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Success of measles virotherapy in ATL depends on type I interferon secretion and responsiveness

Cecilia Parrula¹, Soledad A. Fernandez^{2,4}, Kristina Landes¹, Devra Huey¹, Michael Lairmore^{1,3,4}, and Stefan Niewiesk^{1,3,4}

¹Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio

²Center for Biostatistics, The Ohio State University, Columbus, Ohio

³Center for Retrovirus Research, The Ohio State University, Columbus, Ohio

⁴Comprehensive Cancer Center, The Arthur James Cancer Hospital and Research Institute, The Ohio State University, Columbus, Ohio

Abstract

Adult T cell leukemia/lymphoma (ATL) is a highly aggressive CD4⁺/CD25⁺ T-cell malignancy caused by human T cell lymphotropic virus type 1 (HTLV-1). Previous studies in the MET-1 cell /NOD/SCID mouse model of ATL demonstrated that MET-1 cells are very susceptible to measles virus (MV) oncolytic therapy. To further evaluate the potential of MV therapy in ATL, the susceptibility of several HTLV-1 transformed CD4⁺ T cell lines (MT-1, MT-2, MT-4 and C8166-45) as well as HTLV-1 negative CD4⁺ T cell lines (Jurkat and CCRF-CEM) to infection with MV was tested in vitro. All cell lines were permissive to MV infection and subsequent cell death, except MT-1 and CCRF-CEM cells which were susceptible and permissive to MV infection, but resistant to cell death. The resistance to MV-mediated cell death was associated with IFN β produced by MT-1 and CCRF-CEM cells. Inhibition of IFN β rendered MT-1 and CCRF-CEM cells susceptible to MV-mediated cell death. Cells susceptible to MV-induced cell death did not produce nor were they responsive to IFN β . Upon infection with NDV, MT-1 and CCRF-CEM but not the susceptible cell lines up-regulated pSTAT-2. In vivo, treatment of tumors induced by MT-1 cell lines which produce IFN β demonstrated only small increases in mean survival time, while only two treatments prolonged mean survival time in mice with MET-1 tumors deficient in type I interferon production. These results indicate that type I interferon production is closely linked with the inability of tumor cells to respond to type I interferon. Screening of tumor cells for type I interferon could be a useful strategy to select candidate patients for MV virotherapy.

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Corresponding author: Dr. Stefan Niewiesk College of Veterinary Medicine Department of Veterinary Biosciences The Ohio State University 1925 Coffey Road Columbus, OH 43210-1093 Phone (614) 688-3605 Fax (614) 292-6473 niewiesk.1@osu.edu.

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Keywords

measles virus; virotherapy; HTLV; leukemia; lymphoma; mouse model

1. Introduction

Infection with human T cell lymphotropic virus type 1 (HTLV-1) leads in a minority of patients to adult T cell leukemia (ATL). ATL is invariably fatal and refractory to conventional therapies (Hermine et al., 1998). In the early stages of ATL, infection with HTLV-1 leads to transformation of CD4 + T cells through the Tax protein of HTLV-1. At later stages, additional mutations in cellular genes render CD4+ T cells tumorigenic (Matsuoka and Jeang, 2007). To evaluate novel therapeutic approaches for ATL, a mouse model was established by intraperitoneal inoculation of patient-derived ATL cells (MET-1) into NOD/SCID mice (Phillips et al., 2000) and characterized for the expression of tumor invasion factors and cytokines (Parrula et al., 2009). In this model the level of tumor burden is measured by the amount of secreted human IL-2R α chain in serum of mice. This model has been used to explore the therapeutic potential of antibodies (Phillips et al., 2000), (Zhang et al., 2003a), (Zhang et al., 2003b), (Zhang et al., 2005) and oncolytic measles virus (Parrula et al., 2011). MV is an attractive oncolytic agent because of the existence of a vaccine strain which has been administered to millions of people with an excellent safety record (Fielding, 2005). In the ATL mouse model, inoculation of just two doses of 10^6 pfu of the vaccine strain i.p. once a week was sufficient to strongly reduce tumor burden in mice (Parrula et al., 2011).

