

## ARTÍCULOS ESPECIALES

SCHOOL OF HYGIENE AND PUBLIC HEALTH, JOHNS HOPKINS UNIVERSITY

### Dengue hemorrhagic fever: two infections and antibody dependent enhancement, a brief history and personal memoir

Dr. Scott B. Halstead<sup>1</sup>

**DeCS:** DENGUE/historia; DENGUE/inmunología; FIEBRE DENGUE HEMORRAGICA/historia; FIEBRE DENGUE HEMORRAGICA/inmunología; VIRUS DEL DENGUE/inmunología; AEDES; ANTICUERPOS.

**Subject headings:** DENGUE/history; DENGUE/immunology; DENGUE HEMORRHAGIC FEVER/history; DENGUE HEMORRHAGIC FEVER/immunology; DENGUE VIRUS/immunology; AEDES; ANTIBODIES.

---

#### DENGUE: EARLY HISTORY

The history of dengue since the beginning of World War II has been one of unremitting and challenging expansion, both from the standpoint of human health and science. Key events were the independent recovery of type 1 virus by Japanese and U.S. scientists.<sup>1,2</sup> Although Sabin and Schlesinger are generally credited with having made the first laboratory isolation of dengue type 1 from patients in the Honolulu outbreak of 1943<sup>2</sup>, the publication by Kimura and Hotta of the recovery of virus in mice from patients bled during the 1943 epidemic in Nagasaki preceded the report by U.S. workers.<sup>2</sup> The wartime unavailability of the Japanese medical literature has long obscured this point. Human sera collected from U.S. forces on New Guinea in 1944 led to the isolation of a different virus in mice which was called dengue type 2, the New Guinea B and C strains.<sup>3</sup>

A number of important scientific observations on dengue had been made prior to the first isolation of the virus and without serological evidence. The

description of an outbreak of “bilious remitting fever” by Rush reasonably can be attributed to dengue virus because the characteristic clinical features of dengue fever occurred in adults during the summer months of 1780.<sup>4</sup> With careful reading, the clinical features of Rush’s Philadelphia outbreak can be distinguished from Bylon’s “knokke koorts”, an outbreak of a febrile exanthem with arthralgia which occurred in Batavia, Indonesia in 1779. The latter outbreak reasonably can be attributed to chikungunya, an alphavirus, which, like dengue, is transmitted to human beings by the bite of *Aedes aegypti*.<sup>5</sup> Important pre-World War II observations not confirmed serologically, include the first outbreak of classical dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in North Queensland, Australia in 1897,<sup>6</sup> the first evidence that dengue was a filterable agent and that *Aedes aegypti* was its vector.<sup>7, 8</sup>

Several other pre-War observations have been verified by testing blood obtained from surviving patients or experimental subjects. For example, sera from survivors of the 1928 Greek epidemic, which

---

<sup>1</sup> Physician Doctor.

strongly resembles DHF/DSS, contained dengue 1 and 2 neutralizing antibodies.<sup>9</sup> American soldiers who participated in the clinical trials which established many fundamental facts about dengue disease and epidemiology were caused by dengue 4 (1922 series) and dengue 1 (1929 series).<sup>10</sup> During World War II, combatants and civilians alike were infected in large numbers across the entire Pacific theater of operations. On Saipan, dengue among U.S. Marines threatened the successful outcome of the invasion.<sup>11</sup>

### **DISCOVERY OF HEMORRHAGIC FEVER CAUSED BY DENGUE VIRUSES, 1956-1958**

A comparative lull in reports of dengue activity followed the withdrawal of major foreign forces from tropical Asia at the end of the War. This lull was broken in the mid-1950s by the unexpected recovery of dengue viruses from a “hemorrhagic fever” of children.<sup>12</sup> Hammon, who was in the Philippines to study poliomyelitis, isolated two dengue virus types new to science calling these dengue 3 and 4.<sup>13</sup> Two years later, Hammon and co-workers again recovered dengue viruses from similar cases in children in Bangkok, Thailand labeling these dengue types 5 and 6.<sup>14</sup> In Thailand, there was a complication. A significant fraction of all hospitalized cases were caused by chikungunya, an alphavirus. Many patients with “Thai hemorrhagic fever” had simultaneous serological responses to dengue and chikungunya viruses. The immediate question was “Why were dengue and chikungunya viruses suddenly causing a severe and fatal disease?”

