Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

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The global eradication of smallpox was a tremendous achievement made possible by the development of an effective vaccine. Routine vaccination of the general population is no longer recommended. However, stocks of variola virus, the causative agent of smallpox, still exist in 2 secure laboratories, and permanent disposal has been controversial. In addition, there is speculation that variola virus may exist outside of these 2 facilities, and there is a concern that the threat of smallpox will be used as a bioterrorist weapon. In 2002, this concern led to a vaccination campaign in US military and civilian healthcare workers and first responders. Although the historical live virus vaccine has proven efficacy, it also is associated with serious adverse events and rare fatal reactions, particularly in the setting of immunodeficiency and atopic eczema. In addition, this vaccine was historically produced using animal intermediaries in a process that was prone to contamination and not acceptable for current manufacturing standards. Development of alternative poxvirus vaccines is focused on replication-defective viruses, gene-based vectors, and subunit approaches to improve safety and immunogenicity. The conundrum is that in the absence of an intentional release of variola, efficacy evaluation of new candidate vaccines will be limited to animal model testing, which creates new challenges for the vaccine licensure process. Although motivated by the threat of bioterrorism, the hope is for new poxvirus vaccines to have their greatest utility against other pathogenic orthopoxviruses such as monkeypox and for the development of recombinant poxvirus-based vectors to treat and prevent other diseases. (J Allergy Clin Immunol 2006;118:1320-6.)

Key words: Poxvirus, vaccination, immunization, variola, monkeypox

From the time of its suspected emergence after 10,000 BC to the time of its eradication in 1980, smallpox was a feared disease that claimed hundreds of millions of lives and changed the course of history. Although its origin is unknown, it is hypothesized to have been an animal poxvirus that adapted to be readily transmitted among human beings. Ironically, there is no known animal reservoir of smallpox, and the requirement of variola for person-to-person spread is an important feature that allowed the possibility of eradication through vaccination. Another similar orthopoxvirus (Table I) that is transmissible between human beings, monkeypox, has well established animal reservoirs, making eradication untenable. After exposure to smallpox, individuals are well during the typical 10-day to 12-day incubation period, then enter a pre-eruptive phase of fever, headache, myalgias, nausea, and vomiting. Two to 3 days after the onset of fever, a characteristic rash develops initially as small macules with progression to vesicles and pustules within a week, followed by scab formation and separation over the next 1 to 2 weeks. Patients are most contagious after the rash appears. The mortality rate for the most pathogenic variety of smallpox, variola major, was about 30%. During the eradication campaign, a less pathogenic form of disease, variola minor, was identified with a mortality rate of about 1%. Persons infected with monkeypox can experience a similar syndrome, but with less systemic symptoms. Nevertheless, mortality from monkeypox was as high as 10% to 17% in some regions of the Congo River basin during the 1970s and 1980s.

INTRODUCTION OF VARIOLATION AND VACCINATION

Disfiguring pockmarks were 1 sequela of smallpox survival, and those who bore these scars were observed to be immune to disease recurrence. Although persons who acquired smallpox through a scratch were not completely protected from disease, they had an attenuated disease course. On the basis of these observations, attempts were...
made to expose people to smallpox material, thus inducing a milder form of the disease and protecting against fulminant smallpox on natural exposure. Inoculation with smallpox pus or scabs either by a nasal or cutaneous route, a process known as variolation, was initiated as early as 1000 AD in China.2

The practice of vaccination was introduced centuries later by Edward Jenner in 1796. The term vaccination is derived from vacca, the Latin word for cow. Milkmaids who developed cowpox lesions were observed to be resistant to smallpox. Jenner took material from a cowpox lesion on the hand of milkmaid Sarah Nelmes and inoculated a young boy, James Phipps. Phipps was resistant to smallpox on subsequent exposure. Jenner extended this experiment to other children, with the same outcome. Cowpox inoculation was relatively benign compared with variolation, and Jenner’s practice of vaccination gradually gained acceptance. Initially, vaccination was performed with cowpox virus, but over time, vaccinia virus, the origin of which is unknown, became the preferred virus for vaccination.2