The results obtained with MV therapy in the MET-1 cell/NOD/SCID mouse model contrast with results obtained in mouse models of B-cell lymphoproliferative diseases. In xenograft studies of B-lymphoma (Grote et al., 2001) and myeloma (Peng et al., 2001), MV also caused partial or complete regression of xenografts, but higher MV titers (10^7 plaque forming units (pfu)) per dose were required for treatment. At lower doses the efficacy of MV was substantially reduced. The reason for a 10-fold difference between the MV titers required for treatment of B-cell lymphoproliferative diseases and ATL could be due to intrinsic biological differences between B cells and T cells, or genetic defects in ATL cells targeting intrinsic cellular defenses. Using vesicular stomatitis virus (VSV), it was shown that a number of tumor cell lines do not respond to type I interferon and in consequence are highly susceptible to lysis by VSV in vitro and in a mouse model (Stojdl et al., 2000). Similarly, VSV infection of ATL cells derived from patients lead to rapid destruction in vitro (Cesaire et al., 2006) and defects in the type I interferon pathway unique to ATL cells were hypothesized as the cause for the permissiveness of primary ATL cells to VSV mediated lysis (Cesaire et al., 2006). Based on these reports, the goal of this study was to determine whether success of MV treatment was due to defective type I interferon production and/or responsiveness in ATL cells.

2. Materials and Methods

2.1 Animals

Female immunodeficient NOD/SCID (NOD.CB17-Prkdc^{scid}/J) were purchased from The Jackson Laboratory (Bar Harbor, ME), and inoculated intraperitoneally (i.p.) with 2×10^7 MET-1 cells or MT-1 cells, respectively. Animals were monitored and weighed regularly after inoculation of tumor cells. Mice were sacrificed using CO₂ inhalation as soon as they developed signs of morbidity (more than 20% body weight loss). All animal experiments were approved by the Institutional Animal Care and Use Committee of The Ohio State University.

2.2 Cell lines

Jurkat (Weiss et al., 1984), CCRF-CEM (Foley et al., 1965), MT-1 (Hinuma et al., 1981), MT-2 (Miyoshi et al., 1981), (Yamamoto et al., 1982), (Koyanagi et al., 1984), MT-4 (Koyanagi et al., 1984), (Datta et al., 2001), C8166-45 (Salahuddin et al., 1983) and BJAB (Klein et al., 1974) were maintained in Advanced RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FCS). MET-1 cells were derived from a patient with ATL and expanded in NOD/SCID mice (Phillips et al., 2000). Vero cells (African green monkey) and Vero cells expressing CD150 (Ono et al., 2001) were grown in Advanced MEM medium (Invitrogen) supplemented with 10% FCS.

2.3 Viruses

Molecularly cloned measles virus vaccine strain Ed-NSE (Singh and Billeter, 1999) was grown and titered on Vero cells by plaque forming assay. Briefly, serial 10-fold dilutions of virus stock were incubated with Vero cells in 6-well plates. After incubating cells for 1 hour with viral dilutions, 0.1% agar was overlayed onto the cell monolayer. Individual plaques were counted 7 days post infection after staining cells with neutral red. Measles virus wild type strain (WTFb) was grown in BJAB cells and titered on Vero cells expressing CD150 (Ono et al., 2001). Growth and titration of Newcastle Disease Virus (NDV) has been described (Kim et al., 2011).

2.4 Enzyme-linked immunosorbent assays (ELISAs) for type I interferons

ELISA for the detection of human IFN α and human IFN β was performed using kits acquired from R&D Systems according to the manufacturer's protocol.

2.5 Cell counts after infection with MV

10^6 cells from each cell line were incubated with MV or mock treated (controls) for 2 hours at a moi of 0.1 or 1. After 24 hours, 48 hours or 72 hours post infection viable cells were counted by the trypan blue exclusion method. Assays were performed in triplicates and repeated 3 times.

