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Oncolytic virotherapy of cancer with vesicular stomatitis virus

Review Article

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Abbreviations: adult T-cell leukemia, (ATL); CARD adaptor inducing IFN-β, (CARDIF); chronic lymphocytic leukemia, (CLL); double-stranded, (ds); hepatitis C virus, (HCV); human rhinovirus, (HRV); interferon regulatory factor, (IRF); interferon-β stimulator 1, (IPS-1); Interferon, (IFN); internal ribosome entry site, (IRES); mitochondrial antiviral response, (MAVS); plasmacytoid dendritic cells, (pDCs); retinoic acid inducible gene I, (RIG-I); single-stranded, (ss); virus induced signaling adapter, (VISA)

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Summary

Recent basic and pre-clinical studies have demonstrated that several innocuous, non-disease causing, replication competent viruses can selectively replicate in and kill a large panel of human tumor cells, clear bone marrow of leukemic cells and effectively arrest metastatic spread of tumors, while apparently sparing normal cells. These studies have revived interest in the clinical applicability of these oncolytic (onco=cancer; lytic=killing) viruses. Although it is as yet unknown why oncolytic viruses preferentially target and kill tumor cells, it appears that during the evolution of malignancies, genetic abnormalities accumulate that, while providing the cancer cells with growth and survival advantages, compromise the normal antiviral program of transformed cells. Defects in the Interferon (IFN) antiviral signaling network within transformed cells have been implicated in preferential oncolysis; according to this model, IFN-related defects allow VSV and other oncolytic viruses to replicate to high titers, uninterrupted by the host antiviral response, resulting in high virus production and virus induced lysis. The goal of our research program is to understand the molecular basis of virus-induced oncolysis and to identify important changes in the genetic response to virus infection in tumor cells. Basic, pre-clinical and clinical studies will attempt to harness what we believe is a potent new form of cancer therapy with promise in many areas of cancer treatment.

I. Introduction

An ideal cancer therapeutic will selectively kill malignant cells while leaving normal tissues intact. Unfortunately, the current standards of care for cancer - chemotherapy and radiation therapy - fall well short of this goal. It is imperative that emerging knowledge of the molecular biology of cancer be used to generate novel therapeutics that are targeted specifically to cancer cells. An excellent example of this approach has been the design or selection of replication selective oncolytic viruses that exploit genetic defects commonly found in tumor cells (Bell et al, 2002; Hawkins et al, 2002; Kruyt and Curiel, 2002). These same viruses are unable to replicate efficiently in normal tissues and thus have a superior therapeutic index. Malignant cells, by virtue of the constellation of genes they do or do not express, can be unique niches for the growth of Oncolytic Viruses. As an example, we have evidence that the genetic abnormalities contributing to the malignant phenotype also compromise the innate antiviral programs of these same cells. Malignant cells with defects in: IFN induction (IRF-3 and IRF-7), activation of IFN-inducible genes such as PKR,

and deficiencies in IFN-mediated signaling pathways mediated by STAT1 and IFNAR are susceptible to virus oncolytic activity (Balachandran et al, 2000; Lu et al, 2000; Matin et al, 2001; Jackson et al, 2003; Caras et al, 2004; Murad et al, 2005). Our goals are to understand virus-host cell interactions in a comprehensive manner, exploit this knowledge to selectively target tumor cells, and develop oncolytic viruses as therapeutics.

II. Canadian oncolytic virus consortium

With the support of the National Cancer Institute of Canada, a program to accelerate the development of candidate oncolytic virus therapeutics has been initiated. Candidate viruses at all stages of development are being studied; it is the goal of this consortium to combine scientific and clinical expertise to foster the continuous generation of new information, therapeutics and clinical approaches. The members of the Consortium are: OTTAWA REGIONAL CANCER CENTRE - Drs. John Bell, Earl Brown and Harry Atkins MCGILL UNIVERSITY - Drs. Nahum Sonenberg and John Hiscott TOM BAKER CANCER CENTRE, UNIVERSITY OF CALGARY - Drs. Peter Forsyth and Don Morris ROBARTS RESEARCH INSTITUTE, UNIVERSITY OF WESTERN ONTARIO: Dr. Grant McFadden.

III. Virus platforms

Several replicating virus platforms are being developed concurrently. Some candidates (eg. Myxoma and Human Rhinovirus) are at early developmental stages, others are poised to launch into clinical trials (eg VSV) while a third group has already passed into phase 1 studies (NDV and Reovirus). In the following we present a brief overview of the biology of each of the virus platforms.

A. Vesicular Stomatitis Virus

VSV is an enveloped virus with a single stranded RNA genome that encodes only five proteins. The G or glycoprotein is found on the surface of the virion and mediates cell surface attachment through a ubiquitous cellular receptor that minimally contains sialic acid. The Nucleocapsid protein (N), the phosphoprotein (P) and polymerase or L protein function in unison to bring about the production of viral mRNAs and subsequently constitute the viral genome replication complex (Wagner, 1996). The Matrix or M protein is a sophisticated multi-functional protein that was initially thought to serve only a structural role in virus assembly but is now known to act as a critical player in the oncolytic activity of VSV (von Kobbe et al, 2000; Petersen et al, 2001; Terstegen et al, 2001 Yuan et al, 2001; Ahmed et al, 2003; Stojdl et al, 2003; Faria et al, 2005) **(Figure 1)**. VSV has many virtues that make it an excellent therapeutic candidate: (1) VSV rapidly and effectively kills a wide range of tumor cells; (2) From a commercial point of view, VSV is easy to produce, is physically stable and can be purified in high concentrations; (3) VSV is not a human pathogen and most humans have never been infected nor have pre-existing neutralizing antibodies that could limit clinical application; (4) the virus is amenable to genetic manipulation because of a relatively efficient recombinant system, a feature that can be exploited in the generation of novel recombinant VSV vectors **(Figure 1)**.

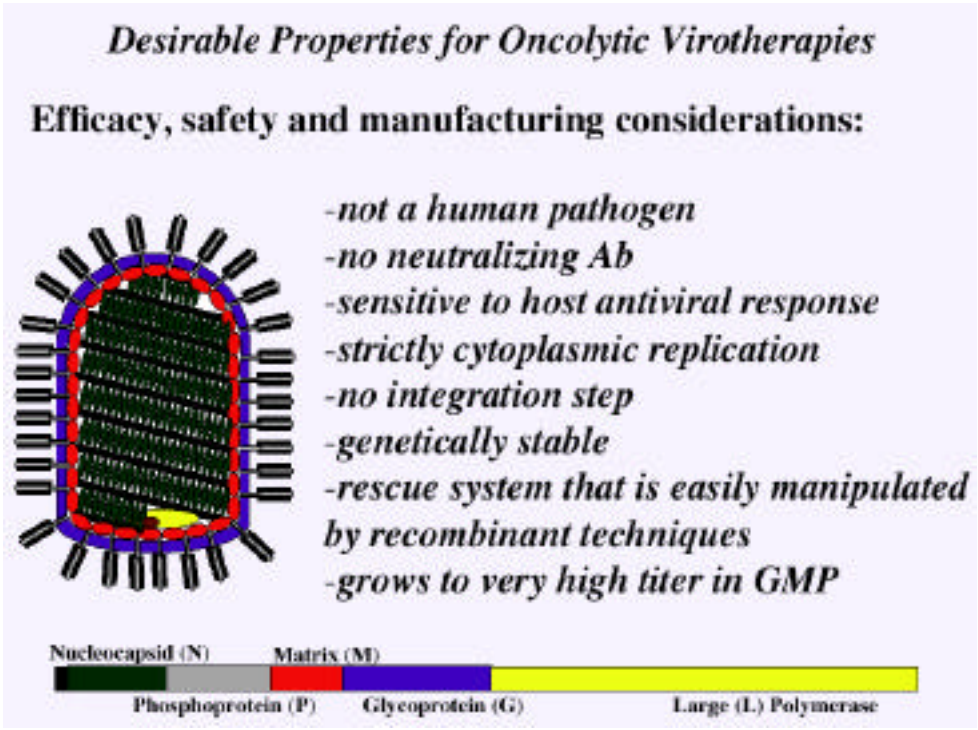

Figure 1. Desirable properties of oncolytic virotherapies and VSV subgenomic mRNA. VSV mRNA's encode for five distinct proteins: nucleocapsid (N), phosphoprotein (P), Matrix (M), Glycoprotein (G), and large (L) polymerase.

