Published in final edited form as:

Future Virol. 2011 January 1; 6(1): 85–99. doi:10.2217/fvl.10.70.

Measles virus neurovirulence and host immunity

Michael Oglesbee¹ and Stefan Niewiesk^{1,†}

¹Department of Veterinary Biosciences, Ohio State University, 1925 Coffey Road, Columbus, OH 43210, USA

Abstract

Measles virus is highly neuroinvasive, yet host immune responses are highly effective at limiting neurovirulence in humans. We know that neurons are an important target of infection and that both IFN- γ and - β expression are observed in the measles virus-infected human brain. Rodent models can be used to understand how this response is orchestrated. Constitutive expression of the major inducible 70-kDa heat-shock protein is a feature of primate tissues that is lacking in mice. This article examines the importance of addressing this difference when modeling outcomes of brain infection in mice, particularly in terms of understanding how infected neurons may activate uninfected brain macrophages to produce IFN- β and support T-cell production of IFN- γ , a mediator of noncytolytic viral clearance. New and historical data suggest that the virus heat-shock protein 70 relationship is key to a protective host immune response and has potential broad relevance.

Keywords

CNS; fever; heat-shock protein; innate immunity; measles virus; neuroinvasiveness; neurovirulence

CNS infection by measles virus & other morbilliviruses in their natural host

Measles virus (MeV) is a negative-strand enveloped RNA virus within the family *Paramyxoviridae*, genus *Morbillivirus*. The natural hosts of MeV are humans and the pathogenesis associated with infection is very similar to that of the closely related canine distemper virus (CDV), a morbillivirus infecting a broad host range that includes dogs, ferrets, minks and raccoons. Aerosol exposure results in the infection of dendritic cells and macrophages associated with airways. Tonsillar tissue has long been viewed as an important primary target, although more recent data in MeV-inoculated nonhuman primates suggests that infection is initiated within the lung, targeting alveolar macrophages and dendritic cells of bronchoalveolar lymphoid tissue [1]. The initial round of replication results in a primary viremia with dissemination of virus to lymphoid tissues throughout the body. Replication within these lymphoid tissues causes a significant amplification of viral burden and in the absence of protective immunity, a high level of secondary viremia. Viremia is associated

© 2011 Future Medicine Ltd

[†]Author for correspondence: Tel.: +1 614 292 5661, Fax: +1 614 292 6473 niewiesk.1@osu.edu.

For reprint orders, please contact: reprints@futuremedicine.com

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

with infected peripheral blood mononuclear cells (both monocytes and lymphocytes) [2], although significant cell-free viremia has also been documented in CDV-infected dogs [3]. The secondary viremia supports multisystemic endothelial infection, where virus is then transmitted to epithelial cells through their basolateral membranes for subsequent shedding into the respiratory, gastrointestinal and urinary tracts [1]. Shedding of virus through respiratory secretions is particularly effective for transmission between hosts, and the contagion of morbilliviruses within an immunologically naive population is among the highest known. Typical incubation times for measles in humans is 10–14 days, at which point fever is induced and cell-mediated immune responses commence, resulting in viral clearance. Clinical manifestations of MeV infection most often reflect secondary microbial infections, the result of virus-mediated immune suppression and virus-induced cytopathic effects on mucosal surfaces [4]. Bacterial bronchopneumonia is the most common cause of MeV-induced mortality [4].

Morbilliviruses exhibit a broad tropism for different endothelial cell types, and not simply those populations that facilitate viral shedding from mucosal surfaces [2]. In this way, viremia results in infection of the integument (i.e., dermis and epidermis). For MeV, infection of the integument combined with the cell-mediated immune response results in the formation of a generalized maculopapular erythematous rash. CDV may persist in the epidermis following viral clearance from other tissue compartments, resulting in abnormal keratinocyte proliferation and keratin formation. The latter gives rise to an entity in dogs and mink known as hard pad disease (i.e., acanthosis and hyperkeratosis affecting the digital pads of the feet). The degree to which infection of the integument contributes to viral transmission is unknown.

Infection of other endothelial populations is a dead end for the virus in terms of transmission – a byproduct of systemic dissemination. CDV infection of brain endothelial cells has been well documented, representing an efficient means of neuroinvasion as virus spreads from endothelial cells to perivascular astrocytes [3]. Nonfenestrated endothelial cells held together by tight junctions are a primary basis for the blood–brain barrier, with endothelial cell infection being an efficient means of circumventing any barrier function. Other means of CNS entry may include passage of infected peripheral blood mononuclear cells through the endothelial lining of the cerebral vasculature, passage of cell-free virus through vasculature in brain regions that normally lack a blood–brain barrier (e.g., circumventricular organs of the diencephalon), infection of the choroid plexus epithelium with subsequent viral shedding into the cerebral spinal fluid pathways, or infection of olfactory epithelium with antegrade transport of virus to the brain via the olfactory nerve [5]. Viral entry into the CNS may or may not result in clinical neurological disease, with outcome being defined by the host immune response and ability of virus to spread from points of entry to achieve a significant brain viral burden. The ability to cause disease (neurovirulence) is determined by both host and viral determinants. Ferrets infected with CDV of canine origin invariably suffer fulminate neuronal infection and death, and a similar outcome was observed in lion populations in East Africa that were exposed to CDV of canine origin in the 1990s [6]. Dogs infected with CDV of canine origin exhibit an age-dependent susceptibility to infection of the brain that is correlated to immune status, including presence of passively acquired neutralizing antibody (e.g., from colostrum) and cell-mediated immune responsiveness [7,8]. Dogs that are immunologically naive and are infected prior to 4 weeks of age suffer fulminate neuronal infection. At older ages, neurological disease is correlated to diminished *in vitro* responses of peripheral blood lymphocytes to phytohemagglutinin. Neuronal support of viral replication is reduced in these animals, with viral tropism shifting more to astrocytes and oligodendrocytes. Viral persistence in these cell types gives rise to demyelinating lesions that become clinically apparent following viral clearance from other organ systems.

Distinct neurotropic viral variants of CDV have long been recognized, although the virological basis for these differences in biological behavior remains to be elucidated.

Measles virus may exhibit a degree of neuroinvasiveness that is comparable with CDV, although the incidence of clinical neurological disease due to viral replication in the CNS is rare. Electroencephalographic changes have been observed in 50% of uncomplicated cases of naturally occurring MeV infection [9], and MeV N transcripts have been detected by reverse transcriptase-PCR in the brains of 18–19.6% of autopsy cases (from a total of 61 and 51 autopsy specimens, respectively) unassociated with neurological disease [10,11]. These studies would benefit from additional confirmatory analyses (e.g., immunohistochemistry). Earlier studies using immunohistochemistry and *in situ* hybridization have argued for a lesser incidence of neuroinvasiveness in cases of acute fatal measles, although this was based upon only four cases in which viral antigen or RNA could be detected in non-neural tissues [12]. While the precise incidence of MeV neuroinvasiveness may be difficult to establish using naturally occurring case series, the multiple pathways for CNS entry that morbilliviruses readily exploit supports the view that MeV neuroinvasiveness is common. Similarly, mumps virus (family *Paramyxoviridae*, genus *Rubulavirus*) is characterized by a high degree of neuroinvasiveness and a relatively low degree of neurovirulence [13]. Clinical neurological sequela of MeV infection includes acute (post-infectious) encephalitis, an immune-mediated process whose progression is independent of active viral replication in nervous tissues. The incidence of acute measles encephalitis is reported as one in 1000 to one in 5000. Fulminate spread of MeV infection among CNS neurons and glia is known as acute measles encephalitis of the delayed type or measles inclusion body encephalitis (MIBE) and is associated with defects in cell-mediated immunity [14]. MIBE is not associated with specific MeV variants and can even be caused by vaccine strains [15], indicating that permissiveness of CNS cell types is not the major barrier to viral replication – it is the host immune response. Virus can also undergo a more chronic persistent infection of neurons and glia, giving rise to a demyelinating disease known as subacute sclerosing encephalitis (SSPE), and this is typically manifest years following childhood exposure [14]. The incidence of SSPE in the USA is reported to be approximately 4–11 cases per 100,000 cases of measles [16]. These persistent viruses adopt numerous secondary mutations that influence viral membrane glycoprotein gene expression and assembly of enveloped viral particles, proteins and/or functions that are nonessential for the direct cell-to-cell transmission that is observed in brain cell populations [17]. These secondary changes hinder attempts to understand viral properties that promote the establishment of persistence. It is also unclear how the host contributes to the establishment of persistence, presumably reflecting shortcomings in innate and adaptive immunity.

Our discussion will focus on the fate of MeV that has entered the CNS, this being the target of regional host immune responses that clear infection or limit brain viral burdens to sub-clinical levels. There are two ways to approach the study of MeV neurovirulence: focus upon the diseases themselves, characterizing MIBE and SSPE or models of these types of infections; or focus upon the reasons why, in the face of a high degree of neuroinvasiveness, MeV neurovirulence is rare. The latter is an underutilized approach that seeks to identify host factors that effectively control brain viral burden, thereby providing the context for addressing MeV-induced neurological disease. Perhaps more important than the relevance to MIBE and SSPE are insights gained that may be applied to other more highly neurovirulent agents, beginning with other negative-strand RNA viral pathogens of the CNS, including Nipah and rabies viruses. This article is thus focused upon model systems that may enhance our understanding of this aspect of virus–host interaction.

