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Vaccinia virus strain LC16m8 defective in the *B5R* gene keeps strong protection comparable to its parental strain Lister in immunodeficient mice

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ABSTRACT

Background: Attenuated vaccinia virus strain, LC16m8, defective in the *B5R* envelope protein gene, is used as a stockpile smallpox vaccine strain in Japan against bioterrorism: the defect in the *B5R* gene mainly contributes to its highly attenuated properties.
Methods: The protective activity of LC16m8 vaccine against challenge with a lethal dose of vaccinia Western Reserve strain was assessed in wild-type and immunodeficient mice lacking CD4, MHC class I, MHC class II or MHC class I and II antigens.
Results: The immunization with LC16m8 induced strong protective activity comparable to that of its parent strain, Lister (Elstree) strain, in wild-type mice from 2 days to 1 year after vaccination, as well as in immunodeficient mice at 2 or 3 weeks after vaccination. These results implicated that the defect in the *B5R* gene hardly affected the potential activity of LC16m8 to induce innate, cell-mediated and humoral immunity, and that LC16m8 could be effective in immunodeficient patients.
Conclusion: LC16m8 with truncated B5 protein has an activity to induce immunity, such as innate immunity and subsequent cell-mediated and humoral immunity almost completely comparable to the activity of its parental strain Lister.
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1. Introduction

The success of smallpox eradication by the World Health Organization (WHO) program in the 1970s is a landmark in the history of biomedical science. Since the terrorist attack on the World Trade Center in New York in September 2001, however, concern about bioterrorism has increased. Among many pathogens that could be used potentially in bioterrorism, variola virus is one of the most recognized and the most feared. Under such circumstances, serious attempts have been made to restart the development of vaccine strains, including a vaccinia ACAM2000 clone established from Dryvax derived from the New York City Board of Health (NYCBH) strain [1,2], modified vaccinia Ankara (MVA) and replication-incompetent viruses derived from the NYCBH strain [3,4]. However, replication-competent vaccine strains derived from the NYCBH strain may have a side effect of inducing myopericarditis [1,2], and replication-incompetent viruses may have the potential problem of relatively poor immunogenicity [3,4].

In the early 1970s, Hashizume et al. [5,6], developed one of the safest replication-competent vaccines, LC16m8 strain, from the Lister (Elstree) strain that was used worldwide in the WHO smallpox eradication program. Freeze-dried live attenuated smallpox vaccine of LC16m8 prepared in cell culture has been the sole smallpox vaccine licensed in Japan since 1975. LC16m8 was selected as a temperature- sensitive small-plaque- and small-pock-forming clone [6]. A rabbit skin proliferation study and a neurovirulence study in which LC16m8 and Lister viruses were inoculated into the thalamus of cynomolgus monkeys showed very low pathogenicity of LC16m8 compared with Lister. A clinical evaluation of 90,000 infants immunized during the initial development of LC16m8 from 1973 to early 1976 showed no encephalitis or other serious adverse events after vaccination. No major differences exist in the immunogenicity of LC16m8 when compared with conventional first generation smallpox vaccines such as its parental vaccine

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strain Lister. The LC16m8 vaccine is now produced by the Chemo-Sero-Therapeutic Research Institute (Kaketsuken), Japan, and is stockpiled as a smallpox vaccine against bioterrorism in Japan. According to the November 2013 conclusions and recommendations of the Meeting of the Strategic Advisory Group of Experts on immunization [7], both licensed ACAM2000 (2nd generation vaccine) and LC16m8 (3rd generation vaccine) are preferred for the WHO stockpile.

A molecular biological study showed that one gene of envelope proteins is defective, contributing to smaller plaque size and host range and was accordingly called as *ps/hr*) gene [8]: the *ps/hr* gene is a homologue of *B5R* gene of Copenhagen (CPN) strain, and now this gene is usually called as *B5R* gene. The *B5R* gene product, B5 protein, is a 42-KDa envelope glycoprotein composed of 317 amino acids. Vaccinia virus produces two typical types of virion from each infected cell called intracellular mature virus and extracellular enveloped virus (EEV) [9]. B5 protein is necessary for the formation of EEV, and therefore LC16m8 does not form EEV resulting in small-plaque size and less spreading ability, which is considered to mainly attribute to its attenuated properties. The analysis of the full-genome sequence of LC16m8 and its parental strain Lister showed that LC16m8 preserved almost of all the open reading frames of vaccinia virus except for the disrupted EEV protein B5 [10].

A truncated B5 protein (approximately 8 kDa) was expressed abundantly in LC16m8-infected cells, and both murine immune sera and human vaccinia virus immunoglobulin recognized the truncated recombinant B5 protein in antigen-specific enzyme-linked immunosorbent assays [11].

