Recombinant Sendai Virus Vector Induces Complete Remission of Established Brain Tumors through Efficient *Interleukin-2* Gene Transfer in Vaccinated Rats

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Abstract

Purpose: Sendai virus (SeV), a murine parainfluenza virus type I, replicates independent of cellular genome and directs high-level gene expressions when used as a viral vector. We constructed a nontransmissible recombinant SeV vector by deleting the *matrix (M)* and *fusion (F)* genes from its genome (SeV/ΔMΔF) to enhance its safety. We also estimated the therapeutic efficacy of the novel vector system against a rat glioblastoma model.

Experimental Design: We administered the recombinant SeV vector carrying the *lacZ* gene or the *human interleukin-2* (*hIL-2*) gene into established 9L brain tumors *in vivo* simultaneous with peripheral vaccination using irradiated 9L cells. Sequential monitoring with magnetic resonance imaging was used to evaluate the therapeutic efficacy.

Results: We found extensive transduction of the *lacZ* gene into the brain tumors and confirmed sufficient amounts of interleukin 2 (IL-2) production by hIL2-SeV/ΔMΔF both *in vitro* and *in vivo*. The magnetic resonance imaging study showed that the intracerebral injection of hIL2-SeV/ΔMΔF brought about significant reduction of the tumor growth, including complete elimination of the established brain tumors. The ^{51}Cr release assay showed that significant amounts of 9L-specific cytotoxic T cells were induced by the peripheral vaccination. Immunohistochemical analysis revealed that $CD4^+$ T cells and $CD8^+$ T cells were abundantly infiltrated in the target tumors.

Conclusion: The present results show that the recombinant nontransmissible SeV vector provides efficient *in vivo* gene transfer that induces significant regression of the established brain tumors and suggest that it will be a safe and useful viral vector for the clinical practice of glioma gene therapy.

Glioblastoma is the most common malignant brain tumor. It is considered incurable despite multimodal approaches of therapy, including surgery, radiotherapy, and chemotherapy (1). The therapeutic application of the gene transfer technique has been expected as a new therapeutic option for glioblastoma (2). The efficacy, however, has been limited by low levels of transgene transduction and poor distribution of the target molecules throughout the tumor tissues (3). The successful gene therapy in clinical practice will depend on the development of novel vector systems capable of wide distribution and efficient transduction of the target genes into the tumor cells.

Recent advances to manipulate the genome of negative-strand RNA viruses have led to the development of a new class of viral vectors for gene transfer approaches (4). Sendai virus (SeV) is a murine parainfluenza virus type I belonging to the family Paramyxoviridae and is a single-stranded RNA virus. The genome of SeV is a linear and nonsegmented negative-strand RNA of ~15.4 kb. It contains six major genes that are arranged in tandem on its genome; it is tightly encapsidated with the nucleoprotein (NP) and is further complexed to phosphoprotein (P) and large protein (L; the catalytic subunit of the polymerase). This viral ribonucleoprotein complex constitutes the internal core structure of the virion. The viral envelope contains two spike proteins, hemagglutinin-neuraminidase (HN) and fusion (F), which mediate the attachment of virions and the penetration of ribonucleoproteins into infected cells, respectively. Matrix (M) protein functions in virus assembly and budding.

For the use of gene therapy vectors, the promising characteristics of SeV are as follows: (*a*) an exclusively cytoplasmic replication cycle without any risk of integration into the genomic DNA, (*b*) transduction efficacy that is not dependent on the cell cycle of target cells, (*c*) no homologous recombination between

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different SeV genomes or to wild-type virus, (*d*) the remarkably brief contact time that is necessary for cellular uptake, (*e*) a high and adjustable expression of virally encoded genes in a broad range of host cells, and (*f*) the lack of association with any disease process in humans (4). Indeed, the SeV vector has been shown to produce 2 to 3 logs higher transfection efficiency than the adenoviral vectors or lipofection (5, 6), and high gene expressions have been noticed in a broad range of tissues, including the airway epithelial cells, vasculature tissues, skeletal muscle, activated T cells, stem cells, and neural tissues (5–12). In addition to these biological features of the SeV vector, we have constructed a nontransmissible recombinant SeV vector by deleting the M and F genes from its genome to enhance its safety (13–15).

We previously verified the efficacy of the intracerebral (i.c.) transplantation of interleukin 2 (IL-2)–producing cells in the rat brain tumor model (16, 17). For clinical application of the cytokine gene therapy strategy, however, the cell-mediated therapy needs a large amount of cytokine-producing cells to cover the whole tumor areas in the human brain. The SeV vector–mediated strategy would be superior in the wide distribution of the transgene products achieved by a small amount of viral solution, and it is expected to be especially suitable for delivery of the secreted proteins, such as cytokines. In the present study, we examined the therapeutic potentials of the nontransmissible recombinant SeV vector carrying *human IL-2* gene in a cytokine gene therapy against brain tumors.