Rodent models of MeV neurovirulence & neuroprotective antiviral immunity

Rodent models of MeV brain infection have provided significant insights into host immune responses that limit viral neurovirulence. These experimental approaches utilize intracranial viral inoculation, which is well suited to the question of ‘what limits viral spread?’ since the level of virus that is introduced into the brain can be made constant. Direct viral inoculation into the CNS is also the basis for the monkey neurovirulence test, used to determine the safety of attenuated vaccines for poliovirus, measles, mumps and rubella viruses [18]. The limitation of rodent models is that measles virus is poorly neurovirulent in weanling animals, with the challenge being one of assuring that neuronal targets of infection provide a level of permissiveness that is similar to humans, so that host immune responses that limit spread can be distinguished from limitations in cell support of viral replication.

Receptor utilization by MeV during infection of the rodent brain & other cellular factors determining susceptibility

Much focus has been placed upon receptor utilization and permissiveness of rodent neurons to MeV infection. The premise is simply that neurons should express receptors to which the infecting MeV strain is adapted in order for those cells to be optimally permissive. MeV utilizes a variety of receptors to support virion attachment via the hemagglutinin (H) glycoprotein, and subsequent fusion to/penetration of the target cells via the fusion (F) glycoprotein. The signaling lymphocyte activation molecule (SLAM, or CD150) is known to support attachment and penetration of vaccine and wild-type strains of MeV, whereas vaccine strains may additionally recognize the complement receptor CD46 [19]. The tissue expression patterns of CD46 and SLAM do not, however, explain MeV infection of human epithelial cells or neurons, since these molecules are not expressed by either cell lineage [19]. Characterization of receptors used by MeV in the infection of neurons and epithelial cells is an active area of investigation, whether that is a natural human or experimental rodent host [20,21].

We do know that Edmonston (Ed) MeV, the prototype vaccine strain, can infect mouse neurons *in vitro* and *in vivo* and support cell-to-cell spread [22,23]. The efficiency of infection can be enhanced by transgenic expression of human CD46 in the target cell populations, and this does result in increased neurovirulence *in vivo* [24]. The same is true of transgenic mice expressing human CD150 infected with wild-type MeV strains [24]. Efficiency of infection may also be enhanced by using rodent brain-adapted MeV (i.e., virus that has been serially passaged in rodent brain, such as CAM/RB). One component of the brain adaptation is the selection of viral H glycoprotein variants that are more likely to efficiently use rodent neuronal receptors. Support for this concept was the generation of an Ed MeV chimera expressing the H glycoprotein of CAM/RB MeV. The viral chimera exhibited increased neurovirulence in mice relative to the parent Ed MeV [25]. What remains unclear is the degree to which increased virus–receptor adaptation influences the initial level of infection postinoculation versus cell-to-cell spread of the infection, either of which could enhance neurovirulence. That is, if the efficiency of cell-to-cell spread is constant, increased levels of the initial infection could ultimately result in increased brain viral burden and/or clinical manifestations of infection. MeV spread (i.e., spread of the encapsidated genome) between neurons appears to be independent of H receptor interaction, utilizing natural cell-to-cell contacts that may include synaptic vesicles and the network of plasmalemmal gap junctions that connect the cytoplasm of large populations of neurons to form functional ‘syncytia’ [26].

Age is another determinant of permissiveness, but the basis is poorly understood. This phenomenon is observed for a variety of viruses in a number of hosts and host tissues, but is particularly well documented in rodents receiving intracranial inoculation of MeV [27].

Neonatal C57BL/6 mice (42 h of age) inoculated with 10^4 TCID₅₀ of Ed MeV will exhibit 15–30% mortality associated with high brain viral RNA burden [23,28], whereas neurovirulence is dramatically reduced for mice infected at 7 days, infections being asymptomatic at later periods and associated with lower brain viral RNA burden. Immaturity of adaptive immune mechanisms in neonates is the most frequent explanation for their enhanced susceptibility, although direct comparison of adaptive immune responses of neonates versus older suckling or weanling mice to intracranial MeV infection has not been performed. If we are to examine immune responses that prevent MeV virulence using mouse (or other rodent) models, we must ensure that we have removed nonimmune impediments to viral replication. We must consider the intrinsic permissiveness of neonatal cells to infection. Study of alphavirus-infected mice show that there are host developmental changes that restrict virus replication within individual infected cells, rendering neonates more permissive to infection [29]. The basis for this enhanced permissiveness is probably multifactorial, contributing to the radiomimetic behavior of numerous viruses in neonatal tissues (i.e., tendency to destroy or lyse rapidly dividing or metabolically active cells of diverse histogenesis), particularly in the CNS. As a model system, neonatal mice thus offer the intrinsic cell support of viral replication that allows us to then identify mechanisms by which the immune system can contain viral spread.

MeV antiviral immunity in the CNS of mice

Measles virus infection of humans is controlled in part by both virus-specific humoral and cell-mediated immune responses, although T cells and IFN- γ appear to play a dominant role during primary infection [30,31]. Both IFN- α/β and IFN- γ are expressed in brain in association with MeV-associated neurological disease [32,33]. Studies in rodent models provide support for the key role of IFN- γ in mediating host protection against MeV neurovirulence, with current investigations focused upon mechanisms of IFN- γ induction and the role of type I interferons (IFNs) in antiviral immunity.

Deletion of the *IFN- γ* gene [34] or antibody neutralization of IFN- γ [35] renders mice more susceptible to brain infection. Neurons express receptors for IFN- γ , and intracellular responses to receptor stimulation include the induction of antiviral proteins, similar to the response to type I IFNs [36]. In this way, IFN- γ mediates noncytolytic viral clearance from neurons [34]. This clearance mechanism is ideally suited to the CNS in that neurons (a nonrenewable cell population) need not be killed in order to suppress or eliminate replicating virus. It is therefore no surprise that IFN- γ also mediates noncytolytic clearance of lymphocytic choriomeningitis virus [37], Sindbis virus [38], mouse hepatitis virus [39], vesicular stomatitis virus [40], Theiler's virus [41], and the LP-BM5 retrovirus [42] from the CNS. IFN- γ also mediates noncytolytic viral clearance outside of the CNS [43].

The source of IFN- γ in the mouse model of MeV infection is virus-specific T cells [35], with both CD4⁺ and CD8⁺ T cells contributing to host protection [34]. Mouse strains exhibit differences in susceptibility to MeV infection that are correlated to their H-2 haplotype and the effectiveness of the immune response to MeV following intracranial challenge [44,45]. The H-2 haplotype-dependent basis for susceptibility has been attributed to differential kinetics in the generation of the MeV-specific T-cell response and IFN- γ production. In resistant mouse strains, CD4 and CD8 T-cell responses are being generated before peak viral titers can be observed in brain tissue [45]. By contrast, susceptible mouse strains generate a weak CD8 T-cell response due to insufficient stimulation by low-affinity peptide/MHC I complexes [44,46]. In addition, IFN- γ secretion is increased in resistant mouse strains carrying the *H-2^d* allele and diminished in susceptible strains carrying the *H-2^b* allele [35] thus explaining the delayed generation of a CD4 T-cell response [47]. The difference may reflect the action of immune regulatory genes affecting cytokine production, since *in vitro* and *in vivo* stimulation of lymphocytes from H-2^b mice (using mitogen or anti-CD3

antibody) results in lower levels of Th1 cytokine production relative to H-2^d mice, including IFN- γ [48].

The MeV N protein is the predominant target of the cell-mediated immune response, with immunization of N protein alone being protective in rodent models of brain infection [49]. Priming of this immune response occurs in the periphery (i.e., non-nervous tissue) when virus is introduced into cerebrospinal fluid during the intracranial inoculation [50]. Cerebrospinal fluid pathways lead to both the systemic circulation and regional lymph nodes. Primed T cells enter the brain, where activation to produce IFN- γ requires viral antigen presentation and type I IFNs, which in the brain is primarily IFN- β [51,52]. The IFN- β assures that activation of the T-cell response occurs only in sites of viral replication. Antigen presentation is mediated by brain macrophages, either resident microglia or those of blood (monocyte) origin. Brain macrophages are not infected unless transgenic expression of human CD46 is employed [53], the infection being restricted to neurons [54]. The problem is that MeV-infected neurons do not express MHC-I, let alone MHC-II, nor do they express IFN- β [34,55]. The essential role of brain macrophages in providing a link between MeV neuronal infection and activation of antiviral T-cell responses was originally proposed by Patterson *et al.* [34]. The question is how does an infected neuron trigger antigen presentation in an uninfected brain macrophage, and what is the source of IFN- β (Figure 1)? To find a possible answer to this question we will now examine studies that address the question of the intracellular determinants that support viral replication and influence the development of antiviral immunity.

Role of heat-shock proteins during viral infection

Heat-shock proteins (HSPs) have been shown over the last few years to influence viral transcription and genome replication, with expression profiles helping to explain interspecies difference in permissiveness. Our work with MeV–HSP interaction suggests that differences in basal levels of HSPs can explain differences in permissiveness between cell lines derived from mouse relative to primate cells. Mouse cells exhibit reduced permissiveness to MeV infection relative to primate cells and lack basal expression of a major inducible 70-kDa HSP that is known to stimulate MeV replication[22].

Cellular HSPs are recognized for their function as molecular chaperones that facilitate protein folding and trafficking [56], and also for their ability to bind native proteins, resulting in altered activity of the substrate [57]. Multiple families of HSPs are recognized and are classified according to their mass, with members of the 70-kDa family being expressed at high basal and/or stress-inducible levels in all mammalian organ systems examined thus far [58]. Cytosolic 70-kDa HSPs include a constitutively expressed isoform (hsc70 or HSP73) and two highly stress-inducible isoforms that differ by only two amino acids and are collectively referred to as HSP70 (also known as HSP72). HSP70 is highly conserved across species, with 50% amino acid sequence homology between human HSP70 and the bacterial homolog DnaK, and 76% homology between human HSP70 and yeast [59]. For both humans and mice, HSP70 is encoded by two closely linked, intronless genes [58]. These genes are located in the MHC-III cluster, suggesting a role in inflammation and/or host defense.

