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Smallpox: Pathogenesis and Host Immune Responses Relevant to Vaccine and Therapeutic Strategies

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1. INTRODUCTION

Recently, smallpox has been a disease of only historical interest since the certification of its eradication by the World Health Organization on May 8, 1980.[1] However, there is a growing awareness and apprehension regarding possible bioterrorist threats with these concerns escalating since the tragedy on September 11, 2001. Accordingly, there is an increased interest in understanding smallpox-induced pathogenesis as well as in the development of new vaccines and therapeutics. This chapter will discuss the history of smallpox infection and its eradication. Discovery of methods for protection against naturally occurring smallpox infection will also be discussed, as well as clinical and epidemiological features of infection, virus structure and pathogenesis as well as host defense and the immune response. An improved understanding of the disease is leading to new methods of prophylaxis and therapy that are discussed in this chapter. In addition, current vaccination strategies will also be reviewed since the development and licensure of novel smallpox vaccines

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that may be safely used to immunize those with exposure and/or risk factors is currently of high priority.

2. HISTORY OF SMALLPOX INFECTION AND ITS ERADICATION

Once prevalent throughout the world as an endemic infection, wherever concentrations of population were sufficient to sustain transmission, smallpox usually found its major reservoir in children because there is no animal reservoir for smallpox. Therefore, the virus had to spread continually from human to human to survive with epidemics occurring when travelers carried the agent to outlying populations that lacked immunity. Smallpox was first described in South Africa by de Korte in 1904 and in the United States by Chapin in 1913, and subsequently became prevalent throughout the United States, parts of South America, Europe as well as some areas of Eastern and Southern Africa.[2] The first evidence that smallpox emerged as a pathogen was some time after the first human agricultural settlements, about 10, 000 B.C., while the first scientific evidence for smallpox was identified in the mummified remains of the 18th Egyptian dynasty and Ramses V.[3] Written descriptions of smallpox typically did not appear until the fourth century A.D. in China and the tenth century in southwestern Asia. However, earlier descriptions, although rare, did appear such as the one by Thucydides in 430 B.C. in Athens.[4] There is no Greek or Latin word for smallpox, although the name *variola*, derived from the Latin *varius*, meaning pimple was first used during the sixth century by Bishop Marius of Switzerland. By the tenth century, the word *poc* or *pocca*, a bag or pouch, was used to describe smallpox and the prefix *small* was used to distinguish variola, the "small pox", from syphilis, the "great pox".[5]

The first immunization procedure was termed "variolation", in which material from pustules or scabs from infected persons were deliberately inoculated into the skin, a method first carried out in India sometime before A.D. 1000.[6] This method resulted in an infection that was usually less severe than an infection acquired naturally by inhalation of droplets. Importantly, the method of variolation was brought to England by Lady Mary Wortley Montague. She had been disfigured by smallpox in 1715 and while in Istanbul with her ambassador husband she became aware of the practice of variolation. She, in fact, had her son and daughter "variolated" which led to some acceptance of this method, which then spread throughout England. Although a small percentage of individuals "purposely" infected by the variolation method did not survive, the mortality was considerably lower than in those naturally exposed and infected with smallpox. In 1796, Edward Jenner discovered that infection with a more benign poxvirus caused by cowpox virus, prevented subsequent smallpox infection. He called the material *vaccine*, from the Latin *vacca*, meaning cow. The process of *vaccination* then began to be employed widely in many countries of Europe, and within a decade, it had been transported to countries throughout

