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Virus-host protein-protein interactions as molecular drug targets for arboviral infections

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Arboviruses have the potential to spread quickly and cause a global health emergency. These are RNA viruses that use RNA-dependent RNA polymerase (RdRp) for their replication. RdRp lacks proofreading activity, leading to high error rates, low replicative fidelity, and more genetic variability. In addition, shorter generation time and faster evolutionary rate of these viruses lead to re-emergence and recurrence of arboviral infections due to the emergence of new variants and the development of antiviral resistance. During the replication inside the host cell through protein-protein interactions (PPIs), these viruses interact with several host factors and utilize the host cellular machinery for their benefit. Besides this, viruses employ several transmission strategies to combat host innate and adaptive immune responses by manipulating the signaling and metabolic pathways of the hosts. Hence, antiviral therapies targeting host-virus PPIs can provide an alternative broad-spectrum strategy against RNA viruses. The approach of targeting virus-specific proteins for developing antivirals is expected to solve the problem of antiviral drug resistance and combat emerging new variants of these viruses. This review focuses on host-virus PPIs of arboviral infections that directly affect the host immune signaling and metabolic pathways. Better understanding of these mechanisms will develop new therapeutic tools to treat viral infections.

KEYWORDS

arboviruses, protein protein interaction (PPI), host factors, broad spectrum antivirals, host immune response

Introduction

Various viral pathogens, including arboviruses, are re-emerging and causing worldwide infections in humans and animals (1). In 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its various variants emerged and caused an acute respiratory disease pandemic called 'Coronavirus disease 2019' (COVID-19). This

pandemic has threatened human health and public safety (2). Most emerging and re-emerging viruses appear to be caused by a change in virus spread due to deforestation, environmental change, microbial adaptation/change, commerce, technology, ecological changes, agricultural development, failure of public health measures, and lack of public health infrastructure (2).

Arboviruses are a diverse group of arthropod-borne viruses that spread from infected to susceptible hosts by arthropod vectors such as mosquitoes, sandflies, ticks, or biting midges (1). Besides insect bites, viruses can also be transmitted through blood transfusion, pregnancy, and childbirth from mother to child (3, 4). Flaviviridae, Togaviridae, Bunyaviridae, and Reoviridae are the prominent families of economically important arboviruses that cause human infections (5–7).

Most infections caused by arboviruses are asymptomatic. Nevertheless, these viruses can show symptoms ranging from a mild flu-like illness to encephalitis, i.e., brain inflammation. Furthermore, the symptoms and clinical characteristics are distributed mainly into two subgroups such as neuroinvasive and non-neuroinvasive (8). Neuroinvasive arboviruses frequently cause meningitis or encephalitis. In addition, neuroinvasive arboviruses symptoms include the rapid onset of fever along with muscle pain, stiff neck, headache, weakness in the arms and legs, confusion or disorientation, and seizures. On the other hand, non-neuroinvasive arboviruses do not affect the nervous system, but they may cause fever, headache, joint pain, muscle aches, vomiting, diarrhea, upset stomach, nausea, and rash (8). To date, no specific treatment is commercially available against arboviruses.

The core of any viral infection is that protein-protein interactions (PPIs) are significant for the biological system (9, 10). Virus-host PPIs play a vital role in running the host's metabolic and signaling pathways and also help activate the host immune system to counter the viral infection. Detailed knowledge of interacting proteins is essential for understanding viral diseases and discovering potential drugs (11).

Therapeutic intervention against infectious viral diseases using small molecule inhibitors either directly acts on viral proteins or modulates the host immune system (12). Few potential host protein targets also act as receptors for the virus,

facilitating viral attachment and entry inside the host cell (13–15). Moreover, host metabolic processes such as glycosylation, autophagy, actin polymerization, fatty acid biosynthesis, and host-mediated proteolytic cleavage are necessary for viral survival making them potential drug targets (13–16). Therapeutic strategies targeting both host and virus commonly aim to suppress viral infection to prevent the disease. This review focuses on host-virus PPIs of arboviral infections that directly affect host immune signaling and metabolic pathways.

General introduction about economically important arboviruses

Arboviruses are maintained in nature through biological transmission between susceptible vertebrate hosts and blood-sucking arthropods. These viruses consist variety of genome types such as positive single-stranded RNA (+ssRNA), negative-sense single-stranded RNA (-ssRNA) and double-stranded RNA (dsRNA), etc. Various families such as Flaviviridae, Togaviridae, Bunyaviridae, and Reoviridae are some crucial families of arboviruses (5–7) (Table 1).

Flaviviridae

Flaviviridae family includes spherical enveloped +ssRNA viruses. *Flavivirus* genus incorporates Dengue virus (DENV), Murray Valley encephalitis virus (MVEV), Japanese encephalitis virus (JEV), Saint Louis encephalitis virus (SLE), West Nile virus (WNV), Zika virus (ZIKV), Tick-borne encephalitis virus (TBEV), Yellow fever virus (YFV), Kyasanur Forest disease virus (KFDV), etc. (7). Phylogenetic analysis of the sequence alignment, generated for individual coding regions and the whole genome sequences of flavivirus species, revealed that the species belonging to the same vector group usually show more similarity than the species of different vector groups (18–20).

Most flaviviral infections remain asymptomatic, but mild to severe symptoms may occur in a few cases. Generally, mild flaviviral infection show symptoms such as fever, conjunctivitis,

TABLE 1 Virus families containing economically important arboviruses.

Family	Morphology and Genome	Virus examples	Vector	Ref.
Flaviviridae	Spherical enveloped +ssRNA	DENV, MVEV, JEV, SLE, WNV, ZIKV, TBEV, YFV, KFDV	Mosquitoes, Ticks	(7)
Togaviridae		CHIKV, EEEV, RRV, VEEV, WEEV, etc		(7)
Bunyavirals order (Formarly known Bunyaviridae family)	Spherical enveloped -ssRNA	RFTV, CCHFV		(5)
Reoviridae	Spherical non enveloped dsRNA	CFTV, IBDV	Ticks	(5, 17)

rash, malaise or headache, and muscle and joint pain. A safe, affordable, and effective vaccine can prevent only a handful of flaviviral infections caused by YFV, JEV, and KFDV (21–23). Overall, no specific antiviral drug treatment or efficacious licensed vaccines are available for flaviviral infection (24–27)

Dengue fever is an ancient disease caused by DENV. *Aedes aegypti* mosquitoes transmit dengue viruses that infect an estimated 400 million globally every year (28, 29). DENV has 4 serotypes, and the severe infection of DENV causes life-threatening illnesses such as dengue shock syndrome and dengue hemorrhagic fever (23). On the other hand, JEV is transmitted by *Culex* species mosquitoes and maintained in a cycle between mosquitoes and vertebrate hosts such as pigs and wading birds. As humans are not primary hosts, they do not harbor high virus titer concentrations in their blood to further pass it on to mosquitoes (22). JEV is endemic in various counties of Asia and the Western Pacific Region. It is estimated that around 68,000 clinical cases per year expose 3 billion people to risk (30). For JEV infection, severe symptoms include sudden onset of high fever, headache disorientation, coma, neck stiffness, spastic paralysis, and seizures. Similarly, ZIKV is transmitted mainly by *Aedes* species mosquitoes. ZIKV infection during pregnancy can cause preterm birth and miscarriage (31). In addition, certain birth defects can occur, where infants born with microcephaly and other congenital malformations, also known as congenital Zika syndrome. Until now, around 86 countries and regions have reported evidence of mosquito-borne ZIKV infection, which can transmit to the additional areas where the *Aedes* species mosquitoes are found (31). Yellow fever is an acute viral hemorrhagic disease caused by YFV infection. It is transmitted by infected *Aedes* or *Haemagogus* mosquitoes. YFV is endemic in tropical areas of Africa and South America (32–34). Similarly, WNV, MVEV, and SLE, also causes various neurological disease and is transmitted by *Culex* species of mosquitoes. WNV, MVEV, and SLE were reported in multiple places worldwide, such as Africa, Europe, the Middle East, North America, and West Asia (35–37). TBEV spreads through the bite of an infected tick. In some cases, TBEV is transmitted through raw dairy product consumption from infected goats, sheep, or cows. It infects the central nervous system of the host. Every year approximately 10 to 12 thousand cases are reported from various regions stretching from Northern and Western Europe to Eastern and Northern Asia. Until now, no cure or vaccine against TBEV is available (38, 39). KFDV was first isolated in 1957 from the diseased monkey of Kyasanur Forest in Karnataka, India. KFDV is transmitted through tick bites and has caused hemorrhagic fever in some patients (26).

