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Review

Role of host cell factors in flavivirus infection: Implications for pathogenesis and development of antiviral drugs

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ABSTRACT

The genus *Flavivirus* contains approximately 70 arthropod-borne enveloped RNA viruses many of which cause severe human and in some cases, animal disease. They include dengue virus, yellow fever virus, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis virus. Hundreds of thousands of deaths due to flavivirus infections occur each year, many of which are unpreventable due to lack of availability of appropriate vaccines and/or antiviral drugs.

Flaviviruses exploit the cytoplasmic cellular machinery to facilitate propagation of infectious progeny virions. They engage in dynamic and antagonistic interactions with host cell membranes and biochemical processes. Following infection, the cells initiate various antiviral strategies to counteract viral invasion. In its defense, the virus has alternative strategies to suppress these host responses to infection. The fine balance between these interactions determines the outcome of the viral infection and disease progression.

Published studies have revealed specific effects of flaviviruses on cellular processes, but the underlying mechanisms that determine the specific cytopathogenetic changes induced by different flaviviruses have not, as yet, been elucidated. Independently of the suppression of the type I IFN response which has been described in detail elsewhere, this review focuses on recent discoveries relating to alterations of host metabolism following viral infection. Such studies may contribute to new approaches to antiviral drug development. The role of host cellular factors will be examined in the context of protection and/or pathogenesis resulting from flavivirus infection, with particular emphasis on West Nile virus and dengue virus.

Contents

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1. Introduction

Flaviviruses are a group of arboviruses belonging to the family *Flaviviridae*. Several members of this genus, such as dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV), are highly pathogenic for humans and constitute major international health problems (Gould and Solomon, 2008; Mackenzie et al., 2004). Many of these viruses are transmitted by mosquitoes and/or ticks leading to flaviviral diseases now global in nature. Most infected humans or animals have either asymptomatic or an undifferentiated febrile illness. However, severe and often fatal infections with haemorrhagic or encephalitic manifestation may arise during infection with DENV, or some tick-borne flaviviruses such as Kyasanur Forest disease virus or Omsk haemorrhagic fever virus. Surprisingly, despite the large number of humans that suffer severe flavivirus infections annually, there are no available antiviral therapies and only three specific vaccines are available against YFV, TBEV and JEV. Although these vaccines are very effective, in practice their utilization encounters limitations and difficulties supporting the contention that there is a real need for antiviral drugs to supplement the health control measures currently available from protective immunisation.

1.1. Yellow fever virus

A major gap in our knowledge of YF is how to manage and treat sick patients infected via mosquito bites or following vaccine-associated adverse events as recently reported (Staples and Monath, 2008). In fact, treatment of the most clinically severe cases of YF by supportive care in most cases is essentially ineffective, and improvements to the vaccine's safety are being sought. Consequently, there is a clear need for safe and effective drugs to treat patients during all stages of the disease.

1.2. Tick-borne encephalitis virus

Despite the availability of at least four inactivated vaccines, tick-borne encephalitis virus also poses a serious and potentially increasing health problem in Western, Central and Eastern Europe (Khasnatinov et al., 2009). In fact, vaccination campaigns have had varying degrees of success, since they are relatively expensive and require repeated administration at about four yearly intervals in order to maintain protective immunity (Juceviciene et al., 2005). Therefore, with no effective therapy for TBEV, treatment is only supportive including the administration of Paracetamol, Aspirin and other nonsteroidal anti-inflammatory drugs (Mansfield et al., 2009).

1.3. Japanese encephalitis virus

Similarly, despite the fact that three different vaccines are available (Paulke-Korinek and Kollaritsch, 2008) and even though JEV is the main cause of encephalitis with about 10,000 fatal cases annually in Asia (WHO, 2007), there is no effective antiviral therapy to treat this infection. However, some reports of systemic and neurological complications have called the live attenuated vaccine's safety into question (Gould et al., 2008). The inactivated vaccines show excellent tolerability but humans in endemic areas need to be boosted every few years to ensure immunity.

1.4. Dengue virus

In terms of morbidity and mortality, the most important flaviviruses are the four dengue virus serotypes (an estimated 2.5 billion people at risk globally which more than 70% reside in Asia Pacific countries, WHO, 2008). Nevertheless, despite their major impact on World Health, no vaccines or antiviral drugs currently exist. Consequently, a single orally administered antiviral treatment has become a major target for the immediate future.

It is therefore clear that whilst the use of flavivirus vaccines has its merits, there are many situations in which effective antiflaviviral drugs could play an important role in health management. For example, non-immune indigenous infants, tourists, visitors and temporarily employed workers from non-endemic regions are particularly vulnerable to flavivirus infections in endemic countries. Immuno-compromised or elderly and malnourished people also constitute high-risk groups. Consequently, available antiviral medication could offer an effective alternative or adjunct to vaccination, both for prophylactic treatment concerning persons localized in endemic areas and to treat severely ill patients.

Today no antiviral therapies are approved for use against flaviviruses. There is thus an urgent need for new molecules that could reduce viraemia during the early stages of infection, block viral replication in the brain in cases of encephalitis, or modulate host responses (Bray, 2008). Antiviral drug discovery protocols rely heavily on screening libraries of compounds and/or small molecules for inhibitory effects and low human toxicity when tested against viral pathogens. Whilst these methods have proven to be moderately successful to reduce the effects of HIV, and hepatitis viruses, at the moment no antiviral drug totally eradicates the infection. Therefore, new approaches are needed. Moreover, it is known that resistance development is a major obstacle to antiviral therapy, and all active antiviral agents have been shown to select for resistance mutations (Nijhuis et al., 2009). Chemotherapy against viral infections can be developed using two strategies, either by blocking virus encoded functions or by blocking the cellular functions needed for viral multiplication. This second approach has the potential complication that it may hamper normal cellular function, but it also has potential advantages in that the therapy should be active against all viruses within the same genus, and emergence of resistance against this type of chemotherapy should be relatively rare. Thus, it is now justified to consider the host cell components as potential therapeutic targets.

This review will therefore focus on the impact of flaviviruses on host cell proteins with a view to identifying potential targets for the development of antiviral drugs. The potential roles of some of these modified cellular proteins belonging to major metabolic pathways are discussed in relation to pathogenesis and the early host antiviral response. We place particular emphasis on DENV and WNV studies, highlighting how new approaches such as proteomics have improved our understanding of the molecular basis of the complex cellular physiological mechanisms associated with these infections.

2. Flavivirus virion, viral genome structure and replication

Flaviviruses are a group of more than 70 enveloped RNA viruses with a single-stranded, positive-polarity 11-kilobase genome encoding a single long open reading frame. The 5′ end of the genome

