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Review

Flaviviruses and their antigenic structure

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ABSTRACT

Flaviviruses comprise important arthropod-transmitted human pathogens, including yellow fever (YF), dengue (Den), Japanese encephalitis (JE), West Nile (WN) and tick-borne encephalitis (TBE) viruses that have the potential of expanding their endemic areas due to global climatic, ecological and socio-economic changes. While effective vaccines against YF, JE and TBE are in widespread use, the development of a dengue vaccine has been hampered for a long time because of concerns of immunopathological consequences of vaccination. Phase III clinical trials with a recombinant chimeric live vaccine are now ongoing and will show whether the enormous problem of dengue can be resolved or at least reduced by vaccination in the future.

Unprecedented details of the flavivirus particle structure have become available through the combined use of X-ray crystallography and cryo-electron microscopy that led to novel and surprising insights into the antigenic structure of these viruses. Recent studies provided evidence for an important role of virus maturation as well as particle dynamics in virus neutralization by antibodies and thus added previously unknown layers of complexity to our understanding of flavivirus immune protection. This information is invaluable for interpreting current investigations on the functional activities of polyclonal antibody responses to flavivirus infections and vaccinations and may open new avenues for studies on flavivirus cell biology and vaccine design.

Contents

1. Human flaviviruses

1.1. Impact of flavivirus diseases

Flaviviruses represent some of the most important human-pathogenic arboviruses worldwide. They form a genus of more

Abbreviations: Den, dengue; JE, Japanese encephalitis; mab, monoclonal antibody; sE, soluble E; TBE, tick-borne encephalitis; WN, West Nile; YF, yellow fever.

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than 70 different viruses in the family *Flaviviridae* and comprise the mosquito-borne yellow fever (YF), dengue (Den), Japanese encephalitis (JE), and West Nile (WN) viruses as well as tick-borne encephalitis (TBE) virus,[1] all of which have a significant impact on public health in their respective endemic and/or epidemic regions.[2] Because of their dependence on specific natural hosts, vectors and ecosystems in general, flaviviruses are not uniformly distributed but have distinct, sometimes overlapping geographical distributions.[2] The dynamic situation of ecological and climatic changes as well as factors associated with urbanization, international travel, trade and the possible adaptation of flaviviruses to new host species increase the potential of flavivirus emergence in previously unaffected regions of the world. This is most dramatically exemplified by the expansion of dengue hyperendemic areas,[3] the introduction of WN virus to New York in 1999 and its subsequent expansion in North- and South-America,[4] increased WN activity in Mediterranean countries[5] and also the detection of new infection sites of TBE virus in Europe.[6]

With respect to global disease incidence, dengue has by far the highest impact, with an estimated 50–100 million infections per year (resulting in 500,000 cases of hemorrhagic dengue fever (DHF) and/or dengue shock syndrome (DSS) with more than 20,000 deaths) and 2.5 billion people living in dengue-endemic tropical and subtropical regions.[3,7,8] In Africa and South-America, dengue areas overlap with those of YF (estimated number of annual cases 200,000[9]) and in South-East Asia with those of JE (estimated number of annual cases 50,000.[10] TBE virus, on the other hand, does not occur in the tropics/subtropics but is endemic in large parts of Europe as well as Central and Eastern Asia.[6,11]

1.2. Laboratory diagnosis

Human flavivirus infections are usually diagnosed by serology using various IgM and IgG immunoassay formats which have replaced previously used hemagglutination-inhibition and complement fixation tests (reviewed in Refs. 12,13). Because of the antigenic relationships between different flaviviruses (see Section 3.1), serological cross-reactions can pose a problem in the specific laboratory diagnosis of flavivirus infections. Especially in the case of sequential infections with different Den virus serotypes, the type-specific serodiagnosis is difficult and may require special immunoassay formats,[14] virus neutralization tests and the analysis of paired sera. Compared to serology, nucleic acid detection assays are very specific and allow precise identification of the infecting virus by sequence analysis. As a drawback of this technology in routine flavivirus diagnosis, in many instances severe symptoms leading to hospitalization develop only at the end of viremia when the virus has already reached undetectable levels.