the world. Several reasons existed for opposition to the use of Jenner's vaccine strategy including being able to find cows infected with cowpox, or in some cases, there was a significant opposition among religious leaders who opposed the principle of infecting humans with an animal-derived serum as being unnatural. Moreover, confidence in the procedure was challenged when some individuals who had been previously "vaccinated" acquired smallpox infection. With the flank of the calf offering an adequate and safer supply, the numbers of vaccinations in Europe increased and subsequently the incidence of smallpox in more industrialized countries diminished more rapidly. However, in less developed areas, smallpox infection continued until the middle of the twentieth century due to the development of a freeze-dried vaccine.[7] With such a valuable vaccine available, a global eradication plan was initiated by the Pan American Sanitary Organization in 1950, followed by a plan in 1958 by the Union of Soviet Socialist Republics proposed to the World Health Assembly that a global smallpox eradication plan should be undertaken. However, it was not until 1966, when the World Health Organization provided more funding that a more intensified program was initiated. Interestingly, in 1967, an estimated 10 to 15 million smallpox cases occurred in 31 countries in which the disease was endemic. Therefore, the campaign carried out massive vaccinations in each country reaching at least 80% of the population, and also developed a system to contain cases and outbreaks. A total of 3, 234 cases of smallpox were reported from Eastern Africa to the World Health Organization[8] in the period January 1–December 6, 1977. The last reported indigenous known case of smallpox occurred in Somalia on October 26, 1977 in the Merca District. The source of this case was a known outbreak in the nearby district of Kurtuware and all 211 contacts were traced, revaccinated, and kept under surveillance. The last known case of smallpox in Ethiopia occurred on August 9, 1976, in El Kere Region, while in Kenya, the last case was on February 5, 1977, in the Mandera District (1977). In 1980, the World Health Organization declared smallpox globally eradicated.[2,9,10]

3. CLINICAL AND EPIDEMIOLOGICAL FEATURES

Smallpox is a viral disease unique to humans, which is spread from person to person by inhalation of air droplets or aerosols. Twelve to 14 days after infection, an average patient has a 2- to 5-day period of high fever, malaise, and prostration with headache and backache followed by the development of maculopapular rash over the face which then spreads to the extremities. These clinical symptoms are listed in Table I as compiled and summarized by the Centers for Disease Control and Prevention (CDC). The rash appears on the mucosa of the mouth and pharynx, the face, and the forearms and spreads to the trunk and legs, and becomes vesicular and then pustular, characterized by round, tense, and deeply embedded in the dermis when crusts begin to

TABLE I
Clinical Symptoms of Smallpox as Described by the CDC

Clinical Stage/Sympton	Duration	Contagious	Description
Incubation Period	7 to 17 days	NO	Following initial exposure, sympton free
Initial Symptoms	2 to 4 days	YES	Fever, malaise, head and body aches, vomiting "prodromal phase"
Early Rash	4 days	YES	Rash emerges as small red spots on tongue and mouth Rash develops into sores that break open Rash then appears on skin, starting on face and spreading to arms and legs, then to hands and feet Within 24 hours, rash spreads to all parts of the body As rash appears, fever falls and person may feel better By the third day, the rash becomes raised bumps By the fourth day, the bumps fill in with thick, opaque fluid and often have a depression in the center. Fever will rise at this time and remain high until scabs form over the bumps
Pustular Rash	5 days	YES	The bumps become pustules, sharply raised, round and firm
Pustules and Scabs	5 days	YES	The pustules begin to form crust and then scab
Resolving Scabs	6 days	YES	Scabs fall off, leaving marks on skin that eventually become pitted scars
Scabs Resolved	—	NO	Scabs have fallen off and person is no longer contagious

form by the 8th or 9th day. Eventually scabs form, which separate and leave pigment-free skin, and pitted scars. In 5 to 10% of smallpox patients, more rapidly progressive, malignant disease develops. Late in the 1st week or during the 2nd week of illness, death occurs due to the effects of overwhelming viremia.[11,12] On occasion, a severe and fatal hemorrhagic form occurs with extensive bleeding into the skin and gastrointestinal tract. Importantly, vaccination before exposure or within 2 to 3 days of exposure offers complete protection against the disease, while vaccination as late as 4 to 5 days post-exposure may protect against death. Although the epidemiology of smallpox infection including morbidity and mortality has been described, the molecular mechanisms of smallpox-induced death are unclear.

