cine and simultaneously administrated with rabies immunoglobulin (RIG). RIG, which is not produced in Japan, is expensive and not readily available. Therefore, almost all PEP implemented in Japan is without using RIG. In foreign countries, it is reported that 76% of PEP-treated overseas tourists are not administrated with RIG after they return back to their home countries [18]. Most of the PEP-treated persons are not administrated with RIG worldwide [19]. WHO issues a recommendation of the RIG-free PEP schedule in the guidelines [3]. The efficiency of the RIG-free PEP should be further verified under evidence-based manner. Under these situations, WHO has appealed to rabies researchers to develop RIG using monoclonal antibodies with neutralizing activities, and studied to use the cocktail preparation. In 2008, Sanofi-Pasteur announced commercialization of human monoclonal antibody cocktails jointly with Crucell of the Netherlands [20]. It is expected that larger amounts of these cocktails are supplied in the future as a replacement for RIG.

In developing countries, most infectious sources of rabies are domestic and stray dogs, and the countermeasures such as vaccination of these dogs and control of the populations are needed. Spreading bait vaccines, which can be orally administered, in the fields is already practiced and producing the effects in European and North American countries [21]. Bait vaccination is expected to be a promising tool for prevention of rabies in wild animals including domestic and stray dogs. Rabies is an enzootic disease, therefore, the reduction in animal rabies certainly results in prevention of human rabies. Rabies could conceivably be eradicated worldwide if these measures are widely practiced in developing countries.

4. Conclusions

In Japan, where rabies has been eradicated, no incidence of human and animal rabies had been reported for 36 years until 2006, the Japanese people forgot awfulness of rabies for a long time. Overseas tourists have been optimistic about risk of rabies virus infection caused by careless contacts with dogs or wild animals, leading to the two imported rabies cases in 2006. It may be rather miraculous that Japan had not even an imported case for 36 years in the midst of growing globalization. People should notice the risk of rabies virus infection even in the developed countries in Europe and North America, which have experienced the sporadic outbreak of rabies. The countermeasures against the imported rabies include 1) dissemination of proper and regular information on rabies to the public, 2) establishment of the prompt and appropriate PEP systems, and 3) increased capacity of rabies vaccine production.

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Case report

Progressive multifocal leukoencephalopathy developed in incomplete Heerfordt syndrome, a rare manifestation of sarcoidosis, without steroid therapy responding to cidofovir

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ABSTRACT

Progressive multifocal leukoencephalopathy (PML) is a severe demyelinating disease of the central nervous system caused by the JC virus; the mortality rate is high and it is usually refractory to treatment. In non-HIV patients, PML occurs as a late consequence of hematologic malignancies or during prolonged immunosuppression for transplantation or autoimmune disease. We describe a 34-year-old PML patient with incomplete Heerfordt syndrome, a rare type of sarcoidosis, who had not received any immunosuppressants, including steroids, at the onset and who was clinically and radiologically responsive to the antiviral drug cidofovir.

1. Introduction

Progressive multifocal leukoencephalopathy (PML) is a rare and devastating disease that typically results in death or an irreversible neurologic insult. Reactivation of JC virus (JCV), a polyomavirus, is the cause of PML with death of oligodendrocytes resulting in demyelinating disease in the central nervous system (CNS). A surge in PML occurred in the 1980s with the onset of AIDS, highlighting the importance of T cells in continued JCV senescence in the human body. PML has also been described in patients with autoimmune disease, including systemic lupus erythematosus (SLE), Wegener's granulomatosis, scleroderma, dermatomyositis, polymyositis, and rheumatoid arthritis. There is no specific proven treatment for PML and its prognosis is poor [1,2].

Sarcoidosis is a systemic granulomatous disorder of unknown etiology that affects the parotid gland in about 6% of cases. If other major symptoms such as fever, uveitis, or facial paralysis are present, the condition is referred to as Heerfordt syndrome [3]. Heerfordt syndrome is an unusual manifestation of systemic sarcoidosis. It is characterized by three major symptoms: enlargement of parotid glands, uveitis, and facial nerve palsy, and is usually associated with fever. If only two of three characteristic symptoms of Heerfordt syndrome are present, it is called as incomplete Heerfordt syndrome. The association of PML and sarcoidosis without immunosuppressive treatment is rare and their relationship is still unclear. In this report, we describe the clinical course of a PML patient with untreated incomplete Heerfordt syndrome, a rare type of sarcoidosis, who was responsive to cidofovir therapy, after which the patient showed neurological improvement and the JCV load in the cerebrospinal fluid (CSF) became undetectable.

2. Case report

A 34-year-old man was seen in our hospital because of weakness in his left leg. At the age of 21, he initially developed enlargement of bilateral parotid glands, uveitis and low-grade fever with skin eruption of bilateral lower legs. Then, he presented to another hospital. At that time, blood test showed angiotensin-converting enzyme (ACE) (31.2 IU/l) was slightly elevated and a purified protein derivative skin test was negative. Gallium scan revealed high uptake of

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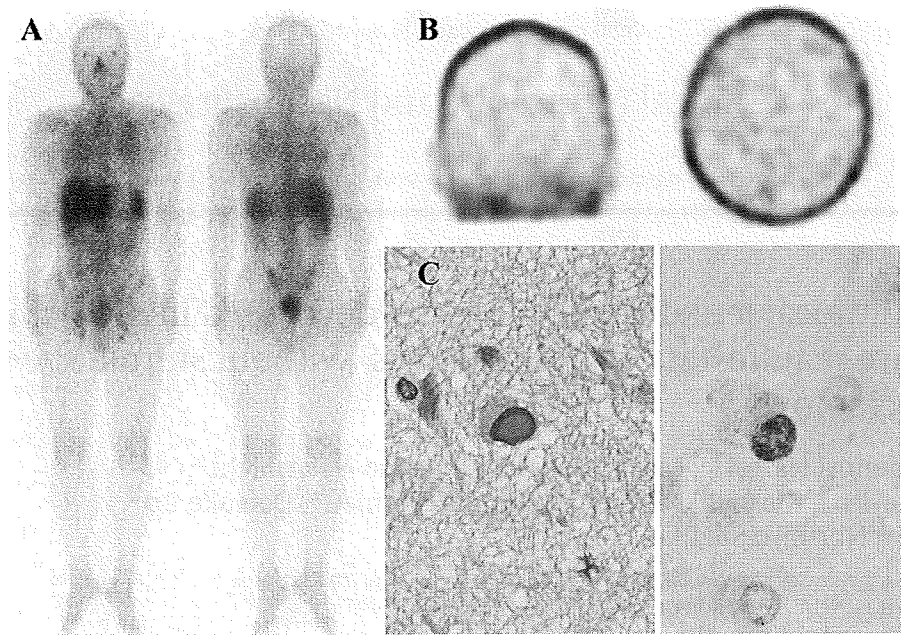

