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Review

Hijacking the Host Immune Cells by Dengue Virus: Molecular Interplay of Receptors and Dengue Virus Envelope

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Abstract: dengue virus (DENV), being one of the lethal pathogens in the hot climatic regions of the world, have been extensively studied to decipher its mechanism of pathogenesis and missing links of its life cycle. With respect to the entry of DENV, multiple receptors have been recognised in different cells of the human body. However, scientists still argue whether these identified receptors are the exclusive entry mediators for the virus. Adding to the complexity, DENV has been reported to be infecting multiple organ types in its human host. Also, more than one receptor in a particular cell has been discerned to take part in mediating the ingress of DENV. In this review, we aim to discuss about the different cells of the human immune system that support DENV infection and their corresponding receptors that DENV deploy to gain access to the cells.

Keywords: DENV; tropism; receptors; entry; immune cells

Introduction

The genus *Flavivirus* includes enveloped viruses (approx 50nm in diameter) containing positive sense, single stranded RNA (approx 11kb in size) genome. Dengue virus (DENV) is one such arbovirus having a genome encoding three structural proteins (C, prM/M, E) and seven non structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [1]. The envelope of the mature virus contains 180 copies of two glycoproteins, prM and E [2]. Depending on the heterogeneity in these two surface proteins, DENV is broadly classified into 4 serotypes and each serotype is further distinguished into different genotypes [3].

DENV, being an arbovirus entirely depends on its insect vectors *Aedes aegypti* and *Aedes albopictus* for circulation in the environment and ultimately reaches its human host for extensive proliferation.

Once DENV gains access to the host, it infects different organs and replicate in multiple cells. DENV exploits various cellular receptors to enter the cells. Although various cellular receptors have

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been identified as receptors for virus entry, none of them has been recognized as an universal receptor for DENV entry. Here, we will discuss about the immune cells that are known to harbour DENV during the disease progression and the corresponding receptors studied so far. It remains an underexplored field and we are yet to nail down the primary receptor/s involved in the entry process. Better understanding of the receptor usage might further help designing specific antiviral candidate/s against DENV infection.

DENV entry receptors in cells of the immune system

Dendritic Cells (DCs)

Broadly, there are two subsets of DCs found in mammalian system: Interferon (IFN) secreting, blood and lymphoid tissue resident plasmacytoid DC (pDC) and antigen presenting, lymphoid and non-lymphoid tissue resident myeloid or conventional dendritic cells (mDCs or cDCs). The antigen presenting property of DC has been exploited by DENV to disseminate from the skin to various lymphoid organs. Also a common monocyte-DC precursor differentiates to give rise to tissue resident macrophages and monocyte derived DCs (moDC) which are non-conventional DCs [1].

The immature DCs (iDCs) particularly in skin (Langerhans cells, dermal cDC and moDC) and in blood have been shown to be more susceptible to DENV infection than mature DC and DENV infect these cells independent of Fcγ receptor [4,5,6]. pDCs are not found to be DENV targets *in vitro* as significantly lower levels of DENV replication was observed when compared to moDC [7,8]. Previous experiments proved LCs in epidermis to be the primary targets of DENV in skin, however, subsequent experiments suggested that DENV is probably released in the dermal layer of the skin affecting its resident cells first [9,10,4]. Hence, the route by which epidermal resident cells (LC and keratinocytes) get infected is still unclear. Studies done by Duangkhae 2018 showed that DENV likely mediates LC migration to the dermis where these cells further get infected [11]. Also, studies done by other groups indicate dermal cDCs and macrophages to play more significant role than LCs in DENV spread [10,12].

The most extensively studied DC receptors are DC-SIGN(CD209) [13,14,4,15], Mannose receptor (MR) [16,17], Langerins [18,19] and Fcγ receptors [20,21,7]. Other potential receptors expressed in DC include TIM3, TIM4 [22,23,24] and AXL [25].

DC SIGN, a C type lectin pathogen recognition receptor, is highly expressed in immature DCs like resident dermal DCs (CD14+), monocyte derived DC in the dermis, DC in lymph node, thymus and lungs, myeloid DCs in blood and also in dermal and alveolar macrophages (7,10,13,15,26,8,27). Although, in presence of Ca^{+2} the 'carbohydrate recognition domain' (CRD) of DC-SIGN has been shown to interact with the high mannose oligosaccharides present in Asn67 residue of DENV E, DC SIGN is also reported to bind to the other branched glycans containing terminal fucose residues [28,29,30,31,32].