#### **CAUSAL HYPOTHESES**

*First impressions, 1958-62.* The natural consequence of Hammon’s discoveries plus other early observations resulted in four hypotheses of the causation of hemorrhagic fever: 1) “*hemorrhagic variants*”, specifically, dengue types 3-6 were responsible; 2) *Role of chikungunya*. In Thailand, as opposed to the Philippines, a non-dengue virus seemed to be causing up to 20 % of

cases – chikungunya. It was thought that simultaneous infections with dengue and chikungunya might account for severe disease.<sup>15</sup> The possibility that chikungunya virus might have gained virulence was underscored by a report that freshly isolated strains produced hemorrhagic enteritis in suckling rodents;<sup>16</sup> 3) *Immune response*. The very first serological studies produced evidence that many patients with Thai and Philippine hemorrhagic fever (THF, PHF) experienced anamnestic or secondary antibody responses to dengue viruses. This meant that these patients had been infected previously with an antigenically related virus. Because the viral epidemiology in these countries was unknown, the initial infection could not be identified; 4) *Human genetic factor*. During the 1962 epidemic in Thailand, predominantly Caucasian resident foreign expatriates, both children and adults, suffered dengue fever, but, not THF.<sup>17</sup> It seemed possible that Caucasians were genetically resistant to severe dengue disease<sup>1</sup>.

Early confusion, 1963-1964. Observations in 1963 and 64 were sometimes contradictory. In 1963, Dasaneyavaja and co-workers reported that chikungunya virus had not been isolated from shock or fatal THF cases.<sup>18</sup> Because THF and PHF were clinically similar, and chikungunya virus did not occur in the Philippines, it seemed unlikely that chikungunya was necessary for hemorrhagic fever to occur. Halstead and Yamarat<sup>19</sup> called attention to earlier episodes of severe and fatal hemorrhagic fever associated with dengue fever outbreaks in Australia and Greece. They concluded from these reports that Caucasians could not be genetically resistant to hemorrhagic fever. Further, they reasoned that failure of Thai dengue strains to cause hemorrhagic fever in Caucasians could only be interpreted to mean that dengue viruses were not inherently virulent. A factor “somehow acquired through continuous exposure to environmental or immunologic conditions of Bangkok”<sup>20</sup> seemed more plausible. Halstead and Yamarat<sup>19</sup> called attention to a small THF outbreak in 1964 in which primary-type antibody responses predominated. Because of these data they characterized the situation as “confusing.” At the 1964 WHO conference, Hammon formulated an “immunological response” hypothesis of THF, but after

considered deliberation, discarded it in favor of the virus virulence hypothesis.<sup>21</sup>

*Increasing clarity.* The WHO Seminar on Mosquito-borne Haemorrhagic Fevers held in Bangkok, 19-24 October 1964, was notable for two events, the introduction by Halstead of the term “*dengue hemorrhagic fever (DHF)*”<sup>22</sup> and an agreement that better case definition would improve etiological classification. This was soon accomplished. In 1966, Cohen and Halstead<sup>23</sup> published their classical study on dengue shock syndrome<sup>2</sup> describing clearly for the first time its underlying pathophysiology as the leakage of fluid and protein through damaged capillaries<sup>2</sup>. This led to the introduction of logical and successful principles of resuscitation.<sup>23</sup> The case definitions made possible by the description of *dengue shock syndrome (DSS)* almost immediately led to a breakthrough in understanding the etiology of DHF/DSS. In their next classical report, Halstead et al.<sup>24</sup> documented the strong correlation between a secondary-type dengue antibody response and dengue shock syndrome. When this paper was read in 1966, immunological research had only recently made it possible to distinguish primary and secondary immune responses based upon immunoglobulin type.<sup>25</sup>

## TWO INFECTIONS DOCUMENTED

In areas where multiple types of dengue viruses are circulating simultaneously, it is possible to obtain extremely solid evidence for the role of infection sequence by comparing the prevalence of secondary-type antibody responses in DHF/DSS with that in milder dengue illnesses as controls. Two important conditions must be applied: 1. cases must demonstrate clinically significant vascular permeability and 2. cases must be one-year and older. The special case of infants less than one year will not be discussed at length in this paper.

