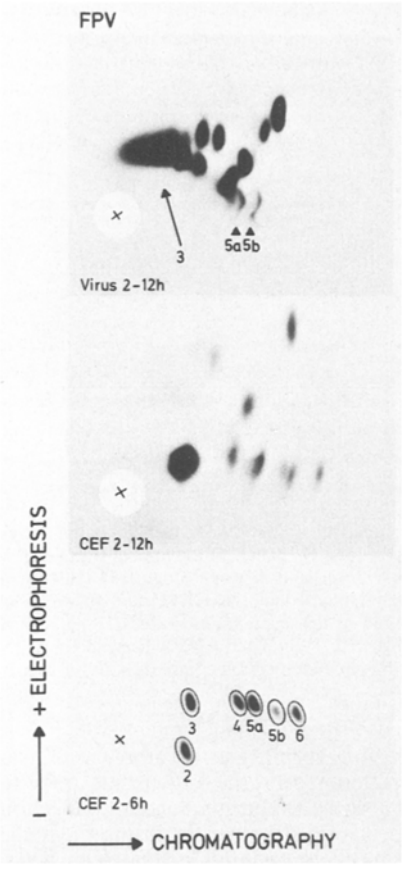

Figure 5. Phosphopeptide fingerprints of the nucleoprotein (NP) of fowl plague virus labeled in chick embryo fibroblasts from 2 to 6 h or 2 to 12 h after infection, and of the NP isolated from purified virus particles. During late labeling the NP isolated from cells contains new phosphopeptides, which predominate in the NP of isolated virus particles, while phosphopeptides 4, 5a, 5b, and 6 are only faintly labeled.

obtaining in the test tube 'sheep in wolves' clothing' by replacing the RNA segments (= genes) of a highly pathogenic avian influenza A virus, the fowl plague virus FPV (H7N1), which are responsible for pathogenicity. For this purpose we have rescued temperature-sensitive (ts) mutants of FPV, with defects in different genes, by double infection with other influenza A strains. The conditions were such that we selected always for the HA of fowl plague virus replacing at least that gene carrying the ts defect[12]. When these specific reassortants were tested for pathogenicity in chickens, several of them were found to be nonpathogenic and to induce neutralizing antibodies against the FPV so that these chickens could withstand the superinfection with the wild-type FPV without exhibiting any symptoms[13]. Such reassortants can be regarded as potential live vaccine strains.

It is possible to change the organ tropism and/or host range by reassortment starting with two parent viruses which are not able to multiply in that organ or host. Thus, if we tested our reassortants of FPV in young mice, several isolates turned out to be highly neurotropic for mice, and in contrast to the parent strains, they also were able to multiply in mouse brain cells cultured in vitro[18]. As an example, the gene constellation of neurotropic reassortants between FPV and another avian influenza A virus (virus N) are shown in table 1. Replacement of the PB1 and/or PA proteins of FPV results in neurotropic reassortants. Replacement of the NP gene counteracts this effect[1]. This is an especially clear example of a change of organ tropism by reassortment paralleled by an increase of pathogenicity. We were unable to protect mice infected intranasally with these neurotropic reassortants by immunization, since the virus was taken up by the nerve endings in the nasal mucosa and spread via the olfactory bulb over the entire brain[11].

This method of creation of highly pathogenic influenza strains seems to happen also under natural conditions. In the winter of 1979/80 many dead seals were found on the east coast of the USA. An influenza A virus of the H7N2 subtype was isolated from the lungs and brains of these animals. This virus was shown to be a reassortant of avian influenza viruses[3, 5]. It is not yet clear whether this strain is highly pathogenic on its own or whether it needs a co-infection with another microorganism to establish the symptoms.

4. A huge reservoir of different influenza A virus strains has been built up in water fowls

It is by no means an advantage for a virus to be highly pathogenic, because such a virus would eliminate itself by eliminating its host. The influenza A viruses, however, can afford the 'synthesis' of such highly pathogenic reassortants, such as the seal virus, because of the existence of a large reservoir of different influenza A viruses in water fowls. Ducks, for example, can distribute the virus strains all over the world. These viruses are non-pathogenic for their natural hosts, because they are restricted to multiplying in a specific region in the gut[24]. Since this reservoir is a very important factor in determining the epidemiological behavior of influenza viruses, we have tried to understand the build-up and maintenance of this reservoir on a molecular level. It is known, mainly from studies in Giessen[9], that the hemagglutinin (HA) of influenza viruses has to be cleaved into the cleavage products HA1 and HA2 in order to start an infection. This cleavage depends on the structure of the HA as well as on the presence of a trypsin-like enzyme in the host cell. Correspondingly, FPV is highly pathogenic for chickens, since its HA is cleaved in many cells so that the virus can start a generalized infection. Other viruses whose HA is cleaved only in a few cell types, will not spread well in the body and therefore will not cause severe symptoms. It has been shown by J. J. Skehel and his colleagues[23] that a cleaved HA undergoes a conformational change at a pH of about 5. This pH is normal in the endosomes, into which the virus is taken up after adsorption. This conformational change is essential to start the fusion between the viral and endosomal membrane. Noncleaved HA does not undergo this conformational change at pH 5 and, therefore, such viruses cannot start an infection.