Materials and Methods

Cells and animals. Rat 9L gliosarcoma, C6 glioma, and rhesus monkey LLC-MK_2 kidney cell lines were maintained in DMEM supplemented with 10% FCS in a humidified atmosphere of 5% CO_2. Male Fisher 344 rats, weighing between 200 and 240 g (7-8 weeks old), were used as indicated in the experiments. These animals were maintained in a specific pathogen-free environment in accordance with the Laboratory Animal Resources Commission Standards.

Recombinant Sendai virus vector. Genome order of the SeV full-length genome used was as follows: the leader (ld) at the 3′-end followed by viral genes, *nucleocapsid (NP)*, *phospho (P)*, *matrix (M)*, *fusion (F)*, *hemagglutinin-neuraminidase (HN)*, and *large proteins (L)*. Finally, a small trailer (tr) sequence was placed at the 5′-end (Fig. 1). We utilized both the *M* and *F* genes–deleted SeV vector (SeV/ΔMΔF) in the experiment. F protein is essential for viral infection and M protein functions in virus assembly and budding (13, 14). Therefore, SeV/ΔMΔF is nontransmissible with loss of particle formation from infected cells (15). SeV/ΔMΔF carrying *human IL-2* gene (hIL2-SeV/ΔMΔF) and *lacZ* gene (lacZ-SeV/ΔMΔF) were constructed as previously described (13). In brief, human IL-2 (accession no. U25676) cDNA was amplified with a pair of *Not*I-tagged primers that contained SeV-specific transcriptional regulatory signal sequences, 5′-ACTTGCGGCCGCGTTTAAACGGCGCGCCATGTACAGGATG-CAACTCCTGTC-3′ and 5′-ATCCGCGGCCGCGATGAACTTTCACCC-TAAGTTTTTCTTACTACGGATTTAAATGGCGCGCCA-3′. The amplified fragment was introduced into the *Not*I site of the parental pSeV18+/ΔMΔF. Thus, the cDNA of hIL2-SeV/ΔMΔF (phIL2-SeV/ΔMΔF) was constructed. The cDNA of lacZ-SeV/ΔMΔF (placZ-SeV/ΔMΔF) was constructed in similar manner using the amplified fragment of lacZ (18). phIL2-SeV/ΔMΔF and placZ-SeV/ΔMΔF were transfected into LLC-MK_2 cells after infection of the cells with vaccinia virus vTF7-3 (19), which expresses T7 polymerase. The T7-driven recombinant hIL2-SeV/ΔMΔF and lacZ-SeV/ΔMΔF RNA genomes were encapsulated by N, P, and L proteins, which were derived from their respective cotransfected plasmids. The recovered SeV vectors were propagated using both M and F protein–expressing packaging cell lines (15). The virus titers were determined using infectivity and were expressed in cell infectious units. The SeV vectors were stored at −80°C until use.

Kinetic analysis of interleukin-2 production. LLC-MK_2 cells (10^6) grown in six-well plates were infected at a multiplicity of infection of 10 for 1 hour with hIL2-SeV/ΔMΔF and incubated in serum-free MEM at 37°C. The culture supernatants were collected every 24 hours, with immediate addition of MEM to the remaining cells. IL-2 protein in the supernatant was quantified by ELISA using the human IL-2 ELISA kit (Biosource International, Inc., Camarillo, CA).

Brain tumor model and treatment. The animals were anesthetized and placed in a stereotaxic apparatus. A burr hole was made at 4 mm posterior to bregma and 3 mm right to midline. A 25-gauge needle was inserted to the point of 3 mm ventral from dura where 1×10^5 syngeneic 9L tumor cells in 10 μL medium were slowly injected. Treatment was started 3 days (day 3) after i.c. inoculation of 9L tumor cells (day 0). The animals received i.c. administration of hIL2-SeV/ΔMΔF or lacZ-SeV/ΔMΔF and/or s.c. vaccination with irradiated wild-type 9L tumor cells. For i.c. administration, 1×10^7 cell infectious units of SeV vector in 10 μL PBS were used in the same stereotactic coordinates. For s.c. vaccination, wild-type 9L cells were irradiated at 30 Gy and 1×10^6 cells in 100 μL medium were injected into the lower abdominal quadrant (16, 17). The animal experimentation was reviewed and approved by the Institutional Animal Care and Use Committee of Chiba Cancer Center Research Institute.

Magnetic resonance imaging study. To estimate i.c. tumor volume sequentially, all the animals were examined with magnetic resonance imaging (MRI) every 7 days started on day 7 after the tumor inoculation. Rats were anesthetized with 50 mg/kg pentobarbital and injected with 0.2 mL gandolinium-diethylenetriaminepentaacetic acid (Gd-DTPA, 0.8-1.0 mL/kg). Coronal T_1-weighted images (TR 500 milliseconds, TE 11 milliseconds, 3 mm thickness, gapless) were obtained with a 1.5-T MR device (Signa Advantage, General Electric, Milwaukee, WI). Tumor volume (mm^3) was calculated as the sum of the Gd-DTPA–enhanced portion of each MR-imaged area (mm^2) times the imaged thickness. The estimated tumor volumes on MRI have a linear correlation with actual tumor weights obtained immediately after the imaging study (20).