Variola virus spreads most readily in the dry and cool winter months but can be transmitted in any climate and in any part of the world. The age distribution of cases depends primarily on the degree of smallpox susceptibility in

the population. In most areas, cases predominated among children because adults were protected by immunity induced by vaccination or previous smallpox infection.[13] In rural areas that had not been previously vaccinated or had smallpox infections, the distribution would be similar to the age distribution of the population.[13] The triumph of the global smallpox eradication has led to an irony in that the ensuing worldwide cessation of vaccination has rendered many of today's population susceptible to infection, which has resulted in smallpox becoming a significant bioterror weapon.

4. VIRUS STRUCTURE AND CLASSIFICATION

Poxviruses are the largest and most complex viruses that infect humans and belong to the genus *Orthopoxvirus*, family Poxviridae, which includes the agents vaccinia, monkeypox, cowpox, camelpox, and ectromelia.[14] Poxviruses are made of a single molecule of double-stranded DNA and have the ability to replicate in the cytoplasm rather than the nucleus of susceptible cells.[15] The linear genome contains approximately 200 genes ranging in size from 130-kb to 260-kb with those in the central region encoding proteins involved in virus uncoating, genome replication or virion structure. The DNA polymerase has conserved the sequences of these genes, and the flanking regions contain genes encoding proteins that modify the intra- and extracellular environment so that virus can replicate and spread. The virions are large and brick-shaped and range in size from 160 nm to 300 nm. Poxvirus replication occurs in the cytoplasmic inclusions, where infecting virions are partly uncoated by cellular enzymes and fully uncoated by viral enzymes released from the virion core. The replication cycle can be divided into functions controlled by early (pre-replicative) gene products and late (post-replicative) gene products. Most virions remain within cells and lack the outer envelope found on naturally released virions. In addition, each infected cell produces two different kinds of virions.[16] The majority of intracellular mature virions remain within necrotic cells and are shed in skin debris or saliva droplets, where they serve as sources of infection. The second type of virion makes up a small percentage that acquire an additional membrane and are transported to the cell surface where they become extracellular enveloped virions that are responsible for cell to cell spread and may participate in systemic dissemination. The membrane antigens may be the targets for humoral immunity and neutralizing antibody responses; however, the core antigens are not expressed on the viral membrane and therefore are only the targets of cellular immunity.

5. PATHOGENESIS, HOST DEFENSE, AND THE IMMUNE RESPONSE

Naturally occurring smallpox infection is initiated by the implantation of variola virus on the oropharyngeal or respiratory mucosa. Replication at the

point of entry is followed by infection of mononuclear phagocytic cells in regional lymph nodes, possibly with further spread through the bloodstream to similar cells in the liver, spleen, and other tissues. Virions in droplets expressed from nasal and oropharyngeal secretions are far more infectious than virions bound in the scab itself. After migration and multiplication in regional lymph nodes, an asymptomatic viremia develops around the 3rd or 4th day, followed by multiplication of virus in the spleen, bone marrow, and lymph nodes. A secondary viremia begins around the 8th day, accompanied by fever and toxemia. This means that the incubation period has ended when the release of inflammatory mediators from infected cells caused fever and other symptoms, and the spread of virus (either within infected monocytes or as free virions) to capillaries in the skin and mucosal membranes initiates the rash. The virus, contained in leukocytes, localizes in small blood vessels of the dermis beneath the oral and pharyngeal mucosa and subsequently infects adjacent cells. In the skin, this process results in the characteristic maculopapular lesions, and later vesicular and pustular lesions. After reaching the skin, disease severity is determined by the ability of host responses to limit viral replication during the incubation period, as reflected by the level of secondary viremia. Secondly, once viral dissemination has occurred, many features of severe illness are the result of host inflammatory responses including the release of chemokine, cytokines, and other mediators into the bloodstream, causing vascular dysfunction, coagulopathy, and multiorgan failure resembling septic shock.[17,18]

Both the humoral and cellular immune responses play important roles in host defense against smallpox infection. Specifically, the humoral response to smallpox infection results in the production of short-lived IgM and persistent IgG, and it may be elicited by inactive virions or viral antigen by non-enveloped or enveloped virus. There are three classes of antibodies important in the host immune response to infection.[16,19–22] The first are antibodies elicited against both non-enveloped virus and enveloped virus resulting in the neutralization of viral infection. The second types of antibodies are those that combine with complement to lyse virus-infected cells. The third are antibodies that combine with circulating antigen to produce immune complexes resulting in some of the toxic symptoms seen in the host during smallpox infection. These classes of antibodies are listed in Table II. Hemagglutinin-inhibiting and neutralizing antibodies could be detected beginning about the 6th day of illness, or about

