Supplementary Methods

Transfection Efficiency and Cytopathic Effect on rBM-DCs

The transfection efficiency of SeV/dF-GFP in rBM-DCs was evaluated. On day 7, immature rBM-DCs propagated in the presence of GM-CSF and IL-4 were collected. rBM-DCs were transfected with SeV/dF-GFP at MOI 1 to 300. Forty-eight hours after SeV/dF infection, rBM-DCs were analyzed by flow cytometry for GFP expression. Cell viability was also assessed with 7AAD to count living cells, as previously described [1].

FITC-Dextran Uptake

FITC-dextran uptake assay was performed to assess the endo-/phagocytotic activity of DCs. DCs were plated in fresh medium (1×10^6 cells/ml) and were incubated with or without rSeV/dF-GFP or LPS (1 ng/ml), and DCs were harvested at different times after stimulation. Acquired DCs were suspended in RPMI 1640 with 10% fetal calf serum and incubated with 1 mg/ml of FITC-dextran (MWt = 40,000; Sigma-Aldrich) for 30 minutes at 4 or 37°C. Cells were washed three times with ice-cold PBS, and mean fluorescent intensity of FITC was analyzed by FACScalibur (BD Biosciences, Franklin Lakes, NJ). The uptake was calculated as the change in mean fluorescent intensity between cell samples incubated at 37°C and 4°C.

Allogeneic Mixed Lymphocyte Reactions

Allogeneic mixed lymphocyte reactions were performed to assess the antigen-presenting ability of DCs. Total splenocytes were obtained from allogeneic Wistar rats. Red blood cells were lysed using VersaLyse Lysing solution and served as responder cells. *In vitro*–generated immature rBM-DCs, as well as rSeV/dF-GFP-DCs and LPS-DCs stimulated on day 7, were collected at day 9. These DCs were irradiated with 3000 rad and then used as stimulator cells. Total splenocytes freshly extracted from Copenhagen rats were also irradiated and used as control stimulator APC. Allogeneic responder cells (1×10^5 cells/wells) were cultured in triplicate in a 96-well round-bottom microplate with different numbers of stimulator APCs (APC–to–T-cell ratios were 1:1, 1:10, and 1:100). Cultures were maintained in a humidified atmosphere at 37°C and 5% CO_2. The thymidine analog bromodeoxyuridine (BrdU) was added on day 3 followed by quantitation of incorporated BrdU after a further 16 hours of culture using an ELISA-based cell proliferation kit (BrdU colorimetric, 1647229; Roche, Mannheim, Germany) according to the manufacturer's protocol.

Supplementary References

[1] Herault O, Colombat P, Domenech J, Degenne M, Bremond JL, Sensebe L, Bernard MC, and Binet C (1999). A rapid single-laser flow cytometric method for discrimination of early apoptotic cells in a heterogenous cell population. *Br J Haematol* **104**, 530–537.

[2] Shibata S, Okano S, Yonemitsu Y, Onimaru M, Sata S, Nagata-Takeshita H, Inoue M, Hasegawa M, Moroi Y, Furue M, et al. (2006). Induction of efficient antitumor immunity using dendritic cells activated by Sendai virus and its modulation of exogenous interferon-β gene. *J Immunol* **177**, 3564–3576.

[3] Yoneyama Y, Ueda Y, Akutsu Y, Matsunaga A, Shimada H, Kato T, Kubota-Akizawa M, Okano S, Shibata S, Sueishi K, et al. (2007). Development of immunostimulatory virotherapy using non-transmissible Sendai virus–activated dendritic cells. *Biochem Biophys Res Commun* **355**, 129–135.

Table W1. Primers Used for Real-time PCR Analysis.