Immunohistochemistry. Tumor-bearing rats were perfused through the ascending aorta with 4% paraformaldehyde and brains were

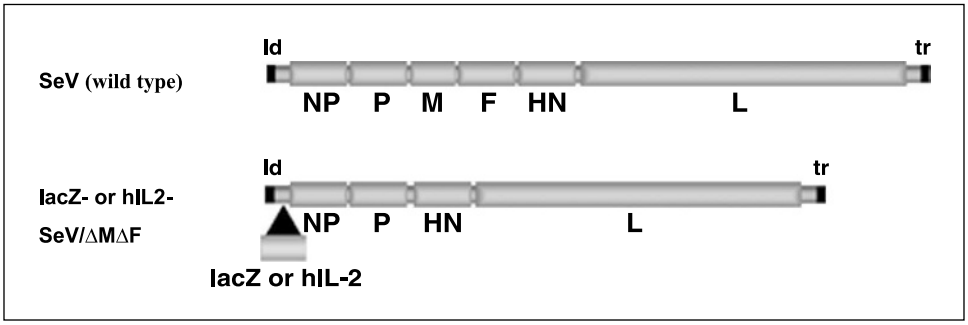

Fig. 1. Schematic genome structures of wild-type SeV and the both *M* and *F* gene–deleted SeV vector carrying *lacZ* or *human IL-2* gene. The open reading frame of the *lacZ* or *human IL-2* gene was inserted with the SeV-specific transcriptional regulatory signal sequences, end and start signals between the leader (*ld*) and the *NP* gene.

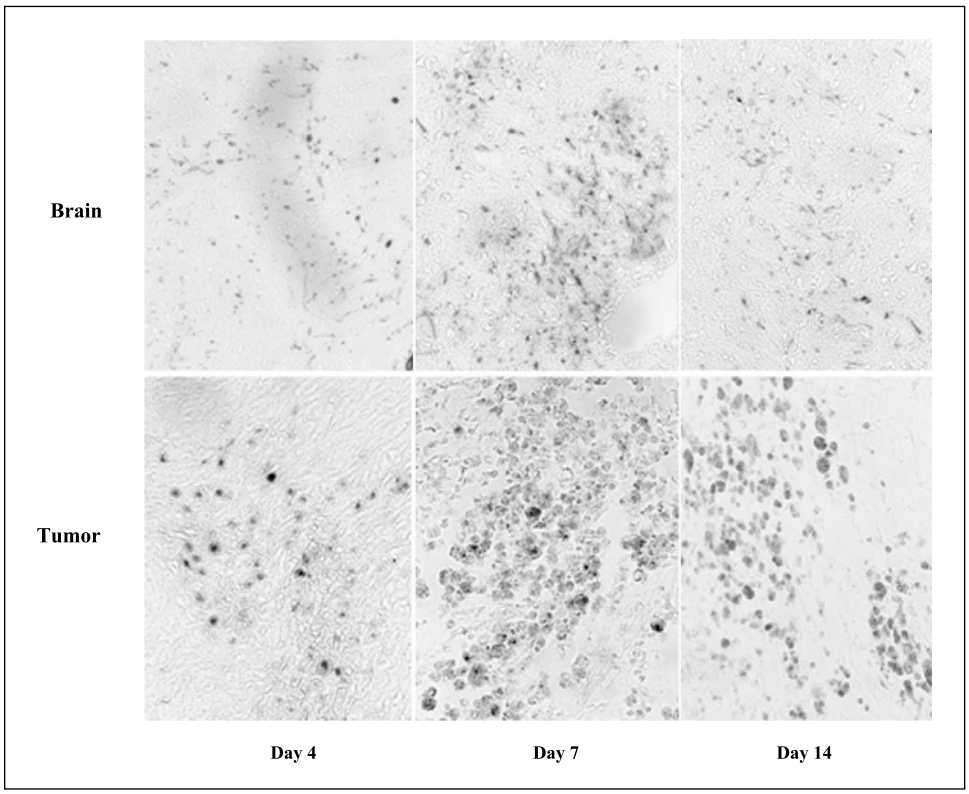

Fig. 2. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining of rat brain tissues (*top*) and 9L brain tumors after 7 days of growth in the brain (*bottom*) administered *in situ* with lacZ-SeV/ΔMΔF. Four, seven, and fourteen days after administration of the vector, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining was done (×200 magnification). Maximal expression or accumulation of β-galactosidase was obtained on day 7 after injection of the vector, and the expression was maintained on day 14 both in the brain tissues and in the brain tumors.

removed. Frozen tissue sections of the brain specimens at 10 μm thickness slices were reacted with anti-CD4 antibody (W3/25, Serotec, Oxford, United Kingdom), anti-CD8 antibody (OX-8, Serotec), and anti-human IL-2 antibody (R&D Systems, Minneapolis, MN). Tissues were then reacted with horseradish peroxidase–conjugated goat anti-mouse IgG and stained with 3,3′-diaminobenzidine tetrahydrochloride (Nichirei, Tokyo, Japan). β-galactosidase expression was detected using β-Galactosidase Staining Kit (Mirus, Madison, WI).