NDV is a single stranded negative sense RNA virus encoding six gene products similar in some ways to VSV described above (de Leeuw and Peeters, 1999). As a member of the paramyxovirus family, it seems likely that NDV derives its oncolytic specificity from tumor specific defects in the IFN pathway. NDV has a long history as an oncolytic virus and perhaps has been administered to more humans in one form or another as a cancer therapeutic than any other virus product. Two recent trials held in Canada (Ottawa and Hamilton) are particularly important. In these studies, both safety and efficacy were attained using high doses of virus administered slowly. NDV is also amenable to genetic manipulation.

C. Reovirus

Reovirus is a double-stranded RNA (dsRNA, non-enveloped) virus with a segmented genome composed of ten individual linear dsRNA molecules (Rux and Burnett, 2004). As the genome is segmented, it is possible to exchange genome segments and therefore biological properties between reovirus strains. Co-infection of cells with two parental strains of reovirus yields progeny viruses, termed reassortants, that possess novel combinations of the ten genome segments and novel biological properties (Nibert et al, 1996). Mammalian reoviruses have broad host ranges, replicating in a variety of human and animal cell lines but are not associated with any human disease.

D. Myxoma virus

Myxoma virus, is a poxvirus (double stranded DNA, enveloped) that induces lethal myxomatosis in rabbits and was utilized in the early 1950s to control rabbit populations in Australia (Cameron et al, 1999). As part of the Australian regulatory process to certify this eradication program, the virus was tested for pathogenicity in a wide range of vertebrates, including the three lead investigators themselves, to prove that the virus was completely rabbit specific (Kerr and Best, 1998). The virulence of this virus in the rabbit model has been extensively studied and a wide variety of virus-encoded immunomodulatory genes have been discovered. It was recently shown that myxoma virus, although unable to replicate in normal human cells, grows to high titer in a number of tumor cell lines, particularly those harboring defects in the STAT signaling pathway (Barrett et al, 2001; Masters et al, 2001).

E. Human Rhinoviruses

HRVs are non-enveloped, positive sense, single stranded RNA viruses. Recent data has demonstrated that exchange of the poliovirus internal ribosome entry site (IRES) with its counterpart from HRV type 2 resulted in attenuation of virulence and slow virus propagation in cells of neuronal origin (Campbell et al, 2005). In contrast, malignant glioma cells maintained either in tissue culture or as xenografts in athymic mice remained highly susceptible to the infection with the recombinant virus (Gromeier et al, 2000). These studies are consistent with tumor-specific function of the HRV IRES and applicability of HRV for cancer therapy. An exciting prospect is that human rhinovirus (HRV, or common cold viruses) may be a novel candidate virus in treating neoplasia. An important consideration in favoring the use of HRV is that this virus is extraordinarily harmless and causes either no symptoms or very mild upper respiratory tract-restricted infections.

F. Molecular virology of VSV

VSV is an arthropod borne virus that primarily infects cattle, swine and horses, although infection of humans and other species can also occur (Mead et al, 2000). Because VSV produces an acute disease in cattle characterized by ulceration of the oral cavity and feet, the pathology mimics the early symptoms of foot and mouth disease virus (Flanagan et al, 2001). Naturally occurring human infections with VSV on the other hand are extremely rare, except in cases where individuals are exposed to infected livestock or in researchers exposed within the laboratory environment. Most VSV infections are asymptomatic in humans or cause mild flu-like symptoms.

VSV contains a single-strand RNA genome of negative polarity (11kb) that is completed protected by viral nucleoprotein (Wagner, 1996). VSV synthesizes five subgenomic mRNAs that encode the five distinct proteins **(Figure 1)**: the nucleoprotein in conjunction with the phosphoprotein, the large polymerase protein and specific host proteins is responsible for both viral transcription and replication; the viral glycoprotein is necessary for budding, viral binding to target cells; and the multi-function matrix protein which consists of 229 aminoacids has an important role in virus assembly, budding, cellular apoptosis and disruption of the host cell innate immunity programs (Barr et al, 2002). The VSV glycoprotein serves to bind to the surface of the host cell and to fuse viral and cellular membranes enabling the release of the viral genome and replicase into the cytoplasm. The glycoprotein binds to phosphatidylserine, a universal component of the cell surface membranes, thus enabling VSV to infect virtually all animal cells (Barr et al, 2002). This extensive tissue tropism therefore enables VSV to be used as anti-cancer agent in all types of tumors, although normal tissues can also be infected.

The matrix-protein of VSV functions in diversified roles to control VSV replication and pathogenesis; matrix protein partially regulates transcription of VSV genes by the virally encoded polymerase and in late infection, catalyzes the generation of inactive RNP cores preparing them for packaging into virions (Carroll and Wagner, 1979; Clinton et al, 1979). In addition to its role in budding, matrix has a crucial role in the early phases of viral infection by helping VSV to avoid the cellular antiviral programs. The interruption of cellular transcription programs and the blockade of mRNA export from the nucleus are both targeted by matrix. The inhibition of cellular transcription by matrix protein has been demonstrated in several ways: matrix-protein expression causes an inhibition of transcription of host genes whether transcribed by RNA polymerase I, II or III (Ahmed and Lyles, 1998; Ahmed et al, 2003). Matrix protein of VSV blocks nucleocytoplasmic transport which involves an interaction between matrix protein and the

cellular Nup98, one of the nucleoporins present at the nucleopore. Interestingly, Nup 98 is an interferon responsive gene and pre-treatment of cells with interferon increases (Petersen et al, 2000, 2001; von Kobbe et al, 2000). Nup 98 expression and reduces the ability of VSV matrix protein to inhibit nucleocytoplasmic transport (Enninga et al, 2002). Mutations in matrix protein that abolish the ability of the protein to block host cell transcription and restore nucleocytoplasmic transport suggest that these two activities of the matrix-protein are not mutually exclusive. Infection of susceptible cells with VSV leads to cell rounding in ultimately to cell death by apoptosis. The induction of apoptosis by matrix protein probably results from the blockage of host cell gene expression since mutations in matrix that abrogate this blockade also reduce its cytotoxicity (Kopecky et al, 2001; Kopecky and Lyles, 2003).

The majority of tumor-derived cells are either non responsive to interferon treatment or develop resistance with time **(Figure 2)**. The natural preference of the virus for transformed cells can be seen both *in vitro* and *in vivo* where VSV will preferentially target and replicate in tumors implanted in rodents (Stojdl et al, 2003). The ability to VSV to infect malignant cells because of the impairment of the interferon response has also been demonstrated for primary cells from patients **(Figure 3)**. In a recent study, we investigated the ability of VSV to lyse primary CD4+ T lymphocytes from patients with adult T cell leukemia (ATL) and compared the ability of VSV to infect and lyse normal CD4+ T cells (Cesaire et al,

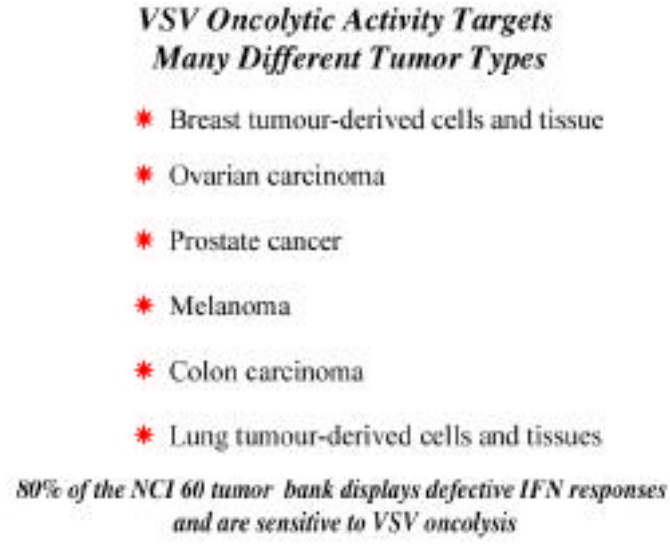

Figure 2. Induction of interferon by VSV protects normal cells from viral infection, whereas transformed cells are eliminated. VSV infection in normal cells generates type interferons that establish an anti-viral state protecting neighboring cells from VSV infection. Transformed cells are non-responsive to interferon due to defects in the interferon pathway and remain susceptible to infection and killing by VSV.

