PERSPECTIVE AND REFLECTION ARTICLE

Loss of stress response as a consequence of viral infection: implications for disease and therapy

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Abstract Herein, we propose that viral infection can induce a deficient cell stress response and thereby impairs stress tolerance and makes tissues vulnerable to damage. Having a valid paradigm to address the pathological impacts of viral infections could lead to effective new therapies for diseases that have previously been unresponsive to intervention. Host response to viral infections can also lead to autoimmune diseases like type 1 diabetes. In the case of Newcastle disease virus, the effects of viral infection on heat shock proteins may be leveraged as a therapy for cancer. Finally, the search for a specific virus being responsible for a condition like chronic fatigue syndrome may not be worthwhile if the disease is simply a nonspecific response to viral infection.

Introduction

Many viral infections leave hosts with chronic fatigue, pain, myalgias, and organ inflammation. These lasting symptoms associated with viral infections may be the result of loss of the host's protein-based stress response. Unlike eukaryotes and bacteria, viruses do not have heat shock proteins (Hsps) and rely on host Hsps for viral protein folding. As a result, processes that regulate host stress proteins are likely targets of strategic manipulation by both viruses and infected hosts.

Some viruses take advantage of a host's activated cellular stress response and are able to increase replication when Hsps are high. Several viruses in fact stimulate a major increase in Hsps toward this end, such as human papillomavirus (Song et al. 2010), adenovirus (Glotzer et al. 2000; Madara et al. 2005), polyomaviruses, and dengue viruses (Reyes-Del Valle et al. 2005). Other viruses, however, suffer a reduction in replication when host Hsps are high, such as human immunodeficiency virus (HIV) and influenza A, where Hsp70 inhibits viral gene expression and replication (Kumar et al. 2011; Li et al. 2011). It is therefore not surprising that some viruses known for inducing chronic fatigue and malaise act to limit the host stress response, including influenza A, West Nile virus, herpes simplex virus, and hepatitis C. Hosts, on the other hand, can respond to viral infections through several defensive maneuvers, including generation of a fever, immunological defense, interferon production, and reduction of protein synthesis—including reduced Hsp synthesis—which set hosts up for adverse symptoms.

This paper will address consequences to hosts when the Hsp stress response is impaired due to viral infection. The background for research in this area is provided by the detailed study of Newcastle disease virus (NDV) over the past three decades, presented in the first section below. The subsequent section then provides a brief review of indolent viral diseases that are associated with an impaired tissue stress response. We then address two conditions—type 1 diabetes and chronic fatigue syndrome (CFS)—both of which may result from viral insult and impaired Hsps. Finally, we propose a mechanism whereby viral blockade

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of the stress response can be used therapeutically to destroy tumors without damaging healthy tissues.

Background: NDV

The potential for major interactions between viruses and host protein machinery is well illustrated by the case of NDV. This paramyxovirus comes in both highly virulent (strain Australia-Victoria, AV) and naturally occurring avirulent strains (strain New Jersey LaSota, strain B1-Hitchner). In addition, a set of mutants called noncytopathic (nc) mutants have been isolated from a chemically mutagenized, cloned stock of NDV-AV (Mandansky and Bratt 1978). Like avirulent strains of NDV, the nc mutants induce reduced levels of viral mRNA in infected chicken embryo cells (Mandansky and Bratt 1981a). Consequently, fewer viral proteins accumulate, and host cell protein synthesis is relatively better preserved in comparison to the virulent parent strain AV.

A study was carried out that compared cellular stress protein Hsp90, Hsp70, Hsp23, and the glucose-regulated protein Grp78 (now known as one of the endoplasmic reticulum stress proteins) after infection by different strains of NDV. The study demonstrated that infection by avirulent B1-Hitchner and avirulent NJ-LaSota markedly raised Hsp protein levels, while the virulent strain AV severely reduced Hsp70 and Hsp23 levels and nearly eliminated Hsp90 and Grp78 levels (see Fig. 1). Peptide mapping comparisons to authenticate cellular stress proteins induced by the arginine analog L-canavanine identified NDV-induced Hsp90, Grp78, Hsp70, and Hsp23 as authentic cellular stress proteins (Collins and Hightower 1982).

At the time of Madansky and Bratt's nc mutant studies in the late 1970s, the ability of NDV avirulent strains to induce cellular stress proteins was not yet known. However, the published gel patterns of the nc mutants clearly show the stimulated production of these proteins. Relative to virulent strains like AV, avirulent strains and the nc mutants have a much reduced capacity for cell killing (in terms of lytic plaque formation in cell cultures) and much longer embryonic death times in infected eggs (Mandansky and Bratt 1981b). Cell and embryo killing correlated directly with viral RNA and protein levels, and inversely correlated with levels of host cell proteins. Although it was not appreciated at the time, extensive cell killing and short mean embryo death times also inversely correlated with cellular stress protein levels (Collins and Hightower 1982).

In retrospect, both the cytosolic/nuclear stress response and unfolded protein response (UPR) were induced by the virulent and avirulent strains. The UPR signature protein BiP (Grp78) is among the induced stress proteins (Fig. 1). We now interpret these responses in light of current understanding as likely caused by competition by viral proteins for molecular chaperones, including viral nucleo-capsid production in the cytosol, and viral glycoprotein production in the endoplasmic reticulum. The virulent strains also induce increased stress protein mRNA levels early in infection, demonstrating that the nuclear signal for activation of stress response genes is generated, but net avian Hsp protein synthesis is blocked at the stage of protein translation (Collins and Hightower 1982). It is clear from these studies that stimulating production of stress proteins is part of the avirulent phenotype, even though we do not know the exact role that these proteins play in countering cell and embryo killing by viruses. One can speculate that inadequate levels of molecular chaperones needed to facilitate the folding and assembly of essential cellular proteins and organelles contributed to the killing by the virulent strain. As we will discuss herein, recent evidence that Hsps modulate major survival pathways such as apoptosis and mitosis is likely part of the answer as well.