CTL assay. To test lymphocytes for their antitumor cytotoxicity, a standard 4-hour ^{51}Cr release assay was done. Spleen cells (1×10^6 cells) were harvested from rats on day 14 after treatment and were cultured in RPMI 1640 supplemented with 10% FCS and 5×10^{-5} mol/L 2-mercaptoethanol. They were stimulated *in vitro* with irradiated 9L cells for 5 days. Syngeneic 9L or allogeneic C6 cells were used as ^{51}Cr-labeled targets and were cultured with the spleen cells at various effector-to-target cell ratios. After 4-hour incubation, radioactivities in the culture supernatants were counted with an automatic γ-counter. Specific cytotoxic activity was calculated as follows: 100 × ([experimental counts per minute − spontaneous counts per minute] / [maximal counts per minute − spontaneous counts per minute]). The maximal counts per minute were released by adding 1% NP40 to wells in experiments.

Statistics. Comparison of tumor volumes in each treatment group was done with the unpaired *t* test. The Kaplan-Meier method was used to estimate the survival rates and the Cox-mantel log-rank test was used to compare the survival differences in each treatment group. All of the statistical analyses were done with the StatView software (SAS Institute, Inc., Cary, NC).

Results

Sendai virus vector–mediated transduction of β-galactosidase gene into glioma tissue. The efficiency of i.c. transduction of the *β-galactosidase* gene by SeV vector was examined in brain tumors and normal brain tissues removed 4, 7, and 14 days after administration of lacZ-SeV/ΔMΔF. When injected into the brain tumor, the typical appearance of the vector-injected tissues was scattered colonies of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside–positive cells, which were composed of transduced tumor cells from the injected lacZ-SeV/ΔMΔF (Fig. 2). Nontransduced tumor cells were seen between the scattered 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside–stained colonies. Maximal expression or accumulation of

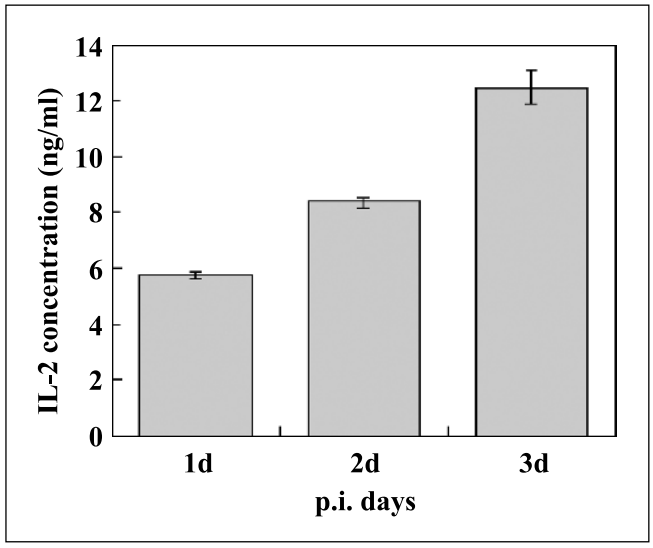

Fig. 3. Kinetics of the expression of IL-2 protein in the cells infected with hIL2-SeV/ΔMΔF *in vitro*. LLC-MK$_2$ cells (10^6) were infected at a multiplicity of infection of 10 with hIL2-SeV/ΔMΔF. The culture supernatants were collected every 24 hours and the amounts of IL-2 protein were quantified by ELISA. Substantial amount of IL-2 was secreted even at 1 day after the infection. The maximum production of 12.5 ng/mL was observed at day 3.

β-galactosidase was obtained on day 7 and the expression level was maintained on day 14 after injection of the vector. Transduction of normal brain surrounding the tumor was scarcely detected with the exception of the choroid plexus. When injected into the normal brain tissues, transductions of neurons and glial cells were also observed at lower efficiency compared with the case of intratumoral injection. Ependymal cells were not transduced by the intraparenchymal injection of the vector.

In vitro ***kinetics of interleukin-2 production by cells infected with hIL2-SeV/ΔMΔF.*** To clarify the transgene expression induced by hIL2-SeV/ΔMΔF, the amount of IL-2 production by the cells infected with hIL2-SeV/ΔMΔF was investigated by ELISA. Sufficient amount of IL-2 protein (5.8 ng/mL) was detected at day 1 after the vector infection and increased to 12.5 ng/mL at 3 days after the infection (Fig. 3).

