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Review

Pathogenesis and potential antiviral therapy of complications of smallpox vaccination

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Abstract

Vaccination against smallpox may result in a variety of complications, ranging in severity from benign to lethal. Universal vaccination was halted in the US in 1972, so almost half the present population has never been vaccinated. Because side effects occur most often in first-time vaccinees, current plans for rapid large-scale vaccination in the event of bioterrorist attack raise concerns about the occurrence of a large number of adverse events. Most complications result from the excessive replication of vaccinia virus, making them potential targets for antiviral therapy. Effective treatment is especially needed for persons with atopic dermatitis or eczema, who are unusually susceptible to the initiation and spread of vaccinia infection because of defects of innate immunity in the skin, and for individuals with defective cell-mediated immunity, who are unable to eliminate vaccinia infection once it has begun. In the past, many complications were treated with vaccinia immune globulin (VIG) and/or the antiviral drug methisazone, but neither was tested in placebo-controlled trials. New antiviral drugs are now available, but have not yet been evaluated for treating vaccinia infections in humans. Both laboratory research and clinical studies are needed to help prevent serious complications in any major vaccination campaign.
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Keywords: Vaccinia virus; Progressive vaccinia; Generalized vaccinia; Eczema vaccinatum; Vaccinia keratitis; Vaccination complications; Smallpox; Orthopoxvirus; Cidofovir; Ribavirin; Interferon; Antiviral therapy

1. Introduction

When progress in the global eradication of smallpox led the US government to discontinue mandatory vaccination for its citizens in the early 1970s, no one could have foreseen that 30 years later the country would be carrying out a crash program of new vaccine production and making plans for large-scale vaccination, in the event that the agent of smallpox (variola virus) is used as a biological weapon (Henderson, 1998; Henderson et al., 1999). Recently announced US government guidelines call for the initial vaccination of half a million health care workers and other "first responders" and a similar number of military personnel, to be followed by a much larger cohort over the next few years. In the event of a smallpox outbreak, many millions of people might have to be vaccinated over a short period of time.

Those planning these vaccination efforts must deal with the fact that smallpox vaccination is associated with a higher incidence of side-effects than any other immunization in current use, and that such complications are roughly 10 times more common in primary vaccinees than in those vaccinated a second or subsequent time (Neff et al., 1967a,b, 2002; Lane et al., 1971; Goldstein et al., 1975; Kemper et al., 2002; Centers for Disease Control, 2003). The only vaccine presently in use in the United States (Dryvax®, Wyeth) is the same material that was used in the 1960s, a lyophilized preparation of a New York City Board of Health (NYCBOH) strain of vaccinia virus. A large amount of a liquid suspension is in frozen storage, and a new cell culture-derived vaccine is in production. Since both are derived from the NYCBOH strain, they may produce the same range of side-effects as Dryvax. Efforts are under way to develop safer smallpox vaccines, but licensure and production are still years away.

As discussed below, most vaccination complications result from the escape of vaccinia virus from the inoculation site and its excessive replication and spread. They are thus potential targets for antiviral therapy. During the 1960s and 1970s, many cases were treated with systemically administered vaccinia immune globulin (VIG) and/or the antiviral

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drug methisazone (Marboran®), as well as with topically applied medications. Since that time, advances in immunology have improved our knowledge of the host response to vaccinia infection, and progress in antiviral drug development has added new medications with antipoxvirus activity. This article reviews our current understanding of vaccination and its complications, summarizes past experience with treatment, describes potential clinical studies aimed at developing improved forms of therapy, and suggests areas for further laboratory research.

2. Origin of smallpox vaccination

Vaccination against smallpox differs markedly from all other immunizations in current use. Instead of being grown under sterile conditions in a laboratory, the vaccine virus was prepared by infecting the shaven flanks of calves, waiting for vesicles to appear, then killing the animals and scraping off the virus-containing fluid. Rather than being injected with a syringe, the virus is jabbed into the skin with a bifurcated needle. In order to understand how this curious procedure came to be in use, it will help to review its origin.

Smallpox is thought to have emerged as a human disease in the cities of the ancient Near East, which for the first time brought together human populations large enough to permit constant transmission of virus in the absence of an animal reservoir (Fenner et al., 1988). In its most common form, *variola major*, the disease had a mortality rate of 20–40%. Many survivors were left scarred or blind.

The observation that people who bore the scars of smallpox were protected against reinfection somehow inspired the first immunization procedure, in which material derived from scabs was inoculated through scratches in the skin. This technique, now termed *variolation*, produced a severe local reaction and occasionally caused disseminated disease, but it provided solid protection against smallpox.

After use for more than 1000 years in Asia, the practice was introduced to Britain in the early 1700s. Near the end of that century, the physician Edward Jenner, who underwent variolation as a child, observed that milkmaids whose hands were scarred by cowpox failed to react when variolated. He modified the traditional inoculation procedure by substituting an inoculum derived from cowpox lesions, producing a much milder skin reaction that still induced immunity to smallpox. Jenner thus empirically discovered the strong cross-protection induced by orthopoxviruses, based on shared antigens. He christened his new method *vaccination*, to denote the bovine origin of the inoculum.

At some time during the 19th century, practitioners of vaccination replaced cowpox with another orthopoxvirus, now designated *vaccinia*, whose natural host is not known. The procedure itself, involving the introduction of virus-containing material through perforations in the skin (*scarification*), has changed relatively little since Jenner's time.

3. Vaccinia virus replication

Vaccinia was long believed to be derived from variola, but the two agents are actually distinct orthopoxviruses. Their large double-stranded DNA genomes encode some 150–200 proteins. Some are required for replication, and are essentially identical in the two viruses. Others that form parts of the virion structure evoke cross-reactive immunity. Yet other proteins are released into the intercellular fluid, where they bind to cytokines and other mediators of the host immune response. The efficiency with which the immunomodulatory proteins encoded by a particular orthopoxvirus block the antiviral responses of a given animal species plays a major role in determining the virus's virulence for that species.

Primary vaccination results in a series of events at the inoculation site known as a "take" reaction. After its introduction into the skin, vaccinia virus replicates in the cytoplasm of keratinocytes in the basal layer and spreads from cell-to-cell, causing necrosis and the formation of fluid-filled vesicles (Fig. 1A). By the end of the first week postinfection, the infiltration of neutrophils, macrophages and lymphocytes and their release of inflammatory mediators transforms the vesicles into a confluent pustule surrounded by reddened, swollen tissues (Fig. 1B). Additional "satellite" pustules sometimes develop on adjacent skin. Vaccinia-specific neutralizing antibodies and cytotoxic T cells are detectable by the second week of infection (Fenner et al., 1988; Demkowicz and Ennis, 1993; Ennis et al., 2002).