Of major concern, however, is whether LC16m8 vaccine has enough immunogenicity to confer strong protection, because LC16m8 lacks normal type of the *B5R* gene product, B5 protein, which is a target epitope of neutralizing antibodies contributing to protection in mice [12]. Furthermore, LC16m8 fails to induce enough anti-B5 antibody responses in the human [13]. Although previous studies showed that in normal animals and humans LC16m8 keeps strong immunological and protective activity comparable to that of its parent strain Lister [6,10,14], whether it keeps such activity also in immunodeficient animals remains to be clarified. This point is important in relation to the problem of whether LC16m8 is usable in areas of Africa where many immunodeficient patients, such as human immunodeficiency virus-infected patients, live.

To address this problem, this study investigated if LC16m8 with truncated B5 protein has immunological and protective activity comparable to that of its parental strain Lister by using wild-type and immunodeficient mice lacking CD4, MHC class I, MHC class II antigen or MHC class I and II antigens. Immunological and protective activity was assessed in intranasally challenged mice with a lethal dose of a highly pathogenic vaccinia virus (VACV) strain, Western Reserve (WR) strain.

2. Material and methods

2.1. Vaccine and virus strains

Freeze-dried smallpox vaccine (LC16-KAKETSUKEN; LC16m8) manufactured under good manufacturing practice (GMP)-compliant condition by Kaketsuken in cell culture using primary rabbit kidney cells was used as the source of LC16m8 VACV strain. The vaccine lot used in this study is one (Lot V06) of the lots of smallpox vaccine stockpiled now in Japan against bioterrorism using smallpox. In the current manufacturing process, the occurrence of *B5R* revertant virus which was reported by Kidokoro et al. [15] was well controlled and the content rate in the Lot V06 is under 1%. The Lister (Elstree) VACV strain, which was used for the WHO smallpox eradication program, and the parental strain of the LC16m8 strain were transferred to Kaketsuken from the Chiba Serum Institute (Chiba) when this institute closed. UV-inactivated Lister virus was prepared by placing 1×10^7 PFU/mL of Lister virus under UV lumps for approximately 18 h and the inactivation was confirmed by standard plaque assay. The WR VACV strain (ATCC VR-1354) used for the challenge was purchased from the American Type Culture Collection (ATCC).

2.2. Mice

Wild-type BALB/c mice (female, 4 or 8-week old) were purchased from Charles River Laboratories Japan (Kanagawa). The following immunodeficient mice (female, 4- to 7-week old) were purchased from the Taconic Farms (New York): Cd4 (001055-MF) CD4-deficient, B2 m (B2MN12-MF) MHC class I-deficient, Abb (ABBN12-MF) MHC class II-deficient, and Abb/β_2 m (004080-MMF) MHC class I and II-double deficient mice.

2.3. Vaccination and WR challenge

Groups of wild-type or immunodeficient mice received a single vaccination at 2.5×10^5 PFU/mouse of LC16m8 or Lister virus by a 15 times-puncture by using standard bifurcated needles at the base of the tail or a single intraperitoneal vaccination with UV-inactivated Lister virus (the virus titer before inactivation was 1×10^7 PFU/mouse). See Table 1 and Table 2 for the number of mice in each experimental group. After vaccination, the mice were challenged intranasally with a dose of 10^5 PFU/mouse or 10^6 PFU/mouse WR viruses, either of which was amply lethal, at appropriate time point after vaccination in order to achieve a highly-lethal condition in the non-immunized animals per each model. The mice were observed and weighed each day for 14 days after the challenge. Mice that had showed severe weight loss and survived to the end of this study, that is, on day 14 after the challenge were euthanized by heart exsanguination under anesthesia. Statistical analysis on mean survival time was done using the log rank method. All mice studies were approved by the Kaketsuken Institutional Animal Care and Use Committee.

2.4. Plaque reduction neutralizing (PRN) assay

The level of neutralizing antibody against vaccinia WR virus in the serum samples was assessed by PRN assay as follows. WR-virus infected Vero E6 cells (ATCC CRL-1586, purchased from ATCC) received three times repeated freeze-thaw and the resultant crude virus fluid was used as a source of WR virus. This virus fluid may contain mainly intracellular mature viruses, with some other virus forms such as intracellular enveloped viruses, cell-associated enveloped viruses and extracellular enveloped viruses [9]. WR virus at 240 PFU in 0.12 mL of Eagle's minimal essential medium (EMEM) (Nissui, Tokyo) containing 1% fetal bovine serum (FBS) (Life Technologies, Maryland) was mixed with 0.12 mL of serially diluted heat-inactivated serum samples collected from test animals and was incubated at 37 °C for 15–18 h. The mixtures were inoculated in VeroE6 cell (ATCC CRL-1586, purchased from ATCC) monolayers in 12-well culture plates and were incubated in EMEM containing 3% FBS and 0.8% agarose ME (Iwai Chemicals Company, Tokyo) after 2 h of adsorption. After 4 days of incubation, the number of observed plaques was counted. The neutralizing antibody titer was calculated based on the plaque number by using the Probit analysis method with Minitab software (Kozo Keikaku Engineering Inc., Tokyo) and was defined as the reciprocal of the dilution level

Table 1
Protection against lethal WR challenge at various points after vaccination in wild-type mice.