Fig. 1. (A) Gallium scan showed abnormal uptake in the bilateral lung fields. (B) The intracranial lesion was not detected by thallium scan. (C) Brain biopsy showing white matter demyelination, and oligodendrocytes with large irregular hyperchromatic nuclei (right); these inclusions were intensely stained with anti-JCV antibody (left) (400×).

bilateral parotid glands, bilateral orbital cavities and bilateral hilar regions. A punch biopsy of skin lesions on the lower legs was performed and it exhibited non-caseating epithelioid cell granulomas. Based on the clinical and the histological findings, he had been diagnosed with incomplete Heerfordt syndrome, a rare syndrome in patients with sarcoidosis. At the time of his first visit to our hospital, he had not received any immunosuppressants for more than 10 years. Brain MRI showed a small non-enhancing diffuse hyperintensive lesion of the white matter in the right frontal and parietal lobes on T2-weighted images. Over a few months, the weakness in his left leg gradually spread to his left arm; T2-weighted MRI also showed that the lesion had enlarged, so he was admitted to our hospital due to clinical and radiological deterioration.

Neurological examination on admission showed the patient's cranial nerves were normal; motor strength was intact in his right arm and right leg, but his left arm and leg showed weakness with hyperreflexia and Babinski's sign. Blood tests revealed ACE (35.3 IU/l) and KL-6 (687 U/ml) to be slightly elevated, but other factors including the number of T cells and CD4 cells were within normal limits, and HIV-1,2 antibodies were negative. CSF had no remarkable findings. Abnormal uptake in the bilateral lung fields was detected by gallium scan (Fig. 1A). Thallium scan demonstrated no uptake within the intracranial lesion (Fig. 1B). Initially, we thought the intracranial lesion had been caused by the underlying disease, because the activity of sarcoidosis persisted subclinically; this was supported by elevated ACE and lung fields, demonstrated by gallium scan. As the intracranial lesion was gallium and thallium negative, the lesion was unlikely to be neoplastic, including lymphoma [4]. Thus, we started steroid therapy and cyclophosphamide.

After initiating immunosuppressant therapy, his weakness spread rapidly to all extremities and the white matter lesion

Table 1
Clinical course and serial measurement of grip power. PSL = prednisolone; mPSL = methylprednisolone.

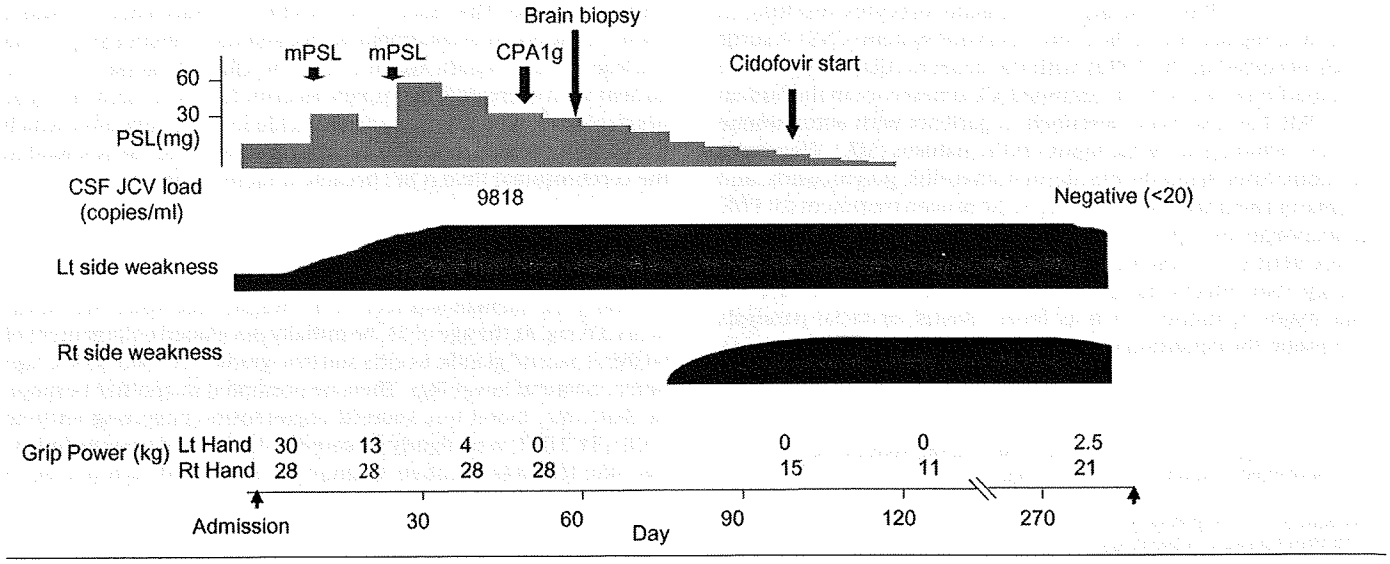

Table 2
Summary of reported cases of PML with untreated sarcoidosis.