TABLE II
Classes of Antibodies Important to Host Immune Responses to Smallpox

Elicited against enveloped and non-enveloped virus resulting in neutralization of virus infection
Those combining with complement to lyse virally infected cells
Those which combine with circulating antigen to produce immune complexes resulting in some of the toxic symptoms noted in the host during smallpox infection

18 days after infection, while complement-fixing antibodies could be identified approximately 2 days later.[23,24]

Moreover, in addition to B cell responses, the cellular response generated following infection also elicits CD4+ T helper and cytotoxic CD8+ T cells, as well as natural killer cells to combat infection.[19,25] The direct effects of T cells on virus-infected cells as well as secreted products including IFN-gamma play a role in host immune responses to smallpox infection. Therefore, it is likely that both humoral and cell-mediated immunity are important in protecting against smallpox. Patients with genetic defects in either B cell or T cell immunity are at increased risk of complications following smallpox vaccination.[26] Moreover, various animal models have also shown that adoptive transfer of either neutralizing antibodies[27–29] or virus-specific T cells[30,31] can provide full protection against vaccinia infection.

6. FEATURES OF SMALLPOX MAKING IT A LIKELY BIOTERROR AGENT

Of the potential biological weapons, smallpox poses by far the greatest threat, albeit because of its clinical and epidemiological properties. Smallpox poses a serious threat because a large segment of the population that has not been previously vaccinated is now susceptible, due to the continued vaccination program being halted several decades ago coincident with the eradication of the disease. It is currently debated by virologists and immunologists as to what percentage of the population vaccinated 30 years ago is protected in terms of morbidity and mortality. It is expected that the case fatality rate after infection with smallpox in the non-vaccinated/non-protected individuals is 30%. Moreover, virus, in an aerosol form, can survive for 24 hours or more and is highly infectious at low doses.[32] Other features of smallpox that make it a likely bioweapon candidate is that it can be produced in large quantities, is stable for storage and transportation, and is spread from person to person.[33]

7. HISTORY AND POTENTIAL OF SMALLPOX AS A BIOWEAPON

Smallpox was first used as a biological weapon during the French and Indian Wars (1754–1767) by British forces in North America. Soldiers distributed blankets that had been used by smallpox patients with the intent of initiating outbreaks among American Indians. Epidemics occurred, killing more than 50% of affected tribes. However, following the global campaign to eradicate smallpox globally in 1977, the World Health Organization required that all countries cease vaccination (1980). The WHO committee later recommended that all laboratories destroy their stocks of variola virus in June 1999.[34] The

deliberate reintroduction of smallpox as an epidemic disease would be an international crime of unprecedented proportions, but it is now regarded as a possibility because the last 4 years have been marked by escalating concerns in the United States about the threat of biological weapons. This is not unconceivable, as Dr. Kenneth Alibek, a former first deputy chief of research and production for the Russian biological weapons program, has reported that smallpox virus had been mounted in intercontinental ballistic missiles and in bombs for strategic use.[35] Former Soviet scientists successfully weaponized many agents and created missile delivery systems for smallpox, while active research was undertaken to engineer more virulent strains. Moreover, with the collapse of the Soviet Union, microbe stocks, including the smallpox virus and other technologies, have possibly found their way into the hands of unknown individuals, increasing the risk of transfer of these materials to terrorists. At least 17 nations are believed to have had offensive biological weapons programs, and scientists with this type of expertise are believed to have been actively recruited by Libya, Iran, Syria, Iraq, and North Korea.[11,12,36,37]

With increasing awareness has come a growing attempt to defend against the possibility of biological warfare and terrorism. One of the best defenses will continue to be vaccines and other treatment options, and this requires the development of new and improved vaccines and treatment against smallpox.