Data from DHF/DSS cases could not answer the question whether severe disease was associated with second, third or fourth dengue infections. This required pre-illness sera. Very few DHF/DSS cases were hospitalized early enough for their sera to retain pre-illness attributes. The first attempt at solving this problem was to compare

observed age specific DHF/DSS hospitalization rates with second, third and fourth dengue age-specific infection rates generated from a mathematical model.<sup>26</sup> Only the second dengue virus infection rate curve fit data for DHF/DSS hospitalizations (fig.1). Of interest, this model predicted there would be 58.5 DHF/DSS cases per 1 000 secondary dengue infections. This is very close to ratios calculated from prospective studies (see below).<sup>27, 28</sup>

It was obvious that only a study format in which children were followed from their first through successive infections could determine if a second, third or fourth infection resulted in DHF/DSS. Pioneer studies were conducted on Koh Samui Island, Thailand, in 1966 and 1967.<sup>29-31</sup> In 1966, 336 children, ages 2-12 years were bled pre- and post-rainy season and their sera tested for dengue HI antibodies. During the interim, cohort children were observed clinically.<sup>29, 30</sup> Six cohort children experienced an illness; 2 and 1 had undifferentiated febrile illnesses with primary and secondary-type infections, respectively. Shock was observed in three, each of whom circulated pre-illness dengue HI antibody. In the study as a whole, no cases of shock were observed in 26 primary infections while 3 DSS cases were observed in 83 secondary dengue infections (36.1 DSS/1000 secondary dengue infections). Dengue type 2 viruses predominated among isolations from DHF/DSS cases.<sup>30</sup> The following year, DHF/DSS broke out in a different part of the island.<sup>31</sup> This time dengue 4 was isolated from cases and again, DSS occurred only among children experiencing a secondary-type antibody response. This was true despite evidence that primary infections occurred more frequently than secondary among the general population. It was still not clear whether DHF/DSS occurred only during a second dengue infection. Many years were to pass before this question was answered by direct observation.

## EXPLANATORY HYPOTHESES: ANTIBODY-DEPENDENT ENHANCEMENT

*Immune Enhancement: Early studies.* Immune enhancement of dengue virus replication was established in two papers published in 1973.<sup>32, 33</sup>

The first described the increased growth of dengue 2 virus in cultures of peripheral blood leukocytes (PBL) obtained from dengue-immune rhesus monkeys<sup>32</sup> (fig. 2). The second described enhanced levels of viremia in monkeys during secondary as compared with primary dengue 2 infections.<sup>33</sup> The mechanism underlying the phenomenon of “immune enhancement of dengue virus infection” was unclear. But, it seemed possible that viruses were replicating in memory T lymphocytes which had been transformed by dengue antigen to form lymphoblasts. The replication of viruses in phytohemagglutinin (PHA) transformed T lymphoblasts was a well documented phenomenon at the time.<sup>34</sup>

Earlier epidemiological evidence had pointed to two groups of human beings who were at risk to DHF/DSS; children experiencing second dengue infections and infants born to dengue-immune mothers who experienced their first dengue infection.<sup>35</sup> The most plausible mechanism which tied together these two observations was antibody which somehow modulated dengue infection. Shortly thereafter, it was demonstrated that dengue antibody, at non-neutralizing concentrations, enhanced dengue infections in cultured human and rhesus PBL.<sup>36</sup> Finally, the unique role played by mononuclear phagocytes in dengue infections was established<sup>4</sup>, first, in supporting dengue virus replication and second, in permitting enhanced infection in the presence of infectious immune complexes.<sup>37</sup> Optimal conditions for in vitro infection enhancement were described.<sup>38</sup> It was possible to enhance dengue 2 viremia in rhesus monkeys circulating small concentrations of passively transferred human dengue antibody.<sup>39</sup> Other laboratories began to study “antibody-dependent enhancement (ADE)”.<sup>40</sup> Although enhanced viremia has been demonstrated in humans during secondary compared with primary dengue 3 infections,<sup>41</sup> the strongest published evidence to date is the correlation between concentrations of dengue antigen-antibody complexes in acute phase sera and the severity of DHF/DSS<sup>42</sup> and viremia titer during the early febrile phase of illness.<sup>43</sup> The modern hypothesis of DHF/DSS pathogenesis assigns enhancing and neutralizing antibodies an afferent role in up- or down-regulating dengue infection in mononuclear phagocytes, while an

efferent role is played by T-cell mediated immunity which is generated to eliminate dengue-infected cells produces cytokines which mediate vascular permeability and abnormal hemostasis.<sup>44</sup> There is wide, if not unanimous, agreement that this is the best explanatory model for the pathogenesis of DHF/DSS.<sup>45</sup>

