Revisiting Smallpox: Is a Now 'Dead' Virus Still a Threat?

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In 1979, the World Health Organization (WHO) announced what is arguably one of the greatest achievements in modern medicine: the world-wide eradication of the virus which causes smallpox (WHO, 1980; Friedman & Isaacs, 2004). The importance of such an accomplishment is realized when one considers that smallpox has resulted in more recorded human deaths than all other infectious diseases combined (McFadden, 2005).

Despite a mortality rate of 30% in unvaccinated individuals, several factors made this eradication of smallpox possible (Friedman et al., 2004; Fenner, Henderson, & Arita, 1988). These factors included the availability of an effective vaccine, the lack of an animal host, and the fact that all those infected with the smallpox virus displayed symptoms of the disease (Friedman et al., 2004; Fenner et al., 1988). Thus, it was possible to identify and isolate all infected individuals while fostering wide-spread immunity through the use of an available and easily produced vaccine developed from a less virulent poxvirus.

It must be noted, simply because the WHO declared this virus 'eradicated' does not mean that live smallpox viruses (termed variola) have disappeared altogether. On the contrary, it is well publicized that two laboratory samples of the variola virus still exist (Enserink, 2005). These samples, present in the Center for Disease Control in the United States and the Russian State Research Center of Virology and Biotechnology, were originally set to be destroyed June 30, 2002 (Friedman et al., 2004). However, post-9/11 fears of bioterrorism has halted this destruction (Friedman et al., 2004) and rekindled debate within the World Health Assembly (WHA) regarding the future uses of these stockpiles (Friedman et al., 2004; Enserink, 2005). Some scientists argue that these stockpiles should be used to support research into new antiviral therapies and safer vaccines. In addition, the destruction of these stockpiles would not necessarily guarantee the complete disappearance of variola (Enserink, 2005). Indeed, current biotechnology has enabled scientists to reproduce the 1918 Spanish Influenza virus from frozen corpses for the purpose of determining the source of its virulence (Tumpey et al., 2005). For similar reasons, the WHA has recently authorized a series of research studies on the smallpox virus, and both the United States and Russia have currently expanded their research programs studying this virus (Enserink, 2005).

Although this virus is currently deemed as eradicated, it is important to examine what threat it may pose should it re-emerge. In addition, if re-emergence does occur, how prepared are national and global health agencies in containing the spread of this agent?

The Biochemistry, Pathogenesis and Pathology of Smallpox

The variola virus, which causes smallpox, is an *Orthopoxvirus* that belongs to the *Poxviridae* family (Flint, Enquist, Racaniello, & Skalka, 2004). Historically,