Togaviridae

This family consists of +ssRNA viruses. The *Alphavirus* genus incorporates numerous viruses such as Chikungunya virus (CHIKV), Eastern equine encephalitis virus (EEEV), Ross River virus (RRV), Venezuelan equine encephalitis virus (VEEV), Western equine encephalitis virus (WEEV), etc. (7). Among alphaviruses most of the protein coding region of the genome is highly conserved, with the variable regions occurring at the end of both protein coding and non coding sections of genome (40).

Chikungunya is a viral infection caused by the CHIKV and is transmitted to humans by infected *Aedes* mosquitoes. Most CHIKV infections remain asymptomatic, but in some cases, mild to severe symptoms such as fever, myalgia, headache, rash, and polyarthralgia can be seen (41). The disease was first reported in 1952 in Tanzania and is now endemic in various countries of Africa, and Asia, with some occasional outbreaks in Europe and America (41–43). Similarly, the Sindbis virus (SINV) causes Sindbis fever. This virus is similar to CHIKV and has caused a few outbreaks in South Africa and Northern Europe (44, 45). EEEV, RRV, VEEV, and WEEV are also some crucial viruses that have caused sporadic cases all around the world. Overall, no treatment or vaccine is available against these viruses (42, 46–49).

Bunyavirals

Bunyavirals, formerly known as the Bunyaviridae family, is an order which consists of various a family's such as Phenuiviridae, Nairoviridae, Hantaviridae, etc. These families incorporate the genome of three segments of -ssRNA. These viruses are enveloped, spherical RNA viruses (5). Bunyaviridae family consists of important arboviruses such as Rift Valley fever virus (RFTV) and Crimean-Congo he morrhagic fever virus (CCHFV). These viruses mainly infect animals, but hemorrhagic fever cases were also reported in humans. RFTV and CCHFV get transmitted *via* mosquitoes and ticks, respectively (5, 50, 51).

Reoviridae

The Reoviridae family consists of dsRNA viruses. It incorporates various viruses such as Rotavirus, Infectious bursal disease virus (IBDV), and Colorado tick fever virus (CFTV) (17). CTFV is a tick-borne rare viral disease from the western United States and Canada. The amino acid in different genera of reoviruses has a sequence identity of less than 30% in RNA-dependent RNA polymerase. Exceptions include Rotaviruses B with 22% identity to other rotaviruses, while *Aquareovirus* and *Orthoreovirus*, show an amino acid sequence identity of up to 42% (52, 53). CFTV has a relatively large genome as compared to other arboviruses. CFTV also has an icosahedral capsid structure. Further, as per literature, no vaccines or medicines are available against CFTV (6, 17).

Common viral targets and antiviral resistance

Ideal antiviral targets are those viral proteins that are essential for the completion of the viral life cycle. Additionally, it should have a lower rate of mutations, an important attribute in the case of RNA viruses like flaviviruses and alphaviruses. The most attractive molecular targets for the development of broad-spectrum antiviral compounds are viral protease or polymerase (54). In Addition to this, extensively studied antiviral targets also include viral surface proteins, which function as an anchor for the attachment and cell entry (55). Several studies are based on small molecule inhibitors targeting structural proteins such as envelop protein (E) of DENV, Spike protein (S) of SARS-CoV-2, and Capsid protein of CHIKV (11, 46–49, 56, 57). Other studies targeting various essential non-structural viral proteins (nsPs) such as non structural protein (nsP5) of DENV, RNA polymerase of ZIKV, Proteases of SARS-CoV-2, viral capping enzyme nsP1 of CHIKV, and two-component nsP2B-nsP3 protease of ZIKV, DENV, and WNV (28, 54–62).

Despite the enormous research, there is a dire need for antiviral therapies or broadly acting vaccines for the treatment of flaviviral and CHIKV infection (28, 54, 58). Only one vaccine is available against the DENV virus, i.e., Dengvaxia® licensed by the pharmaceutical company Sanofi Pasteur. This vaccine has limited use due to the high risk of severe dengue outbreak in vaccinated individuals. Antivirals used to treat viral infections generally act upon either viral or host proteins (61). The antivirals that directly act against the viral proteins are known as Direct-acting antivirals (DAA) (62) (Table 2). Using DAA against viral infection provides a promising approach, as DAA specifically acts upon viral protein and, therefore, generally shows low toxicity and a wide treatment window (72). For instance, Remdesivir, a nucleotide analog, acts upon RdRp of SARS-CoV-2 and inhibits viral replication (70, 71). Apart from that, Chloroquine is approved as an antimalarial drug and is a well-known DAA against CHIKV; it acts by inhibiting the endosomal acidification, thereby preventing the virus entry (64–66). Similarly, Arbidol is an antiviral drug and a potent inhibitor of cell entry and fusion for the influenza virus (67). It also acts in the early stage of CHIKV and blocks the virus attachment and entry (73). Some common bottlenecks of DAA are the high risk for the development of antiviral resistance, short window period of effectiveness, harmful side effects, and ineffectiveness against latent viruses (12, 42, 74). Mutations of the nsp5 protein of CHIKV cause resistance to favipiravir (T-107), a broadly acting antiviral (75). In flavivirus resistance to ciprofloxacin, is reported after seven passages with the drug (76). For CHIKV development of partial resistance is reported after fifteen passages against 4-OHT (4-Hydroxytamoxifin) (77). The RNA viruses have two mechanisms for drug resistance either through specific opposition to drug action, which results in decreased fitness in the absence of the drug, or a general increase in fitness, which is sometimes continued even after drug withdrawal (78–84). Antiviral therapy targeting multifunctional common nodes of interaction occurring between host and viral proteins have the potential to target a wide range of viral infection (12).

Host factors and their importance in the virus life cycle

Proteins or RNA encoded by host genes that either positively or negatively impact an intruding virus are called host factors. The

TABLE 2 List of DAA against viruses.

Active against	Antivirals	Mode of action	Ref.	
CHIKV	Chloroquine	The prevention of endocytosis and/or endosomal acidification	(64–66)	
	Pep-I, Pep-II compounds	Inhibits replication	(56, 57)	
	Picolinic acid (PCA)			
Influenza virus	Arbidol	A potent inhibitor of cell entry and fusion for the	(67)	
DENV	Balapiravir	Nucleotide analog acts upon RdRp	(68)	
	RK-0404678	Targets RdRp protein	(69)	
DENV-2, WNV	Compound 3b	NS3 protease inhibitors	(54)	
WNV, YFV, Vesicular stomatitis virus (VSV), DENV-2	Palmatine	Inhibition of NS2B-NS3 protease and viral propagation	(54)	
HCV	Glecaprevir, Pibrentasvir Sofosbuvir, Velpatasvir, Voxilaprevir	Anti NS5A/NS4A/3 protease	(55)	
SARS-CoV-2	Remdesivir	Nucleotide analog acts upon RdRp	(70, 71)	

host factor that ablates viral survival or dissemination is called restriction or antiviral factors. On the contrary proviral factors or dependency factors promote viral growth and enable viral infection (72, 85). Viruses are gene-poor relative to their host, as the viral genome encodes hundreds of genes, whereas host cells typically encode thousands of genes (74). During viral infection, several host factors play a critical role in every facet of the viral life cycle (86) (Table 3). For instance, viruses utilize various biomolecules present on the host cell surface as an anchor for their attachment and entry inside the host cell. Flaviviruses such as DENV, WNV, JEV, YFV, and ZIKV binds through viral envelop protein (E) via heparan sulphate (HS), dendritic cell-intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), heat-shock proteins (HSPs), and high-affinity laminin receptors, phosphatidylserine receptors (80, 81). In flavivirus, viral genome replication occurs in the host endoplasmic reticulum (ER) and uses ER-associated ribosome for translation (102). Also, in WNV, DENV, and ZIKV, many viral non-structural proteins interact with different host proteins of the reticulon (RTN) family, atlastin (ATL) family, and several transmembranes for ER remodeling and vesicle formation (103–107). Alphaviruses such as CHIKV, EEEV, RRV, VEEV, and WEEV utilize E1 and E2 with HS and DC-SIGN receptors to gain access to the surface and enter inside the host cells (82, 83). Similarly, the virus controls the eukaryotic initiation factors 4F (eIF4F) complex of a host, thereby suppressing the expression of the host gene, ensuring the uninterrupted synthesis of viral proteins in the host cell, and evasion of the host immune system (84) occurs in the endoplasmic reticulum and requires host viral RNA is initiated by nsP1 of the virus to guide the eukaryotic initiation factors 4E (eIF4E) for the identification and initiating translation and shielding the mRNA from cellular exonucleases (108–111). Another example of viral hijacking is the manipulation of host protein complexes to either delay or accelerate the host cell cycle

to promote the replication of the viral genome (112, 113). In DENV, host cellular pathways such as autophagy, actin polymerization, and fatty acid biosynthesis are required for viral replication (114). Additionally, the formation and assembly of the mature infectious viral particle occurred in the endoplasmic reticulum and required the host glycoprotein-processing pathway (115).