HSPs & viral gene expression

Heat-shock proteins of the 70-kDa family play numerous roles in support of viral replication, but those roles differ for constitutively expressed isoforms (i.e., hsc70 and Grp78) and the highly inducible isoforms (i.e., HSP70) (see [60,61] for reviews). In general, hsc70 and Grp78 (Bip) support protein folding/unfolding and assembly/disassembly reactions required for viral uncoating, membrane glycoprotein maturation and nucleocapsid

assembly [62]. By contrast, HSP70 has been primarily linked to direct support of viral transcriptase and/or replicase activity. HSP70 mediates transcription and replication of human papillomavirus and herpes simplex virus type 1 (HSV-1) by promoting assembly of preinitiation complexes on the origin of DNA replication [63,64]. The ability of HSP70 to stimulate DNA virus gene expression may provide a mechanistic basis for the *in vitro* observation that heat shock stimulates reactivation of latent HSV-1 [65]. A role for HSP70 in stimulating gene expression by a RNA virus replicating in the cytoplasm was first shown by our group with CDV and MeV, beginning with the observation that HSP70 forms stable complexes with cytoplasmic nucleocapsid derived from the cytoplasm of infected cells [66,67]. The morbillivirus nucleocapsid consists of the single-stranded negative-sense RNA genome that is packaged by the N protein to form the nucleocapsid template for the virus-encoded RNA-dependent RNA polymerase (L). L is linked to the nucleocapsid through a tetramer of the polymerase cofactor P, where P binds the surface exposed C-terminus of the N protein (N_{TAIL}) [68]. HSP70 binds the same N_{TAIL} motifs that are bound by the P protein [69]. HSP70 can directly enhance transcriptional activity of purified nucleocapsid in cell-free assays [70], both viral transcription and genome replication are enhanced when HSP70 levels are elevated in the cytoplasm of cells and reduced when HSP70 levels are limiting [22,71], and HSP70-dependent effects on viral gene expression are mediated by HSP70 (and P) binding sites on N_{TAIL} [22,69,72].

Several models have been invoked to explain the HSP70-dependent effects on morbillivirus gene expression [61,73]. HSP70 binding to N_{TAIL} may alter interaction of the viral polymerase complex with the nucleocapsid template in such a way that encourages cycles of binding and release required for progression along the template. This could be achieved by direct HSP70 competitive inhibition of P/N_{TAIL} binding, or by more indirect effects on nucleocapsid conformations that are influenced by N_{TAIL}. That HSP70 may be a component of the viral transcriptase is consistent with the ability of antibody against HSP70 to inhibit cell-free nucleocapsid transcriptional activity not only for morbilliviruses, but also for respiratory syncytial virus, a member of the genus *Pneumovirus*, family *Paramyxoviridae* [74]. By analogy, HSP60 has been identified as a component of the transcriptase of vesicular stomatitis virus, a negative-strand RNA virus in the family *Rhabdoviridae* [75]. It is also possible that HSP70-mediated viral gene expression directs nucleocapsids to membrane platforms for the assembly of viral transcriptases and replicases, as has been suggested for respiratory syncytial virus (RSV) and shown for plant viruses [74,76,77].

HSPs & permissiveness of neural cells to MeV

Having established that HSP70 can be an important determinant of viral gene expression, we can now appreciate the potential significance of differences in basal levels of HSP70 that characterize certain species. In addition to being highly inducible, HSP70 is expressed to high constitutive levels in primate cells in contrast to rodents in which constitutive expression is lacking [78,79]. This has been shown in epithelial, mesenchymal and neural cell lines. In tissues, a direct comparison of HSP70 levels in humans and rodents shows that levels in nonstressed human brain are more than tenfold greater than the highest levels observed in brains of heat-shocked rats or mice [80]. We recently compared basal expression levels of HSP70 in tissues from nonstressed humans and mice using an ELISA of brain total protein extracts. No HSP70 is detected in brain extracts from C57BL/6 mice, whereas levels in normal human cerebrum, hippocampus and diencephalon averages 130 ng/mg total protein [Oglesbee M, Unpublished Observation]. If we constitutively express recombinant human HSP70 (i.e., the product of the *HSP70A1A* gene) in mouse neuronal cells, those cells support a dramatic increase in viral transcript and genome levels relative to cells in which constitutive HSP70 expression is lacking. This has been shown *in vitro*, comparing viral infection parameters in mouse neuroblastoma cells that are stably

transfected to express human HSP70 relative to infected vector transfected controls [22]. The ability of HSP70 to enhance permissiveness of mouse neuronal cells to MeV infection has also been shown *in vivo*, using transgenic C57BL/6 mice in which the neuronspecific enolase promoter drives constitutive HSP70 expression [23]. Transgene expression is restricted to neurons based upon results of immunohistochemistry, and levels of HSP70 in brain total protein extracts are low but detectable in these mice (approximately 1 ng/mg total protein). The lower level relative to humans probably reflects the selective expression of a single HSP70 isoform in neurons of the transgenic mice, versus constitutive expression of multiple HSP70 isoforms in humans. In addition, constitutive HSP70 in humans would be observed in neurons, glia and endothelial cells, versus selective neuronal expression in our transgenic mice. Neurons represent only 10% of the total cell population in human brains. The contribution of glia and endothelial cells to total brain HSP70 is illustrated by examining transgenic mice in which HSP70 expression is driven by a β -actin promoter [81]. In those mice, the HSP70 content is 14 ng/mg [Oglesbee M, Unpublished Observation].

Constitutive HSP70 expression in neurons enhances MeV neurovirulence in C57BL/6 mice. Intracranial inoculation of neonatal HSP70 transgenic C57BL/6 mice with 10^4 TCID₅₀ of Ed MeV resulted in 60% mortality between 11 and 20 days postinfection, versus 15% mortality in nontransgenic infected mice [23]. The increased mortality was associated with dramatic increases in brain viral RNA burden (by over one order of magnitude), increased levels of viral membrane glycoprotein transcripts and protein, and increased viral cytopathic effect, including formation of virus-induced syncytia, intranuclear and intracytoplasmic inclusion body formation, and necrosis of virus-infected cells. These cytopathic effects are hallmarks of MIBE in MeV-infected humans.

Viral induction of HSP70 may explain how mouse cells lacking detectable constitutive HSP70 levels can support a low level of permissiveness to MeV. HSP70 is induced by several RNA viruses replicating in the cytosol, including MeV [82], CDV [83], vesicular stomatitis virus [84], Sindbis virus [84], Newcastle disease virus [85] and RSV [86]. Here the mechanism probably involves activation of heat-shock transcription factor (HSF)-1. Both hsc70 and HSP70 bind HSF-1, maintaining the transcription factor in an inactive monomeric form [87,88]. When the cytosolic concentration of proteins requiring a chaperone is increased, the HSPs are titrated away from HSF-1 to the new client protein, allowing HSF-1 to trimerize and undergo phosphorylation activation [87]. Activated HSF-1₃^P then migrates to the nucleus to directly stimulate HSP transcription through binding of heat-shock-responsive promoter elements, resulting in increased intracellular levels of HSP70 [89]. We have shown that MeV induces HSP70 in mouse neuroblastoma (N2A) cells, and this occurs very early in the infection cycle [82]. This timing is consistent with the high affinity of N_{TAIL} for HSP70, the fact that N_{TAIL} is exposed on the surface of nucleocapsid, and the fact that the viral N protein is the first and most abundantly expressed viral protein in the cell [72]. Early induction would thus reflect the limited pools of HSP70 present in the uninfected cell (i.e., levels reflecting a prior protein unfolding response or true constitutive expression below our level of detection), the possibility that N_{TAIL} may bind hsc70 as well as HSP70 in the cytosol, and the abundance of N_{TAIL} at early time points postinfection that can compete with HSF-1 for HSP70/hsc70 binding. Mouse embryo fibroblasts lacking HSF-1 exhibit dramatically reduced levels of MeV gene expression relative to control cells [Oglesbee M *Et AL.*, Unpublished Observation], supporting the importance of virus-induced HSP70 and the HSF-1 pathway to MeV permissiveness of mouse cells. We also cannot exclude the possibility that HSP70 induction is the result of virus-cell surface interactions that trigger as yet uncharacterized signaling pathways, a possibility proposed for HSP70 induction in HIV-infected lymphocytes [90]. Collectively, these observations support the view that HSP70 levels can be limiting to MeV gene expression, that virus can induce levels in mouse cells that provide limited support of

replication, and that primate-like patterns of HSP70 expression confers a primate-like level of permissiveness to MeV infection in mouse cells.

