ORTHOPOXVIRUSES
PATHOGENIC FOR HUMANS
Orthopoxviruses
Pathogenic for Humans

With 112 Figures
Preface

The viruses belonging to the genus *Orthopoxvirus* of the family Poxviridae are among the pathogens heading the list of microorganisms that have had an important role in the interactions between the humankind and infectious agents. Until recently, smallpox, caused by variola virus, was the most dangerous epidemic disease of humans, spreading as a conflagration. The toll of this infection was a tremendous number of human lives. Only in the previous century, smallpox killed about 300 million people. The variola virus is unique in that the only sensitive host of this pathogen is the man; moreover, the case-fatality rate of smallpox may exceed 30%. Variola virus is a strict anthroponosis unable to be retained in wild nature in animal organisms.

Another orthopoxvirus—cowpox virus—occupies one of the most honorable places in the history of medicine. In 1796, already one hundred years before the kingdom of viruses was discovered by Dmitri Ivanovsky in 1892, the famous experiments of Edward Jenner commenced use of cowpox virus for infecting people in order to protect them from smallpox, thereby opening the era of vaccine prevention of communicable diseases.

The origin of vaccinia virus, which substituted cowpox virus during massive vaccinations of humans against smallpox about one hundred years ago under vague circumstances, is a great mystery for the modern science. It is yet unclear whether vaccinia virus is a result of long-term artificial selection of a highly immunogenic against smallpox and lowly reactogenic virus. This virus so far has not been discovered in nature. Nonetheless, the availability of effective live vaccine against smallpox involving vaccinia virus as the major constituent, the lack of natural reservoir of variola virus, and joint efforts of the world medical community under the auspice of the World Health Organization (WHO) allowed the most hazardous infectious
disease, smallpox, to be defeated by 1977 for the first time in the history of humankind. Hitherto, this is a unique example of successful campaign that eventuated in eradication of epidemically dangerous human disease.

As the massive immunization against smallpox was accompanied by side effects and complications, WHO in 1980 recommended to stop the routine vaccination. This resulted in an ever vanishing protection of the majority of the world population against not only smallpox, but also other infections caused by cowpox and monkeypox viruses. Smallpox is beaten; however, the circulation of monkeypox virus, a pathogen capable of infecting a wide range of animals and humans, in the zone of tropical rainforests in Africa arouses the concern of the scientific and medical communities. Moreover, monkeypox virus causes a human disease similar to smallpox in its clinical manifestation and course. Monkeypox virus is less efficient in person-to-person transmission compared with variola virus. Nevertheless, if this virus acquires the capability of highly efficient transmission in the human population as a result of evolutionary changes, the humankind will face the problem more complex than that when it encountered variola virus, as monkeypox virus is zoonotic, making it virtually impossible to eradicate the pathogen and the corresponding disease. Potential penetration of monkeypox virus to other continents also presents a considerable menace. The human monkeypox outbreak in the USA in 2003 was the first recorded outside the African continent. This disease was imported into the USA with Western African animals intended as pets. This was the first alarm for the public health services worldwide.

Thus, the orthopoxviruses pathogenic for humans are still attracting a rapt attention of scientists as well as medical researchers and practitioners. A large amount of information about these viruses has been accumulated recently. This made us consider it timely and necessary to summarize the data, obtained in many laboratories of the world as well as in our laboratories, on biological, ecological, and molecular genetic features of these unique viruses, which have played and continue to play an important role in the history of humankind.
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Chapter 1

SMALLPOX IN HUMAN HISTORY

1.1 Introduction

The role of smallpox in human history has been discussed in detail (Fenner et al., 1988; 1989). These references, while excellent, are out of print, but they remain available at many libraries. In addition, one of these, the superb reference Smallpox and its Eradication (Fenner et al., 1988) is available from the World Health Organization on their WEB site (http://www.who.int/emc/diseases/smallpox/Smallpoxeradication.html).

There is also an excellent historical treatise, which dwells in depth on the role of smallpox in human events entitled Princess and Peasants: Smallpox in History, originally published in 1983 by Donald R. Hopkins. This book was recently reissued under the title of The Greatest Killer—Smallpox in History (Hopkins, 2002). A perusal of any of these sources will readily convince the reader that smallpox has had an enormous impact on human history for over 2000 years. We have relied heavily on these references, as the purpose of this Chapter is to provide the reader with an appreciation of the role of smallpox (variola major) in human history. It is difficult today, when the world has been freed of this dreadful disease for nearly thirty years, to appreciate the past terror and apprehension concerning smallpox, a disease ever present, prior to its eradication, throughout the world. Prior to the third quarter of the 20th century, when eradication succeeded, it was widely known and appreciated that epidemics had occurred and would again, the only variables being when and how severe. Parenthetically, thirty years after eradication, it is tragic that serious worldwide concerns have been rekindled for fear this virus might be intentionally released by terrorists as a bioweapon. The possibility that smallpox, or smallpox-like viruses, such as monkeypox or engineered recombinant poxviruses, might be deliberately released would knowingly undo what is arguably the single most significant
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medical achievement of mankind, namely, the eradication of smallpox from the face of the earth. This possibility poses a potential tragedy of the first order.