Time (after vaccination)	Vaccination	Survival ratio	Mean survival time (days)	Log-rank test (*P*)
2 days	Non-immunized*	0/6	8.5	–
	LC16m8	6/6	>14.0	<0.01
	Lister	6/6	>14.0	<0.01
4 days	Non-immunized*	0/10	3.9	– <0.01
	LC16m8	10/10	>14.0	<0.01 1.00
	Lister	10/10	>14.0	<0.01 –
	Inactivated Lister	5/10	9.5	<0.01 <0.01
3 weeks	Non-immunized*	0/10	4.2	–
	LC16m8	10/10	>14.0	<0.01
	Lister	10/10	>14.0	<0.01
24 weeks	Non-immunized*	0/10	4.9	–
	LC16m8	10/10	>14.0	<0.01
	Lister	10/10	>14.0	<0.01
1 year	Non-immunized*	0/5	4.7	–
	LC16m8	5/5	>14.0	<0.01
	Lister	5/5	>14.0	<0.01

* The mice were inoculated with LC16m8 diluent.

Table 2
Protection against lethal WR challenge at various points after vaccination in immunodeficient mice.

Mouse model	Vaccination	Survival ratio	Mean survival time (days)	Log-rank test (*P*)
CD4-deficient	Non-immunized*	0/4	6.4	–
	LC16m8	4/4	>14.0	<0.01
MHC class I-deficient	Non-immunized*	0/5	8.0	–
	LC16m8	5/5	>14.0	<0.01
	Lister	5/5	>14.0	<0.01
	Inactivated Lister	5/5	>14.0	<0.01
MHC class II-deficient	Non-immunized*	1/5	7.2	–
	LC16m8	5/5	>14.0	0.014
	Lister	5/5	>14.0	0.014
	Inactivated Lister	3/5	11.2	0.064
MHC class I- & II-double deficient	Non-immunized*	0/10	8.8	–
	LC16m8	7/10	13.1	<0.01
	Lister	6/10	13.2	<0.01

* The mice were inoculated with LC16m8 diluent.

resulting in a 50% reduction of total plaques formed by WR virus with no treatment by vaccinated sera.

3. Results

3.1. Protection in wild-type mice by vaccination with LC16m8

Fig. 1 shows the survival and body weight of wild-type mice challenged intranasally with 10^5 PFU of WR virus on day 2 (A) or on day 4 (B), with 10^6 PFU of WR at 3 weeks (C), 24 weeks (D) and 1 year (E) after vaccination. Table 1 shows the survival ratio of each group, in which the number of mice (5, 6 or 10) in each group is indicated.

Both vaccinated and non-vaccinated mice challenged with WR on day 2 after vaccination showed weight loss. Mice vaccinated with either LC16m8 or Lister lost weight from 2 to 6 days after the challenge, but they began to recover weight around days 7 and 8 after the challenge and they all survived. Non-vaccinated mice lost weight continuously, culminating in death by day 11 after the challenge (Fig. 1A). When mice were challenged with WR on day 4 after vaccination, both LC16m8- and Lister-vaccinated mice showed neither severe nor continuous weight loss, but all non-vaccinated mice and half of ultraviolet (UV)-inactivated Lister vaccinated mice lost weight until they died (Fig. 1B). All mice challenged with WR at 3 weeks, 24 weeks and 1 year after single dose vaccination showed almost the same results as for mice challenged on day 4 after vaccination (Fig. 1C–E).

These results (1) showed that both LC16m8 attenuated by the defect of *B5R* gene and its parental strain Lister had comparable abilities to protect wild-type mice from very early stages to 1 year post-vaccination, and (2) implicated that vaccination with both LC16m8 and Lister conferred at least partial protective immunity on day 2 after vaccination, and almost complete protective immunity on day 4 or later until at least 1 year after vaccination.

The neutralizing antibody titers against vaccinia WR virus were almost not detectable on days 2 to 6 after vaccination (data not shown), but then increased rapidly until 3 weeks, reaching a plateau from 8 weeks to 1 year after vaccination in both LC16m8- and Lister-vaccinated mice (Fig. 2).

3.2. Protection in immunodeficient mice by vaccination with LC16m8

Because protection by vaccination with LC16m8 was shown in wild-type mice challenged with WR as early as 2 days after vaccination, we speculated the involvement of other immune mechanism as than humoral immunity in this protection. To study this possibility, we investigated the effect of LC16m8 vaccination by using

various immunodeficient mice. Table 2 shows the survival ratio of each group, in which the number of mice (5 or 10) in each group is indicated.