Antitumor effects of i.c. administration of hIL2-SeV/ΔMΔF. All the naive rats inoculated with 9L cells in the brain developed progressive tumors. We examined the therapeutic effect of i.c. administration of hIL2-SeV/ΔMΔF combined with s.c. vaccination by measuring the tumor volumes with serial Gd-enhanced MRI (Fig. 4A). The tumor volumes on day 21 in the rats treated with the i.c. administration of hIL2-SeV/ΔMΔF and the vaccination (86.5 ± 63.8 mm^3, n = 10) were significantly smaller than the following groups: untreated (286 ± 51.2 mm^3, n = 10, $P < 0.0001$); vaccination alone (197 ± 48.9 mm^3, n = 10, $P = 0.0005$); i.c. administration of lacZ-SeV/ΔMΔF combined with the vaccination (233 ± 73.2 mm^3, n = 6, $P = 0.0012$); and i.c. administration of hIL2-SeV/ΔMΔF alone (256 ± 53.2 mm^3, n = 6, $P = 0.0001$; Fig. 4B). When treated with the combination strategy, all the inoculated tumors became visible by MRI on day 21 and the established brain tumors were completely eliminated in 3 of 10 rats (Fig. 4A). There was no difference in the tumor volumes on day 21 between the three cured tumors (81.6 ± 74.9 mm^3) and the other seven tumors (88.6 ± 67.1 mm^3). Accordingly, the lifetime of the rats treated with i.c. administration of hIL2-SeV/ΔMΔF vector combined with the vaccination was significantly prolonged compared with the untreated control rats or the rats treated otherwise ($P < 0.05$, log-rank test; Fig. 4C). All the cured animals completely rejected

