Supplementary materials and methods

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Splenocytes were treated with scIL-12 (100 pg/ml) and wt-HVJ-E (1.5 x 10^8 particles/ml), and 20 hours later, 10 μg/ml Brefeldin A (Sigma) was added to the splenocytes. Four hours later (24 hours after the addition of scIL-12 and wt-HVJ-E), the splenocytes were fixed and permeabilized with the BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution. The splenocytes were then treated with an anti-mouse CD16/CD32 antibody (BD Biosciences) and stained with FITC-anti-mouse CD11b (BioLegend, San Diego, CA, USA), FITC-anti-mouse CD11c (BioLegend), FITC-anti-mouse CD49b (BioLegend), FITC-anti-mouse CD3ε (BioLegend), PE-anti-mouse CD45R/B220 (BioLegend) and APC-anti-mouse IFN-γ (BioLegend).

Inhibition of the F protein by the anti-F neutralizing antibody

wt-HVJ-E (1.5 x 10^7 particles) was pretreated with an anti-F antibody or normal rabbit IgG (10 μg) in 50 μl OptiMEM (Gibco) for 30 min at 37°C. The pretreated mixture of HVJ-E and antibody was administered to splenocytes (2 x 10^5 cells/100 μl/well) with scIL-12 (10 pg/50 μl), and 24 hours later, the IFN-γ concentration of the supernatant was measured by ELISA.

Measurement of the amount of ZZ-scIL-12 in Fc-HVJ-E

In the Western blotting analysis of scIL-12-HVJ-E (3×10^7 particles) and ZZ-scIL-12 (50, 100, 150, 200 and 300 pg), ZZ-scIL-12 was detected using the HA antibody, and the density of the bands corresponding to ZZ-scIL-12 was measured using the Scion Image software program (Scion Corp., Maryland, USA).

Preparation of dendritic cells (DCs)

Murine bone marrow-derived dendritic cells were generated from female C57BL/6N mice. The bone marrow of the tibia and femur was flushed with culture medium, and the effluent was filtered through a 40-μm mesh sieve. The filtrate was washed, and the cells (1×10^6) were sedimented by centrifugation and seeded in 24-well plates in 1 ml culture medium supplemented with 10 ng/ml recombinant murine GM-CSF (R&D Systems, Inc., Minneapolis, MN, USA). The cultures were nourished every other day by gently aspirating the spent medium and adding fresh medium. Six days later, non-adherent and loosely adherent clusters of proliferating dendritic cells were used for subsequent experiments as immature dendritic cells.

IFN-γ measurement *in vitro*

Bone marrow-derived DCs (1 x 10^5 cells/100 μl/well) were seeded in 96-well plates. wt-HVJ-E, Fc-HVJ-E or scIL-12-HVJ-E in culture medium (0.3, 1.5 and 3.0 x 10^8 particles/100 μl) were added to the cells, and the culture media were collected 24 hours later. The IFN-γ concentrations of the culture media were measured using an IFN-γ ELISA assay (R&D Systems).

Generation of a mouse model of metastatic lung melanoma

A viable F10 melanoma cell suspension (5 x 10^5 cells/200 μl PBS) was intravenously injected into the tail veins of female C57BL/6N mice followed by an injection of 300 μl PBS to avoid embolization of the vessels by the F10 melanomas. On days 3, 4 and 5 after the injections were administered, the lungs were isolated from the mice, and the number of metastatic foci was counted.

Detection of serum IFN-γ

Five days after the mice were intravenously injected with the F10 melanoma cells to generate the metastatic lung melanoma model, wt-HVJ-E, scIL-12-HVJ-E (6 x 10^8 particles/200 μl PBS), ZZ-scIL-12 (500 pg/200 μl PBS) or 200 μl PBS was

intravenously injected into the tail veins of the mice once a day for three consecutive days. Blood was collected from the hearts of the mice 24 hours after the last injections were administered, and the serum was isolated from the blood by centrifugation. The IFN-γ concentration of the serum was measured using a murine IFN-γ Quantikine ELISA kit (R&D Systems).

Neutralization of IFN-γ and ^{51}Cr release CTL assay

The anti-IFN-γ antibody (clone name, R4-6A2) was a kind gift from Dr. Murakami. Mice bearing metastatic lung melanomas were generated by administering F10 melanoma cells, and the anti-IFN-γ antibody (200 μg) was peritoneally administered on days 4, 5, 6, 7, 9, 11, 14 and 17 to neutralize IFN-γ (1). Rat IgG (200 μg) was administered to the mice as a control. scIL-12-HVJ-E (6 x 10^8 particles/200 μl PBS) or PBS (200 μl) was intravenously administered on days 5, 7 and 9, and the spleens were isolated from the mice on day 19. Splenocytes prepared for the ^{51}Cr release CTL assay were mixed with ^{51}Cr-uptaking F10 melanoma cells and a bone marrow-derived stromal cell line (MSC-1) (2), and the amount of ^{51}Cr released from the cells was determined using γ-scintillation counting.

Co-administration of scIL-12 and Fc-HVJ-E

Five days after the intravenous injection of the F10 melanoma cells, PBS (300 μl), scIL-12-HVJ-E (1.5 x 10^9 particles/300 μl PBS) or a mixture of scIL-12 (1250 pg) and Fc-HVJ-E (1.5 x 10^9 particles) (in a total volume of 300 μl in PBS) was intravenously administered into the mice bearing metastatic lung melanomas. Twenty-four hours later, blood was collected from the hearts of the mice, and the serum was isolated from the blood by centrifugation. The IFN-γ concentration of the serum was measured using a murine IFN-γ Quantikine ELISA kit.