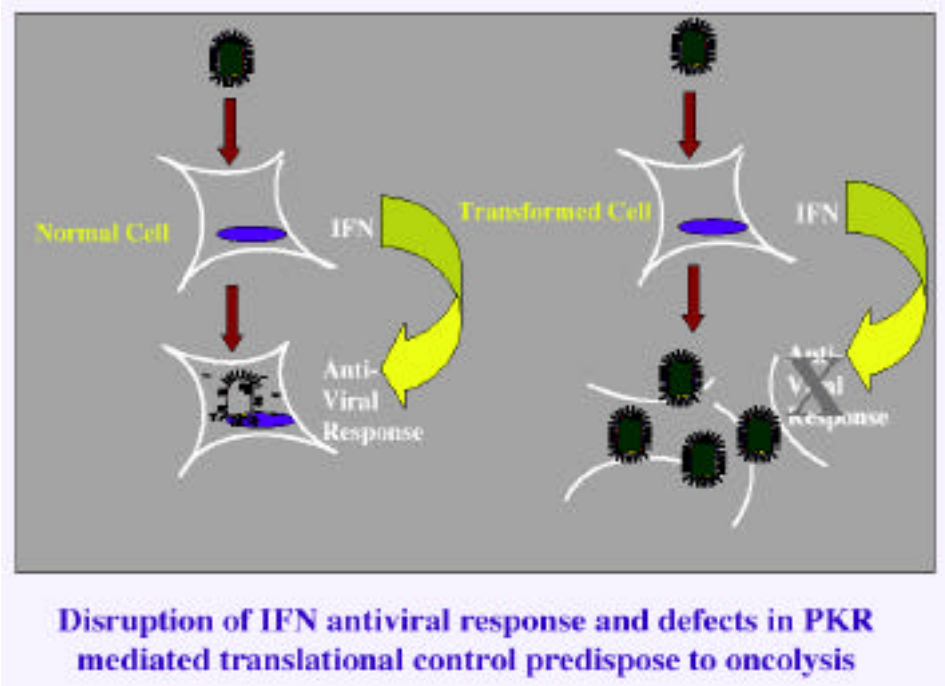

Figure 3. VSV oncolytic activity in different malignant tissue. VSV has the ability to infect and kill malignant cells from different tissue due to various defects in the interferon response in primary cells.

2005). *Ex vivo* primary ATL cells were permissive for VSV and underwent rapid oncolysis in time-dependent manner. Importantly, VSV infection showed neither viral replication nor oncolysis in HTLV-1 infected, non-leukemic cells from patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), in naive CD4+ T lymphocytes from normal individuals or in *ex vivo* cell samples from patients with chronic lymphocytic leukemia (CLL). Interestingly, activation of primary CD4+ T lymphocytes with anti-CD3/CD28 monoclonal antibody, and specifically with anti-CD3, was sufficient to induce limited viral replication and oncolysis. However, at a similar level of T cell activation, VSV replication was increased 4-fold in ATL cells, compared to activated CD4+ T lymphocytes, emphasizing the concept that VSV targets genetic defects unique to tumor cells to facilitate its replication. These findings have provided the first essential information for the development of a VSV-based treatment for ATL (Cesaire et al, 2005). Although treatments for hematological malignancies have improved considerably over the past decade, the growing therapeutic arsenal has not benefited adult T-cell leukemia (ATL) patients who are completely refractory to conventional chemotherapeutic regimens. The possibility of a novel virotherapy approach to hematological malignancies such as ATL illustrates the potential for this technology, particularly under circumstances where delivery and cost effectiveness may be practical issues.

Stojdl *et al* have identified what may be considered "second generation" oncolytic variants of VSV – naturally occurring, interferon inducing VSV mutants originally identified by reduced plaque size on cells able to produce and respond interferon. Mutations to the matrix protein render these viruses interferon-inducing and prevent the matrix protein from blocking nucleocytoplasmic transport and inhibiting host cell transcription. Wild-type and mutant strains of VSV are both able to induce the expression of the gene encoding interferon, but the mutant viruses fail to block the export and translation of the interferon mRNA (Ahmed et al, 2003; Stojdl et al, 2003). The induction of interferon and other antiviral genes by these viruses generates what is been termed a 'cytokine cloud' that protects the host not only from the mutant virus but also from any wild-type virus present in the innoculum. The strategy produces an effective oncolytic virus that is less toxic than a recombinant virus engineered to express interferon.

Many advantages to VSV argue that this virus may be a superior oncolytic virus: VSV grows to high titer in bioreactors and is very well characterized at the molecular level in the mammalian cell cultures. VSV is a relatively innocuous virus that even in its most virulent state causes a very mild disease in cattle and humans. It is unlikely that most humans have come into contact with VSV and therefore do not have pre-existing immunity. The virus replicates quickly *in vivo* and maybe be able to mediate a significant or complete tumor response before the patient develops an acquired immunity to the virus. Cytoplasmic replication and genetic stability also preclude problems associated with integrating viral vectors **(Figure 1)**.

IV. Oncolytic viruses and the IFN antiviral pathway

Direct and indirect lytic effects of some viruses have been exploited recently in a novel virus-based approach to biological therapy of cancer, involving oncolytic viruses (Chiocca, 2002). The enveloped, negative strand RNA virus – vesicular stomatitis virus (VSV) - has been added to a growing list of viruses with nonpathogenic, oncolytic properties that include: Reo, Paramyxo and Rhinoviruses (Chiocca, 2002). VSV infection selectively killed a large panel of human tumor cell lines (**Figure 3**) including 80% of the NCI 60 tumor cell bank**,** cleared bone marrow of leukemic AML cells (Stojdl et al, 2000) and effectively arrested metastatic spread of CT26 lung metastases in immunocompetent animals **(Figures 4 and 5)** (Stojdl et al, 2003). Although VSV fails to replicate efficiently in primary cells that contain a functional interferon system (Stojdl et al, 2000), this virus replicates to high titers in the majority of immortalized and transformed cell lines. The current hypothesis is that aspects of IFN signaling and the action of downstream effectors are compromised in such malignant cells, thus affording a cellular environment that facilitates viral replication - uninterrupted by the host antiviral response - resulting in virus induced lysis.

The Type 1 IFNs - a family of antiviral cytokines composed of IFNβ and several IFNα species - induce a cascade of events through the activation of signaling mediated by the Jak STAT pathway, resulting in the production of hundreds of proteins that function to limit viral replication and signal adaptive immune responses (Malmgaard, 2004). IRF-3 and IRF-7 are critical mediators of IFN gene activation, with complementary roles in the induction of the host antiviral state following virus infection (Au et al, 1998; Lin et al, 1998, 1999a; Marie et al, 1998; Sato et al, 1998, 2000; Wathelet et al, 1998; Weaver et al, 1998; Yoneyama et al, 1998; Mamane et al, 1999). IRF-7 activation is one (of many) steps that may be defective in VSV infected cells, although IFN inducibility can be restored using the attenuated variant of VSV. Other studies demonstrate that translation control downstream of PKR activation, frequently dysregulated in transformed cells, can cooperate with the attenuated IFN antiviral activity to facilitate VSV oncolysis. Elevated levels of eIF2Bε are required for increased permissiveness of transformed cells to VSV replication. Cells transfected with siRNA against eIF2B were almost completely protected against VSV-induced cytolysis and produced approximately 10-fold less virus than control cells (Balachandran et al, 2004) .The oncolytic activity of VSV is effective against tumors exhibiting aberrant p53, Ras, or Myc function (Balachandran et al, 2001).