Protein-protein interactions in the replication cycle of arboviruses

PPIs serve as the foundation of important cellular processes. During viral infections, the structural and functional characteristics of various signaling, metabolic and regulatory pathways of the host are controlled by host-virus PPIs (116). PPIs research has slowly and considerably evolved with technological and conceptual growth in diverse research areas over the past centuries (116). The basic level of PPIs investigation is crucially important because of their involvement in cellular processes like metabolism, signalling and their concerned diseases. Therefore, molecular interaction studies help in establishing molecular-level strategies to ascertain valuable therapeutics (117). Progression of *in-vivo* and *in-vitro* studies includes co-purification of protein complexes, TAP (tandem affinity purification), pull-down assays, proteomics, and Y2H (yeast 2 hybrid) system, etc., which are vital for identifying PPIs. Recent advancements in *in silico* methods like sequence and structure-based approaches, chromosome proximity, gene fusion, *in silico* 2 hybrid, phylogenetic tree, phylogenetic profile prediction, etc. makes it easy to predict and know about PPI-based multifaceted diseases and apply the knowledge towards the cure of these diseases (11). Viruses are sub-microscopic obligate intracellular microbes. They depend

TABLE 3 List of host targeting drugs against different arbovirus.

Virus	Cellular target	Tested host-targeted inhibitors	Ref.
DENV, WNV	Fatty acid synthases	C75,Cerulenin	(87, 88)
	HMG CoA reductase	Lovastatin	(89–91)
	Cholesterol transport	Methyl b-cyclo Dextrin	(90)
	IMP dehydrogenase	ETAR, Mycophenolic acid, Ribavirin, ribavirin triphosphate,	(92–94)
	Proteasome	MG-132, ALLN	(95)
DENV	Cholesterol transport	U18666A	(96)
	Ionophore	Narasin	(97)
WNV	Cholesterol transport	25-hydroxycholesterol	(90, 96)
	OMP decarboxylase	6-Azauridine	(93)
JEV	Ubiquitin Proteasome system	Curcumin	(98)
CHIKV	Fatty acid synthesis and calmodulin signalling	Pimozide, Orlistat and TOFA	(99)
	MAPK Signalling pathway	Berberine	(100)
	Host stress- pathway chaperone (HSP90)	Geldanamycin	(101)

on host cells for their life cycle, which can be divided into three stages containing several steps, Entry (attachment, entry, uncoating), replication (gene expression and replication), and budding (assembly and release). At every step, viruses interact and manipulate host cellular machinery for its replication (118) (Table 4).

Virus entry

Virus attachment to target cells relies upon the interaction between viral surface proteins and cell membrane proteins. Flaviviruses structural protein consists of three proteins designated C (capsid), prM (precursor of membrane), and E (envelope). E protein consists of 3 homodimers and has an essential role in viral attachment, entry, and membrane fusion (139–141). Further, each E monomer is divided into 3 ectodomains (ED) EDI, EDII, and EDIII. These ectodomains are connected *via* flexible linkers. Further, two transmembrane (TM) domains, TM1 and TM2, has a crucial role in fusion (142, 143). HS is a type of glycosaminoglycan (GAG) that play a role of epithelial cell receptor. Several research articles have shown that

flaviviruses interact with host cells by oppositely charged residue interactions between E and GAG (80, 119, 120). Furthermore, in DENV and WNV, DC-SIGN assists the virus in entry into Dendritic cells (15, 123). In addition to this, Fc receptors are also important in antibody dependant enhanced infection of monocytes with DENV (121). Above and beyond, various HSPs, high-affinity laminin receptors, phosphatidylserine receptors, etc., are also important in numerous *Flavivirus* entry mechanisms (13, 81, 122).

Alphaviruses are composed of five structural proteins, namely C, E3, E2, 6K, and E. E1 is a pH-dependent fusion protein, and E2 is a vital protein essential for receptor recognition. Hence, both are crucial proteins for viral attachment and entry into host cells (82). Alphaviruses utilize several cell surface molecules, including HS, DC-SIGN receptors, etc., to carry out an initial attachment to target cells (108–111). Many alphaviruses are internalized *via* clathrin-mediated endocytosis. Later, the clathrin-coated pits are transported inside the cell leading to the dissociation of clathrin molecules and virus delivery to endosomes (83). To mediate the fusion of the viral membrane with the endosomal membrane, at the low-pH environment of the endosomes, E1/E2 glycoproteins undergo conformational changes (83).

TABLE 4 Virus-Host PPIs found in the virus life cycle.

Life cycle stage	Virus	Virus-Host PPI	Function	Ref.
Entry	Flaviviruses	E with HS, Dc-SIGN, Heat-shock proteins, High-affinity laminin receptors, Phosphatidylserine receptors, etc	Viral attachment, entry, and membrane fusion	(13, 15, 80, 81, 119–123)
	Alphaviruses	E1 and E2 with HS, DC-SIGN receptors, etc		(108–111)
Gene expression and replication	ZIKV, DENV	NS4A with SEC62, SEC61γ, and SRPR, NS4A/2B with SEC61β, and NS4B with SEC61α	Translation and stability	(105, 124)
		NS4B with EMC 1 to 4		(125, 126)
		NS1, NS2B, NS3, and NS4B with OST complex	Structural role in ER genome replication	(127, 128)
	Flaviviruses (WNV, ZIKV, JEV, DENV, and YFV)	C-prM, prM-E, E-NS1, and 2 K-NS4B with SPCS1	Polyprotein cleavage	(129)
	WNV	NS4A with RTN family proteins	ER remodeling	(130)
	ZIKV	NS4A with LNP protein with TMEM41B		(105)
		NS4A and NS2B with RTN family proteins		(105, 131)
		NS2A, NS2B3, NS4A, and NS4B with ATL family proteins		(103)
	WNV	NS4B with ATL family proteins		(106)
	TBEV	NS4B with LNP protein		(107)
	ZIKV, YFV	NS4A and NS4B with TMEM41B		(124)
	DENV	NS2B, NS3, and NS5 with ATL family proteins		(104)
		NS4A with vimentin	Anchoring of replication compartments in the ER	(132)
		DENV NS1 with host RACK1	Unknown	(127)
	CHIKV	nsP3 with G3BP	Regulating cellular stress response	(133)
		nsP1 with endosomal and lysosomal membranes	nsP1-Mediated replication complex anchoring	(134–136)
Assembly and release	JEV	E with BST2	Lysosomal degradation	(137)
	CHIKV	nsP1 with BST-2	Poorly Known	(138)

Gene expression and replication: -

In flaviviruses, several host pathways play an important role in the translation of the flaviviral genome into its functional proteins. All viruses initially need host ribosomes to translate its gene into viral polyproteins. However, *Flavivirus* replication remains confined to the ER and utilizes ER-associated ribosomes for its translation (102). Furthermore, numerous host proteases cleave polyproteins into singular proteins. These NS proteins (NS2A, NS2B, NS4A, and NS4B) comprise various TM domains which need to be properly inserted into the ER membrane to be functional (144–148). The signal-recognition particle (SRP), host SEC61 translocon, and ER membrane complex (EMC) were demonstrated to be crucial host factors for the stability and insertion of these proteins in flaviviruses. Additionally, signal peptidase complex (SPCS) and oligosaccharyltransferase (OST) complex were seen to be indispensable for the replication of several flaviviruses (128, 129, 149–151). The flaviviral NS protein also recruits and takes over the functions of other host protein families, including ATL, RTN, and the Lunapark (LNP) protein, Transmembrane Protein 41B (TMEM41B), and Vacuole membrane protein 1 (VMP1), Vimentin, Receptor for Activated C Kinase 1 (RACK1) protein, etc (127, 130, 152–154).