Case	Age, gender	Types of sarcoidosis	Treatment	Clinical coarse
Rosenbloom [5]	59, F	Pulmonary sarcoidosis	None	Died 6 months from the onset
Olindo [6]	47, F	Pulmonary sarcoidosis	None	Died 4 months from the onset
Raedt [7]	43, M	Pulmonary sarcoidosis, uvetitis	Cidofovir	Alive and disease progression stopped
Present case	34, M	Incomplete Heerfordt syndrome	Cidofovir	Alive and neurologically slightly improved

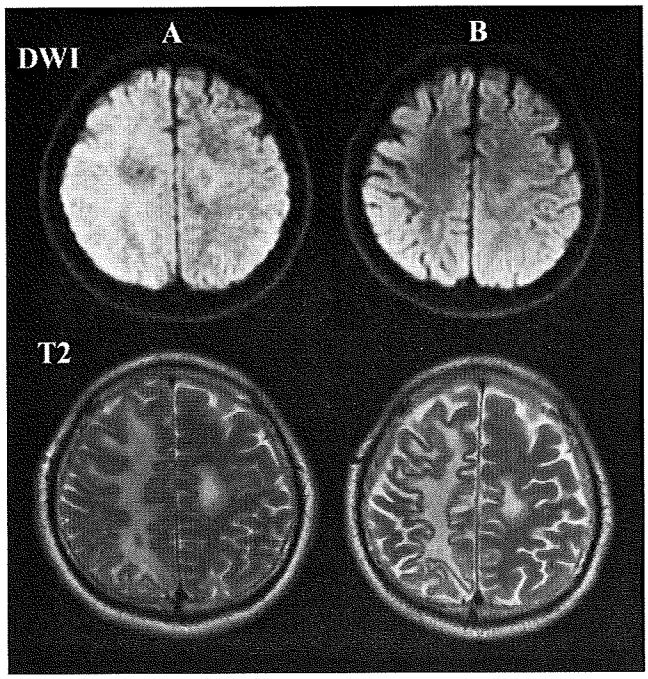

Fig. 2. Diffusion-weighted image (DWI) and T2-weighted image before (A) and after 10 cycles of cidofovir (B). The high intensity area on the DWI disappeared and a part of the lesion was reduced after cidofovir treatment.

spread over the left hemisphere (Table 1). The exacerbation of the intracranial lesion was induced by immunosuppressant therapy. To reconsider the first diagnosis, JCV DNA load was measured in the CSF and a brain biopsy was performed. JCV DNA load of large T gene was 9818 copies/ml. Brain biopsy showed oligodendrocytes with enlarged nuclei due to intranuclear inclusions and astrocytes with pleomorphic nuclei on hematoxylin and eosin stain, and these inclusions were intensely stained with anti-JCV antibody; therefore, PML was diagnosed (Fig. 1C). Immunosuppressant therapy was tapered immediately after diagnosis, and cidofovir, an antiviral drug, was administered intravenously at a dose of 5 mg/kg weekly for the first 2 weeks, followed by the same dose every other week. After a few cycles, the progression of his weakness stopped and MRI also showed the hyperintense lesion of the white matter on T2-weighted image to be slightly reduced. The high lesion on diffusion-weighted image (DWI) disappeared (Fig. 2). Contrast enhancement was absent from MRI images before cidofovir therapy, but after initiation of cidofovir therapy, MRI images with contrast administration (Gd-DTPA) showed some enhancement in the periphery and part of the abnormal brain areas. Clinically, his weakness of extremities ameliorated slightly after the cidofovir therapy and the progression totally stopped. His condition became neurologically and radiologically stable more than 1 year from the onset of the disease. After 12 cycles of cidofovir therapy, JCV DNA was undetectable in the CSF.

3. Discussion

We report a PML patient with sarcoidosis who had a favorable outcome after treatment with cidofovir and discontinuation of immunosuppression. In this case, the patient had not received immunosuppressive treatment at the onset of PML, so PML had not been triggered by immunosuppressive agents. In addition, the disease progression essentially began even before administration of steroids. After the diagnosis of PML by brain biopsy and PCR for JCV in the CSF, we immediately withdrew immunosuppressive agents. Nevertheless, the activity of the disease still remained, because the expansion of white matter lesion was detected by MRI. So, although withdrawal of immunosuppressive agents was effective for the attenuation of the disease progression to some extent, cidofovir basically contributed to the cessation of the disease activity. The reason for the occurrence of PML is fundamentally unclear although it might be due to immune abnormality caused by sarcoidosis. Only four cases of PML associated with untreated sarcoidosis have been reported in the literature since 1983 [5–7] (Table 2). Of the various clinical types of sarcoidosis, this is the first reported case of PML with incomplete Heerfordt syndrome.

The DWI and contrast-enhanced images were found to be useful for evaluating the activity of PML. Radiological study showed that areas of diffusion abnormality correlated with disease progress and high signals on DWI mark the regions of active infection and cell swelling [8]. Moreover, the appearance of contrast enhancement after cidofovir therapy shows clinical remission with virus elimination from the CSF [9]; this was confirmed by the disappearance of JCV DNA from the CSF. The assay of JCV load in the CSF could be useful in determining the prognosis of PML patients and in monitoring the effectiveness of anti-JCV therapies [10]. In this patient, reversal of CSF positivity for JCV DNA meant that the activity of JCV was significantly decreased. These findings confirm response to cidofovir therapy.

Cidofovir is a cytidine nucleotide analog originally used as an intravenous treatment for cytomegalovirus retinitis in AIDS patients. Furthermore, it has been found to have considerable *in vitro* activity against polyomaviruses, including JCV [11]. This antiviral agent has been used in patients with HIV and PML with some favorable outcomes [12,13]. However, one multicenter study found cidofovir to have no significant additional benefit over HAART alone in HIV patients with PML [14]. Furthermore, recent studies showed that a benefit for cidofovir in addition to HAART in HIV-related PML could not be proven [15,16]. There has not been a clinical trial of cidofovir therapy in non-HIV-related PML, and published data of such therapy – including the first evidence for PML responsiveness to cidofovir in a patient with sarcoidosis [7] – is limited to case reports; thus, its efficacy is unknown. There are some case reports of successful outcomes for non-HIV-related PML in other autoimmune diseases such as SLE and destructive polyarthritis [17,18]. PML usually progresses to death in most of non-HIV patients even after withdrawing immunosuppressive agents and there is no proven therapy against PML itself so far. Although further large clinical studies are needed, we suggest that cidofovir could be one of the potent therapeutic options for non-HIV-related PML in addition to the withdrawal of immunosuppressive agents.