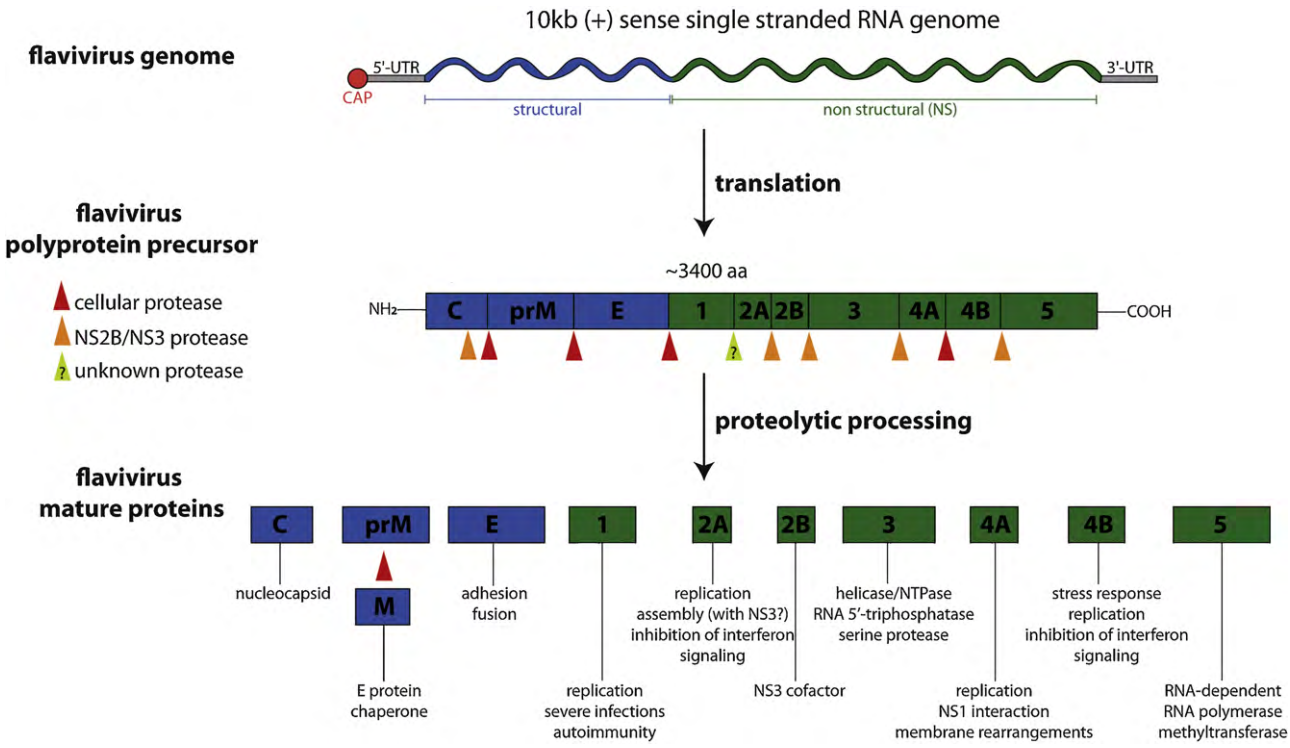

Fig. 1. Flavivirus genome and polyprotein organization. The single open reading frame (~10 kb) is depicted with the structural and non-structural protein coding regions (coloured in blue and green respectively), the 5′-CAP and the 5′ and 3′ untranslated region (UTR). The single open reading frame encodes an immature polyprotein precursor that is co- and post-translationally cleaved into three structural proteins (in blue) and seven non-structural proteins (in green). The cleavage sites for cellular proteases (▲), NS2B/NS3 (▲) and unknown protease (▲) are indicated. Putative functions of these proteins during infection are described. aa, amino acids; C, capsid protein; M, membrane; E, envelope. The amino termini of prM, E, NS1, and NS4B are generated upon cleavage by the host signal peptidase in the ER lumen, while the processing of most of the NS proteins and the carboxyl terminus of the C protein is carried out by the viral NS3 serine protease with the NS2B cofactor in the cytoplasm of the infected cell. NS3 (70 kDa) and NS5 (104 kDa) are the most characterized non-structural proteins, with multiple enzyme activities that are required for viral replication. Mutations that affect each activity impair viral replication (Matusan et al., 2001a,b). NS1 protein (46 kDa) is required for flavivirus replication and is presumably involved in negative-strand synthesis by an unknown mechanism. NS2A (22 kDa) is a small hydrophobic transmembrane protein that is involved in generation of virus-induced membranes during virus assembly (Leung et al., 2008). NS4A (16 kDa) is an integral membrane protein which induces membrane rearrangements to form the viral replication complex (Miller et al., 2007; Roosendaal et al., 2006). NS4B protein (27 kDa) inhibits the type I interferon response of host cells (Munoz-Jordan et al., 2005), and may modulate viral replication via its interaction with NS3 protein (Umareddy et al., 2006).

has a type 1 cap, but the 3′ end lacks a poly-A tail (Fig. 1). The genome is packaged by the viral capsid protein (C) in a host-derived lipid bilayer containing the viral envelope protein (E) that functions in receptor binding, membrane fusion and viral assembly.

Host cells for flaviviral infection include monocytes, macrophages and dendritic cells and the virus attaches to the cell surface, mediated by the E protein, and enters the cell by receptor-mediated endocytosis (Fig. 2) (Barba-Spaeth et al., 2005; Krishnan et al., 2007; Lozach et al., 2005). Low pH in the endosomal compartment triggers fusion of the viral and host cell membrane mediated by structural reorganization of E, which leads to the release of the nucleocapsid and viral RNA into the cytoplasm (Rey et al., 1995). Translation of the genome by the host cell machinery produces a polyprotein that is cleaved co- and post-translationally into the mature proteins comprising the viral structural and non-structural proteins that are required for replication and assembly of new virions. The N-terminal end of the polyprotein encodes the three structural proteins (C–prM–E), followed by seven non-structural (NS) proteins (NS1–NS2A–NS2B-NS3–NS4A–NS4B–NS5) (Fig. 1) (Rice et al., 1985). NS3 (70 kDa) and NS5 (104 kDa) are the most characterized non-structural proteins, with multiple enzyme activities that are required for viral replication. NS3 protein has three distinct activities: serine protease together with the cofactor NS2B, required for polyprotein processing (Assenberg et al., 2009; Aleshin et al., 2007; Bera et al., 2007); helicase/NTPase activity, required for unwinding the double-stranded replicative form of RNA; RNA triphosphatase, needed for capping nascent viral RNA (Bollati et al., 2009). NS5 is the largest and most highly conserved flaviviral protein, with greater than 75% sequence identity across all DENV serotypes. It contains two distinct enzymatic activities, separated by an interdomain region: an *S*-adenosyl methyltransferase and an RNA-dependent RNA polymerase (RdRp) (Davidson, 2009).

Viral RNA replication occurs in the rough endoplasmic reticulum (RER) and in Golgi-derived membranes called vesicle packets (VP) where double-stranded RNA (dsRNA) is concentrated (Mackenzie, 2005; Salonen et al., 2005; Welsch et al., 2009). Assembly of virus particles occurs in the lumen of the RER and maturation of these virus particles occurs in the trans-Golgi network, where prM is cleaved to M by furin, along with conformational rearrangements of E protein (Yu et al., 2008; Heinz et al., 1994). This is an essential step for the virus in the transition from fusion-incompetent and non-infectious virus particles to mature, fusion-competent, and infectious virions. The mature particles eventually exit from the host cell, as packages of virions (Fig. 2).