ZIKV and DENV have shown several PPIs with a host, such as NS4A with SEC62, SEC61γ, and SRPR, NS4A/2B with SEC61β, and NS4B with SEC61α (105, 124). Similarly, both the viruses have demonstrated PPI between NS4B and EMC family proteins (125, 126). Furthermore, SPS1 has shown polyprotein cleavage activity for C-prM, prM-E, E-NS1, and 2 K-NS4B (82). OST complex proteins which have an important role in ER genome replication, show PPI with NS1, NS2B, NS3, and NS4B of ZIKV and DENV (127, 128). In WNV-host PPI, NS4A interacted with RTN family proteins for ER remodeling. On the other hand, for ZIKV-host, NS4A and NS2B interacted with RTN family proteins (105, 130, 131). Additionally, various ATL, LNP, and TMEM41B family proteins have shown PPI with flavivirus NS proteins for ER remodeling (103–106, 124). More on this, DENV NS4A interacts with vimentin, which plays an important role in the anchoring of replication compartments in the ER (153). In addition, DENV NS1 interacts with host RACK1, although its function is unknown (127).

Alphaviral RNA replication is host-dependent and occurs in cytoplasmic vacuoles derived from endosomal and lysosomal membranes (134–136). Alphaviruses comprise four non-structural proteins (nsPs), specifically nsP1, nsP2, nsP3, and nsP4, which are indispensable in the life cycle of the viruses. The nsP1-mediates anchoring of replication complex on these membranes (132). The nsP1 also causes the capping of viral RNA to assist eukaryotic translational initiation factor 4E (eIF4E) in identification and initiating translation and shielding the mRNA from cellular exonucleases (155). Yet, nsP3's precise role in replication complexes is less clear; it interacts with Ras-GAP SH3 domain-binding protein (G3BP), regulating cellular stress response (133).

Assembly and release

Flavivirus assembly occurs in the ER. Then, the virion is further transferred for maturation and furin cleavage in the trans-Golgi-network (TGN), and at the end, the virus is released out of the cell (156). In the virion maturation process, prM protein on the outermost part gets cleaved by host proteases within the Golgi apparatus. An acidic environment in Golgi and secretory vesicles makes the cleavage site accessible (157–159). Bone marrow stromal cell antigen 2 (BST2), also called tetherin, restricts the replication of various viruses. In the case of JEV, BST2-E protein interactions lead to its lysosomal degradation, promoting virus replication (137).

Alphaviral subgenomic RNA is translated to produce structural proteins in the cytoplasm (160). Further, E3-E2-6K-E1/E2-E2-TK translocated into ER for post-translational modification of E2/E1 (160). Later, these proteins get mature through TGN and are deposited on the plasma membrane. Lastly, icosahedral nucleocapsids are formed by the interaction between capsid and genomic RNA, subsequently releasing mature progeny virions (160). Alphaviruses are also dependent on host machinery for the transfer of their E protein to the plasma membrane (161). nsP1 mediated down-regulation of BST-2 expression overcomes the BST-2 facilitated CHIKV and SFV virus release (138).

Host immune response in arbovirus infections

Host immune response plays a critical role during primary infection of arboviruses and the carrier (162). The first line of defense mechanism against any arbovirus infection is Skin. The skin possesses some specialized cells that provide safety against arthropod bite and the relevant agent (163). The Skin has three structural layers, including the uppermost epidermis, lowermost dermis, and the connecting air interface (164). Cells responsible for immunity like T and B cells, dermal macrophages, and dendritic cells exist in the epidermis, while the hypodermis has lesser numbers of immune cells in healthy skin (165). At the time of viral infection, host epidermal cells release cytokines and antimicrobial peptides; however, the inflammation and wounding process is continued by innate and adaptive immune cells (21, 166). Simultaneously neutrophils, monocytes, and other peripheral immune cells secret chemokines that help in the reduction of viral load (163). Additionally, the lymph nodes, the macrophages, and

dendritic cells introduce antigens to B and T cells of adaptive immunity (163, 167). The B and T cells target the infected cells and produce virus-specific antibodies (165, 166). Innate and adaptive immunity work together to improve the healing from infection and tenderness at the bite site (168). Skin immune responses protect the host from arboviral infections, but evolution in arboviruses enables them to hijack protection mechanisms (169). Epidermal cells of the skin are prone to mosquito-borne DENV and WNV infection (170). Whereas the Langerhans cells (LCs) of the epidermis are more prone to infection by CHIKV, DENV, WNV, and TBEV (171). The migratory nature of LCs makes them move from epidermis to dermis and reach lymph nodes. It increases the viral load to the rest of the body parts and directly contributes to the viral-born infection of naive ticks feeding on infected cells (172). Salivary Gland Protein (SGP) secretion also plays a role in suppressing Skin Host Immune Responses (173). Even with evolutionary branching between mosquitoes and ticks, the SGP in both is functionally conserved concerning their interaction with the mammalian immune system (174). The SGP of ticks and mosquitoes suppresses T cell proliferation and inhibits and alters the release of key inflammatory cytokines at the time of viral infection (175). The progression of cytokine production during infection recruits monocytes from the blood. Monocytes inhibit viral propagation to PNS (Peripheral Nervous System) and CNS (Central Nervous System) (172, 176, 177).

The arboviral infection (SINV and WNV) induces a membrane attack complex (MAC), which activates the complement system that initiates the lysis of viral particles/

virus-infected cells (178). The activation of the complement system also helps in producing antibodies that lead to the clearance of viral load (179). DENV and ZIKV mainly target monocytes and decreases vascular permeability through epithelial cell damage (180–183). Therefore, arboviruses assist their access to the remaining hosts by hijacking their dermal immune system, which is crucial for antiviral defense (184).

In the case of arthropods, the immune system protects the host from viral infection. Likewise, mosquitoes adopt an innate immune pathway (JAK-STAT and TOLL receptor) against viral infection (185) (Figure 1). Viral infection initiates a defense system by the formation of a complex consisting of transcription factors, protein kinases, and several regulatory molecules to keep balance on downstream gene expression (186). The regulatory genes are responsible for handling antimicrobial peptides (AMPs) and the effect of immune response on viruses. Mosquitoe express cytokine receptors and tyrosine kinase and together these two are responsible for further phosphorylation and release of transcription activator (STAT) (187). Self-phosphorylation of tyrosine kinase (JAK) leads to phosphorylation of cytosolic STAT (188). PIAS is a negative regulator of the Janus kinase/signal transducers and activators of the transcription (JAK-STAT) pathway (189) (Figure 1). STAT self-dimerize and translocate to the nucleus of the infected cell, which further activates effector genes and makes the immune system functional (190). The Toll pathway is well known for the production of innate immunity against pathogens (191). This immune path is initiated by the degradation of a cytokine ligand (Spätzle or Spz) that can bind to a transmembrane receptor toll-
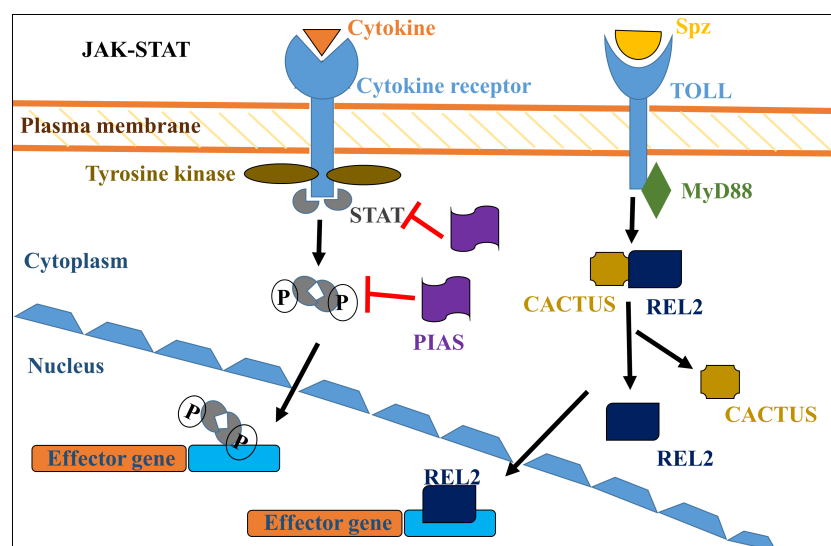

FIGURE 1
JAK–STAT and Toll-signaling pathway responsible for the activation of the immune system in mosquitoes. Activation of the signaling pathway initiates the formation of a multiprotein complex consisting of protein kinases, transcription factors, and the regulatory molecules to control the downstream genes that initiate and stimulate the immune system.

like receptor (Toll) (192). Activation of Toll pass on its signal and further activates MyD88 (adaptor proteins associated with Toll) (Figure 1) (193). CACTUS is a negative regulator of the Toll pathway (194). It gets phosphorylated and degraded (by proteasome) and leads to translocation of the transcription factor Relish 1 (Rel1) from the cytoplasm to the nucleus of the infected cell (195) (Figure 1).