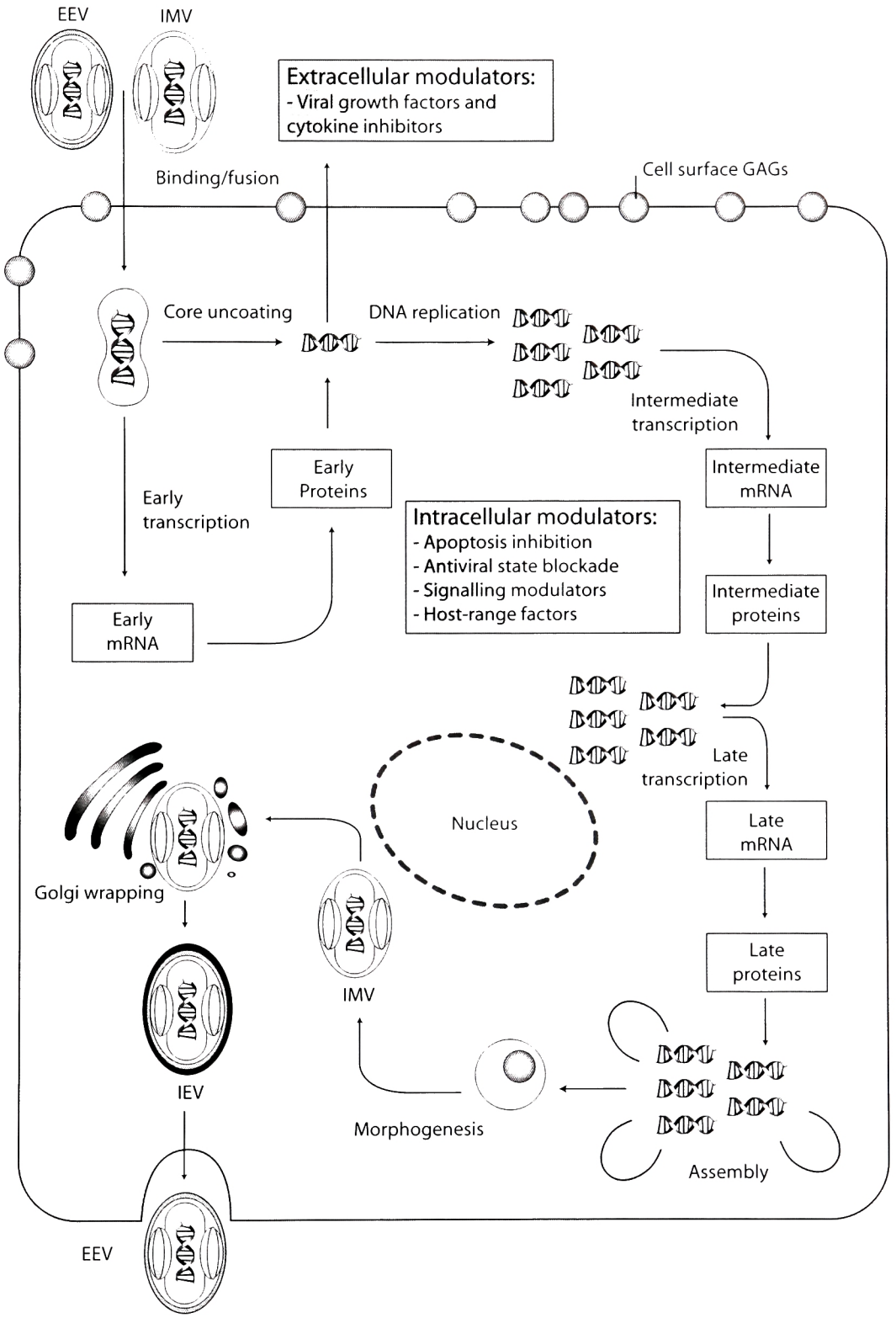

Figure 1: While the mechanisms that determine host tropism in the variola virus are still not understood (McFadden, 2005), the vaccinia virus (the virus which is used to vaccinate individuals against variola) can be used as a template to explore Orthopoxvirus replication. The virus initially fuses with cellular membranes, ultimately releasing the viral core into the cytoplasm. The presence of viral DNA-dependent RNA polymerase and transcription factors results in the production of early mRNA, which produces the early proteins that facilitate genome replication. Once the DNA genome has been replicated, viral initiation proteins and the products of early genes result in the translation of intermediate mRNAs. Once translated, the intermediate proteins help stimulate the production of late mRNA, which codes for structural proteins and additional enzymes that must be packaged within the newly replicated virus. Once assembled, the virus matures into a brick-shaped intracellular mature virion (IMV), which can be released if the cell lyses. If lyses does not occur, the IMV will acquire a second double membrane from the Golgi to form the intracellular enveloped virion (IEV), which fuses with the cell membrane to bud the virus from the cell (Flint et al., 2004).

there are two forms of the virus; variola major and variola minor. These two types are distinguished based on fatality rates, with the major form resulting in mortality in 30% of cases while the minor form is associated with a 1% mortality rate (Centers for Disease Control and Prevention, 2004a). The Poxviridae family consists of double stranded DNA viruses with a virion diameter of 170-200 x 300-450 nm. They also have 130-280 kb genomes, which are large enough to code for all proteins necessary for replication. Consequently, these viruses are minimally dependent on the host cell and are capable of utilizing their own proteins to synthesize DNA and produce viral mRNAs in the cytoplasm (Flint et al., 2004; see Figure 1 for an overview of the *Orthopoxvirus* replication cycle).