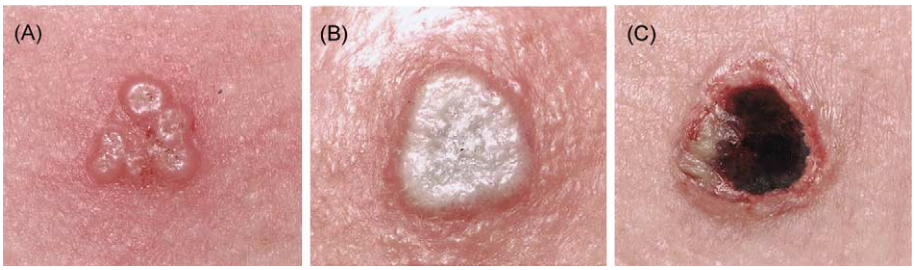

Fig. 1. Normal development of a primary smallpox vaccination lesion. (A) Day 4. Individual vesicles at sites of puncture by bifurcated needle. (B) Day 8. Confluent pustule with erythema and edema of surrounding skin. (C) Day 14. Resolution of inflammation and involution of pustule, with scab formation. (All figures copyright 2002© Logical Images, Inc.)

The inflammatory response usually reaches its peak by day 10–12 and begins to resolve about day 14, with formation of a scab that is shed by day 21 (Fig. 1C). The take reaction following primary vaccination thus mimics the formation of a smallpox pock in a non-immune individual. A successful take is required for the development of antivaccinia antibody and cell-mediated responses (McClain et al., 1997; Ennis et al., 2002; Frey et al., 2002a,b).

In addition to the local reaction, many vaccinees develop tender, swollen axillary lymph nodes, along with fever, malaise, and other constitutional symptoms. Some strains of vaccinia virus commonly disseminate through the bloodstream, infecting lymphoid tissues and producing scattered skin pocks, but the NYCBOH strain reportedly causes only a limited viremia in a small percentage of its recipients during the period of pustule formation (Blattner et al., 1964; Fenner et al., 1988). Involvement of organs other than the skin and lymph nodes is rarely observed.

4. Restriction of vaccinia replication by innate and adaptive responses

Vaccinia virus is of low virulence for humans with normal cutaneous and systemic immune function. In the great majority of primary vaccinees, a combination of innate and adaptive immune responses confine the virus to the inoculation site. Defects in these mechanisms may render an individual susceptible to severe vaccination complications.

The initial response to vaccinia infection consists of the synthesis and release by keratinocytes, macrophages and local dendritic cells (Langerhans cells) of a range of substances that restrict local viral spread and evoke an adaptive immune response. These may include antimicrobial peptides; chemokines that attract neutrophils and macrophages to the site of infection; and types I and II interferons (IFN), interleukin (IL)-12 and other proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, that evoke an antiviral state in local cells, activate natural killer cells, and contribute to the differentiation of Th1 CD4+ T cells, thereby eliciting a cytotoxic T cell response (Engler et al., 2002; Ong et al., 2002; Stanley, 2002).

A series of experiments employing recombinant vaccinia viruses encoding cytokine genes has demonstrated that the balance between Th1 and Th2 responses strongly influences the course and outcome of infection. Thus, athymic nude mice that are normally incapable of controlling vaccinia infection are able to eliminate recombinant viruses if they encode IL-2 or IFN-γ (Kohonen-Corish et al., 1990; Hugin et al., 1993). Similarly, the replication of an IL-2 encoding virus is restricted in nonhuman primates (Flexner et al., 1990). By contrast, recombinant vaccinia or ectromelia virus encoding the Th2 cytokine IL-4 showed markedly increased virulence for mice (Sharma et al., 1996; Jackson et al., 2001).

As discussed later, defects in innate immunity, including a predominantly Th2 cytokine response to cutaneous viral infection, appear to play a critical role in the increased susceptibility of individuals with eczema and other forms of atopic dermatitis to the initiation and rapid spread of vaccinia infection (Engler et al., 2002). Persons with defects of cell-mediated immunity, by contrast, may be able to employ innate antiviral responses to keep vaccinia from establishing a foothold in their skin, but cannot generate cytotoxic T cells to eliminate a focus of infection, once established.

Antibodies appear to be less important than cell-mediated immune responses in eliminating vaccinia infection. Early studies reported that children with agammaglobulinemia responded normally to vaccination (Good et al., 1956), and that the administration of antivaccinia serum after the beginning of lesion development had little effect on the take reaction (Gispen et al., 1956). As discussed later, VIG was reported to be beneficial in the treatment of some vaccination complications, suggesting a role for antibodies in restricting viral dissemination or facilitating antibody-dependent cell-mediated cytotoxicity. However, its efficacy was never proven in controlled trials.

5. Overview of vaccination complications

Most of the adverse effects of smallpox vaccination can be predicted from the nature of the procedure, which essentially employs a small circle of skin as a “culture plate” in which to grow vaccinia virus. Complications can be divided into two categories: those involving excessive viral replication, either at the vaccination site or at other areas on the body, and those brought about through other mechanisms (Table 1).

5.1. Complications involving excessive viral replication

Diagnostic features of these complications are listed in Table 2. The most common is *accidental infection*, in which virus is unintentionally transferred from the vaccination site to other areas on the body of the vaccinee or his close contacts (Fig. 2A). Less frequently, internal viral dissemination through the bloodstream results in *generalized vaccinia*, in which skin pocks appear at randomly scattered sites (Fig. 2B). Both processes may have serious consequences in individuals with eczema and other forms of atopic dermatitis, whose skin is unusually permissive to the initiation and rapid spread of vaccinia infection (*eczema vaccinatum*) (Fig. 2C and D).

Much less commonly, the inadvertent inoculation of an individual with defective cell-mediated immunity leads to *progressive vaccinia*, characterized by the inexorable enlargement of the primary vaccination site and the eventual development of similar lesions elsewhere on the body (Fig. 2E and F). Finally, an extremely rare complication, *fetal vaccinia*, occurs when the vaccination of a pregnant woman is followed by internal dissemination of virus to her fetus.

