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F．研究発表

1．論文発表
・Takahashi K, Shimadzu H. The daily incidence of out-of-hospital cardiac arrest unexpectedly increases around New Year's Day in Japan. Resuscitaion 96: 2015; 156-162.

2．学会発表
・高橋邦彦，島津秀康．年末年始における院外心停止傷病者救急搬送件数急増の検出．第26回日本疫学会学術総会，鳥取，2016年1月22日．
・高橋邦彦，高橋和郎，金谷泰宏．公衆衛生対策におけるスキャン統計量を用いた症候サーベイランスと視覚化の活用．H27年度第2回人流物流ネットワークとその周辺研究会．東京，2016年1月23日．

3．著書
なし

G．知的所有権の取得状況
なし

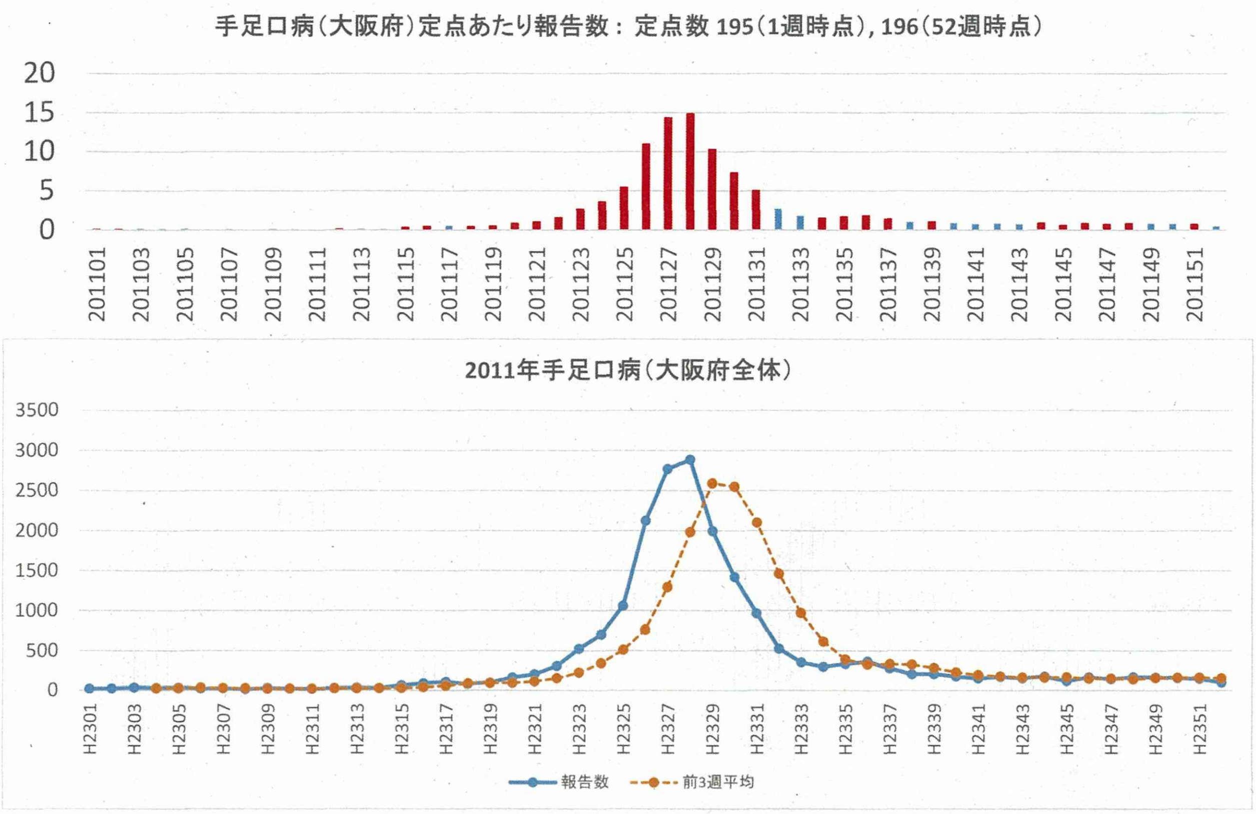

図1. 大阪府手足口病 定点報告（2011年）

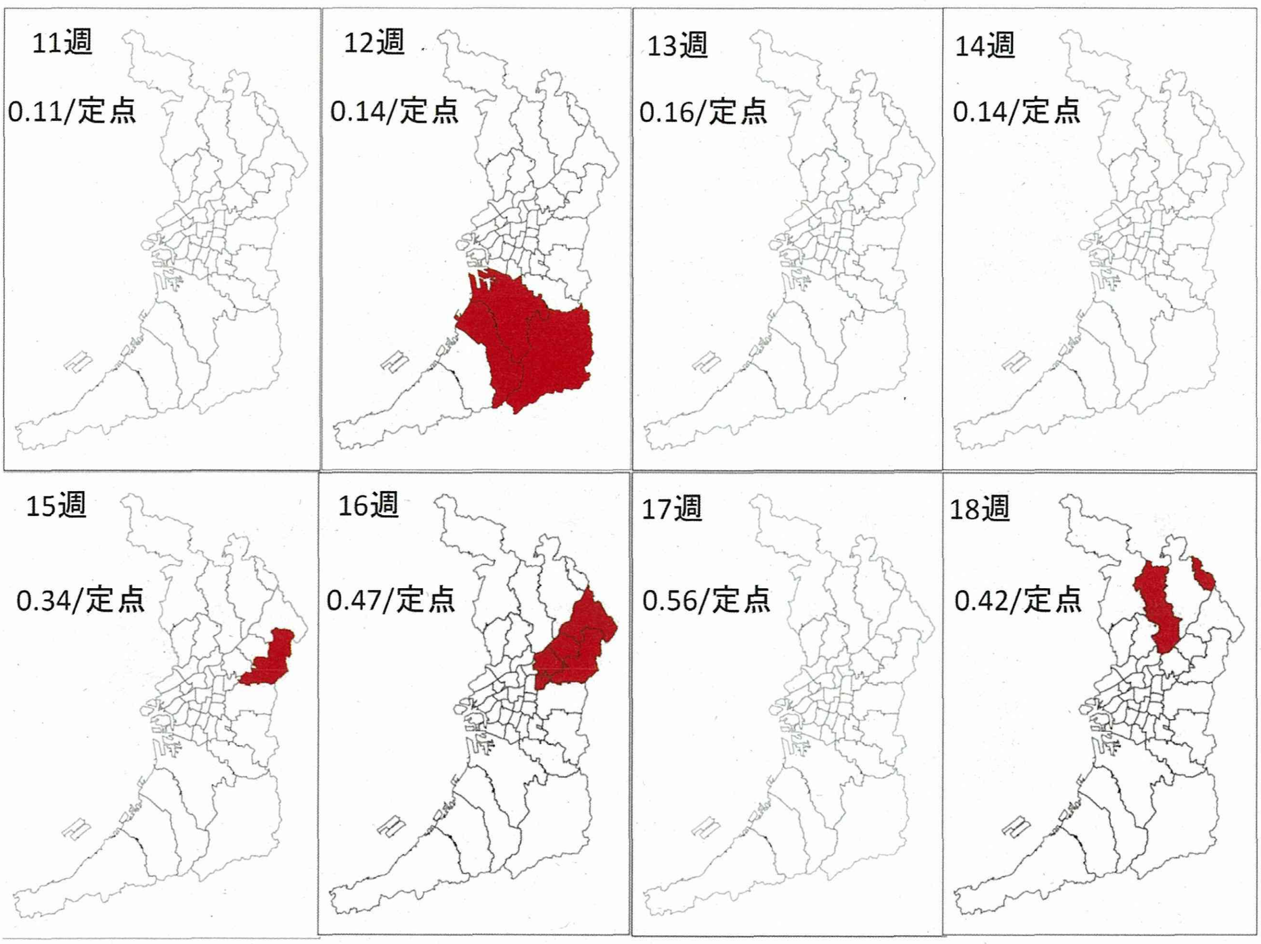

図2. 検出された集積地域

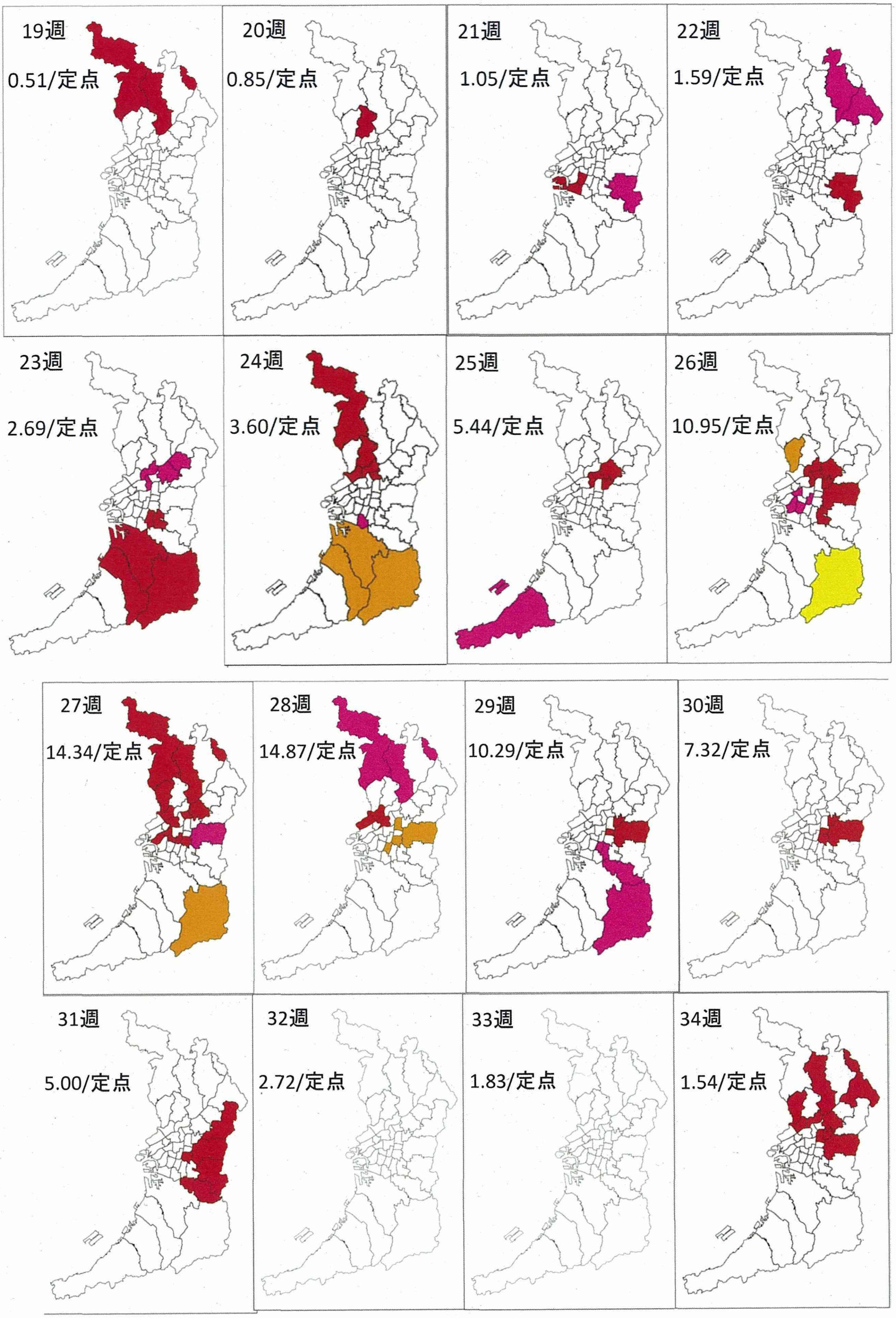

図2（続）．検出された集積地域

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻	ページ	出版年
Nishiyama Y, Matsukuma S, Matsumura T, Kanatani Y, Saito T.	Preparednessfor a Smallpox Pandemic in Japan: Public Health Perspectives.	Disaster Medicine and Public Health Preparedness	9(2)	220-223	2015
Nishiyama Y, Fujii T, Kanatani Y, Shinmura Y, Yokote H, Hashizume S.	Freeze-dried live attenuated smallpox vaccine prepared in cell culture "LC16-KAKETSUKEN":Post-marketing surveillance study on safety and efficacy compliant with Good Clinical Practice.	Vaccine	33(45)	6120-7	2015
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Recent advances in the study of live attenuated cell-cultured smallpox vaccine LC16m8

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ABSTRACT

LC16m8 is a live, attenuated, cell-cultured smallpox vaccine that was developed and licensed in Japan in the 1970s, but was not used in the campaign to eradicate smallpox. In the early 2000s, the potential threat of bioterrorism led to reconsideration of the need for a smallpox vaccine. Subsequently, LC16m8 production was restarted in Japan in 2002, requiring re-evaluation of its safety and efficacy. Approximately 50,000 children in the 1970s and about 3500 healthy adults in the 2000s were vaccinated with LC16m8 in Japan, and 153 adults have been vaccinated with LC16m8 or Dryvax in phase I/II clinical trials in the USA. These studies confirmed the safety and efficacy of LC16m8, while several studies in animal models have shown that LC16m8 protects the host against viral challenge. The World Health Organization Strategic Advisory Group of Experts on Immunization recommended LC16m8, together with ACAM2000, as a stockpile vaccine in 2013. In addition, LC16m8 is expected to be a viable alternative to first-generation smallpox vaccines to prevent human monkeypox.

1. Introduction

LC16m8 is a live, attenuated, cell-cultured smallpox vaccine developed in Japan in the 1970s to generate a vaccine strain with a safer profile than first-generation vaccines, such as the Lister and Dryvax strains, which have rare but severe adverse effects [1]. A clinical study of 10,578 children, among approximately 50,000 children vaccinated with LC16m8, showed no severe adverse events and comparable immunogenicity and efficacy with first-generation vaccines [2].

LC16m8 is a promising vaccine because of its lower virulence and replication competency. A frameshift mutation in B5R, a major extracellular envelope virion (EEV, also called EV) antigen, results in expression of a truncated protein, which attenuates the virulence of the strain [3]. LC16m8 was licensed in 1975, but was not used in the smallpox eradication program because routine smallpox vaccinations were halted in 1976. After the terrorist attacks in the USA in 2001, vaccine production was recommenced in Japan and government stockpiles have been established to prepare for possible bioterrorism [4,5]. The safety and efficacy of LC16m8 has also been explored. Thus, in Japan, vaccination with LC16m8 has been evaluated in 3221 subjects [6] and 268 subjects [7] since 2002, and in the USA, a phase I/II clinical trial was conducted in 153 subjects [8]. Moreover, several experiments have been performed in animal models to examine the safety and efficacy of LC16m8 [3,9–14].

A review on LC16m8 was published by Kenner et al. in 2006, describing the development of LC16m8, its molecular characterization, the then-current knowledge, and comparison to other smallpox vaccines [15]. Here, we describe advances in LC16m8 research since then, including our own clinical studies, animal experiments, long-term effects, and formation of antibodies to B5 protein, since LC16m8 is a B5R-mutant strain. We also discuss possible clinical applications of LC16m8 as a human monkeypox vaccine.

2. Clinical studies

A unique feature of smallpox vaccine research is that safer vaccines are sought by assessing strain immunogenicity in the absence of an endemic outbreak [16–18]. Clear correlations of surrogate markers with protection against smallpox were limited until the