Variola transmission occurs via inhalation of airborne variola virus, which then replicates within macrophages residing in the respiratory tract. In addition, the virus may be transmitted through direct contact with infected lesions, bodily fluids or contaminated objects and surfaces (Constantin et al., 2003). Once infected with the virus, macrophages migrate to lymph nodes where additional viral replication occurs. The presence of virus in lymph nodes and the circulatory system, known as viremia, allows the virus to spread to the rest of the body. Once the infection reaches small dermal blood vessels, it can result in infection of epidermal cells and endothelial swelling. When the infection spreads to the deep vascular inner layer of the skin and sebaceous glands, the patient develops pockmarks which later heal to produce the scars characteristic of smallpox (Friedman et al., 2004).

To become infected with smallpox an individual would only need to be exposed to an initial 10-100 virus particles (FIRSTConsult, 2005). Normally, the time it takes for the virus to establish itself in its host prior to the onset of symptoms is anywhere from 10 to 14 days, during which the infected person is non-contagious (Constantin et al., 2003). In the following prodromal phase, which lasts between 2 to 4 days, initial symptoms appear and the individual may be contagious. Initial symptoms are described as influenza-like and most commonly include a high fever, as well as body and headaches. Once the prodromal phase has passed, red spots appear on the tongue and mouth, which develop into sores. Subsequently, rashes appear on the skin, which then become raised bumps and fill with fluid to become pustules. These pustules will eventually scab and fall off (CDC, 2004a).

Despite the well documented progression of smallpox in the literature, less is known about the biochemical factors which result in the high virulence of the variola virus relative to other members of the *Orthopoxvirus* genus. For example, an analysis of the DNA genome of a Bangladesh variola strain has noted 187 putative proteins which might contribute to pathogenicity (Massung et al., 1994; Massung et al., 1993). Of these proteins, 37 were found to be notably different compared to the less virulent vaccinia virus. These differences may result in the contrasting degrees of virulence between the variola and vaccinia virus, although the specific proteins involved are still largely unknown (Massung et al., 1994; Massung et al., 1993). One protein that has been identified, the smallpox inhibitor of complement enzymes (SPICE), has been shown to be 100 times more potent at inactivating the complement cascade protein C3b relative to the vaccinia counterpart (Rosengard, Liu, Nie, & Jimenez, 2002). In addition, it appears that the SPICE protein is more specific to human complement proteins, inhibiting the formation of the C3/C5 convertases which lead to pathogen destruction and opsonization (Rosengard et al., 2002; Janeway, Travers, Walport, & Shlomchik, 2005).

Though routine vaccination is no longer common practice, the recent threat of smallpox as a bioterrorist agent has resurrected the smallpox vaccine as a potential defensive measure. The United States has been at the forefront of developing a smallpox response plan, outlining immunization procedures and potential health risks (see CDC, 2005).

Smallpox Vaccination

The current licensed vaccine in the United States is Dryvax®, which contains a live vaccinia virus that that produces neutralizing antibodies that are protective against viruses within the *Orthopoxvirus* genus. The Dryvax® vaccine is acquired by draining the lymph nodes of calves infected with the vaccinia virus (Rosenthal et al., 2001). Calf-lymph derived vaccines were used in the global eradication of smallpox (Arita, 2005).

The vaccine is administered using a bifurcated needle, dipped in the vaccine, and pricked on the skin surface in the upper arm (CDC, 2004b). The length of protection has traditionally been thought to vary between three to five years. However, Hammarlund et al. recently found immunity to the vaccinia virus in individuals immunized more than 25 years ago against smallpox, indicating that the vaccine may show effectiveness over longer durations than once thought (2003).

Normal reactions to immunization with the vaccine include fever, body aches, and a scar from a scabbed pustule. Unfortunately, there are concerns over the adverse side effects of Dryvax®. As mentioned above, the smallpox vaccine contains a live form of the vaccinia virus, which poses the risk that the virus may spread to other parts of the body. Specifically, adverse cases are known to occur in persons with eczema, which is a chronic skin condition characterized by hardening of the skin, redness, inflammation, and flaking. In addition, individuals with suppressed or weakened immune systems face the risk of a progressive infection characterized by developing necrosis at the site of vaccination (Hong, 2005; see Figure 2). Pregnant individuals are also advised against immunization since there is a great risk to the fetus (Recommendations of the Advisory Committee on Immunization Practices, 2001).

