Review Article

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Smallpox: can we still learn from the journey to eradication?

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One of the most celebrated achievements of immunology and modern medicine is the eradication of the dreaded plague smallpox. From the introduction of smallpox vaccination by Edward Jenner, to its popularization by Louis Pasteur, to the eradication effort led by Donald Henderson, this story has many lessons for us today, including the characteristics of the disease and vaccine that permitted its eradication, and the obviousness of the vaccine as a vector for other intractable infectious diseases. The disease itself, interpreted in the light of modern molecular immunology, is an obvious immunopathological disease, which occurs after a latent interval of 1-2 weeks, and manifests as a systemic cell-mediated delayed type hypersensitivity (DTH) syndrome. The vaccine that slayed this dragon was given the name vaccinia, and was thought to have evolved from cowpox virus, but is now known to be most closely related to a poxvirus isolated from a horse. Of interest is the fact that of the various isolates of orthopox viruses, only variola, vaccinia and monkeypox viruses can infect humans. In contrast to the systemic disease of variola, vaccinia only replicates locally at the site of inoculation, and causes a localized DTH response that usually peaks after 7-10 days. This difference in the pathogenicity of variola vs. vaccinia is thought to be due to the capacity of variola to circumvent innate immunity, which allows it to disseminate widely before the adaptive immune response occurs. Thus, the fact that vaccinia virus is attenuated compared to variola, but is still replication competent, makes for its remarkable efficacy as a vaccine, as the localized infection activates all of the cells and molecules of both innate and adaptive immunity. Accordingly vaccinia itself, and not modified replication incompetent vaccina, is the hope for use as a vector in the eradication of additional pathogenic microbes from the globe.

Key words Eradication - smallpox - smallpox virus

Introduction

Almost two hundred years after Edward Jenner's landmark publication showing the efficacy of prophylactic immunization for smallpox[1], a worldwide campaign successfully eradicated this dread disease that produced a 30 per cent mortality after infection and resulted in 10 per cent of the world's blind. Much of the success of the worldwide eradication programme is attributable to Donald S. Henderson, the leader of the World Health Organization (WHO)-sponsored effort. He has very succinctly summarized the eradication programme after a Symposium on Smallpox Eradication that was held to commemorate the 30th anniversary of the historic declaration[2]:

"…the world and all its peoples have won freedom from smallpox…a most devastating disease…since earliest

time, leaving death, blindness and disfigurement in its wake and which only a decade ago was rampant in Africa, Asia and South America."

Henderson also has chronicled the eradication effort in a recent book[3], which details the roles of all of those who contributed, and where he tells of the hurdles and hardships that had to be overcome in each country as the ten year campaign unfolded between 1967 and 1977. Accordingly, those who are interested, especially in the public health aspects of this campaign, are referred to these excellent first-hand accounts by those who were there. At this time, one would like to delve into the immunology of this eradication effort, to ascertain why it was possible to rid the planet of this virus by vaccination. In this regard, it must be recalled that in the decade between 1967-1977, the science of immunology was still in its infancy. It was a time before the revolution of molecular immunology was ushered in after 1980. The events leading up to this revolution in our understanding of the workings of the immune system are reviewed elsewhere[4,5]. Thus, this eradication effort required a great deal of chutzpah, and also a great deal of luck, in addition to very very hard work.

The question now before us is how was it possible, and can we reproduce this feat with all of the infectious diseases known to be due to microbes in our environment?

The disease

Since smallpox was eradicated from the globe more than thirty years ago, very few people are alive today who have experienced the actual disease, so that it is worth recounting just how devastating the infection actually was. Henderson wrote in his book[3]:

*"In the last hundred years of its existence, smallpox is thought to have killed at least half a billion people. All of the wars on the planet during that time killed perhaps 150 million. In the contest of Smallpox vs. War, War lost. Smallpox killed roughly one-third of the unimmunized people it infected, and the disease was grisly. Once a person was infected with smallpox (*which generally occurred via inhalation), *there was an incubation period of around ten days before the person became noticeably sick. Then the person got a high fever and severe aching pains. After two to three days, the patient would begin to develop a rash. The rash appeared first on the face, hands, and feet, and quickly rose into pustules. Smallpox pustules were hard, pressurized blisters filled with a clear, faintly opalescent pus. The pain of the smallpox pustular rash was virtually unbearable. If the pustules merged into…. a confluent rash, the patient was very likely to die. They died of shock."*