The importance of DC-SIGN as a DENV entry receptor was highlighted when its expression in various cells lines rendered these cells permissive to DENV infection [13,15,28]. The mechanism by which DC-SIGN mediates DENV entry was further studied by Liu et al 2017. By using live cell imaging on DENV infected MX-DC-SIGN cells, the researchers showed that DC-SIGN and DENV after forming a complex migrate towards clathrin coated pits and get endocytosed. However, the mutants lacking the internalization domain (DC-SIGN-3A) or the one containing a partial cytoplasmic domain (DC-SIGN-Δ35) when expressed in MX-DC-SIGN cells still favoured DENV infection, although to a lesser extent than the intact DC-SIGN. Other groups also found similar results when mutant DC-SIGN (without cytoplasmic tail) in HEK-293T cells could still enhance DENV infection [28,33]. Hence, the role of DC-SIGN in mediating DENV infection has been interjected to be an attachment factor for DENV, which concentrate the virus on the cell surface and present it to a mysterious receptor, further ushering DENV to the endosomal compartment [13].

Studies done by Cerny et al 2014 on single cell suspensions of healthy human skin discovered LCs, dermal macrophages and CD14+DCs to be highly infected as compared to the other subsets of dermal cDCs (CD1c+ and CD141+DCs) [9]. On further study, they showed that CD14+ DC being

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highly susceptible to DENV infection, expressed both DC-SIGN (CD209) and mannose receptor (MR) (CD206). However, CD1c+ DC showing lesser susceptibility to DENV expressed only mannose receptor (CD206) and CD141+ DC being least susceptible expressed none of these receptors. Dermal macrophages also express both DC-SIGN (CD209) and mannose receptor (CD206) on their cell surface and hence are highly permissive to DENV [27]. Hence, both receptors (DC-SIGN and MR) seem to work together to mediate DENV entry in these cells. MR is constitutively internalised via endocytic or phagocytic pathway and hence may act as DENV entry receptor for DC and macrophages, whereas DC SIGN is mostly confined to the cell surface and work as an important attachment factor [17].

Langerin (CD207) is another C type lectin receptor similar to DC-SIGN and is predominantly expressed in Langerhans cells in the epidermis [18,19]. It also specifically recognises mannose and fucose glycans along with GlcNAc moieties on the DENV E protein [34]. DENV uses this receptor to gain access to LCs in skin where it proliferates for further dissemination [4].

TIM3 and TIM4 are another group of receptors that are expressed on the surface of DC and might facilitate DENV entry in these cells [22,23]. TIM3 and TIM4 have been observed to play an important role in phagocytosis of apoptotic cells and TIM-4 was particularly detected in immature DCs and macrophages of spleen [24]. These receptors have been studied to mediate DENV entry in transfected cell lines where TIM3 has been perceived to play a less significant role than TIM1 and TIM4 in mediating the entry process [35]. TAM receptors (TYRO3, AXL and MER) particularly AXL, also involved in the uptake of the apoptotic cells, has been observed to be expressed on the surface of Langerhans cells early during its differentiation and might play a significant role in mediating DENV infection in these cells [25].

Antibody dependent enhancement (ADE) is the mechanism by which the heterologous antibodies (IgG), irrespective of the neutralizing capabilities surround DENV during the secondary infection and present it to an FcγR bearing cells to enhance DENV infection [36,8, 37,38,39,40,41,42,43].

In case of ADE, mature DC and macrophages showed enhanced infectivity at a low concentration of heterologous antibodies. Hence, immature DCs (iDC) and mature DCs (maDC) were observed to play a distinct role in primary and secondary infection and cell tropism was found to be slightly different in two conditions. In primary infection iDC were infected most followed by maDC and macrophage, whereas in secondary infection, in presence of heterologous anti DENV antibodies the macrophage is infected most followed by maDC and iDC [44].

FcγRIIa and FcγRIIb are the two receptors that are expressed on the hematopoietic cells and interact with the opsonising antibodies (IgG) surrounding DENV particle for its enhanced phagocytosis. The LCs, immature moDCs and dermal DCs as they express high levels of langerins (LC) and DC-SIGN (moDCs and dermal DCs) respectively, become primary targets of DENV in absence of enhancing antibodies [4,15,5]. Despite, they do not play any role in ADE even though they express FcγRIIA higly as elevated levels of expressed DC-SIGN plays a dominant role and mediate DENV entry in these cells [7,20]. In contrast to this, mature moDCs gets infected by DENV moderately during primary infection as they express lesser amount of DC-SIGN but inflated levels of FcγRIIA on their surface, showing significantly high capacity for ADE [20,7].

Monocytes and macrophages

Monocytes and macrophages are the primary targets of DENV along with the Dendritic Cells. It has been reported that macrophages in lymphoid and non lymphoid tissues are the major targets of DENV replication during later period of infection. They are also the primary reservoirs of DENV after its dissemination from the skin. DENV was found to replicate in macrophages of different organs namely, kuppfer cells in liver, alveolar macrophages in lungs, macrophages of lymphoid organs (spleen, lymph node and thymus) dermal macrophages, microglial cells and monocytes in peripheral blood [45,46,37,47,9,48].

