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Preventive and therapeutic approaches to viral agents of bioterrorism

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Certain viruses, such as those that cause smallpox and hemorrhagic fevers, have been identified as possible bioterrorism agents by the Centers for Disease Control and Prevention. They have been designated as potential threats because large quantities can be propagated in cell culture, they are transmissible as aerosols and, for the most part, there are only limited vaccine and pharmaceutical strategies for either prevention or treatment of established infection. An additional concern is the potential to genetically modify these agents to enhance virulence or promote resistance to vaccines or identified antivirals. Although the major impact of these agents is human illness, the release of zoonotic agents, such as the Nipah virus, would have consequences for both humans and animals because infected and noninfected animals might need to be sacrificed to control the spread of infection. Continued research is necessary to develop effective strategies to limit the impact of these biological threats.

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▼ NATO has identified biological agents capable of being used as weapons of mass destruction and the Centers for Disease Control and Prevention (CDC; http://www.cdc.gov) has categorized these into three groups based on their potential for production, dissemination and impact on health and public perception. Many of these agents are viruses. In addition, there are only limited strategies for the early detection of pathogens released as a bioterrorism event. In fact, the event will probably only become obvious when victims start to appear in healthcare facilities manifesting signs of the disease. The hours following the detection of a bioterrorism event are crucial for minimizing casualties. It is during this time that antimicrobials and vaccines must be readily available for post-exposure prophylaxis and treatment. This review discusses current concepts for prevention, post-exposure prophylaxis and treatment of some of the viral pathogens that could be released as agents of bioterrorism.

Smallpox

Smallpox is one of the oldest infections, with the earliest descriptions dating back to 10,000 BC [1]. Humans are the only known host and infection is spread by aerosol or direct contact. Smallpox is a serious threat as a bioweapon because infection poses a 30% fatality rate among unvaccinated individuals and no specific, licensed antiviral therapies are available [2]. One of the first recorded uses of smallpox as a potential bioweapon dates back to the French–Indian Wars in the 18th century when the British delivered blankets contaminated with smallpox to the American Indians. As a result of a global vaccination campaign, smallpox was eradicated in the late 1970s. Routine vaccinations ceased in the United States in 1972, leaving more than 40% of the current US population unvaccinated [2]. Although the course of smallpox might be ameliorated in those vaccinated before 1972, it is unlikely that this previous vaccination would protect against smallpox infection. Owing to diminished population immunity against the organism, it is estimated that as few as 50–100 cases of smallpox could lead to a massive epidemic [3]. Compounding this concern is the absence of approved, effective antiviral agents to treat patients infected with smallpox and the problems associated with developing new vaccination strategies.

Prevention

Preparations for a potential release of smallpox include decisions on pre-event and postevent vaccination and the development

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of effective, safe antiviral therapies for those who develop clinical illness. Studies for preventing infection with smallpox have evaluated immunoprophylaxis, chemoprophylaxis and vaccination based strategies [1,4–27]. Kempe and colleagues demonstrated the partial protective efficacy of vaccinia immune globulin (VIG) as post-exposure immunoprophylaxis in humans [4]. Adult and child contacts of smallpox patients were given vaccinia hyperimmune globulin and demonstrated a reduction in the incidence of smallpox compared with controls. Recently, Ramirez *et al.* demonstrated that pretreating mice with a monoclonal antibody directed against a 14 kDa trimeric protein, important in replicating vaccinia virus, protected against intraperitoneal challenge infection with vaccinia, although it had limited therapeutic effect once the infection was established [5]. If given after the challenge infection, the monoclonal antibody delayed time of death but did not improve survival. However, immune sera strategies for post-exposure prophylaxis are complicated by the limited supply of VIG and the need for parenteral administration. VIG use is best suited to manage those patients with severe vaccine reactions or those who are immunosuppressed and, therefore, at greater risk for vaccine complications.