1.2 The Origins of Smallpox

Smallpox, a uniquely human virus, has no known animal reservoirs and therefore must rely on human-to-human transmission to be maintained in the population. Therefore, while considered an ancient disease, it should be appreciated that a requirement for the virus to become endemic was that sufficient numbers of susceptible individuals within a large enough population must exist to allow the virus to be sustained. The true origins of the virus are murky, but it is clear that in addition to a critical population density, dissemination from a point of origin depended on the emergence of commerce between nations and groups and armed conflicts both of which facilitated contact within different populations.

The virus as we know it probably originated in either Egypt or India no later than roughly 1000 B.C. In Egypt, the mummified remains of the pharaoh Ramses V (Ruffer, 1921; Figure 1.1), which could date as early as 1157 B.C., show pustular eruptions consistent with smallpox. Examination of several other mummies dating from this period or even earlier also suggested the presence of pustular lesions again consistent with smallpox (Ruffer & Ferguson, 1910). These mummies date to roughly 3000 years ago, which precedes reliable descriptions of the virus anywhere else by approximately 1000 years and makes a strong argument that the virus originated in Egypt and was then carried to India and Asia by caravans of commerce, ocean going vessels, or traveling armies. Consistent with this notion are reports dating even earlier from the 14th century B.C. describing Hittite attacks that ultimately defeated Egypt. A “pestilence” is described as having broken out among the Hittites, contracted from the Egyptians, which persisted for some 20 years, killing large numbers of people including at least two Hittite leaders. There is some but not-conclusive evidence that this epidemic could have been due at least in part to smallpox.

In India, there are ancient Sanskrit writings “Charka Samhita” and “Susruta Samhita”, which could be as old as 1500 B.C., that also describe a disease consistent with smallpox. It is interesting to note that Hopkins describes writings of an Indian

![Figure 1-1. The mummified head of Ramses V, pharaoh of Egypt who ruled from 1150-1145 B.C., showing facial pustules believed to be consistent with smallpox.](image)
scholar, Dhanwantari, who some 2000 years ago described a preventative procedure astonishingly similar to the procedure described in the last years of the 18th century by Jenner. Dhanwantari writes, "Take the fluid of the pock on the udder of the cow or on the arm between the shoulder and elbow of a human subject on the point of a lancet and lance with it the arms between the shoulders and elbows until the blood appears. Then, mixing this fluid with the blood, the fever of the smallpox will be produced" (Hopkins, 2002). There are also descriptions by Brahmin priests, who describe rituals and prayers directed toward the "Goddess of Smallpox" from 1000 B.C. to the birth of Christ. What is abundantly clear from the writing and descriptions is that smallpox existed in India as well as Egypt well before the birth of Christ.

Therefore, the most reliable evidence would suggest that the disease originated from either Egypt or India, but anecdotal evidence suggests that the virus was present in ancient Greece as well. Hippocrates (400 B.C.) has written references to a disease that could have been smallpox. Better evidence is provided by Thucydides, a resident of Athens, who described the "plague of Athens" that occurred during the Peloponnesian war. This pestilence lasted for a number of years, killed roughly 25% of the Athenian army as well as private citizens, and ultimately resulted in the introduction of the virus into Persia. This again would be consistent with virus that originated in Egypt, but entered Greece through seafarers through the port of Piraeus roughly 30–50 years before this war. Whether this was smallpox is not known with certainty, but the end result was a serious erosion of Athenian strength, which diminished their capacity for later conflict with the Spartans and their ultimate decline (Hopkins, 2002).

China in ancient times would have had the population to allow both epidemics and the virus to become endemic. Scholars estimate that the virus was introduced into China from the North about 250 B.C. An epidemic is described about 243 B.C., which, from descriptions, could have been smallpox. However, the first clinical descriptions date from Ko Hung in 340 A.D. It was roughly 200 years later, before Chinese writings describe the disease in either Korea or Japan with introductions into Korea likely in 583 A.D. and Japan in 585 A.D.