#### COMMENTS

1. To an extent unusual in science, the major observations which established dual and contradictory roles for antibody both in protecting and harming human beings during an infectious disease were made by research groups under the direction of a single individual. To have lived this history has been a privilege, exciting and rewarding. There are still vivid memories of being drafted into the U.S. Army Medical Corps, and receiving an assignment beyond my wildest imagination to a major medical research laboratory located on the outskirts of Tokyo, Japan. It was there that I learned virology at the bench and in the field working at the time with Japanese encephalitis virus and, in due course, dengue 1 and 2 viruses. While in Japan, 1957-59, I learned of reports of a “hemorrhagic fever” occurring in the Philippines in 1956. At the time, it was assumed that this was another outbreak of what was then called Korean hemorrhagic fever – now known to be a hantaviral disease. In 1958, the author’s Department at the 406<sup>th</sup> Medical General Laboratory, received specimens from the hemorrhagic fever cases in Bangkok. The 8<sup>th</sup> U.S. Army had jurisdiction over U.S. Forces throughout the Asia-Pacific theater. There are scenarios which might have resulted in my having been ordered to Bangkok to work up this new disease.

That didn’t happen, in part, because William (Bill) McD. Hammon, a distinguished founder of the field of arbovirology, was a prominent member of the Armed Forces Epidemiology Board (AFEB). The AFEB provided funds to civilian laboratories enabling them to work on infectious disease problems of the military. It was with these funds and through his connections with USAID officials that Bill Hammon arrived in Bangkok in 1958 before the hemorrhagic fever epidemic was over. While I was in Japan, Hammon’s group had isolated and

characterized two new dengue viruses from their earlier studies in the Philippines and named them types 3 and 4. The implication of finding new dengue virus types was clear. Virulent dengue strains were causing a hemorrhagic disease. By 1959, Dr. Hammon reported early evidence that two other new dengue viruses were circulating in Thailand.

Bangkok might never have materialized as an assignment for me except for the coincidence of my friendship with Gene Gangarosa. I met Gene while I was assigned to the Department of Virus and Rickettsial Diseases, Walter Reed Army Institute of Research (WRAIR), Washington, D.C. He was assigned to the Department of Bacteriology, was interested in cholera and had been on temporary duty (TDY) to Bangkok in 1959. There he joined a U.S. Navy team studying the cholera which had broken out in epidemic form in 1958. Gene had brought with him the "Crosby capsule," an intestinal biopsy device invented by Dr. William Crosby, Chief of Hematology at WRAIR. The Crosby capsule allowed Gangarosa to obtain the first intestinal mucosa tissue from living cholera patients. Tissue sections showed scarring of duodenal mucosa and blunting of intestinal villi. Unfortunately, no controls had been biopsied. Dr. Gangarosa needed someone to assist him obtain intestinal biopsies from controls during his planned return trip to Bangkok, May – July 1960. I was available and volunteered to join the group.

While in Bangkok I was introduced to Dr. Charas Yamarat, Chair, Department of Microbiology, School of Public Health, University of Medical Sciences. The School of Public Health, which had been built partly with Rockefeller Foundation money, was located on Rajavithi Road next to Bangkok Children's Hospital and across the street from the Royal Thai Army Institute of Pathology, headquarters of the SEATO Cholera Laboratory. During my stay, I obtained a few acute and convalescent sera from Thai hemorrhagic fever patients and reached a tentative agreement to return to Bangkok as Chief of a new SEATO Virology Department which would be located in the School of Public Health. On my return to Washington, the acute phase sera yielded a chikungunya virus. In the process of preparing a seed virus by inoculating suckling mice

intracerebrally, I accidentally impaled my finger on a 25 guage needle. Three days later, I noted lumbar pain, a macular rash and sudden onset of fever. I was rushed to Walter Reed Army Hospital, because everyone thought I had "hemorrhagic fever." The most memorable aspect of my hospitalization was the 50 ml of blood taken every day to perform clinical and hematological studies. Back in the lab, I discovered that mice, hamsters and rats inoculated with chikungunya developed severe intestinal hemorrhages. This finding was subsequently reported in *Science*.<sup>16</sup> It was difficult at that time not to think that SE Asian viruses had a special hemorrhagic potential.

Once I arrived in Bangkok in September 1961, faculty members of the Department of Microbiology were integrated into a joint research group which was created with funds from the newly established SEATO Medical Research Laboratory (SMRL). Thai physicians, medical technicians and nurses all became members of the joint SPH-SMRL Virology Department. In Bangkok, I began my lifelong friendship with Dr. Suchitra Nimmannitya, then a junior pediatrician at Bangkok Children's Hospital, and now a world authority on clinical aspects of DHF/DSS. We agreed to a longitudinal collaborative study of out-patients, in-patients and surgical patients as controls. We hired nurses who visited the hospital daily to collect blood samples and clinical data. A notable feature of Thai hemorrhagic fever was its local name, "Chinese medicine poisoning" and the fact that foreigners developed not THF, but dengue fever. Because I had set up a virology diagnostic service for Americans who attended the U.S. Embassy Medical Clinic and ultimately extended this service to all expatriates living in Bangkok, we knew a lot about dengue in expatriates.