2.6 Flow cytometry

For detection of CD46 and CD150 proteins on the surface of MT-1 and CCRF-CEM cells, antibodies specific for CD46 (clone E4.3) and CD150 (clone A12) directly conjugated to

FITC and PE, respectively, as well as isotype controls (clones G155-178 and MOPC-21; all from BD Pharmingen) were used for staining for 1 hour. To determine expression of MV hemagglutinin (H) on the cell surface, cells were stained with a mouse monoclonal antibody specific for measles virus H (clone K29 (Liebert et al., 1990)) or an IgG1 isotype control and subsequently stained with donkey antibodies specific for mouse IgG labeled with FITC (Abcam). Propidium Iodide Staining Solution (BD Pharmingen) was used to stain cells according to the manufacturer's protocol. Annexin V coupled to FITC (BioLegend) was used to stain cells according to the manufacturer's protocol. Cells were analyzed with a BD FACSCalibur flow cytometer.

2.7 Transient transfection of MT-1 and CCRF-CEM cells

Transient transfections of MT-1 and CCRF-CEM cells with pCAGGS-NS1 (SAM) (plasmid encoding the NS1 protein from the A/PR/8/34 strain of influenza virus (Talon et al., 2000)) were performed with an Amaxa®Cell Line Nucleofector Kit C (Lonza, Switzerland) according to the manufacturer's protocol. A pmaxGFP plasmid supplied by the manufacturer was used as a control.

2.8 Western blotting

Cells were either pelleted untreated or after overnight infection with Newcastle Disease Virus with a multiplicity of 3. Cells were lysed in 20 mM Tris-HCl (pH 7.50), 150 mM NaCl, 1% Triton, 1 mM Na₂EDTA, 1 µg/mL leupeptin, 1 mM phenylmethanesulfonylfluoride, 1 mM Na₃VO₄ and 1 mM β-glycerophosphate. Lysates were separated by SDS-PAGE electrophoresis and subsequently transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with tris buffered saline (TBS), 0.1% Tween-20 with 10% w/v nonfat dry milk and incubated with an antibody specific for Myxovirus protein (clone D-14, Santa Cruz Biotechnology), 2'-5'- oligoadenylate synthetase (clone 18-K, Santa Cruz Biotechnology), interferon response protein (IRF) 3 (ab138422, Abcam), phosphorylated STAT-2 (ab53132, Abcam) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) control antibody (clone 1D4, Assay Designs) and the respective secondary antibodies labeled with horseradish peroxidase. Blots were developed with Supersignal West Pico Chemiluminescent Substrate (Pierce Biotechnology). For quantification of pSTAT-2 expression, films were scanned and analyzed using Image Studio Software (Li-COR Biosciences). Equal areas containing the band were used to determine the relative density as the signal/background ratio. A blank lane was used as background signal.

2.9 Statistical analysis

Means of the groups of mice inoculated with MT-1 and MET-1 cells were compared using ANOVA models. Survival times (Kaplan Meier curves) between mice with MT-1 and MET-1 cell tumors treated with MV and respective control groups were compared using the log-rank test.

3. Results

3.1 Differences in susceptibility of HTLV-1 transformed CD4+ T cells to MV infection

In mice, treatment of tumors caused by MET-1 cells, a leukemic ATL cell line, caused tumor regression and increased survival of mice at doses of 10^6 pfu (Parrula et al., 2011). This contrasted with previous xenografts studies in B cell lymphoproliferative diseases where MV titers in the order of 10^7 pfu were required to cause regression of tumors. To evaluate further the sensitivity of HTLV-1 transformed cells and consequently the potential of MV treatment as a novel therapeutic approach for ATL, other human CD4+ T cell lines transformed by HTLV-1 including MT-1, MT-2, MT-4 and C8166-45 were tested for their sensitivity to MV (Ed-NSE strain) infection *in vitro*. Jurkat and CCRF-CEM served as two HTLV-1 negative control CD4+ T cell lines. One million cells of each cell line were infected with MV (Ed-NSE) at moi of 0.1, and 72 hours post infection viable cells were counted. MV infection led to a reduction of C8166-45, Jurkat, MT-2 and MT-4 cells (Figure 1). In contrast, CCRF-CEM and MT-1 cells were resistant to MV mediated cell death, and no significant differences were found between the numbers of MV infected and uninfected cells. The same was true when CCRF-CEM and MT-1 cells were infected with a MV wildtype strain (WTF) instead of the MV vaccine-related strain Ed-NSE (data not shown).