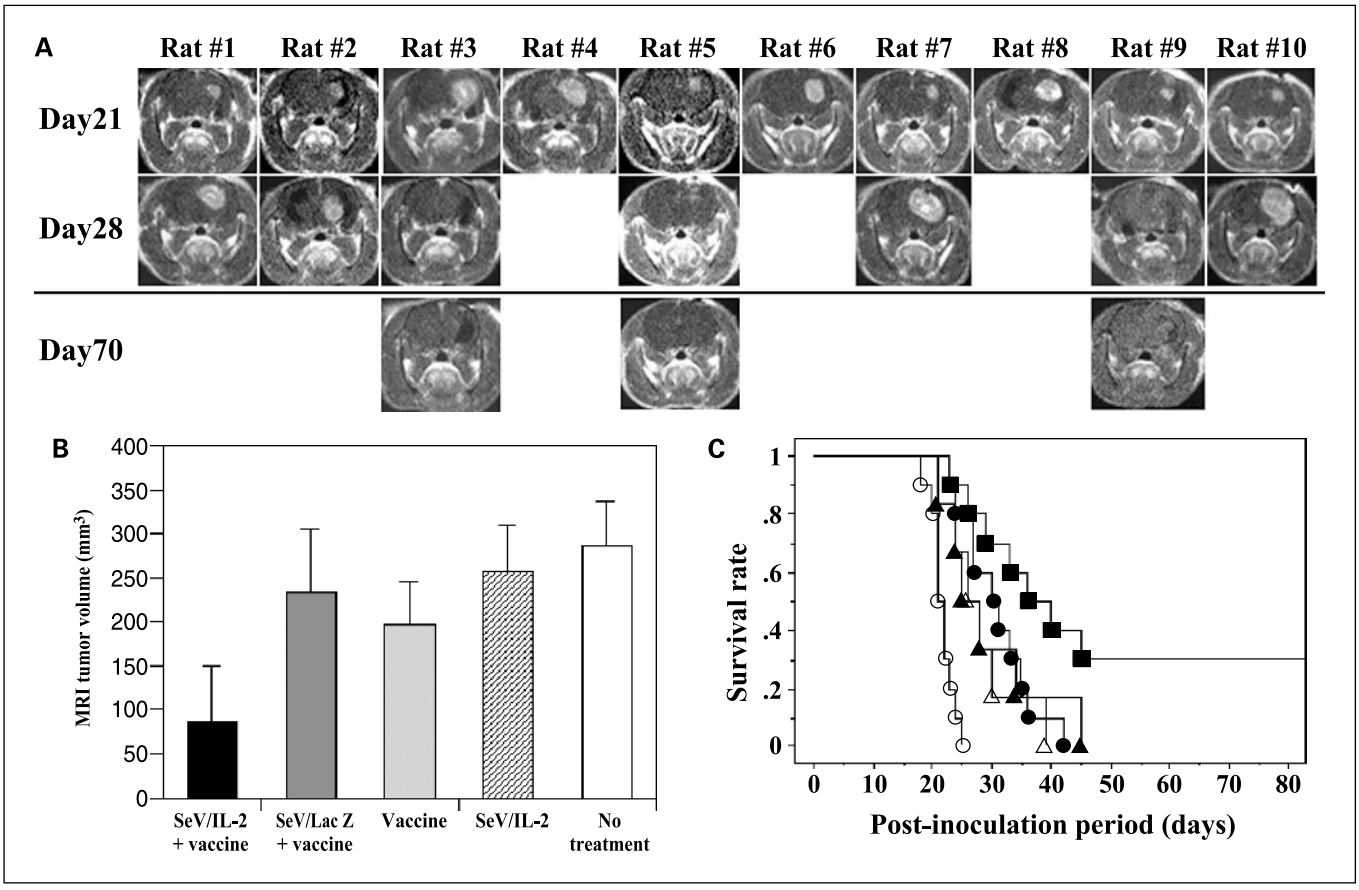

Fig. 4. Antitumor effects of i.c. administration of hIL2-SeV/ΔMΔF. *A*, Gd-DTPA – enhanced MRI images of all the 9L brain tumors treated with i.c. administration of hIL2-SeV/ΔMΔF and s.c. vaccination of irradiated wild-type 9L cells. All the animals were examined with MRI every 7 days, starting on day 7 after tumor inoculation. When treated with the combination strategy, all the i.c. tumors became visible at latest on day 21. The established brain tumors completely disappeared on day 28 in 3 of 10 rats examined (rats 3, 5, and 10). There was no difference in the tumor volumes on day 21 between the three eliminated tumors (81.6 ± 74.9 mm^3) and those of the seven other tumors (88.6 ± 67.1 mm^3). *B*, mean volumes of the 9L brain tumors measured by Gd-enhanced MRI on day 21. I.c. administration of hIL2-SeV/ΔMΔF combined with the vaccination (86.5 ± 63.8 mm^3, n = 10) resulted in significantly smaller volumes than the following groups: untreated (286 ± 51.2 mm^3, n = 10, $P < 0.0001$), vaccination alone (197 ± 48.9 mm3, n = 10, $P = 0.0005$), i.c. administration of lacZ-SeV/ΔMΔF combined with the vaccination (233 ± 73.2 mm^3, n = 6, $P = 0.0012$), and i.c. administration of hIL2-SeV/ΔMΔF alone (256 ± 53.2 mm^3, n = 6, $P = 0.0001$). Columns, mean; bars, SD. *C*, Kaplan-Meier survival analysis of the tumor-bearing rats. ○, untreated; ●, treated with the vaccination alone; ▲, treated with i.c. administration of lacZ-SeV/ΔMΔF and the vaccination; △, treated with i.c. administration of hIL2-SeV/ΔMΔF alone; ■, treated with i.c. administration of hIL2-SeV/ΔMΔF and the vaccination. Statistical analysis with log-rank test showed that the rats treated with i.c. administration of hIL2-SeV/ΔMΔF and the vaccination survived significantly longer than the other treatment groups ($P < 0.05$).

a second challenge of wild-type 9L inoculation in the brain and manifested no adverse effect during the 3-month follow-up period.

Induction of tumor-specific cytotoxic T cells. We used the standard ^{51}Cr release assay to evaluate the cytotoxic activity of spleen cells from the treated rats or those from naive rats against syngeneic 9L cells or allogeneic C6 cells. The cytotoxicity to 9L targets was strongly induced by the spleen cells from the rats treated with s.c. vaccination of the irradiated wild-type 9L cells (Fig. 5). In contrast, the cytotoxicity of the same effector cells to the C6 targets was not observed. The spleen cells from the tumor-bearing rats that were treated with i.c. administration of hIL2-SeV/ΔMΔF alone did not induce significant cytotoxicity against the 9L cells. These data suggest that the irradiated wild-type 9L cells were sufficiently immunogenic in the peripheral tissue and that the s.c. vaccination with these cells could induce 9L-specific cytotoxic T cells.

Immunohistochemical analysis. To ascertain the gene expression at protein level, we analyzed the immunoreactivity of IL-2 protein in the hIL2-SeV/ΔMΔF–injected tumors and confirmed that IL-2 protein was diffusely expressed in the treated tumors (Fig. 6A). We also immunohistochemically examined the presence of $CD4^+$ T cells and $CD8^+$ T cells in the tumors. Diffuse and dense infiltrations of $CD4^+$ T cells and $CD8^+$ T cells were observed in the tumors that were treated with i.c. administration of the hIL2-SeV/ΔMΔF and concurrent s.c. vaccination (Fig. 6B). In the tumors treated either with i.c. administration of the lacZ-SeV/ΔMΔF and the vaccination or i.c. administration of the hIL2-SeV/ΔMΔF alone, infiltrations of these cells were sparsely detected.