I suppose I have always had an aptitude for epidemiology. Field research on Japanese encephalitis (where I measured the ratio between infection and clinical disease for the first time) had schooled me in its methods and enormous value. I soon met a European social scientist who had just helped design the country's first modern census (1960). From this contact, I gained access to census tract maps. Using a random numbers table and census tract numbers, 20 tracts were chosen randomly. By the end of 1961, we had hired a team

of field nurses, secured large scale police maps for each census tract and began to define the study population. Ultimately, we dropped one site, thus, maintaining 19 study areas, each of 200-250 households, for a total study population of 44,000. Every house was assigned a unique number, residents were censused and assigned study numbers. It was planned to bleed a 10% random sample every six months. But, after a chickenpox epidemic broke out in one of the study sites, Chinese residents bolted their doors and wouldn't answer the knocking of our nursing teams. There were even episodes of householders cursing and throwing stones at our nurses. Traditional Chinese believe in the magical properties of blood. Even though we were taking only finger tip blood specimens, they blamed us for causing the chickenpox!

2. Although the Bangkok dengue field study was ultimately extremely useful, two other events really determined the outcome of my studies on Thai hemorrhagic fever. The first was the unexpected arrival in Bangkok in 1963 of Sanford Cohen, M.D., a newly drafted U.S. Army pediatrician trained at Johns Hopkins. "Sandy" had heard about Thai hemorrhagic fever and wanted to see cases for himself. We became friends and I agreed to support a clinical study which Sandy would lead when he returned in 1964. The second important event was the arrival of Dr. Wilbur Downs of the Rockefeller Foundation late in 1963. Wil agreed to call on the Permanent Secretary of the Ministry of Public Health along with Dr. Yamarat and me to ask permission to set up a special hemorrhagic fever clinical research ward. This would be under the medical direction of a foreign physician, a problem under Thai law. Ultimately, we provided Dr. Cohen with the able assistance of two Thai physicians. Both became famous in later life: Dr. Aree Valyasevi, a pediatrician on the faculty of Siriraj Hospital, ultimately, was the founding dean of Ramthibodhi Hospital Medical School and a Magsaysay Award winner and Dr. Chaiyan Kampanartsanyakorn, became among other jobs, Deputy Mayor of Bangkok.

One day in September 1964, while we were compiling data for the October WHO meeting, Sandy came to me and said' "Scott, we have two different clinical syndromes in this study." I went

back to the serology data on these patients analyzing them separately, and the rest is history.

The next experience of importance was in 1966, one year after I had left Bangkok and gone to the Yale Arbovirus Research Unit to work up my data set and write papers. I was greatly bothered by the occurrence of a substantial group of primary-type antibody responses in infants less than one year. I obtained Army orders for TDY to Bangkok specifically to "get rid" of these cases. They were ruining the two-infection data which were so clear in the now analyzed large Children's Hospital data set. In Bangkok, I reviewed some 80-100 clinical charts of infant cases, spoke with pediatricians and at length with the remarkable pathologist at Children's and Women's Hospital, Dr. Kamolwat Vinijchaikul. In those days, high quality medicine was rated by autopsy percentage. Thai medicine, very much under the influence of American academic standards, aspired to high autopsy rates. None were higher than at Children's and Women's Hospitals, a Ministry of Public Health complex, not then affiliated with any medical school. Because autopsy rates were in the high 90 %s during 1962-65, I was able to review many autopsy records on infants less than one year who had died of Thai hemorrhagic fever. Their gross and microscopic findings were identical to those of older children.

Infants less than one clearly developed classical DHF/DSS during a primary dengue infection. The hypothesis had to be expanded. But, how? Experimental work in monkeys provided the answer.