Upon recognition of specific molecular components of viruses or other pathogens, the host cell activates multiple signaling cascades through Toll-like receptor-dependent and -independent pathways, culminating in the

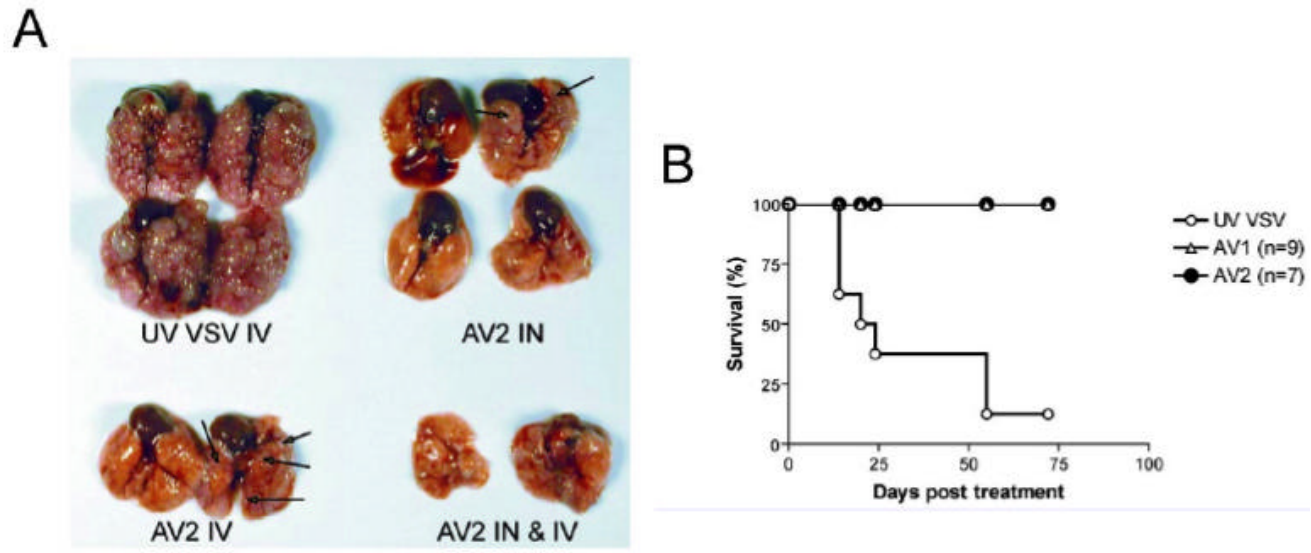

Figure 4. Eradication of CT26 lung metastases following intravenous or intranasal VSV inoculation in mice. A. Lung tumors were established by injecting 3 x 105 CT26 cells into the tail vein of Balb/C mice. Four days after treatment with intranasal and intravenous inoculation with AV1 and AV2, all mice were sacrificed and their lungs were removed. Arrows indicate residual tumors (Stojdl et al, 2003). **B.** Lung tumors were established as described above. On day 12, mice received 5 x 107 pfu of AV1 or AV2 by intranasal instillation every other day for 2 weeks (6 doses total), "n" denotes number of mice in treatment group (Stojdl et al, 2003).

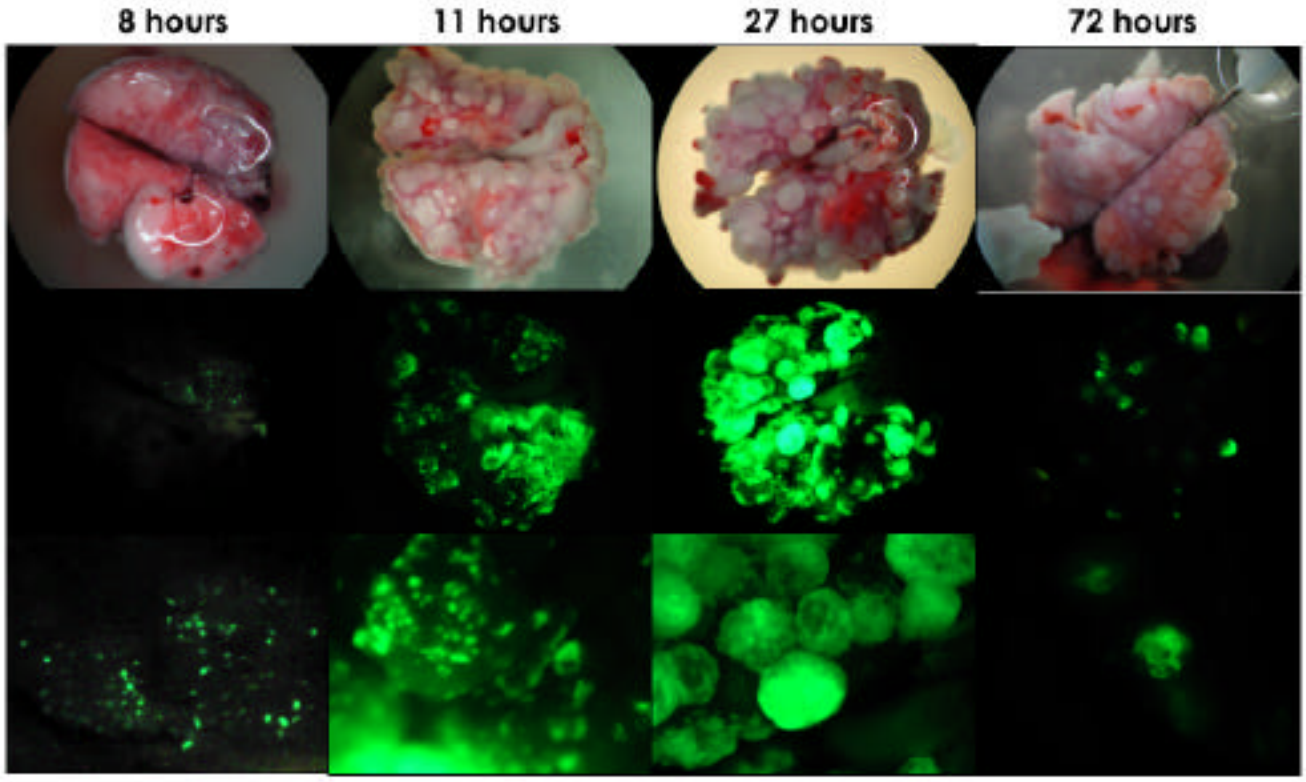

Figure 5. VSV-GFP time course in CT26 lung metastasis model. Mice bearing CT-26 lung tumors were infected with VSV-GFP, and the lungs were removed and visualized at different time intervals by fluorescent microscopy (Stojdl et al, 2003).

production of cytokines and chemokines that disrupt virus replication and initiate innate and adaptive immune responses (Malmgaard, 2004; Samuel, 2001; Sen, 2001). Rapid induction of type I IFN expression is a central event in establishing the innate antiviral response and requires pathogen-inducible, activation of transcription factors that function in a synergistic fashion to induce gene expression (reviewed in (Hiscott et al, 1999; Levy et al, 2002; Mamane et al, 1999; Servant et al, 2002a; Servant et al, 2002b; Taniguchi et al, 2001). Among the members of the interferon regulatory factor (IRF) family, IRF-3 and IRF-7 play essential roles in the virus-induced type I IFN gene activation following virus infection (Au et al, 1998; Lin et al, 1998, 1999a, b; Marie et al, 1998; Sato et al, 1998,

2000; Yoneyama et al, 1998). IRF-3 is activated by C-terminal phosphorylation which promotes dimerization, cytoplasmic to nuclear translocation, DNA binding, association with CBP/p300 histone acetyltransferases and transactivation of downstream early genes such as IFNb. IFNa1 and RANTES. In contrast, IRF-7 protein is synthesized *de novo* upon IFN stimulation and contributes to the expression of delayed-type genes including other IFNα subtypes. As with IRF-3, virus infection induces C-terminal phosphorylation and activation of IRF-7 (Marie et al, 1998; Sato et al, 1998). The IKK-related kinases – IKKε (Peters et al, 2000) and TBK-1 (Pomerantz and Baltimore, 1999; Bonnard et al, 2000; Tojima et al, 2000) – were shown to be essential signaling components required for IRF-3 and IRF-7 phosphorylation (Fitzgerald et al, 2003b; Sharma et al, 2003; McWhirter et al, 2004) **(Figure 6)**.