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Review:

Research on Preparedness for Bioterrorism – Associated Events in Japan: Smallpox Vaccine Preparedness

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The 2001 anthrax attack in the United States made the deliberate release of biological agents a real issue and forced many countries to improve their bioterrorism preparedness. Smallpox is a threat that requires international preparedness because it spreads from human to human and would be expected to have a high level of lethality. In 2001, Japan restarted the production and stockpiling of a medical countermeasure, the smallpox vaccine LC16m8, which is a cell-cultured, live attenuated smallpox vaccine that was developed in the 1970s in Japan. The Smallpox Vaccine Research Group has continuously worked to characterize LC16m8 genetically, demonstrate the efficacy and safety of the LC16m8 vaccine, reduce the cost of stockpiling through shelf-life extension via the presentation of evidence of its stability in long-term storage, and maintain the quality of the stockpiled vaccine that is available in case of emergency. Maintaining the stockpile and production capacity of "orphan" medical countermeasures has contributed to national security and also to global health. This paper reviews smallpox vaccine preparedness and the surrounding policy issues in Japan as a typical and classic case of biopreparedness research activities and discusses achievements, problems, and future directions.

Keywords: smallpox, vaccine, stockpile, preparedness, medical countermeasures

1. Introduction

The proliferation of biological weapons among nations became an international concern before the end of the Cold War, and terrorism using biological weapons became a prominent issue in the early 1990s [1]. The 2001 anthrax mailing attack in the United States [2] makes the deliberate release of biological agents a real issue and has forced many countries to improve their bioterrorism-preparedness (in short, biopreparedness).

Biopreparedness describes proactive efforts to mitigate damage to society caused by a bioterrorism attack, primarily involving early detection and quick response mostly through the comprehensive development of the capacity of the public health system. Biopreparedness begins with developing probable crisis scenarios based on historical, scientific, and epidemiological evidence on pathogens and disease spread as well as terrorists' capacities and intentions as estimated through intelligence activities. Using these scenarios and estimates of consequences, we develop reasonable action plans and guidelines, constitute laws to support those plans, and secure resources, including medical countermeasures and other medical and social infrastructures, to fill the gaps between the current capacity and what is required in the scenarios.

One of the major difficulties in biopreparedness is the preparation of medical countermeasures. Medical countermeasure preparedness is achieved only when drugs and vaccines with certain efficacy against the deliberately released pathogens can be made available to those exposed within the therapeutic window period. Bottlenecks are different for respective pathogens. For example, even though effective and licensed antibiotics for anthrax are available, it would be difficult to save people exposed to deliberately-released aerosolized anthrax spores unless they were administered drugs before they developed symptoms of the inhalational anthrax. This would require the recognition of aerosolized anthrax spore release, the identification of the people exposed, and well-organized logistics for delivering medical countermeasures. On the other hand, effective countermeasures are not yet available for many pathogens listed as possibly being deliberately released in the future, and sometimes we have to simply start with very basic biological research on a particular pathogen. Thus, biopreparedness research involves very basic biomedical research through to public health and national security research.

In this paper, the smallpox vaccine preparedness in Japan and surrounding policy issues are reviewed as a case of biopreparedness research activities for biological agents for which medical countermeasures are already available. Smallpox is one of the primary concerns among bioweapons to be prepared for in the international community. Primary efforts have been to secure the supply of smallpox vaccine – building and maintaining a certain

stockpile and/or maintaining the production capacity of an effective and safe vaccine – because the smallpox vaccine is very effective in eradicating the disease. The stockpiling of resources is an activity that is fundamental to preparedness. The Japanese effort in this area is a classic case for presenting achievements and future directions as well as for providing us with lessons for better public health preparedness.

2. Threat of Smallpox Terrorism and its Response

The smallpox virus can transmit from person to person, and causes a lethal disease. Thanks to a very effective vaccine and an extensive worldwide eradication program led by the World Health Organization, the eradication of smallpox was declared in 1980 [3]. The eradication of this disease in turn made it a primary threat as a biological agent because the population gradually becomes more susceptible and vulnerable with the termination of routine vaccination. The smallpox virus has resided in only two official repositories in the United States and Russia [4] since its eradication; however, there was an attempt to weaponize the smallpox virus in the former Soviet Union [5] and there are fears that some countries keep hidden stocks [6]. A Bush administration intelligence review pointed out that terrorists or certain nations could have obtained the virus [7]. Even an outbreak in a geographically remote area could be a serious threat in this era of jet travel, as was illustrated by the global epidemic [8] of Severe Acute Respiratory Syndrome (SARS).

The most essential preparedness tool for smallpox is the vaccine because currently there are few effective drugs, and the vaccine is expected to prevent infection and alleviate symptoms when administered to a patient within 4 days of contact with the virus [9]. A "surveillance and containment" vaccination program has proven to be a critical strategy in the eradication program [4]. As the administration window is too short in comparison with the lead time for producing the vaccine, which is approximately 4 months from the seed virus [10], the spread of the disease during the lead time would be a serious disaster and difficult to control without a vaccine stockpile or an active and maintained production capacity. As of the year 2005, eight countries had stockpiled the vaccine for all of their citizens; however, the global stockpile covers only 10% of the world's population [11, 12]. A possible conflict in the fair distribution of the stockpile among countries was suggested in the table top exercise Atlantic Storm [11]. In 2005, the World Health Assembly called for a global vaccine stockpile of at least 200 million doses and a minimum manufacturing capacity of 20 million doses at each of at least two facilities [13]. Therefore, for national and global security, smallpox vaccine preparedness – involving the securing of the supply of the vaccine and a rapid and strategic distribution plan – is central to smallpox preparedness.