Table 1
Complications of smallpox vaccination and prognosis of untreated cases

Basis of adverse effect	Mechanism	Complication	Immune status	Severity/prognosis
Excessive viral replication	Local spread of virus	Progressive vaccinia	Cell-mediated immune defect	Serious to fatal
	External transfer of virus	Accidental infection	Normal	Benign to serious (eye)
		Eczema vaccinatum	Defects of innate and adaptive immunity	Benign to fatal
	Internal dissemination of virus	Generalized vaccinia	Normal	Benign
		Eczema vaccinatum	Defects of innate and adaptive immunity	Benign to fatal
		Progressive vaccinia	Cell-mediated immune defect	Serious to fatal
		Fetal vaccinia	Normal (pregnancy)	Mother: benign; fetus: fatal
Other	Contamination of vaccination site	Bacterial superinfection	Normal	Benign
	Unknown (allergic?)	Erythema multiforme	Normal	Benign to fatal
	Unknown (autoimmune?)	Postvaccinial encephalitis	Normal	Serious to fatal

5.2. Other adverse events

The most common complication that is not caused by viral replication is *bacterial superinfection* of the vaccination site. Less often, vaccination evokes a generalized erythematous, urticarial skin reaction, *erythema multiforme*, which is usually accompanied by mild illness with or without fever. Severe forms of this reaction may require steroid therapy.

The most serious, but fortunately the rarest of these complications is *postvaccinial encephalitis*, in which neurologic changes appear 1–2 weeks after vaccination, usually beginning with signs of increased intracranial pressure and often leading to stupor, coma, seizures or paralysis (Roos and Eckerman, 2002). Encephalitis apparently occurs at random in individuals with no known predisposing condition. Fatal cases are characterized by cerebral perivascular mononuclear cell infiltrates with surrounding areas of demyelination, resembling changes that may also occur after measles and other viral infections (Fenner et al., 1988). Vaccinia virus was recovered from the cerebrospinal fluid and from brain tissue at autopsy in some, but not all cases of postvaccinial encephalitis in Russia (Gurvich and Vilesova, 1983). A study in army recruits indicated that the incidence of encephalitis could be reduced by administering VIG at the time of vaccination (Nanning, 1962), but there is no evidence that VIG has any effect once signs of illness have developed.

Table 2
Clinical features of vaccination complications that result from excessive viral replication

Complication	Clinical features
Accidental infection	Small number of new vaccinia lesions appear at the same time or soon after development of the primary pustule or after contact with a recent vaccinee Pocks tend to occur on the face and other areas frequently touched by the hands Areas of damaged skin are particularly vulnerable to infection
Generalized vaccinia	Multiple additional pocks appear during the second or third week postvaccination Randomly scattered lesions range in number from a few to several hundred Pocks undergo accelerated development and resolve with the primary lesion
Eczema vaccinatum	Occurs in individuals with eczema or other forms of atopic dermatitis, whether or not skin disease is active Multiple pocks appear within 1–2 weeks after vaccination or direct contact with a vaccinee Lesions develop on both normal and eczematous skin; when the latter is present, it may become heavily infected and inflamed Extensive spread of vaccinia pocks may create an appearance similar to smallpox
Progressive vaccinia	Occurs in individuals with defects of cell-mediated immunity The enlarging vaccination lesion forms an ulcer with necrotic tissue in its center and a raised, advancing rim containing viral vesicles Infection may evoke little or no local inflammatory reaction and no lymphadenopathy or systemic signs of infection Additional lesions appear one by one at other sites on the body over the course of weeks or months and form ulcers resembling the primary lesion

6. Incidence rates of vaccination complications

Our knowledge of the frequency of adverse events is based on large-scale surveys from the middle third of the 20th century, when tens of millions of inoculations were performed each year in the US and other countries (Table 3). Surveys based on physician reports (e.g. Lane et al., 1969) generally detected only the most serious complications, while those that employed active case finding (e.g. Lane et al., 1970b) uncovered a much larger number of less severe side effects. The incidence rates of complications in the two surveys cited are shown in Table 4. Overall, fatal complications occurred at a rate of less than one per million vaccinees (Lane et al., 1970a). With the exception of progressive vaccinia, all types of adverse event were much more common in primary