3. Pathogenesis of flavivirus infection

Flaviviruses exhibit significant pleiotropism within the vertebrate host and can be broadly grouped into viruses that have the capacity to cause vascular leakage and haemorrhage, including DENV and YFV, and those that cause encephalitis, including WNV, JEV and TBEV (Table 1). They enter through the skin via the bite of an infected arthropod, proliferating locally and spreading to become generalized within a short period of time, usually with a signifi-

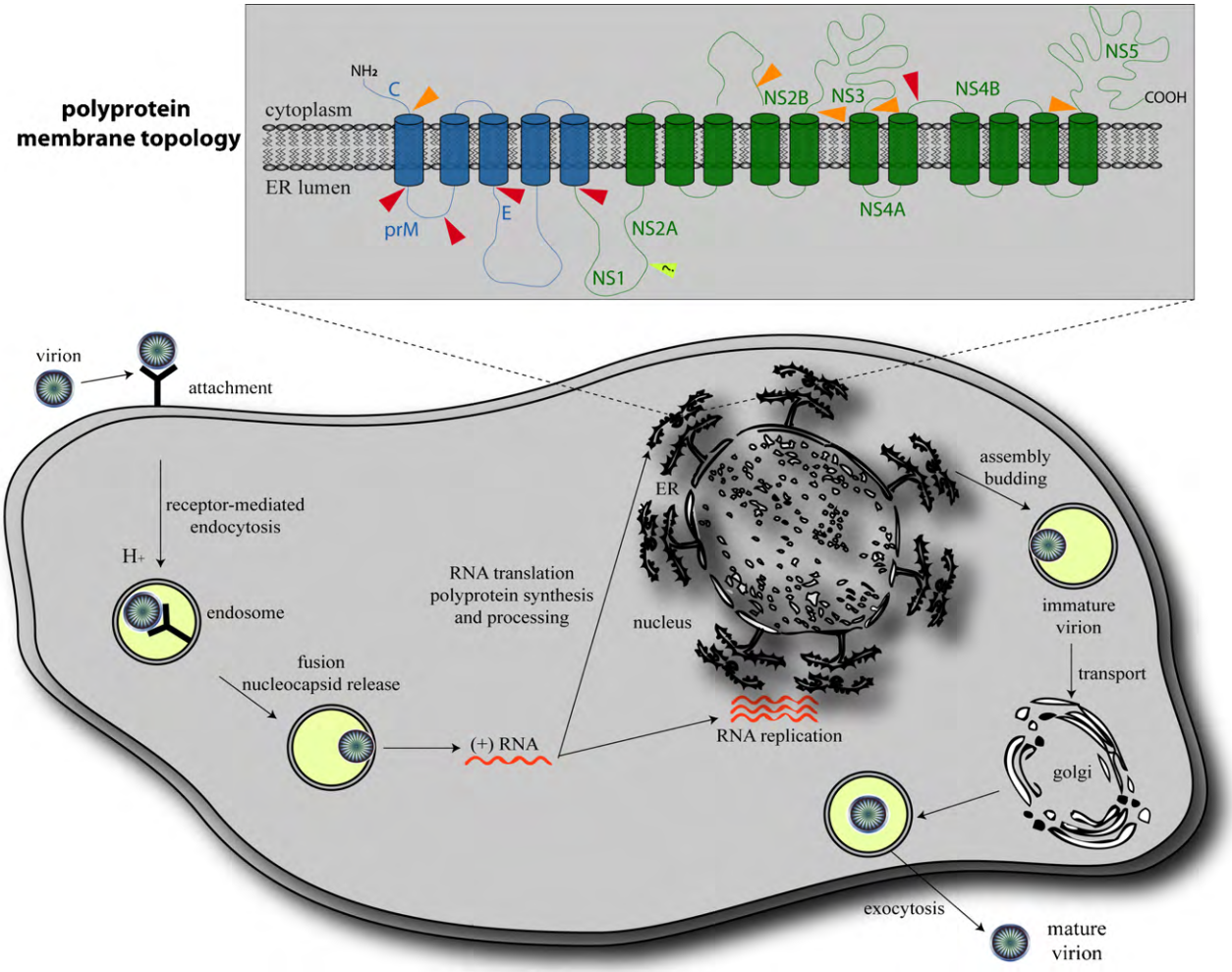

Fig. 2. Flavivirus life cycle and proposed topology of the polyprotein. The infection is initiated when mature virus particles bind to host surface receptors. Virions then enter the host cell via receptor-mediated endocytosis. The low pH in the endosomes mediates fusion of viral membranes with endosomal membranes and the release of the RNA genome into the cytoplasm. The positive-sense RNA is translated into a single polyprotein that is co- and post-translationally processed by viral and host proteases. The cleavage sites and topology of the polyprotein at the ER membrane are illustrated schematically. Genome replication occurs on intracellular membranes. Virus assembly occurs at the surface of the ER when the structural proteins and newly synthesized RNA buds into the lumen of the ER. The newly synthesized viral RNA is extruded in the intermembrane space of the double-membrane VPs, from which it exits into the cytoplasm by an unknown mechanism (Uchil and Satchidanandam, 2003). Assembly of virus particles occurs in the lumen of the RER. The first step in this process is the coating of the newly synthesized viral RNA with the C protein (Khromykh and Westaway, 1996; Perera et al., 2008; Perera and Kuhn, 2008). Next, E and PrM proteins hetero-dimerize and envelope the nucleocapsid, forming an immature virus particle that buds from the lumen of the RER into the Golgi complex (Mackenzie and Westaway, 2001). However, the mechanism of interaction of the C protein within the nucleocapsid is still not clear. Maturation of virus particles occurs in the trans-Golgi network, where prM is cleaved to M by furin, along with conformational rearrangements of E protein (Mukhopadhyay et al., 2005; Yu et al., 2008). Mature virions are subsequently released by exocytosis.

cant viraemia. The viraemia facilitates transmission to non-infected arthropods although non-viraemic transmission is considered to be the major determinant of virus transmission between co-feeding ticks in the tick-borne encephalitis complex (Gould et al., 2003). The route of progression through the vertebrate host has not been clearly established, but it appears that the virus progresses first from the site of the bite to draining lymph nodes, where it replicates and is amplified before (in the case of encephalitic flaviviruses) crossing the blood–brain barrier by as yet undefined mechanisms (Hayasaka et al., 2009).

Most pathogenic flaviviruses are associated with neurologic disease and some are found worldwide being transmitted between vertebrates by competent mosquitoes and/or ticks. The mosquito-borne encephalitic flaviviruses are all antigenically and genetically closely related. They are grouped phylogenetically in the Japanese encephalitis serocomplex and many are transmitted to vertebrate hosts primarily by *Culex* spp. mosquitoes. Most tick-borne flaviviruses cause encephalitis and are found principally in Europe and Asia. These viruses are transmitted by *Ixodes* spp. ticks.

After peripheral amplification, the virus enters the circulation, crosses the blood–brain barrier (BBB) and enters the central nervous system (CNS) through unknown mechanisms. Several hypotheses have been offered for mechanisms of CNS penetration. These include virus penetration as a result of inflammation and damage to vascular integrity (Lossinsky and Shivers, 2004), entry through the olfactory bulb (Cook and Griffin, 2003), toll-like receptor-mediated entry (Wang et al., 2004) and transcytosis across vascular endothelial cells (Lossinsky and Shivers, 2004). Crossing the BBB is an important factor for the pathogenesis and unfavorable clinical outcome of the neurotropic viral infection (King et al., 2007). Some reports suggest macrophages could serve as a reservoir for WNV, spreading the virus from the periphery to the CNS (Cardosa et al., 1986; Rios et al., 2006). Other studies have shown that WNV is able to enter the CNS via anterograde axonal transport (Hunsperger and Roehrig, 2006), whilst JEV virions binding to the

Table 1
Principal flaviviruses pathogenic for humans.