3.2 Surface expression of MV receptors on resistant cells

To investigate the cause of the apparent resistance of CCRF-CEM and MT-1 cells to MV mediated cell death, the expression of CD46 and CD150, the two MV receptors on lymphocytes, was determined on the cell surface of MT-1 and CCRF-CEM cells. Flow cytometry analysis of MT-1 and CCRF-CEM cells demonstrated high expression levels of CD46 on both cell lines, and expression of CD150 also on CCRF-CEM cells (data not shown). These findings indicate that MV infection of MT-1 and CCRF-CEM cells is not impaired at the point of entry of the virus into the cell because MV (Ed-NSE) utilizes both receptors.

3.3 Infection of cell lines resistant to MV-mediated cell death

To measure MV infection, the expression of MV hemagglutinin (H) on the surface of infected cells was analyzed by flow cytometry (Figure 2). All cells expressed MV-H but no correlation between levels of MV-H expression and resistance to MV-mediated cell death was found. All cell lines increased in staining for Annexin V, a marker of apoptosis, after infection with MV. However, the increase in susceptible cell lines (17% in MT-2, 14% in MT-4, 28% in C8166-45 and 47% in Jurkat) was higher than in the resistant cell lines (11% in MT-1 and 2.5% in CCRF-CEM). Subsequently, MT-1 and C8166-45 cells, which represent a resistant and a susceptible cell line respectively, were co-stained for MV-H and propidium iodide (PI) which is a marker of cell death. Flow cytometry analysis 72 hour post infection (Figure 3) showed that all of the MT-1 cells were positive for H, and 6% were double positive for MV-H and PI. This result shows MV is able to infect MT-1 cells but that infection causes little cell death. Flow cytometry analysis of C8166-45 cells showed that approximately 50% of the cells were positive for MV-H, and 20% were double positive for MV-H and PI. In addition, 29% of C8166-45 cells stained positive for PI only, suggesting

that cell death may occur through contact with infected cells expressing the measles virus glycoproteins.

3.4 Secretion of IFN β is responsible for resistance of MT-1 and CCRF-CEM cells to MV mediated cell death

Type I interferons (IFN) are known to induce an antiviral state in cells. To determine if type I interferons are responsible for the differences seen in cell death after MV infection, all cell lines were infected with Newcastle disease virus, which induces high levels of type I interferon. No IFN α was detectable in any of the supernatants (data not shown). IFN β was only detectable in supernatants of MT-1 cells (107 pg/mL) and CCRF-CEM cells (77 pg/mL).

The finding of IFN β in the supernatant of MT-1 and CCRF-CEM cells suggested that IFN β was responsible for the resistance of these cells to MV-mediated death. To confirm this, cells were transfected with a plasmid coding for the NS1 protein of influenza virus A/PR/8/34 (Kochs et al., 2007). NS1 protein inhibits IRF3 and blocks the activation of the type I interferon promoter. Transient transfection of MT-1 and CCRF-CEM cells with a plasmid coding for NS1 followed by infection with MV showed significant differences between infected and mock infected cells at 72 hours (Figure 4). No differences were seen between the numbers of MV infected and uninfected MT-1 and CCRF-CEM cells transiently transfected with a control plasmid expressing GFP. These findings further support the notion that IFN β secretion by MT-1 and CCRF-CEM cells is responsible for resistance against MV infection *in vitro*.