Abbreviations: EEV, extracellular enveloped virion; PRNT, plaque reduction neutralization titer; GMT, geometric mean titers; IMV, intracellular mature virion; PFU, plaque forming unit; WR, Western Reserve; MVA, Modified vaccinia Ankara; SCID, severe combined immunodeficient; LD, lethal dose; SAGE, Strategic Advisory Group of Experts.

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0264-410X/

1970s [16–18]. Therefore, a vaccine was evaluated by comparing the immunological responses of the target vaccine with first-generation vaccine(s) and by assessing the protection conferred in animal challenge models [16–18]. Three clinical studies, including one post-marketing surveillance study of LC16m8, have been performed in the United States and Japan since 2002. In Japan, clinical and immunological responses to LC16m8 were evaluated in 3221 healthy Self-Defense Force personnel aged 18–55 years, including 1529 vaccinia-naive subjects and 1692 who had previously been vaccinated with smallpox vaccine [6]. LC16m8 was administered using a bifurcated needle. Vaccinia-naive subjects received 5 punctures and previously vaccinated individuals received 10 punctures. The major outcomes were the frequency of adverse events up to 30 days post-vaccination, the proportion of major cutaneous reactions, and the plaque reduction neutralization titer (PRNT) in sera.

No severe adverse events and no abnormal ECG findings or symptomatic heart disease were observed in the study [6]. Two mild cases of allergic dermatitis and erythema multiforme, respectively, were suspected to have been caused by vaccination. Two other minor adverse events of pain from a swollen axillary lymph node and groin pain caused by a bacterial infection were also suspected to be related to vaccination. Majority of adverse events consisted of swelling in the axillary lymph nodes and low-grade fever at rates of 15.5% and 2.6% for primary vaccinees (vaccinia-naive subjects) and 3.5% and 1.4% for revaccinees (previously vaccinated individuals), respectively. Adverse events occurred earlier in revaccinees than in naive subjects, with onset of fever at 5.8 and 2.0 days and swelling of axillary lymph nodes at 5.6 and 3.2 days (means) in naive subjects and revaccinees, respectively.

The outcomes of the study included high levels of major cutaneous reactions ("take") and seroconversion [6], with proportions of major cutaneous reactions of 94.4% and 86.6% in vaccinia-naive subjects and revaccinees, respectively. There were no significant differences in these proportions among age groups of revaccinees with different vaccination histories (variations in the dose and strain of routine smallpox vaccine vaccination prior to 1976). The rate of seroconversion based on the level of neutralizing antibodies raised against Dryvax was 90.2% in primary recipients, and the effective boost rate was 60.0% in revaccinees, with no significant difference in the level of PRN geometric mean titers (GMTs) in post-vaccination serum between the two groups.

A further study in 268 Self-Defense Force personnel was conducted in 2005 as a post-marketing study [7]. The subjects were aged 19–52 years, 196 were vaccinia-naive, 71 had been previously vaccinated with smallpox vaccine(s), and 1 had an unknown vaccination history. The number of punctures was identical to that used in the previous study [6]. No severe adverse events occurred [7] and the proportions of major cutaneous reactions were 94.4% and 81.7% in naive and previously vaccinated individuals, respectively. Seroconversion based on PRNT was found in 84.2% of naive individuals at 1 month post-vaccination.

Kennedy et al. [8] compared the safety and immunogenicity of LC16m8 with those of the Dryvax strain in a randomized, double-blind, multicenter phase I/II clinical trial in 153 vaccinia-naive adults aged 18–34 years in the USA. Dryvax or LC16m8 was administered using a bifurcated needle via 15-puncture intraepidermal inoculation. The primary endpoints were the anti-intracellular mature virus (IMV) PRNT 30 days after vaccination and the rate of vaccine-attributable adverse events. Secondary endpoints were the rate of major cutaneous reactions, lesion size at the vaccination site, anti-EEV PRNT, cell-mediated immune responses, viral persistence, and viremia after vaccination. There were no clinically significant abnormalities including cardiac toxicity, and the local and systemic reactions after vaccination were similar for both strains [8]. The major local reactions included axillary lymph node tenderness (Dryvax, 50%; LC16m8, 48%), tenderness at the vaccination site (Dryvax, 46%; LC16m8, 42%), and swollen axillary lymph nodes (Dryvax, 46%; LC16m8, 37%). The rates of swollen axillary lymph nodes and rash were higher in the US study (37% and 2%) than in the Japanese trial (16% and 1%) [6]. This difference may be related to the number of skin punctures, to different vaccine lots, and to the number, training, and experience of observers [8].