Accordingly, now with the hindsight of thirty years of molecular immunology, it is obvious that this was an immunopathological disease of disseminated inflammation, *i.e.* systemic rubor, calor, tumor, and dolor, caused by the adaptive immune response to the virus, which evidently replicated unchecked by the innate response during the prodromal period. As we now know that all of these cardinal signs of inflammation are ascribable to the production and action of pro-inflammatory cytokines (*e.g.* IL-1, IL-6, TNFα), primarily by antigen-presenting cells and T cells, it is quite possible that armed with our new anti-cytokine therapies, it might be possible to mitigate both the severe signs and symptoms of this syndrome, and also to ameliorate the huge mortality rate.

The virus

The smallpox virus or *Variola major* is a member of the orthopoxvirus family. *Variola* has a large, double-stranded DNA genome with approximately 200 genes, and humans are the only known hosts susceptible to infection, which has been attributed to the species specificity of the viral gene products that circumvent innate immunity. Recent data indicate that the severity of poxvirus disease correlates with the lack of host control of viral replication within the 10-14 day incubation phase. In this regard, poxviruses devote a considerable proportion (~ 50%) of their large genomes manipulating the host innate immune defenses[6].

From Jenner to Henderson

There is a remarkable story of how Edward Jenner came to create the world's first effective vaccine[7]. One hundred years before Pasteur introduced the concept that all microbial diseases might be prevented via prophylactic vaccination or cured via therapeutic vaccination[8,9], Jenner relied on his uncanny powers of observation and logic to come up with the methods to produce a vaccine for smallpox using the cowpox virus. He also showed many others how to reproduce his results, and furnished all of those in the world a seed source of cowpox virus that could be used for immunizations.

As noted in the Jenner article, what was originally thought to have originated from cows, the strain of poxvirus that came to called vaccinia in the 19th and

20th centuries was probably actually indigenous to horses[10]. New data from molecular genetics indicate that the vaccinia virus is most closely homologous to an equine poxvirus. Thus, instead of vaccination, we probably should speak of equination when referring to immunization.

Henderson highlighted three parameters that allowed the eradication of smallpox:

(i) An inexpensive, heat-stable effective vaccine and a simple bifurcated needle used to deliver it reproducibly.

(ii) An easily diagnosed disease by virtue of the characteristic rash.

(iii) No animal reservoir or asymptomatic carriers, *i.e.* 100 per cent of those infected contracted the clinical disease.

All of these characteristics made feasible an effective "surveillance-containment" strategy that was instrumental in identifying and containing any outbreaks. Thus, non-medical personnel in the community could be trained to readily identify and report cases of characteristic smallpox, so that all contacts could be traced and vaccinated. This surveillance-containment effort made possible the eradication of smallpox from each community and country without attaining 100 per cent vaccination rates. However, the goal of vaccinating at least 80 per cent of susceptible individuals was a necessary counterpart to the surveillance-containment policy producing 'herd immunity' and thus decreasing the prevalence of the virus in the population.

Dryvax™ was the inexpensive, heat-stable, effective vaccine that was developed from vaccinia in the 1st half of the 20th century. During most of the 19th century, Jenner's live cowpox virus was transmitted from arm to arm in humans all over the world. One can imagine how this practice could lead to problems before microbiology became appreciated in the late 19th and early 20th century. For example, one egregious episode recorded was the transmission of syphilis when pus from an individual with secondary syphilis was transferred to children during a vaccination programme[3]. Thus, in the 20th century the preparation of vaccine using infected calfskin was a significant step forward, in that large amounts of live virus could be prepared. However, it was not until the 1950s that an eradication programme could be made feasible, when the cell-free filtrate of the calfskin vaccine preps were lyophilized (hence the name Dryvax™), so that the vaccine could be transported and stored at ambient temperatures[3]. Also, arrangements were made with Connaught Laboratories in Toronto to serve as a quality-control laboratory, testing batches of vaccine prepared all around the world so that reproducible vaccine could be made available.