Interplay between HSPs & immune response determines neurovirulence

As mentioned previously, resistant mice (H-2^d haplotype) can overcome MeV infection, whereas susceptible mice (H-2^b haplotype) may succumb to infection. These differences in susceptibility were shown using 6–8-week-old mice challenged with the rodent brain-adapted CAM/RB MeV. Differences in susceptibility of neonates were never examined. To test the neurovirulence of MeV infection in mice that are ‘humanized’ in terms of HSP70 expression, H-2^b and H-2^d congenic C57BL/6 that constitutively express HSP70 in neurons were generated [91]. Ed MeV was used since the HSP70 responsiveness of this strain has been extensively characterized. While mortality in 6–8-week-old mice is not observed using this virus, we could exploit the enhanced susceptibility of neonates. Baseline mortality of Ed MeV-infected H-2^b and H-2^d congenic C57BL/6 mice was similar. Failure to detect H-2-dependent differences in susceptibility may reflect the immaturity (and thus inefficiency) of adaptive immune responses at this age. However, complete protection against virus-induced mortality was observed in HSP70-overexpressing (immune competent) H-2^d mice, compared with 35% mortality in nontransgenic H-2^d controls, and 70% mortality in H-2^b HSP70 transgenic mice. There was a significant inverse correlation between survival times and brain viral RNA burden. Complete protection was only observed with virus mounting a transcriptional response to HSP70 overexpression (i.e., parent Ed MeV). This was established by using an Ed MeV mutant lacking a transcriptional response to HSP70 [22,69]. A single amino acid substitution in one of the HSP70 interacting sites on the N protein (N522D) is sufficient to disrupt HSP70 stimulation of transcription without affecting other viral replication parameters, including HSP70-dependent increases in viral genome levels. Survival times were not statistically significantly different between HSP70-overexpressing and control mice challenged with an Ed N522D, although there was a trend towards protection in the transgenic mice [91].

In order to establish the basis for protection against Ed MeV virulence in HSP70-overexpressing mice, infected animals were depleted of CD4⁺ and/or CD8⁺ T cells through biweekly intraperitoneal injection of CD4 and/or CD8-specific monoclonal antibodies [91]. Mortality increased from 35 to 94% in nontransgenic mice in which CD4⁺ T cells were depleted, compared with 63% mortality in CD8⁺ T-cell-depleted mice. Mortality was 100% when both T-cell subsets were deleted. Results are consistent with a greater reliance on CD4⁺ T cells for protection, although both lineages contribute [92]. By contrast, mortality in transgenic mice was only 41% with CD4⁺ T-cell depletion and 27% with CD8⁺ T-cell depletion. Combined CD4 and CD8⁺ T-cell depletion still allowed 27% survival, indicating a significant level of innate immunity that is associated with HSP70.

Infected brains were analyzed at 5 days postinfection in order to gain insight as to the basis for the innate immunity that is associated with constitutive HSP70 expression [91]. Significant lymphocytic infiltrates were not present at this time, based upon evaluation of tissue sections and the low levels of IFN- γ transcripts that were detected in total brain RNA by real-time reverse transcriptase-PCR. Both lymphocytic infiltrates and IFN- γ transcripts are significantly increased by 10 days postinfection, marking the onset of rapidly escalating adaptive T cell immune responses. Transcript levels for IFN- γ were approximately two- to three-fold greater in infected transgenic relative to infected nontransgenic mice at both 5 and 10 days postinfection, although statistical significance was not demonstrated due to the large standard deviations of the means. Transcripts for both CD68 and MHC-II were significantly greater in infected transgenic relative to nontransgenic mice at 5 days postinfection, indicating enhanced activation of macrophages. In addition, transcripts for Toll-like receptor (TLR)2 and TLR4 were significantly elevated in brains of infected transgenic relative to

infected nontransgenic mice. The latter observation suggests that TLR signaling may be a basis for the macrophage activation, although we cannot exclude the possibility that such increases are a consequence of type I IFN signaling.

Model for the efficient induction of host immune responses to MeV infection of brain

HSP70 can be secreted from abnormal or stressed cells and released following cell death, where they may initiate innate immune and/or proinflammatory responses (see review [93]). The proinflammatory effects of extracellular HSPs are attributed to interaction with TLRs of antigen-presenting cells, particularly macrophages [94]. TLRs were originally identified as receptors responding to pathogen-associated molecular patterns, but also recognize both HSP and non-HSP endogenous ligands [95,96]. There are now 13 known TLRs, some expressed on the cell surface and others expressed only within the cell. HSP70 exerts its effects primarily through TLR4 but also through TLR2 [96–98], these being cell-surface TLRs that signal antigen presentation, proinflammatory cytokine release and type I IFN production in macrophages [99,100]. In this way, extracellular HSP70 may serve as a danger signal similar to other proinflammatory cytokines, this being the basis for the term ‘chaperokine’ in reference to extracellular HSP70 and other extracellular HSPs [101]. Secretory release involves mechanisms that are only beginning to be understood, and the pathway is thought to be similar to that of IL-1 [102,103]. Both IL-1 and HSP70 lack leader sequences used by most proteins to enter vesicular transport pathways. Secretory release has been suggested for RSV *in vitro* infections, based upon the observation that viral infection causes redistribution of HSP70 to intracellular membranes of the secretory pathway [74]. Virus-induced cell death is also a potent means of releasing HSP70 from cells, this having been demonstrated in parvovirus infections [104]. Release of HSPs is not limited to HSP70, with contributions by other family members including HSP60 and HSP90. We have shown extracellular release of HSP70 in MeV-infected cell lines, where magnitude of release is proportionate to both the level of viral gene expression and intracellular HSP70 concentration [Oglesbee M, Unpublished Observation]. In addition, RSV infections increase extracellular HSP70 concentrations in airways of infected children [105], supporting the *in vivo* relevance of this phenomenon.

Data from HSP70 transgenic mice suggests how the primate pattern of constitutive HSP70 expression may be a key variable in explaining the ability of humans to mount highly efficient antiviral immune responses that prevent MeV neurovirulence (Figure 2). In an immune competent host, HSP70-mediated increases in viral gene expression would enhance viral cytopathic effects in the initially infected cells, but this would serve to increase extracellular HSP70 release, resulting in activation of uninfected macrophages through TLR2 and TLR4. The result would be increased expression of IFN- β , and enhanced presentation of viral antigen to infiltrating T cells with subsequent amplification of IFN- γ release. It should be noted that microglia can also secrete IFN- γ following TLR4 stimulation [106], and TLR4 signaling induces IFN- γ production by lymphocytes [107], adding to that produced by infiltrating virus-specific T cells. The protective effects of IFN- β would be both indirect through support of T-cell activation and direct. In addition to inducing MHC expression, HSP70 could mediate antigen crosspresentation. HSP70 binds MeV N protein (i.e., N_{TAIL}) with high affinity [72], HSP70 and bound antigen can be internalized by macrophages via CD91 for MHC presentation of the antigen [108], and an immunodominant epitope of MeV N protein (for H-2^d mice) is localized to the HSP70-binding sites within N_{TAIL} [109]. Release of HSP70 from viable neurons would contribute to a viral clearance mechanism that does not require cell loss. But should this fail, virus-induced cell death would certainly provide the signal [110]. Thus, the MeV–HSP70 relationship is adaptive, provided that the host is immune competent. In the face of an immune deficiency, HSP70-

mediated increases in viral gene expression are unchecked, and the result is fulminate viral spread – enhanced neurovirulence.

If HSP70-mediated stimulation of MeV gene expression contributes to the induction of antiviral immunity, then there should be an immune selection pressure against viruses such as Ed MeV that exhibit a transcriptional response to HSP70 overexpression. Indeed, *in vitro* and *in vivo* fitness analyses show that the N protein 522D motif exhibits reduced fitness relative to 522N, the *in vivo* fitness being measured in lungs of cotton rats at 4 days postinfection [22]. The reduced fitness associated with the 522D motif stands in contrast to the fact that it is carried by 94% of all wild-type MeV strains [111,112], whereas 522N is typical of tissue culture-adapted vaccine lineages of which Ed is a prototype [113]. The prevalence of 522D motifs among wild-type viruses, despite the reduced fitness compared with 522N motifs, supports the presence of a selection pressure against 522N. Lack of a viral transcription response to HSP70 does not preclude HSP70 release from infected cells, particularly in natural primate hosts. Given that virus-induced release is proportionate to intracellular levels of HSP70, and that high constitutive HSP70 expression is characteristic of primate cells and tissues, it is likely that wild-type and vaccine viruses differ mainly in the magnitude of HSP70 release, not the release *per se*.

The model explains the well-recognized association between viral infection and a host febrile response. Fever is a defining feature of MeV infection [114], and fever is a potent inducer of HSP70 in brain and other tissues [78,115]. Higher intracellular levels of HSP70 translate into higher potential levels of extracellular HSP70. The phylogenetic conservation of the febrile response suggests that it is adaptive, and the model provides an explanation for how this response can be host protective despite the ability of HSP70 to enhance viral gene expression. In this regard, study of MeV–HSP70 interaction may have broad virological relevance. A review of viruses causing acute neurological disease in humans reveals that members of the herpesvirus [64,116–120], flavivirus [121,122], picornavirus [123], rhabdovirus [124,125] and bunyavirus [126] families utilize HSP70 in some aspect of their replication, although *in vivo* significance of the virus–HSP70 interaction has not been established. Relevance may apply to organ systems outside of the CNS. For example, in lung, RSV causes release of HSP70 in airways of infected children and activates TLR4 signaling pathways in leukocytes [105], and *TLR4*^{−/−} mice exhibit enhanced susceptibility to RSV challenge [127,128], although we have yet to directly link release of HSP70 to antiviral immune responses in this system. Extracellular release of HSP70 in the CNS is an emerging area of study, and extracellular HSP70 has not been evaluated in cases of viral meningoencephalitis in humans. With that said, it has recently been shown that increases in extracellular HSP70 are associated with other CNS inflammatory states, including that associated with ischemia-reperfusion injury in humans and dogs [129,130] and steroid-responsive meningoarteritis in dogs [Oglesbee M et al., Unpublished Observation].