8. SMALLPOX VACCINES AND ANTIVIRAL THERAPIES

The events of September 11, 2001, coincident with the use of anthrax as a bioweapon, underscored the potential for use of biological agents as weapons. This concern prompted the Bush administration to make recommendations for the use of a smallpox vaccine in a pre-event vaccination program. This has prompted revisiting the safety concerns for the existing smallpox vaccine, (Dryvax), as well as the need for developing an efficacious but safe vaccine against smallpox. To that end, eradication of naturally occurring smallpox has not eliminated the need for an improved smallpox vaccine. The current threat posed by potential bioterrorist attacks has brought forth the need for new vaccination strategies due to the large numbers of individuals living in North America who are elderly, women of child-bearing age, non-vaccinia-virus (VACV) vaccinated, and immuno-compromised (HIV infection, transplantation recipients, intravenous drug users as well as other individuals on immunosuppressive therapies). Although the traditional live VACV vaccine for smallpox has proven to be effective in conferring protection where only 1 out of 10, 000 individuals vaccinated experienced significant adverse effects during the 1960s, in today's society, where there are a large number of susceptible individuals, the potential complications arising from adverse events following vaccination are likely unacceptable. It is imperative that instead of abandoning the current live VACV vaccine, leaving us vulnerable to terrorist attacks, a new strategy that improves

the safety of the current vaccine, yet increases its potency, is developed and implemented.

8.1. Smallpox Vaccine Strategies and Related Issues

The currently available smallpox vaccine is a lyophilized preparation of live vaccinia virus (VACV), generated from a New York City calf lymph strain (NYCCL), obtained from infecting cows by scarification, with subsequent lesional removal by scraping.[38] This vaccine is one of the oldest and most successful vaccines ever developed. However, this live virus vaccine also had reports of several adverse complications.[39–53] Dermatologic and central nervous system disorders were the most frequently recognized adverse events, including vaccinia necrosum, a complication with case fatality rates of 75 to 100% that occurred almost exclusively in persons with cellular immunodeficiency.[49] Generalized vaccinia was reported, resulting in rare blood-borne dissemination of virus in normal persons. More rare diseases such as pericarditis,[54] arthritis,[55] and malignant tumors at vaccination scars[56] have been described in case reports. Eczema vaccinatum was associated with case-fatality rates of up to 10% overall and 30 to 40% in children less than 2 years old.[57] Moreover, approximately seven to nine deaths per year were attributed to vaccination. Infants were identified as the highest-risk population, with death resulting from postvaccinal encephalitis.[43,44] Thus, the adverse events associated with the current smallpox vaccine are well documented, and new strategies must be developed to prevent further complications.

An important concern, as indicated earlier, is that there are a significant number of immunocompromised (HIV infected individuals) and elderly populations, as well as pregnant women, intravenous drug users, transplant recipients, and individuals on immunosuppressive drugs, who are at significant risk of developing adverse reactions after smallpox vaccination. These risks groups are listed in Table III. In North America, the fact that an overwhelming number of people, in theory, could be hospitalized due to serious complications, is of major concern, and many people could even die. Currently, several options are

TABLE III
Groups at Risk of Adverse Reactions to Vaccinia-Based Smallpox Vaccine

Pregnant women
Therapeutically induced immunosuppresssion such as in those receiving cancer chemotherapy or anti-organ rejection drugs
Persons with HIV infection
Persons with history of eczema
Intravenous drug users
Potentially the young or elderly due to immune incompetence

available. Firstly, we can go forth and use the currently stockpiled vaccine, and risk a significantly higher rate of complications than occurred in the 1960s. Alternatively, we could design and manufacture a novel efficacious and safe vaccine, disregarding the current one. However, this obviously leaves the country very vulnerable, and without protection, until such a vaccine is developed. A third strategy would be to continue with the current vaccine while pursuing studies to improve on its safety profile, while not interfering with its potent immune responses.