SMALLPOX ERADICATION

At the start of the 19th century, arm-to-arm vaccination was practiced; however, by the close of the century, this practice was being replaced by vaccine produced from the hides of live animals by harvesting lymph. This was primarily done in calves, but donkeys and horses were also used. Although this practice allowed manufacturing capacity to be distributed even to remote areas, it was complicated by the risk of contamination from bacteria, fungi, and other viruses and raised the theoretical risk of transmissible spongiform encephalopathies.4 Strains of vaccinia virus and methods of storage and application of vaccines varied significantly by region after a century of nonstandardized and unregulated smallpox vaccination.2

In 1965, the World Health Organization (WHO) mandated that undiluted smallpox vaccine should contain $1 \times 10^8$ plaque-forming units per milliliter.5 Vaccine was introduced into the epidermis by various methods, but in 1968, the bifurcated needle became the accepted method of vaccination because of its practical advantages. The bifurcated needle was designed to contain about 2.5 µL vaccine suspension, facilitating transfer of the vaccine to the skin, and the flat prongs provided consistent delivery to the right depth during the multiple shallow punctures administered.2

Among the available vaccinia virus strains, some were more commonly used for vaccination during smallpox eradication. Vaccines used in the United States originated from the New York City Board of Health (NYCBH) strain, which was developed from seed virus from England in 1856. In the 1930s, Dr Rivers developed 2 attenuated strains of virus, CVI-78 and CVII, by passaging the NYCBH strain in rabbit testes and chick embryos. Both strains caused less local and systemic reactogenicity than the NYCBH strain when administered as vaccines, but their ability to protect sufficiently against smallpox was questioned. The NYCBH strain was also believed to have given rise to the EM-63 strain which was used for vaccination in the Union of Soviet Socialist Republics. In the late 1960s, vaccine derived from the EM-63 strain was given to the WHO. In China, the Temple of Heaven (Tian Tan) strain was used for smallpox vaccination. In the United Kingdom, the Lister strain was used for vaccination beginning in 1892 and eventually became the strain most

### TABLE I. Classification of poxviruses (adapted from references2,23,38)

| Family       | Genus             | Species               | Host                                      |
|--------------|-------------------|-----------------------|-------------------------------------------|
| Poxviridae   | Parapoxvirus      | Camelpox virus        | Camel                                     |
|              |                   | Cowpox virus          | Rodents, felines, bovines, elephants, humans |
|              |                   | Ectromelia virus      | Laboratory mice, natural reservoir unknown |
|              |                   | Monkeypox virus       | Rodents, primates, humans                  |
|              |                   | Racoonpox virus       | North American raccoon                    |
|              |                   | Taterapox virus       | African gerbil                             |
|              |                   | Vaccinia virus        | No natural reservoir                       |
|              |                   | Buffalopox virus      | Buffalo, cattle, humans                    |
|              |                   | Rabbitpox virus       | Colonized rabbit, no natural reservoir     |
|              |                   | Variola virus         | Humans; eradicated from nature             |
|              |                   | Volepox virus         | California pinon mouse and voles           |

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commonly used to manufacture smallpox vaccine during the Intensified Smallpox Eradication Programme.\textsuperscript{2} Table II lists some commonly used vaccinia virus vaccines.\textsuperscript{2,11,23}

| Parent strain and some derivatives | Comments |
|-----------------------------------|----------|
| NYCBH                             | ● NYCBH was dispersed to multiple laboratories and became known by other names  
                                       ● Variation in strains resulted from different passage techniques |
| NYCBH Rivers                       |          |
| Rivers                             |          |
| Wyeth (Dryvax)                     |          |
| APSV                               |          |
| Lister (Elstree)                   | ● Lister was believed to originate from variola virus  
                                       ● By 1971, the majority of vaccine producers were using Lister |
| L-IVP                             |          |
| LC16m8                             |          |
| Lancy-Vaxina                       |          |
| Copenhagen                         | ● Copenhagen strain had high pathogenicity  
                                       ● NYVAC is attenuated by intentional deletion of virulence genes |
| NYVAC                             |          |
| Temple of Heaven (Tian Tan)        | ● Believed to originate from variola virus  
                                       ● Used predominantly in China |
| Ankara                             | ● Proposed uses of MVA: (1) immunization of general population and boost with traditional live vaccine if needed, (2) vaccination of those with contraindications for traditional vaccine, (3) immunization of laboratory workers against accidental exposure to recombinant vaccinia, (4) immunization of those at risk for monkeypox |
| MVA                               |          |

