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The potential use of influenza virus as an agent for bioterrorism

Robert M. Krug*

Section of Molecular Genetics and Microbiology, Institute for Cellular and Molecular Biology, University of Texas at Austin, 2500 Speedway, Austin, TX 78712, USA

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Abstract

Influenza A virus has been responsible for widespread human epidemics because it is readily transmitted from humans to humans by aerosol. Recent events have highlighted the potential of influenza A virus as a bioterrorist weapon: the high virulence of the influenza A virus that infected people in Hong Kong in 1997; and the development of laboratory methods to generate influenza A viruses by transfection of DNAs without a helper virus. Antiviral drugs that are directed at functions shared by all influenza A viruses constitute the best line of defense against a bioterrorist attack. Consequently, new antiviral drugs need to be developed, and the few currently available antiviral drugs should be stockpiled.
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Influenza A and influenza B viruses, two of the four influenza virus genera, cause epidemics in humans (Lamb and Krug, 2001; Wright and Webster, 2001). Both influenza A and B viruses contain eight genomic RNA segments, and most, but not all, of the proteins encoded by the corresponding genomic RNA segments serve similar functions (Lamb and Krug, 2001). Influenza A viruses, which have been isolated from a wide variety of avian and mammalian species, are responsible for the widespread human epidemics, or pandemics, that have caused high mortality rates (Wright and Webster, 2001). Influenza B virus appears to infect only humans (Wright and Webster, 2001), although influenza B virus has recently been isolated from seals (Osterhaus et al., 2000). As discussed below, influenza A virus has the most potential as a bioterrorist weapon, so that this Chapter will focus solely on influenza A virus.

One of the genomic RNA segments of influenza A virus encodes the hemagglutinin (HA), the major surface protein of the virus against which neutralizing antibodies are produced. The antigenic structure of the HA of human influenza A viruses undergoes two types of changes (reviewed in Wright and Webster, 2001). Antigenic drift involves relatively minor changes in the HA that result from the selection of mutant viruses by antibodies generated against the major HA antigenic type circulating in the human population. As a result, a new strain (HA subtype) appears within a few years, causing epidemic disease until it is replaced by yet another HA subtype. Antigenic shift, which occurs much less frequently, leads to a major antigenic change. This change results from a replacement of the genomic RNA segment encoding HA. The new HA genomic segment is derived from an avian influenza virus, via reassortment with the currently circulating human strain or via direct transmission of the avian virus from birds or an intermediate host. Antigenic shift results in human influenza A viruses that cause widespread epidemics, or pandemics.

Epidemics and pandemics occur because influenza A virus is readily and rapidly transmitted from humans to humans by aerosol (Wright and Webster, 2001). Because new influenza A virus strains appear frequently as a result of antigenic drift in the HA, people are urged to get annual vaccinations. Nonetheless, each year as many as 40,000 Americans die due to influenza virus infection and its complications. The vast majority of the deaths occur among people with underlying disease—lung, heart, and kidney disease, and diseases that result in immunosuppression. Because deaths are largely confined to this subpopulation, many people view influenza virus—the flu—as a temporary annoyance. These people feel miserable for 1–2 weeks, and then return to work or school. “It was only the flu, but I was really miserable for many days.” During the anthrax scare, one of the concerns was to distinguish anthrax infection from “just the flu”.

* Tel.: +1-512-232-5563; fax: +1-512-232-5565.
E-mail address: rkrug@icmb.utexas.edu (R.M. Krug).

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Historical events have provided a less benign view of the potential dangers of human influenza A virus infections. Thus, pandemics, which result from antigenic shifts in the HA of influenza A virus, have caused death rates that are much higher than in non-pandemic years. The most devastating pandemic in 1918–1919—"Spanish flu"—caused 20–40 million deaths worldwide (reviewed in Reid et al., 2001). A large proportion of the deaths occurred among young adults (15–35-year-old), who did not appear to have any underlying disease. Because influenza virus was not identified until 1933 (Smith et al., 1933; Francis, 1934), the 1918–1919 virus itself has not been isolated. However, it has been possible to determine the sequence of several of the genomic RNAs of the 1918–1919 virus by using modern molecular biology approaches to analyze preserved autopsy samples (Reid et al., 2001). These sequences have not yet provided any insight into the severity of the 1918–1919 disease. In fact, it has been hypothesized that immunological factors may have been at least partially responsible for the severity of the disease(Reid et al., 2001). One hypothesis is that people in the 15–35-year-old age group, unlike older people, had never been exposed to a virus that bore antigenic resemblance to the 1918–1919 virus. As a consequence, the naïve immune systems of 15–35-year-olds may have been unable to respond in a timely way to infection by this particular influenza A virus.