Among the eleven members of the human TLR family, TLR3, TLR4, TLR7, TLR8, and TLR9 are involved in the initial sensing of viral components. In mice, viral single- and double-stranded (ds) RNA, fusion protein of respiratory syncytial virus, single-stranded (ss) RNA, and genomic DNA from herpes and cytomegalovirus are recognized by TLR3, TLR4, TLR7, and TLR9, respectively (Kurt-Jones et al, 2000; Alexopoulou et al, 2001; Lund et al, 2003; Diebold et al, 2004; Heil et al, 2004; Krug et al, 2004).

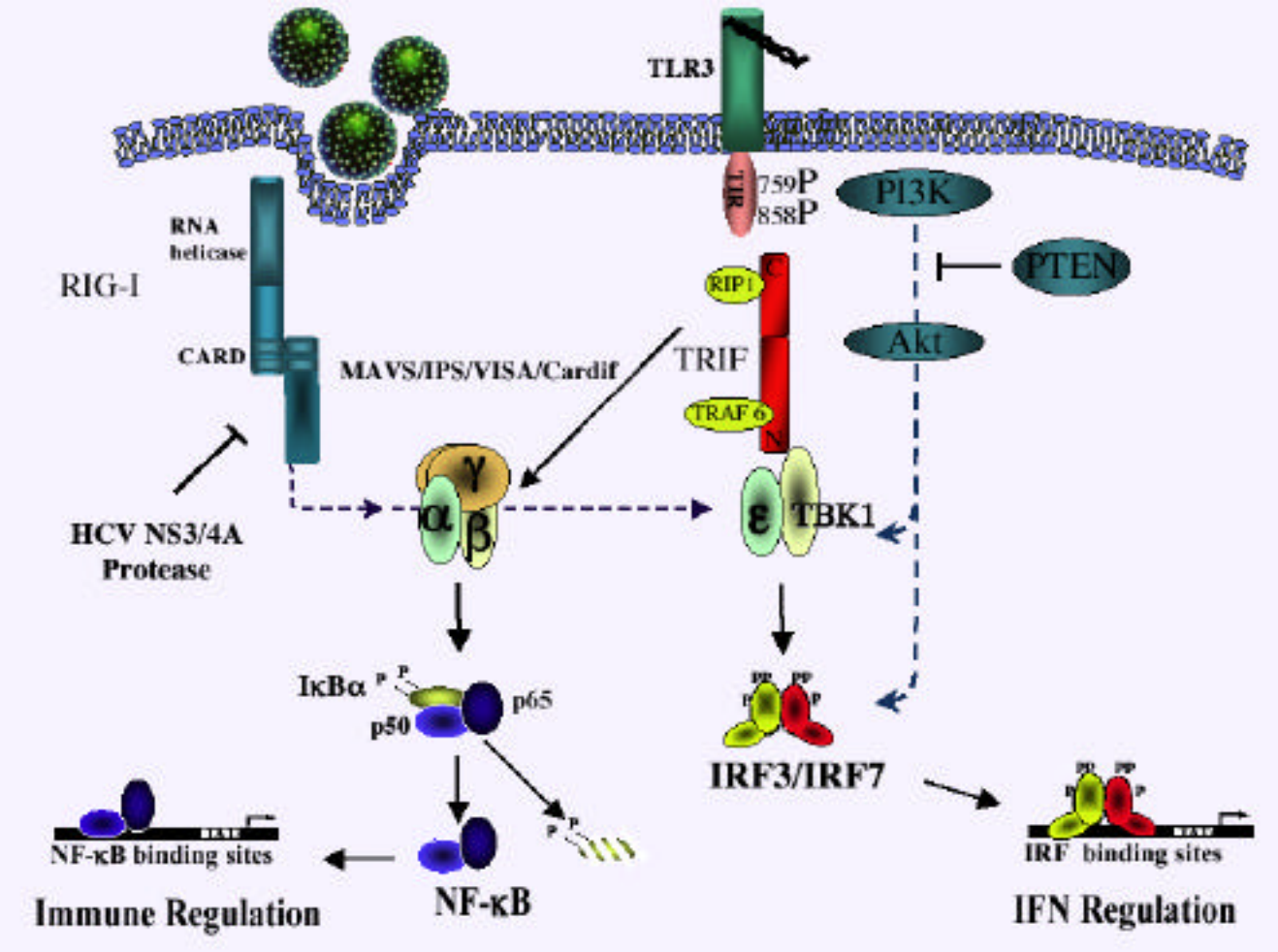

Figure 6. Double strand RNA and virus mediated signals leading to interferon gene activation. Engagement of TLR3 by dsRNA leads to recruitment of the TRIF adapter molecule and the activation of TBK1 and IKKε kinases that phosphorylate IRF3 and IRF7 transcription factors. TRIF can also signal to NF-κB activation, through a TRAF6 or RIP1 dependent mechanism, via the IKKα/β complex which phosphorylates the inhibitory subunit IκBα, resulting in release of NF-κB DNA binding subunits. Recruitment of dsRNA also causes tyrosine phosphorylation of the cytoplasmic tail of TLRs at multiple residues and the recruitment of PI3 kinase (Hiscott, 2004; Sarkar et al, 2004). In the absence of PI3K activation, IRF3 is incompletely phosphorylated and fails to stimulate IFN gene transcription. It remains to be determined at which level PI3K-Akt functions. Virus infection represents a distinct stress to the cell that may utilize TLR3 independent mechanisms. RIG-1 has been shown to stimulate NF-κB and IRF dependent pathways and may be the sensor molecule that initially recognizes incoming cytoplasmic viral ribonucleoprotein complexes. RIG-I contains two N-terminal caspase recruitment domains (CARD) and a C-terminal RNA helicase activity that interacts with viral RNA and is thought to 'sense' intracellular virus particles. A CARD-containing adapter molecule (MAVS/IPS/VISA/Cardif) appears to be the link between sensing of incoming virus, the triggering of downstream kinases and the activation of IRF and NF-κB target genes. Furthermore, MAVS is the physiological target for inhibition by the NS3/4A protease activity of Hepatitis C virus; cleavage of MAVS blocks signaling to the innate immune response and contributes to chronic HCV persistence.

While the adapter molecule MyD88 is commonly used by all TLRs, other adapter proteins including MAL/TIRAP, TRIF/TICAM1 and TRAM/TICAM2 are involved in MyD88 independent pathways (Oshiumi et al, 2003; Yamamoto et al, 2003b). TLR3 and TLR4 engage the adapter TRIF/TICAM-1, leading to TBK1 and IKKε activation, which in turn activates IRF3 and IFNα/β transcription (Fitzgerald et al, 2003a; Sato et al, 2003; Yamamoto et al, 2003a).

A separate signaling pathway utilizes the retinoic acid inducible gene I (RIG-I) to recognize a variety of RNA viruses and trigger the innate antiviral response, independent of the TLR-dependent pathways **(Figure 6)**. RIG-I contains a DExD/H box RNA helicase domain and two caspase recruitment domains (CARD; full-length RIG-I can interact with dsRNA through its DExD/H box within C terminus and augment IFN production in response to viral infection in an ATPase-dependent manner, and the two copies of the CARD at its N terminus transduce signals leading to the activation of IRF-3 and NF-κB. The constitutively active form of RIG-I (CARD domain alone) is capable of activating IRF-3 and NF-κB and stimulating IFN-β production (Yoneyama et al, 2004). Recent studies demonstrated that the hepatitis C virus (HCV) gene product NS3/4A protease complex efficiently blocks RIG-I signaling pathway and contributes to the establishment of HCV persistence (Breiman et al, 2005; Foy et al, 2005; Sumpter et al, 2005). The generation of RIG-I-deficient mice revealed that RIG-I, but not the TLR system, plays an essential role in antiviral responses in various cells, except plasmacytoid dendritic cells (pDCs). Reciprocally, the TLR system, but not RIG-I, was indispensable to IFN secretion in pDCs. (Kato et al, 2005).