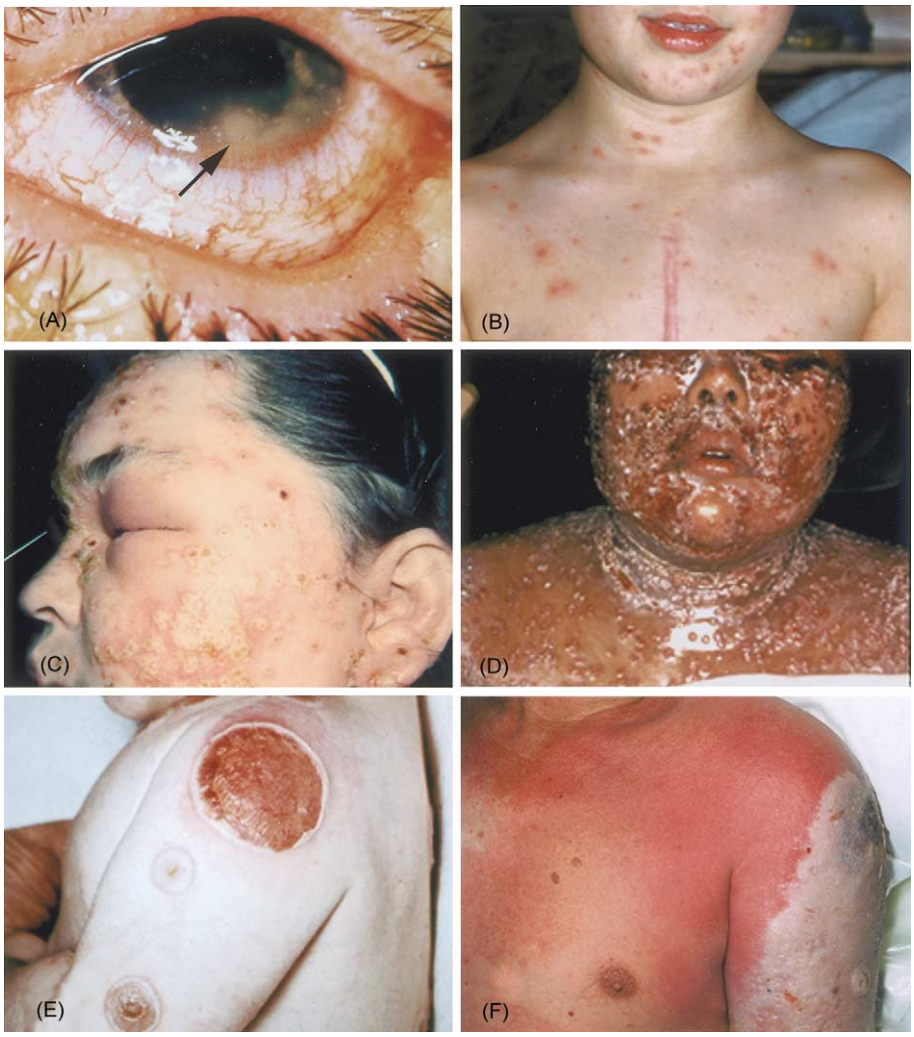

Fig. 2. Complications of vaccination caused by excessive vaccinia virus replication. (A) Accidental ocular infection, with conjunctivitis, vascular proliferation and corneal infiltrates (arrow). (B) Generalized vaccinia in a primary vaccinee, showing randomly scattered small vaccinia pustules. (C) Eczema vaccinatum, showing the development of multiple individual or confluent vaccinia pustules in areas of eczematous skin. (D) Severe eczema vaccinatum, resembling smallpox, in a 22-year-old woman who acquired the infection through contact with her recently vaccinated boyfriend. Treated with methisazone and VIG, survived. (E) Fatal progressive vaccinia in a 3-month-old infant with severe combined immunodeficiency. Note absence of inflammation in skin surrounding the lesions. (F) Fatal progressive vaccinia in a 71-year-old man with lymphosarcoma. Skin below the necrotic vaccination ulcer contains vaccinia vesicles. Treated with VIG and methisazone without response. (All figures copyright 2002© Logical Images, Inc.)

Table 3
Sources of information on the frequency and outcome of adverse effects of smallpox vaccination

Country	Year	Survey method	Number of vaccinations	Reference
Great Britain	1951–1960	Reports of vaccination complications to public health authority	5,061,013	Conybeare, 1964
England and Wales	1962	Questionnaire to dermatologists; reported cases; requests for VIG	3,250,000 (primary)	Copeman and Wallace, 1964
USA	1963	National survey: VIG requests; case reports; death certificates; national questionnaire	14,014,000	Neff et al., 1967a
		Direct survey of all physicians in four states	668,000	Neff et al., 1967b
	1968	National survey: reported complications; requests for VIG or methisazone; vaccine manufacturers' reports; encephalitis surveillance; specimen submissions	14,168,000	Lane et al., 1969
		Direct survey of all physicians in 10 states	1,648,000	Lane et al., 1970b

Table 4
Incidence per million vaccinees in 1968 in two large US surveys of complications following primary or secondary (booster) vaccination

Basis of adverse effect	Complication	Incidence: 1968 national survey (Lane et al., 1969)		Incidence: 1968 10-state survey (Lane et al., 1970b)	
		Primary	Secondary	Primary	Secondary
Excessive viral replication	Accidental inoculation	25.4	0.8	529.2	42.1
	Generalized vaccinia	23.4	1.2	241.5	9.0
	Eczema vaccinatum	10.4	0.9	38.5	3.0
	Progressive vaccinia	0.9	0.7	1.5	3.0
Other	Erythema multiforme	ND	ND	164.6	10.0
	Postvaccinial encephalitis	2.9	–	12.3	2.0
	Other	11.8	1.0	266.2	39.1

ND: not done.

vaccinees than in those receiving the vaccine a second or subsequent time.

7. Antiviral agents of potential therapeutic value

No specific therapy of vaccination complications was available until the 1950s, when VIG and methisazone were introduced into clinical use. Several ophthalmic medications were used to treat vaccinia keratitis over the next two decades. Since universal vaccination ended in the 1970s, two antiviral drugs with activity against orthopoxviruses, ribavirin and cidofovir, have been licensed. Neither is currently approved for the treatment of vaccinia infections.

All published reports of successful treatment with VIG or methisazone are based on physicians' personal impressions of improvement in a patient's condition following treatment; no placebo-controlled trials were performed. However, because the vast majority of vaccination complications resolve on their own without treatment, as a result of the patient's own immune response, the contribution of any type of antiviral therapy to the final outcome may be difficult to measure. Those reading early reports of successful therapy must keep in mind the patient's own immune response as an unmeasured variable.

7.1. Vaccinia immune globulin

VIG was prepared as an approximately 20-fold concentrate of γ-globulin from the pooled plasma of recently vaccinated military recruits (Kempe et al., 1956; Kempe, 1960). At the time it was introduced into clinical use, most vaccination complications were believed to result from defective antibody responses, and the role of cell-mediated immune function in vaccinia infection was still unknown. Despite the absence of proven efficacy, a nationwide distribution system was soon set up through Red Cross blood centers, and after 1960 almost all patients with significant vaccination complications were treated with VIG.

The intramuscular inoculation of 0.6 ml of VIG per kg of body weight was reported to halt the formation of new lesions and to cause rapid clinical improvement in cases of generalized vaccinia and eczema vaccinatum (Kempe et al., 1956; Sussman and Grossman, 1965; Sharp and Fletcher, 1973; Goldstein et al., 1975). Progressive vaccinia was also reported to respond to VIG, but treatment required multiple inoculations over the course of weeks before full resolution was seen. As noted earlier, the improvement observed in some cases may actually have been caused by gradual recovery of the patient's cell-mediated immune function. VIG was also recommended for prophylactic use at the time of vaccination, in a dose of 0.3 ml/kg, when an immediate threat of smallpox necessitated the vaccination of an individual who would otherwise have been deferred. This did not prevent a take reaction (Kempe et al., 1956).

The US stock of VIG is maintained by the Centers for Disease Control and Prevention (CDC), Atlanta, GA. VIG is currently recommended for treating severe generalized vaccinia, eczema vaccinatum, progressive vaccinia and cases of vaccinia infection of large areas of damaged skin. Its role in ocular infections is currently being debated (see the description later). Because the existing material has become discolored during prolonged storage, it is classed as an investigational drug, requiring informed consent. A new stock suitable for intravenous administration is currently being produced.