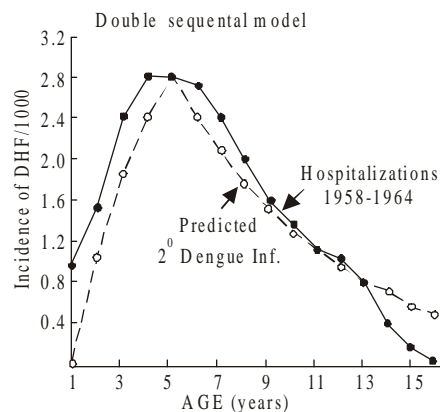
3. My stay at the newly opened Department of Epidemiology and Public Health, Yale University School of Medicine was fortuitous because John Paul and Dorothy Horstmann, of polio fame, owned a collection of rhesus monkeys and had just constructed a large monkey holding facility. I was able to gain access to these monkeys without charge. Soon, I obtained AFEB funding to hire a technician and started to develop a monkey model of DHF/DSS. Monkeys were infected with dengue viruses in different sequences. I used every combination of two sequential infections – there are twelve. Every day for two years, I bled 10-20 monkeys, personally performed hematocrit, platelet and white blood cell counts and saved samples for

liver chemistries and prothrombin times. A portion of each plasma sample was tested for virus titer and ultimately for HI and neutralizing antibodies. One animal infected with DEN 4 then DEN 2 developed laboratory evidence of increased vascular permeability and thrombocytopenia.<sup>30</sup> At the time it seemed that monkeys just didn't develop severe disease following two dengue infections.

In 1968, I left the Army and Yale and went to the University of Hawaii to start a new Department in a new medical school. With no funds whatsoever, it took some time to get back into dengue research. Funds for rubella research and a special grant from the Defense Department got us started. We had access to a large monkey colony used for Navy microwave research. Our plan, this time, was to study the organ and cellular distribution of dengue virus in monkey tissues during first and second infections. One day in early 1973, Dr. Nyven Marchette reported virus isolation and fluorescent antibody results on a monkey with a secondary DEN 2 infection, "I have never seen so much virus in the tissues." This happened virtually on the same day that virus assays were coming off from a pioneering immunology study. Joyce Chow, a PhD candidate in the Department of Tropical Medicine and Medical Microbiology, had reported the absence of thymidine uptake following PHA treatment of peripheral blood leukocyte cultures. The cultures had been inoculated with undiluted live dengue virus. However, blast transformation did occur in PHA-treated PBL previously inoculated with a 1:10 or higher dilution of virus. I wondered if dengue virus might be growing in these cells and destroying their ability to respond to PHA. Assays of the PBL cultures showed that indeed DEN 2 virus was growing. These two results led me back to unanalyzed viremia data for 118 Yale rhesus monkeys, each infected separately with four different dengue viruses and thereafter with various combinations of sequential infections. Data had been entered in painstaking detail on large accounting sheets by Henry Shotwell, my laboratory technician at Yale. DEN 2 viremia titers in secondarily-infected monkeys were higher than in monkeys with a primary infection using the same virus given at the same dose and by the same route!

*The immune enhancement hypothesis was born.* Amazingly, it all happened at once. Enhanced viremia titers were observed in monkeys experiencing secondary dengue 2 infections and DEN 2 virus grew in cultured peripheral blood leukocytes from immune but, not from susceptible monkeys. Everything fit!

4. The final chapter in hypothesis-making was the step by step realization that it was not T or B lymphocytes which supported dengue virus replication, as we originally believed, but, mononuclear phagocytes – monocytes and macrophages. This became clear while I was on sabbatical leave in the laboratory of Dr. Anthony Allison, Clinical Research Centre, Medical Research Council, London, England, in 1975-76. There I was able to use protein-coated silica particles to selectively kill mononuclear phagocytes, a technique pioneered by Dr. Allison. Phagocytic cells ingest silica particle, which dissolve releasing silicic acid that destroys the cell. This fact can be recognized immediately using a vital-stain, acridine orange. It became immediately apparent that IgG antibody, whether derived from an initial dengue infection or transferred from mother to infant, could control the afferent kinetics of dengue virus infection and, ultimately, the severity of the resultant disease. Antibody-dependent enhancement of dengue infection became a unified explanatory hypothesis.



Annual Dengue Trans. = 15%  
 D1/D2/D3 = 1/2/1  
 T=5  
 SF=12.1  
 Rate at Age 16 = .51/1000  
 Width Exp./width Obs. = 0.89

Fig. 1. Predicted age-specific second dengue infection rates compared with observed Bangkok age-specific DHF/DSS hospitalization rates in a model in which three dengue virus types are circulating in a population at an average annual infection rate of 15%. A restriction of two infections occurring within five years was imposed to achieve a best fit. Recent data from Cuba have shown there is no restriction on the period of sensitization following a first dengue infection. Age specific hospitalization rates are shaped by the fact that children to age 15 are inherently more susceptible to DHF/DSS during second dengue infections than are adults (From Fischer DB and Halstead SB. Yale J Biol Med 1970;42:329-49, with permission).