Chemoprevention

Few studies have reported the benefit of antiviral agents for preventing smallpox infection. Methisazone (1-methylisatin-3-thiosemicarbazone) protects mice against challenge infections with *Variola major* but is of equivocal value in preventing infection in humans, and hence is not recommended [6–8]. The benefit appears to be in mice who have partial immunity to smallpox [7]. Recently, the acyclic nucleoside phosphonate, cidofovir (*S*-1–3-hydroxy-2-phosphonylmethoxypropylcytosine) protected mice against challenge infections with vaccinia virus or cowpox virus, thus suggesting potential efficacy against smallpox [9–12]. Cidofovir has broad antiviral activity against several DNA viruses, including orthopoxviruses, in cell culture, thereby making it a potential paradigm drug for smallpox studies [9,10]. Bray and colleagues studied the efficacy of cidofovir in a lethal model of aerosolized cowpox that simulates a potential intentional release of smallpox [11]. Aerosolized or subcutaneous cidofovir given immediately before an aerosolized challenge infection was effective in preventing illness and death. Similar findings were observed by Smee *et al.* who demonstrated that mice treated with cidofovir had greater survival and less severe lung disease than placebo treated mice after vaccinia virus challenge infections [12]. These studies suggest a possible role for cidofovir as chemoprevention for those exposed to the intentional release of smallpox, although these data are investigational and cidofovir has not been approved for this use.

Vaccination

Considerable controversy exists over the wisdom of a pre-event vaccinia virus vaccination strategy involving healthcare workers and members of the military [1,13–18]. The debate centers around the true threat of smallpox, the risks associated with the vaccinia vaccine**,** the duration of immunity provided by the vaccine and the relative value of postevent prophylaxis. Vaccinial immunity is defined as the prevention of smallpox infection and it is estimated that neutralizing antibodies might remain fairly stable over time following revaccination [19]. However, it is not fully understood whether this is sufficient to limit the severity of a smallpox epidemic. Older studies have shown that both the incidence of severe disease and mortality from smallpox might be reduced significantly in those who contract the disease 10–20 years after vaccination [20].

The CDC has supported the recent call for limited pre-event vaccination focusing on healthcare workers and the military [21]. Using a mathematical model that assumes a 30% mortality rate from smallpox and one vaccine related death per 1 million vaccinations, Kaplan and colleagues have called for mass vaccination to prevent deaths from smallpox and to achieve faster epidemic eradication. In calling for mass vaccinations, these authors assume that the potential mortality rate associated with smallpox would be far higher without the aggressive use of the vaccinia vaccine [14]. Bozzette and colleagues modeled vaccine strategies depending on different types of theoretical attacks and argue that pre-event vaccination strategies are likely to save lives in high impact scenarios, such as smallpox release in a major airport [15]. Halloran *et al.* identified the potential limitations of targeted ring vaccination strategies versus mass vaccination and the impact that residual smallpox immunity within the community has on a vaccination strategy [16]. Using a stochastic simulation of the spread of smallpox following an intentional release, the authors argue that pre-event residual immunity against smallpox in the adult community would lower the probability of a major epidemic substantially, and although a ring vaccination strategy following release would have fewer vaccine related complications, mass vaccination following an event might be more effective.

Vaccination strategies

So far, US vaccine based strategies depend on the use of the dried calf lymph type vaccinia vaccine [Dryvax, Wyeth Laboratories (http://www.wyeth.com/)]. Although this vaccine is highly effective in evoking protective immunity, it

has significant side effects for vaccinees and their contacts, including inadvertent transmission to other sites on the vaccinee or to contacts, progressive vaccinia, generalized vaccinia, eczema vaccinatum and postvaccinia encephalitis [1,2,13,22]. The true risk to the vaccinated group is not known, but it could be substantial depending on the predictive model used [15]. A related issue is the overall contagiousness of the vaccinia virus. Although the true risk of intrafamily or nosocomial spread of vaccinia following vaccination is unknown, the historical rate of transmission could range from 9–59% [23]. Owing to the risk of the vaccine and the inability to clearly define the actual risk of smallpox being released as a terrorist event, some argue that a postevent vaccination strategy could be adequate because it is widely believed that the post-exposure protective window for vaccination is 4 days. However, a recent study indicated that vaccination within 4 days of exposure could decrease mortality but might not prevent smallpox, thereby limiting the ability to rapidly control the spread of infection [18]. Previous success in controlling a smallpox outbreak depended on prompt vaccination of the exposed individuals, which usually occurred in a setting of significant population immunity. Today, mass vaccination would be primary and not revaccination, which might decrease the severity of illness but would not necessarily prevent the disease entirely.