There is no doubt about the high virulence of the influenza A virus that infected people in Hong Kong in 1997 (Claas et al., 1998; Suarez et al., 1998; Subbarao et al., 1998; Yuen et al., 1998). An avian influenza A virus that was directly transmitted from chickens to humans killed 30% (6 out of 18) of the infected humans. This virus contains the HA gene segment (H5 HA) from a goose virus (A/Goose/Guandang/1/96-like) and the other seven gene segments from a duck virus (A/Teal/HK/1/97-like) (reviewed in Wright and Webster, 2001). Because this virus contains the N1 neuraminidase (NA) gene, it is classified as an H5N1 virus. Specific sequences in at least two of the encoded proteins, the HA and the PB2 subunit of the viral polymerase, have been implicated in the virulence of this virus (Hatta et al., 2001a). Transmission of this virus from humans to humans did not occur, indicating that the acquisition of this property probably requires mutations and/or reassortment of genes with circulating human influenza viruses. It is thought that reassortment was prevented by the rapid slaughter of the poultry in Hong Kong (Wright and Webster, 2001). Because similar H5N1 viruses continue to circulate in Asian poultry markets (Webster et al., 2002), it is conceivable that a lethal influenza A virus will again be transmitted from chickens to humans, but in this case it may not be possible to prevent the virus from acquiring the ability to be transmitted from humans to humans. Clearly, coping with the rapid transmission of such a lethal influenza A virus would be a major challenge and would require strategies similar to those used to thwart a bioterrorist attack, as described below. In addition, such a naturally occurring lethal virus would be a potent bioterrorist weapon.

Even if such a lethal human influenza A virus does not emerge in nature, it is likely that it can be generated in the laboratory, utilizing the recently developed reverse genetic system, whereby influenza viruses can be generated by transfection of multiple DNAs without a helper virus (Fodor et al., 1999; Neumann et al., 1999). In fact, the pathogenic H5N1 virus has already been generated in at least one laboratory using this reverse genetic system (Hatta et al., 2001a,b). There is every reason to believe that the same recombinant DNA techniques can be used to render this H5N1 virus transmissible from humans to humans. Furthermore, it should be possible to introduce mutations into such a recombinant virus so that it is resistant to currently available influenza virus antivirals (M2 inhibitors: amantadine and rimantadine; and NA inhibitors: zanamivir and oseltamivir) (Hay et al., 1985; Pinto et al., 1992; Air et al., 1999), and so that it posseses an HA antigenic site that is unlike those in recently circulating human viruses. In fact, several viruses with different HA antigenic sites could be generated. The human population would lack immunological protection against such viruses, existing antiviral drugs would not afford any protection, and these viruses could be spread simply by release of an aerosol spray in several crowded areas.

It can be argued that most terrorists would not have the knowledge, facilities and ingenuity to carry out these recombinant DNA experiments. This is probably the case at the present time, but the situation can be expected to change in the future, perhaps after as little as 5–10 years. In addition, it should be kept in mind that the dangers inherent in producing lethal influenza virus strains will probably not be a deterrent, because modern terrorists have shown that they are willing to sacrifice their lives for their causes. The prudent course of action is to take advantage of the window of time that is available to prepare for the possible use of influenza virus as a bioterrorist weapon.

Vaccination will probably be of limited value against an influenza virus bioterrorist attack. Currently it takes about 6 months to prepare a vaccine against a new influenza virus strain. Perhaps reverse genetic approaches can shorten this time somewhat, but there will still be a few months between an influenza virus outbreak and the availability of a protective vaccine. In addition, the vaccine approach can be readily thwarted by bioterrorists who could spread several influenza viruses with different HA antigenic sites.

In contrast, antiviral drugs that are directed at functions shared by all influenza A virus strains constitute the best line of defense against a bioterrorist attack. Currently the NA inhibitors (zanamivir and oseltamivir) are the only such antivirals available (Air et al., 1999). Consequently, it would be prudent to maintain a stockpile of the NA inhibitors while other antiviral drugs are being developed. In fact, it would be advisable to establish this stockpile as soon as possible as a defense measure against the possible

emergence of a naturally occurring H5N1 virus that acquires human-to-human transmissibility.