3.5 The interferon response pathway is partially impaired in IFN β secreting and non-secreting CD4 $^{+}$ T cells

To determine differences in the capacity of susceptible as well as resistant cell lines to respond to type I interferon, the expression of phosphorylated IRF-3 and STAT-2, interferon response genes 2'-5' adenylylase synthetase (OAS) and Myxovirus resistant protein (Mx) 1 was measured by Western blot. Both OAS and Mx 1 were expressed in all cell lines, except in Jurkat which expressed only Mx 1. pIRF-3 expression was not detected in any of the cell lines (data not shown). A difference between resistant and susceptible cell lines was the regulation of pSTAT-2. All cell lines expressed pSTAT-2 but only MT1 and CCRF-CEM up-regulated it after NDV infection (Figure 5). These findings suggested that the interferon response pathway in susceptible cell lines might be impaired.

To determine the functional status of the IFN response pathway all cells susceptible to MV-mediated cell death were treated with either IFN α or IFN β (5000 IU/mL). 24 hours after interferon treatment, cells were infected with MV (Ed-NSE) at a moi of 0.1 and counted one day later. C8166-45 cells were protected by type I interferon treatment (Figure 6). However, Jurkat, MT-2 and MT-4 cells were not protected by type I interferon treatment indicating that they were unresponsive to exogenous interferon.

3.6 Resistance of MT-1 and CCRF-CEM cell lines to MV-mediated cell death is overcome at higher multiplicities of infection

To assess if the resistance of MT-1 and CCRF-CEM cells to MV-mediated cell death could be surpassed with higher viral loads, 10^6 cells of each cell line were infected with MV (Ed-NSE) at a moi of 0.1 and 1. Numbers of viable cells were counted 72 hours post infection and significant differences were found between the numbers of each cell line infected at a moi of 1 and controls (Figure 7). However, similar to previous experiments, no significant differences were found between numbers of MT-1 and CCRF-CEM cells infected at a MOI of 0.1 and controls. These findings indicate that the antiviral effect of IFN β secretion, which is responsible for the resistance of MT-1 and CCRF-CEM to MV-mediated cell death, can be overcome at high viral titers *in vitro*.

3.7 Effect of IFN β expression on survival in ATL xenograft models

To test the influence of type I interferon expression on MV treatment *in vivo*, treatment with MV was compared between immunodeficient mice inoculated with MT-1 or MET-1 cells. MET-1 cells are leukemic cells derived from an ATL patient and grow only in immune deficient mice. They do not secrete IFN α or IFN β (Parrula et al., 2009). After inoculation of tumor cells, mean survival time in mice with MET-1 tumors was 18.2 days, whereas the mean survival time in mice with MT-1 tumors was 19.2 days. Consistent with previous findings (Parrula et al., 2011), two treatments with MV (10^6 pfu MV/treatment) were necessary and sufficient to prolong mean survival time by 44 days to 64.4 days in mice with MET-1 tumors. In mice with MT-1 tumors, two treatments prolonged mean survival time by only 23 days to 43.2 days. Because secretion of IFN β by MT-1 cells interferes with MV-mediated cell death *in vitro*, and a high virus dose is necessary to induce cell death, mice with MT-1 tumors were treated 8 times (twice weekly for four weeks) with 10^6 pfu MV. This treatment also prolonged mean survival time only by approximately 22 days to 42.6 days in comparison to untreated controls (Figure 8). The reduced efficacy of MV treatment in MT-1 tumors compared to MET-1 tumors even though they were treated at a higher total dose (8×10^6 pfu MV in MT-1 tumors versus 2×10^6 pfu MV in MET-1 tumors) further supports that type I interferon is associated with decreased susceptibility to MV-mediated cell death *in vitro* and *in vivo*.

