RECAPITULATIONS ON CHANGES IN DENGUE VIRUS PROPERTIES AND THE AETIOLOGY OF HAEMORRHAGIC FEVER

NELIA P. SALAZAR

Department of Parasitology, Institute of Public Health,
University of the Philippines System, Manila, Philippines.

A number of significant observations were made during studies on dengue virus conducted at the Faculty of Science, Mahidol University in Bangkok, Thailand in 1973. This concerns the behavior of prototype DEN-2 New Guinea C strain in *Aedes albopictus* cell culture. Altogether the observations substantiate the hypothesis that host-induced modification of virus surface antigens occurs based on biological, immunological and electrophoretic analyses of RHA fractions and/or the products of persistently infected mosquito cells, heretofore, referred to as mosquito-adapted or modified virus. (Salazar and Thomson, 1973; Salazar, 1975).

Evidently viral maturation involves a process by which the nucleocapsid core interacts with some membranous element of the cell, thereby acquiring as it is a protein coat or envelope. Host-induced modification, therefore, appears to be the result of incorporation of host-cell membrane constituents in the viral coat.

The initial observation of host-induced modification of DEN-2 is credited to Sinarachatanant and Olson (1973) who postulated that the presence of cellular elements on the virion's surface structure accounts for its altered biological and immunological properties. These authors first demonstrated that modified virus manifest diminished lethality for suckling mice and altered plaquing characteristics. Loss of virulence *in vitro* and *in vivo* in mammalian indicator systems and diminution in immunological reactivity were likewise reported by Salazar and Thomson in the same year. Furthermore, the latter authors observed changes in electrophoretic behavior of variously derived RHA. These are summarized in Table 1.

Table 1

Changes in dengue virus properties in persistently infected mosquito cells.

	Proto type	Modified virus
Biological		
1. Virulence to suckling mice (LD_{50}) and mammalian cells (CPE)	+	–
2. Plague variant	Large	Small
3. Virus interference	–	+
Immunological		
1. CF	+	–
2. HA:pH optimum	6.4	6.0
3. PRNT : NT_{50} titer (monkey antisera)	1:640	1:350
Electrophoretic profile: PAGE analysis (RHA)		
1. V_3 M.W.53,000-59,000		↓
2. V_1 M.W. 7,700- 8,700		↑
3. V_4 ?		↑
4. Relative proportions of V_1 to V_2, & of V_4 to V_3	$V_1 < V_2$ $V_4 < V_3$	$V_1 > V_2$ $V_4 > V_3$

Earlier in these studies small and large plaques variants of DEN-2 were recovered from mosquitoes and only the large plaque variant from mammalian sources. In retrospect, this suggests that:

(i) there is a selection for small plaque and large plaque variants in insect and mammalian cells, respectively;

(ii) the large plaque variant in mosquitoes might represent newly acquired and potentially virulent particles from vertebrate hosts;

(iii) the small plaque variant represents host-modified virions in persistently infected mosquito cells. This variant produces progressively diminishing and ill-defined plaques that eventually disappear in carrier culture or latent virus infection. The apparently non-lethal (or avirulent) products of carrier culture induce virus interference and retain the capacity to react with specific antibody; and

(iv) latent virus, therefore, is not readily demonstrated by conventional methods of isolation i.e. virulence in mice and/or plaque formation. Their presence and identity, however, can be established by combined challenge virus resistance/neutralization tests.

The implication therefrom is that host-induced modification or the acquisition of host cellular antigens might accompany the maturation of endogenous virus in the endothelial and/or lymphoid tissues of man and subsequently elicit the production of antibody directed against these components, possibly along with the development of a viral carrier state. If so, this could predispose either to an auto-immune process i.e. an abrogation of self-tolerance, or in repeated infections with heterologous virus, the formation of antigen-antibody complexes which activate complement and cause the release of vasoactive substances (WHO, 1973).

There are at least three possible avenues which directly or indirectly predispose to the eventual collapse of the vascular system. These are:

(i) the attack by antibody on host cellular elements in the endothelial tissues;

(ii) the activation of complement by circulating antigen-antibody complexes and the release of vasoactive amines by mast cells, basophils, or platelets;

(iii) the elaboration of soluble factor(s) possibly kinins? by sensitized or infected lymphocytes/leukocytes which, in turn, stimulate platelets to release vasoactive amines. Kinins *per se* can cause lowered blood pressure and increased vascular permeability. Furthermore, antigen-antibody complexes have been shown to activate the plasma kinin system.

The hypothesis is illustrated in Fig. 1.

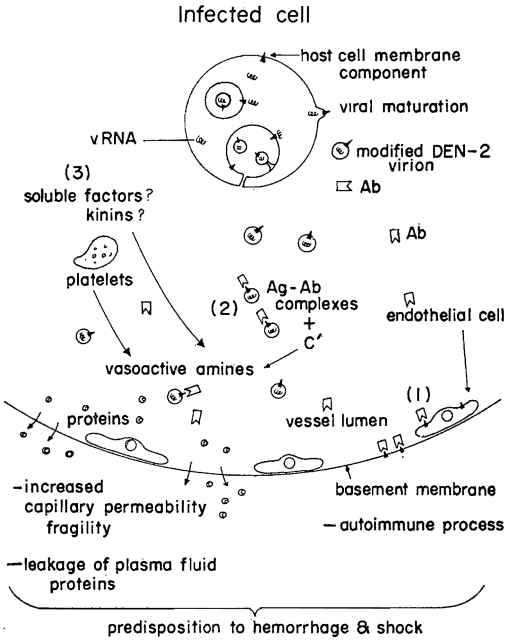

Fig. 1 Host-pathogen interactions in dengue shock syndrome.

Thus, both humoral and cellular factors in the immune response are involved in virus infection and its sequelae. By and large, these mechanisms could serve to explain the tissue damage seen in dengue shock syndrome.

The mystery of dengue haemorrhagic fever is far from being solved. (Tan *et al.*, 1968; Hammon, 1973). Instead it continues to take its toll on human life and resources.

The significance of this present knowledge in understanding the development and/or transmission of dengue virus in nature can not be overemphasized. Once more, the intriguingly diverse behavior of virus in both arthropodan and vertebrate hosts recapitulates on the phenomenon of selective adaptation wherein virus and vector might be the protagonists and man, an innocent if hapless bystander.

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