First, the vaccination effect on CD4-deficient mice was evaluated by challenging with 10^6 PFU WR/mouse at 3 weeks after vaccination (Fig. 3A). By day 6 after the challenge, more than 70% of non-vaccinated mice had severe weight loss and died, while all LC16m8-vaccinated mice survived, suggesting that mice were protected by innate immunity or $CD8^+$ T-cell mediated immunity or both.

Second, the effect on MHC class I-deficient mice was evaluated by challenging with 10^5 PFU WR/mouse at 3 weeks after vaccination (Fig. 3B). All mice vaccinated with LC16m8, live Lister virus or UV-inactivated Lister virus survived; mice vaccinated with Lister and LC16m8 showed almost no weight loss. However, non-vaccinated mice had severe weight loss, and mice vaccinated with inactivated Lister virus had mild weight loss between >5 and 11 days after the challenge. These results suggest that immune mechanisms independent of MHC class I antigens contribute to the elicitation of a protective response in mice vaccinated with LC16m8

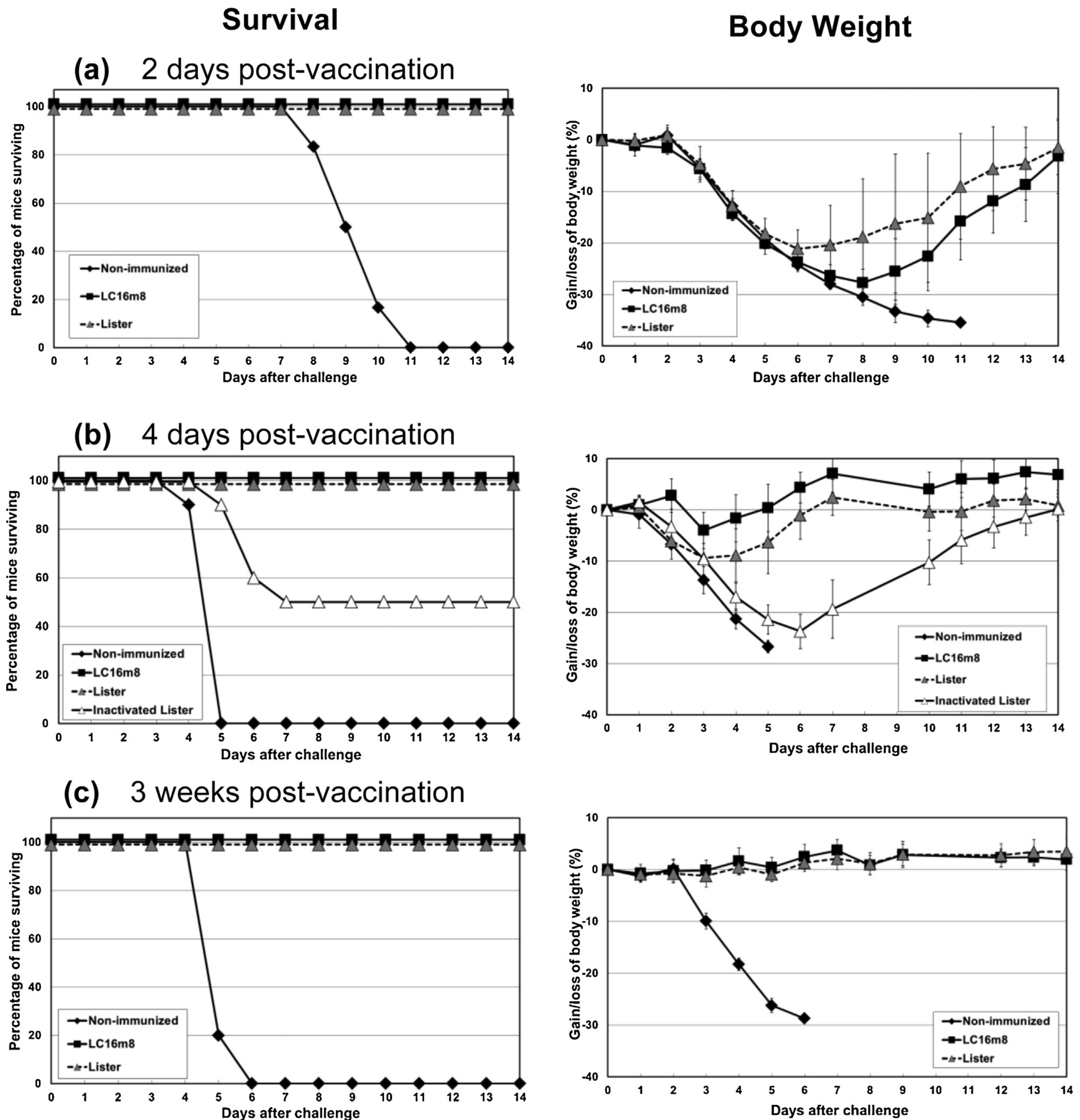