It is clear that while the virus was well established in North Africa, India, China, and Persia, there is no evidence of smallpox in Europe until far later, or roughly the 6th century A.D. Very likely, the virus was introduced during the Islamic invasions, which originated from North Africa and entered Europe via Iberia in the 7th and 8th centuries. In the first millennium, several notable writings were produced. Al-Razi, an outstanding Persian physician and philosopher who lived from 850–925 A.D., has been credited as the first to use animal gut for sutures and plaster of paris for casts. He produced many medically related texts including his most famous A Treatise on the
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Figure 1-2. A translation in 1776 of the Abu Bakr Mohammad Ibn Zakariya al-Razi (864–930 A.D.) treatise on smallpox and measles.

Smallpox and Measles (Figure 1.2). In Japan, Ishinho described smallpox hospitals and the “red treatment”, which was to completely cover rooms with red cloth, similarly outfit patients in red clothing, and then expose them to red light. By 1000 A.D., smallpox was endemic in the more densely populated regions of Europe and Asia encompassing North Africa and Spain in the west to Japan in the east. In some ancient cultures, smallpox was so devastating, that infants were not named until it was clear they had caught the disease and survived.

The establishment of an endemic infection in Europe was aided in great measure by the Crusades, which took place between European countries and those of South West Asia. At the same time, in Africa, trade caravans transversed the Sahara Desert to spread the disease into those countries of West Africa that had sufficient population to sustain the infection. The interior of Africa was largely spared, even though Arabs likely introduced the virus sporadically during these years, because these regions lacked the population necessary to sustain the infection. By the 16th century, smallpox was common throughout Europe but did not become a major problem until the 17th century (Carmichael & Silverstein, 1987). During the 17th and 18th centuries, the London Bills of Mortality provided accurate documentation of the nature and effect of smallpox on Europe. During the 18th century alone, smallpox killed five reigning European monarchs. The spread of smallpox from Egypt and India into Asia and Europe is summarized in (Figure 1.3). Well into the 20th century, epidemics had huge social, economical, and clinical impacts. In 1962, a Pakistani traveler initiated a smallpox epidemic in Rhondda, Wales, in the UK. Ultimately, 25 people contracted smallpox and 6 died. The public demanded vaccination and ultimately 900,000 people in South Wales were vaccinated. One of the last of the major European outbreaks occurred in 1972 in Yugoslavia. The Yugoslavian epidemic was apparently initiated by a Muslim pilgrim named Latin Muzza, who had return from Mecca via Iraq to his home in Kosovo. He likely contracted the disease while in Iraq where smallpox was active. Muzza, upon falling ill in Yugoslavia, visited a number of hospitals before being admitted to a Belgrade hospital. Unfortunately, the epidemic had progressed extensively before definitive diagnosis was made. Strict government measures were implemented in order to control this outbreak, yet there were 175 cases
Figure 1-3. Proposed spread of smallpox within the ancient world (from Fenner et al., 1988; reprinted with permission of the World Health Organization).

reported with 35 deaths before the epidemic ended (World Health Organization, 2004). Officials were so concerned that mass vaccination was employed as a control measure in Belgrade and Serbia with the result that 8,160,000 people were vaccinated out of a total population of 8,437,000. A similar vaccine strategy ensued in Kosovo, which also reported some cases, and 1,200,000 persons were vaccinated out of a total of 1,244,000.

1.3 The Spread and Effect of Smallpox on Naïve Populations

Extensive documentation details the effects of smallpox when introduced into naïve, previously unexposed populations. Up to the 1500s, the disease probably was not present to any significant degree in Southern Africa, the Americas, or Australia. In the Americas, it was probably the Spanish who brought the disease into the Caribbean in 1507 (Figure 1.4). The effect was to completely decimate the native island population, which encouraged the importation of African slaves to fill the population void. There is little doubt that the conquest of both Mexico and Peru by the Spanish was influenced by smallpox. An introduction of the virus in 1520 by Cortes on the mainland was a major factor in the devastation of the Aztecs by the Spanish invading armies. Similarly, Pizarro brought the disease to the Incas, which was a major factor in the downfall of that empire as well. In Brazil, missionaries carried the disease far into the interior of the continent.

In North America, smallpox was a major factor in the pattern of settlement of both the English and French and was a major factor in the political evolution of Canada and the United States (Stearn & Stearn, 1945). Initially, the effects of the disease, while devastating on individual introductions, had less overall effect because of the lesser density of native
Figure 1-4. The spread of smallpox by Europeans into the Americas, Australia, and South Africa (from Fenner et al., 1988; reprinted with permission of the World Health Organization).

populations. The virus was repeatedly introduced by seamen into North America with the result of devastation of the native population mostly at the site of introduction, which involved initially coastal tribes. As the virus spread inland, the Iroquois Nation in upstate New York suffered no less than five separate epidemics during the 17th and 18th centuries. In the 18th and 19th centuries, the Tripara and Mandan Tribes further west were similarly exposed and essentially destroyed with the result that the Sioux tribes annexed their territory as a consequence of a lessened ability to resist.