The case study of NDV showed that a diminished stress protein response in the host correlates with the severity of disease inflicted by the virus. These observations provided an early clue that a diminished capacity to produce Hsps could have a deleterious effect on infected hosts. Research since that time has shown that interactions between viruses and host stress protein machinery can be either highly specific to individual viral proteins and particular Hsps, or part of a more general viral host response. Below we review a variety of cases showing viral-induced deficiencies in Hsp synthesis at the level of translation, as well as deficiencies in the stress response due to direct viral protein–Hsp interactions.

Viruses associated with impaired Hsps

West Nile virus

West Nile virus (WNV) is a member of the Flaviviridae family, the best-known member of which is Yellow Fever Virus. The Flaviviridae are single-stranded RNA viruses in which the virion strand is also the mRNA, and which assemble in the cytoplasm into icosahedral virions composed of one type of capsid protein. While the majority (90 %) of West Nile virus infections in humans are innocuous, some infections produce meningitis and encephalitis, and are noted for long-term convalescence and fatigue (Kramer et al. 2007). The capsid of WNV is a major pathogenic protein that is associated with growth arrest of host cells. Pertinently, WNV capsid protein binds to the substrate-binding domain of Hsp70, both of which are in the cytoplasm of infected cells. Capsid binding to Hsp70 disrupts the functional role of Hsp70 in ensuring protein
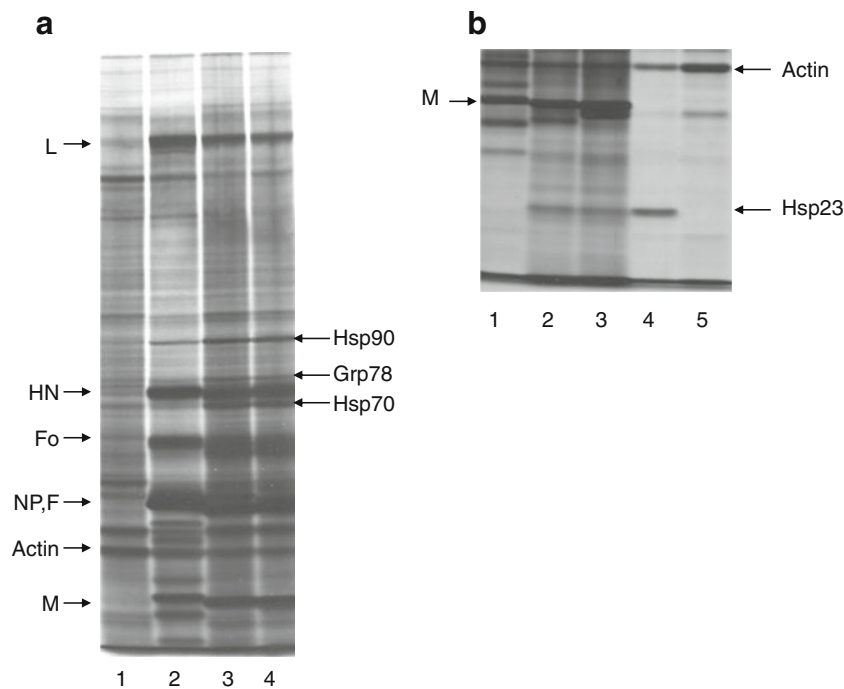
a

b

Fig. 1 Comparison of protein synthesis in uninfected cultured chicken embryo cells and cultures infected at moi=5 PFU/ml with virulent NDV-AV, avirulent NDV strains B1-Hitchner, and NJ-LaSota. The cultures were incubated for 6 h at 40.5 °C, then radiolabeled for 30 min with 35S-methionine. Extracts were prepared in gel sample buffer, and polypeptides were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Extracts of radioactively labeled polypeptides from uninfected and infected cells labeled with radioactive methionine were compared. **a** Fluorogram of a 7 % polyacrylamide gel. *Lane 1* uninfected cultures, *lane 2* strain AV infected, *lane 3* strain B1-Hitchner infected, *lane 4*, strain NJ-LaSota infected. The positions of avian Hsp70, Grp78, and Hsp90 are marked with the *arrows on the right side*. These avian stress proteins were verified by peptide mapping (Collins and Hightower 1982). *Left side arrows* mark major NDV polypeptides: *L* large polypeptide, the transcriptase, *HN* haemagglutinin-neuraminidase glycopolypeptide, *Fo* the precursor to the fusion glycopolypeptide, *F* fusion glycopolypeptide, *NP* nucleocapsid polypeptide, *M* matrix polypeptide. The position of cellular actin is marked as well. **b** Portion below 46 kDa of a fluorogram of a 9 % polyacrylamide gel on which the extracts described in **a** were separated in order to retain the small Hsp23 on the gel for comparison. *Lane 1* strain AV, *lane 2* strain NJ-LaSota, *lane 3* strain B1-Hitchner, *lane 4* cultures treated with the arginine analog canavanine to induce authentic cellular stress proteins (Hsps) including avian Hsp23 (equivalent to mammalian HspB1); *lane 5* uninfected, untreated control cultures. The NDV matrix polypeptide position and that of cellular actin are marked as well

folding. Specifically, the folding function of Hsp70 and Hsp40 complexes in restoring enzyme activity is impaired as viral titers increase. Furthermore, WNV capsid induces cytotoxicity—via caspase-induced apoptosis and mitochondrial dysfunction—that is attenuated by Hsp70. The data support the notion that direct binding of WNV capsid to Hsp70 may mediate illness severity in the host (Oh and Song 2006).

Influenza A

Influenza viruses have a singled-stranded segmented genome. They are called negative strand viruses because the genomic segments are complementary to the viral mRNA. These segments reassort during viral reproduction, allowing influenza viruses to rapidly produce new strains of highly infectious respiratory viruses. Influenza A is often responsible for seasonal epidemics and sporadic pandemics, with symptoms of myalgia, pneumonia, and even death. The influenza

viral protein NS1 inhibits processing of Hsp70 pre-mRNA, thus blocking Hsp70 protein expression (Shimizu et al. 1999). Interestingly, with early infection, Hsp70 RNA in the nucleus increases 25 to 30 times basal levels, and yet only a minimal amount of mRNA enters the cytoplasm for translation. Initially, there is a modest rise in cytoplasmic Hsp70, followed by a drop. The effect of upregulation of Hsp70 is to limit apoptotic effects by binding to Apaf-1, which inhibits apoptosome formation and caspase-9 recruitment. It is thought that the initial high Hsp levels assure low cell death rates that can maximize viral replication. However with time, Hsp70 levels fall and another viral protein M1 binds to Hsp70, resulting in caspase induction of apoptosis, cell lysis, and virus release (Halder et al. 2011).