Conclusion

By considering recent developments in the study of virus–HSP70 interaction and HSP70 biology in general, we may make some sense of historical observations and relationships that are relevant to MeV neurovirulence. In humans, MeV is similar to other paramyxoviruses in being highly neuroinvasive yet poorly neurovirulent, and it would appear that a highly efficient host immune response is responsible for the latter. In humans, IFN- β and IFN- γ are expressed in infected brain, and use of rodent models has helped us understand how these responses are orchestrated and how they can be so effective. Data from mouse intracranial inoculations with MeV show that IFN- γ production is released primarily by virus-specific T cells, where IFN- γ mediates noncytolytic clearance of virus from neurons. In the mouse, virus-infected neurons do not express IFN- β and brain

macrophages are uninfected, raising questions as to the source of IFN- β , its role in innate and adaptive antiviral immunity in brain, and how uninfected microglia cells are activated to support T-cell production of IFN- γ . Consideration of species differences in expression of HSP70 allows us to address these questions. HSP70 supports MeV gene expression and is immune stimulatory when released from infected cells. The high basal levels of HSP70 in primates, compared with lack of such expression in rodents, is therefore a significant difference that should be addressed when using mice to model MeV infection of human brain. Transgenic mice that constitutively express HSP70 overcome limitations in intrinsic cell support of viral gene expression that is otherwise observed in rodents, and in animals with deficient cell-mediated immunity, these transgenic mice support a fulminate form of CNS infection that reproduces key features of MIBE. In animals with efficient cell-mediated immune responses to infection, determined by H-2 haplotype, these same mice mount a highly efficient antiviral immune response that precludes virus-induced mortality. A model for the HSP70 protective effect centers on the relationship between elevated intracellular levels of HSP70, extracellular release of HSP70 from virus-infected cells and widespread activation of uninfected microglia through TLRs. Aspects of this model remain to be proven, but fit with the conservation of HSP70-inducing febrile responses to viral infection and our emerging view of stress proteins as danger signals. The HSP70 transgenic mice are the first to model the biological (*in vivo*) significance of virus–HSP70 interaction, and future studies must address the impact of host age on this phenomenon and the broad–virological relevance.

Future perspective

Advances in any field of study come from insights gained from other areas of investigation. In the case of MeV neurovirulence, or neurovirulence mediated by any number of viral pathogens, advances in HSP biology/immunology alters the way we address antiviral immunity. But the field of virology has been slow to examine the consequences of virus–HSP interaction on viral pathogenesis. Emphasis on virus–HSP interaction has remained largely focused upon how HSPs support viral replication, without asking how that support may influence outcome of infection within an animal host (including humans). The relatively few virological investigations that consider the immune modulatory potential of HSPs have focused upon the ability of HSP70 to enhance the immunogenicity of viral vaccines [131,132], or for virolytic tumor therapies to release HSPs in an attempt to stimulate antitumor immunity [104]. Work with MeV is the first to address how the virus–HSP interaction influences viral virulence, and how that balance may be tipped in favor of the virus or the host. That work highlights the importance of considering the animal model that is used. We must ask how well mice may model antiviral immunity against a human pathogen given the dramatic difference between humans and mice in terms of constitutive HSP70 expression. Use of HSP70 transgenic mice is one means of enhancing relevance of the mouse model, or we may turn to other animal species. Cotton rats exhibit constitutive HSP70 expression in multiple tissues [Oglesbee M, Unpublished Observation], similar to primates, and a similar expression pattern is seen in dogs [133]. Dogs infected with CDV may therefore represent a model of antiviral immunity in neurovirulence that has relevance to MeV in humans. To date, cotton rats have not been used to study brain infection by MeV, although we do know that this species models humans in terms of differential immune responses to vaccine and wild-type viruses following respiratory challenge [134]. Use of the cotton rat should therefore be considered in examining the interaction between HSP70 and vaccine and wild-type strains of MeV in brain, specifically the impact upon antiviral immunity and viral virulence.

We can anticipate increased emphasis being placed upon mechanisms (and consequences) of extracellular HSP70 release, be that secretory or the result of virus-induced cell death.

Levels of extracellular HSP70 release have already become a focus of investigation in CNS inflammatory conditions associated with ischemia–reperfusion [129,130] and metabolic disease [135], and release associated with viral infections is a new area of investigation. This opens the door to a number of questions that must be addressed. From a therapeutic standpoint, enhancing intracellular HSP70 levels that may in turn enhance extracellular levels of HSP70 can be achieved pharmacologically or as a consequence of fever, but our model predicts that the host must be immune competent, otherwise the stimulatory effect of intracellular HSP70 on viral gene expression may proceed unchecked, leading to conditions such as MIBE. A state of immune suppression may be overt, as in a combined immune deficiency syndrome, but it is also possible that MHC haplotype defines virus-specific immune responses that are inadequate to exploit the virus–HSP relationship to the benefit of the host. We must also consider the possibility that high levels of HSP70 trigger inflammatory responses with untoward side effects. HSP70 signaling through TLRs causes release of high levels of proinflammatory cytokines, and the relationship between virus-induced HSP release and postinfectious viral encephalitis must be examined. Finally, by embracing HSPs as a fundamental component of host innate immune responses, we may ask if deficiencies in this response contribute to the establishment of persistent viral infections that may ultimately give rise to disease entities such as SSPE. In short, by viewing historical questions surrounding MeV neurovirulence through the lens of HSP biology, we may be able to advance the field.

Bibliography

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Leonard VH, Sinn PL, Hodge G, et al. Measles virus blind to its epithelial cell receptor remains virulent in rhesus monkeys but cannot cross the airway epithelium and is not shed. *J. Clin. Invest.* 2008; 118:2448–2458. [PubMed: 18568079]
2. de Vries RD, Lemon K, Ludlow M, et al. *In vivo* tropism of attenuated and pathogenic measles virus expressing green fluorescent protein in macaques. *J. Virol.* 2010; 84:4714–4724. [PubMed: 20181691]
3. Krakowka S. Canine distemper virus infectivity of various blood fractions for central nervous system vasculature. *J. Neuroimmunol.* 1989; 21:75–80. [PubMed: 2908882]
4. Duke T, Mgone CS. Measles: not just another viral exanthem. *Lancet.* 2003; 361:763–773. [PubMed: 12620751]
5. Rudd PA, Cattaneo R, von Messling V. Canine distemper virus uses both the anterograde and the hematogenous pathway for neuroinvasion. *J. Virol.* 2006; 80:9361–9370. [PubMed: 16973542]
6. Roelke-Parker ME, Munson L, Packer C, et al. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature.* 1996; 379:441–445. [PubMed: 8559247]
7. Krakowka S, Koestner A. Age-related susceptibility to infection with canine distemper virus in gnotobiotic dogs. *J. Infect. Dis.* 1976; 134:629–632. [PubMed: 1003016]
8. Krakowka S, Long D, Koestner A. Influence of transplacentally acquired antibody on neonatal susceptibility to canine distemper virus in gnotobiotic dogs. *J. Infect. Dis.* 1978; 137:605–608. [PubMed: 659917]
9. Brooks, GF.; Butel, JS.; Morse, SA. Paramyxovirus and rubella virus. In: Butler, JP.; Ransom, J.; Ryan, E., editors. *Adelberg's Microbiology*. Appleton and Lange, Stamford, CT: USA; 1998. p. 507-527.
10. Katayama Y, Hotta H, Nishimura A, et al. Detection of measles virus nucleoprotein mRNA in autopsied brain tissues. *J. Gen. Virol.* 1995; 76:3201–3204. [PubMed: 8847530]