Experiments in mice indicate that after DENV infects skin, the inflammatory Ly6C+ monocytes were recruited to the skin replenishing LCs in epidermis andLy6C+ CD11b+ moDC in the dermis

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which were efficiently targeted by DENV [7,10]. These studies indicate that possibly this observation holds true in case of humans, where blood derived monocytes migrate to the site of infection in skin and act as another reservoir of DENV for replication [9,7,10,49].

Cell surface receptors that help in DENV tropism in monocytes and macrophage include mannose receptor (CD205) [16,17,50], CD14-associated protein [51,52], HSP70/HSP90 [53,54], DC-SIGN(CD209) [7,10,15,50] and CD300a [55,56], AXL and TIM4 [57,22,24] and PD1[58].

Mannose receptor (MR) is another C type lectin, found in both macrophages and DC and has a multi domain structure [16,17,50]. MR binds specifically to the carbohydrate moieties terminating in mannose, fucose and N-acetyl Glucosamine (NAG) residues as found in Asn67 of DENV E glycoproteins [16,17,50]. Mannose receptor has been shown by Miller et al 2008 to be an important receptor for DENV entry in human macrophages. Pretreatment of monocytes with type 2 cytokines enhanced the surface expression of MR and DC SIGN on human monocyte derived macrophages which led to the increased percentage of infected cells. DC-SIGN, which is known to play a role in DENV attactment to DC, also has some role to play in macrophage susceptibility to DENV, probably acting as an additional attachment factor for these cells [17]. However, Dermal and Alveolar macrophages are the only macrophages that possess cell surface DC-SIGN and hence may mediate DENV infection in these cells in cooperation with MR [26,27].

CD14 is a cell surface glycoprotein, expressed predominantly on the surface of monocytes and macrophages and possesses a high affinity for LPS [59]. It remains associated with the low affinity transmembrane proteins that show signal transducing properties [52,59]. *In vitro* infection model studies on monocytes and macrophages have shown a role of CD14 or its associated molecules in DENV mediated infection, as pre treatment of these cells with LPS before DENV infection suppressed the infection markedly [51,52]. The decrease in infection was inspected not due to LPS mediated release of cytokines but due to blockage of CD14 and its associated cell surface molecules by LPS [52]. However, the outcome of LPS pre treatment before DENV infection was perceived to show both strain and cell specific effect on DENV infection [51].

Cell surface proteins like HSP70 and HSP90 are known to be a part of the receptor complex helping in DENV tropism in human monocytes and neurons [53,54]. Hsp90and Hsp70 (74/84 kDa molecule) isolated from neuroblastoma cell line SK-SY-5Y, U937 cells and human peripheral monocytes/macrophages, was observed to interact with DENV-2 strain 16681 E protein and pretreatment with anti Hsp 70/anti Hsp90 antibodies reduced DENV infection in these cells [54].

It has been observed that phospholipid receptors like TIM, TAM and CD300a expressed on the surface of phagocytes recognise Phospholipids like phosphatidyl ethanolamine(PE)and phosphatidyl serine(PS) expressed on the surface of apoptotic bodies and mediate their phagocytosis [24,35,57,25,56].This mechanism has been exploited by DENV to interact with such receptors as the DENV membranes express such phospholipids which it acquires during the process of virus budding from ER [35,55,60]).

The T cell/transmembrane, immunoglobulin, and mucin (TIM) gene family include 3 members in humans (TIM-1, TIM-3, and TIM-4) and these receptors are expressed in different cells with slightly different functions.TIM1is highly expressed on T-helper 2 (Th2) cells and are important for T cell activation, TIM 3 is highly expressed on Th1, Tc1 cells and DC mediating phagocytosis of the apoptotic cells and cross-presentation of antigen and TIM4 is expressed in antigen presenting cells (APC) and has a role in phagocytosis of apoptotic cells and immune tolerance [22,23]

TIM 1, TIM 4 and to a lesser extent TIM3 were found to enhance mosquito-derived DENV2-JAM infection when expressed in 3T3 and Vero cell lines and a direct interaction between TIM receptors and DENV virions was observed in a Ca^{+2} dependent manner [35]. Furthermore, the authors showed that TIM receptors (TIM 1 and TIM 4) expressing 293T cells recognised PS expressed on DENV virion envelope to mediate its entry in the cells. The role of TIM1 in DENV infection was inferred by Meertens et al 2012 and Dejarnac et al 2018 where the significance of TIM1 and its cytoplasmic tail in mediating DENV infection was assessed [35,60]. TIM1 knockout cells like A549 and Huh7.5 showed significantly less DENV2 infection [60] but TIM1 mutant without cytoplasmic tail (TIM-1 Δcyt) had no such effect when transfected in 293T cells [35]. Hence TIM1 is important for enhancing DENV

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infection but its cytoplasmic tail has no or minimal role in enhancing DENV infection. Nevertheless, it plays a vital role in mediating DENV internalization by clathrin mediated endocytosis [60].