Fig. 1. Induction of immediate and long-lasting protective immunity in wild- type mice vaccinated with LC16m8. Groups of mice (4 or 8-week-old, BALB/c, female) were vaccinated at 2.5×10^5 PFU of LC16m8 or Lister by using the multiple-puncture method with standard bifurcated needles at the base of the tail or were vaccinated intraperitoneally with UV-inactivated Lister virus (the virus titer before inactivation was 1×10^7 PFU/mouse). After vaccination, the mice were challenged intranasally with 10^5 PFU of vaccinia-WR strain at 2 days (A) or 4 days (B), and with 10^6 PFU of vaccinia-WR strain at 3 weeks (C), 24 weeks (D), or 1 year (E) after vaccination. The mice were observed and weighed each day for 14 days after the challenge.
The number of mice of each group is shown in Table 1.

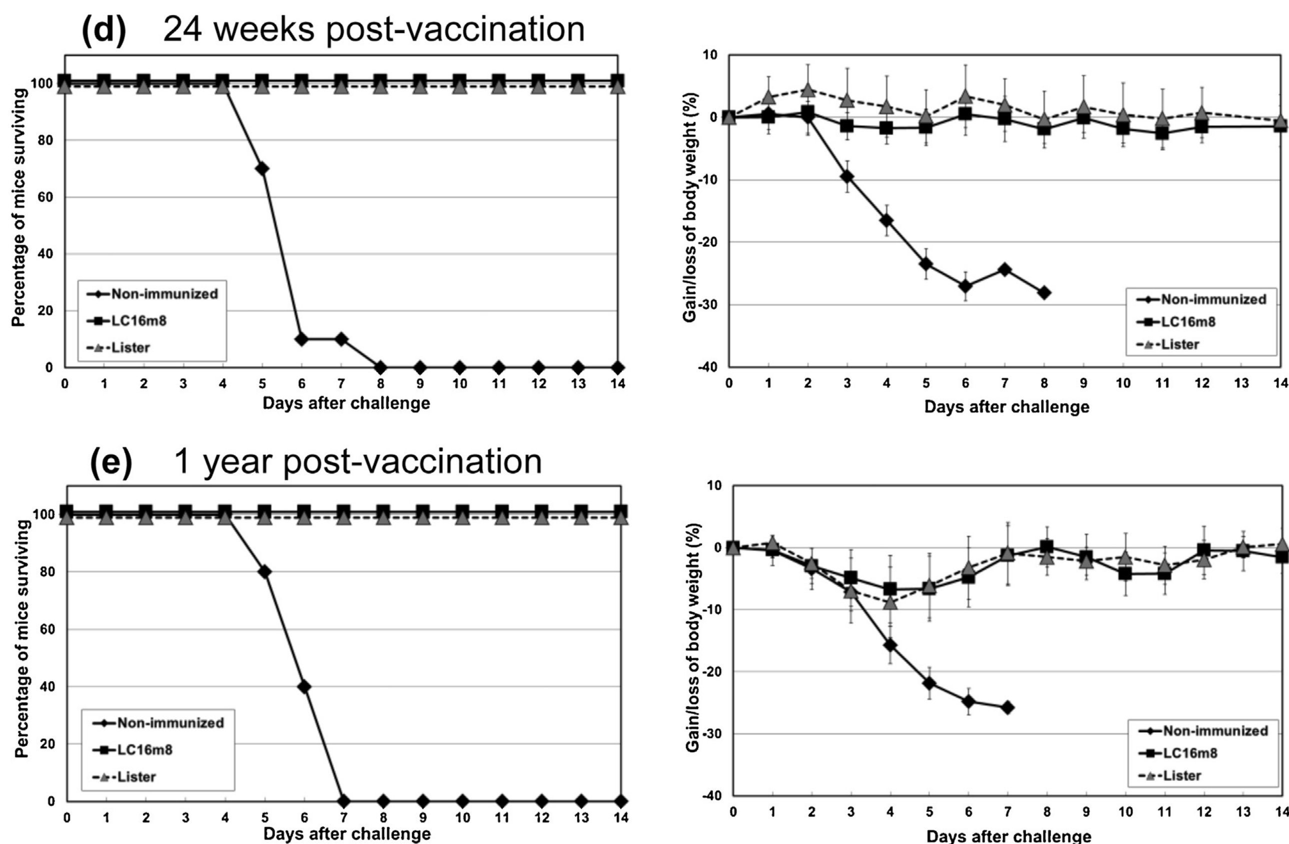

Fig. 1. *(Continued)*

and Lister, and live replicating virus is more effective in protection than inactivated non-replicating virus. Innate immunity or CD4+ T cell-mediated immunity or both are suggested to participate in protection.