The proportions of major cutaneous reactions were 100% in the 125 LC16m8 vaccine recipients and 86% in the 28 Dryvax recipients [8]. In the 4 subjects without a major cutaneous reaction to Dryvax, the vaccine was administered at the same location on the same day. The subjects were not seroconverted, suggesting a problem with vaccine reconstitution. The average size of the major cutaneous reaction was larger with Dryvax than with LC16m8, but LC16m8 induced robust humoral and cell-mediated immunity [8]. The anti-IMV PRNT was assessed against three poxviruses: vaccinia, monkeypox, and variola. Sera from LC16m8 vaccinees had antibody titers >1:40 against all these viruses. The mean GMT PRNT was higher for Dryvax than for LC16m8, but cellular immune responses induced by LC16m8 were comparable to those induced by Dryvax. LC16m8-induced interferon-γ (IFN-γ) measured by ELISPOT was lower than that induced by Dryvax, but LC16m8-induced lymphoproliferation was higher in comparison to Dryvax [8].

These three studies [6–8] indicate that LC16m8 can be safely administered and has comparable efficacy to Dryvax (Table 1). The number of punctures is a potential cause for the differences in the rates of weak and mild adverse events between the studies conducted in the US [8] and Japan [6], as suggested by Kennedy et al. [8].

3. Experiments in animal models

Animal model challenge studies are critically important for evaluation of smallpox vaccines, since it is impossible to evaluate these vaccines in endemic controls [16–19]. Several studies of the safety and efficacy of LC16m8 have been conducted in mouse [3,9,11,12,14], rabbit [3,11], and non-human primates [10,13].

The protective efficacy of LC16m8 was compared with those of its parental strain LC16mO and the grandparental Lister strain in BALB/c mice vaccinated subcutaneously with a single dose of the vaccine or PBS [9]. At 21 days after vaccination, the animals were challenged with 10^6 PFU of pathogenic Western Reserve (WR) strain of vaccinia virus. Clinical signs, survival rate, body-weight loss, and histopathology of the nasal tissue were examined and sera from pre- and 14 days post-challenge were tested for anti-vaccinia and anti-B5 antibody by ELISA, for neutralizing antibody against IMV by PRN assay, and for anti-EEV antibody production by comet inhibition activity. The survival rate of LC16m8-vaccinated animals was 100%, similar to the LC16mO and Lister strains, while PBS-treated control mice died within 9 days after infection, and had clinical symptoms and body-weight loss. Vaccinated mice exhibited only slight, transient body-weight loss after WR challenge and this did not differ between vaccine strains.

The protective efficacy of LC16m8 against lethal WR challenge was also examined in a comparative study with the Dryvax strain in BALB/cByJ mice [12]. Outcomes include humoral immunity (anti-IMV antibody, immunoglobulin isotype, anti-A33 specific antibody, anti-B5 specific antibody, and anti-IMV and -EEV neutralization), cell-mediated immunity (interferon-gamma), and protection against lethal WR challenge. Sera from LC16m8- and Dryvax-vaccinated mice had similar antibody production against vaccinia and neutralizing antibodies for IMV, and similar cell-mediated immunity (IFN-γ production). Sera from LC16m8-vaccinated mice contained anti-B5 antibody, but at much lower levels than in sera from Dryvax-vaccinated mice. Sera from both groups exhibited anti-EEV neutralizing activity *in vitro*. However, possible effects of

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Table 1
Summary of three clinical studies of smallpox vaccine LC16m8.

Study	Saito [6]		Nishiyama [7][a]		Kennedy [8]	
Country	Japan		Japan		USA	
Study design	Clinical study, open-label		Post-marketing surveillance		Clinical trial phase I/II, randomized, double-blind	
Race	Asian (100%)		Asian (100%)		Native American (1%), Asian (6%), African American (2%), Pacific Islander (1%), Caucasian (88%), Multiracial (2%)	
Vaccine	LC16m8		LC16m8		LC16m8	Dryvax
Vaccination status	Naive	Vaccinated	Naive	Vaccinated	Naive	Naive
Number of participants	1529	1692	196	71	125	28
Number of punctures	5	10	5	10	15	15
Major cutaneous reaction ("take") (%)	94.4	86.6	94.4	81.7	100	86[b]
Serious adverse events	No	No	No	No	No	No
Weak or mild adverse events (%)[c]	22.4	6.6	27.0	5.6	82	68
Swollen axillary lymph nodes (%)	15.5	3.5	25.5	2.8	37	46
Swollen cervical lymph nodes (%)	0.4	0.2	–[d]	–	–	–
Fever (%)	2.6	1.4	1.0[e]	2.8[e]	–	–

Three studies are summarized based on data from references [6–8].
[a] The vaccination history of one participant in the Nishiyama study was unknown.
[b] See text.
[c] Rate for local reactogenicity in the Kennedy 2011 study.
[d] Not described.
[e] Fever was recorded for two participants for both Naive and Vaccinated groups.

revertant B5 protein detected at low levels by western blot analysis could not be excluded [12].

The protective efficacy of a B5-deletion strain has also been compared to that of MVA and Dryvax in BALB/c mice challenged with virulent WR [3]. Vaccine strains were administered intramuscularly and WR challenge was given intranasally. The protective efficacy of the B5-deficient strain was similar to that of Dryvax and superior to that of Modified vaccinia Ankara (MVA), based on survival rate and loss of body weight. The B5-deficient strain also induced a neutralizing antibody response against WR. Results in severe combined immunodeficiency (SCID) mice also showed that LC16m8 and B5-deficient strains could be used safely in these immunodeficient animals.

The safety of LC16m8 vaccination with Lister and LC16mO strains has also been compared in suckling mice, SCID mice, and cyclosporin-A-treated mice [14]. Suckling mice received intracerebral inoculations and SCID mice were inoculated intraperitoneally. The survival rates of suckling mice after intracerebral inoculation with $10^{3.3}$ PFU vaccine were 10% with both Lister and LC16mO strains and 70% with LC16m8 in an observation period of 21 days. The mean survival times were 6.3, 6.1, and 17.1 days for the Lister, LC16mO, and LC16m8 strains, respectively. The survival rates of SCID mice were 0% for Lister and LC16mO strains, and 100% for LC16m8, with mean survival of 30.8, 24.5, and >120 days, respectively. Cyclosporin-A-treated mice developed severe vaccinia-related symptoms, including pock formation and rash, whereas LC16m8-inoculated mice displayed no clinical symptoms.

Empig et al. evaluated the efficacy of LC16m8 in protection against orthopoxvirus challenge in mice and rabbits [11], as reviewed by Kenner [15]. In rabbits, the protective efficacy of LC16m8 and Dryvax against rabbitpox virus was examined. This animal model was used to evaluate the anti-EEV efficacy of LC16m8 because rabbitpox virus produces high levels of EEV. LC16m8 and Dryvax gave similar protection against rabbitpox virus, whereas non-vaccinated animals did not survive lethal doses of the virus. Anti-IMV neutralizing antibodies were significantly higher in sera from LC16m8-vaccinated rabbits than in those vaccinated with Dryvax. Anti-EEV neutralizing antibodies in sera from LC16m8-vaccinated rabbits were lower than those in Dryvax-vaccinated rabbits, but the difference was not significant.

In non-human primates, the efficacy of LC16m8 against monkeypox virus in cynomolgus monkeys was compared with that of the Lister strain [10]. Vaccine or PBS was administered by the multiple puncture method with a bifurcated needle in macaques, similar to the vaccination procedure for humans. For virulent monkeypox virus challenge, two models were used: at 5 weeks post vaccination, monkeypox virus was administered intranasally (Liberia strain) or subcutaneously (Zr-599 strain). The proportions and sizes of major cutaneous reactions were assessed after immunization. In the intranasal challenge model, LC16m8- and Lister-vaccinated animals had no clinical symptoms, whereas non-vaccinated animals developed typical symptoms [10]. In the subcutaneous challenge model, LC16m8-vaccinated animals had no clinical symptoms, but exhibited an ulcer at the vaccination site. Lister-vaccinated animals were protected against virulent challenge, while control animals developed typical symptoms and died.

The safety and efficacy of LC16m8 and Dryvax strains were compared in cynomolgus macaques administered with vaccine or saline, and injected with monkeypox virus (Zaire 79 strain) 60 days later [13]. The animals were protected by both vaccines. To investigate the mechanism underlying the local containment of vaccinia-induced skin lesions, animals were depleted of host B cells or T cells before vaccination with Dryvax or LC16m8 [13]. B-cell depletion had no effect on the size of skin lesions in animals vaccinated with either vaccine, whereas T-cell depletion caused progressive vaccinia only in Dryvax-vaccinated animals.

These results indicate that LC16m8 is safer than Dryvax for use in immunocompromised individuals.

Thus, studies in three animal models (mouse, rabbit, and macaque, Table 2) have shown that LC16m8 protects the host against viral challenges [3,9–13] and has a good safety profile, including low neurotoxicity, consistent with findings of studies conducted in the 1970s [1]. Notably, LC16m8 can be administered to immunodeficient animals without severe adverse events, which is markedly different from the first-generation vaccine (Table 3) [3,13,14].

4. Long-term immunity conferred by LC16m8

Long-term immunity is a prerequisite for a good vaccine. Previous reports have demonstrated the decades-long efficacy of anti-smallpox vaccination [20–23]. Routine smallpox vaccination was conducted until 1976 in Japan and conferred long-term immunity, as confirmed by recent studies on anti-vaccinia antibodies and neutralizing antibodies [6–7,24].

In a study of the effect of LC16m8 for up to 7 months post-vaccination in humans [7], GMT was decreased, but the seroconversion rate in primary vaccinees based on PRNT was still 75% at 7 months. Cell-mediated immunity was evaluated up to 180 days post-vaccination for 9 Dryvax vaccinees and 38 LC16m8 vaccinees in a US study [8], and lymphocyte proliferative responses were observed in 97% of LC16m8 and 89% of Dryvax vaccinees. Five LC16m8 vaccinees were negative for IFN-γ at 180 days post-vaccination, whereas all Dryvax vaccinees were positive.