TABLE III. Contraindications to live virus vaccine for nonemergency use\textsuperscript{39}

| Contraindications to live virus vaccine for nonemergency use |
|-------------------------------------------------------------|
| Eczema (past or present) or other acute, chronic, or exfoliative skin conditions |
| Household contacts of such persons |
| Immunodeficiency or immunosuppression |
| Caused by acquired or congenital diseases |
| HIV/AIDS, solid organ or stem cell transplant, generalized malignancy, leukemia, lymphoma, defects of cellular and/or humoral immunity |
| Household contacts of such persons |
| Caused by treatments |
| Radiation, high-dose corticosteroids, immunosuppressive drugs (eg, alkylating agents, antimetabolites) |
| Household contacts of such persons |
| Pregnancy and household contacts of pregnant women |
| Allergic reaction to smallpox vaccine, any of its components, or reagents used in manufacturing |
| Polymyxin B sulfate, dihydrostreptomycin sulfate, chlorotetracycline hydrochloride, neomycin sulfate |
| Infants <12 months of age |
| Heart disease |
| Known cardiac disease or other heart conditions being treated by a doctor |

commonly used to manufacture smallpox vaccine during the Intensified Smallpox Eradication Programme.\textsuperscript{2} Table II lists some commonly used vaccinia virus vaccines. Although vaccination is safer than variolation, it is not without complications and serious adverse reactions. Vaccine complication rates are dependent on the pathogenicity of the vaccinia virus strain. The Lister strain and strains derived from the NYCBH strain were less reactogenic.\textsuperscript{2} However, in the absence of disease exposure, live virus vaccines are contraindicated in a number of people (Table III). With an increase in the prevalence of atopy, more available immunosuppressive medications, and the advent of HIV, there are even more people today in whom the vaccine is contraindicated because of the associated risks.

Persons with immunodeficiencies are at risk of developing progressive vaccinia, a frequently fatal disease characterized by progressive extension of the lesion at the vaccination site and development of similarly expanding lesions elsewhere. Eczema vaccinatum presents with constitutional symptoms and a vaccinal rash at current or previous eczematous locations, although healthy skin can also be affected. Prognosis is dependent on the extent of skin involvement, and severe cases should be treated like burns.\textsuperscript{2,6} Vaccinia immune globulin, made from pooled human plasma of previous smallpox vaccinees, was used to treat eczema vaccinatum and progressive vaccinia, although there were no controlled clinical trials supporting its use.\textsuperscript{6} Primary vaccinees are at greater risk of developing generalized vaccinia marked by a disseminated rash attributed to viremia. The rash develops 6 to 9 days after vaccination and is composed of lesions that follow a similar progression as the lesion at the vaccination site. Generally, no treatment is necessary. The most common complications of vaccination, autoinoculation and inadvertent infections of vaccinee contacts, are generally
self-limiting. In otherwise healthy persons, the most serious complication is postvaccinial encephalitis.2

The last naturally occurring case of smallpox was in Somalia in 1977, and the WHO declared the achievement of global eradication in 1980.2 In spite of smallpox eradication, the potential use of variola virus as a bioweapon has remained a threat. In the 1980s, all variola virus stocks were either destroyed or consolidated into 1 of 2 WHO-sanctioned laboratories, the Centers for Disease Control and Prevention (Atlanta) and the Institute of Virus Preparations (Moscow). However, accounts arose of mass smallpox production by the Soviet Union in the 1980s as part of a bioweapons research program. The dissolution of the Union of Soviet Socialist Republics and departure of scientists and other resources led to speculation that more countries might possess stocks of variola virus or the means to acquire it.7

CURRENT VACCINES

The only US Food and Drug Administration (FDA)–licensed vaccinia virus vaccine derived from NYCBH strain (Wyeth Laboratories, Inc., Marietta, Pa). It is lyophilized calf lymph and comes with a diluent containing 50% glycerol and 0.25% phenol in Sterile Water for Injection, USP (Wyeth; package insert). Vaccination of the general public stopped in the United States in 1972, and production of this vaccine stopped in 1982.8 Recent studies were performed evaluating clinical and immunologic responses to diluted vaccine in volunteers who had not previously been immunized to determine whether this stock vaccine could safely be diluted to provide more available doses. At dilutions of 1:5 or 1:10 (10^{-7.0} plaque-forming units), the vaccine retained its potency and was able to elicit adequate immune responses.8-10 Although the Lister strain is not used in this country, trials similar to the Dryvax dilution studies were conducted in South Korea with Lancy-Vaxina, a derivative of the Lister strain.11