11. Katayama Y, Kohso K, Nishimura A, et al. Detection of measles virus mRNA from autopsied human tissues. *J. Clin. Microbiol.* 1998; 36:299–301. [PubMed: 9431973]
12. Moench TR, Griffin DE, Obriecht CR, et al. Acute measles in patients with and without neurological involvement: distribution of measles virus antigen and RNA. *J. Infect. Dis.* 1988; 158:433–442. [PubMed: 3042879]
13. Rozina EE, Kaptsova TI, Sharova OK, et al. Study of mumps virus invasiveness in monkeys. *Acta Virol.* 1984; 28:107–113. [PubMed: 6145343]
14. Katz M. Clinical spectrum of measles. *Curr. Top. Microbiol. Immunol.* 1995; 191:1–12. [PubMed: 7789153]
15. Bitnun A, Shannon P, Durward A, et al. Measles inclusion-body encephalitis caused by the vaccine strain of measles virus. *Clin. Infect. Dis.* 1999; 29:855–861. [PubMed: 10589903]
16. Bellini WJ, Rota JS, Lowe LE, et al. Subacute sclerosing panencephalitis: more cases of this fatal disease are prevented by measles immunization than was previously recognized. *J. Infect. Dis.* 2005; 192:1686–1693. [PubMed: 16235165]
17. Komase K, Rima BK, Pardowitz I, et al. A comparison of nucleotide sequences of measles virus *L* genes derived from wild-type viruses and SSPE brain tissues. *Virology.* 1995; 208:795–799. [PubMed: 7747453]
18. Poirier B, Morgeaux S, Fuchs F. The assessment of OPV vaccines by the monkey neurovirulence test: why and how to qualify the experts in histology of central nervous system. *Vaccine.* 2002; 21:115–119. [PubMed: 12443669]
19. Yanagi Y, Takeda M, Ohno S, et al. Measles virus receptors. *Curr. Top. Microbiol. Immunol.* 2009; 329:13–30. [PubMed: 19198560]
20. Watanabe A, Yoneda M, Ikeda F, et al. CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells. *J. Virol.* 2010; 84:4183–4193. [PubMed: 20147391]
21. Makhortova NR, Askovich P, Patterson CE, et al. Neurokinin-1 enables measles virus trans-synaptic spread in neurons. *Virology.* 2007; 362(1):235–244. [PubMed: 17434199]
22. Carsillo T, Zhang X, Vasconcelos D, et al. A single codon in the nucleocapsid protein C terminus contributes to *in vitro* and *in vivo* fitness of Edmonston measles virus. *J. Virol.* 2006; 80:2904–2912. [PubMed: 16501099]
23. Carsillo T, Traylor Z, Choi C, et al. HSP72, a host determinant of measles virus neurovirulence. *J. Virol.* 2006; 80:11031–11039. [PubMed: 16971451] • Constitutive expression of heat-shock protein (HSP)70 in neurons of the mouse support a fulminate pattern of spread in the brain when the host is immune compromised.
24. Sellin CI, Horvat B. Current animal models: transgenic animal models for the study of measles pathogenesis. *Curr. Top. Microbiol. Immunol.* 2009; 330:111–127. [PubMed: 19203107]
25. Duprex WP, Duffy I, McQuaid S, et al. The *H* gene of rodent brain-adapted measles virus confers neurovirulence to the Edmonston vaccine strain. *J. Virol.* 1999; 73:6916–6922. [PubMed: 10400789]
26. Young VA, Rall GF. Making it to the synapse: measles virus spread in and among neurons. *Curr. Top. Microbiol. Immunol.* 2009; 330:3–30. [PubMed: 19203102]
27. Griffin DE, Mullinix J, Narayan O, et al. Age dependence of viral expression: comparative pathogenesis of two rodent-adapted strains of measles virus in mice. *Infect. Immun.* 1974; 9:690–695. [PubMed: 4132617]
28. Sakae H, Kohase M, Kurata T, et al. Isolation of a measles virus variant: protection of newborn mice from measles encephalitis by 24 h prior intracerebral inoculation with the variant. *Arch. Virol.* 1997; 142:1937–1952. [PubMed: 9413503]
29. Ryman KD, Gardner CL, Meier KC, et al. Early restriction of alphavirus replication and dissemination contributes to age-dependent attenuation of systemic hyperinflammatory disease. *J. Gen. Virol.* 2007; 88:518–529. [PubMed: 17251570]
30. Uytendaele FG, van Binnendijk RS, Kenter MJ, et al. Cytotoxic T lymphocyte responses against measles virus. *Curr. Top. Microbiol. Immunol.* 1994; 189:151–167. [PubMed: 7924435]
31. Gans HA, Maldonado Y, Yasukawa LL, et al. IL-12, IFN- γ , and T cell proliferation to measles in immunized infants. *J. Immunol.* 1999; 162:5569–5575. [PubMed: 10228039]

32. Joncas JH, Robillard LR, Boudreault A, et al. Letter: interferon in serum and cerebrospinal fluid in subacute sclerosing panencephalitis. *Can. Med. Assoc. J.* 1976; 115:309. [PubMed: 953897]
33. Hofman FM, Hinton DR, Baemayr J, et al. Lymphokines and immunoregulatory molecules in subacute sclerosing panencephalitis. *Clin. Immunol. Immunopathol.* 1991; 58:331–342. [PubMed: 1900461]
34. Patterson CE, Lawrence DM, Echols LA, et al. Immune-mediated protection from measles virus-induced central nervous system disease is noncytolytic and γ interferon dependent. *J. Virol.* 2002; 76:4497–4506. [PubMed: 11932415]
35. Finke D, Brinckmann UG, ter Meulen V, Liebert UG. γ interferon is a major mediator of antiviral defense in experimental measles virus-induced encephalitis. *J. Virol.* 1995; 69:5469–5474. [PubMed: 7636992]
36. Robertson B, Kong G, Peng Z, et al. Interferon- γ -responsive neuronal sites in the normal rat brain: receptor protein distribution and cell activation revealed by Fos induction. *Brain Res. Bull.* 2000; 52:61–74. [PubMed: 10779704]
37. Tishon A, Lewicki H, Rall G, et al. An essential role for type 1 interferon- γ in terminating persistent viral infection. *Virology.* 1995; 212:244–250. [PubMed: 7676639]
38. Binder GK, Griffin DE. Interferon- γ -mediated site-specific clearance of alphavirus from CNS neurons. *Science.* 2001; 293:303–306. [PubMed: 11452126]
39. Parra B, Hinton DR, Marten NW, et al. IFN- γ is required for viral clearance from central nervous system oligodendroglia. *J. Immunol.* 1999; 162:1641–1647. [PubMed: 9973424]
40. Komatsu T, Bi Z, Reiss CS. Interferon- γ induced type I nitric oxide synthase activity inhibits viral replication in neurons. *J. Neuroimmunol.* 1996; 68:101–108. [PubMed: 8784266]
41. Rodriguez M, Zoecklein LJ, Howe CL, et al. γ interferon is critical for neuronal viral clearance and protection in a susceptible mouse strain following early intracranial Theiler's murine encephalomyelitis virus infection. *J. Virol.* 2003; 77:12252–12265. [PubMed: 14581562]
42. Koustova E, Sei Y, McCarty T, et al. Accelerated development of neurochemical and behavioral deficits in LP-BM5 infected mice with targeted deletions of the *IFN- γ* gene. *J. Neuroimmunol.* 2000; 108:112–121. [PubMed: 10900344]
43. Guidotti LG, Rochford R, Chung J, et al. Viral clearance without destruction of infected cells during acute HBV infection. *Science.* 1999; 284:825–829. [PubMed: 10221919]
44. Neumeister C, Niewiesk S. Recognition of measles virus-infected cells by CD8⁺ T cells depends on the H-2 molecule. *J. Gen. Virol.* 1998; 79:2583–2591. [PubMed: 9820133]
45. Weidinger G, Czub S, Neumeister C, et al. Role of CD4⁺ and CD8⁺ T cells in the prevention of measles virus-induced encephalitis in mice. *J. Gen. Virol.* 2000; 81:2707–2713. [PubMed: 11038383]
46. Niewiesk S, Brinckmann U, Bankamp B, et al. Susceptibility to measles virus-induced encephalitis in mice correlates with impaired antigen presentation to cytotoxic T lymphocytes. *J. Virol.* 1993; 67:75–81. [PubMed: 8093223]
47. Weidinger G, Ohlmann M, Schlereth B, et al. Vaccination with recombinant modified vaccinia virus Ankara protects against measles virus infection in the mouse and cotton rat model. *Vaccine.* 2001; 19:2764–2768. [PubMed: 11282186]
48. Matthews VB, Christiansen FT, Price P. Lymphocytes from H2^b mice produce lower levels of several cytokines than congenic H2k or H2^d mice. *Immunol. Cell Biol.* 2000; 78:247–253. [PubMed: 10849112]
49. Bankamp B, Brinckmann UG, Reich A, et al. Measles virus nucleocapsid protein protects rats from encephalitis. *J. Virol.* 1991; 65:1695–1700. [PubMed: 1825854]
50. Stevenson PG, Hawke S, Sloan DJ, et al. The immunogenicity of intracerebral virus infection depends on anatomical site. *J. Virol.* 1997; 71:145–151. [PubMed: 8985333]
51. Pien GC, Nguyen KB, Malmgaard L, et al. A unique mechanism for innate cytokine promotion of T cell responses to viral infections. *J. Immunol.* 2002; 169:5827–5837. [PubMed: 12421964]
52. Slifka MK, Rodriguez F, Whitton JL. Rapid on/off cycling of cytokine production by virus-specific CD8⁺ T cells. *Nature.* 1999; 401:76–79. [PubMed: 10485708]
53. Roscic-Mrkic B, Schwendener RA, Odermatt B, et al. Roles of macrophages in measles virus infection of genetically modified mice. *J. Virol.* 2001; 75:3343–3351. [PubMed: 11238860]

