ROLES OF HUMAN AND VECTOR DERIVED PHENOTYPES OF DENV IN CAUSING HUMAN DISEASE- CAN MOSQUITO MEDIATION BE BYPASSED?

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ABSTRACT

BACKGROUND

Plasma membrane of midgut epithelial cells of the mosquito differs from that of human dendritic cells in composition with respect to protein and lipid content and posttranslational modifications, viz. glycosylation. Virus acquires its envelope from the host cell membrane. Expectedly therefore, such differences are reflected in the construction of its envelope, which may influence virulence. Lipid composition and glycosylation pattern of envelope protein E1 (with important roles in viral entry) are different in the virus grown in insect cell lines and mammalian cells. As consequence, they have 'different modes' of cell entry each with role at different stages in disease course. Virions that initiate primary infection are mosquito derived; but then on, it is a phenotype of human cell origin that multiplies and spreads in the host.

MATERIALS AND METHODS

In a hospital-based yearlong prospective study conducted at our institute, we have tried to highlight (indirectly albeit), all important role of antibody mediated cell infection by DENV in the human host and how it modified disease process.

RESULTS

Mediation of the biological vector thus is required essential in 'initiation' of primary infection (emphasising the role of vector control as numero uno strategy for disease control); in the human tissues, thereafter, antibody mediated cell infection seems to take the lead role.

CONCLUSION

Mediation of the biological vector mosquito is required in natural cycle of transmission of DNV from man to man. Unique features of the envelope of 'mosquito derived' virions enabling them to enter human cells nonpermissive to human derived phenotype maybe capitalised and such mechanisms be targeted in designing vaccine or drugs against dengue and besides this emphasises the relative importance of vector control in dengue control.

KEYWORDS

Human and Vector, DENV, Virions.

HOW TO CITE THIS ARTICLE: Payyappilly RJ, Athilat D. Roles of human and vector-derived phenotypes of DENV in causing human disease- Can mosquito mediation be bypassed?. J. Evid. Based Med. Healthc. 2017; 4(26), 1514-1518. DOI: 10.18410/jebmh/2017/296

BACKGROUND

Dengue is the most common vector-borne disease affecting man. About 40% of the world's population is living in areas endemic for the disease with ~390 million established cases, more than 100 million infections occurring every year- 1 in 2000, resulting in death.[1]

Infection maybe asymptomatic. A recent study estimated that in 2010, there were 96 million apparent and 294 million unapparent dengue infections worldwide.[2] The disease may range from a self-limited febrile illness to classical Dengue Fever (DF) with high pyrexia and severe joint pain, which may proceed to 'severe dengue', with thrombocytopenia and vascular leakage- Dengue Haemorrhagic Fever (DHF) or circulatory collapse- Dengue Shock Syndrome (DSS).[3]

Dengue virus (DENV) is a Flavivirus belonging to family Togaviridae. It is a 50 nm enveloped virus with an icosahedral capsid in T4 symmetry enclosing a 11 kbp single stranded positive sense RNA genome, coding for envelope proteins (E and M/Pre M) and nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5).

There are four serotypes of the virus, DEN-1, DEN-2, DEN-3 and DEN-4 with ~ 60-65% genomic sharing between them. The latter is responsible for serological cross reactions between the serotypes.[4]

The antibodies may even 'cross protect' during initial period until class switch occurs from IgM to IgG, which whereas confers lifelong immunity against homologous type, can only bind, but are unable to neutralise heterologous serotypes. This forms the basis of 'Antibody Dependent Enhancement' (ADE) leading to DHS/DSS during secondary infection with a different strain.