Dryvax was used to vaccinate military personnel and a select civilian population beginning in 2002. In these highly screened individuals, there were fewer adverse events than anticipated on the basis of the historical data, and no cases of progressive vaccinia or eczema vaccinatum.12,13 However, a new finding of cardiac complications became a cause for concern. Although European and Australian literature from the 1950s, 1960s, and 1970s reported fatal and nonfatal postvaccinial cardiac complications, such reports were rare in the United States. At the time, this difference was believed to have been related to the less reactogenic strain of vaccinia virus used in the United States.14 However, the findings from the military and civilian vaccination programs indicate those with cardiac disease should not receive vaccinia in nonemergent settings. Of the 38,885 civilian smallpox vaccines administered between 2002 and 2003, there were 21 cases of myopericarditis and 10 ischemic cardiac events, of which 2 were fatal.12 In the military program as of June 2006, there were more than 1 million vaccinations and 120 cases of myopericarditis. The 16 cases of ischemic heart disease were consistent with rates in unvaccinated military recruits of the same age. The investigation of 8 fatalities after vaccination determined 1 death from an acute lupuslike illness may have a causal relationship to vaccine. In addition, vaccination was thought possibly to contribute to the sudden death of a 26-year-old military recruit 16 days after he received smallpox and influenza vaccinations. However, autopsy revealed myocarditis with parvovirus B in the cardiac muscle and no evidence of vaccinia virus.15

Besides the lyophilized Dryvax formulation, during routine vaccination in the United States, a second vaccine also derived from the NYCBH vaccinia strain was used. This vaccine, Aventis Pasteur smallpox vaccine (APSV; Sanofi-Aventis, Bridgewater, NJ), was manufactured from 1956 to 1957 and was maintained as a frozen preparation. Similar to the Dryvax studies, undiluted APSV was tested along with 1:5 and 1:10 dilutions to compare vaccination success rate and reactogenicity. A total of 340 vaccinia-naïve volunteers were enrolled. APSV was determined to be effective even at diluted doses. Relative to trials of vaccinia-naïve volunteers vaccinated with the lyophilized preparation, APSV was more reactogenic. Recipients experienced larger mean lesion sizes, more episodes of fever, increased lymphadenopathy, and more satellite lesions. The APSV study was prematurely terminated because of the 2003 FDA suspension of all vaccinia trials after unanticipated adverse cardiac events noted in the military and civilian vaccination campaigns. In the APSV trials, 5 persons reported cardiac symptoms, all of which resolved without sequelae. Retrospective data review indicated no evidence of moderate or severe myopericarditis or myocardial ischemia.16

In addition to unacceptable side effects and problems related to production, although the vaccine is effective, it is unclear how long it provides protection. Data suggest vaccine-specific memory B cells may persist for more than 50 years after vaccination, but not knowing which immunologic responses determine protection makes it difficult to define the duration of protective immunity.17 Alternative vaccine strategies designed to be safer than the presently available live virus vaccines are being pursued.

ALTERNATIVE VACCINE APPROACHES

Smallpox vaccines currently being investigated can be divided into 4 groups: replication-competent and replication-deficient viruses (Table IV), gene-based vectors including plasmid DNA, and recombinant proteins. Among the replication-competent virus options, there has been renewed interest in LC16m8, derived from the Lister strain. This vaccine was administered to >100,000 children, including those with a history of eczema, in Japan between 1973 and 1975.18 It had a good safety profile but unproven efficacy, because smallpox was no longer present in Japan, and animal challenge studies were not performed at that
Clinical trials have also been conducted using the NYCBH-derived ACAM1000 and ACAM2000 vaccinia virus-based vaccines. ACAM2000 was prepared from ACAM1000 master seed stock and produced in Vero cells to address the need for rapid large-scale vaccine production. On the basis of animal studies, ACAM2000 is believed to be less neurovirulent than Dryvax. ACAM1000 and ACAM2000 were similar to Dryvax in their ability to induce immune responses and in reactogenicity in phase I trials. During phase II and phase III clinical trials, cases of myopericarditis were associated with both ACAM2000 and Dryvax in vaccinia-naive volunteers.\textsuperscript{21,22}

Although manufacturing vaccines in cell culture can avoid some problems associated with first-generation vaccines produced on the hides of animals, replication-competent virus still has the potential for serious adverse events. Thus, replication-defective virus vaccines have been evaluated as a strategy to achieve a safer vaccine option.