The parallel evolution of the viral infection strategies with the host immune system is responsible for viral evasion from host immune cells (196). Viral translational machinery is inhibited by interferon (IFN) as host antiviral responses (197), but due to evolution in viral infection strategies, IFN induction is hampered by the virus (198). The capability of many viral proteins for immune evasion makes them a potential target for antiviral therapies (9). The nsP2 of alphaviruses has a virulence feature mainly responsible for inhibiting transcription and translation in infected host cells (199). Likewise, non-strucrural proteins of the Flaviviridae family also help in immune escape for the viruses (200). WNV nsP1 acts *via* the Toll-like receptor 3 (TLR3) pathway, and DENV nsP2 based STimulator of INterferon Genes (STING) cleavage results in a decrease in Interferon β (IFNβ) level (201). It leads to the inhibition of antiviral molecules by viral proteins at ease. DENV nsP4 inhibits IFN-mediated phosphorylation of signal transducer and activator of transcription (STAT) (202). Therefore, viruses can malfunction the antiviral activity. Understanding Immune system evasion provides target protein for the development of potent antiviral drug molecules in the future (10).

Role of host-virus protein-protein interactions in the signaling pathway

Arboviruses have a unique mode of life cycle as they require hosts (mostly humans, small mammals, and birds) for their replication and arthropods (commonly mosquitoes) as a vector for their transmission (203, 204). Similar to other viruses, arboviruses also induce host cell infection *via* cellular responses like unfolded protein response (UPR), apoptosis,

and autophagy (205). In prolonged stress conditions, UPR and autophagy are both directly connected to programmed cell death (apoptosis) by Bcl2 family proteins and pro-apoptotic proteins like CHOP and Beclin-1 to maintain cellular homeostasis (205).

Unfolded protein response

Various kinds of cell stress like heat shock/UPR and programmed cell death play a crucial role in protecting other cells in different physiological conditions (206) (Table 5). However, stress response like autophagy is principally involved in the denaturation and recycling of proteins and cell organelles (211). The host cell endoplasmic reticulum (ER) is mainly responsible for the proper folding and maturation of proteins (205). During cell stress conditions, UPR stimulates ER membrane stress transducer, leading to activation of stress sensors like inositol-requiring enzyme I (IRE1), protein kinase RNA-activated-like ER kinase (PERK), and transcription factor6 (ATF6) (207). The mechanism of UPR regulation is quite different in similar viruses, such as flaviviruses that encourage the host protein UPR response, whereas WNV activates UPRs transcription and translation pathway (212–214). There are three pathways for UPR response. Among these three UPR pathways, proteasomal degradation of ATF and the PERK pathway play an essential role in the phosphorylation of eIF2α and induce the pro-apoptotic protein CHOP (215). This protein eventually leads to CHOP-dependent cell death to inhibit WNV infection (216). The ATF6 signaling pathway is critically responsible for innate immune response inhibition, which also helps in cell survival (217). The other arboviruses like SINV, CHIKV, and DENV also control the host's UPR system through different mechanisms (205). SINV induces premature cell death due to less control on UPR by inhibition of ER chaperones, increased eIF2α phosphorylation, and subsequently activation of CHOP protein (218). CHIKV suppresses eIF2α phosphorylation and PERK pathway and activates the IRE1-ATF6 cascades, whereas DENV triggers XBP1 signaling and enhances cellular apoptosis, leading to virus-induced cytotoxicity (171).

TABLE 5 Arboviruses and UPR pathway.

Viruses	UPR Pathway	Ref.
WNV	Activates UPR *via* ATF6/IRE1 pathways	(207)
	Activation of CHOP-dependent cell death	(208)
SINV	Failure of ER chaperones synthesis	(151)
	Increased phosphorylation of eIF2α and activation of CHOP	(151)
CHIKV	Activates the ATF6 and IRE1 cascades	(151)
	Suppresses the PERK pathway	(151)
	Suppresses the eIF2α phosphorylation	(151)
DENV	Activates XBP1 pathway	(209)
	Enhanced cellular apoptosis	(210)

Apoptosis

Programmed cell death is termed apoptosis (219). Apoptosis regulates virus pathogenesis and mortality in viral infection (220). Apoptosis plays a vital role in the activation and initiation of the immune system, which is essential for arbovirus replication, infection, and pathogenesis (216, 221) (Table 6). In general, viral infection can use intrinsic and extrinsic apoptotic pathways. Flaviviruses include; DENV and WNV, which can delay apoptosis by activating several cell survival pathways (132). CHIKV uses external factors/ligands like tumor necrosis factor (TNF) as an extrinsic signal to activate caspases-8, which further triggers the activation of caspases-3, -6, and -7 (227). WNV and DENV both follow the intrinsic pathway of apoptosis *via* stimulation of P53 (228). The activation of P53 facilitates mitochondria-dependent apoptosis of cells (133). Viral infection initiates apoptosis by inducing dsRNA-dependent protein kinases (PKR) as it regulates eIF2a and activates effector caspases (229).

The apoptosis process induced by SINV is vital for its pathogenicity (230). A double-stranded RNA intermediate is formed into the host cell at the time of SINV entry. PKR helps in the identification of invaded viral particles (231). PKR kinase protein phosphorylates eukaryotic translation initiation factor 2A (eIF2A) to block cellular translation and inhibition of Mcl-1 (Bcl2 family anti-apoptotic protein) biosynthesis (232). PKR-mediated insulin receptor substrates (IRS1) phosphorylation of c-Jun N-terminal kinases (JNK) activates 14-3-3 (phosphorylation-dependent regulatory/effector proteins), which provides substrate access to kinases (Bad) (233). This process induces the interruption of the complex between anti-apoptotic proteins from the Bcl2 family, B-cell lymphoma-extra-large (Bcl-xl), Bak, and the release of Bad (234). Bax and Bak (pro-apoptotic proteins) are members of the Bcl-2 family and core regulators of the intrinsic pathway of apoptosis (234). Further, Bak replaces Bad and Bik (pro-apoptotic protein) from Mcl-1. The displacement of Bad by Bak oligomerization and release of cytochrome c induces apoptosis (235).

Apoptosis is essential for CHIKV replication as the virus elicits the apoptosis process to escape from the immune system of the host and can infect neighboring cells (236). CHIKV infection triggers apoptosis in two different manners: intrinsically, by helping in replication along with caspase-9 activation, and extrinsically, by activation of caspase-8 (mediators of apoptosis) (171). CHIKV uses external factors/ligands like tumor necrosis factor (TNF) as an extrinsic signal to activate caspases-8, which further triggers the activation of caspases-3, -6, and -7 (227). Eventually, both actuate caspase-3 and finally lead to cell death and expediting virus discharge and its spread (232). The histopathological studies of DENV-2 infection in the liver of mice provided an insight into that activation of the mitogen-activated protein kinase (MAPK) pathway blocks phosphatidylinositol 3 kinase (PI3K), which is an intracellular regulatory kinase (226). MAPK cascades are key signaling pathways that regulate a wide variety of cellular processes. Therefore, no impact was seen on DENV virus particle production. DENV infection also induces a cytopathic effect that triggers the process of apoptosis in infected cells (203). Similar to SINV, WNV also follows the analogous mechanism to uphold their pathogenesis using apoptosis (204). These viruses trigger signaling for cell survival through the PI3K-AKT signaling pathway (221). The PI3K-Akt pathway is an intracellular signaling pathway that promotes cell growth, angiogenesis, cell metabolism, proliferation, and cell survival, in response to extracellular signals (71). Anti-apoptotic activities have also been seen in some viruses, such as; DENV and WNV (207, 217). The regulation of cell cycle machinery by apoptosis and related signaling pathways are important for the determination of disease and identification of potential drug molecules for future arboviral infections (212).