Table 2. Summary of properties of H3N2 influenza viruses isolated from humans, pigs or birds

Property under study	Virus isolated from			
	Human, all isolates	Pigs, all except Sw126 and SW127	Poultry, all isolates	Pigs, only SW126 and Sw127
Ability to replace the NP-gene of FPV (rescue)	–	–	+	+
Hybridization against RNA segment 5 of WSN, % RNase-resistance	28–31%	28%	17%	17–18%
Binding of monoclonal antibodies against the NP of WSN; monoclones bound	3/1, 5/1, 7/3	3/1, 5/1, 7/3	3/1, 5/1, 7/3, 150/4, 469/4	3/1, 5/1, 150/4

If virus with cleaved HA is treated in the test tube at pH 5, its HA also undergoes this conformational change; however, under these conditions it leads to an irreversible inactivation of infectivity. As shown in figure 3, virus with noncleaved HA is stable to treatment of at least pH 4.4, but this virus needs a short incubation with external trypsin to start an infection[19]. Influenza viruses can survive in slightly acidic lake water[4] in spite of the fact that the virus is excreted from water fowls via the feces, which, because of the special nitrogen excretion in birds, contain large amounts of uric acid (pK of 5.4). Therefore, we have to assume that the virus which survives in lake water has a noncleaved HA. Since water fowls can become infected via the cloaca we now can put these facts together in order to understand how this virus reservoir is built up. The virus which survives in lake water with a noncleaved HA cannot start an infection without pretreatment with an external trypsin-like protease. After infection via the cloaca the virus reaches a site in the gut where trypsin comes in from the pancreas. Only here can the virus start an infection, which would explain the restricted site of multiplication in the gut of water fowls. This also explains why the virus does not spread further, because at other sites the virus can undergo only a single cycle of infection and is released with a noncleaved HA. This seems to be the reason why these viruses are not pathogenic for their hosts[19].

RNA viruses in general have an extremely high mutation rate, which leads to a relatively large number of variants in each virus population, and it is only a matter of the selection pressure we use on such a population to isolate the variant we are interested in. An example of how a highly pathogenic variant can emerge from a relatively harmless virus population was seen in the sudden outbreak of fowl plague in chicken farms in Pennsylvania in October 1983. In April of 1983 an almost non-pathogenic H5N2 virus with a noncleavable HA was isolated from chickens in that area which were laying fewer eggs than expected. Five months later a H5N2 virus was again isolated, this time from dead animals. This virus was shown to be highly pathogenic and to contain now a cleavable HA. This change in cleavability, and pathogenicity, was due to a single amino acid replacement in the HA[6]. If such a variant, able to multiply without the aid of external trypsin, were to emerge from the influenza virus reservoir in water fowls this would be disastrous and would not only wipe out most of the water fowls but also the influenza virus reservoir. Therefore, we have to assume that water fowls always select against highly pathogenic variants by the same mechanism as outlined above; a variant with a cleaved HA would immediately be destroyed during its excretion via the feces, at the low pH.

5. The nucleoprotein as a major factor in determining species specificity

Another important epidemiological problem concerns the question as to how viruses can cross the species barrier if two large reservoirs exist in different species. What determines the species specificity? It should be a virus protein that cooperates with most of the other viral components during virus replication and which might be specifically modified by the host cell so that the virus becomes vulnerable to a transfer from one cell type or organism to another. The nucleoprotein seems to fulfill these requirements, since it has to interact early in the infectious cycle with the P proteins of the polymerase complex, and later during virus assembly with membranes modified by the M protein. Furthermore, the NP is specifically phosphorylated by host protein phosphokinases, as shown in figure 4. If different influenza A viruses are labeled in the same cell, they exhibit a strain-specific phosphopeptide fingerprint. Furthermore, if the same virus is labeled in different cells, the fingerprints are different[7].