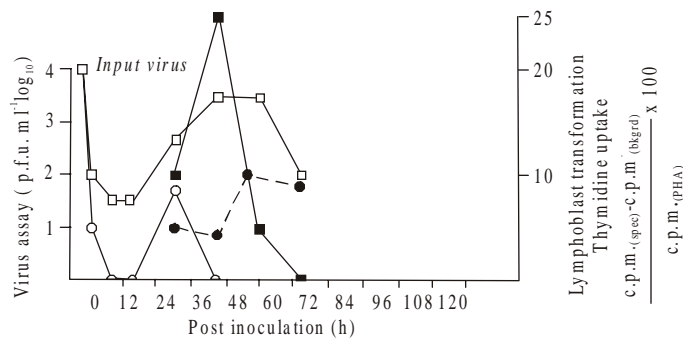


Fig. 2. Immunological enhancement of dengue infection in peripheral blood leukocytes. Dengue-2 growth (open squares) and lymphoblast transformation of leukocytes (solid squares) from a dengue-4-immune rhesus monkey compared with the same monkey before infection (open and closed circles). (From Halstead SB *et al.* Nature New Biology 1973;243:24-6, with permission).

## REFERENCES

- Kimura R, Hotta S. On the inoculation of dengue virus into mice. *Nippon Igaku* 1944;(3379):629-33.
- Sabin AB, Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice. *Science* 1945;101:640-2.
- Schlesinger RW, Frankel JW. Adaptation of the New Guinea B strains of dengue virus to suckling mice and to adult Swiss mice. *J Immunol* 1956;77:352-63.
- Rush B. An account of the bilious remitting fever as it appeared in Philadelphia, in the summer and autumn of the year 1780. *Med Inquires & Observations*;1789, pp 89-100.
- Pepper OHP. A note on David Bylon and dengue. *Ann. Med History* 1941;3:363-8.
- Hare FE. The 1897 epidemic of dengue in North Queensland. *Australasia Med Gaz* 1898;17:98-107.
- Ashburn PM, Craig CF. Experimental investigations regarding the etiology of dengue fever. *J Inf Dis* 1907;4:440-75.
- Bancroft TL. On the etiology of dengue fever. *Australasian Med Gaz* 1906;25:17-8.
- Halstead SB, Papaevangelou G. Transmission of dengue 1 and 2 viruses in Greece in 1928. *Amer J Trop Med Hyg* 1980;29:635-7.
- Halstead SB Etiologies of the experimental dengues of Siler and Simmons. *Amer J Trop Med Hyg* 1974;23: 974-82.
- Capps RB. Dengue in Internal Medicine in World War II. VII. Infectious Diseases Coates JB Jr, Havens WP (eds). Washington DC:Office of the Surgeon General, Department of Army; 1963:59-78.
- Hammon WMcD, Rudnick A, Sather GE. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. *Science* 1960;131:1102-3.
- Hammon W McD, Rudnick A, Sather G, Rogers KD, Morse LJ. New hemorrhagic fevers of children in the Philippines and Thailand. *Trans Assoc Amer Phys* 1960; 73:140-55.
- Hammon WMcD, Sather GE. Virological findings in the 1960 hemorrhagic fever epidemic (dengue) in Thailand. *Amer J Trop Med Hyg* 1964;13:629-41.
- Hammon WMcD. Discussion. SEATO Medical Research Monograph No. 2. Symposium on Haemorrhagic Fever, 110-11 August, 1961. SEATO Bangkok, 1961. pp 125-6.
- Halstead SB, Buescher EL. Hemorrhagic disease in rodents infected with virus associated with Thai hemorrhagic fever. *Science* 1961;134:473-6.
- Halstead SB, Yamarat C, Scanlon JE. The Thai hemorrhagic fever epidemic of 1962. A preliminary report. *J Med Assn Thailand* 1963;46:449-66.
- Dasaneyavaja A, Robin Y, Yenbutra D. Laboratory observations related to prognosis in Thai hemorrhagic fever. *J Trop Med Hyg* 1963;66:35-41.
- Halstead SB, Yamarat C. Recent epidemics of hemorrhagic fever in Thailand. Observations related to pathogenesis of a "new" dengue disease. *Amer J Pub Health* 1965;55:1386-95.
- Halstead SB, Yamarat C. Hemorrhagic fevers of Southeast Asia. *Proc Seventh Int Cong Trop Med Mal.* Vol III. Rio d Janeiro: 1963:297-8.
- Hammon WMcD. Immunological response: Possible role of human response as an etiologic factor. *Bull WHO* 1966;35:55-6.
- Halstead SB, Thirayodhin P, Olsson RA. Inquiry into hereditary factors in the pathogenesis of dengue haemorrhagic fever: A preliminary note. *Bull WHO* 35:56-57, 1966 (presented WHO Seminar, 19-24 October 1964).
- Cohen SN, Halstead SB. Shock associated with dengue infection. I. Clinical and physiologic manifestations of dengue hemorrhagic fever in Thailand, 1964. *J Ped* 1966;68:448-56.
- Halstead SB, Nimmannitya S, Yamarat C, Russell PK. Hemorrhagic fever in Thailand: Newer knowledge regarding etiology. *Jap J Med Sci Biol* 1967;20S:96-102.
- Russell PK, Udomsakdi S, Halstead SB. Antibody response in dengue and dengue hemorrhagic fever. *Jap J Med Sci Biol* 1967;20S:103-8.
- Fischer DB, Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. V. Examination of age specific sequential infection rates using a mathematical model. *Yale J Biol Med* 1970;42:329-349.
- Guzmán MG, Kourí G, Valdés L, Bravo J, Halstead SB. Enhanced severity of secondary dengue 2 infections occurring at an interval of 20 compared with 4 years after dengue-1 infection. *PAHO J Epidemiol* 2002;81:223-7.
- Guzmán MG, Kourí G, Valdés L, Bravo J, Halstead SB. Effect of age on outcome of secondary dengue 2 infections. *Int J Infect Dis* 2002;6:118-24.
- Winter PE, Yuill TM, Udomsakdi S, Gould E, Nantapanich S, Russell PK. An insular outbreak of dengue hemorrhagic fever. I. Epidemiologic observations. *Amer J Trop Med Hyg* 1968;17:590-9.
- Russell PK, Yuill TM, Nisalak A, Udomsakdi S, Bould DJ, Winter PE. An insular outbreak of dengue hemorrhagic fever. II. Virologic and serologic studies. *Amer J Trop Med Hyg* 1968;17:600-8.
- Winter PE, Nantapanich S, Nisalak A, Udomsakdi S, Dewey RW, Russell PK. Recurrence of epidemic dengue hemorrhagic fever in an insular setting. *Amr J Trop Med Hyg* 1969;18:573-9.