Among the replication-defective vaccines, modified vaccinia Ankara (MVA) has been studied most extensively. MVA has an excellent safety profile and could be used in groups in whom Dryvax is currently contraindicated.\textsuperscript{23} MVA was given to 120,000 people in Germany in the 1970s, followed by vaccination with live virus Elstree (Lister).\textsuperscript{24} Similar to LC16m8, MVA was safe but was not field-tested because smallpox was not present in Europe at that time. MVA has since been evaluated in

| Strain     | Parent virus | Replication ability | Cell substrate | Comments |
|------------|--------------|---------------------|----------------|----------|
| ACAM1000   | NYCBH        | Competent           | Cloned vaccine grown in MRC-5 human diploid cell line | Never field-tested but closely related to field-tested efficacious vaccines |
| ACAM2000   | NYCBH        | Competent           | Cloned virus grown in Vero cells | Live virus vaccines with associated risks |
| CCSV       | NYCBH        | Competent           | Adapted to replicate in MRC-5 cells | Limited data suggest similar immunogenicity and reactogenicity to Dryvax |
| LC16m8     | Lister       | Competent           | Serial passage in rabbit kidney cells at low temperature | Never field-tested |
| MVA (TBC-MVA, IMVAMUNE, ACAM3000) | Ankara | Deficient in mammalian cells | Derived from CVA dermovaccinia; >570 serial passages in primary chick embryo fibroblast cultures; multiple DNA deletions and loss of ~15% of parent genome, including genes specific for viral host range regulation and immune evasion | Good safety record |
| NYVAC      | Copenhagen   | Deficient           | Deletion of 18 ORFs from a plaque-cloned isolate of Copenhagen strain; grows only in primary cells | Limited data available |
| dVV-L      | Lister       | Deficient           | Deletion of uracil DNA glycosylase gene necessary for viral replication; produced in an immortalized cell line that complements UDG activity | Does not require primary cells or eggs as a substrate, Limited preclinical data |

NHP, Nonhuman primate; ORFs, open reading frames.
animal models and in human studies. Monkeys that received 2 doses of MVA or 1 dose of MVA before Dryvax developed antibody and T-cell responses that were the same as or greater than Dryvax alone. Animals immunized with only MVA developed a few transient skin lesions after monkeypox virus challenge, but did not develop overt clinical disease.25 MVA can also protect mice from vaccinia challenge.26 In addition, mice immunized with MVA were protected against lethal infection with a more virulent form of vaccinia virus altered to coexpress IL-4. IL-4 diminishes the cytolytic capacity of CD8+ T cells, resulting in delayed viral clearance and increased virulence.27

In phase I human clinical trials, MVA was found to be safe and immunogenic on its own28 and to prime for greater immune responses and attenuate lesion formation if given in advance of Dryvax vaccination.40 MVA is also being evaluated in persons with contraindications to live virus vaccine such as atopic dermatitis and immunosuppression. In immunodeficient animal models, MVA immunization of CD8 T-cell–deficient or B-cell–deficient mice is safe and protective against intranasal lethal challenge with the Western Reserve (WR) strain of vaccinia virus. However, CD4 or MHC class II knockout mice immunized with MVA were poorly protected, whereas mice with low CD4 and CD8 counts were completely unprotected from the vaccinia challenge.29

Another replication-defective virus vaccine under consideration as an alternative smallpox vaccine is NYVAC (Sanofi-Pasteur, Toronto, Ontario, Canada). NYVAC was derived from the Copenhagen strain and developed by selective deletion of 18 open reading frames. In immunodeficient macaques with CD4 counts <300, NYVAC, and MVA before Dryvax were safe. However, neither attenuated vaccine administered with Dryvax nor Dryvax alone provided protection against lethal monkeypox virus challenge in the setting of impaired immune function.30 These studies suggest that the best way to protect immunocompromised subjects from poxvirus infection may be through herd immunity established by vaccination of the general population.