54. Abdullah H, Earle JA, Gardiner TA, et al. Persistent measles virus infection of mouse neural cells lacking known human entry receptors. *Neuropathol. Appl. Neurobiol.* 2009; 35:473–486. [PubMed: 19490430]
55. Dhib-Jalbut S, Xia J, Rangaviggula H, et al. Failure of measles virus to activate nuclear factor- κ B in neuronal cells: implications on the immune response to viral infections in the central nervous system. *J. Immunol.* 1999; 162:4024–4029. [PubMed: 10201924]
56. Hartl FU. Molecular chaperones in cellular protein folding. *Nature.* 1996; 381:571–579. [PubMed: 8637592]
57. Gamer J, Multhaup G, Tomoyasu T, et al. A cycle of binding and release of the DnaK, DnaJ and GrpE chaperones regulates activity of the *Escherichia coli* heat shock transcription factor sigma32. *EMBO J.* 1996; 15:607–617. [PubMed: 8599944]
58. Dugaard M, Rohde M, Jaattela M. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* 2007; 581:3702–3710. [PubMed: 17544402]
59. Hunt C, Morimoto RI. Conserved features of eukaryotic *HSP70* genes revealed by comparison with the nucleotide sequence of human *HSP70*. *Proc. Natl Acad. Sci. U.S.A.* 1985; 82:6455–6459. [PubMed: 3931075]
60. Mayer MP. Recruitment of HSP70 chaperones: a crucial part of viral survival strategies. *Rev. Physiol. Biochem. Pharmacol.* 2005; 153:1–46. [PubMed: 15243813]
61. Oglesbee M. Nucleocapsid protein interactions with the major inducible 70 kDa heat shock protein. In: Longhi S, editor. *Measles Virus Nucleoprotein*. Nova Science Publishers, NY: USA; 2007. p. 53–98.
62. Perez-Vargas J, Romero P, Lopez S, et al. The peptide-binding and ATPase domains of recombinant hsc70 are required to interact with rotavirus and reduce its infectivity. *J. Virol.* 2006; 80:3322–3331. [PubMed: 16537599]
63. Liu JS, Kuo SR, Makhov AM, et al. Human HSP70 and HSP40 chaperone proteins facilitate human papillomavirus-11 E1 protein binding to the origin and stimulate cell-free DNA replication. *J. Biol. Chem.* 1998; 273:30704–30712. [PubMed: 9804845]
64. Tanguy Le GN, Boehmer PE. Activation of the herpes simplex virus type-1 origin-binding protein (UL9) by heat shock proteins. *J. Biol. Chem.* 2002; 277:5660–5666. [PubMed: 11711536]
65. Halford WP, Gebhardt BM, Carr DJ. Mechanisms of herpes simplex virus type 1 reactivation. *J. Virol.* 1996; 70:5051–5060. [PubMed: 8764012]
66. Oglesbee M, Ringler S, Krakowka S. Interaction of canine distemper virus nucleocapsid variants with 70K heat-shock proteins. *J. Gen. Virol.* 1990; 71:1585–1590. [PubMed: 2374009]
67. Vasconcelos D, Norrby E, Oglesbee M. The cellular stress response increases measles virus-induced cytopathic effect. *J. Gen. Virol.* 1998; 79:1769–1773. [PubMed: 9680141]
68. Bankamp B, Horikami SM, Thompson PD, et al. Domains of the measles virus N protein required for binding to P protein and self-assembly. *Virology.* 1996; 216:272–277. [PubMed: 8615002]
69. Zhang X, Bourhis JM, Longhi S, et al. HSP72 recognizes a P binding motif in the measles virus N protein C-terminus. *Virology.* 2005; 337:162–174. [PubMed: 15914229]
70. Oglesbee MJ, Liu Z, Kenney H, et al. The highly inducible member of the 70 kDa family of heat shock proteins increases canine distemper virus polymerase activity. *J. Gen. Virol.* 1996; 77:2125–2135. [PubMed: 8811012]
71. Vasconcelos DY, Cai XH, Oglesbee MJ. Constitutive overexpression of the major inducible 70 kDa heat shock protein mediates large plaque formation by measles virus. *J. Gen. Virol.* 1998; 79:2239–2247. [PubMed: 9747734]
72. Zhang X, Glendening C, Linke H, et al. Identification and characterization of a regulatory domain on the carboxyl terminus of the measles virus nucleocapsid protein. *J. Virol.* 2002; 76:8737–8746. [PubMed: 12163594] • HSP70-binding site is defined on the C-terminus of the measles virus nucleoprotein that mediates HSP70-dependent stimulation of viral transcription.
73. Bourhis JM, Canard B, Longhi S. Structural disorder within the replicative complex of measles virus: functional implications. *Virology.* 2006:94–110. [PubMed: 16364741]
74. Brown G, Rixon HW, Steel J, et al. Evidence for an association between heat shock protein 70 and the respiratory syncytial virus polymerase complex within lipid-raft membranes during virus infection. *Virology.* 2005; 338:69–80. [PubMed: 15936795]

75. Qanungo KR, Shaji D, Mathur M, et al. Two RNA polymerase complexes from vesicular stomatitis virus-infected cells that carry out transcription and replication of genome RNA. *Proc. Natl Acad. Sci. U.S.A.* 2004; 101:5952–5957. [PubMed: 15069200]
76. Jiang Y, Serviene E, Gal J, et al. Identification of essential host factors affecting tombusvirus RNA replication based on the yeast Tet promoters Hughes Collection. *J. Virol.* 2006; 80:7394–7404. [PubMed: 16840320]
77. Nagy PD. Yeast as a model host to explore plant virus-host interactions. *Annu. Rev. Phytopathol.* 2008; 46:217–242. [PubMed: 18422427]
78. Morrison-Bogorad M, Zimmerman AL, Pardue S. Heat-shock 70 messenger RNA levels in human brain: correlation with agonal fever. *J. Neurochem.* 1995; 64:235–246. [PubMed: 7798918]
79. Carsillo T, Carsillo M, Niewiesk S, et al. Hyperthermic pre-conditioning promotes measles virus clearance from brain in a mouse model of persistent infection. *Brain Res.* 2004; 1004:73–82. [PubMed: 15033421] • Febrile temperatures induce HSP70 in the mouse brain and this induction is correlated to enhanced viral clearance following measles virus challenge in an immune competent host.
80. Pardue S, Wang S, Miller MM, et al. Elevated levels of inducible heat shock 70 proteins in human brain. *Neurobiol. Aging.* 2006; 28:314–324. [PubMed: 16413087] • Humans express abundant constitutive HSP70 in brain, in contrast to rodents.
81. Plumier JC, Krueger AM, Currie RW, et al. Transgenic mice expressing the human inducible HSP70 have hippocampal neurons resistant to ischemic injury. *Cell Stress Chaperones.* 1997; 2:162–167. [PubMed: 9314603]
82. Oglesbee MJ, Pratt M, Carsillo T. Role for heat shock proteins in the immune response to measles virus infection. *Viral. Immunol.* 2002; 15:399–416. [PubMed: 12479391]
83. Oglesbee M, Krakowka S. Cellular stress response induces selective intranuclear trafficking and accumulation of morbillivirus major core protein. *Lab. Invest.* 1993; 68:109–117. [PubMed: 8423670]
84. Garry RF, Ulug ET, Bose HR Jr. Induction of stress proteins in Sindbis virus- and vesicular stomatitis virus-infected cells. *Virology.* 1983; 129:319–332. [PubMed: 6312678]
85. Collins PL, Hightower LE. Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. *J. Virol.* 1982; 44:703–707. [PubMed: 7143579]
86. Brasier AR, Spratt H, Wu Z, et al. Nuclear heat shock response and novel nuclear domain 10 reorganization in respiratory syncytial virus-infected a549 cells identified by high-resolution two-dimensional gel electrophoresis. *J. Virol.* 2004; 78:11461–11476. [PubMed: 15479789]
87. Snoeckx LH, Cornelussen RN, Van Nieuwenhoven FA, et al. Heat shock proteins and cardiovascular pathophysiology. *Physiol. Rev.* 2001; 81:1461–1497. [PubMed: 11581494]
88. Mosser DD, Duchaine J, Massie B. The DNA-binding activity of the human heat shock transcription factor is regulated *in vivo* by HSP70. *Mol. Cell Biol.* 1993; 13:5427–5438. [PubMed: 8355691]
89. Mandrekar P, Catalano D, Jeliaskova V, et al. Alcohol exposure regulates heat shock transcription factor binding and heat shock proteins 70 and 90 in monocytes and macrophages: implication for TNF- α regulation. *J. Leukoc. Biol.* 2008; 84(5):1335–1345. [PubMed: 18689673]
90. Furlini G, Vignoli M, Re MC, et al. Human immunodeficiency virus type 1 interaction with the membrane of CD4⁺ cells induces the synthesis and nuclear translocation of 70K heat shock protein. *J. Gen. Virol.* 1994; 75:193–199. [PubMed: 7906708]
91. Carsillo T, Carsillo M, Traylor Z, et al. Major histocompatibility complex haplotype determines HSP70-dependent protection against measles virus neurovirulence. *J. Virol.* 2009; 83:5544–5555. [PubMed: 19321604] • Primate pattern of constitutive HSP70 expression in mouse neurons is host protective in immune competent mice (defined by H-2 haplotype). Our model predicts that it is the release of HSP70 from infected cells that mediates a protective immune response and that such release is enhanced when viral gene expression is increased by HSP70.
92. Finke D, Liebert UG. CD4⁺ T cells are essential in overcoming experimental murine measles encephalitis. *Immunology.* 1994; 83:184–189. [PubMed: 7835934]
93. Quintana FJ, Cohen IR. Heat shock proteins as endogenous adjuvants in sterile and septic inflammation. *J. Immunol.* 2005; 175:2777–2782. [PubMed: 16116161]