Third, the effect on MHC-class II-deficient mice was evaluated by challenging with 10^6 PFU WR/mouse at 2 weeks after vaccination (Fig. 3C). All mice vaccinated with LC16m8 or live Lister survived, but 40% of the mice vaccinated with inactivated Lister and 80% of non-vaccinated mice had severe weight loss and died. These results strongly suggest that innate immunity or CD8+ T-cell mediated immunity or both participate in protection by vaccination with LC16m8 and Lister.

Fourth, the effect on MHC class I- and II-double deficient mice was evaluated by challenging with 10^5 PFU WR at 2 weeks after vaccination (Fig. 3D). All non-vaccinated mice died, while 70% and 60% of mice vaccinated with LC16m8 and Lister, respectively, survived until 14 days after the challenge. All groups of mice had continuous weight loss until 14 days after the challenge, and especially non-vaccinated mice had the severest weight loss. Notably, live LC16m8 and Lister showed significant partial protection, strongly suggesting that innate immunity contributes to protection.

In this series of experiments, neutralizing antibodies to VACV WR were undetected at 14 days after WR challenge in immunized MHC class II-deficient and immunized MHC class I and II-double deficient mice (limit of detection, 1:8) (data not shown).

4. Discussion

This study showed that vaccine strain LC16m8 with truncated surface B5 protein has protective activity completely comparable to that of its parental strain Lister in all experiments using wild-type, CD4-deficient, MHC class I-deficient, MHC class II-deficient or MHC

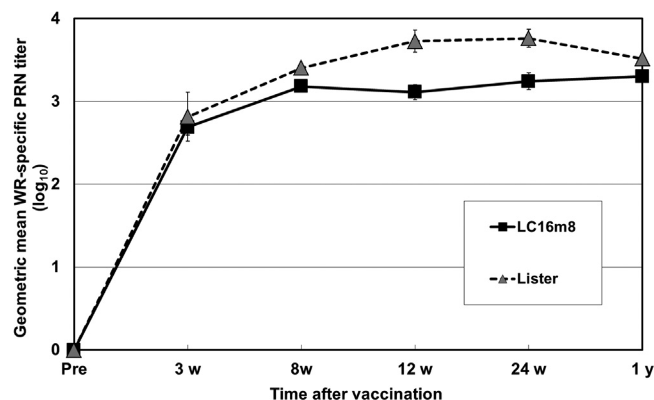

Fig. 2. Induction of long-lasting neutralizing antibodies in wild-type mice vaccinated with LC16m8. Five or ten mice of each group (4-week-old, BALB/c, female) were vaccinated at 2.5×10^5 PFU of LC16m8 or Lister by using the multiple-puncture method with standard bifurcated needles at the base of the tail. The sera collected from the immunized mice at 3 weeks ($N=10$), 8 weeks ($N=10$), 12 weeks ($N=10$), 24 weeks ($N=10$) or at 1 year ($N=5$) after vaccination were tested for vaccinia WR-specific neutralizing antibody titers by PRN assay.

class I- and II- double deficient mice. Notably, no difference existed between total neutralizing antibody titers induced with LC16m8 and Lister in wild-type mice (Fig. 2), MHC class II-deficient and MHC class I- and II- double deficient mice, despite the failure of LC16m8 to induce anti-B5 neutralizing antibody that is protective in mice [12].

Recently, we studied the safety of LC16m8 in three immunodeficient mouse models (suckling mice, severe combined immunodeficient mice, and wild-type mice treated with cyclosporine) and showed that LC16m8 showed extremely low virulence in each of these mouse models when compared with its parental strains,