Financial or Other, Competing Interest: None.
Submission 23-02-2017, Peer Review 28-02-2017,
Acceptance 20-03-2017, Published 28-03-2017.
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DOI: 10.18410/jebmh/2017/296

Aedes aegypti is the primary vector of DENV. Aedes albopictus, albeit its wider distribution and better survival has only a secondary role to the former - a matter of vector compliance than compatibility per se.[5] Humans and mosquitoes are only known host for the virus. Humans thus serve as host and the reservoir for the virus. The mosquito remains infected for life and may sustain the virus through Transovarial Transmission (TOT).

The virus replicates in the midgut of the mosquito (the biological vector) and enters its haemocoel to finally reach the salivary glands where it continues multiplying before ready to transmit the virus to human. The virions so transmitted, needless to say, will have its envelope derived from plasma membrane of the insect cell and proteins with posttranslational modifications that occurred during while.

Envelopes of virions produced in mosquito cells differ from those budding out from human cells in their lipid disposition and glycosylation pattern. N-glycosylation in mammalian cells is of the complex type with a lot of processing enzymes adding diversity. Glycans produced in insect cells are less complex because of less diversity in processing enzymes and usually contain more high mannose and paucimannose type of glycans.[6]

The first step in cell entry of DENV is binding to 'attachment factors' that concentrate the virions in conducive locations on the plasma membrane.[7,8,9]

Viruses utilise conserved cellular pathways subserving biological functions for their entry into cells- not surprisingly involving components of the innate immune system that 'recognise' them. Putative attachment sites for DENV like DC-sign ('dendritic cell specific ICAM-3 grabbing non-integrin') on immature dendritic cells[10,11,12,13] and its analogue L-sign (liver and lymph node specific ICAM-3 grabbing non-integrin), CLEC5A (C-type lectin, family 5, member A)[14] and MR (mannose receptors) on macrophages and monocyte derived DCs[15] are all C-type lectins that function as pattern recognition molecules in the innate immune system. All of them sensing 'mannose rich' N-glycans, which are present on the E-protein of 'mosquito derived' DENV that initiate 'primary infection.'[16,17]

Virions of both phenotypes may gain entry into dendritic cells and M2 type macrophages via Fc gamma receptors, when (if) carried in as immune complexes by non-neutralising class switched IgG antibodies. This happens during 'secondary infection' with a heterologous serotype, which may lead to ADE.

'Fc alfa/mu receptors' are also present on macrophages (and platelets) that allow cell entry of virions bound to non-neutralising IgM antibody likewise that may occur during primary infection.[18] This has been cited as a cause of autoimmune damage to platelets though not thought to cause ADE.

Immature virions with uncleaved precursor envelope protein (PrM), which form ~30-40% of the virion population are mostly responsible for production of non-neutralising antibodies. They become infectious on combining with the antibody, which carries it in via Fc receptors.[19]

Another reason cited for FcR mediated cell entry is a post zone phenomenon with subthreshold level of antibody. The M2 macrophages so infringed produce cytokines that mount a type 2 response allowing the virions multiply inside them.[20]

Other putative receptor/attachment sites involved in DENV cell entry after primary infection or during secondary infection include CD14-HSP70/HSP90 complex in monocytes, Heparan Sulfate (HS), Glucose-Regulated Protein (GRP-78) and neolactotetraosylceramide in liver (targeted during DHF/DSS) and TIM and TAM transmembrane receptors (involved in phagocytosis of apoptotic cells).

Virions are internalised by a receptor-mediated endocytosis via clathrin-coated pits or sometimes in a clathrin-independent manner. Direct translocation of the genome by a C-dependent mechanism has also been described.[21] Fusion and release of RNA into the cytosol occurs from the late endosome that requires 'protection' of cholesterol.[22] After replication and assembly, daughter virions are released by budding, when they acquire their envelopes from the cell membrane.

Direct translocation of the genome into neighbouring cells also has been described.

Lipid composition and organisation in cell membrane is important for viral entry. FcγR is known to associate with lipid rafts upon IgG binding. Membrane composition is important in other steps of viral entry such as membrane fusion and genome release into the cytosol. Direct translocation of genome across cell membrane as well is influenced by lipid composition of latter.