In a comparison of the long-term effects of LC16m8 and the Lister strain in BALB/c mice [25,26], both groups of vaccinated mice were protected when challenged with a lethal amount of virulent WR after 1 year, whereas non-vaccinated mice died. An evaluation of serum antibody profiles by protein array 1.5 years after vaccination with LC16m8 [27] showed that antibody titers and neutralizing activities were substantially lower in comparison to 30-day sera, but that the LC16m8-vaccinated serum possessed antibodies common to Lister-vaccinated serum.

5. LC16m8 and formation of antibodies to B5 protein

B5 is a major antigen of EEV and antibodies against B5 are important for EEV neutralization [28,29]. B5 is a 42-kDa glycosylated type I membrane protein located in the EEV membrane [30,31]. After translation, B5 is transferred to the Golgi network and is internalized to the plasma membrane of the infected cell and exposed at the cell surface [31–33]. B-cell epitopes of anti-B5 monoclonal antibodies are localized to amino acid residues 56–84 and 254–275 [34]. LC16m8 has a point mutation in the B5R sequence, resulting in truncation to amino acids 1–91 of the original protein [35]. Given that this mutant lacks a C-terminal transmembrane domain, it is unlikely to be localized in the EEV [10]. In LC16m8-infected cultured cells, the truncated B5R product is recovered in cell extracts or supernatants [3,12]. Anti-truncated B5 antibodies are produced in mice upon vaccination with LC16m8 [12]. With regard to whether LC16m8 vaccination can induce the production of antibodies against the B5 protein in humans, Johnson et al. reported that the anti-B5 antibody did not increase upon vaccination of vaccinia-naive individuals, as determined by ELISA [36]. The neutralizing ability of sera from LC16m8-vaccinated humans was lower than that of sera from Dryvax-vaccinated humans, based on 30% EEV plaque reduction activity [8]. This is consistent with the results of Johnson et al. [36].

Table 2
Animal model challenge studies of smallpox vaccine LC16m8.

Study	Morikawa [9]	Kidokoro [3]	Empig [11]	Meseda [12]	Empig [11]	Saijo [10]	Gordon [13]
Host animal	BALB/c mouse	BALB/c mouse	A/NCR mouse	BALB/cByJ mouse	Rabbit	Cynomolgus macaque	Cynomolgus macaque
Vaccine	LC16m8, LC16mO, Lister	LC16m8, LC16m8 derivatives, Dryvax, MVA	LC16m8, Dryvax	LC16m8, Dryvax	LC16m8, Dryvax	LC16m8, Lister	LC16m8, Dryvax
Administration route	Subcutaneous	Intramuscular	Scarification	Scarification	Bifurcated needle scarification	Bifurcated needle scarification	Bifurcated needle scarification
Administration dose (PFU)	10^5, 10^7	10^4–10^6	2×10^5	10^6	2×10^5	$>1 \times 10^8$	2.5×10^5
Challenge Virus	Vaccinia WR strain	Vaccinia WR strain	Ectromelia	Vaccinia WR strain	Rabbitpox	Monkeypox Liberia strain Monkeypox Zr-599 strain	Monkeypox Zaire 79 strain
Inoculation route	Intranasal	Intranasal	Aerosol	Intranasal	Intradermal	Intranasal Subcutaneous	Intravenous
Inoculation dose	10^6 PFU	10^6 PFU	1–2 × LD_{50}	100 × LD_{50} (3.2 × 10^6 PFU), 250 LD50 (8 × 10^6 PFU)	1 × LD100 (200 PFU), 5 × LD100 (1000 PFU)	10^6 PFU 10^6 PFU	5×10^7 PFU

Animal model challenge studies are summarized based on data from references [3,9–13].
PFU, plaque forming unit; WR, Western Reserve; MVA, Modified vaccinia Ankara; LD, lethal dose.

Table 3
Studies of smallpox vaccine LC16m8 in immunodeficient animals.

Study	Kidokoro [3]	Yokote [14]			Gordon [13]
Immunodeficient animal	SCID mouse	Suckling mouse	SCID mouse	Cyclosporin-A treated mouse	T-cell, B-cell depleted rhesus macaque
Vaccine	LC16m8, LC16m8 derivatives, Dryvax, MVA	LC16m8, LC16mO, Lister	LC16m8, LC16mO, Lister	LC16m8, Lister	LC16m8, Dryvax
Administration route	Intraperitoneal	Intracerebral	Intraperitoneal	Intraperitoneal	Scarification
Administration dose (PFU)	10^3–10^7	$10^{3.3}$–$10^{5.3}$ (LC16m8), $10^{1.3}$–$10^{3.3}$ (LC16mO, Lister)	$10^{5.3}$	10^7	2.5×10^5

Immunodeficient animal studies are summarized based on data from references [3,13,14].
SCID mouse, severe combined immunodeficient mouse; MVA, Modified vaccinia Ankara; PFU, plaque forming unit.

6. Discussion

Since 2002, repeated evaluations of LC16m8 have consistently demonstrated its excellent safety profile [6–8]. No severe adverse events attributable to the vaccine were reported in studies conducted in the 1970s [1] or in the three relatively recent studies presented here [6–8]. These results indicate that LC16m8 is safer than first-generation smallpox vaccines, although the absence of rare adverse events cannot be conclusively confirmed because of the relatively small number of vaccinated subjects. Furthermore, LC16m8 can be safely administered to immunodeficient animals [3,13,14]; however, the safety of the administration of LC16m8 to immunocompromised patients has yet to be evaluated.

Vaccination with LC16m8 produces a high proportion of major cutaneous reactions [6–8], which is consistent with studies performed in the 1970s [1]. LC16m8 vaccination stimulates the production of neutralizing antibodies against orthopoxviruses, vaccinia, monkeypox, and variola viruses in human sera [8] and demonstrates host protection in animal-model challenge studies [3,9–13]. As experiments conducted in animal models are critical for research on smallpox vaccines [16–19], a comprehensive evaluation of clinical studies, *in vitro* experiments, and animal model studies suggests that the efficacy of LC16m8 is similar to that of the first-generation smallpox vaccines.

The LC16m8 vaccine induces both humoral and cellular immunity [3,6–14]. The efficacy of this vaccine has been debated because LC16m8 lacks an intact B5 protein [37]. However, LC16m8 has been shown to clearly protect the host in challenge studies conducted in mice [9,11,12] and other animal models [10,11,13]; recent reports suggest that even B5-deleted viruses can confer protection against virulent virus challenges in mice [3,38]. A protein array examination [39] of the immunogenicity of sera after vaccination with smallpox vaccine revealed the induction of multiple antibodies in a redundant manner [40–46]. This redundancy may account for LC16m8-conferred protection, which is similar to that observed in other vaccinia strains [40–46]; however, the role and importance of the truncated B5 protein in LC16m8-conferred protection remains unknown.

The WHO Strategic Advisory Group of Experts (SAGE) on Immunization have recommended LC16m8, together with ACAM2000, as a stockpile vaccine in 2013 [47]. To select the vaccine strain for stockpiling, SAGE conducted a systematic review of vaccines, which included the number of doses that should be stockpiled and the population to be targeted in case of smallpox re-emergence or as a preventive strategy [48]. SAGE made its recommendations based on expert consultations regarding the safety, immunogenicity, and efficacy of the vaccine and the practical properties that may be advantageous in the event of a smallpox outbreak, including lyophilized storage, administration via bifurcated needles, and production of major cutaneous reactions.

LC16m8 has been shown to confer protective effects against monkeypox in macaques [10,13], and sera from LC16m8-vaccinated humans exhibit anti-monkeypox neutralizing abilities [8]; these findings raise the possibility of the use of LC16m8 against human monkeypox. Surveys conducted in the Democratic Republic of the Congo revealed that a lack of smallpox vaccination caused a 5.2-fold higher risk of human monkeypox in non-vaccinated individuals [49]. Thus, LC16m8 is a viable alternative to first-generation smallpox vaccines to prevent human monkeypox, at least in high-risk populations [49]; however, its clinical efficacy against human monkeypox is yet to be evaluated.

7. Conclusions

Compared to first-generation smallpox vaccines, LC16m8 has a safer profile and similar efficacy. LC16m8 also has advantageous properties for practical use, such as possible administration with bifurcated needles. Vaccination with LC16m8 produces a high proportion of visible major cutaneous reactions. They are indicators of successful vaccination and the production of immunity. By using an established freeze-drying production method, LC16m8 can be easily stocked and administered, and the success of vaccination can be evaluated. This balance between safety and efficacy makes this vaccine valuable for use in the event of a smallpox outbreak. Further studies are expected to evaluate the use of LC16m8 against human monkeypox and in immunocompromised populations.

Conflict of interest

H. Y. is employed by the Chemo-Sero Therapeutic Research Institute. All other authors report no potential conflicts of interest.