Virus	Abbreviation	Location of isolation	Geographic distribution	Principal vector species	Human disease
Alkhurma	ALKV	Saudi Arabia	?	ticks	Haemorrhagic fever
Dengue 1	DENV-1	Hawai	Tropics, subtropics	*Aedes aegypti*	Fever, rash, vasculopathy, haemorrhagic fever
Dengue 2	DENV-2	New Guinea	Tropics, subtropics	*Aedes aegypti*	Fever, rash, vasculopathy, haemorrhagic fever
Dengue 3	DENV-3	Philippines	Tropics, subtropics	*Aedes aegypti*	Fever, rash, vasculopathy, haemorrhagic fever
Dengue 4	DENV-4	Philippines	Tropics, subtropics	*Aedes aegypti*	Fever, rash, vasculopathy, haemorrhagic fever
Kyasanur Forest disease	KFDV	India	India	*Haemaphysalis spinigera*	Haemorrhagic fever
Omsk haemorrhagic fever	OHFV	Russia	Western Siberia	Dermacentor pistus	Haemorrhagic fever/encephalitic
Yellow fever	YFV	Ghana	Sub-Saharan Africa, South America	*Aedes and Haemagogus* spp	Pantropic
Powassan virus	POWV		Western United States, Western Canada, Siberia	*Ixodes spp*, *Dermacentor variabilis*, *I. persulcatus*, *H. neumanni*, *H. consinna* and *D. silvarum*	Encephalitis
Japanese encephalitis	JEV	Japan	Asia	*Culex tritaeniorhynchus*	Encephalitis
Langat	LGTV	Malaysia	Malaysia, Thailand, Siberia	*Ixodes granulatus*	Encephalitis
Louping ill	LIV	Scotland	UK, Ireland	*Ixodes* spp	Encephalitis
Murray Valley encephalitis	MVEV	Australia	Australia, New Guinea	*Culex annulirostris*	Encephalitis
St Louis encephalitis	SLEV	USA	South and Central America	*Culex* spp	Encephalitis
Tick-borne encephalitis	TBEV	Russia	Europe, Asia	*Ixodes* spp	Encephalitis
West Nile	WNV	Uganda	Old World and New World	Many mosquito/tick spp	Encephalitis

endothelial surface of the CNS have been shown to be internalized by endocytosis (Liou and Hsu, 1998).

The mechanisms by which encephalitic flaviviruses induce neuronal injury *in vivo* remain largely unknown. However, *in vitro* studies have begun to elucidate the pathways involved in WNV-induced cell death. It has been demonstrated that WNV infection triggers apoptosis in different transformed cell lines, resulting in caspase 3 activation, cytochrome *c* release, and exposure of phosphatidylserine on the outer leaflet of the plasma membrane (Chu and Ng, 2003; Parquet et al., 2001). The cellular outcome of WNV replication depends on interactions between host and viral factors. UV-inactivated WNV failed to induce cell death, suggesting that viral replication is required to trigger apoptosis (Parquet et al., 2001). Several WNV proteins may contribute directly to this process. Ectopic expression of the WNV NS3 protein or its helicase or protease domain induced apoptosis and activation of caspase 3 and 8 (Ramanathan et al., 2006). Expression of WNV capsid protein either *in vitro* or in the striata of mouse brains also triggered apoptosis downstream of caspase 3 and caspase 9 activation (Yang et al., 2002). It is known that programmed cell death could have opposing functions during viral infection: it may be antiviral by inducing the death of infected cells, or it may enhance viral spread and progeny release. Cell death can also be pathological if it occurs in non-renewing cell populations, such as neurones. Thus, it has been postulated that virus-induced apoptosis may contribute to neuronal death *in vivo* and the pathogenesis of encephalitic flaviviruses (Parquet et al., 2001; Raung et al., 2001; Weissenbock et al., 2004; Yang et al., 2002). However, direct evidence for this mechanism has been lacking, and the pathways involved in the flavivirus-mediated death of neurones are not well understood.

Other pathogenic flaviviruses, principally DENV and YFV, belong to the viral hemorrhagic fevers group (VHFs) causing a syndrome of fever and malaise, 'capillary leak' with loss of plasma volume, and coagulation defects which can lead to bleeding. The 15–20 different types of viral haemorrhagic fever appear to share a similar pathogenesis, in which macrophages and dendritic cells, rather than acting as barriers to an invading pathogen, serve as the principal sites of viral replication, supporting the rapid spread of infection (Bray, 2005; Bray and Geisbert, 2005; Geisbert and Jahrling, 2004). Although direct cytopathic effects can also contribute to disease severity (hepatic injury in case of YFV infection), most features of illness are often caused by innate immune responses, as the systemic spread of virus to macrophages and dendritic cells leads to the release of mediators that modify vascular function and have procoagulant activity.

DENV is a mosquito-borne flavivirus of global public health importance; an estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually (Halstead, 2007). The economic importance of dengue in the developing world is also a major concern (Clark et al., 2005). After the bite of an infected mosquito vector, primarily *Ae. Aegypti* (but other *Aedes* spp. are also competent to transmit the virus), DENV can cause a mild and self-limiting infection but may induce more severe symptoms such as dengue fever (DF) or it may progress to a potentially lethal dengue haemorrhagic fever (DHF) and in the worst cases, dengue shock syndrome (DSS). The mosquito vectors are currently most prevalent in tropical and subtropical regions of the world but *Aedes albopictus* in particular is dispersing more northerly and in the future this could lead to DENV becoming prevalent in southern Europe as the impact of climate change takes greater effect.

The characteristic features of DHF are, increased capillary permeability without morphological damage to the capillary endothelium, thrombocytopenia, altered number and functions of leucocytes, altered haemostasis and liver damage. The pathogen-

esis of DHF is not fully understood despite extensive work carried out in recent decades, due mainly to the absence of an appropriate animal model. Various mechanisms have been suggested for DHF enhancement including a role for non-neutralizing enhancing antibodies in secondary dengue infections with heterologous serotypes, memory T-cell-mediated pathogenesis, immune complex disease or complement and its products, anti-NS1 antibodies that cross-react with vascular endothelium. A cytokine Tsunami and other soluble mediators such as high concentrations of soluble IL-2 receptor, soluble CD4, soluble CD8, TNF receptors, IL-10 and macrophage migration inhibition factor are also considered important factors in dengue pathogenesis. Finally, selection of virulent virus strains (South East Asian type DENV-2) and host genetic polymorphism are others important factors largely described and certainly involved in DHF (Noisakran and Perng, 2008; Martina et al., 2009).

4. Antiflaviviral therapy and drug targeting of host metabolism

Pathogenic viruses exploit host cell pathways and enzymes during their replicative life cycle. Thus, it seems reasonable to expect that inhibiting such cellular processes might have an inhibitory effect on virus reproduction. It is now widely demonstrated, both *in vitro* and *in vivo*, that this strategy can be effective.

At the present time, interest in developing inhibitors is limited to viruses that cause chronic disease, viruses that have the potential to cause large-scale epidemics, or ubiquitous viruses for which the treatment of acute infection would be beneficial even if the infection was ultimately self-limiting. In practice, the use of antiviral drugs in otherwise healthy adults and children is not generally recommended. Therefore, the utility, target populations and optimal duration of preventive antiviral treatment must be determined by examining the groups most at risk and the severity of complications. In fact, antiviral drugs are not an alternative to available vaccination, but may be a useful adjunct in some situations. It is probably wisest to limit their use to short-term prophylaxis of vulnerable persons in situations where the risk of contracting the virus infection is high.

In the example of flavivirus infections, it is likely that reducing the initial acute viremia may be sufficient to provide clinical benefit without compromising the immune response. Rapid diagnosis and suppression of the virus replication are particularly important determinants of clinical outcome for several reasons:

- to identify the pathogen precisely since treatment decisions should be a balance between potential benefit and toxicity related to antiviral therapy
- to reduce the risk of brain injury which has only limited potential for repair
- to modulate the host response which may contribute to pathogenesis
- to reduce the potential for transmission between susceptible hosts when viral burden is low.