The bioterrorism threat necessitates the evaluation of potentially safer vaccines to prevent smallpox and antivirals to treat those individuals with infection. Alternatives to the Dryvax vaccine include attenuated, nonreplicating vaccinia strains such as the modified vaccinia Ankara (MVA) and LC16m8. MVA is a nonreplicating, live vaccinia virus that is immunogenic in humans and possibly safer for the general public and those with immunodeficiency or atopic dermatitis [24]. MVA evokes protective immune responses in transgenic mice challenged with vaccinia virus [25]. The LC16m8 strain is a temperature sensitive, attenuated variant of the Lister strain of vaccinia virus that has a take rate equivalent to Dryvax, but produces a lower antibody response in humans [26,27]. It appears to have a lower fever rate and neurovirulence and so might be a safer vaccine [27]. However, the protective efficacy of either vaccine in humans is not fully known because they have not been tested during periods of smallpox occurrence.

Antiviral strategies for smallpox treatment

Antiviral therapy for those individuals with smallpox infection or a complication resulting from vaccinia vaccination is limited. Adenine arabinoside and cytosine arabinoside have activity against vaccinia and cowpox viruses in cell culture, but not in animal models [2,28]. These agents are no longer considered for treatment of smallpox infections. Based on laboratory data, inactive or ineffective agents also include the thymidine kinase inhibitors, such as acyclovir, the nucleoside or non-nucleoside reverse transciptase inhibitors, zidovudine, didanosine and efavirenz, and protease inhibitors [29,30].

Several agents are being investigated as potential therapeutics for smallpox infection [10]. These studies depend on the activity of the antiviral drug against certain orthopoxviruses in either cell culture or in animals as a predictive model for their potential use against smallpox. Of those tested, the most promising agent is the acyclic nucleoside phosphonate, cidofovir [30]. Cidofovir has been approved for the treatment of cytomegalovirus retinitis. This drug has good activity against several orthopoxviruses in cell culture systems and animals models [11,12,29–34]. In animal studies, cidofovir delayed viral replication in the lungs and the time of death in severe combined immunodeficient mice challenged with vaccinia virus [33]. Bray and colleagues demonstrated that aerosolized cidofovir prevented lung injury and death in weanling Balb/C mice challenged with cowpox virus, a related orthopoxvirus [11]. Although premature, the authors suggest that cidofovir might be effective as a pre-exposure or immediate post-exposure agent for humans that could be delivered by a dried aerosol powder similar to the neuraminidase inhibitor, zanamivir. However, they indicate that parenteral cidofovir would be the more appropriate choice for established infection. In another study, subcutaneously administered cidofovir reduced respiratory tract viral titers and death following infection with vaccinia virus [12]. To date, there have only been individual case reports in which cidofovir was used to treat humans infected with the molluscum contagiosum virus or orf virus, both of which are poxviruses.

Although cidofovir has excellent *in vitro* activity against orthopoxviruses, its use might ultimately be limited by its nephrotoxicity and poor oral bioavailability. Alkoxyalkyl esters of cidofovir and cyclic cidofovir have been compounded to address issues of bioavailability. Kern and colleagues tested these antiviral analogs in fibroblast cell cultures infected with cowpox or vaccinia virus [34]. The analogs are significantly more active than the parent drugs but might be more cytopathic in cell culture. Preliminary studies have reported on one of the analogs, 1–0-hexadecyloxypropyl-cidofovir (HDP-CDV), in mice [32]. This compound, and octadecycloethyl-cidofovir (ODE-CDV) are licensed by Chimerix (http://www.chimerix-inc.com) and should be tested in nonhuman primates in the future.

Other licensed agents that inhibit orthopoxvirus replication experimentally include gemcitabine, trifluridine and idoxuridine [29,30,35]. Gemcitabine is licensed as an antineoplastic drug and has activity in cell culture against vaccinia and cowpox viruses, but elicits a significant

cytopathic effect that potentially limits its utility [29]. Trifluridine and idoxuridine are licensed to treat herpes simplex keratitis and both have activity against vaccina and cowpox viruses in cell culture [29]. Idoxuridine reduces vaccinia lesions and delays death in mice following vaccinia virus challenge infections [35].