In addition to the adverse side-effects of Dryvax®, there is a need to find a replacement for the limited stocks of this vaccine, since its manufacture is no longer acceptable. The reasons for this include: the development process of Dryvax® lacked controls, the risk of bacterial contamination in isolating the virus from calves, and the risk of diseases like bovine spongiform encephalitis from a bovine intermediary (Greenberg et al., 2005).

Establishing a Response Plan

With the many risks involved with vaccination, one might ask how prepared is a nation like the USA in dealing with a potential re-emergence of smallpox? At present the United States has enough stock of Dryvax® to immunize its current population (CDC, 2004c). The Centers for Disease Control and Prevention (CDC) Smallpox Response Plan is a set of guidelines to contain an outbreak of smallpox. The effectiveness of this plan in containing smallpox rests on the ability of the CDC to mobilize and deliver vaccines and personnel to areas of confirmed outbreak. Being able to isolate and vaccinate individuals with confirmed and suspected smallpox infection (a process called ring vaccination), along with those at high-risk of contracting the disease, is part of the plan's general strategy. The CDC realizes that certain barriers exist in the implementation of the above response plan. Low residual immunity, lack of routine vaccination, health personnel's unfamiliarity with the disease and a growing population are factors that may contribute to the rapid spread of smallpox, making its containment difficult (CDC, 2004c; CDC, 2005).

Figure 2: Progressive vaccinia is a potential adverse effect due to smallpox vaccination. In this immunodeficient child, the vaccination site expanded with rapid necrosis instead of healing normally (Hong, 2005).

This defensive initiative moves beyond the stockpiling of vaccines to include support for research initiatives. Many scientists now claim that genetic modifications of the variola virus could speed up the development of more effective vaccines and antiviral drugs (BBC News, 2005). Facilitating such therapeutic and preventative developments is a growing understanding of the biochemical pathways involved in a smallpox infection.

Recently, Yang et al. published an article which explores host cellular pathways facilitating smallpox viral replication (2005). Here, they note that Smallpox Growth Factor (SPGF) binds to host receptors of the tyrosine-kinase superfamily. In particular, binding of SPGF to Erb-1 receptors stimulates intracellular signal pathways that ultimately aids in viral pathogenesis. This particular study examined the effects of a tyrosine-kinase inhibitor known as CI-1033 in variola and vaccinia infected monkey liver cells. The inhibitor had no effect on the overall quantity of newly made virus in experiments in which all cells were infected simultaneously. However, it did have an effect in the transmission of virus in the experiments that infected a single cell in culture. Plaques were minimized, indicating that the single infected cell was hampered in its ability to transmit the virus to unaffected cells (Yang et al., 2005). Although this example highlights some of the antiviral research being conducted, more work needs to be done before the results of biochemical studies may be applied in a clinical setting.

Conclusion

Craig Venter, co-founder of Celera Genomics Corporation and adviser to former US president Bill Clinton, addressed the American Association for the Advancement of Science on the on the threat of genetically engineered weaponry. During this speech, he suggested that cracking the genetic code of every bacteria and virus was no longer just a biomedical inquiry, but a threat to national security (Ellis, 1999). Although the variola virus no longer occurs naturally, its genetic sequence is well known and studied. This information may potentially be utilized to recreate the virus, and in the wrong hands it provides access to a lethal biological weapon. As we have outlined above, some countries, including the US, have become apprehensive and developed a series of policies and procedures to be followed and implemented in the event of a biological attack.

Although the potential to use smallpox as a weapon exists, it would be impossible to accurately predict the damage the variola virus would inflict if reintroduced. With variables like method of dispersal, epidemiology of viral spread, effectiveness of vaccination or potential for antiviral interventions (Weiss et al., 2004), any such predications would be clouded in uncertainty or prone to erroneous assumptions. Also, given the fact that the great majority of the population has never been exposed to smallpox, and that there is still debate as to whether past vaccination still provides immunological protection, past estimates of viral spread may also be misleading (Weiss et al., 2004). Nonetheless, the old maxim that 'it is better to be safe then sorry' holds true when evaluating the smallpox threat. It is by exploring the many different biological agents which may be purposely used to take lives, and designing protocols to address these threats, that a country may best protect the health and wellbeing of their citizens in the gravest of circumstances. M

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