Vaccinia, the prototypic live attenuated vaccine

According to Henderson[3], only variola, vaccinia and monkeypox viruses are transmissible to humans, and only the variola viruses cause widespread, disseminated infections. Consequently, in recent times vaccinia has been enlisted as a vector in the creation of vaccines for other microbes, such as HIV. However, to generate vaccines that can be delivered safely to normal individuals, the one characteristic of vaccinia that made it such an effective vaccine for smallpox, *i.e.* its ability to replicate in human cells, was crippled via genetic engineering. Thus, gene-deleted modified vaccinia strains unable to replicate have been developed, *e.g.* Modified Vaccinia Ankara (MVA) and New York Vaccinia (NYVAC), as well as avian pox (canarypox and fowlpox) strains unable to replicate in mammalian cells are under development for use as vectors to deliver genes from other microbes. In this regard it is noteworthy that Louis Pasteur introduced the concept of attenuating virulent microbes to create live vaccines[8,11]. Pasteur hypothesized that the live, replication-competent microbes depleted the host of vital trace nutrients, thereby rendering the host incapable of supporting the viability and reproduction of the virulent pathogenic organisms[8,11]. Accordingly, Pasteur was totally naïve of how the immune system functions, by actively recognizing and engaging pathogenic microbes, as well as attenuated vaccines.

Pasteur was correct that live attenuated microbes made for the best vaccines, but he was right for the wrong reasons. Live, replication-competent vaccines are more effective than replication-incompetent vaccines, because these establish a self-limited local infection that efficiently activates all of the immune cells and molecules involved in immunity, both innate as well as adaptive immunity. Thus, a self-limited infection ensures microbial peptide presentation via molecules encoded by both class I and class II major histocompatibility complex (MHC) genes, thereby promoting the activation of both class I and class II restricted T cell receptors (TCRs). Also, a replication competent, attenuated microbe ensures the generation of a high enough concentration of microbial peptides, so that there will be a 'strong' TCR activation,

enough to overcome negative feedback loops in place that attenuate a meaningful immune response[12]. In essence, a successful adaptive immune response is one that leads to the production of antibodies, as well as all of the cytokines that promote maximal cellular immune responses, mediated by both cytolytic and helper T cells. Moreover, a replication-competent attenuated vaccine can be delivered in smaller doses than a replication-incompetent vaccine, an important point when a worldwide vaccination programme is under consideration and billions of people must be vaccinated.

Thus, Jenner's observation was that cowpox virus resulted in an attenuated local infection, and not a systemic infection. But why is this possible? The answer to this question is perhaps the most important in vaccinology today. With regard to smallpox, the hallmark of the disease is the 1-2 wk latent period followed by a generalized type IV delayed type hypersensitivity (DTH) reaction to a disseminated very high viral load. Apparently, the virus goes undetected by the first line of defense, *i.e.* the innate immune system[6,13], so that when the adaptive immune system finally reacts the viral load is huge, so that the immunopathology is also huge. From the virus' viewpoint, this sort of an infectious cycle ensures that enough progeny will be produced and disseminated that ensures sustained viability.

By comparison, vaccinia infection remains localized to the site of inoculation, so that this virus is not disseminated systemically. However, the local infection does result in viral replication, albeit limited to the local tissues. Vaccinia then, represents the prototype of vaccines. One needs to identify how vaccinia differs from variola, and logic dictates that variola has genes encoding molecules that circumvent innate immunity, thereby allowing rapid viral replication and dissemination before the adaptive immune response takes hold. By comparison, probably due to species differences of gene products, vaccinia cannot effectively circumvent innate immune responses, which makes for a locally contained infection, and not widespread dissemination.