7.2. Methisazone

During the 1950s, a number of thiosemicarbazone derivatives were found to inhibit the replication of vaccinia virus. One of them (methisazone, Marboran®) became the first antiviral drug to be introduced into clinical use (Bauer, 1965; Driscoll, 2002). Its mode of action appears to involve a block in protein synthesis during a late stage of virion maturation (De Clercq, 2001). Methisazone was fairly toxic when administered systemically, but nevertheless it was quickly applied to the therapy of vaccination complications. Several reports claimed that it hastened the resolution of eczema vaccinatum and was beneficial for progressive vaccinia (Bauer, 1965; Rao et al., 1965; Brainerd et al., 1967; Jaroszynska-Winberger, 1970; McLean, 1977), but the lack

of controlled trials makes it difficult to judge whether treatment actually played a role in recovery. The drug is no longer in use.

7.3. Ribavirin

The broad-spectrum antiviral drug ribavirin (Virazole®, Rebetol®) inhibits the replication of vaccinia and other orthopoxviruses (Baker et al., 2003). The drug was active in a model of vaccinia keratitis in rabbits (Sidwell et al., 1973) and in a tailpox model in mice (De Clercq et al., 1976). It has been used once to treat a vaccination complication, a case of progressive vaccinia in a leukemia patient (see the description later).

7.4. Cidofovir

A compound with stronger antipoxvirus activity than ribavirin, but greater potential for systemic toxicity, is the phosphonate analog of cytosine, cidofovir (Vistide®), which is licensed for treatment of cytomegalovirus infections (De Clercq, 2001, 2002). The drug must be administered intravenously, accompanied by probenecid and hydration to avoid renal toxicity (Naesens et al., 1997). Cidofovir's remarkably long intracellular half-life permits infrequent dosing. Modified forms that can be taken by mouth are currently under development (Kern et al., 2002).

Cidofovir has not been used to treat orthopoxvirus infections in humans, but has been tested extensively in laboratory animals. It protected immunocompetent mice against lethal vaccinia infection (Smee et al., 2001) and delayed the death of SCID mice (Neyts and De Clercq, 1993). A combination of cidofovir and VIG eliminated vaccinia infection in athymic nude mice (Hanlon et al., 1997). Topically administered cidofovir has shown a beneficial effect against two other poxviral infections of the skin, molluscum contagiosum and orf (Calista, 2000; Geerinck et al., 2001), suggesting that similar therapy would be useful in treating localized areas of vaccinia infection, such as the enlarging vaccination lesion in progressive vaccinia.

7.5. 5-Iodo-2′-deoxyuridine

5-Iodo-2′-deoxyuridine (idoxuridine, Stoxil®) is a thymidine analog that is phosphorylated by cellular thymidine kinase and acts as a DNA polymerase inhibitor. It was first used to treat ocular vaccinia infections in the early 1960s (Kaufman et al., 1962). Systemically administered idoxuridine is effective against vaccinia infection in mice (Neyts et al., 2002), but the drug is too toxic for such use in humans (Driscoll, 2002).

7.6. Adenine arabinoside

Adenine arabinoside (vidarabine, Ara-A®, Vira-A®) is an adenosine analog that is phosphorylated by both viral and cellular kinases, and acts as a DNA polymerase inhibitor. It is active topically against vaccinia keratitis (Hyndiuk et al., 1976a). Vidarabine prevented death from vaccinia infection in immunosuppressed mice (Worthington and Conliffe, 1977), but its systemic use in humans is limited by toxicity.

7.7. Trifluorothymidine

Trifluorothymidine (trifluridine, Viroptic®) is a thymidine analog with a mode of action similar to idoxuridine. Used topically, it is effective against ocular herpes and vaccinia infections (Hyndiuk et al., 1976b; Lee et al., 1994; Pavan-Langston, 1997). Trifluridine appears at present to be the most widely available medication for the treatment of ocular vaccinia infections.

7.8. Other nucleoside analogues

Concern over possible bioterrorist use of variola virus has stimulated efforts to develop new drugs for the treatment of orthopoxvirus infections, in particular the attempt to devise orally available cidofovir derivatives. The reader is referred to articles by De Clercq (2001), Baker et al. (2003), and Kern (2003) for information on these compounds. Recent work by Neyts and De Clercq (2001) and Smee et al. (2002) has shown that 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine (S2242), which has potent antiherpesvirus activity, is also active against vaccinia and cowpox viruses. The work of Snoeck et al. (2002) in using organotypic "raft" cultures to evaluate the antivaccinia activity of a range of acyclic nucleoside phosphonate derivates should assist drug development efforts.

7.9. Interferon and interferon inducers

Early studies showed that vaccinia keratitis in humans responded rapidly to IFN treatment (Jones et al., 1962), and that murine IFN prevented the development of tail pocks in vaccinia-infected mice (De Clercq and de Somer, 1968). IFN-α and -β have been used in gel form to treat herpetic lesions in humans (Glezerman et al., 1988), but have not been tested for the treatment of vaccination complications. However, intradermally injected IFN-β blocked the development of vaccination lesions in human volunteers (Scott et al., 1978), suggesting that topical therapy would restrict excessive vaccinia replication.

The double-stranded RNA preparation poly(ICLC), alone or in combination with VIG, showed significant activity against vaccinia infection in immunosuppressed mice (Worthington and Baron, 1973). PolyICLC ointment applied to the skin of rabbits induced local IFN production, prevented the formation and spread of large vaccinia lesions, and markedly reduced the maximum lesion size when applied after vaccination (Levy and Lvovsky, 1978). It has not been used to treat vaccination complications in humans.

7.10. Other immunomodulators

A group of low molecular weight compounds, the imidazoquinolinamines, possess antiviral activity through their ability to potentiate innate antiviral responses (Stanley, 2002). One of them, imiquimod (Aldara®, R-837, S-26308) is in clinical use as a topical therapy for human papillomavirus infections, and has shown efficacy against molluscum contagiosum (Miller et al., 2002). A more soluble and more potent analogue, resiquimod (R-848, S-28463) is currently undergoing Phase III evaluation for the treatment of genital herpes. The compounds appear to act through the Toll-like receptor 7 of keratinocytes, Langerhans cells and macrophages, stimulating them to release IFN-α, IFN-γ, IL-12 and other Th1 cytokines, thereby promoting the development of Th1 CD4+ T cells and biasing adaptive immunity in favor of a cell-mediated immune response (Wagner et al., 1999; Hemmi et al., 2002; Miller et al., 2002).