3. The Recommencing of Smallpox Vaccine Preparedness Around the Globe

In the late 1990s, after the bioweapon program in the former Soviet Union was uncovered [14], the United States launched an anti-bioweapon program and began discussing the reproduction and stockpiling of the smallpox vaccine. In 1998, the world stockpile was estimated to be approximately 70 million doses, and no countries possessed vaccines for all of their citizens [15]. At that time, stockpiled or licensed smallpox vaccines around the world except for the LC16m8 vaccine in Japan were the calf lymph-derived vaccine grown on calf skin. This was used in the eradication program but did not fulfill the current General Manufacturing Protocol and was at risk of being contaminated by adventitious agents [16]. In 2000, the United States signed a contract for the purchase of 40 million doses of a novel cell-cultured smallpox vaccine [17].

On the contrary, Japan was the only country in the world to possess a licensed, cell-cultured smallpox vaccine. The then Ministry of Health and Welfare reestablished a study group to investigate the smallpox vaccine in 1998 and carried out the test production of 10,000 doses of smallpox vaccine. Those were for young scientists who handled orthopox viruses but had not been part of the regular vaccination program in the past, thus were not for bioterrorism preparedness. The Japanese government did not launch a reproduction program until the threat of bioterrorism attack became ongoing after the anthrax attack in the United States in 2001.

4. Development of Live Attenuated Smallpox Vaccine LC16m8 in Japan Before 2001

LC16m8 is an attenuated vaccinia virus strain that was obtained through serial passage of the Lister strain, one of the major vaccinia strains used in the eradication era, in primary rabbit kidney cells [18]. Dr. Hashizume of the Chiba Serum Institute in Japan in the 1970s developed it to reduce severe adverse events after smallpox vaccination. In the early 1960s, even though no new cases had been reported since 1956, more than 20 cases of post-vaccinial encephalitis per million naive vaccinations and approximately 10 deaths due to severe post-vaccinial complications had been reported in Japan [19]. However, due to the risk from imported cases of smallpox, several attenuated vaccinia strains were developed and tested so that the vaccination program could be continued. After a clinical trial involving approximately 10,000 children, the proportion of takes and presence of the neutralizing antibody titer in vaccinia naive children given LC16m8 vaccine showed a level of immunogenicity comparable with that achieved with the original Lister vaccine [19], and LC16m8 was given a conditional license in 1975 (an unconditional license was given in 1980). Japanese health authorities administered the LC16m8 vaccine to

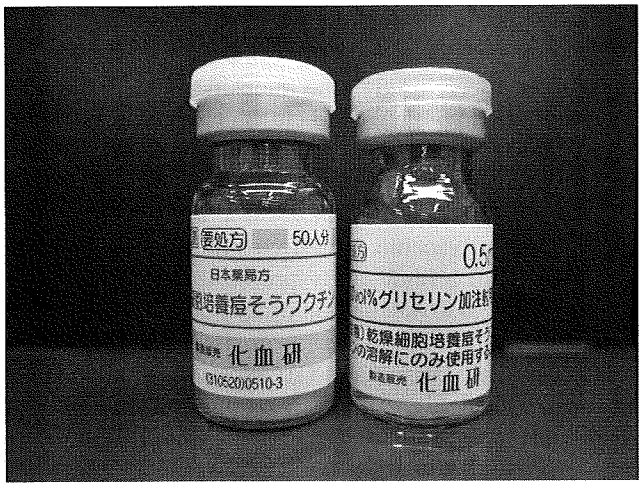

Fig. 1. Smallpox Vaccine LC16m8 (Kaketsuken).

more than 100,000 infants between 1973 and the beginning of 1976, the end of the regular smallpox vaccination program, without serious adverse events [18,19]. The preclinical and clinical research on the LC16m8 vaccine by the Smallpox Vaccine Research Group (SVRG) formed by the then Japanese Ministry of Health and Welfare has been extensively reviewed elsewhere [20]. Vaccine production ceased in 1980 and 130 million doses of vaccine, most of which were calf lymph vaccine, were stockpiled and maintained [21].

5. Rebuilding the Stockpile of LC16m8 Vaccine after the 9/11 Attack, 2001

Immediately after the anthrax attack in the United States, the Japanese government budgeted about 1 billion yen from the supplemental budget of FY2001 to purchase 2.5 million doses of smallpox vaccine from the Chiba Serum Institute, which owned the vaccine seed for the LC16m8 vaccine [22]. This was the first production since the termination of the regular vaccination program except for a test production in 1998. After the delivery of 2.5 million doses of the LC16m8 vaccine in March 2002 [23], the vaccine seed and license were transferred to the Chemo-Sero-Therapeutic Research Institute (Kaketsuken), Kumamoto, Japan, in July 2002 [24] (**Fig. 1**) because the Chiba Serum Institute owned by Chiba prefecture was closed. In fiscal year 2003, the government stockpiled an additional 7.5 million doses [25]. The then Defense Agency also purchased vaccines from 2002 and vaccinated select personnel in the Japan Self-Defense Forces for International Peacekeeping Operation activities of the United Nations Disengagement Observer Force [26]. In March 2005, a task force for smallpox vaccine preparedness funded by the Ministry of Health, Labour and Welfare (MHLW) recommended 56 million doses as an appropriate national stockpile, as that number of doses would cover the vaccinia-naive population in Japan [10, 27]. Consequently, the government began increasing the stockpile to achieve this number of doses; however, the amount of vaccine currently stockpiled is not open to the public for security reasons. Kaketsuken has built a new bulk facility with an annual capacity of approximately 80 million doses [28]. This fulfills the manufacturing capacity expected by the World Health Organization [13].

Table 1. Factors in Considering the National Smallpox Vaccine Stockpile.

