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Dengue: An Emerging Health Challenge ?

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Definition of emerging and re-emerging infection

An "emerging infection" is defined as a new infection that has never appeared before or a known infection that has recent increase in prevalence.[1] The classic examples of emerging infectious diseases are the HIV pandemic and the severe acute respiratory syndrome (SARS)-associated Coronavirus outbreak in the 1980's and 2003, respectively. A "re-emerging infection" is a familiar infection that recurs such as tick borne encephalitis virus and the hemorrhagic fever viruses in Europe.[2] These emerging and re-emerging infectious diseases (EIDS) attract our attention because of their impact on public health and economic consequences.

II. Determinants for the increase in emerging and re-emerging viral infectious diseases

One of the important questions on EIDS of viral etiology is "Where do these viruses come from?" Three main driving forces for the emergence of these EIDS are viral factors, human factors and ecological factors.

Viral factors: Most of the viral agents that cause EIDS are RNA viruses.[1] These types of viruses are very dynamic through three fundamental mechanisms which are point mutation, recombination and reassortment. Point mutation or antigenic drift introduces a high mutation rate and is common for all types of RNA-virus. This phenomenon is likely attributable to the poor fidelity of the RNA-dependent RNA polymerase and RNA-dependent DNA polymerase (reverse transcriptase). The lack of a 3'→5' proofreading exonuclease activity generates a mutation rate of approximately 10^{-4} to 10^{-5}.[1] This rate results in 2 mutations in every 5 new genomes produced.[3] Anti-HIV drug resistance is a result of this process.

Recombination: The SARS Coronavirus is a good representative virus that uses this mechanism. Crossover between two RNA molecules occurs during co-infection, producing a hybrid genome molecule.[4-5] This mechanism requires a large genome, discontinuous transcription and transcriptionally active full and sub-genomic length RNAs.[1]

Reassortment: This mechanism is specific for viruses that harbor fragmented genomes. Co-infection with this group of virus yields progeny viruses that contain a genome set derived from the multiple parent viruses. This strategy, which is known as "antigenic shift", causes dramatic changes in the virus phenotype. The emergence of an influenza virus is a classic example of this mutation strategy.

The combination of these three mutation strategies results in a mutation rate in RNA viruses some six orders of magnitude higher than the rate in DNA viruses and eukaryotes.[6,7] Therefore, the constitution of these mutations and the immense population size makes RNA viruses evolve rapidly.

Human factors: Human factors are actually the most potent factors driving diseases emergence since microbes introduced into a new region may have a great impact depending on the susceptibility of the host population or, to a lesser extent, the maintenance of the microbes in the population.[1]

Ecological factors: Climate variability such as global warming and the El Nino Southern Oscillation are extreme climatic conditions that can be associated with habitat modification and the explosion of viral vector populations.[8,9]

III. Dengue viruses and genetic diversity

Dengue viruses are members of the family Flaviviridae, genus Flavivirus. Their genomes are organized as 5'-untranslated region-viral structural protein genes (capsid-premembrane/membrane-envelope) -viral nonstructural protein genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) -3'-untranslated region. Dengue viruses exist as four antigenically distinct serotypes, DENV1-4. Each serotype can be further phylogenetically classified into subtypes or genotypes.

Dengue disease is the most common vector-borne viral disease of humans with *Aedes* mosquitoes as the principal vector, particularly *Aedes aegypti*. The majority of infections, particular primary infections, are asymptomatic infections.[10] There are roughly 500,000 cases of DHF/DSS requiring hospitalization each year. Dengue viruses have a great impact on public health as well as an economic impact such as medical care, mosquito control, lost working hours and reduced tourism. All four serotypes can cause a severe form of life-threatening infection DHF/DSS. This form of infection is believed to be regulated by immunological factors including cross-reactive enhancing antibodies, memory T cells, cytokines/chemokines etc.[11] These factors can theoretically enhance infectivity, interfere with infected cell elimination as well as damage the vascular system.

Is dengue infection an emerging infectious disease?

There is no definitive evidence on when dengue first appeared in the human population. However, by the late

18th century, epidemics of a dengue-like fever were reported in Asia and the Americas, and by the late 19th and early 20th centuries the virus probably spread throughout the tropics and subtropics.[12] Shortly after World War II, dengue hemorrhagic fever and dengue shock syndrome, DHF/DSS, were endemic in Southeast Asia. The first well documented outbreak of DHF/DSS occurred in Manila in 1953/1954, followed by a larger outbreak in Bangkok in 1958. These severe forms of the disease have become endemic in all countries in Southeast Asia and dengue has continued to expand to more than 100 countries in the tropics and subtropics.[13] Therefore, dengue is considered a typical "emerging" disease.