Orally administered resiquimod induced serum cytokine elevations in cynomolgus monkeys, suggesting that these compounds may find applications for systemic therapy (Wagner et al., 1997). Oral formulations of both imiquimod and resiquimod have been evaluated in humans. Neither substance has been used to treat vaccinia infections, but their efficacy in the experimental treatment of herpesvirus infections and molluscum contagiosum suggests that they would be of benefit. Their ability to induce expression of interferon and Th1 cytokines might make them beneficial in treating eczema vaccinatum (see the description later).

8. Specific complications and potential modes of therapy

8.1. Accidental infection

Diagnostic features of accidental infection are listed in Table 2. This complication only poses a significant threat to persons with normal cutaneous and systemic immunity when it involves the eye or extensive areas of traumatized skin. Lesions are most severe in those not previously vaccinated (Neff et al., 2002; Sepkowitz, 2003).

8.1.1. Ocular vaccinia

The frequency of hand-eye contact makes the orbit one of the most common sites of accidental vaccinia infection (Fig. 2A). A review of 348 cases of ocular vaccinia found that 70% occurred in primary vaccinees, more than half of whom were children under 5 (Ruben and Lane, 1970). Most cases involved only the eyelids and conjunctiva, and few resulted in corneal infection (vaccinia keratitis). Four of 22 cases with vaccinia keratitis ended up with corneal scarring, while only 2% of the 322 remaining cases had residual damage (usually eyelid scarring). Another survey found corneal involvement in 5 of 48 cases of ocular vaccinia infection (Sussman and Grossman, 1965).

8.1.1.1. Potential therapy. Topical therapy with a variety of antiviral compounds has proven effective in arresting ocular vaccinia infections. During the 1960s, vaccinia keratitis was usually treated with topical idoxuridine (Kaufman et al., 1962), but subsequent comparative studies showed that trifluridine was more effective (Hyndiuk et al., 1976b). A recent report describes a case of keratouveitis treated with trifluridine and reviews the diagnosis and therapy of ocular vaccinia infections (Lee et al., 1994). Topical cidofovir is as effective or more effective than trifluridine for the treatment of experimental acute herpetic keratitis (Kaufman, 1999; Romanowski et al., 1999), suggesting that it should be tested for efficacy in experimental ocular vaccinia infection.

Although many authors over the years have recommended VIG as a component of therapy for ocular vaccinia infections (e.g. Pavan-Langston, 1997), its use is now a matter of debate, because of an early report suggesting that its administration might lead to the formation of antigen–antibody complexes with enhanced corneal clouding and worsened scarring (Fulginiti et al., 1965). The reader should consult CDC publications for current recommendations.

8.1.2. Other accidental infections

Injured skin is unusually permissive to the initiation and spread of vaccinia infection, perhaps because of the presence of increased numbers of immature keratinocytes. Examples cited in the past include healing wounds, burns and multiple foci of skin damage from acne, scabies or chickenpox (Sepkowitz, 2003). Infection may occur either through the external transfer of virus or via bloodstream dissemination.

8.1.2.1. Potential therapy. VIG has traditionally been recommended for the treatment of vaccinia infection of extensive areas of injured skin (Sussman and Grossman, 1965, and others). There are no case reports of vaccinia wound or burn infections in the English-language literature, but vaccinia-infected burns were reported in some patients in Germany (Nimpfer, 1936) and a number of herpesvirus infections of burns have been described (Foley et al., 1970). Although rare, such infections would clearly offer a target for investigational antiviral therapy. Although topical cidofovir might be employed for this purpose, caution should be employed in applying it to extensive areas of injured skin, because absorption may result in renal toxicity (Bienvenu et al., 2002).

8.2. Generalized vaccinia

This condition occurs almost exclusively in primary vaccinees (Fig. 2B). During the era of routine vaccination, generalized vaccinia was thought to result from delayed production of anti-vaccinia antibodies, but the concept remains unproven. However, it is reasonable to suppose

that the developing immune response affects the extent of bloodstream dissemination and the size and number of the resulting pocks. In most cases, the lesions resolve quickly, without scarring.

8.2.1. Potential therapy

Generalized vaccinia usually resolves without treatment. During the 1950s and 1960s, individuals who developed a large number of lesions and became seriously ill were sometimes treated with VIG, which was reported to halt the development of new lesions and produce rapid clinical improvement (Kempe et al., 1956; Sussman and Grossman, 1965; Sharp and Fletcher, 1973). Treatment with methisazone was also said to result in a rapid response (McLean, 1977). Clinical studies might be useful in determining whether VIG actually provides a benefit, but the benign nature of the condition does not appear to justify systemic therapy with antiviral drugs.

8.3. Eczema vaccinatum

The terms "eczema" and "atopic dermatitis" refer to a variety of skin conditions that generally begin in infancy, are characterized by recurrent areas of reddened, scaly, itchy skin, and are frequently accompanied by asthma, hay fever or other types of allergy (Rudikoff and Lebwohl, 1998; Leung, 2000). Patients often have a family history of eczema and other allergic diseases. The prevalence of such conditions has been increasing over recent decades, and it is now estimated that some 10–15% of the population may be diagnosed with atopic dermatitis at some time during their lives (Engler et al., 2002). Eczema vaccinatum is therefore of great concern to those planning large-scale vaccination campaigns in the event of a smallpox attack. Because persons with eczema are highly susceptible to infection through contact with a recent vaccinee (Neff et al., 2002; Sepkowitz, 2003), current guidelines call for the deferral from vaccination both of persons with eczema and of anyone with a household contact with the condition.

The characteristic features of eczema vaccinatum are listed in Table 2. A large survey of eczema vaccinatum complicating mass vaccination in Great Britain showed that most patients with atopic dermatitis suffered no ill effects from vaccination (Copeman and Wallace, 1964), and it is likely that many individuals develop only a limited extension of their vaccinia infection that resolves on its own, without therapy. However, occasional individuals develop a profuse rash resembling smallpox (Fig. 2C and D). This extensive infection of multiple sites was once thought to result from external transfer of virus, but it now seems clear that it is caused by the dissemination of virus through lymphatic channels and the bloodstream. Individuals at risk of eczema vaccinatum are also susceptible to severe infection by naturally occurring pathogens, including cowpox virus (Czerny et al., 1991), herpes viruses (Mooney et al., 1994) and staphylococci (Ong et al., 2002). Herpes simplex virus may produce a disseminated infection, eczema herpeticum, that closely resembles eczema vaccinatum; both are included under the eponym "Kaposi's varicelliform eruption" (Mooney et al., 1994).