32. Halstead SB, Chow J, Marchette NJ. Immunologic enhancement of dengue virus replication. *Nat New Biol* 1973;243:24-6.
33. Halstead SB, Shotwell H, Casals J. Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *J Inf Dis* 1973;128:15-22.
34. Wheelock EF, Edelman R. Specific role of each human leukocyte type in viral infections. 3. 17D yellow fever virus replication and interferon production in homogeneous leukocyte cultures treated with phytohemagglutinin. *J Immunol* 1969;103:429-36.
35. Halstead SB, Nimmannitya S, Cohen SN. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J Biol Med* 1970;42:311-28.
36. Halstead SB, O'Rourke EJ. Antibody enhanced dengue virus infection in primate leukocytes. *Nature* 1977;265:739-41.
37. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 1977;146:201-17.
38. Halstead, O'Rourke EJ, Allison AC. Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection *J Exp Med* 1977;146:218-29.
39. Halstead SB. In vivo enhancement of dengue infection with passively transferred antibody. *J Infect Dis* 1979;140:527-33.
40. Porterfield JS. Antibody-dependent enhancement of viral infectivity. *Adv Virus Research* 1986;31:335-55.
41. Gubler DJ, Suharyono W, Lubis I, Eram S, Sulianti Saroso J. Epidemic dengue hemorrhagic fever in rural Indonesia. I. Virological and epidemiological studies. *Am J Trop Med Hyg* 1979;28:701-10.
42. Halstead SB. Pathogenesis of dengue: challenge to molecular biology. *Science* 1988;239:476-81.
43. Vaughn DW, Green S, Kalayanaroj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;181:2-9.
44. Halstead SB. Immunological parameters of togavirus disease syndromes. In *Togaviruses: Biology, Structure, Replication*, Schlesinger RW (ed). New York:Academic Press; 1980. pp. 107-73.
45. Kurane I, Ennis FA. Cytokines in dengue virus infections: role of cytokines in the pathogenesis of dengue hemorrhagic fever. *Sem Virol* 1991;5:443-8.

Recibido: 23 de abril de 2002. Aprobado:19 de julio de 2002.

Dr. *Scott B. Halstead*. Senior Scientist. Department of Molecular Microbiology and Immunology. School of Hygiene and Public Health, Johns Hopkins University 615 N. Wolfe St Baltimore, MD 21205.