1.3. Flavivirus vaccines

In principle, flavivirus diseases can be effectively prevented by vaccination, exemplified by the live attenuated YF vaccine,[9] both live- and inactivated JE vaccines,[10] as well as inactivated TBE vaccines,[15] all of which are in widespread use. For dengue, however, – despite its enormous public health impact – no vaccine has yet become available on the market. The major obstacle are long-standing concerns that vaccination may predispose to an exacerbation of infection by immunological enhancement phenomena also observed in sequential infections with different dengue serotypes[16,17] (see Section 3.3). This problem of dengue immunopathogenesis has been investigated extensively but is still not completely resolved.[16] Nevertheless, great efforts were made in the last decades for the development of dengue vaccines which should ideally induce life-long protection against all four serotypes. The approaches include live-attenuated, inactivated whole virus, recombinant protein, DNA as well as vectored vaccines.[17–20] Currently, the most advanced of these candidate vaccines is a tetravalent recombinant chimeric live vaccine (Chimerivax, Sanofi Pasteur) based on the yellow fever strain 17D backbone combined with the structural proteins of all four dengue serotypes.[21] Ongoing phase III clinical trials[22] will hopefully provide conclusive evidence for protection in the absence of adverse effects and eventually lead to an effective means for the immunoprophylaxis of dengue.

2. Flavivirus structure

Flaviviruses are small enveloped viruses with only three structural proteins, designated E (envelope), prM/M (precursor of membrane or membrane, respectively) and C (capsid). The first assembly products are non-infectious immature virions that contain complexes between E and prM in the viral membrane and are formed by budding into the endoplasmic reticulum (Fig. 1A, left).[23] Upon transport of these particles through the exocytotic pathway of the infected cell, prM is cleaved by the cellular protease furin in the trans-Golgi network,[24] finally resulting in the release of mature infectious viruses (Fig. 1A, right) into the extracellular fluid.

Molecular details of the flavivirus structure were resolved by X-ray crystallography of soluble forms of E and cryo-electron microscopy of immature and mature virus particles (reviewed in Ref. 25). In immature virions, E is associated with prM and forms 60 spikes of trimers of prM-E heterodimers (Fig. 1A, left).[26] The processes of virus maturation (prM cleavage) result in a complete rearrangement of E proteins in the viral envelope[27] and the formation of smooth-surfaced particles with a herringbone-like icosahedral lattice of antiparallel E dimers (Fig. 1B).[28] The ectodomain of the E dimer (soluble E; sE) lacks the trans-membrane anchor and a membrane-associated element called 'stem'; Fig. 1A, right). It is composed of three distinct domains (DI, DII, DIII), forming an elongated rod that is gently curved to accommodate the shape of the viral surface (Fig. 1C and D).

During cell infection, the E protein not only mediates receptor-binding but also fusion of the viral membrane with endosomal membranes after uptake by receptor-mediated endocytosis.[25,29,30] In this low pH-triggered process, the E dimer dissociates, exposes the highly conserved fusion peptide at the tip of DII (Fig. 1C), rearranges its domains to form a hairpin-like structure and is converted into a trimer.[31,32] Because of its essential functions in virus entry, the E protein is the major target of flavivirus neutralizing antibodies which block infection by inhibiting cell attachment, endocytosis and/or membrane fusion.[33]

In certain cell types, the cleavage of prM may be quite inefficient – especially in the case of Den viruses – resulting in the release of varying proportions of immature, partially mature, and mature particles.[34,35] The finding that partially mature virus particles are infectious[36] and that even completely immature particles can infect cells when taken up by antibody- and Fc receptor-mediated endocytosis[37] suggested a possible role of maturation cleavage in the pathogenesis of flaviviruses.[34]

3. Molecular antigenic structure

3.1. Antigenic relationships

Originally, the flaviviruses (former group B arboviruses) were grouped together on the basis of cross-reactions observed in hemagglutination inhibition assays using polyclonal sera.[38] Virus neutralization is more specific and allowed the definition of serocomplexes containing more closely related flaviviruses,[39] as displayed in Fig. 2A. The E proteins of viruses from different serocomplexes share only about 40% identical amino acids, concentrated in the interior of the protein, so that their exposed