94. Vabulas RM, Wagner H, Schild H. Heat shock proteins as ligands of Toll-like receptors. *Curr. Top. Microbiol. Immunol.* 2002; 270:169–184. [PubMed: 12467251]
95. Akira S, Takeda K. Toll-like receptor signalling. *Nat. Rev. Immunol.* 2004; 4:499–511. [PubMed: 15229469]
96. Kakimura J, Kitamura Y, Takata K, et al. Microglial activation and amyloid- β clearance induced by exogenous heat-shock proteins. *FASEB J.* 2002; 16:601–603. [PubMed: 11919167]
97. Vabulas RM, Ahmad-Nejad P, Ghose S, et al. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. *J. Biol. Chem.* 2002; 277:15107–15112. [PubMed: 11842086]
98. Chen T, Guo J, Han C, et al. Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. *J. Immunol.* 2009; 182:1449–1459. [PubMed: 19155492] ■ HSP70 that is released from cells is a potent stimulus for dendritic cell activation and subsequent immune responses.
99. Toshchakov V, Jones BW, Perera PY, et al. TLR4, but not TLR2, mediates IFN- β -induced STAT1 α / β -dependent gene expression in macrophages. *Nat. Immunol.* 2002; 3:392–398. [PubMed: 11896392]
100. Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J. Immunol.* 2004; 173:3916–3924. [PubMed: 15356140]
101. Asea A, Kraeft SK, Kurt-Jones EA, et al. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nat. Med.* 2000; 6:435–442. [PubMed: 10742151]
102. Mambula SS, Calderwood SK. Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. *J. Immunol.* 2006; 177:7849–7857. [PubMed: 17114456]
103. Mambula SS, Stevenson MA, Ogawa K, et al. Mechanisms for HSP70 secretion: crossing membranes without a leader. *Methods.* 2007; 43:168–175. [PubMed: 17920512]
104. Moehler M, Zeidler M, Schede J, et al. Oncolytic parvovirus H1 induces release of heat-shock protein HSP72 in susceptible human tumor cells but may not affect primary immune cells. *Cancer Gene Ther.* 2003; 10:477–480. [PubMed: 12768193]
105. Wheeler DS, Chase MA, Senft AP, et al. Extracellular HSP72, an endogenous DAMP, is released by virally infected airway epithelial cells and activates neutrophils via Toll-like receptor (TLR)-4. *Respir. Res.* 2009; 10:31. [PubMed: 19405961] ■ Evidence of virus-induced extracellular release of HSP70 in humans.
106. Kawanokuchi J, Mizuno T, Takeuchi H, et al. Production of interferon- γ by microglia. *Mult. Scler.* 2006; 12:558–564. [PubMed: 17086900]
107. Spiller S, Elson G, Ferstl R, et al. TLR4-induced IFN- γ production increases TLR2 sensitivity and drives Gram-negative sepsis in mice. *J. Exp. Med.* 2008; 205:1747–1754. [PubMed: 18644971]
108. Basu S, Binder RJ, Ramalingam T, et al. CD91 is a common receptor for heat shock proteins gp96, HSP90, HSP70, and calreticulin. *Immunity.* 2001; 14:303–313. [PubMed: 11290339]
109. Giraudon P, Buckland R, Wild TF. The immune response to measles virus in mice. T-helper response to the nucleoprotein and mapping of the T-helper epitopes. *Virus Res.* 1992; 22:41–54. [PubMed: 1371370]
110. Basu S, Binder RJ, Suto R, et al. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF- κ B pathway. *Int. Immunol.* 2000; 12:1539–1546. [PubMed: 11058573]
111. Rota PA, Bloom AE, Vanchiere JA, et al. Evolution of the nucleoprotein and matrix genes of wild-type strains of measles virus isolated from recent epidemics. *Virology.* 1994; 198:724–730. [PubMed: 8291252]
112. Kreis S, Vardas E, Whistler T. Sequence analysis of the nucleocapsid gene of measles virus isolates from South Africa identifies a new genotype. *J. Gen. Virol.* 1997; 78:1581–1587. [PubMed: 9225033]
113. Rota JS, Wang ZD, Rota PA, et al. Comparison of sequences of the *H*, *F*, and *N* coding genes of measles virus vaccine strains. *Virus Res.* 1994; 31:317–330. [PubMed: 8191786]

114. Hutchins SS, Papania MJ, Amler R, et al. Evaluation of the measles clinical case definition. *J. Infect. Dis.* 2004; 189:S153–S159. [PubMed: 15106104]
115. Su CY, Chong KY, Chen J, et al. A physiologically relevant hyperthermia selectively activates constitutive HSP70 in H9c2 cardiac myoblasts and confers oxidative protection. *J. Mol. Cell Cardiol.* 1999; 31:845–855. [PubMed: 10329212]
116. Young P, Anderton E, Paschos K, et al. Epstein–Barr virus nuclear antigen (EBNA) 3A induces the expression of and interacts with a subset of chaperones and co-chaperones. *J. Gen. Virol.* 2008; 89:866–877. [PubMed: 18343826]
117. Peng CW, Zhao B, Chen HC, et al. HSP72 up-regulates Epstein–Barr virus EBNA1P coactivation with EBNA2. *Blood.* 2007; 109:5447–5454. [PubMed: 17341665]
118. Ohgitani E, Kobayashi K, Takeshita K, et al. Biphasic translocation of a 70 kDa heat shock protein in human cytomegalovirus-infected cells. *J. Gen. Virol.* 1999; 80:63–68. [PubMed: 9934685]
119. Nozawa N, Yamauchi Y, Ohtsuka K, et al. Formation of aggresome-like structures in herpes simplex virus type 2-infected cells and a potential role in virus assembly. *Exp. Cell Res.* 2004; 299:486–497. [PubMed: 15350546]
120. Ohgitani E, Kobayashi K, Takeshita K, et al. Induced expression and localization to nuclear-inclusion bodies of HSP70 in varicella-zoster virus-infected human diploid fibroblasts. *Microbiol. Immunol.* 1998; 42:755–760. [PubMed: 9886148]
121. Das S, Laxminarayana SV, Chandra N, et al. Heat shock protein 70 on Neuro2a cells is a putative receptor for Japanese encephalitis virus. *Virology.* 2009; 385:47–57. [PubMed: 19068261]
122. Oh WK, Song J. HSP70 functions as a negative regulator of West Nile virus capsid protein through direct interaction. *Biochem. Biophys. Res. Commun.* 2006; 347:994–1000. [PubMed: 16854374]
123. Macejak DG, Sarnow P. Association of heat shock protein 70 with enterovirus capsid precursor P1 in infected human cells. *J. Virol.* 1992; 66:1520–1527. [PubMed: 1310763]
124. Sagara J, Kawai A. Identification of heat shock protein 70 in the rabies virion. *Virology.* 1992; 190:845–848. [PubMed: 1325709]
125. Lahaye X, Vidy A, Pomier C, et al. Functional characterization of Negri bodies (NBs) in rabies virus-infected cells: evidence that NBs are sites of viral transcription and replication. *J. Virol.* 2009; 83:7948–7958. [PubMed: 19494013]
126. Kang S, Sim C, Byrd BD, et al. *Ex vivo* promoter analysis of antiviral heat shock cognate 70B gene in *Anopheles gambiae*. *Virol. J.* 2008; 5:136. [PubMed: 18986525]
127. Haynes LM, Moore DD, Kurt-Jones EA, et al. Involvement of Toll-like receptor 4 in innate immunity to respiratory syncytial virus. *J. Virol.* 2001; 75:10730–10737. [PubMed: 11602714]
128. Kurt-Jones EA, Popova L, Kwinn L, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 2000; 1:398–401. [PubMed: 11062499]
129. Awad H, Suntres Z, Heijmans J, et al. Intracellular and extracellular expression of the major inducible 70kDa heat shock protein in experimental ischemia–reperfusion injury of the spinal cord. *Exp. Neurol.* 2008; 212:275–284. [PubMed: 18511046] • Extracellular HSP70 is documented in CNS inflammatory processes.
130. Hecker JG, Sundram H, Zou S, et al. Heat shock proteins HSP70 and HSP27 in the cerebral spinal fluid of patients undergoing thoracic aneurysm repair correlate with the probability of postoperative paralysis. *Cell Stress. Chaperones.* 2008; 13:435–446. [PubMed: 18418731]
131. Chen W, Lin Y, Liao C, et al. Modulatory effects of the human heat shock protein 70 on DNA vaccination. *J. Biomed. Sci.* 2000; 7:412–419. [PubMed: 10971139]
132. Pack CD, Kumaraguru U, Suvas S, et al. Heat-shock protein 70 acts as an effective adjuvant in neonatal mice and confers protection against challenge with herpes simplex virus. *Vaccine.* 2005; 23:3526–3534. [PubMed: 15855011]
133. Oglesbee MJ, Alldinger S, Vasconcelos D, et al. Intrinsic thermal resistance of the canine brain. *Neuroscience.* 2002; 113:55–64. [PubMed: 12123684]
134. Pfeuffer J, Puschel K, ter Meulen V, et al. Extent of measles virus spread and immune suppression differentiates between wild-type and vaccine strains in the cotton rat model (*Sigmodon hispidus*). *J. Virol.* 2003; 77:150–158. [PubMed: 12477820]

135. Hoffman WH, Casanova MF, Cudrici CD, et al. Neuroinflammatory response of the choroid plexus epithelium in fatal diabetic ketoacidosis. *Exp. Mol. Pathol.* 2007; 83:65–72. [PubMed: 17335802]

Figure 1. Viral infection of neurons must trigger microglial antigen presentation and production of IFN- β in order to activate virus-specific T cells

The latter are the predominant source of IFN- γ that is responsible for noncytolytic viral clearance in the mouse model of brain infection. The mediator of antigen cross-presentation and the source of IFN- β in measles virus infections in the mouse model are unknown.

Figure 2. MeV–HSP70 interaction within infected neurons can stimulate IFN- β production and cross-presentation of viral (N) antigen in uninfected microglia, thereby fulfilling the requirements for activation of virus-specific T cells to produce IFN- γ

HSP70-mediated stimulation of MeV gene expression should enhance extracellular release of HSP70, either through a stress-activated secretory mechanism or as a consequence of cell death. Extracellular HSP70 can activate TLR4, resulting in the transcriptional induction of IFN- β via IRF3, or proinflammatory cytokines and class I antigen-presenting molecules (H-2 in the mouse and MHC in humans) via NF- κ B. Extracellular HSP70 that is bound to immunogenic N peptides may also deliver those peptides into class I antigen-presenting pathways via CD91.

HSP: Heat shock protein; MeV: Measles virus; TLR Toll-like receptor.