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Current Research on Dengue

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Introduction

Dengue is the most important arbovirus disease of humans, in terms of both morbidity and mortality (1). Since the end of World War II, the incidence of dengue disease has increased greatly. Coincident with that increase has been the emergence and spread of a severe and fatal form of the disease, dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), as a major public health problem in many areas of the tropics. Today DHF is a leading cause of hospitalization and death among children in many countries of Southeast Asia (2), and in recent years it has become increasingly important in the Pacific Islands and the Americas.

Three factors are primarily responsible for the increased epidemic dengue activity. First, there has been a continuous trend toward urbanization in many areas of the tropics, especially in Asia, since World War II (3). Second, there has been a near complete breakdown of effective mosquito control in these same urban centers, making conditions ideal for transmission of domestic mosquito-borne diseases. Last, with the advent of the jet airplane in the early 1960s, there has been a consistent increase in air travel by man, and this has provided the ideal mechanism for transportation and spread of dengue viruses between population centers of the world. Thus, viremic humans are primarily responsible for introduction of new dengue virus strains and serotypes into areas made permissive by lack of mosquito control. It is unlikely that this set of circumstances, which is so favorable to the spread of epidemic dengue, will change in the near future. Consequently, the prospects are good for continued and frequent epidemic dengue and DHF in most populated areas of the tropics.

The increased importance of dengue as a public health and economic problem in recent years has led to an expansion of research on this disease. Major advances have been made in our knowledge of the epidemiology

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of dengue infection, in diagnostic methodology, and in molecular virology. There is still considerable controversy, however, about what the important risk factors are in the pathogenesis of DHF. In the area of prevention and control, most effort has been directed toward developing vaccines as well as more effective surveillance that can provide early warning for epidemic transmission. Space limitations prevent a comprehensive review of research on dengue viruses in this chapter. Rather, the purpose is to highlight briefly areas that are important to our understanding of dengue viruses and to development of more effective methods for diagnosis, prevention, and control.

Epidemiology

Natural History of Dengue Viruses

Early work in the Philippines demonstrated that nonhuman primates were infected with dengue viruses in nature, leading to speculation that the natural maintenance cycle of dengue viruses involved nonhuman primates and forest mosquitoes in the jungles of Asia (4, 5). Subsequently, extensive field work was done in Malaysia over a 20-year period, eventually documenting that dengue viruses were maintained in the forests of Malaysia in a cycle involving canopy-dwelling *Aedes (Finlaya) niveus* complex mosquitoes and monkeys (5–7). Evidence for this jungle cycle was as follows: (1) A high percentage of wild monkeys (68%) had flavivirus antibodies, primarily against dengue and zika viruses, (2) natural dengue virus infection was demonstrated in sentinel monkeys in the forest, both by isolation of virus (dengue 1, 2, and 4), and by seroconversion (dengue 1, 2, and 3), (3) dengue 4 virus was isolated from a pool of *Ae. (F.) niveus* collected from the canopy of the forest. It was concluded that dengue in peninsular Malaysia exists in a silent enzootic jungle cycle involving canopy-dwelling mosquitoes of the *Ae. (F.) niveus* complex and monkeys, in a rural endemic cycle involving *Ae. (Stegomyia) albopictus* and humans, and in an urban cycle involving *Ae. (S.) aegypti* and humans.

Further evidence of a forest maintenance cycle for dengue viruses has been obtained by French workers in Vietnam and Africa. In Vietnam, dengue virus of an unknown serotype was isolated from *Ae. (F.) niveus niveus* collected in a forest area (8). In West Africa, the evidence is stronger. Over 300 dengue 2 viruses were isolated from wild-caught mosquitoes in 1980 and 1981 (1, 9, 10). The isolates were from five species of mosquitoes, *Ae. (Stegomyia) africanus, Ae. (S.) leuteocephalus, Ae. (S.) opok, Ae. (Diceromyia) taylori,* and *Ae. (D.) furcifer*. Furthermore, two of the isolates were from pools of male mosquitoes, suggesting that transovarial transmission may play a role in the natural maintenance of dengue viruses in certain situations. This aspect of dengue ecology is discussed in more detail below.

In the Americas, evidence of a forest maintenance cycle for dengue viruses is more circumstantial because field studies have not yet been carried out. Nevertheless, dengue 2 neutralizing antibody has been detected in Ayoreo Indians living in the remote Rincón del Tigre area of Bolivia. These persons had not traveled outside the area, and *Ae. aegypti* was not present in that part of the country (11). These infections may be part of a forest cycle similar to that observed in Asia and West Africa. Other, more circumstantial evidence suggests that *Ae. (Gymnometopa) mediovittatus* may be involved in a natural maintenance cycle for dengue on some Caribbean islands (12). This species is a forest mosquito that has moved into the peridomestic environment and shares many larval habitats with *Ae. aegypti*. *Ae. mediovittatus* is a very common mosquito in rural and suburban communities of Puerto Rico where dengue virus transmission has been maintained continuously for over 11 years (San Juan Laboratories, CDC, unpublished data). Field studies have shown that *Ae. mediovittatus* feed avidly on humans, and their biting activity cycle is similar to that of *Ae. aegypti,* with peaks of activity in the morning right after daybreak and late in the afternoon. Laboratory studies have shown that *Ae. mediovittatus* has a significantly higher susceptibility to oral infection with dengue viruses than *Ae. aegypti,* and that they can transmit the virus both horizontally by bite to vertebrate hosts, and vertically, through the eggs to their progeny (12).