Dengue virus most likely arose from sylvatic strains which circulated among non-human primates and mosquitoes and only caused sporadic outbreaks in humans.[14] Cross-species transmission from monkeys to humans did not occur until a few hundred years ago when the rapid increase in human population, widespread urbanization and modern transportation may have contributed to sufficient numbers of hosts (both humans and mosquitoes closely associated with humans) to sustain transmission in humans. At this point, the virus effectively broke-free of its sylvatic cycle and established itself as the endemic disease in human that we see today. It is believed that the early evolution of dengue virus was a continued lineage extinction, as the virus jumped to humans but then burnt out from lack of susceptible hosts. Success in cycle shifting may be partly due to the fact that dengue viruses cause a relatively benign disease in human. Therefore, a relatively low number of infected humans are required to sustain the cycle and mild/asymptomatic-viremic travelers can serve as "Trojan Horses" for global spread.

The cause of genetic variation in dengue virus.

Based on the phylogeny of NS-5 gene sequences, *Flaviviruses* can be grouped into 3 major groups which are viruses that transmitted by ticks, mosquitoes transmitted viruses and viruses with no known vector. Dengue viruses are placed in the group of mosquito-borne viruses.[15] Eventhough the closest relative of dengue virus has never been documented with certainty, evidence suggestive of this is available from reconstruction of the molecular time-scale of it evolution. Using substitution rates, the origin of the dengue virus was found to be remarkably recent, at approximately 1,000 years ago. The molecular evolution of dengue virus started about 200 years ago when DENV-4 was the first to diverge. The current diversity of DENV-2 probably arose within the last 120-200 years,[16] and DENV-1 and DENV-3 split about 100 years ago.[27] The split of DENV into 4 distinct lineages or serotypes may be due to geographic partitioning in different primate population so that the four serotypes evolved independently. Whether these genotypic diversities benefit initiation of an outbreak in certain geographical location remains unclear.

Like many other RNA viruses, dengue exhibits substantial genetic diversity, each serotype of dengue virus presents an intra-serotypic genetic diversity which can be further grouped into several genotypes. These diversities were first unfolded by Rico-Hesse in 1990 when she demonstrated a number of distinct genotypes of DENV-1 and DENV-2 using the E/NS1 region.[18] Since then the scope of genotypic diversity has greatly expanded. Currently, DENV-2 is subgenotyped into 6 genotypes which are Asian 1, Asian 2, American, American/Asian, Cosmopolitan and sylvatic genotypes.[17] Surprisingly, these genotypes often have different geographical distributions. For example, the genotypes that are found in Asian populations are the Asian 1 and 2 genotypes while the American genotype distributed mainly in the Americas and the Cosmopolitan genotype is spread across the tropics and the subtropical world.[19] Two major factors that underpin this biodiversity are mutation and recombination. Dengue virus is highly mutagenic: its RNA-dependent RNA polymerase is thought to produce approximately one error per round of genome replication.[7] The substitution rate of DENV-1 and DENV-3 is 4.55×10^{-4} and 9.01×10^{-4} per site, per year, respectively.[17] Analysis of the genome-wide substitution rate indicate that the RNA polymerase of DENV-2 introduces approximately 10 mutations per genome per year or a rate of 8.53×10^{-4} substitutions per site per year.[16] In addition to mutation, intra-serotypic recombination may be another cause of genetic diversity for dengue virus. Recombination in dengue virus most likely occurs through a copy-choice mechanism in which the polymerase switches between parental viral molecules during replication.[20] This process is facilitated by mosquitoes since *A. aegypti* is known to engage in multiple feeding and there is evidence of mixed infections.[21] Even though there is evidence that recombination can occur among dengue viruses, it is uncertain how common it really is since a high recombination rate would cause more sequence scrambling.

Although biodiversity can be generated through these two mechanisms, only variants passing through natural selection can enter populations where their genomes will be circulated and maintained. DENV circulation in various regions is characterized by frequent episodes of introduction, expansion, apparent extinction, and reintroduction of the same or different viral genotypes. This epidemiologic pattern can be regulated by at least 3 possible mechanisms.

1. Strain extinction and replacement: Phylogenetic analysis of the sequences of DENV-1 genomes confirmed that three distinct clades (from two genotypes) have circulated in Myanmar since the early 1970s. However, one of these clades (Clade A, genotype III) became extinct after 1998 and was replaced by viruses from two other clades (B and C) assigned to genotype I. Phylogenetic analysis has shown that positive natural selection at the amino acid, nucleotide or RNA secondary structure levels was not responsible for this clade extinction, but was most likely related to seasonal variation in the size of the mosquito vector population.[22] Similar rapid extinction and appearances of new genotypes of DENV-2 and -3 have been described in Thailand during inter-epidemic periods.[23,24]

2. Viral population growth: It has been reported that the growth rate of DENV differs between serotypes and this may have a significant impact on the invasion and maintenance of dengue virus types 2 and 4 in the Americas.[25] DENV-4 was first reported in the Americas in 1981 and caused epidemics of dengue fever through out the region. It should be mention that in the same year DHF caused by an Asian strain of DENV-2 was also reported. Strikingly, viral lineage numbers increased far more rapidly for DENV-4 than DENV-2, suggesting a more rapid rate of exponential growth in DENV-4 or a higher rate of geographic dispersal which allowed this virus to spread effectively among localities. The difference in viral dynamics is probably related to host immunity since in 1981 herd immunity to DENV-4 should not have existed as this serotype had not been reported in the Americas until this time. As a consequence, DENV-4 would be able to spread very rapidly due to the higher number of DENV-4- suscep-

tible individuals.[26]