The above-cited survey by Copeman and Wallace identified 185 cases of eczema vaccinatum, including 11 fatalities, that occurred during the course of a campaign that vaccinated more than 6 million people in 1962. Some 80% of these individuals suffered from "atopic" eczema, while the remainder had seborrheic eczema or other conditions. Only one-third had active skin disease at the time of their vaccinia infection. Roughly half of the cases, including eight of the deaths, occurred in children under 5 years of age. About two-thirds of the patients had been deferred from vaccination, but became infected through contact with a recent vaccinee. Four adults acquired the infection from their children.

The susceptibility of individuals with eczema and other forms of atopic dermatitis to the rapid, simultaneous development of large numbers of vaccinia lesions implies a defect in innate immunity that makes it easier for the virus to establish a foothold in the skin. A number of factors may play a role, including failure of neutrophils to migrate to sites of infection, decreased natural killer cell activity, and insufficient production of antimicrobial peptides (Engler et al., 2002). In addition, a constitutive increase in Th2 cytokine expression in the skin of atopic individuals may weaken the cytotoxic T cell response. However, in all but the most overwhelming vaccinia infections, patients are eventually able to develop adaptive responses that eliminate the virus and render them resistant to re-infection.

8.3.1. Potential therapy

During the era of universal vaccination, many investigators reported that treatment with VIG hastened the resolution of eczema vaccinatum and appeared to reduce the overall mortality rate (Kempe et al., 1956; Lundstrom, 1956; Sussman and Grossman, 1965; Sharp and Fletcher, 1973). A single inoculation was often followed within 24 h by a halt in formation of new lesions and within 48 h by the termination of fever and improvement in other clinical indices. Prophylactic administration of VIG was said to be effective in preventing the condition in eczematous individuals exposed to recent vaccinees. However, no controlled trials were performed.

The vast overgrowth of vaccinia virus that occurs in some cases of eczema vaccinatum makes it a prime target for antiviral therapy. Early studies with methisazone appeared to show benefit, but no controlled trials were performed (Turner et al., 1962; Bauer, 1965; Jaroszynska-Winberger, 1970; McLean, 1977). Modern investigative approaches to therapy would focus on the use of cidofovir. The imidazoquinolinamines, imiquimod and resiquimod, might also be of benefit when applied topically as adjunctive therapy, because of their ability to induce a strong Th1 response.

Other approaches are also needed to prevent the occurrence of eczema vaccinatum. Although it is clear that

children with active eczema must be deferred from vaccination, there is currently no way to identify those adults with a past history of skin disease who are susceptible to this complication (Engler et al., 2002). Studies of the biological basis of atopic dermatitis and associated susceptibility to viral infection should therefore focus on developing a simple test to identify individuals at risk.

The improved understanding of the immune defects responsible for eczema vaccinatum should also be directed toward the development of specific forms of prophylaxis or therapy. An animal model of eczema vaccinatum is greatly needed; some initial work might be performed using currently available mouse models of atopic dermatitis, such as mutant NC/Nga mice (Suto et al., 1999; Nakamura et al., 2002), or transgenic mice that constitutively overexpress IL-4 in the skin (Chan et al., 2001).

8.4. Progressive vaccinia

This condition, also known as *vaccinia necrosum* or *vaccinia gangrenosa*, is characterized by the inexorable enlargement of the primary vaccination lesion and the eventual appearance of similar foci of infection on other areas of the body (Fig. 2E and F) (Fulginiti et al., 1968; Bray and Wright, 2003). Additional diagnostic features are listed in Table 2. The slow but inexorable spread of virus through the tissues causes extensive necrosis and osteomyelitis, often with bacterial superinfection, leading to death weeks or months after vaccination.

Early studies attributed progressive vaccinia to an inability to produce anti-vaccinia antibodies (Kempe et al., 1956), but by the mid-1960s it became clear that the syndrome was almost always the result of a congenital or acquired defect in cell-mediated immunity (Fulginiti et al., 1968). In contrast to individuals with eczema vaccinatum, progressive vaccinia patients developed a relatively small number of "metastatic" lesions (compare Fig. 2C and D with E and F), suggesting that their innate immune responses were sufficient to restrict the initiation of vaccinia infection in the skin. Also in contrast to eczema vaccinatum, and perhaps for the same reason, only a single reported case of progressive vaccinia is known to have resulted from contact infection, rather than vaccination (MacKenzie et al., 1969).

During the era of universal vaccination, the condition occurred almost exclusively in two groups of individuals at opposite ends of the age spectrum. The first consisted of infants congenitally lacking cell-mediated immune function (Fig. 2E). When inadvertently vaccinated, they developed relentlessly progressive infections that were almost invariably fatal. The number of such cases was eventually reduced by deferring vaccination to the second year of life, allowing time for immunodeficient infants to be identified.

The second group was made up of adults over 50 with acquired immune deficiency secondary to chronic lymphocytic leukemia, lymphoma or connective tissue disorders, most of whom were receiving corticosteroids and/or antimetabolite therapy (Fig. 2F). In contrast to infants, most adults eventually succeeded either in eliminating the virus, or in at least partially resolving their lesions before dying from other causes. The spectrum of illness must also have included many other chronically ill individuals with milder degrees of cell-mediated immune deficiency, who experienced delayed healing of their vaccination lesions, but recovered without therapy and went unreported.

8.4.1. Potential therapy

VIG was introduced into clinical use at a time when progressive vaccinia was believed to result from a defect in antibody production (Kempe et al., 1956). The subsequent realization that the condition resulted from a lack of cell-mediated immune function would seem to have weakened the rationale for its use, but nevertheless almost all progressive vaccinia patients continued to be treated with VIG. In 1963, for example, it was used to treat all 9 reported cases in the USA, all of whom survived their infections, while in 1968, 10 of 11 cases were treated with VIG, and 6 of them survived (Neff et al., 1967a,b; Lane et al., 1970a,b).