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Freeze-dried live attenuated smallpox vaccine prepared in cell culture "LC16-KAKETSUKEN": Post-marketing surveillance study on safety and efficacy compliant with Good Clinical Practice

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ABSTRACT

Background: In Japan, production of smallpox vaccine LC16m8 (named LC16-KAKETSUKEN) was restarted and was determined to be maintained as a national stockpile in March 2002.
Objective: To conduct a post-marketing surveillance study of the vaccination of freeze-dried live attenuated smallpox vaccine prepared in cell culture LC16-KAKETSUKEN using attenuated vaccinia strain LC16m8. The study complied with Good Clinical Practice, focusing on a comparison between primary vaccinees and re-vaccinees.
Method: 268 personnel (261 males and 7 females) of the Japan Ground Self-Defense Force were inoculated with LC16-KAKETSUKEN and thereafter adverse events and efficacy were evaluated.
Results: Among 268 vaccinee participants, the following vaccinees showed adverse events, none serious: 53 of 196 primary vaccinees (without previous smallpox vaccination), 4 of 71 re-vaccinees (with previous smallpox vaccination) and 1 vaccinee with unknown previous vaccination history. A breakdown of adverse events observed in this study (total 268 vaccinees) showed the following minor or mild adverse events: 52 (19.4%) swelling of axillary lymph node, 4 (1.5%) fever, 2 (0.7%) fatigue, 1 (0.4%) of rash, 14 (5.2%) erythema at the inoculation site, 1 (0.4%) swelling at the inoculation site and 1 (0.4%) autoinoculation. The incidence of adverse events for primary vaccinees (53/196; 27.0%) was significantly higher than for re-vaccinees (4/71; 5.6%). However, the proportion of vaccine take was significantly higher for primary vaccinees (185/196; 94.4%) than for re-vaccinees (58/71; 81.7%). Although the proportion of vaccine take of re-vaccinees was significantly lower than for primary vaccinees due to preexisting immunity by previous vaccination, no significant difference was found in neutralizing antibody titers between primary vaccinees and re-vaccinees at 1, 4 and 7 months after LC16-KAKETSUKEN vaccination.
Conclusion: The present post-marketing surveillance study compliant with Good Clinical Practice demonstrated the efficacy and safety of the smallpox vaccine LC16-KAKETSUKEN in an adult population. LC16-KAKETSUKEN is the sole currently available licensed smallpox vaccine for both adult and pediatric populations.

1. Introduction

In the early 1970s, Hashizume et al. [1,2], then at the Chiba Serum Institute (CSI), Chiba, Japan, developed an attenuated tissue-cultured smallpox vaccine, LC16m8, from the Lister (Elstree) original strain that was used worldwide in the World Health Organization (WHO) smallpox eradication program [1,2]. LC16m8 was vaccinated into more than 50,000 children in Japan during 1973–1974, without showing problematic adverse events, such as postvaccinal encephalitis, progressive vaccinia, and skin complications, including autoinoculation, postvaccinal exanthems and eczema vaccinatum. Based on these studies, the vaccine strain LC16m8 was licensed in 1975 in Japan, and its freeze-dried vaccine preparation was also licensed in 1980. Because of the success of

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the smallpox eradication program, and followed by the declaration of its success by WHO in 1980, a regular vaccination program was ended in 1976 and smallpox vaccination was legally abolished in 1980 in Japan. Therefore, the LC16m8 strain is unlikely to show its effectiveness against endemic smallpox. However, the situation surrounding smallpox vaccination has changed dramatically after the incidents of September 2001 in the U.S.A., because concern about bioterrorism has risen. Among many pathogens, variola virus is one of the most feared.

Under such a situation, serious attempts have been made, both at home and abroad, to restart the development of smallpox vaccines with lower virulence compared with conventional vaccines. A vaccinia ACAM1000 clone established using cell cultures from the Dryvax vaccine may induce myocarditis [3,4]. Modified vaccinia virus Ankara and NYVAC (modified Copenhagen strain) replication-incompetent viruses are certainly safer, but may require high vaccine doses, multiple doses or boosting with replication-competent vaccines [5,6]. LC16m8 vaccine has drawn renewed attention as a promising vaccine against bioterrorism because it is a highly attenuated live vaccine prepared in cell cultures. Of note, a 2013 WHO Strategic Advisory Group of Experts Meeting on Immunization recommended both licensed ACAM2000 (2nd generation vaccine) and LC16m8 (3rd generation vaccine) as preferred WHO stockpile vaccines [7].

The history of LC16m8 vaccine after its licensing in 1975 in Japan is briefly as follows. Kaketsuken started in March 2002 in Japan to manufacture national stockpile vaccine recently named LC16-KAKETSUKEN as a medical countermeasure against bioterrorism as a result of the incident of September 11, 2001. Kaketsuken is manufacturing LC16-KAKETSUKEN from the master virus seed (MVS) transferred from CSI by using the modified manufacturing procedure of CSI as described in Section 3. Its efficacy and safety was shown in mice, rabbits and monkeys [8–13]. A study on the safety and efficacy in adult populations was done by using the vaccine manufactured by CSI (licensed LC16m8 vaccine, lot No. Chiba 02): the LC16m8 vaccine was inoculated from 2002 into personnel of the Japan Ground Self-Defense Force (JGSDF), showing its good efficacy and safety [14].

The series of clinical studies of this post-marketing surveillance (PMS) study were done under Good Clinical Practice (GCP) compliant conditions to confirm the safety and immunogenicity of LC16-KAKETSUKEN in members of JGSDF with detailed background information and with an extended follow up of neutralizing antibody titers until 7 months after vaccination. Clinical data on adult populations in the Package Insert of LC16-KAKETSUKEN were also used.

2. Material and methods

This study was done from June 6, 2005, through to March 31, 2010, under the initiative of principal investigator Tatsuya Fujii, MD in the Department of Internal Medicine, Self-Defense Forces Central Hospital.

2.1. Vaccine and vaccination

LC16-KAKETSUKEN vaccine, a freeze-dried live attenuated smallpox vaccine prepared in cell culture, containing a suspension of greater than 1×10^8 pock-forming units (pfu)/mL of the LC16m8 strain as the only virus component, was used. The process of establishing LC16-KAKETSUKEN vaccine by modifying the method of CSI is described in Section 3. This vaccine was reconstituted with 0.5 mL of the packaged vaccine diluent (water for injection, containing 20, volume per volume % of glycerin) and 0.01 mL was inoculated into the skin. Inoculation was done by physicians who received the instructions and training of the vaccination method by using designated bifurcated needles. Pressure pricking (puncture) was done 5 times for primary vaccinees (without previous smallpox vaccination) and 10 times for re-vaccinees (with previous smallpox vaccination).

2.2. Participants

The Self-Defense Forces Central Hospital planned and organized the smallpox vaccination program for selected personnel in the JGSDF. Participants were JGSDF personnel who were scheduled for deployment in International Peacekeeping Operation activities of the United Nations Disengagement Observer Force [15], except for personnel who did not comply with the implementation guidance of smallpox vaccine inoculation of JGSDF and personnel who were described as ineligible for vaccination or as being contraindicated according to the Package Insert of the LC16-KAKETSUKEN. The following items were confirmed before vaccine inoculation: no abnormality in the blood, biochemical and urea tests, negativity of hepatitis B virus antigens and hepatitis C virus antibodies, and no infection with human immunodeficiency virus. Vaccine inoculation was contraindicated for participants who were pregnant, under immunosuppression or with eczema, and participants who receive live vaccine within 30 days. The participants were 268 volunteers who were vaccinated with LC16-KAKETSUKEN in this PMS study. All the participants were given instructions to avoid contact with pregnant women and newborn babies within one month after

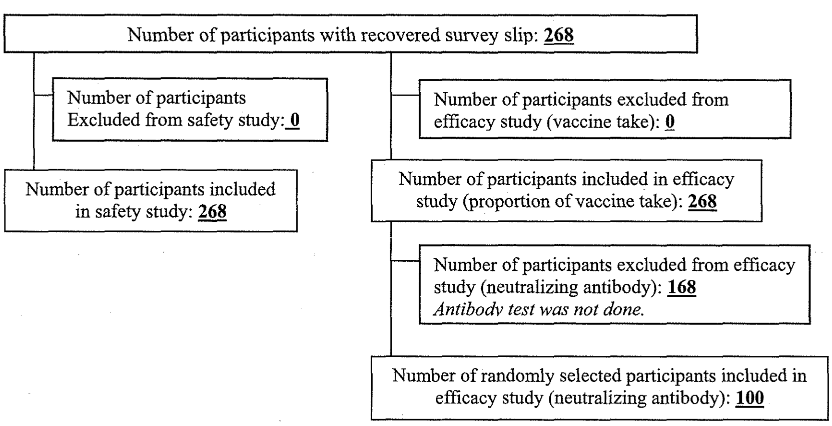

Fig. 1. Flow of participants through the study. This figure shows the flow of participants in safety and efficacy studies. Efficacy was evaluated by the proportion of vaccine take of LC16-KAKETSUKEN and by neutralizing antibody titers in the serum after vaccination with the vaccine. The 100 participants included in efficacy study were selected randomly from 268 participants.

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inoculation. All the participants were distributed individually with adhesive tape for the inoculation site.

Fig. 1 shows the flow of participants through the study. The total number of participants was 268: 261 males and 7 females. The average age was 29.2 (19–52): 9 were born before 1961, 43 were born from 1962 to 1968, 57 were born from 1969 to1975 and 159 were born after 1976 (see also Table 3). Because smallpox vaccination continued until 1976 in Japan, participants born before 1976 had a high possibility to have had previous vaccination (re-vaccinees). The discrimination between primary vaccinees and re-vaccinees was done by taking into account the age and the presence or absence of a vaccination scar. Among 268 vaccinee participants, 71 (26.5%) were judged to be re-vaccinees, 196 (73.1%) to be primary vaccinees and 1 (0.4%) was unknown. An examination for allergy showed that 10 (3.7%) experienced some sort of allergic history, such as pollen allergy. No participants with immune deficiency were found and most of them had a history of receiving vaccination for hepatitis A, B and other-non-hepatitis vaccines.

2.3. Blood pressure and clinical laboratory test results

Immediately before and one month after inoculation, the following tests were done and the obtained data were investigated and analyzed: blood pressure, blood tests (numbers of leukocytes and red cells, hemoglobin value, hematocrit value, number of blood platelets), biochemical tests (total cholesterol, whole protein, albumin, urea nitrogen, uric acid, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, lactase dehydrogenase, alkaline phosphatase, C-reactive protein, blood glucose and urinalysis (protein, glucose)).

2.4. Vaccine take, examination of adverse events and local findings

At 10–14 days after inoculation, physicians who had received the required training engaged judged the vaccine take and examined participants for the presence of adverse events and local findings.