Aims and Objectives of the Study

Our aim is to demonstrate how the differences between mosquito derived and human cell derived virions and the different mechanisms of cell entry involved applied in different stages of disease with primary infection as well as in secondary infections.

The objectives were to provide-

1. Statistical evidence for primary infection requiring mediation of the biological vector, arguing against other transmission modes, viz. mother to child, transfusion transmitted or sexual.
2. Epidemiological data suggesting important role of 'antibody mediated' cell entry involved in multiplication and spread of the virions in human host after initiation of primary infection and in secondary infections.

MATERIALS AND METHODS

Statistical surveys were carried out with documental data on neonatal dengue and children under six months admitted to neonatal and paediatric wards in our hospital during the period 2011 to 2016. Follow up data on TTIs recorded by our blood bank from 2008-2016 was likewise scrutinised for transfusion transmitted dengue.

A study on clinical/laboratory profile of patients with clinical suspected dengue admitted in our hospital from 2011-2013 was conducted. Clinical information and

laboratory findings from time to time were obtained from the patient case records and diagnostic serological tests were performed in the microbiology department. Data on incidence, progress and outcome of the disease was analysed in patients' segregated sex and age wise.

Initial screening of suspected cases was done using rapid diagnostic kit made by J. Mitra Diagnostics Ltd., Delhi. All subjects included in our study were positive for DEN NS1 antigen. IgM and IgG antibody levels were estimated and compared using MAC ELISA and GAC ELISA kits manufactured by Panbio and J. Mitra Ltd., respectively, following manufacturer's instructions.

Epi info 7 version was used for statistical analysis and frequencies, proportions and inferential statistics namely Chi-square was done keeping p <0.05.

RESULTS

No admission with neonatal dengue has been recorded in our newborn ward during period 2011-2016 nor has there been any patient under 6 months of age treated in our paediatric wards during that period.

Blood bank in our hospital (which does not screen donors for dengue) has an average turnover of more than 1500 transfusions from ~1000 donors per year. No case of transfusion transmitted dengue has been recorded in the follow up register in the last 10 years. 28 subjects in our study received transfusion with blood products. But, all of them had 'already' developed severe dengue and comparison of disease progression between groups who did and who did not receive transfusion was of no use.

Epidemiological data collected, however, has something to say. Of 1214 patients who attended 'fever clinics' at ACME in 2014, 157 were found positive for dengue IgM. 110 of them were found requiring hospitalisation who formed subjects of our study. Half of them (32 males, 23 females) had classical DF and were discharged after 2-3 days. The other half (24 males and 31 females) were admitted with or developed DHS while in hospital, 6 (2 males and 4 females) of whom going into shock (DSS). We lost two (1 male, 1 female) of them.

All subjects included in our study were positive for DEN NS1 antigen by card test.

All samples tested for IgM by ELISA were found positive (OD>0.4) (except 2 giving equivocal values) indicating 'primary infection'. OD values given by IgM and IgG were compared to assess risk of ADE. 40 out of 55 cases who developed severe dengue gave an IgM/IgG ratio below threshold 1.4 compared to 28 out of 55 in the uncomplicated DF category.

The difference is significant, Chi-square value is 5.54, p=0.01. Male and female distribution of cases, and importantly, their respective ratios in different age groups diagnosed as DF or DHS/DSS (severe dengue) are depicted in graphs given below.