dVV-L, which is also replication-defective, has been evaluated as a poxvirus vaccine. This vaccine strain was created from the Lister strain by deleting a gene necessary to encode the uracil-DNA-glycosylase (UDG) enzyme, which is essential for a complete cycle of viral replication. In a murine model, dVV-L immunization induced vaccinia virus-specific cytotoxic T lymphocyte (CTL) responses. In addition, when given to immunodeficient mice, dVV-L was well tolerated.31 One great advantage of this approach is that the attenuated virus can be manufactured in a cell line that complements the UDG deficiency rather than in primary cells or eggs that are often needed for other replication-defective viruses, resulting in an improved safety profile and increased capacity for rapid production.

DNA vaccine strategies have been investigated in animal models. A DNA vaccine composed of 4 vaccinia virus genes protected rhesus macaques from severe disease, with the animals exhibiting mild clinical and laboratory abnormalities, after challenge with a lethal dose of monkeypox virus. When vaccinated with a single gene (L1R), macaques developed severe, but not fatal, disease.32 Heterologous prime-boost strategies have also been evaluated. Priming BALB/c mice with DNA vaccine resulted in greater immune responses after boosting with live vaccinia virus compared with controls.33

In addition, protein subunit vaccines have been evaluated in mice. Recombinant proteins of the outer membranes of intracellular mature virus (IMV) and EEV forms of vaccinia virus were used individually or in combination to immunize mice before intranasal challenge with a lethal dose of the WR strain of vaccinia virus. Vaccination with the individual proteins afforded partial protection; complete protection was achieved with 3 doses of the 3-protein (A33, B5, and L1) IMV–EEV combination vaccine.34

**FUTURE DIRECTIONS**

Although several alternative smallpox vaccines look promising, further studies are needed in human subjects, particularly in those with contraindications to vaccination with live virus vaccine. As new vaccine approaches are being developed, ensuring compatibility with potential utilization strategies should be considered. For example, in the event of a bioterrorist attack, the rapidity with which an immune response can be generated may be prioritized over the development of long-term immunity, and accordingly, a prime-boost vaccination strategy may not be as viable as a single-dose vaccination schedule.

Vaccine development for a disease that does not naturally exist is fraught with challenges and limitations. Determinants of immunity remain undefined, and the newer vaccines cannot be field-tested to demonstrate efficacy in human beings. To address issues related to approval of drugs and biological products for which efficacy trials in human beings are not feasible, the FDA has adopted the Animal Rule. This rule permits approval of products in which human safety and immunogenicity have been demonstrated, and appropriate animal studies have established a correlate of efficacy that can be used as a surrogate endpoint for human studies.35 Regardless of the data obtained from animal studies, in the absence of field-testing, live virus vaccines will continue to have an important advantage for use in the setting of an outbreak.

Although developing safer immunogenic vaccines to defend against the potential deliberate release of smallpox is a focus of national security, these vaccines have other potential utility, namely to afford protection against other poxviruses. Laboratory personnel who work with poxviruses, such as vaccinia, are at risk of accidental infection and would thus be a target population for an alternative vaccine. Including variola and vaccinia, there are 9 poxviruses that are capable of causing disease in humans (Table I). Aside from variola, infection with monkeypox virus (particularly strains from the Congo River basin) results in the most severe systemic disease and deserves significant attention. Monkeypox has been predominantly confined to central and western Africa; however, it did...
surface in the United States in 2003 when disease was transmitted from infected prairie dogs and rodents that had been imported from western Africa. Successful evaluation of newer vaccines in the setting of naturally occurring poxvirus infections, such as monkeypox, will increase confidence in their use as a countermeasure against smallpox. As the world faces the challenges of emerging and re-emerging infectious diseases and the threat of bioterrorism, vaccine development will continue to play an important role in protection, a concept that was first recognized centuries ago.

We thank Julie E. Martin, Joseph P. Casazza, and Ingelise J. Gordon for their critical review of the manuscript.

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