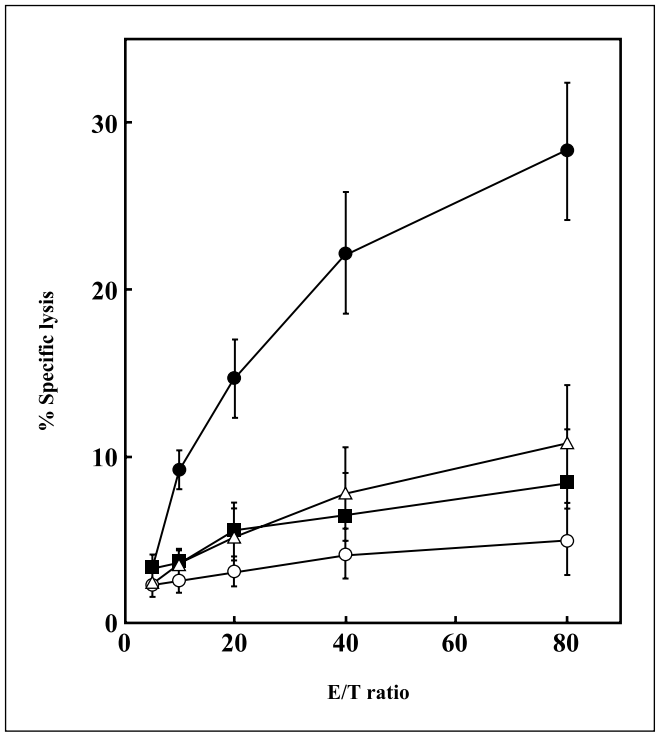

Fig. 5. Cytolytic activity by spleen cells from the treated rats was assessed with a standard ^{51}Cr-release assay against syngeneic 9L or allogeneic C6 targets. ○, spleen cells from naive rats against 9L targets; ●, spleen cells from the rats s.c. vaccinated with irradiated 9L against 9L targets; ■, spleen cells from the vaccinated rats against C6 targets. △, spleen cells from rats treated with i.c. administration of hIL2-SeV/ΔMΔF alone. The vaccination with irradiated 9L cells could induce 9L-specific cytotoxic T cells.

Discussion

We herein first showed that the nontransmissible recombinant SeV vector could transfer genes efficiently into the glioma cells *in vivo*, and this directly correlated with the therapeutic efficacy against the established brain tumors. Even complete elimination of the established brain tumors could be achieved in some cases by the gene therapy strategy using i.c. administration of SeV vector carrying *human IL-2* gene with s.c. vaccination. The SeV vector provided substantive expression of IL-2 protein in the glioma tissues, which would have reached a level necessary to induce significant proliferation and expansion of the peripherally activated tumor-specific T cells.

We have previously reported that transplantation of the IL-2–producing cells into glioma tissues could eliminate approximately half of the established brain tumors in animals immunized with an irradiated whole tumor cell vaccine (16). Although the amount of IL-2 produced by the cells transduced with hIL2-SeV/ΔMΔF was several times greater than that of the IL-2–producing cells utilized in the previous experiment, the cellular infiltration and the cure rate obtained with the hIL2-SeV/ΔMΔF treatment were comparable with those of the cell-mediated therapy. This result would partly be explained by the kinetics of the IL-2 expression in the animals treated with hIL2-SeV/ΔMΔF. The *in vivo* protein expression would have reached its peak 4 days or later after injection of the vector. However, because the tumor doubling time of human glioblastoma is presumed to be longer than the experimental 9L gliosarcoma model, the time lag to reach maximum IL-2 production is not considered to be a critical factor in clinical practice (21). In contrast, the main advantage of virus vector application would be the wide distribution of the transgene products achieved by a small amount of viral solution to be i.c. injected compared with the cell-mediated therapy that needs a large amount of therapeutic cells to cover the whole tumor areas in the human brain.

For the clinical application of this strategy, it would be important to verify the reasons for the difference in the therapeutic efficacy of hIL2-SeV/ΔMΔF among individual animals. This experimental brain tumor model using 9L cells has a quite stable property; the animals are 100% fatal between 18 and 25 days after inoculation and all the untreated tumors are visible by MRI on day 7 with a mean size of 8.0 ± 6.0 mm^3 (16, 17). When treated with hIL2-SeV/ΔMΔF and the vaccination, the observed tumor volumes on day 21 were not different between the eliminated tumors and the other progressing tumors. The pretreatment status of the tumors is not considered to affect the therapeutic outcome. Instead, the presumable differences in the vector injection site (i.e., the center or periphery of the tumor) would affect the efficacy because the stereotactic coordinates used for the tumor inoculation would not necessarily indicate the center of the established tumor. When used in clinical practice, we can precisely identify the center of the brain tumors by computed tomography–guided stereotactic apparatus or navigation system. Another important factor contributing to the difference in therapeutic efficacy may be the expression level of sialic acids.