Several results suggested that an unidentified adapter molecule may link RIG-I with the downstream IKK-related kinase complexes. Four groups recently identified a CARD domain containing adapter molecule - mitochondrial antiviral response (MAVS), interferon-β stimulator 1 (IPS-1), virus induced signaling adapter (VISA), CARD adaptor inducing IFN-β (CARDIF) - involved in RIG-I-dependent signaling to the IKKα/β complex and to TBK1/IKKε (Kawai et al, 2005; Seth et al, 2005). MAVS locates in the mitochondria via a C-terminal mitochondrial transmembrane domain and triggers antiviral responses via activation of NF-κB and IRF3. MAVS also appears sufficient to recruit IKKε activation and insertion into the mitochondrial membrane appears to be sufficient to trigger mitochondria depolarization and apoptosis, as well as activation of IRF3 and the antiviral cascade. In addition, it was found that CARDIF is targeted and cleaved at its C-terminal end by NS3/4A, a serine protease from HCV known to block IFN-β production (Meylan et al, 2005). Furthermore, overexpression of IKKε resulted in strong inhibition of both negative and positive replicative strands of the HCV replicon, suggesting an important role for IKKε in the RIG-I pathway downstream of CARDIF and in suppression of HCV replication (Breiman et al, 2005). Thus, the molecular signaling mechanisms that are essential for the development of the innate immune response are being delineated rapidly. It is intriguing that a direct relationship between VSV-induced apoptosis and the initiation of the IFN antiviral state has been uncovered in a variety of differentiated cell types. The details of the RIGI dependent and -independent signaling mechanisms thus appear central to the direct and indirect oncolytic functions of VSV.

V. Clinical trials with replication competent oncolytic viruses

As of yet, there have been no clinical trials initiated with VSV in cancer patients. However, Phase I trials with NDV and Reovirus have been completed in patients with advanced solid cancers that were unresponsive to standard therapy (Pecora et al, 2002). Phase I Trials with PV701, a replication-competent strain of NDV on seventy-nine patients with advanced cancer displayed promising results: 1) a biopsy conducted on one patient demonstrated budding of virus particles from cancerous cells through electron microscopy and infiltration of the tumor mass with mononuclear inflammatory cells; 2) side effects were confined to flu-like symptoms; 3) 14 patients displayed a halt in progression of cancer, and 7 patients had measurable tumor reductions (Pecora et al, 2002). The latter results were obtained with sub-optimal doses of the PV701 strain, but collectively these results warrant further investigation into the potential of NDV as new cancer therapy.

In addition, two clinical trials with Reovirus have been completed. In the Phase I trial, results indicate that patients with advanced cancer had no toxicity to various administered doses of Reovirus, highlighting the efficacy of this oncolytic virus. Furthermore, 62% of patients demonstrated tumor regression ranging from 32-100% (Morris, 2002; Norman and Lee, 2005). This led to Phase II trials by Oncolytic Biotech Inc. on Reovirus-treated human prostate cancer. Following intraprostatic injection of Reovirus, tumor cell death was observed in the prostate gland in 4 of 6 patients and there were no observable signs of toxicity in the subjects (Norman and Lee, 2005). In all cases, multiple inoculations of oncolytic virus were used; given that immunity to virus builds rapidly over time, it is not clear whether subsequent virus inoculations are effective, or whether the strategy may ultimately require sequential administration of different viruses. These and many other issues warrant further investigation.

VI. Conclusions

Many advantages to VSV argue that this virus may be a superior oncolytic virus: 1) VSV can be grown to high titers in bioreactors and is easily amenable to genetic manipulation by recombinant techniques; 2) VSV causes very mild disease in cattle and humans; 3) because most humans have not come into contact with VSV, pre-existing immunity that could hamper the therapeutic effect of VSV does not exist and a significant or complete tumor response could be achieved before the patient develops an acquired immunity. Defects in the IFN antiviral signaling network within transformed cells have been implicated in preferential VSV oncolysis; new research has delineated important regulatory pathways that impact on virus

replication and the host innate response. The challenge now is to understand the range of human cancer that will respond to oncolytic virotherapy.

References

Ahmed M and Lyles DS (**1998)** Effect of vesicular stomatitis virus matrix protein on transcription directed by host RNA polymerases I, II, and III. **J Virol** 72, 8413-8419.

Ahmed M, McKenzie MO, Puckett S, Hojnacki M, Poliquin L and Lyles DS (**2003)** Ability of the matrix protein of vesicular stomatitis virus to suppress β interferon gene expression is genetically correlated with the inhibition of host RNA and protein synthesis. **J Virol** 77, 4646-4657.

Alexopoulou L, Holt AC, Medzhitov R and Flavell RA (**2001)** Recognition of doublestranded RNA and activation of NF-κB by Toll-like receptor 3. **Nature** 413, 732-738.

Au WC, Moore PA, LaFleur DW, Tombal B and Pitha PM (**1998)** Characterization of the interferon regulatory factor-7 and its potential role in the transcription activation of interferon A genes. **J Biol Chem** 273, 29210-29217.

Balachandran S, Porosnicu M and Barber GN (**2001)** Oncolytic activity of vesicular stomatitis virus is effective against tumors exhibiting aberrant p53, Ras, or myc function and involves the induction of apoptosis. **J Virol** 75, 3474-3479.

Balachandran S, Roberts PC, Brown LE, Truong H, Pattnaik AK, Archer DR and Barber GN (**2000)** Essential role for the dsRNA-dependent protein kinase PKR in innate immunity to viral infection. **Immunity** 13, 129-141.

Balachandran S, Thomas E and Barber GN (**2004)** A FADD-dependent innate immune mechanism in mammalian cells. **Nature** 432, 401-405.

Barr JN, Whelan SP and Wertz GW (**2002)** Transcriptional control of the RNAdependent RNA polymerase of vesicular stomatitis virus. **Biochim Biophys Acta** 1577, 337-353.

Barrett JW, Cao JX, Hota-Mitchell S and McFadden G (**2001)** Immunomodulatory proteins of myxoma virus. **Semin Immunol** 13, 73-84.

Bell JC, Garson KA, Lichty BD and Stojdl DF (**2002)** Oncolytic viruses: programmable tumour hunters. **Curr Gene Ther** 2, 243-254.

Bonnard M, Mirtsos C, Suzuki S, Graham K, Huang J, Ng M, Itie A, Wakeham A, Shahinian A, Henzel WJ, Elia AJ, Shillinglaw W, Mak TW, Cao Z, Yeh WC (**2000)** Deficiency of T2K leads to apoptotic liver degeneration and impaired NF-κB-dependent gene transcription. **Embo J** 19, 4976-4985.

Breiman A, Grandvaux N, Lin R, Ottone C, Akira S, Yoneyama M, Fujita T, Hiscott J and Meurs EF (**2005)** Inhibition of RIG-I-dependent signaling to the interferon pathway during hepatitis C virus expression and restoration of signaling by IKKε. **J Virol** 79, 3969-3978.

Cameron C, Hota-Mitchell S, Chen L, Barrett J, Cao JX, Macaulay C, Willer D, Evans D and McFadden G (**1999)** The complete DNA sequence of myxoma virus. **Virology** 264, 298-318.

Campbell SA, Lin J, Dobrikova EY and Gromeier M (**2005)** Genetic determinants of cell type-specific poliovirus propagation in HEK 293 cells. **J Virol** 79, 6281-6290.

Caras I, Grigorescu A, Stavaru C, Radu DL, Mogos I, Szegli G and Salageanu A (**2004)** Evidence for immune defects in breast and lung cancer patients. **Cancer Immunol Immunother** 53, 11461152.