4.1. Targeting host cell functions

Resistance to viral inhibitors is a particularly serious problem for the medically important RNA viruses, such as HIV, HCV and influenza virus, because they demonstrate much higher mutation rates than those recorded for DNA viruses. In this way one potential advantage of targeting host metabolism using antivirals, is the multiplicity and diversity of putative candidates in the host whilst evading viral escape arising via the viral RNA dependent RNA polymerase (RdRp) which has no proof-reading activity. In this strategy, the side effects caused by inhibition of the primary function of the metabolite can be a problem and to minimize these undesirable effects, inhibition needs to be targeted with pinpoint accuracy. This concept offers some advantages compared with the viral inhibitor approach: host cells offer a wealth of proven, drugable targets. By targeting common cellular pathways that are required for the life cycle of different viruses, 'broad-spectrum', 'silver-bullet' antiviral drugs could potentially be developed to treat multiple viral diseases. Moreover, because all existing licensed drugs that target a human disease process affect the functioning of a cell or organ system in some way, a ready-made pharmacy of antimicrobial agents with defined safety-data profiles and clinical-use histories is available requiring only assessment for new or 'off target' second use.

The evidence that the approach of inhibiting host cell functions works now extends across many diverse virus types, including poxviruses (Reeves et al., 2005), herpesviruses (Jenner et al., 2003; Moses et al., 2002; Zhu et al., 2002), retroviruses (del Real et al., 2004), hepadnaviruses (Bordier et al., 2002, 2003) and flaviviruses (Hirsch et al., 2005). These putative metabolic antiviral targets have at least three possible direct or indirect cellular functions; facilitating efficient virus replication, causing disease pathogenesis or leading to pathogen clearance. Targeting genes or molecules in the first and second examples should lead to reduce virus replication and attenuated disease pathogenesis. In contrast, inhibiting genes or molecules involved in pathogen clearance should be avoided because it is likely to increase viral replication and disease progression.

Table 2 gives an overview of some effective antiviral drugs targeting host cell functions that are active *in vitro* and/or *in vivo*. These inhibitors interact with specific molecular cell factors belonging to many metabolic pathways.

For example, it is known that the cholesterol-rich lipid raft plays an important role in virus entry, replication, and assembly and it has been reported that lovastatin, one of the HMG–CoA reductase inhibitors, inhibited HCV RNA replication in HCV replicon-harboring cells (Aizaki et al., 2008). Drugs lowering the cholesterol cell level prove also to be efficient against HIV-1 replication. Thus, the potential antiviral and immunological properties of statins warrant a randomized clinical trial addressed to explore the clinical use of these molecules in HIV-1 infected patients (Montoya et al., 2009).

Hsp90 is another interesting cellular target for viral replication inhibition. Geldanamycin and Radicicol Hsp90 inhibitors showed potent anti-HCV activity both in an HCV replicon system and in a humanized liver mouse model infected with HCV. In fact, Hsp90 could interact directly or indirectly with any of the flavivirus proteins, from NS3 through NS5B, to regulate viral replication and its inhibition may provide a feasible therapeutic strategy for the treatment of HCV infection (Okamoto et al., 2006; Nakagawa et al., 2007). Hsp90 inhibitors may also represent a new class of antiviral compounds against influenza viruses. Recently, the cellular chaperone Hsp90 was shown to play a role in nuclear import and assembly of the trimeric polymerase complex and its inhibition could lead to reduction in viral RNP assembly (Chase et al., 2008).

Valproic acid (VPA) inhibits class I and II HDAC (histone deacetylases) enzymes directly and gives rise to hyperacetylation of gene promoters, and altered expression of approx. 2% of the cell genome. It has been shown that HDAC is a critical regulator of HIV latency by its action at the viral promoter resulting in HIV gene expression quiescence in infected resting CD4þ lymphocytes. A recent study found that three of four patients treated with valproic acid in addition to highly active antiretroviral therapy (HAART) showed a mean 75% reduction in latent HIV infection (Lehrman et al., 2005). So it is plausible that HIV infection could be cured by treatment with VPA or more potent analogues in combination with more effective antiretroviral drugs.

Table 2
Overview of some inhibitors of infection that target host factors.

Virus species	Host cell target	Drug	References
HCV	PKR	Nitazoxanide	Khattab (2009)
HCV	IMPDH	VX-497	Markland et al. (2000)
HCV	TLR7	ANA245	De Francesco and Migliaccio (2005)
HCV	HSP90	Geldanamycin/Radicicol	Ikeda and Kato (2007)
HCV	PKA	Metformin	Khattab (2009)
HCV	Cyclophilin B	NIM811	Khattab (2009)
HCV	VLDL	Microsomal	Khattab (2009)
HCV/RSV/WNV	Capping enzyme/IMPDH	Ribavirin	Ghosh and Basu (2008), McHutchison and Poynard (1999)
HIV	Receptors or co-receptors	Maraviroc, Vicriviroc, Pro-140	Abel et al. (2009), Klibanov (2009), Trkola et al. (2001)
HIV	HMG-CoA reductase	Lovastatin	del Real et al. (2004)
HIV	ATM kinase	KU-55933	Lau et al. (2005)
HIV	Histone deacetylase	Valporic acid	Lehrman et al. (2005)
HBV	hnRNP K	siRNA	Ng et al. (2005)
WNV	CyPA	Cyclosporine	Qing et al. (2009)
WNV	Src family kinases	PP2/SU6656	Hirsch et al. (2005)
WNV	IMPDH	Mycophenolic acid	Ghosh and Basu (2008)
WNV	OMPDC	Pyrazofurin	Ghosh and Basu (2008)
YFV/DENV	Dihydroorotate	Brequinar	Ray and Shi (2006)
DENV-1/DENV-2/JEV	α glucosidase	Castanospermine	Ghosh and Basu (2008)
Vaccinia virus	Abl tyrosine kinase	Gleevec	Reeves et al. (2005)
KSHV	Vitamin D receptor	EB 1089	Jenner et al. (2003)
HSV-1	EIF-2α	Salubrinal	Boyce et al. (2005)
HCMV	COX-2	BMS-279652/Indomethacin/Aspirin	Zhu et al. (2002)
HDV	Farnesyltransferase	FTI-277/FTI-2153	Bordier et al. (2002, 2003)

The *Poxviridae* family members vaccinia and variola virus produced cell-associated enveloped virions (CEV) which use Abl- and Src-family tyrosine kinases for actin motility. Release of CEV from the cell requires Abl and this step was shown to be blocked by STI-571 (Gleevec), an Abl-family kinase inhibitor used to treat chronic myelogenous leukemia in humans (Reeves et al., 2005). In fact, STI-571 reduces viral dissemination and promotes survival in infected mice, suggesting possible use for this drug targeting host tyrosine kinases in treating smallpox or complications associated with vaccination.

Hepatitis delta virus (HDV) is an important cause of acute and chronic liver disease with no current medical therapies. An essential step in the virus assembly process involves the post-translational lipid modification of a specific HDV protein, namely prenylation of large delta antigen. Preventing prenylation abolishes virus particle formation *in vitro* but also *in vivo* and drugs capable of specifically inhibiting prenylation like FTI-277 or FTI-2153 have been developed for use in humans (Bordier et al., 2003; Glenn, 2006). These agents represent a new class of antiviral agents, with HDV as a first target.