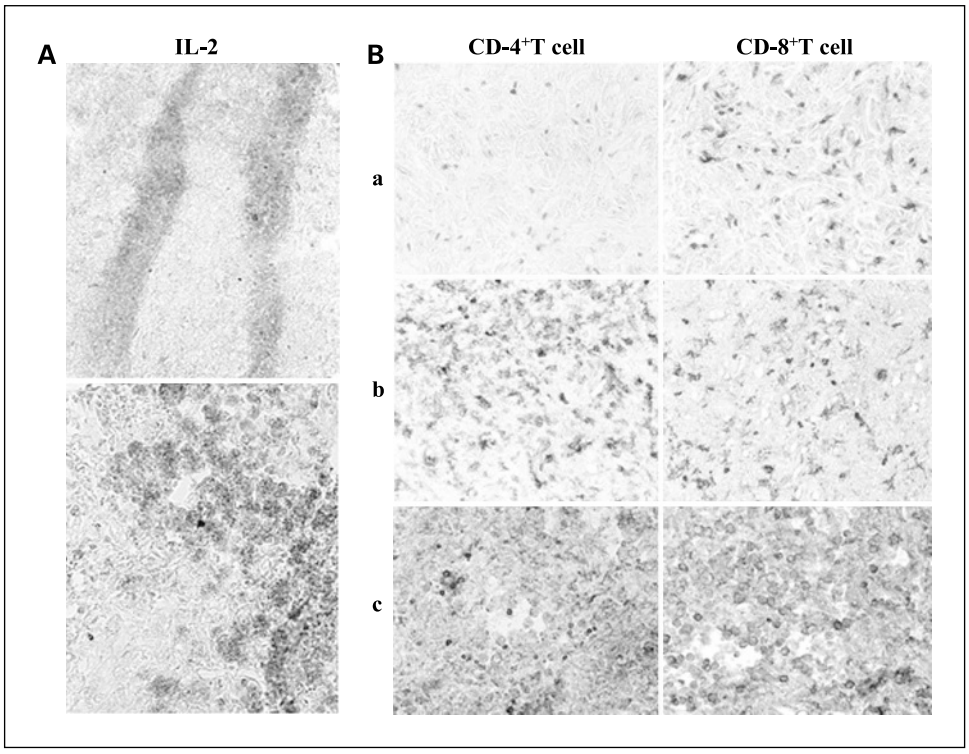

Fig. 6. *A*, immunohistochemical analysis of the expression of IL-2 in the 9L brain tumors treated with i.c. administration of hIL2-SeV/ΔMΔF. IL-2 protein is diffusely expressed. *B*, immunohistochemical analysis of the expression of CD4 and CD8 antigens in rats treated with i.c. administration of lacZ-SeV/ΔMΔF and s.c. vaccination of irradiated 9L cells (*a*), i.c. administration of hIL2-SeV/ΔMΔF alone (*b*), and i.c. administration of hIL2-SeV/ΔMΔF combined with the vaccination (*c*; ×200 magnification). Diffuse and dense infiltrations of $CD4^+$ T cells and $CD8^+$ T cells were observed in the tumors that were treated with i.c. administration of hIL2-SeV/ΔMΔF and the vaccination.

The receptor for SeV is sialic acid bound to gangliosides, which is present on most cell types including the human glia and glioma cells (22, 23). It may be altered by infiltration of the host cells in the xenograft models (24) and this probably affects the infection efficiency of hIL2-SeV/ΔMΔF and resultant efficacy.

Regarding the safety in clinical application, the biological features of SeV, such as the lack of integration into the cellular genome and the lack of homologous recombination between different SeV genomes, are desirable. Furthermore, we utilized both *M* and *F* genes–deleted SeV (SeV/MF) to enhance its safety. We previously constructed an *F* gene–deleted SeV (SeV/F; ref. 13) and an *M* gene–deleted SeV (SeV/M; ref. 14). *F* gene deletion made the SeV vector nontransmissible and *M* gene deletion worked well to make SeV incapable of forming particles from the infected cells. Although simultaneous deletions of these two genes in the same genome resulted in combining both advantages and contributed to increase the safety of the SeV vector, SeV/ΔMΔF still retains high levels of infectivity and gene expression *in vitro* and *in vivo* (i.e., similar to the wild-type SeV; ref. 15). These characteristics of SeV/ΔMΔF are considered suitable for the clinical application in gene therapy. In contrast, a theoretical obstacle for the clinical application could be the presence of antibodies against the human parainfluenza virus type I, which are known to cross-react with SeV HN proteins (4). However, it is currently not known whether a respiratory infection with human parainfluenza virus type I in the past will interfere with the transduction process of SeV vector at distant site, such as the central nervous system.

In conclusion, the present study showed that the nontransmissible recombinant SeV vector provided efficient transduction of the target genes into i.c. glioma cells. The i.c. administration of hIL2-SeV/ΔMΔF could induce a substantial production of IL-2 protein to induce the proliferation and expansion of peripherally activated, tumor-specific T cells. The therapeutic efficacy obtained by sufficient gene transfer with wide distribution and the high-grade safety of the nontransmissible recombinant SeV vector warrants clinical trials to evaluate its usefulness for human glioblastoma.

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