The TAM protein family is a group of three receptor protein tyrosine kinases that recognises PS expressed on the surface of apoptotic cells indirectly via TAM ligands (Gas6 and ProS) and are expressed on phagocytes particularly DC and macrophages [35,57,61,55]. TYRO3 and AXL are two such TAM receptors that are known to recognise apoptotic cells and also enhance DENV infection [35]. Furthermore, TIM1 and AXL have been observed to be expressed in DENV permissive cell lines like A549, Vero, Cos-7 and Huh7 5.1 cells but are absent in cells which are non permissive to DENV like 293T, U937, or RAJI cells [35]. Hence both the receptors (TIM1 and AXL) may act cooperatively and complementarily to positively influence DENV binding in the cells.

Recently a phospholipid receptor CD-300a, expressed on the surface of mast cells, monocytes and monocyte derived macrophages (MDM) has been observed to act as a receptor of DENV [62, 55]. It directly interacts with PE and to a lesser extent with PS expressed on the surface of DENV and enhances its entry in these cells [55,56]. On expression of CD300a in HEK 293T cell line and in HeLa cells, the DENV2 infection was highly enhanced suggesting its importance as an attachment receptor but does not play a major role in mediating DENV entry [55]. In the same study it was shown that although CD300a was expressed in monocytes, mast cells and monocyte derived macrophages, CD300a could increase DENV infection only in MDM and was ineffective in case of other two cells suggesting its cell specific action.

Other chaperones like Protein disulphide isomerise (PDI) has also been shown to enhance the DENV binding to the cell surface THP-1 and also in Endothelial cells [58,63]. PDI is an ER resident chaperone but has been found to be localised in various other cellular regions like nuclear envelope, cytoplasm, Golgi, secretory vesicles, and plasma membrane [64]. PDI was found to be upregulated during DENV infection in THP1cell lines and was observed to be associated with the lipid rafts for an efficient interaction with DENV. Hence, PDI plays a role in mediating DENV interaction with the susceptible cells but further studies are needed to decipher its exact role in DENV tropism as the authors failed to show direct interaction of PDI with DENV E protein [58].

Although Two Fcγ receptors particularly FcγRI(CD64) and FcγRII(CD32) have been shown to mediate ADE in phagocytic cells *in vitro*, FcγRIIA(CD32) was found to enhance DENV infection more efficiently than FcγRIA (CD64) [65,37,66]. However, cells which facilitate ADE particularly primary monocytes, express both the cell surface receptors [37]. FcγRII has two subsets that play different roles in ADE by either activating or inhibiting the process, particularly FcγRIIA which enhances ADE and FcγRIIB which abolishes ADE and both these molecules are known to be expressed in ADE supporting cells- monocytes, macrophage and moDC (mature and immature) [7,20]. Nonetheless, it has been perceived that these cells, specifically mature moDC enlarge the ratio of FcγRIIA/FcγRIIB to facilitate DENV entry during ADE [20]. ADE hypothesis has been widely studied for Monocytes, macrophages, mature DC (moDC) and mast cells but there is a high possibility of B cells and Endothelial cells being involved in ADE , as they express FcγR on their cell surface [67,68,42,43,69]. Monocytes play a less significant role as primary targets for DENV since they express lesser amount of DC-SIGN compared to DCs but has a major role to play in secondary infection during ADE, due to high levels of FcγR expression [7,10,37].

Mast cells and Basophils

Mast cells found in dermis of the skin are important for the surveillance of the immune system and on encountering DENV it gets degranulated, secreting various cytokines (IL-1, IL-6, TNF-α,IFN-α) and chemokines(CCL5, CXCL12, and CX3CL1). This further leads to an antiviral state in nearby cells, generating an inflammatory response and cells like NK and NKT get recruited to combat DENV [70]. Various reports indicate the susceptibility of mast cells to DENV tropism and hence, mast cells are one among other cells that support DENV entry and replication [71,11]. Primary human skin mast cells were identified to support DENV2 infection as seen by qRTPCR and are also among the initial targets of DENV in skin [71]. Furthermore, skin explants when infected with DENV2 showed mast cells to be among the susceptible cell population showing productive infection in the dermis

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[11]. DENV2 was also observed in the secretory granules of these cells and were highly infectious to the uninfected cells [71]. Moreover, when these extracellular granules were injected in mice footpad, the DENV containing granules travelled through lymph to draining lymph nodes (DLN) and spleen leading to further virus dissemination. Hence, they discovered a novel mechanism used by DENV to spread in the host, starting from the skin to the various lymphoid organs, apart from being carried by the infected immune cells.