Epstein–Barr virus

Epstein–Barr virus (EBV) is another member of the herpesvirus family that forms circular DNA molecules that reside

in the cell nucleus and rarely integrate into the host genome. Humans are the natural hosts wherein only two cell types, squamous epithelial cells and B lymphocytes, are known to be infected. Squamous epithelial cells undergo a lytic infection with progeny virus production and cell death. Infection causes mononucleosis and Burkett's lymphoma. Mononucleosis often produces fatigue and malaise that may persist for months and years. In the past, it was thought by some investigators to be a major cause of CFS. However, presently it is not considered *the* cause of CFS because it does not meet criteria for Koch's postulates (i.e., criteria to establish causal relationship between infection and disease). With respect to Hsps and EBV, Hsp70 is bound by Epstein–Barr nuclear protein antigen-LP, an antigen that is high early in infections. The protein–protein interaction is observed both in vivo and in vitro. It is possible that the sequestration of Hsp70 impairs the stress response, and therefore contributes to the complex of symptoms associated with this infection. The protein–protein complex also contributes to immortalization of B lymphocytes, which primes the cells to malignant transformation. Thus, the total effect of this viral–Hsp interaction may not just be a loss of the stress response but also promotion of oncogenesis—in this case the development of Burkett's lymphoma (Kitay and Rowe 1996).

Herpes simplex virus

Human herpes simplex viruses have double-stranded linear DNA genomes capable of integrating into the host cell genome. Herpes simplex virus type 1 (HSV-1) is transmitted orally and causes cold sores and fever blisters. Herpes simplex virus type 2 (HSV-2, commonly known as genital herpes) is sexually transmitted and has been linked to cervical cancer. HSV-2 is ubiquitous and contagious, and can be fatal when passed to the placenta and fetus. In a study of herpes exacerbation during pregnancy, placentas from medically terminated pregnancies demonstrated low Hsp70 levels in placental homogenates and were associated with high herpes antibody titers. Low Hsp70 levels also corresponded with high caspase-3 levels and apoptotic nuclei. The findings suggest that the viral infection suppresses Hsp70 levels, thereby promoting caspase activation and apoptosis (Lutsenko et al. 2010).

It was also recently demonstrated that HSV-1 inhibits the unfolded protein response (UPR) during the early stages of infection of cultured human cells. Viral inhibition of UPR frees the virus from fidelity control of protein synthesis and can increase the efficiency of viral replication 1,000-fold (Burnett et al. 2012; Lutsenko et al. 2010). Loss of UPR disrupts endoplasmic reticulum homeostasis and can thereby lead to a number of pathological sequelae (see below).

Hepatitis C

Hepatitis C virus (HCV) is a noncytopathic hepatotrophic virus that infects only humans and chimpanzees. It has a positive sense single-stranded RNA genome and reproduces in the cytoplasm of cells before undergoing membrane budding. HCV infection is associated with cirrhosis, liver failure, and hepatocellular carcinoma. In a study of liver tissue biopsied from patients with HCV infection, reductions in GRP78 levels—a member of the Hsp70 family key to effecting the UPR in the endoplasmic reticulum—were observed compared to normal liver tissue. As with HSV1, the researchers proposed that loss of this stress protein might encourage viral replication and thus maximize viral load. Interestingly, loss of UPR was not associated with inflammation or fibrosis in this study. However, reduced UPR induced by HCV infection may expose the endoplasmic reticulum to stress, leading to the development of a suite of diseases—diabetes, cancer, and neurodegenerative disease—all of which show increased prevalence in subjects with HCV infection (Engin and Hotamışlıgil 2010; McPherson et al. 2011).

Double-stranded RNA-dependent protein kinase (PKR), interferon, and type 1 diabetes

In order to limit viral exploitation of host protein production, hosts have evolved defensive strategies to limit all protein synthesis and particularly Hsp protein synthesis. PKR is an intracellular pathogen stress sensor that responds to double-stranded RNA and interferon. PKR also responds to endoplasmic reticulum stress and inflammation due to nutrient excess (Nakamura et al. 2010; this topic is discussed further in "Implications and extensions" section). Activated PKR arrests protein synthesis and Hsp translation via phosphorylation of translation initiation factor eIF2α (Van Der Kelen et al. 2009; see Fig. 2). In an animal model of colitis, injection of interferon and TNF-α activated PKR, and resulted in markedly impaired Hsp25 and Hsp70 protein synthesis at the translational level; Hsp25 and Hsp70 were in fact nearly absent in the gut mucosa, while the mRNA of these proteins remained unaffected. The authors observed that the cytokines blocked Hsp70 synthesis twice as much as they blocked β-actin, suggesting that while there is a global reduction in protein translation, Hsp70 is blocked more aggressively (Hu et al. 2007). In the case of NDV, however, reduction in protein translation was more global, and not specific to Hsps. More studies are needed to clarify if and when cytokines' blockade of protein synthesis pertains to all host proteins, or Hsps more specifically.