Autophagy

Autophagy is a process of eliminating damaged cells and facilitating the regeneration of new and healthy cells (237). In

TABLE 6 Arboviruses and apoptosis pathway.

Viruses	Apoptotic Pathway	Ref.
WNV	Akt phosphorylates Bad (during viral replication)	(222)
SINV	Induces virus pathogenesis and mortality	(204)
	Expression of Reaper pro-apoptotic gene in Mosquitoes	(223)
	Activates PKR pathway	(224)
CHIKV	Triggers apoptosis	(171)
	Uses both intrinsic and extrinsic apoptosis pathways	(171)
	Facilitates virus release and spread	(171)
DENV	Blocks PI3K pathway and induce apoptosis	(225)
	Cytopathic effects of DENV-2 infection lead to cell death	(226)

arbovirus infection, autophagy is an antiviral immunological cellular response that activates innate immune response with the assistance of pattern recognition receptor (PRR) based interferon production (238) (Table 7). It also shifted towards adaptive immunity when the virus delivers the antigen T-lymphocytes (212). Some studies on WNV infection showed that the replication of WNV is independent of autophagy; however, autophagy was induced (214). It is reported that autophagy can directly influence pro-viral mechanisms such as; entry, replication of the virus, lipid metabolism, and inhibiting innate immune responses, leading to cell death (213). In the case of DENV-2 infection, amphisomes (fusion vacuoles) play a vital role in the survival of the virus in host cells, while DENV-3 shows interaction with amphisomes as well as autophagolysosomes (202). In both fusion vacuoles, viral translation/replication occurs because these vacuoles are composed of viral RNA and proteins (243). CHIKV infection also facilitates autophagosome formation at the aggregation site of viral replication/transcription complex that helps in viral entry (171). Autophagy regulates the degradation of cytosolic triglycerides during lipid metabolism, and these triglycerides accumulate in the form of lipid droplets (244). At the time of DENV infection, the virus also uses these lipid droplets as an energy source for replication (114). Lysosomes convert lipid droplets to free fatty acids (FFAs) from triglycerides (114). β-oxidation of FFAs takes place in mitochondria, and viruses utilizes the released energy for viral replication (114). Therefore, the formation of amphisome is supposed to be advantageous for the virus to host cell access and reproduction (245). The autophagosome is also important for the replication of some viruses (216). Viruses follow the autophagy path to break lipid droplets into free fatty acids, and the generated energy is utilized as a source of energy/ATP for viral replication (246). Therefore, viruses use autophagy to disrupt the host immune response and eventually improve the replication rate and enhance their survival (247, 248).

Role of host-virus PPIs in the metabolic pathway

Understanding arbovirus infection and developing innovative ideas for their control is very important to develop

effective therapeutics against them. We are aware that viruses lack metabolic enzymes and their machinery. They are dependent on the host for their reproduction and survival (249). Here, polyamines are essential in their life cycle from viral infection to viral replication (250, 251). Polyamines (putrescine, spermidine, and spermine) are positive, small amine units found in mammalian cells. They are helpful in virus survival and indulged in the immune system response of different organisms (250). Metabolism-related studies are essential to detect alterations in host physiology during infection. Earlier studies demonstrate that the evolutionarily conserved pathways, including glucose, fatty acid, protein, and nucleotide metabolism, are mainly manipulated by viral infection to increase their replication (252–254). Targeting metabolic and signaling pathways provides the central understanding of the host-virus interactions and further helps in biochemical research and therapeutics to treat viral infections (255). Togaviruses and flaviviruses depend on the β-oxidation of fatty acids or glycolysis process for energy production as they need it for their multiplication (256, 257). In contrast, DENV upregulates β-oxidation to fulfill energy requirements for other anabolic processes (173, 258). The formation of fatty acids and glycerophospholipids from acetyl-CoA as a byproduct of the β-oxidation process induces membrane architecture for viral replication (258, 259). Membrane-bound vesicles play an essential role in cell entry, replication, assembly, and exit of the virus (260). After arbovirus infection, the level of glycerophospholipids increases in mosquito cells and human host cells because their level is vital for viral replication and virus genome protection (260, 261). A decrease in the level of fatty acid formation leads to a decrease in DENV and WNV formation (114).

Regulation of host metabolism is essential for the survival and replication of arboviruses. Studies explain that host lipid metabolism plays a crucial role in the viral life cycle (262). Likewise, manipulating glucose metabolism using the intracellular Phosphatidylinositol 3-kinase (P13K) or AMP-activated protein kinase (AMPK) signaling pathway controls virus survival and replication (263). Virus replication is also activated in host cells through a proto-oncogene (Myc), sterol regulatory-element binding proteins (SREBPs), and the hypoxia-

TABLE 7   Arboviruses and autophagy.

Viruses	Autophagy Pathway	Ref.
WNV	Upregulated during viral replication	(239, 240)
ZIKV	AXL (phosphatidylserine receptor) is ZIKV entry receptor	(241)
	Enhances Cellular autophagy in permissive cells	(241)
CHIKV	Up-regulation of viral replication in infected cells	(242)
	Increases autophagosome formation as a site for aggregation of viral translation/replication complexes	(215)
	Autophagy postpones apoptosis	(215)
	Promotes CHIKV propagation by inducing the IRE1α–XBP-1 pathway in conjunction with ROS-mediated mTOR inhibition	(236)
DENV	Cell-specific (Monocytes)	(146)
	Up-regulating PI3K-dependent autophagy	(203)

inducible factor 1-alpha (HIF-1α) ubiquitination signaling pathway (264). Inhibition of defined signaling pathways was found responsible for viral replication inhibition *in vitro* and *in vivo* (265). Detailed understanding of the immune responses, signaling pathways, host factors, and different phases of the viral life cycle are prerequisites for providing molecular insights for improving broad-spectrum antiviral therapy against repetitive viral infections (266).

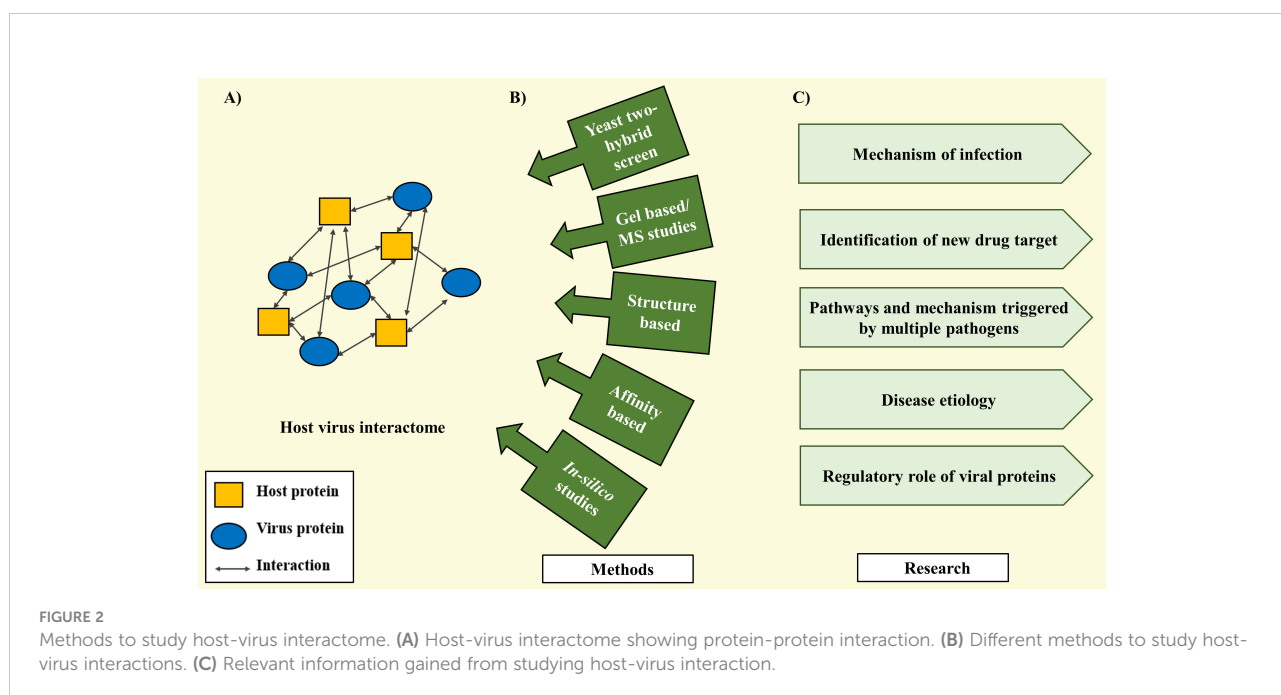## Methods to identify host protein interaction:

Affinity purification

In this technique, the target protein is immobilized on the resin, and then cell lysate/extract is passed through the column, and the binding of proteins present in cell lysate to the target protein is detected by mass spectrometry (MS) by comparing the profile of protein retained in column with the target to the protein retained in the negative control sample (267). MS is used to determine the molecular weight of proteins by calculating the mass to-charge ratio (m/z) (268). There are three components of the mass spectrometer, an ion source, an ion detection system, and a mass analyzer. The overall process has three main steps for the analysis of proteins a) Ionization of proteins and transformation to gas-phase ions, b) separation of ions in the analyzer compartment based on m/z values, and c) finally separated ions were detected, and m/z values were measured for each species (268, 269). A combined proteomic study based

on affinity purification-mass spectrometry (AP-MS) and RNAi screening to study virus-host interactions of DENV and ZIKV with humans and mosquitoes revealed important interactions (Figure 2) (105).

2D-polyacrylamide gel electrophoresis & (MS)

The effect of the viral infection on host cells can be understood by conducting various proteomic studies (Figure 2). A comparative 2D polyacrylamide gel electrophoresis is a study of whole cell lysate taken before and after infection and identification of detected protein through mass spectrometric studies. These studies can provide the degree of changes in the expression pattern of host proteins upon viral infection and also shed light on the regulatory role played by the viral protein. For instance, if the level of specific host proteins increases upon infection, this shows viral infection contributes to the enhanced production of mRNA, increased translation, and enhanced stability for certain proteins (270). Proteome-based studies coupled with mass spectroscopic studies were used for the detection of viral components inducing changes in host cellular proteome and identification of host-virus interactions (267). A study using 2D-polyacrylamide gel electrophoresis and mass spectrometry led to the identification of a total of 14 host proteins that were binding with the core protein of Hepatitis C virus (HCV) (271). Using a 2-dimensional electrophoretic approach followed by Liquid Chromatography-Mass Spectroscopy LC-MS/MS identified 15 differentially

FIGURE 2
Methods to study host-virus interactome. **(A)** Host-virus interactome showing protein-protein interaction. **(B)** Different methods to study host-virus interactions. **(C)** Relevant information gained from studying host-virus interaction.

phosphorylated proteins present in host signaling pathways involved in secondary infection of DENV. Further, in this study, pyruvate kinase M2 (PKM2) was validated as a potential target, and it was shown that inhibitors of PKM2 were able to decrease infection and viral load (272).

GST pull-down assay

Pull-down assays are a common approach in which one of the interacting partners, i.e., purified protein, acts as bait and uses the second partner, i.e., cellular lysate or purified protein, as the prey (Figure 2). Among all the GST pulldowns in which bait proteins are expressed as a GST- tagged chimeric protein are very common. This is a low-cost, easy-to-use method for analysis of protein-protein interaction, in which one can adjust the concentrations of purified proteins for better sensitivity and can use different concentrations for the estimation of binding (273). The major drawbacks of this technique are Cloning, expression, and purification of interacting protein partners with GST tag is time-consuming, and the binding of GST tag can cause changes in the overall folding of proteins. The expression of proteins with GST tags can cause the accumulation of inclusion bodies which hinders the purification of active proteins. Moreover, the isolation of protein from their natural environment can lead to improper folding and eventually affects the interaction with other protein (274).

GST pull-down assay is successfully used for the analysis of the interactome of HIV-1 Tat protein and Hepatitis B virus HBx protein; for this, the researcher identified a protein involved in the metabolism of lipid and cholesterol known as apolipoprotein A-I (apoA-I) as a strong binder (248). The researcher further showed that increasing the apoA-I protein by transfection imparts a protective effect on mice infected with HBV, and this suggests apoA-I can be used as a possible target for the development of anti-HBV; therapeutics (275). For the Chikungunya virus multifunctional domain AUD (Alphavirus unique domain) of nsP3 protein is critical for the life cycle (276). Utilizing yeast two-hybrid system of human proteins interacting with CHIKV AUD was identified SNAP associated protein (SNAPIN)) and NEDD4-binding protein 2-like 1 (N4BP2L2). Further, their interaction was validated using GST pull-down assay, SNAPIN is involved in intracellular vesicle trafficking linked with autophagy, and N4BP2L2 has kinase activity. Still, its role in viral infection is unknown (277).

Yeast two-hybrid screen

This method is useful for screening protein libraries to find the binding of multiple proteins to a single protein of interest (278) (Figure 2). After the identification of Protein-Protein

interaction, this assay can be further employed to map the interface of interacting proteins by making a set of chimeric bait and prey combinations from the fragments of interacting proteins (274). Shortcomings of this method are cloning of bait and prey is time-consuming; also, the transcription factor domain fusion to protein (bait) may induce structural changes that can alter the binding interface. Colocalized binding of bait and prey protein in the nucleus is necessary for the expression of the reporter gene, and this forced colocalization leads to the occurrence of false binding events (279). The yeast-two hybrid method is only suitable for soluble protein, and the interaction involving transmembrane or membrane protein is difficult to screen using this method. Analysis of the interactome of membrane-bound proteins is done using the split ubiquitin membrane yeast two-hybrid (MYTH or MbYTH) method. In this, one chimeric protein binds to N- the terminal region of a ubiquitin moiety (Nub), and another chimeric protein binds with the C-terminal region (Cub) linked to a reporter protein. The binding of two proteins will result in the joining of Nub and Cub regions and the formation of complete ubiquitin moiety that can bind with ubiquitin-specific proteases and will cleave the reporter protein from the protein-protein complexes that are interacting and producing positive results (258, 280). Proteome mapping of Flavivirus NS3 and NS5 proteins using a yeast two-hybrid screen identified 108 human proteins that were interacting either with nsP3 or nsP5 or with both proteins (278). Another study identified human proteins SNAPIN and N4BP2L2 interacting with the nsP3 protein of CHIKV (277).

Structure-based proteomics

Structural information provides knowledge about the biological function and physiological role of proteins. Structural proteomics studies are based on the 3D structure determination using cryo-EM, X-ray, or NMR and high throughput characterization of protein complexes (281) (Figure 2). Structural studies are important to interpret the multifunctional nature of proteins involved in viral infection and to understand how different protein interacts to form active complexes (282). Structural studies of DENV proteins revealed valuable information about conformational changes occurring in E protein and the unique fold present in prM protein (158, 283, 284). Another structural analysis of WNV and Dengue NS3 protease clarifies the mechanism of NS3pro activation and identifies the critical residue for substrate binding (285). Using structural proteomics approaches, thousands of protein structures have been determined and submitted to the protein data bank (PDB) (286). Determining the structure of viral and host protein-protein complexes are attractive targets as their structure reveals essential information relating to the molecular description of the interface and key residue of interaction (287).

Computational study of virus-host interactions

Computational prediction methods and strategies to map the protein-protein interaction are attractive, cost-effective, less laborious, and comparatively fast alternatives to experimental methods. Also, computational methods can be utilized to map the pairwise associations of proteins into a complete network relating to their different functional levels (288). The first published computational study was on 190 pathogen strains to identify the host-pathogen interaction, the data generated from experiments and databases had integrated to identify 10,477 human-pathogen interactions (289).