Mast cells are FcγR bearing cells that express all the FcγRs (FcγRI, FcγRII, and FcγRIII), as observed in various cells like human cord blood derived mast cells (CBMCs) that express all the 3 FcγRs, while FcγRII was prominently expressed on the surface of human mast cell lines HMC-1 and KU812 [42]. Experiments done by various groups in human mast cell-like line HMC-1 and KU812 successfully inferred the importance of heterologous antibodies to mediate DENV entry in human mast cells via FcγRII receptors [72,42,43]. These cells were infected with DENV alone or DENV with antibodies and a significant infection was observed in presence of antibodies [42,43].

Apart from Fc receptors, TIM1and TIM3 are also found to be expressed in mouse peritoneal mast cells and bone marrow–derived cultured mast cells (BMCMCs) [22,73]. These can be potential receptors used by DENV for its entry in mast cells.

T cells and B cells

B cells and T cells have been studied by various laboratories to ascertain their roles in supporting DENV replication, but contradictory results leave it an open question to address. *In vitro* studies done on B cell lines (Raji cells,Wil 2WT, BM and LK63, Daudi and 8866) and primary B cells derived from healthy human Peripheral blood mononuclear cells (PBMC) strongly state its potential role in DENV replication, both in presence and absence of heterologous antibodies [74,75,51,76]. Also, blood samples of DHF patients revealed the presence of DENV antigen in B lymphocytes [77] but mere presence of DENV antigen does not prove DENV replication in these cells. In the humanized mouse model study, lymphocytes (both B and T cells) were DENV infected at an earlier stage of infection (1-2 dpi and 3 dpi respectively) and B cells produced important proinflammatory cytokines (IL-6 and TNF-a) similar to monocytes and macrophage [39]. Furthermore, *in vivo* experiment done on DENV infected BALB/c mice revealed the presence of NS3, E and prM in B cell follicles particularly in the germinal center (GC), B cells of DLN, indicating viral replication in these cells [67]. Some groups of researchers found naive primary human T cells (CD8+, CD4+) derived from PBMCs of healthy individuals and human Th and Tc clones (JK44, JK49, CB2.8 and CB6.17,HSB-2 ,Molt-4 and Jurkat) to be DENV permissive, producing new infectious virions from the infected cells [79,80,51,76].

In contrast to this, other groups found no evidence of DENV replication in primary B and T cells in healthy human PBMCs or in splenic B and T cells as seen by FACS analysis, RT PCR and plaque assay [46,37]. *In vivo* studies found no DENV antigen in B or T cells of infected human tissues as detected via Immuno-histochemistry (IHC) and *in situ* hybridization (ISH) techniques [45]. Viral NS3 specific immune staining of infected AG129 mice and human tissue samples also indicated absence of the DENV specific protein in lymphocytes of all the tissues [81]. According to Kou et al 2008, T or B cells are not DENV permissive both in absence or presence of facilitating anti-E antibodies. The same group showed it to be true for spleen derived mononuclear cells where splenic macrophages were infected both in presence and absence of anti-DENV antibodies but T or B cells were uninfected [46]. Theofilopoulos et al 1976 also reported similar results, where T cell lines lymphoblast MOLT-4 and primary T cells from healthy human PBMC were not found to support DENV replication but DENV was observed to adsorb on the cell surface early after infection. Hence, the author proposed T cells to possess the DENV receptor but due to some other intrinsic or extrinsic factors, DENV failed to enter or replicate in these cells. Schmid et al 2014b further studied the epidermal CD45+ γδT cells in Ifnar–/– mice after intra dermal inoculation of DENV2 and found these cells to be non-permissive to DENV.

Contradictory reports have also been obtained regarding receptor usage of DENV for T cell entry. Heparan sulphate was identified to be the putative receptor on T cells that mediated DENV

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entry [79] but Ohmann et al 2000 found no significant role of heparan sulfate in promoting DENV binding to these cells [51].

Work done on T helper (Th) cells revealed the involvement of cell surface PDI in facilitating HIV entry in these cells [82]. Studies done on PDI in relation to DENV entry have indicated it to likely play a role in DENV tropism, making it a yet another putative receptor for T cells [63] that needs further research. TIM1 and TIM 3 have been noted to be expressed primarily on the surface of Th2 cells and Th1 /Tc1 cells respectively, having a role to play in both their activation and apoptosis [22,23,24,35]. Hence, these receptors might play a role in DENV entry in human T cells. No receptor has yet been identified to be responsible for DENV entry in B cells. However, FcγR might be utilized by DENV to gain access in these cells [67,69].

Discussion

The human immune cells that have been most extensively studied to support DENV are DC, monocytes and tissue macrophages [4,5,6,45,46,37,47,9,48]. However, recent studies have identified mast cells to be the new targets of DENV in humans and their potential in acting as viral replicating machinery [71,11]. Mast cells may be inferred to be among the early targets of DENV in skin along with the known hosts of DENV: DCs and dermal macrophages [71,11]. Future studies will elaborate our understanding of DENV tropism and explore other unknown targets of DENV in the host. Controversial role of lymphoid cells (T and B) in DENV tropism and replication also needs further exploration.