In addition to the apparently beneficial effect on overall survival, physicians often reported that new lesions ceased to form and existing ones began to heal after antibody treatment, suggesting a therapeutic effect. However, in many cases these apparent "responses" may actually have resulted from improvement in the patients' cell-mediated immune function. In particular, the successful cure of progressive vaccinia in some patients with chronic leukemia or lymphoma may have owed more to a decrease or discontinuation of their steroid or antimetabolite therapy than to their physicians' therapeutic efforts.

A much smaller number of progressive vaccinia patients were treated with methisazone, or with methisazone plus VIG, and some survived their infections (Bauer, 1965; Brainerd et al., 1967; Van Rooyen et al., 1967; Douglas et al., 1972). No controlled trials were performed. More recently, ribavirin was used in the initial treatment of an elderly cancer patient who developed progressive vaccinia after receiving experimental therapy (Kesson et al., 1997; Wills et al., 2000). New lesions continued to form while the patient was on ribavirin alone, but none appeared after VIG was added to his regimen.

Progressive vaccinia infection obviously presents a target for antiviral therapy, which currently means treatment with systemically administered cidofovir. Since VIG remains the standard of care, investigative treatment protocols would probably call for initial treatment with VIG, followed by cidofovir if the former failed to produce a response. Given the slowly progressive nature of the disease and the long half-life of cidofovir, significant benefit might be obtained from a single infusion. Clinical protocols might also determine whether the direct application of cidofovir gel to an enlarging primary vaccination lesion would halt viral replication and further spread.

8.5. Fetal vaccinia

This rare complication did not pose a direct threat to the pregnant woman, but was lethal for the fetus (Suarez and Hankins, 2002). In the past, when vaccination was performed in the setting of a smallpox epidemic, it was believed that the risk of fetal vaccinia could be reduced by administering VIG at the time of vaccination, but the efficacy of this practice was never proven (Goldstein et al., 1975).

9. Vaccination complications and HIV infection

Because routine smallpox vaccination was discontinued before the human immunodeficiency virus (HIV) emerged in the early 1980s, little is known about its risk for HIV-infected people. The only reported complication occurred in a soldier vaccinated in 1984, who developed disseminated vaccinia lesions 4 weeks after vaccination (Redfield et al., 1987). Although he appeared to be in good health, he was in fact severely immunocompromised, since he almost simultaneously came down with cryptococcal meningitis, and was found to have a T helper cell count less than $25\,\mu l^{-1}$. The vaccinia lesions resolved after 12 weekly inoculations of VIG; a concurrent increase in the T cell count probably played a role in recovery.

Retrospective studies indicate a prevalence of HIV infection of 1–2 per thousand among the more than 900,000 people who joined the US armed forces in 1983–1985 (Burke et al., 1987; Bray and Wright, 2003), so it is apparent that at least several hundred people in the early stages of HIV infection underwent vaccination without suffering serious adverse effects. In contrast, individuals who have become severely immunocompromised are vulnerable to severe complications, as shown by two AIDS patients with CD4 counts less than $50\,\mu l^{-1}$ who developed relentlessly expanding lesions after being injected with a preparation derived from vaccinia-infected cells (Guillaume et al., 1991).

10. Conclusion: areas for further research

The cessation of universal smallpox vaccination effectively halted research on the treatment of vaccination complications, and many basic questions remain unanswered. Suggested areas for laboratory and clinical research are listed in Table 5. The efficacy of VIG in treating any vaccination complication has never actually been proven in a controlled trial. If it is beneficial, what is its mechanism of action? Could the same effect be mimicked or improved upon by monoclonal antibodies? In terms of other immediately available types of antiviral therapy, the use of systemic cidofovir for the treatment of the most severe complications is clearly justified, but any evaluation will have to be performed in the setting of a clinical research protocol. A more convenient and less toxic approach that should be studied in cases of progressive vaccinia is the direct application of cidofovir gel to the enlarging primary vaccination lesion.

In addition to the requirement for clinical research, there is a pressing need to develop appropriate animal models for human vaccination complications. In particular, currently available models of atopic dermatitis in mice should be studied for their ability to simulate eczema vaccinatum. The pathogenesis and treatment of progressive vaccinia could be studied in nonhuman primates rendered immunodeficient through retrovirus infection, irradiation, or treatment with antimetabolites or antilymphocyte serum.

In the absence of actual vaccination complications, two surrogates can be used to test novel approaches to therapy. The first consists of herpesvirus infections in immunodeficient individuals and in persons with atopic dermatitis or eczema. It will be important to determine whether treatment of these infections with acyclic nucleoside phosphonate derivatives or with immunomodulators is predictive of their efficacy for the therapy of vaccinia infections in the same risk groups. The second surrogate model consists of the experimental prophylaxis or therapy of developing vaccination lesions in normal human volunteers. This system

Table 5
Suggested laboratory research and clinical studies aimed at clarifying the pathogenesis and improving the treatment of complications of smallpox vaccination

Vaccination complication	Laboratory research	Clinical studies
Accidental infection	–	Evaluation of the effect of topical cidofovir or immunomodulators on take reaction in normal vaccinees
Generalized vaccinia	–	Detection of viremia, evaluation of antibody response
Eczema vaccinatum	Evaluation of vaccinia virus infection in mouse models of atopic dermatitis (NC/Nga or IL-4 transgenic mice)	Development of blood test or skin assay predictive of risk of eczema vaccinatum Determination of efficacy of VIG Evaluation of i.v. cidofovir Evaluation of adjunctive therapy with topical immunomodulators: imiquimod, resiquimod
Progressive vaccinia	Development of model in immunodeficient nonhuman primates through retrovirus infection, irradiation or treatment with immunosuppressive drugs or antilymphocyte serum	Determination of efficacy of VIG Evaluation of i.v. cidofovir Evaluation of topical cidofovir

could be used for the preliminary evaluation of topical or systemic medications, based on their ability to suppress the normal take reaction.

Research over the past two decades has led to a great increase in our understanding of the mechanisms by which the skin resists infection by a variety of pathogens. These new concepts of cutaneous immunity should now be translated into new types of prophylaxis and therapy of cutaneous viral infections, including vaccinia. We also possess a sophisticated understanding of the role of cytokines and cytotoxic T cells in the recognition and elimination of vaccinia-infected cells. This knowledge should be applied to the control of vaccinia replication and to the prophylaxis and therapy of more virulent orthopoxvirus infections.

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