National Security Issues
Risk of smallpox attack/outbreak
Potential scenario of deliberate reemergence
Scientific Issues
Epidemiology of smallpox
Vaccine efficacy, effectiveness, and safety
Size of the susceptible population
Residual immunity among those vaccinated in the past
Size of the contraindicated population
Vaccination strategy
Regulatory and Logistic Issues on Vaccines
Licensure
Production capacity
Lead time for vaccine production
Shelf life
Public Health Issues
Capacity for early detection and response
Capacity for distributing vaccines
Political & Financial Issues
Procurement and maintenance cost
Compensation for severe adverse events
Priorities among various stockpiles and other public health investment
International cooperation (i.e., shared stockpiles)
Social Issues
Preventing panic due to public anxiety over shortages

6. Background of the Proposal for the Appropriate Size of the Stockpile

The size of the vaccine stockpile is a critical factor in smallpox preparedness because it determines which

strategies the nation can choose to contain the disease. The task force for smallpox vaccine preparedness was organized in fiscal year 2004 to review Japan's smallpox vaccine preparedness [10]. In planning the appropriate size of the stockpile, not only scientific evidence, such as that from mathematical modeling based on known epidemiologic factors, but also surrounding issues listed in **Table 1** should be considered along with potential scenarios.

The task force tentatively proposed in its final report [10] that the appropriate number of doses in the stockpile be the number of unvaccinated citizens. The unvaccinated population, based on the vaccination registration records for 1962-1976 (the then Ministry of Health and Welfare), was roughly estimated as the sum of the population born after 1976 (approximately 38 million people), when the regular vaccination program ceased, and 20% of those born before 1976 (approximately 17.6 million people).

The task force assumed the outbreak scenario would involve the infection of several hundred people, rather than several thousands of infections in a devastating large-scale aerosol attack, and hence a search and containment strategy would be better than mass vaccination of the whole population. The mathematical epidemic model proposed by the task force took into account the current public health resources in Japan in terms of the number of vaccines needed, which depended on the basic reproduction number, number of people initially exposed, and the starting day of intervention employing the vaccination strategy [29]. The study suggested that the introduction of mass vaccination might be better than ring vaccination in the initial phase only when the number of initial exposures is large (>1000) and/or the agent is highly infectious. The amount of vaccine needed under the ring vaccination strategy was approximately 3 million doses for the moderate case scenario, and even in the worst case, it was less than the size of the susceptible population, which was estimated to be as large as the unvaccinated population. A pilot survey in a city in northeastern Japan indicating that approximately 20% of the population may have contraindications to the administration of the smallpox vaccine [10] supports the statement that mass vaccination of the entire population, especially pre-event, will not be a good option.

7. Reevaluation of the Smallpox Vaccine LC16m8

When the SVRG was reestablished in 1998 with funding from the then Ministry of Health and Welfare, most of its research activity addressed the maintenance and evaluation of the potency, stability, and safety of stockpiles made in 1980 and the LC16m8 vaccine newly produced on a trial basis [30]. After the task force for smallpox vaccine preparedness in fiscal 2004 recommended the reevaluation of the LC16m8 vaccine to assure its immunogenicity and safety [10], the SVRG was reorganized to include clinicians and epidemiologists.

Most countries stockpile conventional calf-lymph vaccines, which were used in the Intensified Smallpox Eradication Program of the World Health Organization [3] , or a cell-cultured, non-attenuated strain such as ACAM2000 (Acambis, Cambridge, Massachusetts), whereas Japan is unique in that it stockpiles solely the live attenuated tissue-cultured vaccine. The LC16m8 vaccine has been shown to have a high safety profile in preclinical studies [18, 31] and a vaccination program for children [19], and it is already licensed in Japan. However, there were several concerns about the LC16m8 vaccine at that time: efficacy in the field and safety in adults and people having contraindications (i.e., the immunocompromised).

7.1. Reevaluation of the Efficacy of the Smallpox Vaccine LC16m8

The LC16m8 vaccine has not been tested against smallpox (i.e., the variola virus) in the field because it was developed at the end of the eradication era. Smallpox vaccines developed after the eradication will never have the opportunity to be tested in the field unless there is an unexpected outbreak, although the best evidence to demonstrate vaccine efficacy or effectiveness is derived from clinical studies. Furthermore, there are no definite serologic indices known to correlate with protection, and knowledge of protective immunity against smallpox is limited. Therefore, to evaluate the protective immunogenicity, the LC16m8 vaccine needs to be compared with vaccine strains field-tested in the eradication era in terms of virological (genetic) characteristics, immunogenicity, and efficacy in animal challenge models and the results extrapolated to the protection of humans against the variola virus.

The major genetic difference between LC16m8 and the parental LC16mO strain is the truncating mutation of the B5R gene, which encodes the envelope protein of the extracellular enveloped virion [32]. Although the B5R is the neutralizing antibody (Ab)-inducing antigen [33], even the LC16m8 with the whole B5R gene deleted protected mice in a lethal challenge test [34]. Thus, attenuated LC16m8 has a sufficient genetic component for inducing protective immunity. Several studies have shown the immunogenicity of LC16m8 in humans. As for the humoral immunity, a level of raised neutralizing Ab titer in children vaccinated with LC16m8 was comparable to that for the original Lister strain [19]. In adults, our group demonstrated a high level of seroconversion and booster response in a plaque-reduction neutralizing Ab test in vaccinia-naive and previously vaccinated participants [26]. The other index of immunogenicity is the "take." Take was the most reliable index of successful immunization in the eradication era [3] and is correlated to the vaccinia virus-specific T-cell and B-cell responses [35]. Both in children and adults, LC16m8 showed high levels of vaccine take (95% and 94% respectively) in primary vaccinees [19, 26]. The variola virus only infects human beings. Natural infection is unobservable

and challenge test in humans is ethically unacceptable. Thus, efficacy in the animal challenge test using orthopox viruses that the animal is susceptible to provides the best evidence for the protective immunity of the LC16m8 vaccine against the variola virus in humans. Cross-protection among orthopox viruses [3] supports the extrapolation of animal challenge study results to the protective immunity for humans. Several groups, mostly the SVRG, have reported successful preclinical challenge studies of LC16m8 vaccine in mice [32, 34, 36, 37], rabbits [36] and monkeys [38, 39]. Such evidence from multiple animal models fulfills the license requirements of the Federal Drug Agency in the United States for vaccines for which human efficacy trials are not ethical or feasible (21 CFR Parts 314 and 601). Combining these evidences, it is reasonable to suggest that the LC16m8 vaccine will protect humans from variola virus infection.