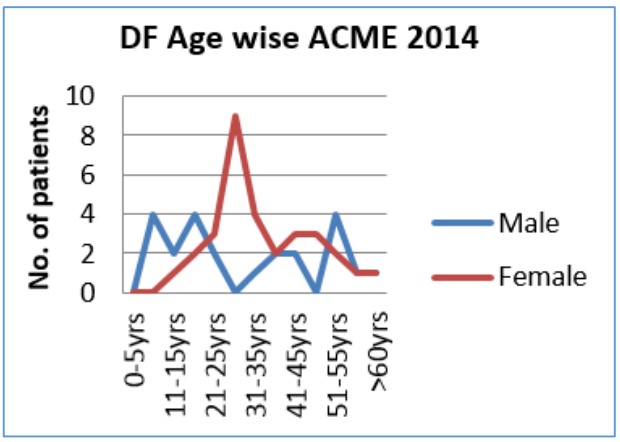

Figure 1. DF Age Wise ACME 2014

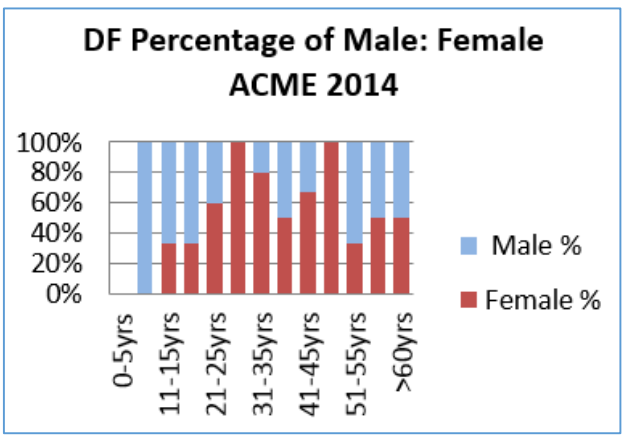

Figure 2. DF Percentage of Male-Female ACME 2014

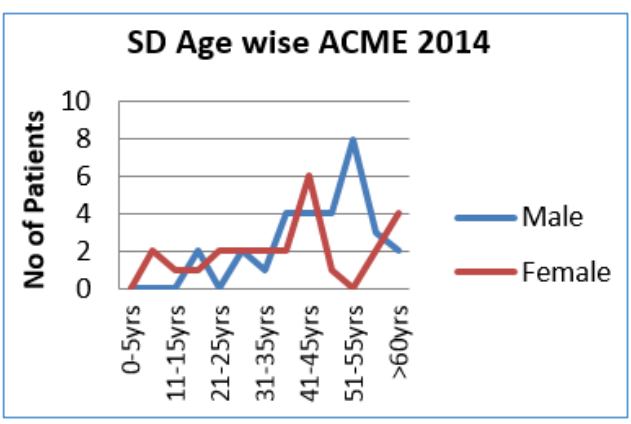

Figure 3. SD Age Wise ACME 2014

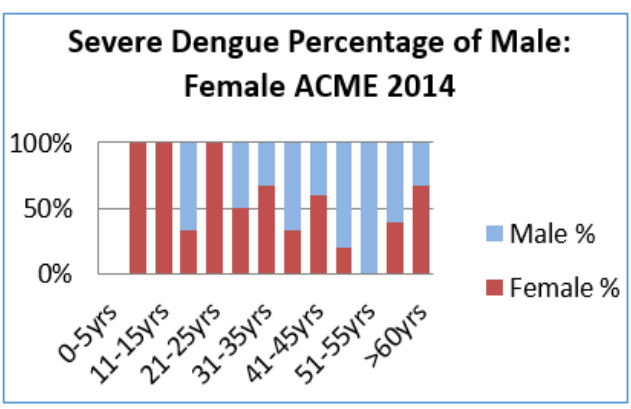

Figure 4. Severe Dengue Percentage of Male-Female ACME 2014

There seems to be a clear preponderance of females in reproductive age groups (from puberty to perimenopause) in both categories of the disease, DF as well as DHF/DSS.

The difference in proportion of males and females in the premenopausal, reproductive and perimenopausal categories in DF is significant, Chi-square value being 38.49, p=0.0001 and also the difference in proportion of males and females in the premenopausal, reproductive and perimenopausal categories in severe dengue is significant, Chi-square value being 61.62, p<0.0001.