2.5. Neutralizing antibody assay

Neutralizing antibody assay was done for blood samples collected 1, 4 and 7 months after inoculation from 100 participants selected randomly. Neutralizing antibody titers against Lister vaccinia virus strain were assessed by the plaque reduction neutralization test assay [14,16]. The neutralizing antibody titer was defined as the reciprocal of the dilution level resulting in a 50% reduction of total plaques formed by Lister vaccinia virus strain with no treatment by vaccinated sera. Vaccinees showing 4 or more in the ratio of maximal post-inoculation titers to pre-inoculation titers were judged as a positive conversion.

2.6. Data analysis

Significant differences in the frequency of adverse events and vaccine take between primary vaccinees and re-vaccinees were determined using the Steel test and the Wilcoxon rank sum test. The Fisher exact probability test and the Steel test were used to compare geometric mean titers of neutralizing antibodies. Statistical analyses were done using SAS/Base/Stat, version 8.0.2. or the updated version.

2.7. Ethical notice

This study was done according to the guidelines of clinical studies and was approved by the institutional review board of the JGSDF Central Hospital (No. 16-004; August 30, 2004: No. 18-022; December 21, 2006).

3. Results

3.1. Improvement of vaccine production process

The amount of MVS of the LC16m8 strain transferred from CSI was limited and therefore we started to produce LC16-KAKETSUKEN by a procedure modified as follows. The number of passages from MVS to vaccine product was extended from 2 to 3 to make production more efficient, but this modification resulted in generating some revertant mutants that form slightly larger plaques than plaques formed by the original MVS. Because the contents of these revertant mutants in the primary passage preparation affected their contents in the final products, the method was modified to produce a primary passage preparation that contains a smaller amount of revertant mutants to a level similar to that of the original MVS.

Table 1
Summary of adverse events.

	Previous smallpox vaccination history			Total
	No	Yes	Unknown	
Number of participants	196	71	1	268
Number of participants with adverse events	53	4	1	58
Number of adverse events	71	5	1	77
Incidence of adverse events[a]	27.0%	5.6%	100%	21.6%
Type of adverse event	Incidence of each adverse event (%)			
Autoinoculation following vaccination[b]	0	1 (1.4)	0	1 (0.4)
Swelling of axillary lymph node	50 (25.5)	2 (2.8)	0	52 (19.4)
Rash	0	0	1 (100.0)	1 (0.4)
Systemic disorders				
Fatigue[b]	2 (1.0)	0	0	2 (0.7)
Fever	2 (1.0)	2 (2.8)	0	4 (1.5)
Local disorders at inoculation site				
Erythema at inoculation site[b]	14 (7.1)	0	0	14 (5.2)
Swelling at inoculation site[b]	1 (0.5)	0	0	1 (0.4)
Complications associated with vaccination[b]	2 (1.0)	0	0	2 (0.8)

Coding by MedDRA Ver.12.1.
[a] Number of participants with adverse events/number of participants × 100.
[b] Adverse events unpredictable from PRECAUTIONS.

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3.2. Incidence of adverse events

Table 1 summarizes the adverse events, and Table 2 shows adverse events for each background of vaccinees. Among 268 vaccinee participants, the following vaccinees showed adverse events: 53 of 196 primary vaccinees, 4 of 71 re-vaccinee and 1 vaccinee participant with unknown previous vaccination history. Swelling of the axillary lymph node was observed for 50 of 196 primary vaccinees and 2 of 71 re-vaccinees. Thus, primary vaccinees showed a higher incidence of adverse events, including lymphoadenopathy, than re-vaccinees. The breakdown of adverse events of a total 58 of 268 vaccinees was: 52 vaccinees with swelling of the axillary lymph node, 4 vaccinees with fever, 2 vaccinees with fatigue, 1 vaccinee with a rash, 14 vaccinees with erythema at the inoculation site, 1 vaccinee with swelling at the inoculation site and 1 vaccinee with autoinoculation. Complications with vaccination were observed for 2 primary vaccinees. One complication was allergic dermatitis that was not eczema vaccinatum and the vaccinee recovered without supportive care. The other case was a rash unrelated to the vaccination.

All the adverse events were mild and no serious events (related to cardiovascular disorder, encephalitis, accessory vaccinia or satellite lesions, or progressive vaccinia) were observed. Electrocardiography examination was done for all participants, who also received both chest X-ray and electrocardiogram examinations. Adverse events related to cardiovascular disorder were not observed, except for 2 vaccinees with electrocardiography examination (1st degree atrioventricular block). Both of these vaccinees were re-vaccinees; one of them had no special past and present disorder history, while the other had a history of allergy history. However, their disorder was mild and therefore a causality between this disorder and the vaccination was denied. Adverse events related to encephalitis or those related to accessory vaccinia and progressive vaccinia was not observed. No subject died during this research.

3.3. Blood pressure and clinical tests (data not shown)

Some vaccinees showed changes in total cholesterol, glutamic-pyruvic transaminase and number of leukocytes before and after vaccination. However, all changes were considered to be due to changes in daily habit or transiently catching a cold, and were judged to be not due to vaccination. Abnormality of blood pressure accompanied by vaccination was also not observed.

3.4. Vaccine take

Table 3 shows the examination of vaccine take for each background of vaccinees. All 268 vaccinees were analyzed. Vaccine take was positive for 244 (91.0%) vaccinees and was negative for 24 (9.0%) vaccinees. Primary vaccinees showed a statistically higher proportion of vaccine take (94.4%; 185/196) than that shown by re-vaccinees (81.7%; 58/71) (Odds ratio at 95% confidential interval = 0.265; 0.113–0.624). The diameter of erythema was significantly larger ($P < 0.001$, Wilcoxon rank sum test) for primary vaccinees than for re-vaccinees, and the diameter of a blister was larger for primary vaccinees than for re-vaccinees without statistical significance ($P = 0.277$, Wilcoxon rank sum test) (data not shown).

Notably, vaccinees with erythema less than 10 mm in diameter had a lower proportion of vaccine take (87.5%) compared with other vaccinees with erythema 10 mm or more, all showing 100% proportion of vaccine take (Table 3). Younger vaccinees tended to show a relatively higher proportion of vaccine take than older vaccinees, probably because most younger vaccinees were primary vaccinees (Table 3). We observed no clear difference in proportion of vaccine take dependent on the history of previous disorders, current medical history, history of allergy and side effects, history of other vaccinations and usage of drugs in combination.

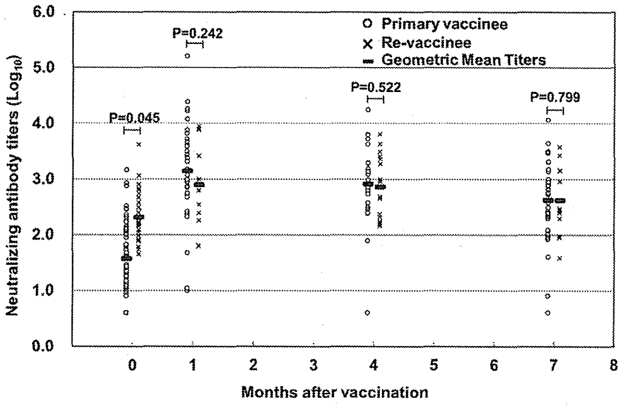

Fig. 2. Time course of neutralizing antibody titers in the serum after vaccination. The time course of neutralizing antibody titers was examined for both primary vaccinees and re-vaccinees. Of note, the titer of one primary vaccinee changed from seropositive to seronegative (<8) at 7 months after inoculation. See the method for titration in Section 2.

3.5. Level of neutralizing antibody responses

Fig. 2 and Table 4 show the results of neutralizing antibody titers and seroconversion rate, respectively. Among 268 vaccinees, the neutralization antibody titer in the serum was determined for 100 randomly selected vaccinees (Fig. 2) done as follows: 99 vaccinees before vaccination (0 month), 51 vaccinees at 1 month, 46 vaccinees at 4 months and 53 vaccinees at 7 months after vaccination. While primary vaccinees showed a statistically higher seroconversion rate at 1, 4 and 7 months after vaccination than that shown by re-vaccinees, primary vaccinees tended to show equivalent or lower antibody titers without exception at 0, 1, 4 and 7 months after vaccination compared with re-vaccinees (Fig. 2 and Table 4). However, both vaccinees kept higher antibody titers even at 7 months after vaccination than those vaccinees at 0 month (Fig. 2). Younger vaccinees tended to show a higher seroconversion rate than older vaccinees, probably because most older vaccinees still had a high neutralizing antibody titer elicited by previous vaccinations (Table 4 and Fig. 2). A statistically significant positive correlation ($P < 0.001$, Fisher exact probability test) was observed between vaccine take and seroconversion (data not shown).

4. Discussion

We showed that LC16-KAKETSUKEN is safe and effective in an adult population at a level similar to that of the previous LC16m8 vaccine (Lot No. Chiba 02) manufactured by the CSI [14]. The following points are especially noted. (1) A clinical study on LC16-KAKETSUKEN, produced by using the improved manufacturing process, was done for the first time strictly compliant with GCP under the approval of the Ministry of Health, Labor and Welfare (MHLW). (2) To evaluate the safety of LC16-KAKETSUKEN, the background of the participants was examined and was described in detail for more than 15 items, such as history of allergy and vaccination with various vaccines, including smallpox vaccine, previous diseases and existence or non-existence of immune deficiency. Such a detailed background of patients was not described in our previous clinical study [14]. (3) Neutralizing antibody titers were monitored until 7 months after LC16m8 vaccination, much longer than in the previous study [14]. A detailed comparison between

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Table 2
Adverse events and vaccine take for each background of vaccinees.