There are two additional concerns regarding efficacy from an operational point of view. One is the long-term immunity, as LC16m8 is an attenuated strain. The SVRG has addressed this issue in two animal models. In a mouse study, the research group demonstrated that mice immunized with LC16m8 vaccine maintained a high level of neutralizing antibody comparable to that for the mice immunized with Lister, and the protective efficacy of LC16m8 was similar to that of Lister in the lethal challenge for 1.5 years after immunization [37]. A study in which a non-human primate model undergoes a monkeypox virus challenge was successful after 1 year of vaccination with the LC16m8 vaccine, as was observed in a challenge study after 6 weeks of vaccination [39, 40]. The other concern is protection by post-exposure administration. Although an animal model for post-exposure vaccination has not yet been established, the challenge studies for early exposure within 2 or 3 days post-vaccination were successful in a mouse model [41] and a monkey model, even when the specific antibody had not yet been raised [42]. Considering the faster disease development in an animal model for orthopox virus infection than that in human for variola virus infection, we may expect some levels of protection from post-exposure administration of LC16m8 vaccine with these observations.

7.2. Reevaluation of the Safety of Smallpox Vaccine LC16m8 in Adults

In preclinical studies, LC16m8 was found to have low proliferation in the peripheral skin [18], poor ability to invade the central nervous system [18, 31], and low proliferation and neurotoxicity in the central nervous system [31, 43, 44]. These three phenomena may explain the low pathogenicity of LC16m8 [45]. Other preclinical studies suggested that LC16m8 has lower pathogenicity than conventional strains such as the New York City Board of Health strains [34, 46]. In clinical settings, past studies of more than 100,000 children did not find severe adverse events, though there have been few reports of LC16m8 vaccinations of adults.

During the vaccination program for the Japan Self-Defense Forces starting in 2002, an elevated rate of myopericarditis following smallpox vaccination was reported by the United States smallpox vaccination program [47, 48], and a subsequent analysis suggested a strong causal relationship between vaccinia inoculation and myopericarditis [49]. In response to this report, the SVRG monitored 3,221 adults in the Japan Self-Defense Forces vaccinated with the LC16m8 vaccine for adverse events for 30 days post-vaccination between 2002 and 2005. No severe adverse events, one case of allergic dermatitis and another of erythema multiforme (both of which were mild and self-limited), and no abnormal electrocardiogram traces or symptomatic heart disease were observed. However, the sample size was limited to sufficiently verify the absence of severe adverse events and myopericarditis [26]. To assure the safety of LC16m8 vaccine by increasing the number of cases, the SVRG continues to closely review the adult vaccination program for the Self-Defense Forces with a detailed cardiological study [50].

7.3. Reevaluation of the Safety of Smallpox Vaccine LC16m8 for Those with Contraindications

People showing contraindications, such as immune suppression or atopic dermatitis, are at potentially high risk when vaccinated with a proliferative vaccine. On the basis of a pilot survey in a northeastern city in Japan in 2004 indicating that 20% of the population may have some kind of contraindication [10], the task force recommended a further study on the safety and immunogenicity of the LC16m8 vaccine for that part of the population. The SVRG has presented several pieces of supportive evidence for its safety; a preclinical study using a murine atopic dermatitis model indicated safety and immunogenicity [51], and studies using immunodeficient mouse models, a severe combined immunodeficiency disease (SCID) mouse and cyclosporine A-treated mouse, demonstrated a high safety profile [52]. In a clinical study on adults, individuals with atopic dermatitis were vaccinated if their lesions were stable, and no features suggesting eczema vaccinatum were observed [26].

To address the safety of those with contraindications, some countries, such as the United States, will consider a nonproliferative strain, the modified vaccinia Ankara, which was originally developed in Germany [53, 54]. Currently in Japan, the policy is to consider the LC16m8 vaccine for people with contraindications when they are at high risk of variola infection. This policy takes into account the several supportive studies mentioned above and the advantage of the LC16m8 vaccine in operation: demonstration of successful LC16m8 vaccination via the visible major skin reaction take by single dermal scarification with a low chance of severe adverse effects. However, further risk assessments of the LC16m8 vaccine in the immunocompromised by the research group are expected.

8. Issues Related to the Stability of the Stockpile and Shelf-life Extension

Cost is a consideration in building and maintaining a national stockpile. The shelf life in particular directly influences the stockpile cost. Under the Pharmaceutical Affairs Law in Japan, the shelf life for the freeze-dried smallpox vaccine used to be 3 years; however, after extensive discussion between the regulatory bureau and the SVRG, which includes the vaccine manufacturer and the National Institute of Infectious Diseases, the shelf life was extended to 4 years in 2006 (Kanpou (The Official Gazette) No.4486, 18 December 2006, Government of Japan). The major problems in extending the shelf life were the potency and stability of the stockpile. The SVRG demonstrated that the virus titer in LC16m8 vaccine stored in freeze-dried form at less than $-20°C$ was stable for at least 5 years, and the packaging is sufficiently air-tight to keep the moisture content stable for 4 years. From experience with the long-term stockpile of the calf lymph vaccine, it is assumed that the potency of the stockpile is stable for more than 20 years, and the evaluation of potency is relatively easily estimated by the virus titer. However, the stability of the vaccine and the rubber-capped glass vial for long-term storage in the freezer is uncertain, and how the stability might affect vaccine safety cannot be predicted for the longer term. Currently, the SVRG is developing a testing method of assuring the stability and potency for longer-term storage [50].