Multiple receptors associated with the DENV tropism in host and vector has been studied to better understand their individual role in facilitating DENV entry. However, all studies indicate that DENV seems to utilise multiple receptors/co receptors to gain access to the host cells and that not a single receptor can be conclusively declared to be solely involved in DENV ingress. These observations raise multiple possibilities regarding DENV-cell interaction at the entry point and the different modes of entry inside the cells (fig 1). A) DENV either attaches to the cell surface via its receptors/ co receptors followed by the dissolution of the membrane (cellular and virion) at the attachment site, which further leads to the entry of the nucleocapsid in the cytoplasm directly, without involvement of any vesicles [83,84] (fig 1a). B) DENV binds to the receptor/co receptors forming DENV–receptor complex first, followed by its diffusion to the pre formed clathrin coated pit for endocytosis [85] (fig 1b). C) DENV first interacts with multiple low affinity co receptors like DC-SIGN, Langerin, CD300A, CD-14, Heparan sulphate, PDI etc via roll over mechanism to toughen its hold on the cells, followed by its interaction with the less abundant but high affinity receptor located near the pre-existing clathrin coated pit or within the pit after which DENV enters possibly via receptor mediated endocytosis [85] (fig 1c). D) Interaction with the receptors/co receptors in lipid rafts might mediate signal transduction to enhance the availability of the main receptor on the cell surface or might lead to caveolae mediated endocytosis (fig 1d). E) DENV might also follow the dynamin dependent, clathrin independent mode of endocytosis to enter the cells [86] (fig 1e). There also lies a possibility that DENV deploys all these receptors in a cell specific manner to approach the cell for a productive infection and as such does not depend on any one receptor for the entry. If this is true then it would be intriguing to know what the underlying factors behind such cell specific interaction are. How does DENV recognize different receptors in a cell specific way?

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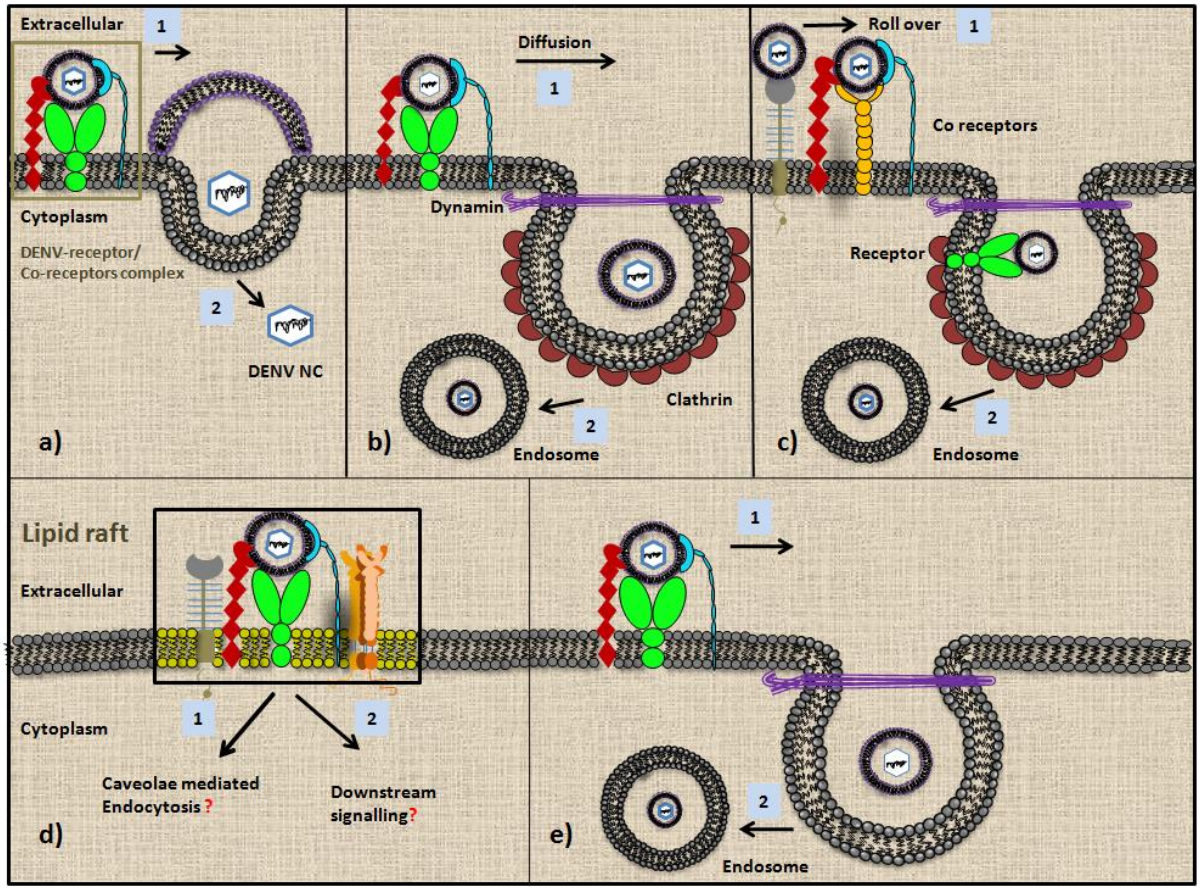