Several vaccine-related issues need to be addressed to ensure public safety. These include the need for a modern alternative to the live animal-produced stock and to determine immune correlates relevant for the twenty-first century in order to test new, safer vaccine candidates. Hammarlund *et al.*[58] provide evidence that vaccine-induced immunity persists for many years, perhaps lifelong. This is an interesting finding since millions worldwide, and about 90% of individuals in the United States over the age of 35, were vaccinated before the end of the mass vaccination campaigns. Although the status of their immunity against smallpox has been under debate,[38,59–61] Hammarlund *et al.*[58] measured T cell immunity against vaccinia virus in 306 vaccinees, up to 75 years after their last vaccination. Interestingly, within the first 7 years after vaccination, CD4+ and CD8+ T cell responses remained high and then declined slowly over decades, with the decline in CD4+ T cells occurring more slowly. Yet even between 41 and 75 years after vaccination, most vaccinees showed some CD4+ and some CD8+ T cell immunity. Conversely, the humoral immune response in these cohorts showed that most maintained stable antibody responses for up to 75 years after vaccination, suggesting lifelong immunity. Studying the usefulness of additional vaccination for people later in life and the expansion of their T cell responses is important. The persistent immune responses observed by Hammarlund *et al.*[58] suggest that side- effects of vaccination, such as eczema vaccinatum, should occur infrequently in revaccinated individuals because most side-effects of vaccinia are observed upon primary series of immunization. Other implications from this study are that many people in the United States over 40 years of age are likely have some immunity to smallpox, aiding in "herd immunity", therefore, the focus should be on the young population and the immunocompromised for development of new vaccines as they are unprotected.

Weltzin *et al.*[62] developed a new tissue culture method for producing smallpox vaccine that bypasses the methodology requiring scraping the hides of cows infected with vaccinia virus, resulting in replacement stocks for those without immunity. This is important because the current vaccine stocks would probably not fulfill the demands of unvaccinated individuals in the United States.[38,59–61] Weltzin *et al.*, adapted the existing DryVax vaccine, which is derived from the crossprotective vaccinia virus, to a human cell line for production in tissue culture. In a small clinical study in humans, 100% subjects vaccinated with the new vaccine candidate (ACAM-100) versus 97%

DryVax- vaccinated subjects exhibited the hallmark of vaccine take, a significant cutaneous reaction at the site of inoculation/scarification. The vaccines had similar safety profiles with each participant experiencing at least one mild to moderate adverse event. Moreover, DryVax induced higher antibody titers, while ACAM-100 vaccination seemed to result in stronger CD4+ T cell responses. Taken together, the two aforementioned studies provide new strategies toward the goal of development of next-generation vaccines.

Modified vaccinia virus ankara (MVA) was generated by more than 500 passages of vaccinia virus in chick embryo fibroblasts, during which it acquired multiple deletions and mutations and lost the capacity to replicate efficiently in human and most other mammalian cells.[63–66] MVA is being considered as a replacement for the present smallpox vaccine for those with a high risk of adverse complications because immune responses elicited by one or two doses of MVA should approach, although not necessarily equal, those of the licensed smallpox vaccine, or for more general use as a pre-vaccine since MVA should reduce the reaction to a subsequent smallpox vaccination without blocking the resulting immune response. Earl *et al.*[67] compare the highly attenuated MVA with the licensed DryVax vaccine in a monkey model, since licensing includes comparative immunogenecity and protection studies in non-human primates. After two doses of MVA or one dose of MVA followed by DryVax, antibody binding and neutralizing titers as well as T cell responses were equivalent or higher than those induced by DryVax alone. After the challenge with monkeypox virus, non-immunized animals developed more than 500 pustular skin lesions and became gravely ill or died, whereas vaccinated animals were healthy and asymptomatic. These findings of similar humoral and cellular immune responses to MVA and DryVax in non-human primates and substantial protection against a monkeypox virus challenge are important steps in the evaluation of MVA as a replacement vaccine for those with increased risk of severe side-effects from the standard live vaccine, or as a pre-vaccine. Perhaps one approach would be to vaccinate with MVA before a smallpox threat, with the thought that standard vaccine or MVA would be used as a boost. However, further experiments would need to be carried out to determine the longevity of protection, and the consequences of delayed boosting and dosage effects as well as, most importantly, other approaches to be used in the case of immunocompromised individuals. An important study by Wyatt *et al.*[68] examines the safety of MVA in immune-deficient mouse models and shows that overlapping immune responses protect immune-competent and immune-deficient mice against a lethal intranasal challenge with a pathogenic vaccinia virus.