Smallpox was a major issue that factored into the British, French, and American strategies during the American Revolutionary war. For example, during the American siege of Boston from June 1775–March 1776, the length of the siege was protracted because of George Washington’s reluctance to enter Boston, which was known at the time to have smallpox, and fear that the virus would be introduced into his American Army. Indeed, when the British finally departed, the city was initially occupied by troops who had survived smallpox. In Canada, smallpox was a major factor in determining that Canada would eventually come under British rule. In the winter of 1775–1776, the Americans were attempting to liberate Quebec province from British rule. The Americans captured Montreal and moved to attack Quebec City. Although the situation for the Americans looked promising, the British commander had citizens recently recovered from smallpox fraternize with the Americans. Smallpox broke out among the American troops and about half of the 10,000 soldiers ultimately fell ill followed by a retreat because the forces were too weakened to continue the battle.
Historically, one of the greatest of American Presidents was a victim of smallpox. Lincoln, who gave his famous Gettysburg address on April 19, 1865, fell acutely ill two days later. It is likely in retrospect that he was symptomatic the day he gave the address. While Lincoln survived, his death could have altered the course of American history as the Nation was in the midst of a civil war.

Introduction of the disease into Southern Africa was into Angola by the Portuguese. The virus was introduced into South Africa in 1713 via contaminated bedding and resulted in the decimation of the native Hottentots. Despite these introductions, the disease did not become endemic in Central Africa until the late 19th century.

Introduction of the virus into Australia, despite being an isolated continent, occurred within one year of European arrival in the city of Sydney. From 1829–1831, the disease broke out among the aborigines of Southeast Australia, which clearly facilitated European settlement of this region.

Smallpox raged throughout areas of the world from the 17th to mid-20th centuries despite the monumental discovery of vaccination by Jenner in 1796, which had long been recognized as being effective. It was only through the concerted efforts of the world community through the United Nations that smallpox was effectively eradicated with the last natural case occurring in October 1977, the patient being Ali Maow Maalin, a hospital cook in the town of Merak, Somalia.

1.4 Smallpox as a Historical Bioweapon

Given the influence of smallpox on world events and the common knowledge that once infected, a survivor was immune to the disease, it is not surprising that smallpox has been considered as a possible weapon. A couple of illustrations suffice. During the French and Indian wars, the British commander, Lord Jeffrey Amherst, for whom the City and University in the US State of Massachusetts are named, deliberately introduced the virus into the warring Indian factions. Amherst, in a letter to one of his officers, Colonel Henry Bouquet, in 1763 stated, “Could it not be contrived to send the smallpox among these disaffected tribes of Indians? We must on this occasion use every stratagem in our power to reduce them”. Bouquet replied, “I will try to inoculate the Indians with some blankets that may fall into their hands and take care not to get the disease myself” (Duffy, 1951).

During World War II, both the British and Americans considered using smallpox as a deliberate weapon. One factor mitigating this was the fact that there was available a good vaccine, which was widely distributed. Therefore, further consideration was abandoned. In 1969, President of the United States, Richard M. Nixon officially banned development of any biological
weapon. In 1972, the UK, US, and Soviet Union all signed the Biological Weapons Convention, superficially ending consideration of smallpox, by this time near eradication, as a weapon of war. Over the years, unfortunately, it appears that efforts to weaponize smallpox were not universally abandoned. In 1972, a Soviet defector, Dr. Kanatjan Alibekov (Ken Alibek) claimed to have been in charge of a bioweapons program designed to develop smallpox into an offensive weapon. Another soviet scientist, Dr. Vladimir Pasechnik, who died in 2001, also supported the thesis that the former Soviet Union had intensified development and perfection of an aerosolized form of smallpox through a company Biopreparat, which had been established in 1973 and which was reportedly active until the end of the Gorbachov era (Alibek, 1999). These activities have since been abandoned. While there is no official acknowledgment of efforts to weaponize smallpox, heightened terrorist activities and concern about rogue Nations have led to renewed efforts to develop better vaccines and ameliorative measures against smallpox infections.