Carroll AR and Wagner RR (**1979)** Role of the membrane (M) protein in endogenous inhibition of in vitro transcription by vesicular stomatitis virus. **J Virol** 29, 134-142.

Cesaire R, Oliere S, Sharif-Askari E, Loignon M, Lezin A, Olindo S, Panelatti G, Kazanji M, Aloyz R, Panasci L, Bell JC, Hiscott J (**2005)** Oncolytic activity of vesicular stomatitis virus in primary adult T-cell leukemia. **Oncogene**. **In press.**

Chiocca EA (**2002)** Oncolytic viruses. **Nat Rev Cancer** 2, 938-950.

Clinton GM, Burge BW and Huang AS (**1979)** Phosphoproteins of vesicular stomatitis virus: identity and interconversion of phosphorylated forms. **Virology** 99, 84-94.

de Leeuw O and Peeters B (**1999)** Complete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. **J Gen Virol** 80 (Pt1)*131-136.

Diebold SS, Kaisho T, Hemmi H, Akira S and Reis e Sousa C (**2004)** Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. **Science** 303, 1529-1531.

Enninga J, Levy DE, Blobel G and Fontoura BM (**2002)** Role of nucleoporin induction in releasing an mRNA nuclear export block. **Science** 295, 1523-1525.

Faria PA, Chakraborty P, Levay A, Barber GN, Ezelle HJ, Enninga J, Arana C, van Deursen J and Fontoura BM (**2005)** VSV disrupts the Rae1/mrnp41 mRNA nuclear export pathway. **Mol Cell** 17, 93-102.

Fernandez M, Porosnicu M, Markovic D and Barber GN (**2002)** Genetically engineered vesicular stomatitis virus in gene therapy: application for treatment of malignant disease. **J Virol** 76, 895-904.

Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM and Maniatis T (**2003a)** IKKε and TBK1 are essential components of the IRF3 signaling pathway. **Nat Immunol** 4, 491-496.

Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM and Golenbock DT (**2003b)** LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. **J Exp Med** 198, 1043-1055.

Flanagan EB, Zamparo JM, Ball LA, Rodriguez LL and Wertz GW (**2001)** Rearrangement of the genes of vesicular stomatitis virus eliminates clinical disease in the natural host: new strategy for vaccine development. **J Virol** 75 6107-6114

Foy E, Li K, Sumpter R, Jr, Loo YM, Johnson CL, Wang C, Fish PM, Yoneyama M, Fujita T, Lemon SM and Gale M, Jr (**2005)** Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling Regulating Intracellular Antiviral Defense and Permissiveness to Hepatitis C Virus RNA Replication through a Cellular RNA Helicase, RIG-I. **Proc Natl Acad Sci U S A** 79, 2689-2699.

Gromeier M, Lachmann S, Rosenfeld MR, Gutin PH and Wimmer E (**2000)** Intergeneric poliovirus recombinants for the treatment of malignant glioma **Proc Natl Acad Sci U S A** *97* 6803-6808.

Hawkins LK, Lemoine NR and Kirn D (**2002)** Oncolytic biotherapy: a novel therapeutic plafform. **Lancet Oncol** 3, 17-26.

Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H and Bauer S (**2004)** Species-specific recognition of single-stranded RNA via tolllike receptor 7 and 8. **Science** 303, 1526-1529.

Hiscott J (**2004)** Another detour on the Toll road to the interferon antiviral response. **Nat Struct Mol Biol** 11, 1028-1030.

Hiscott J, Pitha P, GÇnin P, Nguyen H, Heylbroeck C, Mamane Y, AlgartÇ M and Lin R (**1999)** Triggering the interferon response: the role of IRF-3 transcription factor. **J Interferon Cytokine Res** 19, 1-13.

Jackson DP, Watling D, Rogers NC, Banks RE, Kerr IM, Selby PJ and Patel PM (**2003)** The JAK/STAT pathway is not sufficient to sustain the antiproliferative response in an interferon-resistant human melanoma cell line. **Melanoma Res** 13, 219-229.

Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, Tsujimura T, Takeda K, Fujita T, Takeuchi O and Akira S (**2005)** Cell type-specific involvement of RIG-I in antiviral response. **Immunity** 23, 19-28.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O and Akira S (**2005)** IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. **Nat Immunol** 6, 981-8

Kerr PJ and Best SM (**1998)** Myxoma virus in rabbits. **Rev Sci Tech** 17, 256-268.

Kopecky SA and Lyles DS (**2003)** The cell-rounding activity of the vesicular stomatitis virus matrix protein is due to the induction of cell death. **J Virol** 77, 5524-5528.

Kopecky SA, Willingham MC and Lyles DS (**2001)** Matrix protein and another viral component contribute to induction of apoptosis in cells infected with vesicular stomatitis virus. **J Virol** 75, 12169-12181.

Krug A, French AR, Barchet W, Fischer JA, Dzionek A, Pingel JT, Orihuela MM, Akira S, Yokoyama WM and Colonna M (**2004)** TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. **Immunity** 21, 107-119.

Kruyt FA and Curiel DT (**2002)** Toward a new generation of conditionally replicating adenoviruses: pairing tumor selectivity with maximal oncolysis. **Hum Gene Ther** 13, 485-495.

Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT anderson LJ and Finberg RW (**2000)** Pattern recognition receptors TLR4 and CD14 mediates response to respiratory syncytial virus. **Nature Immunol** 1, 398-401.

Levy DE, Marie I, Smith E and Prakash A (**2002)** Enhancement and diversification of IFN induction by IRF-7-mediated positive feedback. **J Interferon Cytokine Res** 22, 87-93.

Lin R, Heylbroeck C, Genin P, Pitha PM and Hiscott J (**1999a)** Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription. **Mol Cell Biol** 19, 959-966.

Lin R, Heylbroeck C, Pitha PM and Hiscott J (**1998)** Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. **Mol Cell Biol** 18, 2986-2996.

Lin R, Mamane Y and Hiscott J (**1999b)** Structural and functional analysis of interferon regulatory factor 3: localization of the transactivation and autoinhibitory domains. **Mol Cell Biol** 19, 2465-2474.

Lu R, Au WC, Yeow WS, Hageman N and Pitha PM (**2000)** Regulation of the promoter activity of interferon regulatory factor-7 gene. Activation by interferon snd silencing by hypermethylation. **J Biol Chem** 275, 31805-31812.

Lund J, Sato A, Akira S, Medzhitov R and Iwasaki A (**2003)** Toll-like receptor 9- mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. **J Exp Med** 198, 513-520.

Malmgaard L (**2004)** Induction and regulation of IFNs during viral infections. **J Interferon Cytokine Res** 24, 439-454.

Mamane Y, Heylbroeck C, Genin P, Algarte M, Servant MJ, LePage C, DeLuca C, Kwon H, Lin R and Hiscott J (**1999)** Interferon regulatory factors: the next generation. **Gene** 237, 1-14.

Marie I, Durbin JE and Levy DE (**1998)** Differential viral induction of distinct interferonα genes by positive feedback through interferon regulatory factor-7. **Embo J** 17, 6660-6669.

Masters J, Hinek AA, Uddin S, Platanias LC, Zeng W, McFadden G and Fish EN (**2001)** Poxvirus infection rapidly activates tyrosine kinase signal transduction. **J Biol Chem** 276, 4837148375.

Matin SF, Rackley RR, Sadhukhan PC, Kim MS, Novick AC and Bandyopadhyay SK (**2001)** Impaired α-interferon signaling in transitional cell carcinoma: lack of p48 expression in 5637 cells. **Cancer Res** 61, 2261-2266.

McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT and Maniatis T (**2004)** IFN-regulatory factor 3-dependent gene expression is defective in Tbk1-deficient mouse embryonic fibroblasts. **Proc Natl Acad Sci U S A** 101, 233-238.

Mead DG, Ramberg FB, Besselsen DG and Mare CJ (**2000)** Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice. **Science** 287, 485-487.

Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R and Tschopp J (**2005)** Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. **Nature**, in press

Morris DGea (**2002)** A phase I clinical trial evaluating intralesional Reolysin (reovirus) in histologically confirmed malignancies. Abstract presented at: American Society of Clinical Oncology Annual Meeting.

Murad JM, Tone LG, de Souza LR and De Lucca FL (**2005)** A point mutation in the RNA-binding domain I results in decrease of PKR activation in acute lymphoblastic leukemia. **Blood Cells Mol Dis** 34, 1-5.

Nibert ML, Margraf RL and Coombs KM (**1996)** Nonrandom segregation of parental alleles in reovirus reassortants. **J Virol** 70, 7295-7300.

Norman KL and Lee PW (**2005)** Not all viruses are bad guys: the case for reovirus in cancer therapy. **Drug Discov Today** 10, 847-855.

Oshiumi H, Sasai M, Shida K, Fujita T, Matsumoto M and Seya T (**2003)** TIRcontaining adapter molecule (TICAM)-2, a bridging adapter recruiting to toll-like receptor 4 TICAM-1 that induces interferon-β. **J Biol Chem** 278, 49751-49762.

Pecora AL, Rizvi N, Cohen GI, Meropol NJ, Sterman D, Marshall JL, Goldberg S, Gross P, O'Neil JD, Groene WS, Roberts MS, Rabin H, Bamat MK, Lorence RM (**2002)** Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. **J Clin Oncol** 20, 2251-2266.

Peters RT, Liao SM and Maniatis T (**2000)** IKKε is part of a novel PMA-inducible IκB kinase complex. **Mol Cell** 5, 513-522.

Petersen JM, Her LS and Dahlberg JE (**2001)** Multiple vesiculoviral matrix proteins inhibit both nuclear export and import. **Proc Natl Acad Sci U S A** 98, 8590-8595.

Petersen JM, Her LS, Varvel V, Lund E and Dahlberg JE (**2000)** The matrix protein of vesicular stomatitis virus inhibits nucleocytoplasmic transport when it is in the nucleus and associated with nuclear pore complexes. **Mol Cell Biol** 20, 8590-8601.

Pomerantz JL and Baltimore D (**1999)** NF-κB activation by a signaling complex containing TRAF2, TANK and TBK1, a novel IKK-related kinase. **Embo J** 18, 6694-6704.

Rux JJ and Burnett RM (**2004)** Adenovirus structure. **Hum Gene Ther** 15, 1167-1176.

Samuel CE (**2001)** Antiviral actions of interferons. **Clin Microbiol Rev** 14, 778-809.

Sarkar SN, Peters KL, Elco CP, Sakamoto S, Pal S and Sen GC (**2004)** Novel roles of TLR3 tyrosine phosphorylation and PI3 kinase in double-stranded RNA signaling. **Nat Struct Mol Biol** 11, 1060-1067.

Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, Nakao K, Nakaya T, Katsuki M, Noguchi S, Tanaka N and Taniguchi T (**2000)** Distinct and Essential Roles of Transcription

Factors IRF-3 and IRF-7 in Response to Viruses for IFN-α/β Gene Induction. **Immunity** 13, 539-548.

Sato M, Tanaka N, Hata N, Oda E and Taniguchi T (**1998)** Involvement of the IRF family transcription factor IRF-3 in virus- induced activation of the IFN-β gene. **FEBS Lett** 425, 112-116.

Sato S, Sugiyama M, Yamamoto M, Watanabe Y, Kawai T, Takeda K and Akira S (**2003)** Toll/IL1 receptor domaincontaining adaptor inducing IFNβ (TRIF) associates with TNF receptorassociated factor 6 and TANKbinding kinase 1, and activates two distinct transcription factors, NFκ B and IFN regulatory factor3, in the Tolllike receptor signaling. **J Immunol** 171, 4304-4310.

Sen GC (**2001)** Viruses and interferons. **Annu Rev Microbiol** 55, 255-281.

Servant MJ, Grandvaux N and Hiscott J (**2002a)** Multiple signaling pathways leading to the activation of interferon regulatory factor 3. **Biochem Pharmacol** 64, 985-992.

Servant MJ, ten Oever B and Lin R (**2002b)** Review: Overlapping and Distinct Mechanisms Regulating IRF-3 and IRF-7 Function. **J Interferon Cytokine Res** 22, 49-58.

Seth RB, Sun L, Ea CK and Chen ZJ (**2005)** Identification and Characterization of MAVS, a Mitochondrial Antiviral Signaling Protein that Activates NF-κB and IRF3. **Cell** 122, 669-682.

Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R and Hiscott J (**2003)** Triggering the interferon antiviral response through an IKK-related pathway. **Science** 300, 1148-1151

Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N and Bell JC (**2000)** Exploiting tumorspecific defects in the interferon pathway with a previously unknown oncolytic virus. **Nat Med** 6, 821825.

Stojdl DF, Lichty BD, tenOever BR, Paterson JM, Power AT, Knowles S, Marius R, Reynard J, Poliquin L, Atkins H, Brown EG, Durbin RK, Durbin JE, Hiscott J, Bell JC (**2003)** VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. **Cancer Cell** 4, 263-275.

Sumpter R, Jr, Loo YM, Foy E, Li K, Yoneyama M, Fujita T, Lemon SM and Gale M, Jr (**2005)** Regulating Intracellular Antiviral Defense and Permissiveness to Hepatitis C Virus RNA Replication through a Cellular RNA Helicase, RIG-I. **J Virol** 79, 2689-2699.

Taniguchi T, Ogasawara K, Takaoka A and Tanaka N (**2001)** Irf family of transcription factors as regulators of host defense. **Annu Rev Immunol** 19, 623-655.

Terstegen L, Gatsios P, Ludwig S, Pleschka S, Jahnen-Dechent W, Heinrich PC and Graeve L (**2001)** The vesicular stomatitis virus matrix protein inhibits glycoprotein 130-dependent STAT activation. **J Immunol** 167, 5209-5216.

Tojima Y, Fujimoto A, Delhase M, Chen Y, Hatakeyama S, Nakayama K, Kaneko Y, Nimura Y, Motoyama N, Ikeda K, *et al* (**2000)** NAK is an IκB kinase-activating kinase. **Nature** 404, 778-782

von Kobbe C, van Deursen JM, Rodrigues JP, Sitterlin D, Bachi A, Wu X, Wilm M, Carmo-Fonseca M and Izaurralde E (**2000)** Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98. **Mol Cell** 6, 1243-1252.

Wagner RRaR, JK (**1996)** Rhabdoviridae: The Viruses and Their Replication, In Fields Virology, B.N. Fields, Knipe, D.M., Howley, P.M., ed (Philadelphia*PA: Lippincott-Raven), pp. 561-575.

Wathelet MG, Lin CH, Parakh BS, Ronco LV, Howley PM and Maniatis T (**1998)** Virus infection induces the assembly of coordinately activated transcription factors on the IFNγ enhancer in vivo. **Molecular Cell** 1, 507518.

Weaver BK, Kumar KP and Reich NC (**1998)** Interferon regulatory factor 3 and CREBbinding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. **Mol Cell Biol** 18, 1359-1368.

Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K and Akira S (**2003a)** Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. **Science** 301, 640-643.

Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K and Akira S (**2003b)** TRAM is specifically involved in the Toll-like receptor 4- mediated MyD88-independent signaling pathway. **Nat Immunol** 4, 1144-1150.

Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S and Fujita T (**2004)** The RNA helicase RIG-I has an essential function in doublestranded RNA-induced innate antiviral responses. **Nat Immunol** 5, 730-737.

Yoneyama M, Suhara W, Fukuhara Y, Fukada M, Nishida E and Fujita T (**1998)** Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. **EMBO Journal** 17, 1087-1095.

Yuan H, Puckett S and Lyles DS (**2001)** Inhibition of host transcription by vesicular stomatitis virus involves a novel mechanism that is independent of phosphorylation of TATAbinding protein (TBP) or association of TBP with TBP-associated factor subunits. **J Virol** 75, 4453-4458.

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