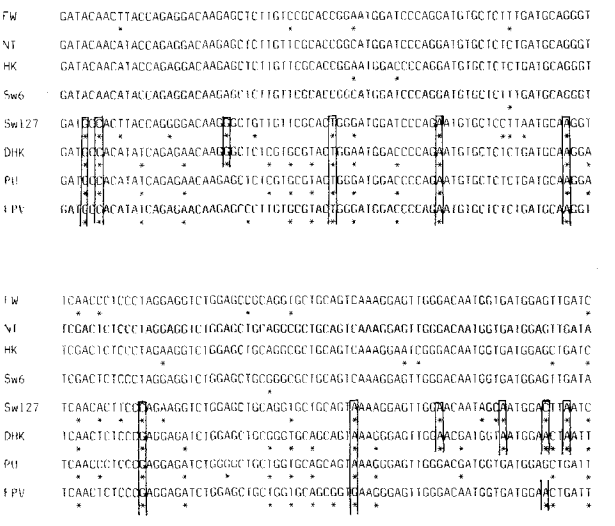

Figure 6. Comparison of the base sequences of the nucleoprotein (NP) genes from positions 477 to 627 of the human strains A/FW/50 (H1N1), A/NT/60/68 (H3N2), and A/Hong Kong/5/83 (H3N2); of the porcine strains A/swine/Hong Kong/6/76 (H3N2), and A/swine/Hong Kong/127/82 (H3N2); and of the avian srains A/duck/Hong Kong/7/75 (H3N2), A/parrot/Ultster/73 (H7N1) and A/FPV/Rostock/34 (H7N1). The asterisks indicate base replacements in comparison to the human A/NT/60/68 strain.

The NP undergoes a subtle sequence of phosphorylation and dephosphorylation steps (fig. 5) during the replication cycle before it is incorporated into infectious particles[8]. When we tried to replace the NP of fowl plague virus (H7N1), which has a complex phosphopeptide fingerprint, by the NP of the Hong Kong virus (H3N2, simple fingerprint) by using the rescue technique as mentioned above, we did not succeed when using chick embryo cells (CEC), but we succeeded when using dog kidney (MDCK) cells. The reassortants carrying the cleavable HA of FPV and the NP of the Hong Kong strain were unable to plaque in chick embryo cells and were non-pathogenic for chickens. Thus, we had succeeded in changing the host range by this type of replacement. Other genes of FPV could be replaced without changing the host range[16]. We have used this technique of rescue of FPV ts mutants with a defect in the NP gene in CEC to investigate many H3N2 strains isolated at about the same time from the same region of South China but from different species (table 2). All avian H3N2 isolates were able to rescue our NP ts mutants of FPV, while all human H3N2 viruses were not. The pig isolates could be placed into two groups in this respect. The genetic analysis using the hybridization technique revealed that the one group of pig isolates had a 'human-like' NP and the other pig viruses had an 'avian-like' NP[20]. These data have been strengthened recently by sequencing of the NP genes. The NP sequences are species-specific[2] (fig. 6). Studies with monoclonal antibodies are confirming these observations more or less. We interpret our results to mean that human H3N2 viruses cannot be transmitted to birds and presumably also not the other way around (avian H3N2 viruses to humans), without prior reassortment in pigs. The pig seems to be tolerant in receiving H3N2 viruses from humans as well as from birds. These species restrictions are determined by the NP (and possibly by the M protein), since rescue of other ts mutants of FPV by the Hong Kong virus is possible. The surface glycoproteins HA and NA seem to cross the species barrier relatively easily, since they were found in all viruses studied, independent of the species derivation[20].

The age-old agricultural techniques practised in South China, where humans, pigs and ducks live always in close contact, might explain why all human influenza virus pandemics start from that region[22]. The pigs seem to be the 'mixing vessels', where the new pandemic strains were created.

6. Concluding remarks

The particular structure of the main immunological component, the hemagglutinin, enables the influenza A viruses to 'drift' rapidly and to create variants which can escape the immune response of the host. This antigenic drift allows these viruses to spread to a certain extent and to cause relatively mild epidemics, since there is still some immunological cross reactivity to the foregoing strain. The segmented structure of the influenza A virus genome, however, can give rise to the creation of a virus with completely new surface antigens, which can then start a severe pandemic, because of a complete lack of immunity in the population. Furthermore, by replacement of other genes, the pathogenic properties might be severely influenced. For the antigenic shift a large reservoir of different viruses in another species is necessary, which contribute to the new surface antigens. The molecular mechanism by which such reservoirs can be built up and maintained in water fowls has been described, and also those which by reassortment led to new pandemic strains. This knowledge does not give us much hope that we will be able to control influenza efficiently in the near future. Therefore, it might be necessary to develop completely new ideas about how to prevent the spread of influenza viruses, and how to overcome the disease.

The epidemiological behavior of influenza A viruses could be explained by the particular structure of their genome and of their hemagglutinin. Therefore, generalizations with respect to other viruses are not permissible. Each virus type has developed its own strategy in order to infect an organism, to multiply there and to spread and to be transmitted to other individuals.

Acknowledgment. The work performed in the author's laboratory was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 47, and by the Fonds der Chemischen Industrie. I thank Dr B. Simpson for helpful discussions during the preparation of the manuscript.