3. Host immune responses: Both T and B cells play a major role on selection of circulating genotypes since sporadic positive selection site in the E gene sequence of DENV-3 and DENV-4, in some genotype of DENV-2 but not in DENV-1 were located in or near B- or T-cell epitopes, suggesting that the selection pressure was related to immune evasion.[19] In addition, immunological determinants are found to affect a transmission cycle of dengue virus.[27]

The burgeoning genetic diversity of DENV is probably a function of the hyperendemicity of the virus and this will also facilitate the production of strains with varying phenotypic properties, including virulence and transmissibility. The naturally selected variants can enter the population and have broad geographical distribution via migration or gene flow by hosts and vectors. All together, these processes may generate and facilitate geographical distributions of biological novelty.

IV. Genotypic variation and virulence of dengue virus

The prevalence of all four dengue serotypes has increased dramatically in recent years, accompanied by an increase in genetic diversity within each serotype. A rise in genetic diversity may also have important phenotypic implications, such as the emergence of viruses with altered antigenicity, virulence, or tissue tropism. Although it is not possible to establish, at this moment, a clear correlation between a particular DENV genotype and the severity of disease outcome, there are indications that certain DENV genotypes are associated with DHF more than DF and vice versa.[28,29] The classical examples are the American and Asian genotypes of dengue 2 virus. Whereas the Asian genotypes are well accepted to introduce DHF in various regions, the native American genotype is associated only with DF.[28] Another example was the epidemic of DHF in Sri Lanka in 1989 which correlated with the appearance of new variants of DENV-3 genotype III. This variant likely spread from the Indian subcontinent into Africa in the 1980 and into Latin American in the mid-1990s.[30] This variant is phylogenetically distinct from DENV-3 isolates from the outbreak in Dhaka, Bangladesh in 2000 and differs from Thailand and Myanmar isolates.[31] Another interesting example is the correlation between severe disease and evolution of dengue virus in Mexico which is another hyperendemic area with multiple introductions of foreign strains. However, the severity of dengue in Mexico increased twice, in the mid-1990 when the epidemic DHF was reported for the first time, and then again in 2001. More importantly, several dengue serotypes have been co-circulating in Mexico since the early 1980s when DHF was rare. This suggests that hyperendemicity and the simultaneous circulation of several serotypes are not sufficient by themselves to result in epidemic DHF in the absence of virulent strains.[32]

How does genotypic variation contribute to the increasing of the severity of dengue virus infection?

The lack of an appropriate experimental model for dengue viral infection has limited our understanding of sequence variation and the severity of infection. However, indirect evidence discloses at least four possible associations between genetic variation and increasing pathogenesis.

1. Genotypic variation creates variants which replicate better in target cells. The best example is the replication of the Thai DENV-2 strain (Asian genotype) in human monocyte-derived macrophage and dendritic cells as compared to replication of the native American genotype. The Thai DENV-2 strain replicates in these two cell types to higher titers than American genotype DENV-2 strain.[33,34] The difference correlates with a mutation at amino acid position 390 of the E gene.

2. Mutation generates progenies that can be transmitted by different mosquito vectors. Phylogenetic analysis showed that new waves of dengue virus in the various part of Tropics often originate from Southeast Asia, this suggests that the Southeast Asia genotype is able to adapt itself better in both sylvatic and domestic mosquitoes.[35]

3. New genotypes have a greater ability to be enhanced by heterotypic antibodies and be able to evade the immune system. The antibody enhancement hypothesis is well accepted as one of the explanation for severe dengue pathogenesis. There is some evidence that DHF-associated strains of DENV-2 cross-reacts more with a panel of DENV-4 monoclonal antibodies than DENV-2 strains associated with DF.[36] Phylogenetic analysis of DENV-2 from a severe dengue epidemic in Cuba in 1997 demonstrated various nucleotide substitution in nonstructural proteins, NS1 and NS5. It is possible that these substitutions generated CTL-escaped variants.[37]

4. Genotypic variants have a greater impact on global gene expression of the host cells. This observation comes from the investigation using genetic engineering derived dengue virus. The live attenuated vaccine candidate, DEN-2 PDK53, containing nine nucleotide mutations, exhibits small plaques, decreased replication in LLC-MK2 and C6/36 cells, lacks of neurovirulence in newborn mice, and exerts less effect on cytokines/chemokines gene expression in DENV-infected PBMC, relative to the wild-type 16681. [38,39]

In conclusion, despite the fact that dengue is one of the most prevalent viral infections in humans, the mechanisms responsible for its pathogenesis remain uncertain. Evolutionary studies of dengue virus reveal that its genetics diversity is increasing. This, coupled with the evidence that viral strains could naturally differ in virulence, suggests that in the future we might be exposed to viruses with an expanded range of pathogenic properties. Another problem that should be kept in mind is the response of these variants to future vaccination programs. Given the increasing size and mobility of the human population and the current lack of an effective vaccine, it is likely that dengue in all its forms will represent an important public health problem for many years to come.

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