Microbial Pathogenesis 69-70 (2014) 45–52

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Review

Role of intracellular events in the pathogenesis of dengue; An overview

Bhawana Jain [a,2], Umesh C. Chaturvedi [a,b,3], Amita Jain [a,*]

[a] Department of Microbiology, K.G. Medical University, Lucknow, India
[b] Indian Council of Medical Research, DHR, New Delhi, India[1]

ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form
17 March 2014
Accepted 20 March 2014
Available online 29 March 2014

Keywords:
Dengue
Autophagy
Stress granules
Processing bodies
Lipid droplets

ABSTRACT

Dengue is one of the most important mosquito-borne viral diseases that are relentlessly spreading in newer areas in the tropical and subtropical regions of the World. In last fifty years, in spite of intensive and extensive investigations, pathogenesis of dengue is still not clearly understood. Recently, the research focus is on studying the role of intracellular events in pathogenesis of viral infections. Entry of virion in the host cell is followed by quick succession of events, unfolded protein response, lipid bodies and lipophagy, endoplasmic reticulum stress and recent demonstration of autophagy. The turbulence caused by these events may result in clearance of the virus/enhanced replication and survival of the host cell/apoptosis. Both, increased virus load and apoptosis of host cell may have pathological effects on the host. In the present review, we have summed up the role of various intracellular events in viral infections with special emphasis on Dengue virus infection.

1. Introduction

Dengue virus (DENV) affects human of any age group, worldwide, including India [1]. According to a recent estimate, there are around 390 million (95% credible interval 284–528) dengue infections per year, of which 96 million (95% credible interval 67–136) manifest apparently [2]. Dengue viruses occur as four antigenically related but distinct serotypes, transmitted to humans by *Aedes aegypti* mosquitoes. These viruses generally cause either a benign syndrome; dengue fever (DF), or a severe capillary leakage syndrome; dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) [3]. According to WHO 2009 classification severe Dengue has been differentiated into three subcategories; severe vascular leakage, severe bleeding, and severe organ dysfunction [4]. The cardinal feature of DHF/DSS is increased vascular permeability without morphological damage to capillary endothelium. This results in extensive plasma leakage in various serous cavities of the host including pleural, pericardial and peritoneal cavities and tissue spaces in patients with DHF, who may go into profound shock (DSS) [3,5]. A number of mechanisms of pathogenesis of DHF/DSS have been discussed including, enhancing antibodies [6,7], T cell-mediated response [8] including regulatory T cells [9], various soluble mediators including a unique Cytotoxic Factor [10,11] and cytokines [8,12,13], immune complex disease, antibodies cross-reacting with vascular endothelium [3], complement and its products, selection of virulent strains, viral virulence and role of host genetics [14]. Among these the most plausible ones are the enhancing antibodies, shift from Th1 to Th2 cell response and the memory T cells in a secondary infection, resulting in a cytokine tsunami [5]. Extensive research has been done for more than fifty years in area of dengue pathogenesis; still, the precise mechanism of DHF/DSS is not well understood. In the last ten years or so, the research focus has shifted to intracellular events during viral infections including DENV that may be translated to understand the pathogenesis of severe disease. This review presents an overview of the role of intracellular events during viral infection with special reference to dengue infection.

2. DENV replication and intracellular events

Biologically diverse cell types, starting from insect cells to highly evolved mammalian cells like endothelium and hepatocytes can be infected by DENV. The first interaction of DENV with its host cell occurs via several putative receptors. They play an important role in

* Corresponding author. Tel.: +91 9415023928.
E-mail addresses: drbhawana_kgmu@hotmail.com (B. Jain), uchaturvedi201@gmail.com (U.C. Chaturvedi), amita602002@yahoo.co.in, amita60@gmail.com, amita602002@yahoo.com (A. Jain).
[1] UCC is a Scientific Consultant of Indian Council of Medical Research, New Delhi.
[2] Tel.: +91 9450391235.
[3] Tel.: +91 9450913506.

http://dx.doi.org/10.1016/j.micpath.2014.03.004
0882-4010/

capturing, concentrating and transmitting infectious virions, leading to a cascade of events that trigger the virus–cell membrane fusion [15,16]. A low pH-triggered conformational change of Envelope (E) protein in endosomes leads to virus entry into the host cell through endocytosis and uncoating. Capsid is released into the cell cytoplasm, where it dissociates and release viral genome. Genome is a single RNA molecule of positive polarity and contains single open reading frame (ORF), which is translated into a single large polyprotein. Polyprotein is targeted to the endoplasmic reticulum (ER), where it is processed by virus and host encoded proteases to form three structural proteins (Capsid protein C, a precursor for the membrane protein PreM and envelope protein E) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5), which help in replication, polyprotein processing and virion assembly [17]. The replication takes place in virus induced vesicular membrane structures associated with ER. Capsid protein covers the copies of genomes. Immature virions having preM and E proteins on the surface bud into ER lumen [18] and then transported through the trans-Golgi network where cleavage of pre/M occurs making the virions infectious and mature and is transported out of the cell by exocytosis [19].