Several other drugs demonstrate potential promise. Adefovir dipivoxil, approved for treating chronic hepatitis B infection, has good activity against vaccinia and cowpox viruses in cell culture and has favorable oral bioavailability [29]. Its activity against variola has not been reported. Certain S-adenosylhomocysteine hydrolase inhibitors have excellent activity against some of the orthopoxviruses but have had limited testing in animal models, so should be considered as investigational [10,28,30].

Perhaps understanding how an orthopoxvirus interferes with natural antiviral defenses might allow for the development of new treatment strategies. For example, the mouse poxvirus genome encodes for a soluble CD30 homolog of mammalian CD30 that inhibits γ-interferon production and Th-1 cytokine mediated inflammation [36]. Theoretically, further understanding of this antiviral defense mechanism could have implications for treatment of these viral infections.

Hemorrhagic Fever viruses

Hemorrhagic Fever viruses (HFV) are small lipid enveloped RNA viruses belonging to four taxonomic families. These include filoviruses (Ebola and Marburg), bunyaviruses (Hantaan, Rift Valley Fever and Crimean–Congo Hemorrhagic Fever), flaviviruses (Yellow Fever and tick-borne encephalitis viruses), and arenaviruses (Junin, Machupo, Tacribe, Sabia, Guanarito and Lassa). Several of these viruses represent potential threats because they can be produced in large quantities and aerosols can infect humans [37]. The threat might be even greater for the arenaviruses because the genome is subject to inter- or intrasegmental reassortments that could give rise to enhanced virulence [38].

Some HFV have been turned into weapons by researchers in the former Soviet Union, USA and possibly North Korea [37]. Humans are incidentally infected following exposure to contaminated excretions of animals, insect bites or, less commonly, from human to human transmission. The clinical illness is usually nonspecific early on with influenza-like symptoms. Later stages of illness include manifestations of hemorrhagic diathesis including thrombocytopenia, disseminated intravascular coagulation and circulatory collapse. The fatality rate varies depending on the organism involved, with the highest mortality seen in those infected with certain Ebola strains [37].

Immune sera for prevention and treatment of HFV infection

The threat of HFV as bioweapons is augmented by the knowledge that there are limited strategies to prevent or treat these infections. Immune plasma or whole blood transfusion has reduced mortality in humans infected with Junin (Argentine Hemorrhagic Fever) and Ebola [39,40]. Immunoprophylaxis has been demonstrated in animal models of Ebola infection [41,42]. Human or primate immune plasma demonstrated a dose dependent, strain specific protection in monkeys challenged with Lassa Fever virus [43]. In these studies, cynomolgus monkeys inoculated with Lassa virus received infusions of high titer plasma on days 0, 3 and 6. Monkeys treated with immune plasma had a case fatality of only 5% compared with 87% of untreated control animals. Recently, Xu and colleagues demonstrated protection of mice by murine monoclonal antibodies directed against the envelope glycoprotein G2 of the Hantaan virus [44]. Although antibodies directed against the G2 glycoprotein were protective, those raised against viral nucleocapsid protein were not. Takada and colleagues identified the protective epitopes on Ebola virus glycoprotein and demonstrated the efficacy of monoclonal antibodies in the passive protection or treatment of mice challenged with Ebola virus [45]. These studies suggest an adjunctive role for immune sera in certain HFV infections, although this use is limited by the need for parenteral administration, limited availability and the lack of definitive clinical trials.

Vaccination

The current status of vaccines to prevent HFV infections is disappointing. The only licensed vaccine is the 17D strain Yellow Fever vaccine. A prospective, randomized, placebo controlled trial reported by Maiztegui *et al.* demonstrated the strain specific protective efficacy of an attenuated live viral vaccine (Candid I) against Junin virus in agricultural workers residing in an endemic region [46]. Agricultural workers living in a Junin endemic region received the Candid I vaccine and no serious side effects were reported. Of the 23 laboratory confirmed cases of Junin, only one occurred in a vaccine recipient, demonstrating the protective efficacy of the vaccine [46]. It is not known whether the Candid I vaccine will provide cross-protection against infection by other arenaviruses.

Several potential vaccine constructs have shown efficacy in animals infected with Lassa Fever and Ebola viruses [47–49]. Fisher-Hoch and colleagues reported the protective efficacy of a Lassa Fever vaccine consisting of a vaccinia virus expressing structural proteins of Lassa virus in a macaque model [47]. Wilson and Hart demonstrated protection in mice challenged with Ebola virus by a Venezuelan equine encephalitis replicon vaccine that encodes for Ebola virus nucleoprotein [48].