Rat IFN-α: amplicon size = 130 bp
Forward: 5′-CCTGCCTCATACTCATAACC-3′
Reverse: 5′-GCCATCCACCTTCTCCAA-3′
Rat IFN-β: amplicon size = 84 bp
Forward: 5′-CTTTGCCATTCAAGTGATGCTC-3′
Reverse: 5′-ACAATAGTCTCATTCCACCAGTG-3′
Rat IL-1β: amplicon size = 235 bp
Forward: 5′-ATGGTCGGGACATAGT-3′
Reverse: 5′-GTGGTTGCCTGTCAGA-3′
Rat IL-12 p35 subunit: amplicon size = 235 bp
Forward: 5′-GACTTGAAGATGTACCAGTCA-3′
Reverse: 5′-GCATTAGCTCATCAATAGCC-3′
Rat IL-12 p40 subunit : amplicon size = 210 bp
Forward: 5′-TCATCAGGGACATCATCAAACC-3′
Reverse: 5′-CGAGGAACGCACCTTTCTG-3′
Rat IL-6: amplicon size = 151 bp
Forward: 5′-CAAAGCCAGAGTCCATTCAGAGC-3′
Reverse: 5′-GGTCCTTAGCCACTCCTTCTGT-3′
Rat ICAM-1/CD54: amplicon size = 71 bp
Forward: 5′-AAACGGGAGATGAATGGTACCTAC-3′
Reverse: 5′-TGCACGTCCCTGGTGATACTC-3′
Rat CCR7: amplicon size = 307 bp
Forward: 5′-TGGTTATCATCCGCACTCTG-3′
Reverse: 5′-CAGCCCAAGTCCTTGAAGAG-3′
GAPDH: amplicon size = 177 bp
Forward: 5′-TGCACCACCAACTGCTTAG-3′
Reverse: 5′-GGATGCAGGGATGATGTTC-3′