Large-scale computational studies were conducted on five viruses Influenza virus (H1N1), Human immunodeficiency virus-1 (HIV), Epstein-Barr virus (EBV), Human papillomavirus (HPV), and Hepatitis C virus (HCV) by integrating available knowledge about human – virus PPIs and human complexes, led to fundamental analysis and identification of complexes targeted by viruses (VTCs) (288). During the host cell cycle, a dynamic expression study for the subunits of host protein complexes targeted by viruses shows the manipulative tendency of viruses toward host protein complexes viruses (288). They also developed a web portal called VTcomplex (http://zzdlab.com/vtcomplex/index.php) which contains significant information about the virally-targeted human protein complexes in the form of text and graphics for easy understanding and a brief overview of complexes (288).

Database for virus-host interactions

Detailed knowledge of the cellular interactions occurring between virus and host proteins is needed to understand the physiological changes and consequences of viral infection in the host. To make the information regarding PPIs readily accessible,

researchers have curated the information in publically available databases (Table 8). Virus molecular interactions (VirusMINT) database collects virus-host (human) protein-protein interaction data from the available literature and integrates this data in an interaction network of proteins. VirusMINT presently has a collection of more than 5000 interactions with over 490 interactions exclusive for viral protein from different viral strains (297). Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a specific database curated for PPIs of virus-virus and virus-host. The interactions involved are either physical (direct) or functional (indirect) associations. The sources of these interactions are genomic context predictions, high-throughput lab experiments, (conserved) co-expression, automated text mining, and previous knowledge. STRING currently has around 9,643,763 proteins from 2,031 organisms and a total of 1,380,838,440 interactions (298, 299). VirHostNet is a knowledge-based bioinformatics database with integrated information for virus-host, virus-virus, and host-host PPIs. This information system is committed to the biocuration of data from original literature, the integration of data, and the visualization of Virus/Host protein-protein interactions Networks based on graph theory (248).

Broad-spectrum antivirals targeting the host-virus protein-protein interaction

The trademark for RNA viruses is a heavy dependence on the host cell machinery for the replication of the viral genome, shorter generation time, and fast evolutionary time (300, 301). RNA viruses have high mutation rates, approximately 6 times higher than their cellular host (301). Spontaneous mutation per site per generation for DENV 2.64×10^{-5}, E. coli 2×10^{-10}, and yeast 3.3×10^{-10} (302–304). The rising number of viral

TABLE 8   Description of different databases used to study protein-protein interaction.

Databases	Description	/URLs	Ref.
Database of Interacting Proteins	Database catalogues experimentally determined interactions between proteins	https://dip.doe-mbi.ucla.edu/dip/Main.cgi	(290)
BioGRID	Biomedical interaction repository	https://thebiogrid.org	(291)
IntAct Molecular Interaction Database	Database for system and analysis tools for molecular interaction data	https://www.ebi.ac.uk/intact/home	(292)
Biomolecular Interaction Network Database (BIND)	Archives biomolecular interaction, reaction, complex and pathway information	http://bind.ca	(293)
HPIDB 3.0	Helps annotate, predict and display host-pathogen interactions (HPI)	https://hpidb.igbb.msstate.edu/index.html	(294)
DenHunt	Comprehensive and integrated database solely dedicated to dengue-human molecular interactions	http://proline.biochem.iisc.ernet.in/DenHunt	(295)
VTcomplex	Virally-targeted human protein complexes and the associated information.	http://zzdlab.com/vtcomplex/index.php	(288)
mPPI	Protein-protein interaction (PPI) databases for PPI structural visualization	http://bis.zju.edu.cn/mppi	(296)

emergence and onset over the last few years created the demand for novel, widely acting antivirals to reduce the burden of these infections on health and commerce. It is well-known that viruses exploit the host's translational assembly and protein folding machinery (149, 305). Developing antiviral compounds targeting pathways in host cells that facilitate viral replication is an appealing method of antiviral drug discovery, particularly with novel viruses. This approach would provide an attractive alternative for developing broadly acting antivirals with a lower risk of antiviral resistance (306) (Table 9).

Screening for antiviral through an image-based phenotypic technique and large-scale target identification led to the discovery of broad-spectrum antiviral having inhibitory activity against different RNA viruses like SARS-CoV-2, CCHFV, and Ebola virus (EBOV), target host proteostasis pathways and interfere with interactions of host cellular HSP70 complex and viral proteins (311). Obatoclax, an antagonist of the B-cell lymphoma 2 (Bcl-2) family of regulator protein that regulate cell death, has reached clinical trials as an anticancer therapy. It is reported to be active against flaviviruses, alphaviruses, and influenza viruses. It acts upon endosomal acidification and thus prevents viral membrane fusion and entry (307). In another study, bioactive compounds and FDA-approved drugs were screened using a CHIKV replicon cell line that identified Abamectin, Ivermectin, and Berberine as a potential inhibitors of CHIKV replication. Moreover, these compounds were also active against other alphaviruses such as SINV and SFV, and Abamectin and Ivermectin were also active against YFV.

Further studies on berberine reported that it acts on the MAPK (mitogen-activated protein kinase) signaling pathway during CHIKV infection (308). HSP-90, host-stress pathway chaperone, has been identified as a crucial factor for viral replication (312). HSP-90 is reported to interact with nsP3 and nsP4 proteins of CHIKV to help in viral replication. Also, during CHIKV infection Akt pathway is activated through the interaction of HSP90 *via* phosphorylation of mTOR to

regulate the translation of viral mRNA I (101, 313). The inhibitors for HSP-90 have been well studied for anticancer therapy, and drugs acting against HSP 90 could decrease inflammation and viral titers upon CHIKV infection (313). The fusion of CHIKV and MAYV depends on the sphingolipid and cholesterol in the host cell membrane. Fatty acid synthase (FASN) and Stearoyl-CoA desaturase-1 (SCD1) were identified as conserved druggable host factors against Alphavirus. Inhibiting the enzyme Fatty acid synthase using cerulenin and orlistat and enzyme (SCD1) using CAY10566 decreased viral replication (99, 309). Moreover, orlistat also acts against other mosquito-transmitted viruses such as JEV, DENV, ZIKV, and CHIKV (310). An interesting study identified a small molecule inhibitor RK-0404678 of RdRp, i.e., NSP5 of dengue virus. The crystal structure revealed that RK-0404678 was able to bind at two different sites in the RdRp domains of DENV (69). The common structural features of these sites are cysteine residues. Moreover, these cysteine residues present in NS5 proteins are conserved across the DENV, JEV, WNV, and ZIKV, indicating that RK-0404678 could also bind to these flavivirus RdRps (69, 314, 315).

Conclusion

Arboviral diseases have a substantial impact on humans. The emergence of resistance towards antivirals is either due to the prolonged use or the error-prone replication cycle of RNA viruses. The use of therapeutic approaches targeting host factors interacting with viral proteins could be a promising alternative therapy. To perform replication, the arboviral genome requires host-virus PPI. Some of these PPIs seem to be broadly conserved among flaviviruses and alphaviruses. These viruses use various host proteins and physically interact with another cellular signaling, metabolomic pathways, and immune responses, which are critical to virus replication. Hence virus-host PPIs can be significant in the development

TABLE 9 Broad-spectrum antiviral acting against arboviruses.

Drug	Active against	Mode of action	Previous reports	Ref.
7-Deaza-2′-C-methyl adenosine (7DMA)	WNV, YFV, DENV-2,TBEV, and ZIKV	Acts as nucleoside inhibitors	Potent HCV inhibitor	(54)
Galidesivir (BCX4430)	YFV, ZIKV, and RVFV	Acts as an adenosine nucleoside analog	In clinical trials for Marburg and filovirus	(54)
Obatoclax	Alphaviruses and Flaviviruses	Inhibits endosomal acidification; prevents viral fusion	In clinical trials for Anticancer therapy	(307)
Abamectin and Ivermectin	CHIKV, SINV, SFV, and YFV	Inhibits viral RNA and protein accumulation, affecting replication.	Anti-parasitic agent	(308)
Orlistat	CHIKV, MAYV, JEV, DENV, ZIKV	Targets fatty acid synthesis	Anti-obesity drug	(99, 309, 310)
Berberine	CHIKV, SINV, SFV	Targets MAPK signaling pathway of host	Anti-diabetic drug	(100, 308)

of pathogenesis. The use of therapeutic approaches targeting host factors interacting with viral proteins could be a promising alternative therapy. PPI biology has great potential in identifying potential drug molecules to combat future viral infections.

Author contributions

MB, VS, PD, and ST reviewed the literature, critically analyzed it, and authored the article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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