Supplementary references

1. Lee J, Nakagiri T, Oto T, Harada M, Morii E, Shintani Y, et al. IL-6 Amplifier, NF-κB–Triggered Positive Feedback for IL-6 Signaling, in Grafts Is Involved in Allogeneic Rejection Responses. J Immunol. 2012;189:1928–36.

2. Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, et al. PDGFRα-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. Proc Natl Acad Sci U S A. 2011;108:6609–14.

Supplementary figure legends

Supplementary Figure S1: FACS analysis of IFN-γ-positive splenocytes.

(A) Splenocytes were treated with scIL-12 and wt-HVJ-E, and the fraction of IFN-γ-positive cells in each population [macrophage (CD11b), DC (CD11c), NK cell (CD49b), T cell (CD3ε) and B cell (B220)] was analyzed by FACS. **(B)** The percentage of each population in IFN-γ-positive splenocytes was calculated from the FACS data.

Supplementary Figure S2: Inhibition of the F protein by the anti-F neutralizing antibody, and IFN-γ induction upon treatment with scIL-12-HVJ-E.

(A) wt-HVJ-E (1.5 x 10^7 particles) was pretreated with the anti-F antibody or normal rabbit IgG (10 μg), and the pretreated mixtures of HVJ-E were then added to splenocytes (2 x 10^5 cells) with scIL-12 (10 pg) for 24 hours. The concentration of IFN-γ in the supernatant was measured by ELISA. **(B)** Bone marrow cells were isolated from female C57BL/6N mice, and DCs were obtained from the cells after treatment with GM-CSF. wt-HVJ-E, Fc-HVJ-E and scIL-12-HVJ-E (0.3, 1.5 and 3.0 x 10^8 particles) were added to the bone marrow-derived DCs (1 x 10^5 cells) for 24 hours, and the amount of IFN-γ secreted from the cells was measured using an ELISA assay.

All data are presented as the mean ± SD (n = 3). ** P < 0.01, Tukey-Kramer test.

Supplementary Figure S3: Sucrose gradient sedimentation of ZZ-scIL-12 and wt-HVJ-E.

ZZ-scIL-12 and wt-HVJ-E were treated to fractionation by 25%-50% sucrose gradients, and the ZZ-scIL-12 and wt-HVJ-E in each fraction were detected by Western blotting.

Supplementary Figure S4: Measurement of the ZZ-scIL-12 on Fc-HVJ-E.

(A) scIL-12-HVJ-E (3 x 10^7 particles) and ZZ-scIL-12 (50, 100, 150, 200 and 300 pg) were detected using Western blotting analysis. **(B)** The densities of the bands corresponding to ZZ-scIL-12 in (A) were measured using the Scion Image software program, and the relative ratio of ZZ-scIL-12 was calculated based on the value of scIL-12-HVJ-E. **(C)** A standard curve was constructed using the relative value of ZZ-scIL-12 in (B), and the amount of ZZ-scIL-12 on Fc-HVJ-E was then calculated.

Supplementary Figure S5: Tissue localization of ΔHN-HVJ-E following intravenous injection, and generation of the murine F10 metastatic lung melanoma model.

(A) ^{125}I-labeled wt-HVJ-E or ΔHN-HVJ-E was intravenously injected into the murine

tail vein. Various tissues (brain, lungs, heart, liver, kidneys, spleen, muscles and blood) were isolated from the treated mice 24 hours after the injection, and the level of ^{125}I in the respective tissues was measured using a γ-scintillation counter. **(B)** F10 melanoma cells (5 x 10^5) were intravenously injected via murine tail veins on day 0, and the lungs were isolated from the mice on days 3, 4 and 5. On day 5, multiple metastatic foci of F10 melanomas were observed in the lungs (arrowheads).

Supplementary Figure S6: Serum IFN-γ levels induced upon the systemic administration of scIL-12-HVJ-E and the scIL-12 and Fc-HVJ-E mixture.

(A) Mice bearing metastatic F10 lung melanomas were intravenously injected once a day for three consecutive days with wt-HVJ-E, scIL-12-HVJ-E, ZZ-scIL-12 or PBS, and the serum IFN-γ levels were measured by ELISA one day after the last injections were administered. All data are presented as the mean ± SD (n = 4). Tukey-Kramer test. **(B)** scIL-12-HVJ-E (1.5 x 10^9 particles) or a mixture of scIL-12 (1250 pg) and Fc-HVJ-E (1.5 x 10^9 particles) were systemically administered to the mice bearing lung metastases, and the serum IFN-γ levels were measured by ELISA. All data are presented as the mean ± SD (n = 3). ** $P < 0.01$, Tukey-Kramer test.

Supplementary Figure S7: Measurement of scIL-12-HVJ-E-mediated CTL activation in the mice bearing lung metastases following treatment with the IFN-γ-neutralizing antibody.

Mice bearing lung metastases were generated by the intravenous injection of F10 melanoma cells, and 5 days after the inoculation, PBS (200 μl) or scIL-12-HVJ-E (6 x 10^8 particles/200 μl PBS) was intravenously injected via the tail vein for a total of three times every other day. The anti-IFN-γ neutralizing antibody or control IgG (200 μg) was intraperitoneally administered on days 4, 5, 6, 7, 9, 11, 14 and 17, and the splenocytes were isolated from the mice on day 19. The CTL activity of the splenocytes against (A) F10 melanoma cells and (B) the bone marrow-derived stromal cell line MSC-1 was measured by a ^{51}Cr release CTL assay. (PBS, n = 5; scIL-12-HVJ-E, + IFN-γ antibody and + control IgG, n = 4). All data are presented as the mean ± SD. * $P < 0.05$, Tukey-Kramer test.