Pancreatic beta cell destruction in type 1 diabetes may be tied directly to interferon disruption of the Hsp response.
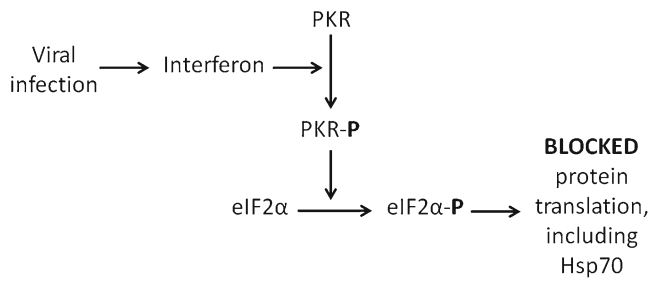
Fig. 2 Interferon, PKR, and eIF2α mediate the effect of viral infection on protein translation including Hsp 70

Evidence for this is provided by the following observations: first, interferon is present at high levels in beta cells of patients that died at the onset of type 1 diabetes (Foulis et al. 1991); second, interferon levels are elevated in both animal models of type 1 diabetes and in patients with new-onset diabetes (Kallmann et al. 1997); third, increased serum interferon concentrations are associated with rapid disease progression (Kaas et al. 2011); fourth, the Hsp70 response to heat shock is markedly impaired in newly diagnosed type 1 patients; fifth, interferon blocks the Hsp70 response in white blood cells in a similar manner to the defect observed at the onset of type 1 diabetes (Burkart et al. 2008); and finally, interferon combined with TNF-α and IL-1β blocks GRP78 translation, thus impairing the UPR in pancreatic beta cells of a mouse model of type 1 diabetes (NOD; Tersey et al. 2012). The net effects of this virally induced disruption of the beta cell stress response are endoplasmic reticulum stress, NF-κB activation, and beta cell death. Additionally, impaired folding of major beta cell proteins (insulin, GAD65, IA-2, ZnT-8, and chromogranin A) initiates the production of neo-self antigens, triggering autoimmunity in individuals with a genetic predisposition to diabetes—an idea recently proposed by O'Sullivan-Murphy and Urano (2012). Parallel virally induced reductions in Hsp25/27 (as noted in Fig. 1) could increase presentation of MHC class 1 autoantigen and lead to beta-cell lysis by CD8(+) T cells (Nagarja et al. 2012). Ultimately, the viral infection sets up a one-two punch—first making beta cells vulnerable, and then immunologically eliminating them.

Consistent with these effects, an increased prevalence of autoimmune disease is observed when interferon is used to treat diseases like hepatitis B, hepatitis C, or multiple sclerosis. Reports observe that interferon therapy is associated with an increase in autoimmune thyroid disease, type 1 diabetes, membranous nephropathy, and other self-antigen-induced diseases (Mammen et al. 2012; Radhakrishnan et al. 2005; Scavone et al. 2010; Tosone et al. 2007; Tsai et al. 2012).

Therapeutically, one would predict that raising the stress response might mitigate the autoimmune destruction of the beta cell. Indeed, mild hyperthermia once per week for 32 weeks in the genetic mouse model of type 1 diabetes (NOD) eliminated the development of type 1 diabetes during the hyperthermic period, with lasting effects such that only a few animals developed the disease in the 20 weeks after the therapy stopped (Capitano et al. 2008). We conclude that modulation of Hsps by diverse stimuli like interferon or heat shock may promote or prevent autoimmunity, depending upon whether they repress or augment Hsps.

Chronic fatigue syndrome

Chronic fatigue syndrome (CFS), also known as myalgic encephalomyelitis, is a condition with no specific etiology, but which has been associated with a host of viral and bacterial infections. Examples include EBV, described previously, and xenotropic murine leukemia virus-related virus, which recently received international press when key papers implicating the latter virus were retracted. These events serve to emphasize the difficulties encountered in associating CFS with a distinct pathogen.

We propose that CFS is not the result of a specific infection but rather a more general response of the body to infection. In particular, infectious impairment of the stress response, as the examples we have described herein suggest, could make subjects vulnerable to even minor stresses and injury. Indeed, subjects with CFS are not resilient to life stresses: a strikingly minimal stress like walking five blocks can leave a person with CFS feeling exhausted for several days. In studies comparing subjects with CFS to healthy control subjects, the stress protein response to exercise is significantly blunted. A study comparing serum Hsp27 and Hsp70 of CFS patients versus control subjects before and after an incremental exhaustive bicycle session found lower baseline Hsp 70 levels in the CFS group compared to the control group. Hsp27 and Hsp70 also failed to rise as high and as fast in the CFS group as in their healthy control counterparts (Jammes et al. 2009). A more recent study of CFS patients divided subjects into those that had postinfection CFS and those that did not. Hsp27 and Hsp70 levels failed to rise with exercise in subjects with infection-associated CFS, and in fact decreased from baseline—which may explain why CFS subjects often complain of exhaustion after moderate exercise (Jammes et al. 2011). Furthermore, CFS muscle biopsies contain mitochondria with functional and morphological defects consistent with an impaired intracellular stress defense (Myhill et al. 2009). Finally, studying aging subjects, Mets and colleagues have reported that monocyte and lymphocyte Hsp27 correlates positively with fatigue resistance (Beyer et al. 2012).

While the etiology of CFS is presently regarded as multifactorial, prior infections—particularly viral infections—are likely major CFS triggers. In support of an infectious

culprit, a study of CFS versus control subjects observed elevated titers of PKR protein and interferon, with minimal overlap of values with the control group (Vojdani et al. 2007). Elevated interferon titers were also detected in veterans ill with chronic fatigue associated with the Gulf War (Zhang et al. 1999). Interferon administration itself produces fatigue and has been used as model to study CFS (personal communication, J. Jones, CDC)

If CFS symptoms reflect an impaired Hsp state, then improving the Hsp response should improve the condition. Indeed, a nutraceutical product (ADAPT-232 forte), which combines extracts from three herbs (*Eleutherococcus senticosus*, *Schizandra chinensis*, and *Rhodiola rosea*), raises Hsps in response to exercise. This same agent is effective in reducing fatigue and improving performance in a placebo-controlled trial of patients with CFS (Panossian et al. 2009).

Viral infection for cancer therapy

Surprisingly, recent research has established that some viral infections can be used as oncolytic agents to treat cancer. In particular, NDV has been found to induce tumor cell death with little or no harm to noncancer cells of the host. So in addition to poultry, NDV can also kill cancer, while sparing healthy human tissue. Currently, there are 600 citations that support the use of NDV in cancer therapy. Zamarin and Palese (2012) have published a very thorough and useful review of this literature.