1.5 Monkeypox Virus and Engineered Viruses: The Future of Smallpox and Smallpox-like Infections

Formally, the world is certified as "smallpox-free”, as the last natural case occurred in 1977. However, there are two aspects of smallpox that merit vigilance for the future. First, there is monkeypox virus, which can cause serious disease in both monkeys and man. Then, there is the possibility of genetically engineering of monkeypox virus, clandestine strains of smallpox, or even the more attenuated orthopoxviruses to exacerbate or create a smallpox-like disease.

Monkeypox virus, an indigenous disease of rodents in equatorial Africa, causes a disease in monkeys and humans indistinguishable from smallpox. Fatality rates of humans in central Africa infected with monkeypox virus are similar to those of smallpox caused by variola major virus. However, monkeypox virus is less transmissible from human to human. Nevertheless, unlike variola strains, which are present in closely guarded deposits only in Russia and the United States, monkeypox virus is ever present in zoonotic reservoirs and poses a continuing threat to human populations, as the territory allocated to wildlife continues to shrink, thereby increasing the likelihood of contact between humans and wild animals.

Monkeypox was first recognized in 1958 at the State Serum Institute in Copenhagen (von Magnus et al., 1959) in a colony of monkeys. The disease was noted in 1970 in humans living in tropical rainforest areas in western and Central Africa (Lourie et al., 1972; Marennikova et al., 1972a). The first case of human monkeypox was reported in Zaire in 1972 (Ladnyi et al.,
1972). It is now recognized that monkeypox is a zoonotic disease of rodents with transmission to both monkeys and man. Human infections are generally seen when humans increase their contact with forests where the natural animal reservoirs of the virus are located. Between February 1996 and October 1997, there were some 511 suspected cases in the Democratic Republic of the Congo (formerly Zaire). The increased frequency of the disease, while related in part to population movements resulting from the political instability of the region, also generated concern that the virus had somehow changed or mutated into a more virulent form. There were also some concerns that the virus might be a progenitor of smallpox virus; however, molecular studies have clearly indicated that this is not the case. The two viruses are distinct, and it is virtually impossible that monkeypox virus could naturally evolve into smallpox virus (Douglass & Dumbell, 1992; Shchelkunov et al., 2001).

The aspects of human monkeypox that have garnered the most attention are the clinical similarities to smallpox and the similar fatality rates. The virus is classified as a possible emerging pathogen by the CDC in Atlanta, USA, and has received considerable attention. A perusal of the map of Africa showing the case distribution of monkeypox virus indicates two "pockets" of the virus, one in Central and the other in West Africa (see Figure 5.11; Jezek & Fenner, 1988). Evidence now suggests that fatalities are much more prevalent in Central than West Africa. Indeed, it now appears that there are two variants of the virus in circulation, and that the strain circulating in West Africa is considerably attenuated as compared to the Central African (Zairian) strain.

Further appreciation of the concerns about monkeypox by Public Health Officials is highlighted by a recent outbreak of the disease in the United States. In early June 2003, monkeypox was reported among several people in the United States. Ultimately, 72 cases were reported but no deaths. It is likely that humans became infected after coming in contact with wild prairie dogs. The prairie dogs in turn in all likelihood became infected after coming in contact with the Gambian giant pouched rat imported as part of a shipment of animals into the US on April 9 for use as pets. Since that time, importation of the Gambian rat has ceased as has importation of tree squirrels, rope squirrels, dormice, brush-tailed porcupines, and striped mice. Interestingly, unlike what was reported for monkeypox infections in the Congo, there were no deaths in the United States. A possible explanation for this dichotomy is that the strain imported into the US was the more attenuated West African strain of monkeypox virus. A second concern highlighted by the American outbreak, was the fear that the virus might become zoonotic in North American rodents. At the present time, at least for this outbreak, this does not appear to be the case, although the zoonotic potential of the virus cannot be ignored.
Another current concern stems from data in the scientific literature that suggest that poxviruses can be engineered in the laboratory to partially overcome the immune response. Ectromelia virus is an orthopoxvirus of mice and hence a member of the same virus family as smallpox. In certain strains of mice (BALB/c), the virus is virtually uniformly lethal, whereas in other strains (C57BL/6) the animals are relatively resistant to the virus. However, when a recombinant ectromelia virus engineered to express the cytokine IL-4 is used to infect mice, all animals including those of the normally resistant C57BL/6 strain develop systemic disease with uniformly high mortalities. A second equally sobering finding was the report that ectromelia expressing IL-4 was able to overcome the effects of vaccination (Jackson et al., 2001). While more recent data suggest that vaccination can protect animals against most of the effects of the ectromelia IL-4 virus (R.M. Buller, personal communication), the concept of engineering poxviruses of increased virulence is a significant issue that argues for continued worldwide vigilance.