Given that females in that stage of lifecycle mount a Th2 type of response to infections and are prolific produces of antibody, this indirectly albeit strongly suggests the role of a FcR mediated infection in both forms of the disease.

DISCUSSION

It transpires thus that the mechanism of human cell entry of DENV in initial infection via receptors on dendritic cells of skin and MCDCs that sense high mannose residues on its E protein is tailor made for 'mosquito derived' virions. Human dendritic cells for instance are not directly permissive to virions produced in them since albeit rich in N-linked complex glycans, they lack in mannose-rich glycosylation[23] and hence not recognised by DC-sign. So, also are macrophages expressing mannose receptors on them.

Fc gamma (and/or Fc alpha/mu) receptor mediated entry of virions requires opsonisation by non-neutralising antibodies, which occurs in secondary infections (or late during primary infection).

Other cells that are targeted later during disease like liver, platelets or endothelium maybe infected via receptors that don't differentiate between the phenotypes.

Human cell derived virus has little role in transmission of dengue among humans (bypassing the biological vector), except in rare situations.

Mother to child transmission of DENV in utero is a possibility, though rare, dreaded should it occur.[24] Here, the virions are of 'human cell origin' supplied by the mother. Concomitant presence of non-neutralising IgG to a heterologous serotype that might have caused an earlier infection in the mother, may cross the placenta, to mediate infection of the foetus, via Fc receptors and lead to ADE. L-sign molecules (liver/lymph node-specific ICAM-3 grabbing non-integrin) are found in placenta relevance of which is to be elucidated.

Passively transferred maternal antibody similarly, may account for ADE and severe disease in early paediatric age group.

There has been one or two reports of DENV infection following organ transplant,[25] even needle stick injury.[26]

Although, AABB (American Association of Blood Banks) has placed DENV in top red level among potential emerging infectious agents that may possibly pose threat to blood transfusion (2009) only 5 cases have been reported in the last 8 years since world over.[27]

This despite more than half the planets population living in dengue endemic areas and most of the infections (up to 75% occur being a symptomatic and may pass donors screening).

Retrospective studies have found up to 0.3% blood products used for transfusion with DENV RNA.[28]

This may be due to the fact that most of the 'receptors' utilised by DENV for cell entry, 'sense' mannose rich glycans on its EIII domain and a feature of the mosquito-derived phenotype. 'Severe dengue' involving infection of internal targets occur mainly via FcR requiring non-neutralising IgG or IgM (e.g. in platelets infection).

However, presence of 'non-neutralising heterologous memory IgG' in the recipient is a possibility that cannot be ignored in a hyperendemic area like ours and apprehension of clinicians to use platelets concentrates and plasma in treatment of DHS maybe justified.

Transmission of DENV through sexual route maybe negated on same grounds mentioned above. At the other extreme, popular notion about 'sex curing dengue', improving platelet counts, immunomodulation by hormones etc. is outside scope of this manuscript.

CONCLUSION

Dengue is not a 'contagious' disease and requires mediation of the biological vector mosquito to transmit the disease from man to man. Primary infection in man is initiated by mosquito cell derived virions with mannosylated envelopes. Thereafter, cell-to-cell spread and infection of internal target organs as well as 'secondary infection', are mainly mediated by Fc receptors with antibody participation though it may also occur via any of the other receptors mentioned above even by direct 'transfection'.

Differences between mosquito-derived and human-derived virions and their cell entry modes maybe capitalised in formulating drugs to treat primary infection, (say, with mannose analogues? or soluble AsNs?). Similarly, non-neutralising antibodies that purport ADE maybe targeted (for instance, by competitive inhibition of 'PreM' binding?). So, even in designing a vaccine that prevented natural infection with all the four serotypes of virus, e.g. targeting a common site for glycosylation on CDIII (viz., AsN67?). Vector control, to conclude, albeit Augean is strategy numero uno for dengue control.

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