Figure 1. An overview of the different routes of DENV entrance in susceptible cells via multiple receptors/co receptors: a) Direct entry of DENV in cells. a1) DENV first forms a complex with receptors/co receptors and then a2) fuse with the cell membrane releasing the nucleocapsid (DENV NC) in the cytoplasm. b), c) Receptor mediated endocytosis of DENV in cells in which b1) DENV after interacting with receptors and co receptors diffuse along the membrane and enter through preformed clathrin coated pit further leading to the b2) endosome formation or c1) DENV might possibly interact with co receptors by rolling over the cell surface until it reaches to the main receptor present near or within the preformed clathrin coated pit after which it gets c2) endocytosed. d) Lipid rafts may also play an important role in DENV tropism by providing the platform for DENV interaction with multiple receptors and co receptors, which in turn may lead to d1) caveolae mediated endocytosis or d2) downstream signalling to enhance the receptor expression at the cell surface. e) Non classical, dynamin dependent but clathrin independent endocytosis is another route of entry that DENV might follow for ingress in cells.

Furthermore, as mentioned in figure 2 and Table 1, DENV interacts with the different receptors in a cell specific manner which further complicates the identification of a possible common receptor mandatory for the DENV entry process. MR in monocytes/macrophages and DC and recently TIM1 receptors have been shown to be important for DENV entry in these cells and these might not just be acting as co receptors/attachment factors for DENV [8, 15,17,35,60]. Further studies are needed to confirm their universal role in the ingress of DENV in susceptible cells and the detailed mechanism of the process.

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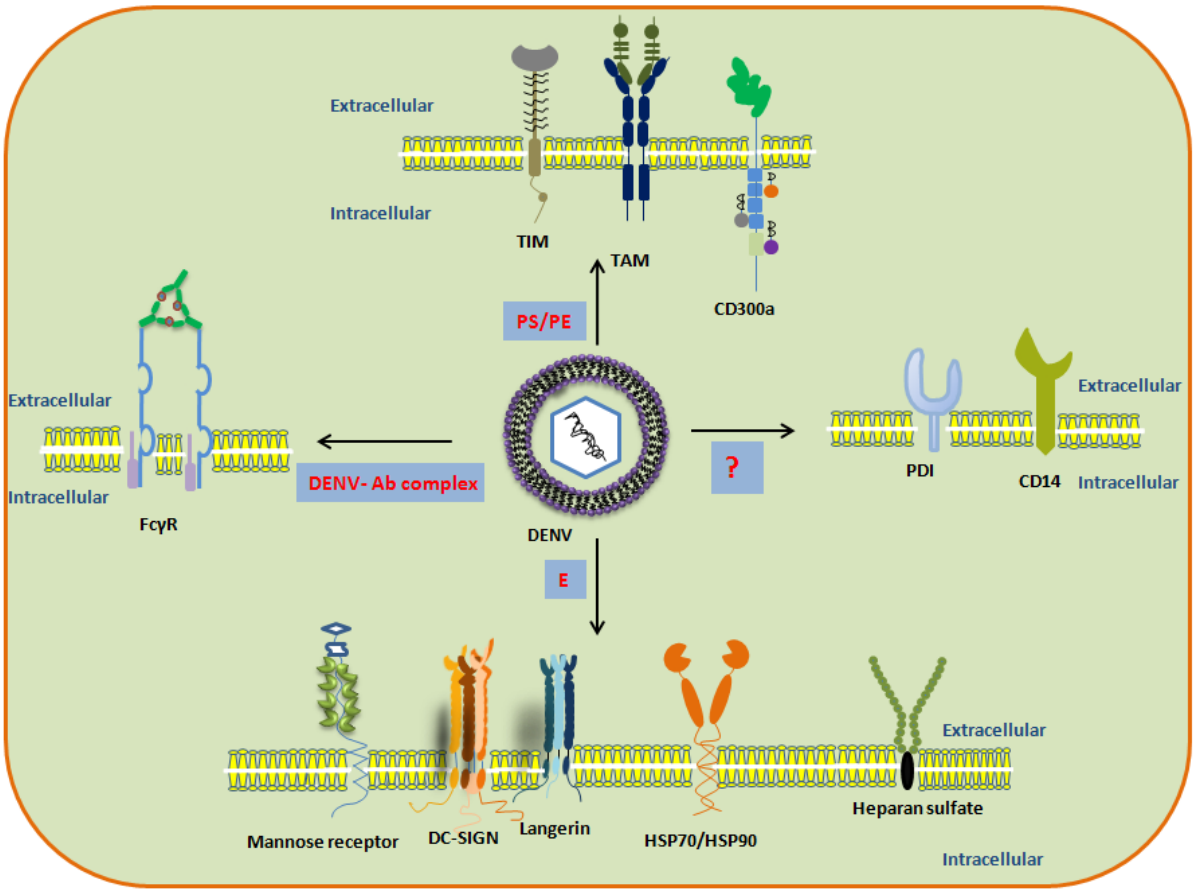