5. Screening the interaction between flavivirus and host cell

An Australian strain of WNV, Kunjin virus (KUNV), represents one of the best studied models of intracellular changes induced following flavivirus infection. Flavivirus replication can cause extensive rearrangement of host cell cytoskeletal and membrane compartments leading to "cytopathic effects (CPE)" in cells of human, primate, rodent and insect origin (Netherton et al., 2007). During the infectious cycle, highly complex membrane structures are induced, which act as platforms for viral replication (Mackenzie et al., 1999; Westaway et al., 2002). How these membranes facilitate viral RNA replication, virion assembly and the virus–cell interactions involved in inducing these specialized membrane subdomains is currently unknown (Mackenzie, 2005). For DENV infection, several studies have demonstrated that dengue-infected human endothelial cells selectively secrete proinflammatory chemokines/cytokines (e.g., RANTES, IL-6 and IL-8) in response to dengue virus infection, resulting in endothelial cell activation, cell damage, and altered transendothelial permeability through cytoskeletal reorganization (Kanlaya et al., 2009). In fact, alteration of these proteins may be involved in the molecular mechanisms underlying the increased vascular permeability in DHF/DSS.

Due to the public health importance of mosquito-borne virus diseases and also to our need to understand the mechanism of virus replication and pathogenesis, increasing numbers of studies have examined host metabolism alterations following flavivirus infection (Fernandez-Garcia et al., 2009) (Table 3). Innovative strategies combining computational biology, genomics, transcriptomics, proteomics, and forward and reverse "chemical genetics" have improved the identification of propathogen host factors. The potential roles of some of these altered cellular factors belonging to major clusters in response to flavivirus infection are discussed below in relation to pathogenesis and early host antiviral response (Table 3).

5.1. Cytoskeleton networks and cell trafficking (Table 3.1)

Microtubules and microtubule-associated proteins are known to play an important role in the intracellular trafficking of viral components as well as virions in the infected host cell (Greber and Way, 2006). The actin filaments were shown to be involved in mediating the internalization of WNV particles into mammalian cells by clathrin-dependent endocytosis (Chu and Ng, 2004). Moreover, it has been demonstrated that actin filaments also play a key role in the release of West Nile (Sarafend) virions (Chu et al., 2003).

Microtubules are cytoskeletal polar structures constituted of α and β-tubulin subunits which perform general functions including organelle movement and cargo transport in all kinds of cells. A previous study has shown that JEV infection induced ultrastructural changes in Vero cells with microtubule rearrangement and redistribution (Chiou et al., 2003). This phenomenon could facilitate the transport of viral proteins from the RER and Golgi apparatus to the convoluted membrane (CM), which may serve as a reservoir for viral proteins during JEV multiplication. Furthermore, as tubulin exhibits the functions of a chaperone (Manna et al., 2001), overexpression of its cellular component may also help viral proteins to maintain their conformation in the CM. Recently, α tubulin has been shown to be up-regulated in WNV-infected cells and in peripheral blood mononuclear cells of patients with DF and DHF (Pastorino et al., 2009; Thayan et al., 2009).

Table 3
Principal host factors involved in flavivirus cell infection.

Table 3.1 Cytoskeleton networks and cell trafficking

Host proteins	Function	Viral function	Reference
MCT4 Plasma membrane transporter of monocarboxylic acids	Transporter	Delay transition into replication	Krishnan et al. (2008)
Nuclear receptor binding	ER-Golgi trafficking	Virus-induced membranes	Chua et al. (2004)
α and β-tubulin	Cytoskeleton networks	Virus trafficking	Pastorino et al. (2009), Thayan et al. (2009)
Importin a/b	Intracellular transporters	RdRp nuclear import	Pryor et al. (2007)
Vimentin	Cytoskeleton networks	Virus trafficking	Pastorino et al. (2009)
Annexin A2/I	Cytoskeleton remodeling	Virus entry; egress and secretion?	Pastorino et al. (2009)
TSG 101	Multivesicular body biogenesis	Virus-induced convoluted membranes	Chiou et al. (2003)

Table 3.2 Stress response, protein modification and degradation

Host proteins	Function	Viral function	Reference
TIA-1 and TIAR	Stress granules assembly	Stress granules inhibition	Emara and Brinton (2007)
Hsp90	Protein homeostasis	Viral replication?	Pastorino et al. (2009)
Hsp70	Protein homeostasis	Viral replication enhancement?	Chavez-Salinas et al. (2008), Padwad et al. (2009)
CBLL1	Ubiquitin ligase	Endocytosis	Krishnan et al. (2008)
Ubc9	SUMOylation	?	Chiu et al. (2007)
Furin	Convertase	Virus maturation	Stadler et al. (1997)

Table 3.3 RNA processing machinery and host translation pathway

Host proteins	Function	Viral function	Reference
Nucleolin	Synthesis and maturation of ribosomes	Virus replication?	Pastorino et al. (2009)
YB-1	Translation, transcription, mRNA stability	Inhibit viral translation; antiviral response?	Paranjape and Harris (2007)
Polypyrimidine-tract-binding protein (PTB)	Splicing regulator, mRNA stability	Virus replication	Anwar et al. (2009)
Poly(A)-binding protein (PABP)	mRNA translation	Virus translation?	Polacek et al. (2009)
La autoantigen (La)	RNA metabolism	Virus replication	Vashist et al. (2009)
EF-1a	Translation elongation factor	Minus-strand RNA synthesis	Blackwell and Brinton (1997)
eEF1A/eEF-2/eIF3	Protein synthesis	Translation of viral RNA	Pastorino et al. (2009), Davis et al. (2007), Pattanakitsakul et al. (2007)
EF-Tu	Protein synthesis	Translation of viral RNA	Pattanakitsakul et al. (2007)
Nucleolar phosphoprotein B23	Transcriptional regulation Histone chaperone activity	JEV C protein nuclear import	Tsuda et al. (2006)
DEAD-box RNA helicase DDX42	RNA helicase; antiviral response	Prevent virus-induced-antagonism of IFN response	Lin et al. (2008)
hnRNP C1/C2/L/U	mRNA biogenesis, RNA binding	?	Noisakran et al. (2008), Pastorino et al. (2009)

Table 3.4. Apoptosis

Host proteins	Function	Viral function	Reference
GAPDH	Glycolysis/transcription/apoptosis	Virus replication?	Yang et al. (2009)
PDCD8/AIF	Apoptosis	Antiviral response?	Liew and Chow (2006), Pastorino et al. (2009)
gp96	Unfolded protein response and apoptosis	Antiviral response?	Liao et al. (1997), Pastorino et al. (2009)
TRAF1	Apoptosis	Antiviral response?	Koh and Ng (2005), Pastorino et al. (2009)
Caspase-1	Apoptosis	Antiviral response	Nasirudeen and Liu (2009)
ROS/RNS	Apoptosis	Antiviral response?	Yen et al. (2008)

Table 3.5. Signal transduction, cell signaling and receptors

Host Proteins	Function	Viral function	Reference
Heparan sulfates	Cell growth factor and differentiation;extracellular matrix	Attachment factor	Chen et al. (1997)
Regulator of G-protein signaling	Cell signaling	?	Pastorino et al. (2009)
Retinol dehydrogenase 10	Cell cycle	?	Pattanakitsakul et al. (2007)
c-Src Tyrosine kinase:	Signal transduction	Budding of the nucleocapsid into the ER lumen	Chu and Yang (2007)
CLEC5A	Inflammatory response	Binding; signaling	Chen et al. (2008)
c-Yes	Signal transduction	Virus trafficking through the secretory pathway	Hirsch et al. (2005)
DC-SIGN (CD209) L-SIGN (CD209L)	Cell adhesion; antigen presentation	Attachment factor	Tassaneetrithep et al. (2003), Davis et al. (2006)
Mannose receptor	Antigen internalization	Binding	Miller et al. (2008)
avb3 integrin	Cell adhesion	Internalization; signaling?	Chu and Ng (2004)
Jab1	COP9 signalosome	Virus degradation; nuclear export	Oh et al. (2006)