4. Discussion

Type I interferons (IFN α and IFN β) are a major components of the innate anti-viral immune response (for review (Zuniga et al., 2007)). They induce an antiviral state which interferes with transcription and translation of viral proteins and consequently viral replication. During tumorigenesis many mutations in cellular genomes arise which may also affect the interferon system. The results of this study show that all cell lines demonstrated some defects in their type I interferon pathways. However, susceptible cells had more defects and these were functionally important. MT-1 and CCRF-CEM up-regulated pSTAT-2, produced IFN β and were resistant to MV-mediated cell death. In contrast, C8166-45, Jurkat, MT-2 and MT-4 cells did not up-regulate pSTAT-2, produced neither IFN α nor β and were susceptible to MV cell death. These results are comparable to those obtained in sarcoma cell lines differing in their susceptibility or resistance to MV infection in tissue culture. It was

found that resistant cell lines strongly up-regulated pSTAT-1 whereas susceptible cell lines exhibited no or a weak response (Berchtold et al., 2013). Similar to our results, some of the susceptible cell lines became resistant to MV infection after interferon treatment. However, it was not evaluated how these *in vitro* differences relate to the *in vivo* situation.

It has been reported that wild type MV strains are less sensitive to the antiviral effects of type I interferon (for review (Fontana et al., 2008)), but in this study no differences were seen after infection with MV vaccine and wild type strains of MT-1 and CCRF-CEM cells. However, a ten-fold increase in vaccine virus load was able to overcome protection by type I interferon *in vitro*. Based on the *in vitro* data, animal experiments were designed to treat tumors after inoculation of an IFN β secreting cell line (MT-1) and non-secreting (MET-1) ATL leukemic cells. Mice with both MET-1 and MT-1 tumors showed prolonged survival after treatment with MV. However, even after eight treatments with MV, the survival in mice with MT-1 tumors was shorter than in mice with MET-1 tumors treated only twice with MV. These results show that type I interferon interferes with MV treatment *in vivo* as well as *in vitro*. Type I interferon secretion may also be responsible for the reported requirement of higher MV doses to induce regression of B-cell lymphomas and myelomas in mice, since the DoHH2 and Raji B lymphoma cell lines (Garbuglia et al., 2007), and the ARH 77 (Hoshino et al., 1983), (Haralambieva et al., 2007) and RPMI 8226 (Adolf and Swetly, 1982), (Haralambieva et al., 2007) myeloma cell lines used in previous studies have been reported to produce type I interferon which might lead to resistance to MV treatment.

The current study demonstrates that not all HTLV-1 transformed CD4⁺ T cell lines are equally susceptible to MV treatment, and supports that susceptibility depends on type I interferon secretion. MV treatment of tumor cells which lack type I interferon secretion is more efficient *in vitro* and *in vivo*. Therefore screening of tumor cells from patients for the expression of type I interferon (as a marker for overall type I interferon responsiveness) might be a good strategy to select candidate patients for virotherapy using measles virus.

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Highlights

CD4 T cell tumor lines differ in their type I interferon responsiveness

Functionally, this leads to differences in susceptibility to measles virus virotherapy

These differences are not only observed in vitro but are also reflected in a mouse model

These findings promise to help to refine virotherapy by analyzing leukemic cells from patients

Figure 1. Effect of MV infection on human CD4⁺ T cell lines transformed with and without HTLV-I

The graph shows the percentage of viable cells ($\pm$ SD) at 72 hours post MV infection relative to controls which are set as 100% (representative of four experiments). The numbers of Jurkat, MT-2, MT-4 and C8166-45 cells infected with MV (Ed-NSE, moi of 0.1) were significantly reduced at 72 hours post infection but no significant differences were found in the numbers of CCRF-CEM and MT-1 cells. * indicates a difference of $p < 0.05$ between infected and uninfected cells. Virus growth in all cell lines was similar ($1-3 \times 10^5$ pfu/ml after 72 hours, with the exception of MT-4 (7×10^5 pfu/ml after 72 hours)).

Figure 2. Analysis of measles virus hemagglutinin expression on human CD4⁺ T cells lines resistant and susceptible to MV-mediated cell death

MV hemagglutinin expression was found on the surface of cell lines which are susceptible to MV mediated cytolysis (C866-45, Jurkat, MT-2, MT-4) as well as cells which were resistant (CCRF-CEM and MT-1). Cells had been infected with MV (moi 0.1) for 72 hours. The experiment was performed twice with similar results.