Although vaccinia virus is highly immunogenic and is known to confer long-lasting protective immunity to smallpox, the adverse events associated with current vaccine strategies pose a significant obstacle to successful vaccination campaigns. Another novel strategy for improved safe vaccines against smallpox includes the use of DNA vaccines. DNA vaccines induce antigen-specific immune responses following the direct injection of non-replicating

plasmids into a host target tissue.[69] Once injected, plasmids drive the synthesis of specific foreign proteins within the inoculated host and mimics natural infection. The host provides post-translational modifications to antigen that faithfully reproduce native conformations. These host-synthesized viral proteins then become the subject of immune surveillance via both the MHC class-I and class-II pathways. These processes lead to elicitation of protective immunity against an infectious agent, or pathogen, primarily by activating both the humoral and cellular arms of the immune system.[70–73] Moreover, DNA vaccines can be constructed to function with many safety features as well as the specificity of a subunit vaccine, there is little risk of reversion to a disease-causing form, and there is no risk for secondary infection as the material injected is non-replicating, and non-infectious. In addition to their added safety, DNA vaccines are highly flexible; encoding genes for immunologic inhibition, or cross-reactivity (autoimmunity) can be altered or deleted altogether. DNA vaccines possess greater stability, and can be easily manufactured on a large scale. The unique features of nucleic immunization make it well suited as an immunization/immune therapy strategy especially when safety in immunocompromised individuals is a concern.

Gene gun-delivered-DNA vaccine approach used to test several vaccinia genes and gene combinations for immunogenicity and protective efficacy in mice resulted in 100% protection of those mice challenged with a lethal dose of vaccinia.[74,75] The authors then moved on to a study of a DNA vaccine comprised of four vaccinia virus genes (L1R, A27L, A33R, and B5R) administered by gene gun in rhesus macaques and were able to first demonstrate significant immunogenicity of the plasmid in this animal model, and later demonstrated protection from severe disease following challenge with monkeypox virus.[75,76] The authors selected these four immunogens due to their role as targets of neutralizing or otherwise protective antibody responses.[27–29,77–79] Animals vaccinated with a single gene L1R, which encodes a target of neutralizing antibodies, developed severe disease but survived with clinical symptoms of the monkeys challenged with a four-fold lower dose of virus. These data support the notion that a subunit (gene- or protein-based) poxvirus vaccine has the potential to mimic the protection afforded by live vaccinia administered by scarification. Such a vaccine would contribute greatly to vaccination strategies aimed at reducing the health hazards of the present smallpox vaccine.

8.2. Smallpox Antiviral Therapies

Vaccination against variola has clearly been responsible for the elimination of naturally occurring smallpox infections in the world. However, the concern that bioterrorists may use smallpox as a bioweapon has stimulated the interest to characterize and develop antiviral agents against this poxvirus as an alternative or adjunct to vaccination. In addition, effective antiviral agents are important for the treatment of the potentially serious and life-threatening complications that can occur from smallpox vaccination. Currently, the only available

treatment of infectious complications resulting from vaccination against smallpox is vaccinia immune globulin (VIG) that is generated from hyperimmune sera from vaccinees.[80,26,81,82,47] Novel antiviral agents against variola will therefore be useful as both a therapy against infection as well as an alternative/adjunct to VIG for the treatment of vaccine-induced complications.