Background of vaccinees	Primary vaccinees			Re-vaccinees		
	No. of vaccines	No. of adverse events	Rate of adverse events (%)	No. of vaccines	No. of adverse events	Rate of adverse events (%)
Total	196	53	27.0	71	4	5.6
Gender						
Male	190	53	27.9	70	4	5.7
Female	6	0	0	1	0	0
Age at vaccination with LC16-K[a] vaccine (year)						
≥50	0	0	0	1	0	0
40–49	6	1	16.7	20	0	0
30–39	31	5	16.1	50	4	8.0
20–29	158	46	29.1	0	0	0
<20	1	1	100	0	0	0
Body weight						
<65 kg	51	19	37.3	13	1	7.7
65–70 kg	47	16	34.0	7	1	14.3
70–75 kg	42	8	19.0	17	2	11.8
≥75 kg	40	10	25.0	17	0	0
Unknown	16	0	0	17	0	0
History of previous disorder						
No	195	53	27.2	71	4	5.6
Yes	1	0	0	0	0	0
Current medical history						
No	195	53	27.2	68	3	4.4
Yes	1	0	0	3	1	33.3
History of allergy and side effects						
No	188	52	27.7	69	4	5.8
Yes	8	1	12.5	2	0	0
Breakdown of allergy and side effects						
Drugs	1	1	100	1	0	0
Others	7	0	0	1	0	0
Breakdown of vaccination history						
Hepatitis A	195	52	26.7	71	4	5.6
Hepatitis B	195	52	26.7	71	4	5.6
Rabies	195	52	26.7	71	4	5.6
Tetanus toxoid	178	52	29.2	54	4	7.4
Polio	193	53	27.5	68	4	5.9
Abdominal typhus	192	51	26.6	69	4	5.8
Cerebral meningitis	190	51	26.8	69	4	5.8
Yellow fever	53	21	39.6	26	2	7.7
Japanese encephalitis	39	14	35.9	13	2	15.4
Drugs used in combination						
Allopurinol	0	0	0	1	0	0
Vaccine take of LC16-K[a].						
No	11	1	9.1	13	1	7.7
Yes	185	52	28.1	58	3	5.2
Local findings of LC16-K[a] vaccine take						
Blister	88	48	54.5	12	1	8.3
Ulcer	12	6	50.0	0	0	0
Crust	29	14	48.3	10	1	10.0
Swelling	15	9	60.0	3	0	0
Others	14	4	28.6	3	0	0
Unknown	79	0	0	36	1	2.8
Breakdown of LC16-K[a] vaccine take – erythema						
<10 mm	6	1	16.7	2	0	0
10–30 mm	54	27	50.0	10	2	20.0
≥30 mm	25	14	56.0	1	0	0
Unknown	3	0	0	1	0	0
Breakdown of LC16-K[a] vaccine take – blister						
≤5 mm	34	13	38.2	6	0	0
6 mm	4	3	75.0	0	0	0
7 mm	4	3	75.0	3	1	33.3
8 mm	14	9	64.3	1	0	0
≥9 mm	20	15	75.0	1	0	0
Unknown	12	5	41.7	1	0	0

[a] LC16-KAKETSUKEN.

participants with and without previous vaccination of smallpox vaccine confirmed the previous results [14]. Based on these results, Kaketsuken could describe the present results of adults, along with existing clinical data collected from a pediatric population, in the Package Insert of LC16-KAKETSUKEN under the approval of MHLW. In the package insert, the summary of this PMS study (162 words) was newly added, including explanation about participants, and the results of vaccine take and antibody titers, as well as adverse events.

All studies on humans done hitherto, including this study, support the safety of LC16m8 vaccine, both in children [17] and adults [14]. Also, animal studies on monkeys, rabbits and mice [2,8,10,12,13] support the safety and protective efficacy of LC16m8 vaccine. Notably, LC16m8 vaccine and its genetically modified

Table 3
Proportion of vaccine take for each background of vaccinees.

Background of vaccinees	Vaccine take (number) Yes	No	Proportion of vaccine take (%)	Odds ratio (95% confidential interval) or comparative test[a,b,c]
Total	244	24	91.0	(0.876–0.945)
Gender				
Male	238	23	91.2	0.580 (0.067–5.027)
Female	6	1	85.7	
Age at vaccination with LC16-K[d] vaccine (year)				
≥50	1	0	100.0	Steel test
40–49	20	6	76.9	≤29 vs ≥50 <0.001 (<0.001–>999.999) $p = 0.994$
30–39	72	10	87.8	≤29 vs 40–49 5.663 (1.781–18.003) p = **0.004**
20–29	150	8	94.9	≤29 vs 30–39 2.622 (0.993–6.924) $p = 0.129$
<20	1	0	100.0	Wilcoxon rank sum test $Z = -2.989$, p = **0.003**
Body weight				
<65 kg	63	1	98.4	
65–70 kg	49	5	90.7	Wilcoxon rank sum test $Z = 1.567$, $p = 0.117$
70–75 kg	54	6	90.0	
≥75 kg	52	5	91.2	
History of previous disorder				
No	243	24	91.0	>999.999 (<0.001–>999.999)
Yes	1	0	100.0	
Current medical history				
No	240	24	90.9	>999.999 (<0.001–>999.999)
Yes	4	0	100.0	
History of allergy/side effects				
No	234	24	90.1	>999.999 (<0.001–>999.999)
Yes	10	0	100.0	
Breakdown of allergy/side effects				
Drugs	2	0	100.0	–
Foods	0	0	–	
Breakdown of vaccination history				
Hepatitis A	243	24	91.0	
Hepatitis B	243	24	91.0	
Rabies	243	24	91.0	
Tetanus toxoid	216	17	92.7	
Polio	239	23	91.2	–
Abdominal typhus	239	23	91.2	
Cerebral meningitis	238	22	91.5	
Yellow fever	72	8	90.0	
Japanese encephalitis	48	5	90.6	
History of previous smallpox vaccination				
No	185	11	94.4	
Yes	58	13	81.7	**0.265 (0.113–0.624)**
Unknown	1	0	100.0	
Drugs used in combination				
No	243	24	91.0	>999.999 (<0.00–>999.999)
Yes (Allopurinol)	1	0	100.0	
Local findings of LC16-K[d] vaccine take				
Blister	100	0	100.0	
Ulcer	12	0	100.0	
Crust	40	0	100.0	–
Swelling	19	0	100.0	
Others	15	2	88.2	
Breakdown of LC16-K[d] vaccine take/erythema				
<10 mm	7	1	87.5	
10–30 mm	64	0	100.0	–
≥30 mm	26	0	100.0	
Breakdown of LC16-K[d] vaccine take/blister				
≤5 mm	40	0	100.0	
6 mm	4	0	100.0	
7 mm	7	0	100.0	–
8 mm	15	0	100.0	
≥9 mm	21	0	100.0	

The bold value signifies that the P value is under 0.05 or 0.01 and the 95% confidential interval of Odds ration is under 1.0.
[a] The calculation of odds ratio or comparative test was not done for unspecified items, such as "unknown" and "others".
[b] The Steel test was used as a multiple comparative technique.
[c] Odds ratio (95% lower limit of confidence interval–95% upper limit of confidence interval).
[d] LC16-KAKETSUKEN.

virus induced no serious adverse events in immunodeficient animals [2,13,18]. The protective ability of LC16m8 vaccine against lethal challenge with the highly pathogenic vaccinia WR virus accompanied by induction of neutralizing antibodies to WR was shown for mice [12,16]. LC16m8 also protected monkeys from monkeypox [8,13]. These animal studies provide supportive evidence for the safety and efficacy of LC16m8 vaccine, including LC16-KAKETSUKEN, in humans.

Table 4
Time course of seroconversion rate depending on smallpox vaccination history and age at vaccination.

Background of vaccinees	Seroconversion rate (%)[a]			Maximum seroconversion rate after vaccination[b]	Steel test[c]				
	After vaccination (1 month)	After vaccination (4 month)	After vaccination (7 month)		Compared with 0 month			Compared with 1 month	
					0 month vs 1 month	0 month vs 4 month	0 month vs 7 month	1 month vs 4 month	1 month vs 7 month
Total	72.5 (37/51)	71.7 (33/46)	62.3 (33/53)	72.7 (72/99)	***p* < 0.001**	***p* < 0.001**	***p* < 0.001**	*p* = 0.994	*p* = 0.432
History of previous smallpox vaccination									
No	84.2 (32/38)	89.3 (25/28)	75.0 (30/40)	86.8 (59/68)	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*p* = 0.783	*p* = 0.506
Yes	33.3 (4/12)	44.4 (8/18)	16.7 (2/12)	40.0 (12/30)	***P* = 0.003**	***P* < 0.001**	*P* = 0.066	*p* = 0.768	*p* = 0.546
Unknown	100.0 (1/1)	– (0/0)	100.0 (1/1)	100.0 (1/1)					
Fisher exact probability test (No vs Yes)	***P* = 0.002**	***P* = 0.002**	***P* = 0.001**	***P* < 0.001**					
Age at vaccination with LC16-KAKETSUKEN (year)									
≥50	– (0/0)	– (0/0)	– (0/0)	– (0/0)	Test im.[b]	Test im.[b]	Test im.[b]	*p* = 0.312	*p* = 0.432
40–49	66.7 (4/6)	28.6 (2/7)	33.3 (2/6)	46.2 (6/13)	***P* = 0.004**	*P* = 0.129	*P* = 0.089	*p* = 0.312	*p* = 0.432
30–39	40.0 (6/15)	60.0 (9/15)	35.3 (6/17)	50.0 (16/32)	***P* = 0.001**	***P* < 0.001**	***P* = 0.001**	*p* = 0.451	*p* = 0.948
20–29	89.7 (26/29)	91.7 (22/24)	82.8 (24/29)	92.5 (49/53)	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*p* = 0.957	*p* = 0.672
<20	100.0 (1/1)	– (0/0)	100.0 (1/1)	100.0 (1/1)	Test im.[b]	Test im.[b]	Test im.[b]	Test im.[b]	Test im.[b]
Steel test									
≤29 vs ≥50	Test im.[d]	Test im.[d]	Test im.[d]	Test im.[d]					
≤29 vs 40–49	*P* = 0.251	***P* = 0.001**	***P* = 0.022**	***P* < 0.001**					
≤29 vs 30–39	***P* = 0.001**	***P* = 0.036**	***P* = 0.002**	***P* < 0.001**					

The bold value signifies that the *P* value is under 0.05 or 0.01 and the 95% confidential interval of Odds ration is under 1.0.
[a] Antibody titer after vaccination/before vaccination (0 month) ≥4 was considered as positive seroconversion.
[b] Portion of the participants who seroconverted at one time point at least after vaccination.
[c] Steel test was conducted to compare the seroconvertion rate between the indicated time points.
[d] Test-im.: Test was impossible.