DENV exists as four heterologous serotypes termed serotypes 1, 2, 3 and 4 (DENV 1–4). People once infected with one serotype of dengue virus are usually protected lifelong from subsequent infection with the same serotype (homotypic infection) [6]. DHF occurs mostly in persons infected with a second DENV serotype after an initial "primary" DENV infection with a different serotype. The antibodies from the previous infection bind to the virus and enhance its uptake by certain Fc receptor bearing monocytes/macrophages cells resulting in high levels of viremia. This phenomenon is known as antibody dependent enhancement (ADE) [6,7,20]. Secondary infections cause forty times more DHF cases than primary infections. There are many mechanisms suggested for DHF/DSS but nothing elaborates the pathogenesis precisely. Recent studies have reported important findings of intimate interaction between DENV and the host cell, which can be helpful in elucidating pathogenesis, like; a) autophagy; b) lipid droplets and Lipophagy; c) unfolded protein response; d) Stress Granules and Processing bodies. Further, strategies used by the virus to resist innate antiviral responses have been discovered. Various mechanisms with reference to DENV infection will be reviewed here.

2.1. Autophagy

Autophagy is an intracellular catabolic system which degrades cytoplasmic components within lysosomes. It has a specific role in elimination of micro-organisms, clearance of intracellular proteins and organelles, cell death, and antigen presentation etc. [21]. The process of autophagy is initiated when cell is subjected to pathogen infection. The signaling pathway; Phosphatidyl Inositol 3-kinase (PI3K) and Beclin 1, gets activated. Beclin 1 first gets separated from Beclin 2 which is a known anti apoptotic as well as anti autophagic protein, and then induces autophagy. Kovacs et al. have shown that inhibition of autophagy via Beclin 1 gene deletion in T cells results into extensive apoptosis of these cells upon T cell Receptor (TCR) stimulation [22]. Beclin 1-deficient animals do not mount autoreactive T-cell responses. This pathway leads to the formation of autophagosome, which is a key organelle of autophagy. Certain autophagy related proteins (Atg) help in autophagosome formation. Autophagosomes are formed in response to a number of stimuli, including host- and pathogen-derived molecules, including toll-like receptor ligands and cytokines. Autophagy can itself regulate the production and secretion of cytokines [23]. Autophagosome fuses with lysosomes where lysosomal hydrolase degrades the inner membrane of autophagosome and contain cytoplasm derived materials, known as autolysosomes or autophagolysosomes [24]. Bhattacharya and Eissa have reviewed the role of autophagy and autoimmunity and have concluded that autophagy, could modulate the induction or exacerbation of autoimmune processes [25].

2.1.1. role of autophagy in viral infection

Autophagy has been studied in a large number of viruses [26] (Tables 1 and 2). Viruses adopt different mechanisms to induce or inhibit autophagy. Autophagy induction takes place by following mechanisms i) by increase in autophagic flux, ii) by binding to the surface of host cells, iii) by other intracellular events e.g., Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR) (Table 1). Autophagy is inhibited (Table 1) by interfering with either autophagosome formation, maturation and degradation or by degrading autolysosomes.

Consequently autophagic induction/inhibition leads to either virus degradation or promotion of viral replication. Autophagy

Table 1
Impact of viral infection on autophagy.

Mechanisms	Viruses	Viral molecules responsible for autophagy	Host molecules affected	References
A. Autophagy induction				
by increase in autophagic flux	Sindbis virus	–	Increases LC3 II	[27]
	Hepatitis C virus	–	Increases LC3 II	[28]
by binding to the surface of target cells	Human Herpes Virus 6	Gp H-L-Q complex	Binds to CD46	–
	Adenovirus B and D	trimeric fiber knob domain	Binds to CD46	–
	Measles Virus	gp41 subunit of viral envelope protein	Binds to CD46	[29]
	HIV 1	–	Binds to CXCR4 (CD4+) T cells	[30]
	Vesicular Stomatitis Virus	Gp VSV-G	binds to TLR 7	[31]
by Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR)	Hepatitis C virus	–	Induces PERK, ATF6, IRE1	[32]
	Dengue Virus	NS4A	–	[33]
B. Autophagy inhibition				
by interfering with autophagosome formation	Kaposi Sarcoma Herpes Virus	vFLIP	target Beclin-1	[34]
	Murine Gamma Herpes Virus 68	M11	encode viral Bcl 2 homolog	[35]
	Herpes Simplex Virus 1	ICP34.5	encode viral Bcl 2 homolog	[36]
by interfering with autophagosome maturation or degradation	HIV 1	Nef	interact with Beclin-1	[37]
	Influenza A virus	Matrix (M2)	interact with Beclin-1	[38]
by autolysosomal degradation	Coxsackie Virus B3	–	–	[39]
	Polio Virus	–	–	[40]

LC3 II = microtubule-associated protein 1 light chain 3; TLR = Toll like receptor; PERK= Protein Kinase R like eIF2α kinase; ATF6 = Activating Transcription Factor 6; IRE 1 = Inositol Requiring Enzyme 1; NS= Nonstructural; NEMO= NF κ B essential modulator; IRGM= Immunity associated GTPase family M; CD = cluster of differentiation.