Another drug utilized for the treatment of vaccine-induced complications was methisazone,[83] which although toxic was reported to accelerate the resolution of eczema vaccinatum and was beneficial against progressive vaccinia.[83,84] However, the lack of properly controlled studies using this drug made it difficult to access the specific efficacy of this drug. As such, methisazone is no longer in use. Several other antivirals have been used to treat vaccinia infection in animals, but many of them have proven to have too much systemic toxicity for human use. These antivirals include ribavirin,[85,86] cidofovir,[86–89], 5-iodo-2′-deoxyuridine,[90,91] 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine[88] adenine arabinoside[92,93] and trifluorothymidine.[94,95] Of these ribavirin, cidofovir, and trifluorthymidine have had some clinical use for the treatment of vaccinia vaccine-induced conditions as well as having utility against other disorders. Other agents with anti-vaccinia activity have included various nucleoside analogues[96] as well as interferon.[97,98] Although some of these pharmacologics have been demonstrated to have anti-vaccinia activity, the accepted standard for the treatment of vaccine-induced complications has been the VIG preparation. However, it is clear that a more efficient and standardized antibody/antisera preparation is needed particularly if widespread vaccination/re-vaccination is required to be implemented in the future. Generation of a cocktail of human or humanized monoclonal antibodies against vaccinia would potentially be useful as an alternative or replacement for the VIG preparation.

In addition to the development of antiviral and immune-based approaches to treat smallpox vaccine-induced adverse events, it is likewise important to develop new prophylactics and therapeutics for smallpox infection itself. Safer vaccines are needed, which have a lower incidence of induction of adverse events that are associated with the current vaccinia-based vaccine preparation. Such novel vaccines would utilize non-live attenuated preparations such as DNA vaccines. In terms of the development of novel antivirals against variola it is important to have a comprehensive knowledge of the cell and molecular biology of poxviruses. Novel prophylactic/therapeutic targets would include variola enzymes[99] as well as viral.[100] Byrd and colleagues have recently generated a structural model of the vaccinia virus 17L proteinase using a homology-based bioinformatics approach and a large library (excess of 50, 000) of chemical compounds some of which have shown some antiviral activity.[100] To date, however, the only drug accepted to possess potential directly against smallpox has been cidofovir ((S)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine = HPMPC). This drug has been previously established to possess antiviral activity against cytomegalovirus (CMV) and is approved for clinical use for the treatment of CMV retinitis in AIDS patients[101]. In

addition, cicofovir has been shown to have biological activity against other herpes viruses including human herpes viruses types 6, 7, and 8 as well as against varicella zoster virus and some polyoma, papilloma, and adenoviruses.[86] Notably, and importantly, it has been demonstrated that cidofovir could successfully be used as a preventative and therapy against lethal vaccinia infection in severe combined immune deficient (SCID) mice.[88,102,103] In addition, it has been shown to have efficacy against cowpox infections in mice and monkeys.[104–106] Lastly, cidofovir has been demonstrated to show efficacy against poxvirus infection in humans, i.e., molluscum contagiosum and orf (sheep pox).[107–110] These observations establish cidofovir to currently have the greatest potential as an antiviral agent against variola infection. It is anticipated that for clinical use against variola in humans cidofovir could be used in cases where infected individuals are unable to obtain a dose of the vaccine within 4 days after the initial contact with the dsisease.

9. CONCLUSIONS

The presence of resurgent smallpox infection is always a concern, especially given the enormous efforts that have been made to eradicate what has been characterized as one of the most devastating of all diseases. Unfortunately, smallpox as a bioterrror agent is a legitimate threat, with safety issues with the current vaccine stocks being of major concern. Moreover, the manufacturing process used to create the smallpox vaccine previously used is not suitable for today's vaccine production standards. MVA is a likely first next-generation approach for novel smallpox vaccines; however, there are major issues as to what the correlate of protection is and what should be in the boost injection for these vaccines. Importantly, immune-deficient individuals would continue to be a high-risk group for these live attenuated-based vaccines. Due to safety and manufacturing issues, DNA vaccine strategies and other recombinant strategies are likely important tools for the approaching development of novel vaccines for smallpox, particularly in the immunocompromised. Most critical to the development of smallpox strategies is the development of quantitative cellular immunological assays and determination of baseline immune responses to facilitate vaccine development and possible use as surrogate correlates. In addition, the discovery of new sources of non-immune/vaccine-based therapies outside of vaccines is important and these studies are currently underway.

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