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In the past, two severe cases of adverse events possibly caused by vaccination with LC16m8 vaccine clinically were reported [14]. One was a 26-year-old male primary vaccinee who experienced rash onset on day 3 after vaccination. The patient was hospitalized for 20 days after vaccination. A skin biopsy from the rash was consistent with allergic dermatitis, which did not disprove a causal relationship with vaccination. The other case was 29-year-old male primary vaccinee who developed a rash on his trunk on day 10 after vaccination and was diagnosed with erythema multiform.

One of the major concerns with adult smallpox vaccination has been myopericarditis observed in the United States vaccination program [19]. Inflammatory cardiac disease was recognized in adult recipients of Dryvax and ACAM2000 vaccine in the United States, but a study since then [14] and this PMS study showed no serious abnormality by electrocardiography examination.

In January 2011, based on the research findings of this PMS study, the section of "Precautions for Use" in the Package Insert for LC16-KAKETSUKEN was modified under the approval of MHLW to ensure appropriate use for not only pediatric populations, but also for adult populations. Notably, according to the Package Insert and Product Information for ACAM2000 and IMVANEX (smallpox vaccine derived from MVA strain, Bavarian Nordic), vaccination of pediatric populations is not authorized by national regulatory authorities now. Therefore, LC16-KAKETSUKEN is the sole currently available smallpox vaccine for both adult and pediatric populations.

5. Conclusion

From the safety and efficacy results of this PMS study that complied with GCP, smallpox vaccine LC16-KAKETSUKEN was confirmed to be a highly useful and excellent vaccine for both primary vaccinees and re-vaccinees as judged from the absence of serious adverse events, proportion of vaccine take and increase in neutralizing antibody titers.

Conflicts of interest

TF received funds to their hospital from Kaketsuken to support their work in the PMS study and he did not receive any direct funds. YS and HY are employees of Kaketsuken. YN, YK and SH declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.09.067.

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PERSPECTIVE

Preparedness for a Smallpox Pandemic in Japan: Public Health Perspectives

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ABSTRACT

Smallpox is an acute, febrile, contagious disease caused by the Variola virus, which is a member of the Poxviridae family. Until the 1970s, smallpox had been a pandemic disease for more than 3000 years, endemic in tropical and developing areas and periodically epidemic worldwide. The World Health Organization declared smallpox to be completely eradicated in 1980 as the result of global vaccination efforts. At that time, all routine vaccination programs were terminated, given the success of this monumental eradication. Although smallpox remains fully eradicated, uncertainty exists regarding the possibility of recurrent smallpox outbreaks. At the end of the Cold War, concerns regarding unstable international security and the feasibility of terrorism with weapons of mass destruction have been highlighted. The potential threat of intentional release of smallpox has forced regional health authorities to reconsider their political landscape and create preparedness plans to protect the community in the event of biological attacks. Here we present current countermeasures to this biological threat in Japan and discuss methods for strengthening public health preparedness both domestically and internationally. These methods include infection control, vaccination policy, and international partnerships to help deter or contain a contagious smallpox pandemic. (*Disaster Med Public Health Preparedness*. 2015;9:220-223)
Key Words: smallpox, bioterrorism, public health, pandemics, biological warfare agents

Bioterrorism is a public health threat that is designed to cause panic in the community through the intentional dispersal of a pathogenic virus, microbe, or toxin. Biological attacks, both worldwide and in Japan, have been attempted multiple times over the past century.[1] In Japan, counterterrorism measures have been developed, largely as the result of a series of national and international events.[2] International security concerns, including the experimental launching of ballistic missiles by North Korea in 1998, the tragic September 11 terrorist attacks, and subsequent anthrax exposures in 2001 in the United States have all impacted Japanese policy. In Japan, the chemical and biological attacks conducted by the Aum Shinrikyo religious cult group in 1992 further highlighted the need for preparedness measures. Shoko Asahara (head of Aum Shinrikyo during the Tokyo subway gas attack) and his followers have also visited Zaire to obtain samples of the Ebola virus for the development of biological agents.[3]

Unfortunately, bioterrorism remains a concern, given that the development of biological agents was sponsored at a national level (eg, the former Soviet Union) and this technology is readily obtainable. Current biological weapons possess pandemic potential given the population density of urban areas, rapid inter-region transportation, and the enclosed environments of modern buildings. Smallpox is well known as an acute and highly contagious disease that is typically spread by direct and face-to-face contact and that has devastated populations throughout history. The fatality rate is estimated at up to 30%, and a rapid airborne outbreak facilitated by air circulation systems has been observed in the past.[4] In 1980, the World Health Organization (WHO) declared that "there is no evidence that smallpox will return as an endemic disease."[5] As routine vaccinations have been terminated, immunity has decreased over time in the older vaccinated populations, and the younger unvaccinated population is left completely unprotected.

In response to these vulnerabilities, smallpox is now considered a significant bioterrorism threat, and health authorities are forced to strengthen their ability to sustainably manage the potential public health emergency.[6] The purpose of this article was to introduce current activities that have been developed to identify and suppress potential contagious bioterrorism threats in Japan. As well, the contribution of

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public health preparedness to domestic and international health security is discussed.

NARRATIVES

The Impact of "Dark Winter" and the Establishment of a Domestic Framework for Managing Bioterrorism

In June 2001, a simulation exercise entitled "Dark Winter" was carried out in the United States to allow senior policy makers to develop a response to a hypothetical biological attack. Dark Winter simulated the deliberate introduction of smallpox in several states during the winter of 2002, with 300,000 individuals becoming infected within 3 weeks of initial exposure. Owing to low supplies of the smallpox vaccine, Dark Winter estimated that over 3,000,000 individuals would eventually become infected. These results revealed that the general population's lack of immunity would render it highly susceptible to smallpox infection, as more than 30 years had passed since vaccination was discontinued. Therefore, a local biological attack with a contagious pathogen could rapidly become a national or international crisis and could cripple the target country.[7] After Dark Winter, researchers analyzed outbreak patterns for populations with or without immunity.[8] These estimations form the basic reference for a possible smallpox pandemic and are useful for calculating the number of vaccinations needed. The results of Dark Winter have had important policy implications, with additional studies regarding pandemic simulation using the basic model described in this exercise.

After the September 11 attacks, the Chemical, Biological, Radiological and Nuclear (CBRN) countermeasure model was established to create a multidisciplinary and cross-sectional platform for collaboration between various authorities to ensure a standardized national response system for all threats. In this system, local government is largely responsible for management at the site of the incident in collaboration with police, fire, and other emergency services. Various tabletop exercises have been performed regarding pandemic simulations, and health care workers of the local health office, quarantine station, and community hospital are regarded as first responders. These individuals are the first to contact infected patients, and their protection (including vaccination) must be considered a first priority.

Japanese national research has also supported the development of a pandemic simulation model to estimate the impact of biological attacks. The Japanese government, in particular the Ministry of Health, Labour, and Welfare, is largely responsible for medical activities in cases of public health emergency and coordinates with other security authorities to manage outbreaks. If a major national incident were to occur, accurate intelligence might not be readily available, highlighting the importance of health authorities, law enforcement, and other agencies sharing the information. Planning at the individual, family, community, and national levels is critical to creating the relationships necessary for an effective response.

Regulations for Control of Infectious Diseases and Biological Incidents

The frequent occurrence of emerging avian influenza in Southeast Asia has been critical in drawing attention to the need for countermeasures for infectious disease in Japan. In response, the Japanese government promulgated the Infectious Diseases Control Law to create a comprehensive management system for such highly contagious pathogens. The objective of this law is to reduce the possibility of a pathogen's accidental or intentional release by improving surveillance, laboratory safety, pathogen collection, and sample transportation. Government officials are legally entitled to enter all relevant facilities to investigate the proper storage of pathogens. In addition, this law requires local governments to implement 4 unique measures: enforcement of an active epidemiological survey, preparation of an action plan in case of a serious infectious disease outbreak, education of government officials and health care workers regarding biological weapons, and designation of a hospital as a fixed observation point during outbreaks.[9] The spread of emerging infection, transmitted by multiple travelers, resulted in the introduction of a rigorous quarantine and isolation system.

National Institutes Play a Central Role in Infectious Disease Control

The Japanese biodefense program has largely been driven by contributions from several national institutes. The National Institute of Infectious Diseases (NIID) has played an important role as a central institute in Japan. Major functions of the NIID are basic research, reference services, surveillance programs, sample collection, data analysis, distribution of information, quality control for vaccines, and international collaboration. The NIID has also organized the Infectious Diseases Surveillance Center (IDSC), which plays a central role in Japan's surveillance system. Serving as a member of the WHO, the NIID has made efforts to enhance international partnerships, agreeing to a global memorandum to promote research collaboration, human resource development, and sharing of information.

Under the Infectious Diseases Control Law, the NIID conducts routine surveillance of infectious diseases. The 2008 Japanese G8 summit provided an opportunity to upgrade Japan's biodefense surveillance system. During this event, the IDSC conducted syndrome surveillance monitoring by using drug marketing data. Although there was no notable outbreak during the event, the importance of an automated surveillance system was realized as a result of this event.[10]

On the other hand, the National Institute of Public Health (NIPH) conducts research, human resource development, and public health initiatives in Japan. The NIPH has made important contributions to the prevention of incidents affecting public health, including pandemic disease. The NIPH holds a training program called the "Health Crisis