The cytotoxicity of cancer chemotherapeutic agents is often brought about by mitochondrion-initiated cell death. The outer membrane of the mitochondrion becomes permeable, resulting in the release of cytochrome c into the cytoplasm, which triggers caspase proteases to activate apoptosis (Reed 2011). Hsps play a key role in modulating apoptosis and cell death. For example, Hsp27 (HSPB1) is a negative regulator of apoptosis. It binds directly to cytochrome c released from mitochondria, which prevents the binding of Apaf-1 to procaspase-9, inhibiting its activation. Through this relatively direct and specific blockade, Hsp27 interferes with mitochondria-mediated caspase-dependent cell death (Bruey et al. 2000). Hsp70 also blocks stress-activated apoptosis by several different pathways. Under stress conditions for example, Hsp70 (HSPA1A) inhibits Bax activation, which is required for release of proapoptotic factors from mitochondria (Stankiewicz et al. 2005). In addition to a major role in facilitating protein folding pathways, there is evidence that Hsp70 can block stress-induced apoptosis by a mechanism independent of chaperone function (Chow et al. 2009).

Another link between stress proteins and tumor growth involves chaperone-mediated autophagy (CMA), a process distinct from the more commonly studied macroautophagy.

As malignant cells are associated with robust activity of CMA, evidence demonstrates tumor growth and metastasis can be decreased and apoptosis stimulated by reducing CMA (Kon et al. 2011). We propose that viral modulation of the levels of molecular chaperones (i.e., stress proteins) directly modulates CMA in tumor cells. It is further anticipated that the virulence of NDV strains is inversely correlated with CMA levels.

Low Hsp25/27 induction by virulent strains of NDV increases tumor necrosis and will increase antigen presentation and activate cytotoxic CD8(+) T cells (Nagarja et al. 2012; see Fig. 1b, in which the avian version of the small Hsps is Hsp23). Thus, inducing cell death by reducing the level and functionality of Hsps in cancer cells is an attractive strategy for cancer therapy (Galluzzi et al. 2006; Jego et al. 2010). Zamarin and Palese (2012) noted that virulence of NDV strains in birds correlates directly with their oncolytic properties. We suggest that there is also a direct correlation between the ability of virulent strains to inhibit host protein synthesis—including cellular stress protein synthesis—and the oncolytic properties of NDV. In general terms, these proteins, especially when intracellular, establish a cytoprotected state in cells and tissues that is antiapoptotic, anti-inflammatory, and at least transiently antimitotic. Hsp70 levels are often used as a marker for the cytoprotected state. The observation that virulent strains of NDV impair Hsp70 translation (Collins and Hightower 1982) predicts that these strains could be effective in blocking the establishment of a cytoprotected state and consequently promote apoptosis. Additionally, the oncolytic effect of NDV is enhanced by influenza protein NS1 (Mansour et al. 2011). The NS1 protein of the highly pathogenic avian H5N1 influenza virus is currently believed to be responsible for the increased virulence of the strain (Li et al. 2006). Relevantly, NS1 inhibits cleavage of Hsp70 premRNAs, blocking the formation of mature mRNAs (Shimizu et al. 1999). Furthermore, NS1 binds to Hsp90, promoting an association of Apaf-1 with cytochrome c and activating the cytotoxic caspase cascade (Zhang et al. 2011). Thus, NDV alone and NDV enhanced with viral Hsp-blockade proteins like NS1 are efficacious in treating a host of malignancies—breast, lung, prostate, colon, melanoma, gliablastoma, neuroblastoma, and sarcoma (Ravindra et al. 2009).

Interferon has been used directly as an oncolytic agent for decades in human malignancies. Clinically beneficial therapeutic activity of interferon has been demonstrated in hairy cell leukemia, Kaposi's sarcoma, CML, B- and T cell lymphomas, melanoma, myelomas, and renal cell carcinoma (Gutterman 1994). Interferon has been thought to be effective by enhancing the immune response to cancer, but it also activates apoptosis (Chawla-Sarkar et al. 2002). As noted above, like NDV, interferon may function as an oncolytic agent by activating PKR and ultimately impairing Hsp translation in the tumor. In

fact, data suggest that NDV induction of interferon may contribute to its oncolytic activity (Mansour et al. 2011).

Acting in the opposite direction, certain viral infections that induce Hsps may promote oncogenesis. Two examples of viral induction of Hsps associated with cancer are EBV and Burkitt's lymphoma, mentioned above, and human papillomavirus (HPV) and cervical cancer. An oncogene of HPV, 16 E7, is associated with increased expression of Hsp70 (Liao et al. 2005), and further induction of Hsp70i (HSPA1A/B) with heat shock enhances viral replication in HPV-infected keratinocytes (Song et al. 2010). Song and coworkers propose that increased Hsp 70i may be a key factor promoting oncogenesis. They note that HPV alone does not produce cancer, but that added factors like smoking, chronic inflammation, multiparity, and oral contraceptives are associated with nitric oxide induction of Hsp70i and genetic mutations. High Hsp70 expression correlates epidemiologically as a determining factor in cervical cancer (Ciocca and Calderwood 2005). In cervical cancer lesions, Hsp70 levels correlate with lesion severity (Castle et al. 2005). Thus, viruses can act as oncolytic agents by knocking out Hsps, or as oncogenic agents when they stimulate aberrantly high levels of Hsps.

Implications and extensions

Many chronic illnesses—diabetes, myocarditis, nephritis, arthritis, encephalitis, asthma, bronchitis, and chronic fatigue syndrome—are often preceded by a nonspecific viral illness. In fact, Hotamışlıgil and colleagues have proposed that PKR acts as a pathogen sensor that sparks inflammatory processes basic to metabolic disease pathogenesis. They suggest that PKR activation plays a key role in initiating both type 1 and 2 diabetes, and note that PKR activation in hepatitis C may contribute to the high prevalence of diabetes associated with this infection (Nakamura et al. 2010). Relevantly, both types of diabetes are associated with impaired cellular stress response, and are ameliorated by restoration of the stress response (Hooper 2007; van Eden et al. 2005; Wieten et al. 2010). Therefore we ask: "Is a viral impairment of the cellular stress response a major contributor to the pathogenesis of numerous diverse diseases?"