Table 3 (*Continued*)

Table 3.6. Mevalonate and complement pathways, pH homeostasis process

Host proteins	Function	Viral function	Reference
3-hydroxy-methylglutaryl-CoA reductase	Cholesterol biosynthesis	Virus-induced membranes	Mackenzie et al. (2007)
Vacuolar ATPase	Acidification of intracellular organelles	Virus entry; egress and secretion	Duan et al. (2008)
Factor H	Complement activation	Prevent complement dependent lysis of infected cells	Chung et al. (2006)
Clusterin	Inhibitor of the terminal pathway of the complement system	Plasma leakage?	Kurosu et al. (2007)
Interferon-inducible transmembrane proteins (IFITM)	Immune cell signaling	Virus entry	Brass et al. (2009)

Vimentin is another important host cytoskeleton protein. Rearrangements of vimentin, often with the formation of a cage structure, could represent a protective response by the cell during virus infection (Sodeik, 2002). Indeed, the vimentin cage may have a cytoprotective role by preventing the diffusion of viral components into the cytoplasm. Vimentin has been shown to be down-regulated in WNV-infected cells but further elucidation is needed to determine whether or not the flaviviral NS2B-NS3 protease can cleave vimentin, resulting in strongly decreased levels and the collapse of the vimentin network (Pastorino et al., 2009). On the other hand, vimentin is also implicated in the regulation of cell death via its caspase mediated cleavage (Byun et al., 2001; Ivaska et al., 2007) and the observed down-regulation of the protein in WNV-infected Vero cells could also be related to the induction of apoptosis (Pastorino et al., 2009).

5.2. Stress response, protein modification and degradation (Table 3.2)

The 70 kDa heat shock protein (Hsp70) was found to be highly expressed while the 90 kDa heat shock protein (Hsp90) was down-regulated in WNV-infected Vero cells (Pastorino et al., 2009). Eukaryotic Hsp70s are highly abundant cytosolic and nuclear molecular chaperones that play essential roles in various aspects of protein homeostasis (Morimoto et al., 1994). Moreover, the antiviral activity of some drugs has been associated with the induction of the specific Hsp70 (Baroni et al., 2007; Endo et al., 2007). Additionally, it has been shown that Hsp70 was able to prevent cytotoxic effects induced by WNV capsid protein, suggesting a protective cell function for this molecular chaperone against WNV infection (Oh and Song, 2006). For DENV, a role of Hsps in the positive modulation of viral replication in monocytic cells has also been suggested (Chavez-Salinas et al., 2008; Padwad et al., 2009).

Hsp90 involvement in viral replication has been reported for many other viruses and it has been demonstrated that its inhibition blocks viral replication (Hung et al., 2002). Recently, a role for Hsp90 in the control of hepatitis C, flock house and influenza virus polymerase function has been shown (Kampmueller and Miller, 2005; Momose et al., 2002; Nakagawa et al., 2007; Okamoto et al., 2006). Thus, it was proposed that Hsp90 is a major host factor with crucial importance for viral replication of many RNA viruses (Connor et al., 2007). The chaperone Hsp90 has also been identified as an essential factor in the folding and maturation of picornavirus capsid proteins (Geller et al., 2007) and its regulation has been observed after Vero cell WNV infection (Pastorino et al., 2009). The importance of the role of Hsp90 in the replication of a variety of viruses opens up interesting possibilities for developing new antiviral therapies that do not induce selection of drug-resistant viruses (Solit and Chiosis, 2008).

Cyclophilins (CyPs) are cellular peptidyl-prolyl isomerases (PPIases) which catalyze the isomerization of peptide bonds from *trans* to *cis* form at proline residues and facilitate protein folding. It has been shown that host CyP plays a role in flavivirus replication with the identification of Cyclosporine (Cs) as an inhibitor of flavivirus replication in cell culture. In fact, it seems that Cs directly blocks the interaction between cellular CyPA and WNV NS5 protein suggesting that CyPs represent a potential target for flavivirus antiviral development (Qing et al., 2009).

It has been shown that inhibition of cellular ER glycosidase caused by castanospermine directly blocked the secretion and infectivity of DENV particles in BHK cells. This antiviral effect was in part due to misfolding of structural glycoproteins. It was also shown to be effective *in vivo* by improving survival rates in infected mice (Courageot et al., 2000; Whitby et al., 2005).

5.3. RNA processing machinery, host translation pathway and base metabolism (Table 3.3)

Human hnRNP C1/C2 proteins are involved in mRNA biogenesis and contain important conserved motifs essential for RNA binding, protein–protein interaction and nuclear localization. Several RNPs, hnRNP Q, A1, A2/B, H and YB-1 have been found to bind specifically to the DENV 3′-UTR, suggesting that these molecules may play a biologically significant role in the DENV life cycle (Paranjape and Harris, 2007). Moreover, YB-1 is directly involved in repression of DENV translation and can participate in early innate immune responses during DENV infection. A specific association has also been demonstrated between HnRNP C1/C2 and dengue virus NS1 proteins which may be favorable for virus survival in host cells (Noisakran et al., 2008). HnRNP L and hnRNP U were also identified as being significantly up-regulated in WNV-infected Vero cells, suggesting that these two specific host cellular proteins play a key role in WNV replication (Pastorino et al., 2009).

Other cellular proteins interacting with the 5′- and/or 3′-UTR of flaviviral RNA have been identified highlighting the role of host proteins in the regulation of viral RNA synthesis and translation (Paranjape and Harris, 2010). Among them, the polypyrimidine tract-binding protein (PTB) has been reported to bind to the untranslated region of the DENV RNA leading to a modulation of the viral replication (Anwar et al., 2009). Human La autoantigen (La) have also been shown to interact with both 5′- and 3′-UTR of DENV and JEV genomes with a role in the viral RNA synthesis in cultured cells (Vashist et al., 2009). Recent work has demonstrated that Poly(A)-binding protein (PABP) binds the non-polyadenylated 3′ end of DENV RNA and this cellular factor could enhance the viral translation (Polacek et al., 2009).

Another important regulating protein, nucleolin, was found to have increased abundance in WNV-infected cells (Pastorino et al., 2009). Nucleolin is one of the most abundant nonribosomal proteins in the nucleolus, and it shuttles between nucleoli, nucleoplasm, cytoplasm, and the cell surface (Bugler et al., 1982). Previous studies have reported that nucleolin plays a role in virus replication via direct interaction with the hepatitis C viral RNA-dependent RNA polymerase or overexpression in herpes simplex virus infected

cells (Calle et al., 2008; Hirano et al., 2003). Based on these data, up-regulation of nucleolin in WNV-infected Vero cells suggests that the host protein plays an important part in flavivirus biology, making it a possible target for future drug development.

It is reported that flaviviruses do not shut-off host protein synthesis and must compete with the cellular translation machinery for limiting factors (Emara and Brinton, 2007). In this context, the eukaryotic translation elongation factors 1A (eEF1A) have been identified as up-regulated proteins or important host cell factors for viral RNA replication during DENV or WNV infection (Davis et al., 2007; Pattanakitsakul et al., 2007). An increase in the eukaryotic translation elongation factor 2 (eEF-2) level was observed in WNV-infected Vero cells suggesting that this protein may also play an important role in the translation of WNV RNA. Since flaviviruses prevent the shutoff of host cell translation, the decrease in eukaryotic translation initiation factor 3 (eIF3) was not expected in WNV-infected Vero cells. However, eIF3f has been identified as a regulator of the translation of virus-induced genes at late stages of the virus infection cycle (Xiao et al., 2008).