Figure 3. Analysis of measles virus infection and cell death in MT-1 and C8166-45 cells
Co-staining of MT-1 and C8166-45 cell lines for MV hemagglutinin (H) and propidium iodide (PI, marker of apoptosis) after MV (Ed-NSE) infection (moi of 0.1) for 72 hours relative to mock infected cells. Although all of the MT-1 cells are positive for MV-H, only 6% are simultaneous positive for MV-H and PI. In contrast, a high percentage of infected C8166-45 cells also express PI, as does a high percentage of non-infected cells. The experiment was performed twice with similar results.

Figure 4. Type I interferon inhibition renders MT-1 and CCRF-CEM cells susceptible to MV-mediated cell death

MT-1 and CCRF-CEM cells were transfected with a plasmid expressing green fluorescent protein (GFP) or the nonstructural protein 1 of influenza virus A (NS1). Transfection efficacy was monitored by GFP expression (50-70%) and Western blot for NS1 (not shown). At 24 hours post transfection cells were infected with MV (Ed-NSE with an moi of 0.1) and at 48 and 72 hours post infection cell viability (expressed as percentage $\pm$ SD of mock infected controls at 0 hours) was evaluated. Transfection with NS1 led to significant reduction in cell viability at 72 hours after infection with MV in CCRF-CEM and MT-1 cells (* indicates $p < 0.05$). One of three similar experiments.

Figure 5. CD4⁺ T cell lines susceptible to MV-mediated cell death do not upregulate pSTAT-2 after NDV infection

All cell lines were either left untreated (–) or infected (+) overnight with NDV, and subjected to Western blot analysis for pSTAT-2 expression. CCRFCEM and MT1 cells up-regulated pSTAT-2 expression after NDV infection ($p < 0.05$ or lower), whereas C8166-45, Jurkat, MT-2 or MT-4 cells down-regulated pSTAT-2 ($p < 0.05$ or lower). Loading of samples was done according to protein content and confirmed by glyceraldehyde 3-phosphate dehydrogenase Western blot (not shown). 5a shows one Western blot, 5b the densitometry analysis of three blots. For densitometry, densities were normalized to GAPDH and MT-2 was arbitrarily set as 100%.

Figure 6. CD4⁺ T cell lines susceptible to MV-mediated cell death do not respond to type I interferon treatment

The graph shows the percentage of viable cells ($\pm$ SD) relative to controls which are set as 100% (representative of three experiments). C8166-45, Jurkat, MT-2 and MT-4 were treated with either IFN α or IFN β (5,000 IU/mL) for 24 hours. Subsequently, a slight (not significant) decrease in MV-mediated cell death (Ed-NSE, moi of 0.1 for 24 hours) was observed for C8166-45 cells but not for Jurkat, MT-2 or MT-4 cells (* indicates a significant reduction in cell numbers through infection; $p < 0.05$).

Figure 7. MT-1 and CCRF-CEM cells are susceptible to measles virus infection at high multiplicity of infection

MT-1 cells and CCRF-CEM cells were infected with MV (Ed-NSE) at a moi of 1. After 72 hours post infection cell numbers ($\pm$ SD) were significantly lower (* indicates $p < 0.05$) then in cells infected with MV (Ed-NSE) at a moi of 0.1 or mock-infected cells (one of two experiments).

Figure 8. MV treatment is less efficient in mice inoculated with tumor cells expressing type I interferon

Mice (5 per group) were inoculated intraperitoneally with MT-1 or MET-1 tumor cells and either infected intraperitoneally with MV (Ed-NSE; black line) or left untreated (dashed line). Treatment with MV significantly increased survival time in mice with MT-1 and MET-1 tumors when compared to no treatment controls (p-value = 0.0026, p-value = 0.0022 and p-value = 0.0021, log-rank test). In untreated mice with MET-1 tumors the mean survival was 18.2 days, and the mean survival in mice treated with two MV treatments (10^6 pfu/treatment) was 64.4 day. Mean survival in untreated mice with MT-1 tumors was

19.2 days, whereas the mean survival in mice treated with two times with MV was 43.2 days and in mice treated eight times (10^6 pfu/treatment) was 42.6 days.