Not only did the vaccine protect mice against challenge infections, but also cytotoxic T lymphocytes derived from vaccinated mice and transferred to unvaccinated mice were protective. Recently, Rao and colleagues demonstrated protection against Ebola virus in laboratory animals vaccinated with liposome encapsulated irradiated Ebola virus [49].

Antiviral therapy

Antiviral therapy for HFV infections is limited and usually effective against only a limited number of viruses. Reports have focused on the activity of drugs on specific viruses using cell culture assays. For example, chlorpromazine and trifluoperazine (phenothiazines licensed as an antiemetic and antipsychotic, respectively) are inhibitory in cell culture against Junin and Tacribe viruses (arenaviruses), but are untested in animal models or humans [50]. Several agents are in the early stages of evaluation. Disulfide based compounds, including intermolecular aromatic disulfides and sulfur containing dithianes, have activity against Junin and Tacribe in cell culture but their efficacy *in vivo* remains unknown [51]. These agents quickly inactivate the viruses in culture in a concentration and time dependent manner. Several inosine monophosphate dehydrogenase inhibitors have been evaluated, including ribamidine and EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-carboxamide), each of which have efficacy against arenaviruses in cell culture and animal models [38]. Few antivirals have reported activity against filoviruses. The experimental *S*-adenosylhomocysteine hydrolase (SAH) inhibitor, carbocyclic-3-deazaadenosine, is active against Ebola virus in cell culture and has had limited positive results in animal models [38,52–54].

A promising agent is the inosine monophosphate inhibitor, ribavirin, that is licensed for the treatment of hepatitis C virus and respiratory syncytial virus infections. It has broad activity against HFV except filoviruses and flaviviruses and is effective in cell cultures, animal models and humans [38,55–58]. Jahrling *et al.* demonstrated increased survival of Lassa virus infected monkeys when treated with ribavirin [58]. Treatment with ribavirin, if given within the first 4 days, significantly decreased viremia and death, but had only a modest protective effect if given after day 7. Kilgore *et al.* reported on three individuals infected with Machupo virus, an arenavirus, who were treated with ribavirin, two of whom recovered significantly [55]. The efficacy of ribavirin might be enhanced by coadministration of immune sera [57,58]. Weissenbacher and colleagues demonstrated improved survival of marmosets challenged with Junin, an arenavirus, when treated with ribavirin and immune sera derived from marmosets immunized with an attenuated strain of Junin [57]. In a monkey model of Lassa virus infection, animals treated with ribavirin and immune sera demonstrated a higher survival rate compared with untreated controls or animals treated with ribavirin alone [58]. If a bioterrorism event is caused by viruses other than filoviruses or flaviviruses, ribavirin is recommended under an investigational new drug protocol for empiric treatment of those who manifest clinical symptoms [37]. However, post-exposure prophylaxis with ribavirin is not recommended in those who are exposed but asymptomatic.

Other potential viral agents

Other viruses might emerge as potential bioterrorism agents. Chief among these is influenza A because this organism is readily transferable between humans, might possess significant virulence and could be genetically modified to enhance virulence or resist currently available antivirals [59]. Nipah virus, a recently described, deadly paramyxovirus, causes significant neurological and respiratory disease in swine and severe encephalitis in humans [60]. It is attractive as a bioterrorism agent because it has a mortality rate of 40%, readily infects humans and animals and there are limited treatment options (ribavirin). The control of infection in Malaysia required the culling of 1.1 million pigs from 900 farms, demonstrating the significant economic and political impact than an intentional release would generate.

Conclusions

Viruses possess the attributes necessary to serve as potential agents of bioterrorism. As a group they are virulent and transmissible to a greater or lesser extent, and there are only limited strategies for prevention or treatment of infection. Encouraging studies indicate a greater understanding of these organisms and potential methods to protect humans from infection and to treat those with established infections. Continued research is needed to identify safe and effective vaccines and develop antiviral agents that can be used for post-exposure prophylaxis and treatment. Consistent public health policy will also be necessary to ensure preparation for the intentional release of a biological weapon of mass destruction.

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