The $64 question is whether vaccinia itself can be used as a vector to deliver the gene products of other organisms, instead of genetically modified vaccinia, which circumvents the capacity of the virus to replicate in human cells. Experience with the modified vaccinias as well as the avian poxviruses indicates that although these are safe, these are relatively weak immunogens[14].

Because of the recent threat of bioterrorism and the news that the Soviets had a large bioterrorist research and development programme focused on variola, the U.S. government contracted to stockpile >200 million doses of vaccinia smallpox vaccine[15,16]. The previously licensed smallpox vaccine in the U.S., Dryvax® (Wyeth Laboratories Inc.) had a questionable safety profile, because it consisted of a pool of vaccinia virus strains with varying degrees of virulence. Thus, six individual clones of viruses were isolated by plaque purification in tissue culture from a pool of 30 vials (3,000 doses) of Dryvax®, NYCBH, and were tested for immugenicity and virulence by comparison with Dryvax®. A test for immunogenicity included the diameter of erythema and lesions on day eight after scarification of rabbit skin. The rabbit scarification model mimics the vaccine *"take"* observed following human vaccination with Dryvax® because of local replication of vaccinia, which activates a typical adaptive DTH cellular immune response[4,5]. The virulence test measured survival time and viral replication in brain tissue after intracerebral injection of suckling mice. One of the six clones selected for further testing was found to be less virulent than Dryvax® and just as immunogenic. A pilot lot of 750,000 doses of the vaccinia clone propagated on a human lung fibroblast cell line was prepared and tested in 100 volunteers compared with 30 subjects who received Dryvax®. The tissue culture derived clone elicited a 100 per cent take-rate, 100 per cent seroconversion, and a comparable T cell response, monitored via cytotoxic T lymphocyte (CTL), proliferation, and γ-interferon (IFNγ) ELISPOT[16]. Of note, further testing of this clone showed that a full-length copy of the IFN-αβ receptor and the TNFα receptor were both absent whereas these 'virulence factors' were intact in other isolates[16].

To enhance preparedness in the event of a possible variola terrorist attack, a 2002 Presidential initiative recommended vaccination of enlisted military personnel and civilan health care workers who might become first responders. By June 2004, 39,566 civilians and by September 2006, > 1 million soldiers were vaccinated[16]. The appearance of adverse events (AE) was carefully monitored. Cases of progressive vaccinia, eczema vaccinatum, and foetal vaccinia were completely avoided by careful screening of potential vaccinees. One serious AE noted in Dryvax® vaccinations was a myopericarditis that appeared within 1-2 wk post vaccination. When carefully monitored with EKG and cardiac enzyme tests, the cloned vaccine gave an incidence of 0.5 per cent (seven cases in 1307 subjects), compared with 0.8 per cent of individuals who received

Dryvax® (three cases in 363 subjects), a difference not statistically significant[16]. However, most of these cases were subclinical or asymptomatic. Moreover, no cases of myopericarditis occurred among 1819 vaccinia-experienced subjects vaccinated with either vaccine[16].

Accordingly, given these data, which represent an extensive testing of the safety and efficacy of a vaccine in humans, it can be concluded that this strain of plaque-purified vaccinia could serve as a safe and effective vector for many other dangerous microbes, such as HIV. The regulatory authorities, especially of those countries where HIV is endemic at a high prevalence, should be advised to carefully consider the risk/benefit ratio of replication-competent vaccinia as a vector to reduce and perhaps eradicate HIV and other intractable infectious diseases.

Conclusions

Smallpox virus was eradicated from the globe because of its unique virological and immunological characteristics. Whether a similar eradication effort can have similar results with other microbes remains to be seen. Malaria is still endemic in many developing countries despite all efforts, due in large part to the lack of an effective vaccine. Polio incidence has been reduced considerably worldwide thanks to an effective vaccine combination, but vaccination efforts have been thwarted in countries like Pakistan and Nigeria due to civil unrest and the execution of vaccinators by factions distrustful of modern medicine. Even so, the characteristics of variola vs. vaccinia infections may well point the way to the development of a vaccinia-based replication-competent vector that could be used to engineer vaccines useful for the prevention of many of the diseases that plague us today, including HIV infection.

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