Figure 2: Multiple receptors used by DENV for entry in the host. PS/PE: phosphatidyle serine and phosphatidyl ethanolamine on DENV membrane. prM: precursor membrane of DENV. E: Envelope protein of DENV.

Receptors mediating DENV tropism.	Susceptible cells in humans expressing the receptor	References
DC-SIGN	• Dermal Dendritic cells (CD14+) • Dermal macrophages • Alveolar macrophages • Dendritic cells in lungs • Dendritic cells in lymph nodes • Myeloid DCs in blood	• Schaeffer et al 2015, Cerny et al 2014 • Schaeffer et al 2015 • Soilleux et al 2003 • Soilleux et al 2003 • Soilleux et al 2003 • Sun et al 2009, Tassaneetrithep et al 2003,Wu et al 2000
Langerin	• Langerhans cells	• Wu et al 2000
Mannose receptor	• Dermal Dendritic cells (CD14+, CD 1c+) • Macrophages	• Cerny et al 2014 • Schaeffer et al 2015,Miller et al 2008
TIM	• Dendritic cells • Macrophages • T cells	• Freeman et al 2010,Manzanet et al 2009, Kobayashi et al 2007 • Freeman et al 2010, Manzanet et al 2009 • Freeman et al 2010, Manzanet et al 2009, Kobayashi et al 2007, Meertens et al 2012
TAM	• Langerhans cells • Macrophages	• Bauer et al 2012, Lemke et al 2008 • Lemke et al 2008
CD-300a	• Mast cells • Monocyte derived macrophages • Blood monocytes	• Borrego et al 2013,Carnec et al 2015 • Borrego et al 2013,Carnec et al 2015 • Borrego et al 2013,Carnec et al 2015

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FcγR	• Mast cells • Macrophages • Blood monocytes • Conventional DC • B cells	• Brown et al 2006, King et al 2000, Brown et al 2010 • Boonnak et al 2008, Schmid et al 2014b • Kou et al 2008,Boonnak et al 2008, Schmid et al 2014b • Boonnak et al 2008, Schmid et al 2014b • Gergely et al 1977
Protin Disulfide Isomerase (PDI)	• Blood Monocytes • T cells	• Diwaker et al 2015 • Barbouche et al 2005
Heat shock proteins (HSP70,HSP 90)	• Blood monocytes	• Valle et al 2005
Heparan Sulfate	• T cells	• Silveira et al 2018, Ohmann et al 2000
CD-14	• Macrophages • Blood monocytes	• Wright et al 1990 • Wright et al 1990

Table 1. Outline of the various receptors that have been studied in context of DENV entry in susceptible immune cells. .

In relation to DENV tropism, researchers emphasized on the importance of E protein in interacting with the host receptors, but the role of M protein has long been neglected. M protein, another important component of the DENV envelope, lies in close proximity to E protein and act as its chaperone to maintain proper E protein confirmation and govern its antigenicity [87,88]. Furthermore, the glycosylation pattern of M protein has not been studied in great detail but glycosylation at Asn64-69 residues have been detected in different DENV serotypes, particularly at Asn 68 residue [89,16]. Hence, there lies a possible role of M protein in mediating DENV interaction with the host cell receptors either indirectly or directly. If it does perform any role in DENV-receptor interaction then what exactly is its role? Does it interact with any host receptor/co receptor directly to strengthen the interaction of DENV with the target cell or it indirectly facilitates DENV E interaction with the receptors?

The true potential of DENV E in recognizing the host receptors needs to be further explored. Studies done on mosquito derived DENV E stated that apart from mannose residues at Domain II (DII) Asn67 residue, fucose, sialic acid, GalNAc and GlcNAc have also been observed at this position along with sialic acid at Domain I(DI) Asn153 site [90]. Therefore, role of these glycan moieties in recognizing host receptors need further investigation and their binding partners may play an equally important role in attaching DENV to the cell surface. What other residues in E get glycosylated and how that affects DENV tropism in host and vector is still an open question to address.

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