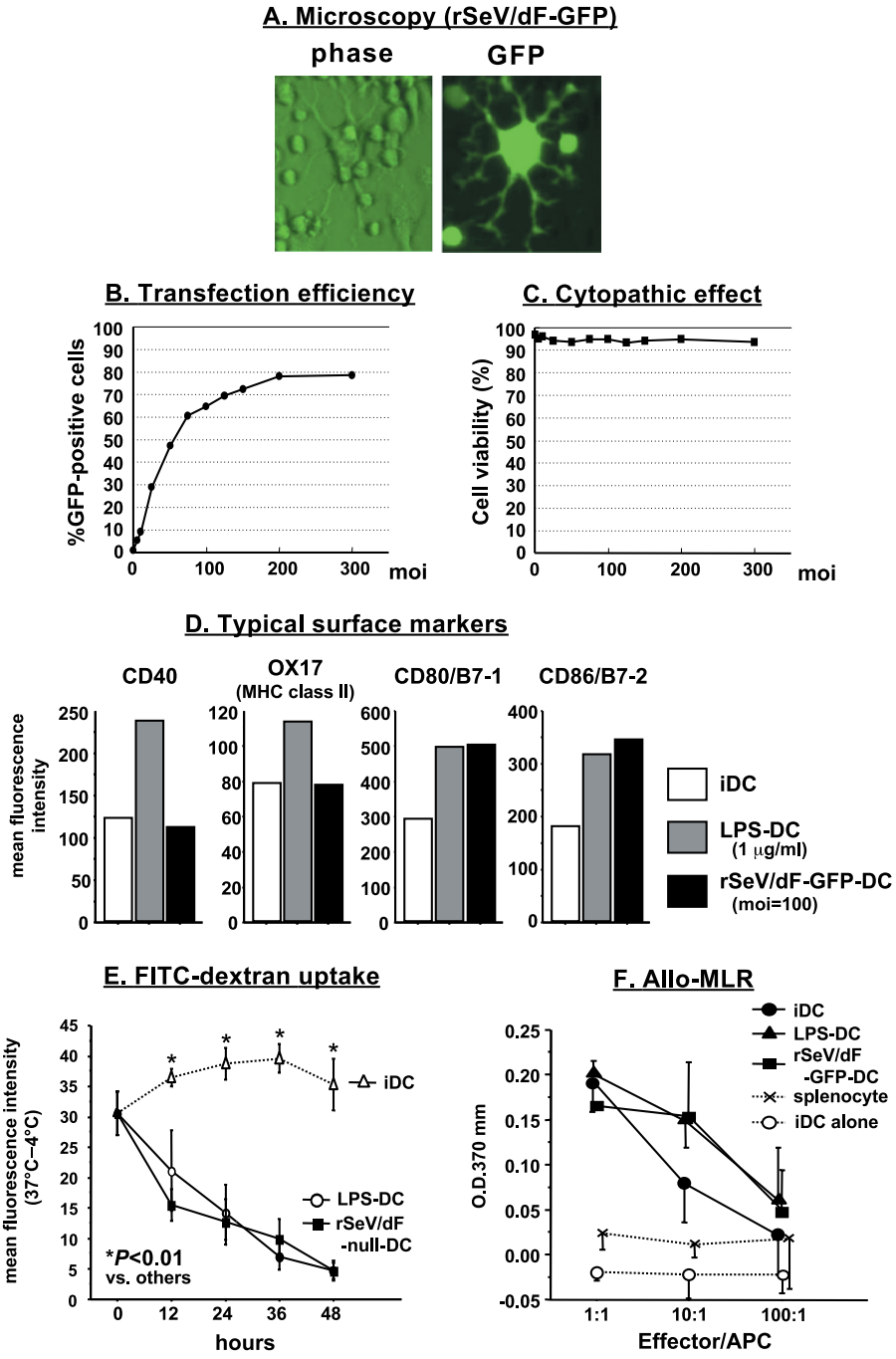

Figure W1. Characterization of rBM-DCs transfected by nontransmissible rSeV/dF-GFP *ex vivo*. Seven days after cultivation under the presence of rat IL-4 and GM-CSF for differentiating iDCs, fresh medium with rSeV/dF-GFP was added, and cells were examined for the following examinations. Panels A to D were done more than three times each, showing similar results. (A) Spontaneous maturation of rBM-DCs by transfection of rSeV/dF-GFP. Two days after transfection, cells were examined under a fluorescent microscope. Larger cells showing dendrites positive for GFP were demonstrated. (B and C) Dose-dependent transfection efficiency (B, %GFP-positive cells) and cytopathic effect (C, %cell viability assessed by 7AAD assay). Approximately 80% of iDCs could be transfected by rSeV/dF-GFP, showing no apparent cytopathic effect under MOI = 0 to 300. (D) Spontaneous up-regulation of typical surface markers (CD80/B7-1 and CD86/B7-2) 2 days after transfection of rSeV/dF-GFP (MOI = 100). LPS (1 μg/ml) was used for a positive control. Both CD80/B7-1 and CD86/B7-2 were upregulated by rSeV/dF-GFP treatment, whereas the expression of neither CD40 nor OX17 was significantly enhanced by rSeV/dF-GFP, findings that were similar to those observed in murine DCs treated with replication-competent rSeV in our previous study [2,3]. (E) Time course of FITC-dextran uptake of iDCs, LPS-DCs, and rSeV/dF–null-DCs to assess endo-/phagocytotic activity of DCs. Note the use of rSeV/dF–null instead of rSeV/dF-GFP to avoid background fluorescence. Each group contained n = 3. rSeV/dF treatment caused the loss of the endo-/phagocytotic activity of rBM-DCs, a conflicting result that was observed in case of murine BM-DCs [3], probably because of the species' specificity. (F) Allostimulatory activity of DCs. Allogeneic T cells were cultured with each DC for 48 hours, at which point allostimulation was determined by proliferation as described in Materials and Methods. No significant difference was observed among iDC, LPS-DC, and rSeV/dF-GFP-DC. Each group contained n = 3. Allo-MLR indicates allogeneic mixed lymphocyte reaction.