Most importantly, new antiviral drugs directed against other viral targets should be developed. One promising target is the viral RNA-dependent RNA polymerase, which requires cellular primers to initiate viral messenger RNA (mRNA) synthesis (Krug et al., 1989). A viral endonuclease that is intrinsic to the polymerase cleaves cellular nuclear capped RNAs to produce capped fragments 10–13 nucleotides in length that serve as primers for viral mRNA synthesis. In the past, many research groups have screened for compounds that specifically inhibit the catalytic activity of the cap-dependent endonuclease, based on the premise that this reaction is unique to the virus and is not shared by cellular enzymes. A few compounds have so far been identified that appear to possess such specific inhibitory activity; these compounds have not yet been tested in animal or human studies (Tomassini et al., 1994, 1996). However, recent results from the author's laboratory (Li et al., 2001) argue against the possibility of identifying antiviral compounds that specifically target the catalytic activity of the viral cap-dependent endonuclease without adversely affecting cellular enzymes. We have shown that the viral cap-binding and endonuclease active sites are similar to the active sites of cellular enzymes: the viral cap-binding sequence has homology with the cap-binding site of the cellular eIF4E translation initiation factor; and the endonuclease active site is similar to the active sites of cellular enzymes that, like the viral endonuclease, cut polynucleotides to produce 3′-OH ends. Consequently, the toxicity of antiviral compounds directed against the activity of the viral cap-dependent endonuclease may be unacceptably high.

However, the influenza virus polymerase has another distinctive property that can be targeted. This polymerase lacks the catalytic activity for producing capped RNA primers unless the 5′- and 3′-terminal sequences of the viral genome RNA sequentially bind to specific amino acid sequences in the polymerase (Hagen et al., 1994; Cianci et al., 1995; Li et al., 1998, 2001). These activation steps are potential targets for the development of antiviral drugs. In addition, other viral proteins are potential targets. For example, the virus entry steps mediated largely by the HA could be targeted (Lamb and Krug, 2001). Another promising target is the virus-encoded non-structural (NS1) protein, particularly its interaction with two cellular proteins that are required for the 3′-end processing of cellular pre-mRNAs (Nemeroff et al., 1998; Chen et al., 1999; Li et al., 2001; Kim et al., 2002).

The development of these new antivirals can be carried out with the many non-lethal influenza A viruses currently used in many laboratories. Consequently, these experiments can be carried out under the approved existing biosafety conditions (BSL2), thereby expediting this research and enabling many laboratories to participate. At the same time, the few laboratories that have the facilities to carry out research on H5N1 viruses (approved BSL3+ biosafety conditions) should continue to elucidate the molecular mechanisms responsible for the virulence of these viruses. Such information on H5N1 viruses will be expected to provide new approaches for the development of antiviral drugs.

How would such antiviral drugs be used? First, it will be necessary to detect the appearance of a lethal influenza virus strain(s) and to distinguish influenza virus from other pathogens that produce similar symptoms. Currently, there are kits that can distinguish influenza A virus from other pathogens, but we need more sensitive tests that will also establish whether new influenza A virus strains have appeared. Such technology, i.e. biosensor technology, is in fact being developed (Scheller et al., 2001; Chaubey and Malhotra, 2002; O'Sullivan, 2002). Biosensors contain an immobilized biologically active compound, e.g. an antibody or an RNA aptamer, which specifically interacts with a target. This interaction results in a physical–chemical change in the immobilized biologically active compound, and this change is rapidly converted into an output signal. Biosensors should be more sensitive and rapid than other detection methods. In addition, biosensors can be designed to detect the appearance of an HA subtype that differs from the currently circulating HA subtype, thereby providing rapid evidence for the appearance of a new HA subtype. Undoubtedly, development of such biosensors should be accelerated. Indeed, biosensors are important components of the defense against all bioterrorist weapons.

Once a bioterrorist release of new pathogenic influenza A virus is detected, antiviral drugs could be administered. Because influenza A virus is so contagious, the most prudent course of action would be rapid administration of the antivirals to as many people as possible, not only to those with symptoms and their immediate contacts, but also to people in surrounding areas. Consequently, it is essential that a stockpile of antiviral drugs be maintained. Of course, vaccine development should also be initiated to provide a later, secondary means of defense.

Even with a concerted, coordinated effort, the development of new safe and effective anti-influenza virus drugs is a long-term project, one that will probably take at least 5–10 years. Accordingly, it is essential to start such a program now, and take advantage of the window of time before influenza virus becomes a viable weapon for bioterrorists. It should be emphasized that the results of these efforts will also be of great use for the control of future naturally occurring influenza A virus pandemics.

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