A remarkable postviral disease that lacks pathogenic explanation is Reye's syndrome. This acute catastrophic illness typically occurs in children who are treated with aspirin during a viral illness. The child rapidly becomes ill and develops multi-organ failure. Low Hsps are observed in sepsis and acute respiratory distress syndrome (Singleton and Wischmeyer 2007; Weiss et al. 2000). Does the fact that aspirin activates eIF2α, the same molecule activated by interferon and viral illness, lead to a doubled impact of impaired Hsp protein translation and loss of tissue defenses? (Silva et al. 2007). Indeed, an intramitochondrial defect in

mitochondrial enzyme processing has been observed in Reye's syndrome, and a reduced Hsp state has been proposed to be responsible for this abnormality (Van Coster et al. 1991).

Given the interplay between viral infections and the host stress response, one might predict that a compound that raises Hsps may alter the clinical impact of a viral infection. Indeed, Salidroside—an extract of *R. rosea* that is in Adapt-232 mentioned above—has been observed to reduce the severity of coxsackie myocarditis. Salidroside administration reduces myocardial inflammation and apoptosis, preserving myocardial function in coxsackie-infected animals (Wang et al. 2009). Similarly, geranylgeranylacetone (GGA), an Hsp inducer, is effective as an antiviral in treating influenza A infection. GGA limits viral replication, blocks synthesis of the virulent viral protein NS1, and limits weight loss and pulmonary infiltration (Unoshima et al. 2003). GGA has been used clinically for decades to treat gastric ulcers, is safe in humans, and inexpensive.

In addition to influenza A, other viral infections that are impaired by high Hsp expression—and which may therefore be sensitive to Hsp-raising agents—are caused by rhinovirus (Conti et al. 1999), rotavirus (Pavlovic et al. 1992), poliovirus (Conti et al. 1996), vesicular stomatitis virus (Rossi et al. 1996), Sindbis virus (Mastromarino et al. 1993), leukemia virus type 1 (D'Onofrio et al. 1994), and HIV1 (Kumar et al. 2011). To have agents that could treat diseases as ubiquitous as the common cold, as fatal as HIV, or as high impact as influenza pandemics would be a boon to mankind. Could an Hsp inducer be administered when type 1 diabetes is first diagnosed, and thereby limit beta cell destruction? On a similar note, Parkinson's disease symptoms have been observed following seasonal influenza (Toovey et al. 2011). As it is known that Hsps have a protective role in limiting the impact of Parkinson's (Aridon et al. 2011), Hsp-inducing medications could limit the ultimate downstream impacts of influenza infections on health. It is worth noting, however, that while raising Hsps may be protective in the case of some viral infections, in other cases, increasing Hsps could, theoretically, augment viral replication.

Studying the interplay between virus and host as it relates to stress proteins can reveal insights that generate a new vision of disease causation and presentation. We hope that the thoughts introduced in this perspective will generate novel hypotheses that can be tested and which can lead to efficacious therapies for a wide range of disease states.

References

Aridon P, Geraci F, Turturici G, D'Amelio M, Savettieri G, Sconzo G (2011) Protective role of heat shock proteins in Parkinson's disease. Neurodegener Dis 8:155–168

Beyer I, Njemini R, Bautmans I, Demanet C, Bergmann P, Mets T (2012) Inflammation-related muscle weakness and fatigue in geriatric patients. Exp Gerontol 47:52–59

Bruey J-M, Ducasse C, Bonnlaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo A-P, Kroemer G, Solary E et al (2000) Hsp27 negatively regulates cell death by interaction with cytochrome c. Nat Cell Biol 2:645–652

Burkart V, Germaschewski L, Schloot NC, Bellmann K, Kolb H (2008) Deficient heat shock protein 70 response to stress in leukocytes at onset of type 1 diabetes. Biochem Biophys Res Commun 369:421–425

Burnett H, Audas T, Liang G, Lu R (2012) Herpes simplex virus-1 disarms the unfolded protein response in the early stages of infection. Cell Stress Chaperones 17(4):473–483

Capitano ML, Ertel BR, Repasky EA, Ostberg JR (2008) Fever-range whole body hyperthermia prevents the onset of type 1 diabetes in non-obese diabetic mice. Int J Hyperthermia 24:141–149

Castle PE, Ashfaq R, Ansari F, Muller CY (2005) Immunohistochemical evaluation of heat shock proteins in normal and preinvasive lesions of the cervix. Cancer Lett 229:245–252

Chawla-Sarkar M, Leaman DW, Jacobs BS, Borden EC (2002) IFN-beta pretreatment sensitizes human melanoma cells to TRAIL/Apo2 ligand-induced apoptosis. J Immunol 169:847–855

Chow AM, Steel R, Anderson RL (2009) Hsp72 chaperone function is dispensable for protection against stress-induced apoptosis. Cell Stress Chaperones 14:253–263

Ciocca DR, Calderwood SK (2005) Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10:86–103

Collins PL, Hightower LE (1982) Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. J Virol 44:703–707

Conti C, Mastromarino P, Tomao P, de Marco A, Pica F, Santoro MG (1996) Inhibition of poliovirus replication by prostaglandins A and J in human cells. Antimicrob Agents Chemother 40:367–372

Conti C, de Marco A, Mastromarino P, Tomao P, Santoro MG (1999) Antiviral effect of hyperthermic treatment in rhinovirus infection. Antimicrob Agents Chemother 43:822–829

D'Onofrio C, Franzese O, de Marco A, Bonmassar E, Amici C (1994) Antiproliferative activity of cyclopentenone prostaglandins in early HTLV-1 infection is independent of IL-2 and is associated with HSP70 induction. Leukemia 8:1045–1056

Engin F, Hotamışlıgil GS (2010) Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases. Diabetes Obes Metab 12:108–115

Foulis AK, McGill M, Farquharson MA (1991) Insulitis in type 1 (insulin-dependent) diabetes mellitus in man–macrophages, lymphocytes, and interferon-gamma containing cells. J Pathol 165:97–103

Galluzzi L, Larochette N, Zamzami N, Kroemer G (2006) Mitochondria as therapeutic targets for cancer chemotherapy. Oncogene 25:4812–4830

Glotzer JB, Saltik M, Chiocca S, Michou A-I, Moseley P, Cotten M (2000) Activation of heat-shock response by an adenovirus is essential for virus replication. Nature 407:207–211