1 Bonin, J., and Scholtissek, C., Mouse neurotropic recombinants of influenza A viruses. Archs Virol. *75* (1983) 255–268.
2 Gammelin, M., et al., manuscript in preparation.
3 Geraci, R., St. Aubin, D.J., Barker, I.K., Webster, R.G., Hinshaw, V.S., Bean, W.J., Ruhnke, H.L., Prescott, J.H., Early, G., Baker, A.S., Madoff, S., and Schooly, R.T., Mass mortality of harbor seals: Pneumonia associated with influenza virus. Science *215* (1982) 1129–1131.
4 Hinshaw, V.S., Webster, R.G., and Turner, B., Waterborne transmission of influenza A viruses. Intervirology *11* (1979) 66–68.
5 Hinshaw, V.S., Bean, W.J., and Webster, R.G., Biologic and genetic characterization of an influenza A virus associated with epizootic pneumonia in seals, in: Genetic Variation among Influenza Viruses, pp. 515–524. Ed. D.P. Nayak. Academic Press, New York–London–Toronto–Sydney–San Francisco 1981.
6 Kawaoka, Y., Naeve, C.W., and Webster, R.G., Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? Virology *139* (1984) 303–316.
7 Kistner, O., Müller, H., Becht, H., and Scholtissek, C., Phosphopeptide fingerprints of nucleoproteins of various influenza A virus strains grown in different host cells. J. gen. Virol. *66* (1985) 465–472.
8 Kistner, O., et al., manuscript in preparation.
9 Klenk, H.-D., and Rott, R., Cotranslational and posttranslational processing of viral glycoproteins. Curr. Top. Microbiol. Immun. *90* (1980) 19–48.
10 Palese, P., The genes of influenza virus. Cell *10* (1977) 1–10.
11 Reinacher, M., Bonin, J., Narayan, O., and Scholtissek, C., Pathogenesis of neurovirulent influenza A virus infection in mice. Route of entry of virus into brain determines infection of different populations of cells. Lab. Invest. *49* (1983) 686–692.
12 Scholtissek, C., Harms, E., Rohde, W., Orlich, M., and Rott, R., Correlation between RNA fragments of fowl plague virus and their corresponding gene functions. Virology *74* (1976) 332–344.
13 Scholtissek, C., Rott, R., Orlich, M., Harms, E., and Rohde, W., Correlation of pathogenicity and gene constellation of an influenza A virus (fowl plague). I. Exchange of a single gene. Virology *81* (1977) 74–80.
14 Scholtissek, C., The genome of the influenza virus. Curr. Top. Microbiol. Immun. *80* (1978) 139–169.
15 Scholtissek, C., Rohde, W., von Hoyningen, V., and Rott, R., On the origin of the human influenza virus subtypes H2N2 and H3N2. Virology *87* (1978) 13–20.
16 Scholtissek, C., Koennecke, I., and Rott, R., Host range recombinants of fowl plague (influenza A) virus. Virology *91* (1978) 79–85.
17 Scholtissek, C., The genes coding for surface glycoproteins of influenza A viruses contain a small conserved and a large variable region. Virology *93* (1979) 594–597.
18 Scholtissek, C., Vallbracht, A., Flehmig, B., and Rott, R., Correlation of pathogenicity and gene constellation of influenza A viruses. II. Highly neurovirulent recombinants derived from non-neurovirulent or weakly neurovirulent parent virus strains. Virology *95* (1979) 492–500.
19 Scholtissek, C., Stability of infectious influenza A viruses to treatment at low pH and heating. Archs Virol. *85* (1985) 1–11.
20 Scholtissek, C., Bürger, H., Kistner, O., and Shortridge, K.F., The nucleoprotein as a possible major factor in determining host specificity of influenza H3N2 viruses. Virology *147* (1985) 287–294.
21 Scholtissek, C., Molecular biological background of the species and organ specificity of influenza A viruses. Angew. Chem. int. Ed. *25* (1986) 47–56.
22 Shortridge, K.F., and Stuart-Harris, C.H., An influenza epicentre? Lancet *2* (1982) 812–813.
23 Skehel, J.J., Bayley, P.M., Brown, E.B., Martins, S.R., Waterfield, M.D., White, J.M., Wilson, I.A., and Wiley, D.C., Changes in the conformation of the influenza virus hemagglutinin at the pH optimum of virus mediated fusion. Proc. natl Acad. Sci. USA *79* (1982) 968–972.
24 Webster, R.G., Yakhno, M., Hinshaw, V.S., Bean, W.J., and Murti, G., Intestinal influenza: Replicaiton and characaterization of influenza viruses in ducks. Virology *84* (1978) 268–276.

0014-4754/87/11/121197-05$1.50 + 0.20/0