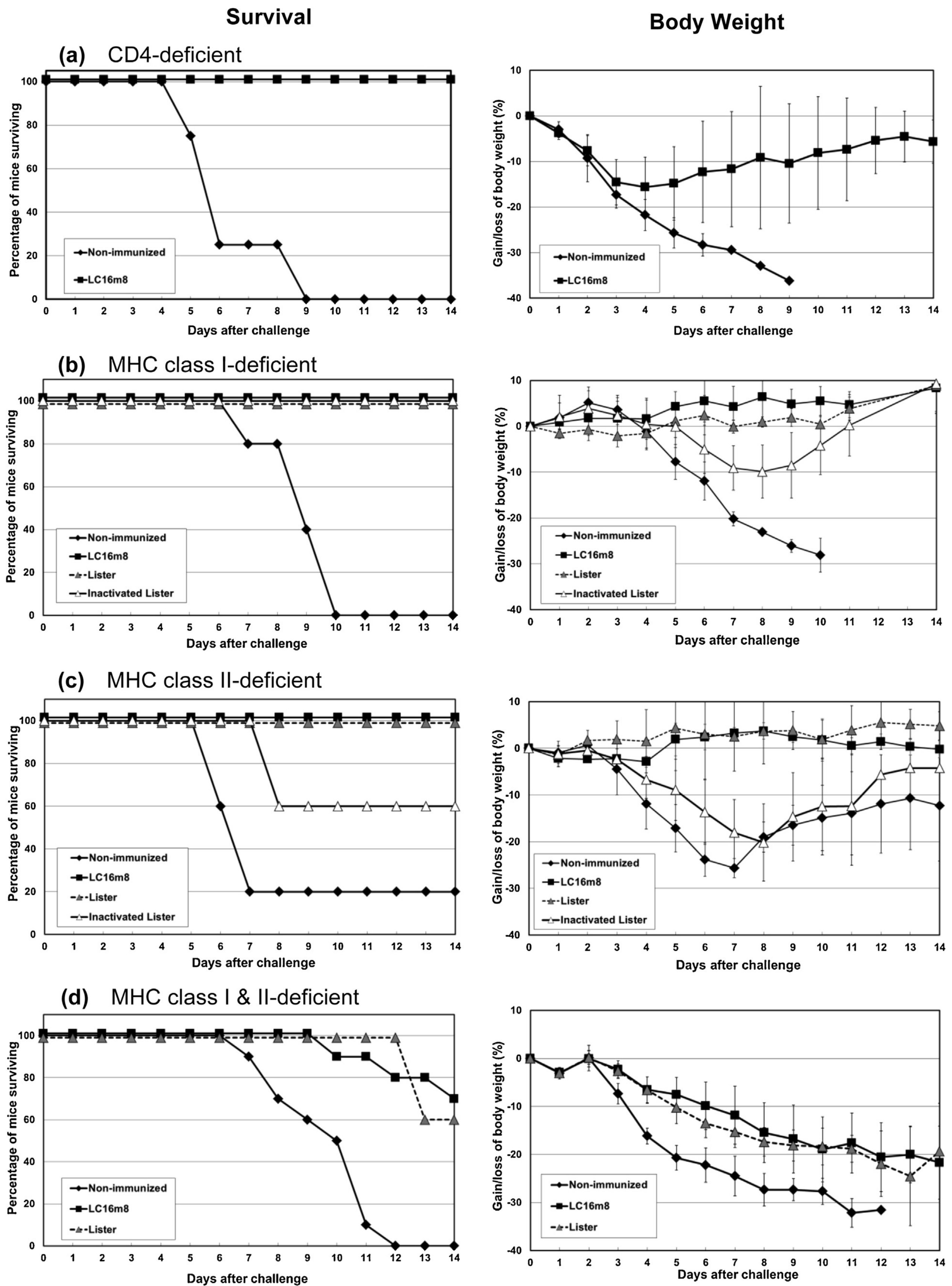

Fig. 3. Induction of strong and comprehensive protective immunity in immunodeficient mice vaccinated with LC16m8. Groups of mice (4 to 7-week-old, female, CD4-deficient (A), MHC class I-deficient (B), MHC class II-deficient (C), or MHC class I and II-double deficient (D)) were vaccinated at 2.5×10^5 PFU of LC16m8 or Lister by using the multiple-puncture method with standard bifurcated needles at the base of the tail or were vaccinated intraperitoneally with UV-inactivated Lister virus (the virus titer before inactivation was 1×10^7 PFU/mouse). After vaccination, mice of CD4-deficient (A), MHC class I-deficient (B), MHC class II-deficient (C), or MHC class I and II-double deficient (D) were challenged intranasally at 10^6, 10^5, 10^6 or 10^5 PFU of vaccinia-WR strain at 3, 3, 2 or 2 weeks after vaccination, respectively. The mice were observed and weighed each day for 14 days after the challenge. The number of mice of each group is shown in Table 2.

Lister and LC16mO [16]. These results, together with the results obtained in this study, open the way to use LC16m8 in immunodeficient patients. Relevant to this, a notable wide epidemic of monkeypox was reported in areas of west and central Africa where a high prevalence of human immunodeficiency virus infection exists [17].

The *B5R* gene defect is the major factor in attenuation of LC16m8 [8,10]. Of concern is that LC16m8 with truncated B5 protein might have less immunogenicity and poor protective activity. People vaccinated with LC16m8 induce neither extracellular enveloped virus-neutralizing antibody nor other kinds of antibody to B5 protein, even though after vaccination the neutralizing antibody to B5 protein is boosted in previously vaccinated individuals [13]. This boosting effect might be due to an abundant expression of a truncated B5 protein in LC16m8 as mentioned previously but the possibility of the occurrence of a revertant virus due to repair of the mutation in the B5R gene [15] is not completely excluded. However, we use a vaccine preparation with a limited number of propagation to lessen this risk.