Gutterman JU (1994) Cytokine therapeutics: lesson from interferon α. PNAS 91:1198–1205

Halder UC, Bagchi P, Chattopadhyay S, Dutta D, Chawla-Sarkar M (2011) Cell death regulation during influenza A virus infection by matrix (M1) protein: a model of viral control over the cellular survival pathway. Cell Death Dis 2:e197

Hooper PL (2007) Insulin signaling, GSK-3, heat shock proteins and the natural history of type 2 diabetes mellitus: a hypothesis. Metab Syndr Relat Disord 5:220–230

Hu S, Ciancio MJ, Lahav M, Fujiya M, Lichtenstein L, Anant S, Musch MW, Chang EB (2007) Translational inhibition of colonic epithelial heat shock proteins by IFN-gamma and TNF-alpha in intestinal inflammation. Gastroenterology 133(6):1893–1904

Jammes Y, Steinberg J, Delliaux S, Brégeon F (2009) Chronic fatigue sydrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses. J Intern Med 266:196–206

Jammes Y, Steinberg J, Delliaux S (2012) Chronic fatigue syndrome: acute infection and history of physical activity affect resting levels and response to exercise of plasma oxidant/antioxidant status and heat shock proteins. J Intern Med 272(1):74–84

Jego G, Hazoumé A, Seigneuric R, Garrido C (2010) Targeting heat shock proteins in cancer. Cancer Lett. doi:10.1016/j.canlet.2010.10.014

Kaas A, Pfleger C, Kharagjitsingh AV, Schloot NC, Hansen L, Buschard K, Koeleman BP, Roep BO, Mortensen HB, Alizadeh BZ (2012) Association between age, IL-10, IFNγ, stimulated C-peptide and disease progression in children with newly diagnosed type 1 diabetes. Diabet Med 29(6):734–741

Kallmann BA, Hüther M, Tubes M, Feldkamp J, Bertrams J, Gries FA, Lampeter EF, Kolb H (1997) Systemic bias of cytokine production toward cell-mediated immune regulation in IDDM and toward humoral immunity in Graves' disease. Diabetes 46(2):237–243

Kitay MK, Rowe DT (1996) Protein-protein interactions between Epstein–Barr virus nuclear antigen-LP and cellular gene products: binding of 70-kilodalton heat shock proteins. Virology 220:91–99

Kon M, Kiffin R, Koga H, Chapochnick J, Macian F, Vartikovski L, Cuervo AM (2011) Chaperone-mediated autophagy is required for tumor growth. Sci Transl Med 3:109–117

Kramer LD, Li J, Shi P-Y (2007) West Nile virus. Lancet Neurol 6:171–181

Kumar M, Rawat P, Khan SZ, Dhamija N, Chaudhary P, Ravi DS, Mitra D (2011) Reciprocal regulation of human immunodeficiency virus-1 gene expression and replication by heat shock proteins 40 and 70. J Mol Biol 410:944–958

Li Z, Jiang Y, Jiao P, Wang A, Zhao F, Tian G, Wang X, Yu K, Bu Z, Chen H (2006) The NS1 gene contributes to the virulence of H5N1 avian influenza viruses. J Virol 80:11115–11123

Li G, Zhang J, Tong X, Liu W, Ye X (2011) Heat shock protein 70 inhibits the activity of influenza A virus ribonucleoprotein and blocks the replication of virus in vitro and in vivo. PLoS One 6: e16546

Liao WJ, Fan PS, Fu M, Fan XL, Liu YF (2005) Increased expression of 70 kD heat shock protein in cultured primary human keratinocytes induced by human papillomavirus 16 E6/E7 gene. Chin Med J(Engl Ed) 118:2058–2062

Lutsenko MT, Dorofienko NN, Andievskaya IA (2010) Morphofunctional characteristics of syncytiotrophoblast and content of heat shock protein 70 in it during exacerbation of herpesvirus infection in pregnant women. Bull Exp Biol Med 150:149–152

Madara J, Krewet JA, Shah M (2005) Heat shock protein 72 expression allows permissive replication of oncolytic adenovirus dl1520 (ONYX-015) in rat glioblastoma cells. Mol Cancer 4(1):12

Mammen JS, Ghazarian SR, Pulkstenis E, Subramanian GM, Rosen A, Ladenson PW (2012) Phenotypes of interferon-α-induced thyroid dysfunction among patients treated for hepatitis C are associated with pretreatment serum TSH and female sex. J Clin Endocrinol Metab. doi:10.1210/jc.2012-1026

Mandansky CH, Bratt MA (1978) Noncytopathic mutants of Newcastle disease virus. J Virol 26:724–729

Mandansky CH, Bratt MA (1981a) Noncytopathic mutants of Newcastle disease virus are defective in virus-specific RNA synthesis. J Virol 37:317–327

Mandansky CH, Bratt MA (1981b) Relationships among virus spread, cytopathogenicity, and virulence as revealed by the noncytopathic mutants of Newcastle disease virus. J Virol 40:691–702

Mansour M, Palese P, Zamarin D (2011) Oncolytic specificity of Newcastle disease virus is mediated by selectivity for apoptosis-resistant cells. J Virol 85:6015–6023

Mastromarino P, Conti C, Petruzziello R, de Marco A, Pica F, Santoro MG (1993) Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis. Antiviral Res 20:209–222

McPherson S, Powell EE, Barrie HD, Clouston AD, McGuckin M, Jonsson JR (2011) No evidence of the unfolded protein response in patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol 26:319–327

Myhill S, Booth NE, McLaren-Howard J (2009) Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med 2(1):1–16

Nagarja GM, Kaur P, Neumann W, Asea EE, Bausero MA, Multhoff G, Asea A (2012) Silencing hsp25/hsp27 gene expression augments proteasome activity and increases CD8+ T cell-mediated tumor killing and memory responses. Cancer Prev Res 5:122–137

Nakamura T, Furuhashi M, Li P, Cao H, Tuncman G, Sonenberg N, Gorgun C, Hotamışlıgil GS (2010) Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell 140:338–348