9. Smallpox Vaccine Usage and Distribution Plan in Japan

Guidelines for a smallpox outbreak in Japan are provided by the Department of Tuberculosis and Infectious Disease in the MHLW [55]. The preparedness and response plan for bioterrorism has three levels. The current situation in Japan is at level I, with no specific threat being recognized and smallpox vaccination not provided in principle. When a high probability of bioterrorism attack is recognized or a patient is found outside the country and the Health Crisis Management Commission declares a level II situation, first responders, such as medical staff, police, firefighters and air/seaport staff (approximately 100,000 people) will be vaccinated, but not the general population. When a smallpox patient is found within Japan, the preparedness level is raised to level III, and a search and containment strategy is primarily applied and all people found to have come into contact with patients in the active surveillance are vaccinated. If the outbreak level exceeds the public health capacity for a search and containment strategy, targeted mass vaccination is conducted to cover the outbreak area. This immunization plan is also supported by law. The vaccination will be administrated under the Preventive Vaccinations Act as an "extraordinary vaccination," in which the prioritization of vaccination for first responders and people at high risk of infection is explicitly stated in the enforcement order. When the mass vaccination strategy is adopted, giving priority to those unvaccinated is recommended by the Infectious Disease Subcommittee of the Health Science Advisory Board of the MHLW [56] because people vaccinated in the past are expected to have residual immunity against smallpox.

The pitfall in this current preparedness plan is the lack of a detailed logistic plan for the mass distribution of vaccines. The following issues are not yet resolved: the distribution of the right amount of limited stockpiles to a vaccination station, the mobilization of medical staff, and the securing the vaccination station and the mass screening of those having contraindications. Simulations and exercises for the search and containment strategy scenarios have often been carried out; however, few exercises have been conducted for the quick mass distribution of medical countermeasures for a larger scale outbreak or attack. The secrecy of the amount and location of the vaccine stockpile, which is important for national security, sometimes seems to be an obstacle to preparedness planning. Currently, the preparedness and response plan for smallpox is under review and will be updated in detail [57]. In this revision, the common approach with the plan for an influenza pandemic will be incorporated. Lessons learned from the exercises for an influenza pandemic and the outbreak of the 2009 H1N1 influenza underway are expected to contribute to the improvement of the smallpox preparedness plan.

10. Summary and Future Directions of Smallpox Vaccine Preparedness Research

Maintaining the stockpile and production capacity of "orphan" medical countermeasures is a part of national security and a huge contribution to global health. The SVRG in Japan has conducted research since it was reestablished in 1998, and it collaborates closely with the regulatory agency, a manufacturer, and scientists from academia and national institutes. Smallpox vaccine preparedness in Japan was mostly achieved through close communication among these stakeholders. The group has demonstrated the quality of a national LC16m8 vaccine by characterizing the LC16m8 strain at a molecular level, showing the efficacy and safety of the LC16m8 vaccine in preclinical and clinical studies, addressing stockpile costs through shelf-life extension via providing evidence of stability in long-term storage and maintaining the quality of the stockpile vaccine that is available in case of emergency.

Three future directions for smallpox vaccine preparedness research are proposed. The first is to address remaining issues in vaccine efficacy and safety and consolidate its position as a global standard. For example, the long-term immunity in humans, as already demonstrated in animal studies [37, 40], would be of particular benefit to first responders. Whether first responders who were vac-

cinated after the 2001 attack in some countries need additional vaccination when they face an emergency is a problem of operation in the field. Revealing the protection mechanisms in the early exposure after vaccination [41, 42] and establishing an animal model for evaluating postexposure vaccination would address the concern regarding the efficacy of postexposure vaccination. The efficacy and safety in the immunocompromised and people with atopic dermatitis are still issues of concern. A smallpox vaccine would be applicable not only to biodefense, but also to a current threat: monkeypox, which is present in some countries and has approximately 1-10% lethality in humans [58]. The LC16m8 vaccine would be sufficiently safe for use in a regular vaccination program. A clinical trial is worth consideration in the endemic area. The clinical experience would be meaningful for biopreparedness as well. A comparative test with conventional vaccines such as Dryvax® and Lister, in animal models would be of great use.

Second, research by the SVRG has focused too much on vaccine development. The vaccine itself is just a part of smallpox vaccine preparedness and the overall biopreparedness. Unless the vaccine is available to affected people during the window period, we will never fully benefit from a high-quality vaccine stockpile. We should overview the whole process from vaccine production, procurement, stockpiling and distribution to administration in terms of the whole biopreparedness process and identify and intervene where there is a bottleneck in vaccine preparedness.

Third, we need to establish how to evaluate the stockpile and manufacturing capacity for the smallpox vaccine. In contrast to a "visible" ongoing threat, such as the new 2009 H1N1 influenza and other emerging/reemerging diseases, the benefits of biopreparedness tend to be undervalued and inadequately budgeted for. More work is needed to adequately evaluate the cost and "hidden" benefit of stockpiling and being one of few countries that has cell-cultured smallpox vaccine production facilities, not only as a deterrent in a national security context but also as a public good for the well-being of the world's population.

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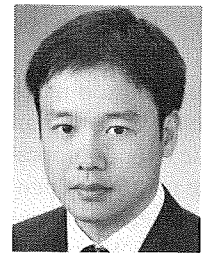

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- T. Saito, T. Fujii, Y. Kanatani, M. Saijo, S. Morikawa, H. Yokote, T. Takeuchi, N. Kuwabara, "Clinical and Immunological Response to Attenuated Tissue-Cultured Smallpox Vaccine LC16m8," JAMA, 2009, Vol.301, No.10, pp. 1025-1033, Mar., 2009.
- T. Saito, M. Nishi, M. I. Lim, B. Wu, T. Maeda, H. Hashimoto, T. Takeuchi, D. S. Roos, and T. Asai, "A Novel GDP-Dependent Pyruvate Kinase Isozyme From *Toxoplasma gondii* Localizes to Both The Apicoplast And The Mitochondrion," Journal of Biological Chemistry, 2008, Vol.283, No.20, pp. 14041-14052, May., 2008.

Academic Societies & Scientific Organizations:
- The Japanese Society for Vaccinology
- Japanese Society of Public Health