In addition to LC16m8 showing protective activity completely comparable to that of its parental strain Lister, the following immunological evidence strengthens the idea that the B5 protein defect does not affect all the protective activity of LC16m8. Not only humoral immunity, but also cell-mediated immunity, contribute to protection against poxvirus infection [18,19]. Antigens directed for protective immunity are considered to be numerous because of the large size of VACV [19]. A total of 49 epitopes recognized by $CD8^+$ T-cells from 35 different VACV antigens were identified by screening with peripheral blood mononuclear cells of 31 vaccinees [20]. Fewer studies have been done to identify targets of VACV-specific $CD4^+$ T-cells in the murine system (C57BL/6). By using a random peptide library, 14 epitopes recognized by $CD4^+$ T-cells were identified [21]. Five known neutralizing antibody target antigens exist: L1, H3, A27, D8 and B5. Interestingly, antibodies against the A33 surface protein are protective, but are not neutralizing [19]. To conclude, even if LC16m8 fails to induce a neutralizing antibody against B5 protein, both cell-mediated and humoral immune responses directed to many antigens other than B5 protein of large-size VACV should fully compensate the defect of LC16m8 because of truncated B5 protein.

From the results that CD4-deficient mice, MHC class I-deficient mice and MHC class II-deficient mice were protected for at least 14 days after the challenge (Fig. 3A–C), one or a combination of humoral, $CD4^+$ T-cell-mediated and $CD8^+$ T-cell-mediated immunity is suggested to be enough for protection. Poor protection of MHC class I and II-double deficient mice (Fig. 3D) suggests that either $CD4^+$ T-cell-mediated immunity or $CD8^+$ T-cell-mediated immunity is essential for protection. Although neutralizing antibody titers in CD4-deficient mice and MHC class I-deficient mice were not examined in the present study, if these results are obtained in the future, the results may strengthen our hypothesis.

Other clinical studies support our view. Administration of LC16m8 to healthy adults who were vaccinia-naïve is associated with high levels of vaccine take and seroconversion (Saito et al. [22]). Previously vaccinated healthy adults yield an effective booster response: a phase I/II clinical trial of LC16m8 vaccine achieved anti-vaccinia, anti-variola, and anti-monkeypox neutralizing antibody titers >1:40, and induced robust cellular immune responses as assessed by a lymphocyte proliferation test [23].

Gordon et al. [24] investigated the immunologic basis of the containment of vaccinia in the skin with the goal to identify safer vaccines for smallpox. Macaques were depleted systemically of T or B cells and vaccinated with either Dryvax or an attenuated vaccinia vaccine, LC16m8. B cell depletion did not affect the size of skin lesions induced by either vaccine. However, while depletion of both $CD4^+$ and $CD8^+$ T cells had no adverse effects on LC16m8-vaccinated animals, it caused disseminated vaccinia in macaques immunized with Dryvax. Furthermore, as both Dryvax and LC16m8 vaccines protect healthy macaques from a lethal monkeypox intravenous challenge, their data identify LC16m8 as a safer and effective alternative to ACAM2000 and Dryvax vaccines for both healthy and immunocompromised individuals. This study strongly support the idea that $CD4^+$ and $CD8^+$ T cell- immunity plays crucial roles in protective ability, and that the lack of normal B5 protein does not affect this ability.

Our study also strongly suggested that LC16m8 induced innate immunity partially contributing to protection at the early stages after vaccination because vaccination with LC16m8 protected wild-type mice challenged on 2 to 4 days after vaccination (Fig. 1A, B): humoral, as well as $CD4^+$ and $CD8^+$ T-cell mediated, immune responses, if any, may not be induced or are very poor in these stages. Vaccination with LC16m8 or Lister induced comparable partial protection in MHC class I- and II-double deficient mice lacking both T-cell- and B-cell-mediated immunity and strongly supports the above speculation of the contribution of innate immunity to immediate protection (Fig. 3D).

Mice infected with various viruses, including VACV, induce natural killer (NK) cell activity on day 1 to day 10 after vaccination [25] and is consistent with the speculation from our results that protective activity of LC16m8 observed at the early stages after vaccination in wild-type mice and in various immunodeficient mice is given by innate immunity (Figs. 1, 3). Microorganisms, like viruses that invade a vertebrate host, are initially recognized by the innate immune system through pattern-recognition receptors. Several classes of these receptors, including Toll-like receptors and cytoplasmic receptors, recognize distinct microbial components and induce the release of interferon-α and interferon-β [26].

Induction of innate immunity by live LC16m8 virus is considered to activate cell-mediated and humoral immunity, as shown by good protection of mice against lethal challenge by at least 1 year after vaccination (Figs. 1, 2). VACV antigens present on the cell surface induce acquired immunity, including humoral and T-cell-mediated immunity, whereas VACV antigens inside cells contribute to the production of interferons, pro-inflammatory cytokines and chemokines, and NK cells responsible for innate immunity [27,28].

5. Conclusion

This study strongly suggested that LC16m8 with truncated B5 protein has an activity to induce innate immunity and subsequent cell-mediated and humoral immunity almost completely comparable to the activity of its parental strain Lister.

Conflict of interest statement

HY, YS, TK, SM, MK and HM are employees of Kaketsuken. SH declares that he has no conflicts of interest.

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