Mycophenolic acid (MPA) is a nucleoside triphosphate inhibitor reported as having anti-WNV and anti-DENV-2 activity (Takhampunya et al., 2006; Ghosh and Basu, 2008). MPA inhibited DENV-2 replication in monkey kidney (LLC-MK2) cells leading to an increase in defective viral RNA production and a dramatic reduction of intracellular viral replicase activity. Guanosine reversed the inhibition of MPA, suggesting that one mode of antiviral action of MPA is by inhibition of inosine monophosphate dehydrogenase (IMPDH) and thereby depletion of the intracellular GTP pool.

Brequinar is another antiviral agent shown to inhibit YFV and DENV in *in vitro* studies (Ray and Shi, 2006). This drug acts as an inhibitor of cellular dihydroorotate dehydrogenase, an enzyme that catalyzes the fourth step in the *de novo* biosynthesis of pyrimidine. However, *in vivo* efficacy of the compounds remains to be determined.

Some nucleoside triphosphate inhibitors reported as having anti-flavivirus activity include compounds like pyrazofurin. These types of molecule inhibit oritidine monophosphate decarboxylase (OMPDC) involved in the synthesis of GTP, UTP, and TTP and demonstrate effective anti-WNV activity in Vero cells.

5.4. Apoptosis (Table 3.4)

The programmed cell death eight protein or apoptosis-inducing factor (PDCD8/AIF) is a flavoprotein localized in the mitochondrial intermembrane and its translocation to the nucleus induces chromosome condensation and fragmentation (Yu et al., 2002). Up-regulation of this protein in WNV-infected Vero cells reflects the induction of virus-mediated apoptosis (Pastorino et al., 2009).

It is likely that flavivirus infection triggers perturbation of ER-homeostasis and also the unfolded protein response (UPR), as demonstrated by substantial induction of the expression of several chaperones (Su et al., 2002). During ER stress, tumor rejection antigen, also known as gp96, is one of the important molecular chaperones involved in UPR and apoptosis (Bando et al., 2004; Lu et al., 2007). A decrease in protein levels of gp96 in WNV-infected Vero cells has been observed (Pastorino et al., 2009). As already described for JEV infection (Liao et al., 1997), this phenomenon accelerates ER stress-induced apoptosis and may represent a host defense mechanism to limit viral replication.

Transcriptional-profiling analysis of WNV-infected cells demonstrated upregulation of selected apoptosis-related genes, including the tumor necrosis factor receptor-associated factor TRAF1, although the physiologic relevance of these observations is unclear (Koh and Ng, 2005). Recently, it has been demonstrated that gene silencing of caspase-1 or inhibition of caspase-1 activity led to reduction in DENV-induced apoptosis with minimal effect on virus replication (Nasirudeen and Liu, 2009).

Another study demonstrated that DENV and TNF together induce endothelial cell apoptosis through the production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Yen et al., 2008). The role of ROS in antiviral defense is thought to be mainly mediated by apoptosis (Skulachev, 1998).

5.5. Signal transduction, cell signaling and receptors (Table 3.5)

The src family kinase (SFK) c-Yes has also been identified as a cellular protein involved in WNV assembly and egress (Hirsch et al., 2005). WNV-infected cell lines treated with the SFK inhibitor PP2 demonstrated a decrease in viral titers related to inhibition of virion transit through the secretory pathway. These results identify c-Yes as a potential novel cellular target for therapeutic intervention.

5.6. Mevalonate and complement pathways, pH homeostasis process (Table 3.6)

Genetic and pharmacological modulation of cholesterol biosynthesis have been shown to regulate dengue and JEV virus replication *in vitro* (Rothwell et al., 2009; Lee et al., 2008). In fact, cholesterol mainly affected the early step of the flavivirus life cycle. Its presence during viral adsorption significantly blocked JEV and DENV-2 infectivity suggesting that flavirial entry, probably at fusion and RNA uncoating steps, was hindered by cholesterol.

In the same way, the interferon-inducible transmembrane proteins (IFITM), which represent about 30 cellular membrane proteins, have been shown to inhibit the early replication of WNV and DENV-2 by preventing the viral endocytosis or fusion steps (Brass et al., 2009).

Because flavivirus NS1 is a secreted glycoprotein that binds to cell surfaces after secretion in a soluble form and thus accumulates in serum, it has been speculated to have an immune evasive function. This hypothesis was confirmed when Chung et al. (2006) showed that NS1 protein inhibits complement activation both in solution and on cell surfaces. The authors demonstrated that soluble and cell-surface-associated NS1 protein binds to and recruits the complement regulatory protein factor H, resulting in decreased complement activation in solution and attenuated deposition of C3 fragments and C5b–9 membrane attack complexes on cell surfaces. More molecular understanding of the interaction between WNV NS1 protein and factor H would facilitate the development of inhibitors that block immune evasion.

By screening a human liver cDNA library, a vacuolar ATPase (V-ATPase) was also identified as a novel interacting partner of DENV prM protein. This association seemed to be critical for efficient virus egression and represented another promising avenue to follow in the development of new antiflaviviral drugs (Duan et al., 2008).

6. Conclusion

Flaviviruses are arthropod-borne viruses (arboviruses) and one can argue that appropriate measures to control relevant arthropods could effectively eradicate flaviviruses and other pathogenic arboviruses. Indeed, this strategy has been demonstrated to reduce disease incidence due to YFV in many regions of Latin America. However, such control strategies are not feasible on a worldwide scale, particularly in countries where the health infrastructure is insufficiently developed. Moreover, despite the demonstrated success of the YF 17D vaccine to control infections due to YFV, this virus has a natural reservoir in the forests of Africa and South America, thus, if these forests survive it will never be eradicated. In terms of vaccine development, DENV presents several significant problems, the most important being that this virus exists as four serotypes, each of which will require a serotype specific vaccine if the disease

is to be controlled. Also, the DENV are known to circulate in the sylvatic environment, rarely encountering humans. One of the other major problems in the context of DENV is the delivery of the vaccine to the humans that are most likely to be exposed. This would be a major undertaking, and it remains to be seen if it will prove possible in areas where dengue epidemics arise frequently. Clearly, therefore, there is a case to be made for the development of effective antiviral therapies that can be administered directly in the face of an epidemic.

Future antimicrobial therapies may combine conventional targeting of microbial virulence factors (Clatworthy et al., 2007) with host directed drug therapy including the enhancement of protective host factors (Cayrol et al., 2008; Krieg, 2006; Pulendran, 2004, 2005; Rothfuchs et al., 2007; Schwegmann et al., 2007; Yadav and Schorey, 2006). Following this argument, the identification of host susceptibility and resistance factors is crucial to provide a comprehensive molecular portrait of flavivirus–human cell interactions. In a recent study approximately 300 host proteins were identified that are involved in WNV infection demonstrating the recruitment of a wide variety of molecules and cellular pathways for successful infection as well as both overlapping and unique interaction strategies between flaviviruses with host cells (Krishnan et al., 2008).

The development of innovative technologies for drug targeting exploits high-throughput screening, resulting in accelerated identification of hits, greater sensitivity, increased application, and identification of more targets. The next phase in the search for novel antimicrobial drugs may involve the integration of epigenetic studies with host directed drug targeting strategies. As illustrated in this review, the understanding of flavivirus–host cell interactions has advanced greatly in recent years with the identification of key host factors in regulatory networks that are important for pathogen survival and can be targeted. Further comparative studies of virus–host interactions, on medically important flaviviruses will improve our knowledge of the crucial mechanisms underlying disease pathogenesis and increase the discovery of potential antiviral cell targets.

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