Oh W, Song J (2006) Hsp70 functions as a negative regulator of West Nile virus capsid protein through direct interaction. Biochem Biophys Res Commun 347:994–1000

O'Sullivan-Murphy B, Urano F (2012) ER stress as a trigger for β-cell dysfunction and autoimmunity in type 1 diabetes. Diabetes 61:780–781

Panossian A, Wikman G, Kaur P, Asea A (2009) Adaptogens exert a stress-protective effect by modulation of expression of molecular chaperones. Phytomedicine 16:617–622

Pavlovic J, Haller O, Staeheli P (1992) Human and mouse Mx proteins inhibit different steps of the influenza virus multiplication cycle. J Virol 66:2564–2569

Radhakrishnan S, Upadhyay A, Mohan N, Dhar A, Walia HK, Zubaidi G (2005) Late development of diabetes mellitus after interferon-alfa and ribavirin therapy for chronic hepatitis C: a case report. Med Princ Pract 14:281–283

Ravindra PV, Tiwari AK, Sharma B, Chauhan RS (2009) Newcastle disease virus as an oncolytic agent. Indian J Med Res 130:507–513

Reed JC (2011) Priming cancer cells for death. Science 334:1075–1076

Reyes-Del Valle J, Chávez-Salinas S, Medina F, Del Angel RM (2005) Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. J Virol 79:4557–4567

Rossi A, Elia G, Santoro MG (1996) 2-Cyclopenten-1-one, a new inducer of heat shock protein 70 with antiviral activity. J Biol Chem 271:32192–32196

Scavone G, Zaccardi F, Manto A, Caputo S, Pitocco D, Ghirlanda G (2010) A case of chronic hepatitis C developing insulin-dependent diabetes, thyroid autoimmunity and stiff-person syndrome as complications of interferon therapy. Diabetes Res Clin Pract 89:e36–e38

Shimizu K, Iguchi A, Gomyou R, Ono Y (1999) Influenza virus inhibits cleavage of the HSP70 pre-mRNAs at the polyadenylation site. Virology 254:213–219

Silva AM, Wang D, Komar AA, Castilho BA, Williams BR (2007) Salicylates trigger protein synthesis inhibition in a protein kinase R-like endoplasmic reticulum kinase-dependent manner. J Biol Chem 282:10164–10171

Singleton KD, Wischmeyer PE (2007) Glutamine's protection against sepsis and lung injury is dependent on heat shock protein 70 expression. Am J Physiol Regul Integr Comp Physiol 292:R1839–R1845

Song H, Moseley PL, Lowe SL, Ozbun MA (2010) Inducible heat shock protein 70 enhances HPV31 viral genome replication and virion production during the differentiation-dependent life cycle in human keratinocytes. Virus Res 147:113–122

Stankiewicz AR, Lachapelle G, Foo CPZ, Radicioni SM, Mosser DD (2005) Hsp70 inhibits heat-induced apoptosis upstream of mitochondria by preventing Bax translocation. J Biol Chem 280:38729–38739

Tersey S, Nishiki Y, Templin A, Cabrera S, Stull N, Colvin S, Evans-Molina C, Rickus J, Maier B, Mirmira R (2012) Islet β-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. Diabetes 61:818–827

Toovey S, Jick SS, Meier CR (2011) Parkinson's disease or Parkinson symptoms following seasonal influenza. Influenza Other Respi Viruses 5:328–333

Tosone G, Borgia G, Gentile I, Cerini R, Conte MC, Orlando R, Piazza M (2007) A case of pegylated interferon alpha-related diabetic ketoacidosis: can this complication be avoided? Acta Diabetol 44:167–169

Tsai MS, Chen JH, Fang YW, Yang AH, Chang CH (2012) Membranous nephropathy induced by pegylated interferon alpha-2a therapy for chronic viral hepatitis B. Clin Nephrol 77:496–500

Unoshima M, Iwasaka H, Eto J, Takita-Sonoda Y, Noguchi T, Nishizono A (2003) Antiviral effects of geranylgeranylacetone: enhancement of MxA expression and phosphorylation of PKR during influenza virus infection. Antimicrob Agents Chemother 47:2914–2921

Van Coster RN, De Vivo DC, Blake D, Lombes A, Barrett R, DiMauro S (1991) Adult Reye's syndrome: a review with new evidence for a generalized defect in intramitochondrial enzyme processing. Neurology 41:1815–1821

Van Der Kelen K, Beyaert R, Inzé D, De Veylder L (2009) Translational control of eukaryotic gene expression. Crit Rev Biochem Mol Biol 44:143–168

van Eden W, van der Zee R, Prakken B (2005) Heat-shock proteins induce T-cell regulation of chronic inflammation. Nat Rev Immunol 5:318–330

Vojdani A, Ghoneum M, Choppa PC, Magtoto L, Lapp CW (2007) Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA. J Intern Med 242:465–478

Wang H, Ding Y, Zhou J, Sun X, Wang S (2009) The in vitro and in vivo antiviral effects of salidroside from Rhodiola rosea L. against coxsackievirus B3. Phytomedicine 16:146–155

Weiss YG, Bouwman A, Gehan B, Schears G, Raj N, Deutschman CS (2000) Cecal ligation and double puncture impairs heat shock protein 70 (HSP-70) expression in the lungs of rats. Shock 13:19–23

Wieten L, van der Zee R, Goedemans R, Sijtsma J, Serafini M, Lubsen NH, van Eden W, Broere F (2010) Hsp expression and induction as a readout for detection of immune modulatory components in food. Cell Stress Chaperones 15:25–37

Zamarin D, Palese P (2012) Oncolyitc Newcastle disease virus for cancer therapy: old challenges and new directions. Future Microbiol 7:347–367

Zhang Q, Zhou X-D, Denny T, Ottenweller JE, Lange G, LaManca JJ, Lavietes MH, Pollet C, Gause WC, Natelson BH (1999) Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome. Clin Vaccine Immunol 6:6–13

Zhang C, Yang Y, Zhou X, Yang Z, Liu X, Cao Z, Song H, He Y, Huange P (2011) The NS1 protein of influenza a virus interacts with Hsp90 in human alveolar basal epithelial cells: implication for virus-induced apoptosis. Virol J 8:181