The role of transovarial transmission in the maintenance cycles of flaviviruses has been reinforced in recent years by laboratory and field studies that have shown that Koutango, dengue, yellow fever, Japanese encephalitis, Kunjin, Murray Valley encephalitis, and St. Louis encephalitis viruses can all be transmitted transovarially by certain species of mosquitoes (13–19). Of special interest is the fact that transovarial transmission of dengue 2 and dengue 4 has been documented in nature. The first evidence of vertical transmission in nature was obtained by French workers in West Africa. Dengue 2 was isolated from a pool of male *Aedes (furcifer) taylori* collected in the forest of Ivory Coast (9). According to Rosen *et al.* (19), these same French workers also isolated dengue 2 from another pool of *Ae. taylori* collected in Senegal. In Burma, dengue 2 was isolated from 5 of 199 pools of *Ae. aegypti* larvae (13,930 mosquitoes) collected from natural breeding containers in Rangoon (20). Two of the isolates were from pools of male mosquitoes that had been reared to adult stage with the sexes separated before testing. In Trinidad, dengue 4 was isolated from 1 of 158 pools (10,957 mosquitoes) of adult *Ae. aegypti* collected as eggs (21). By contrast, no dengue virus was isolated from over 8000 larvae (80 pools) collected from breeding containers in six locations of Jakarta, Indonesia in 1977 (D.J. Gubler, unpublished data) or from over 5000 larvae collected in Bangkok, Thailand (22).

In the laboratory, it has been shown that although transovarial transmission of dengue viruses occurs in *Ae. aegypti,* the filial infection rate is very low compared to that of other species of *Aedes* (19). Also, this

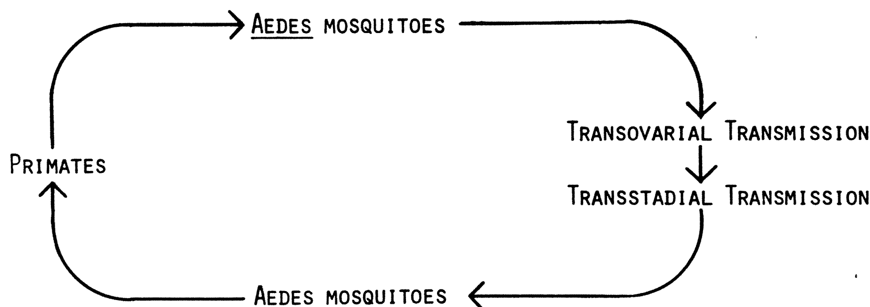
species has a lower oral susceptibility to dengue viruses than other species, including *Ae. albopictus* and *Ae. mediovittatus* (12, 23–26). Collectively, the data suggest that *Ae. aegypti* is a less efficient host for dengue viruses than certain species of forest *Aedes* (19).

There is no doubt that *Ae. aegypti* is the most important epidemic vector of dengue and DHF, primarily because of this species' highly domesticated habitats and close association with humans. Because of its lower susceptibility to oral infection with dengue viruses, however, *Ae. aegypti* must feed on persons with high viremia to become infected. It has been documented that considerable variation exists among strains of dengue viruses in their ability to produce viremia (27–30), and there is some evidence that high human viremia may be associated with epidemic strains of dengue virus, while lower viremia is associated with endemic dengue virus strains (29, 30). Furthermore, even with the same strain of virus, there appears to be considerable variation in the duration and magnitude of viremia among individuals. With the low oral susceptibility to dengue virus infection of *Ae. aegypti,* it is likely that only those viruses associated with high human viremia would be transmitted by this species, while those viruses causing low human viremia would probably not be transmitted. It can be speculated that this virus–vector relationship may be a major factor in selecting and propagating epidemic strains of dengue viruses in urban situations. By contrast, viruses in rural areas or in forests could be maintained by more efficient *Aedes* spp. in a cycle combining transovarial transmission with periodic amplification in humans or monkeys.

Rosen *et al.* have suggested that the four dengue virus serotypes may have evolved in association with different mosquito species in different geographic areas (19). Thus, the natural maintenance cycles of dengue viruses may be associated with the forests of tropical Asia, Africa, or perhaps America in a cycle similar to that presented in Figure 2.1. Important questions remain to be answered: (1) do rural and/or forest maintenance cycles exist in the Americas, and (2) what role do variation in vector competence and natural maintenance cycles for dengue viruses play in the distribution and spread of epidemic dengue and DHF?

Risk Factors Associated with Dengue Hemorrhagic Fever

Controversy still exists concerning the risk factors associated with DHF/DSS, even though considerable research has been done in this area. One important question that remains to be answered is why some countries, such as Thailand and Indonesia, have major epidemics of DHF every few years, while other areas, such as India and Sri Lanka, which also have high endemicity for multiple dengue virus serotypes, have only sporadic cases of DHF. Evidence, primarily from Thailand, suggests that secondary infection with dengue 2 is the most important risk factor for DHF/DSS (31–33). The work leading to this conclusion has been summarized (32, 33) and is the basis for the secondary infection hypothesis for DHF. Briefly,



AFRICA : AEDES (DICEROMYIA)
AEDES (STEGOMYIA)

ASIA : AEDES (FINLAYA)
AEDES (STEGOMYIA)

AMERICAS: AEDES SP.

RE 2.1. Proposed sylvatic maintenance cycle for dengue viruses.

. is hypothesized that mononuclear phagocytes are primary sites of dengue virus replication, that nonneutralizing heterologous dengue antibodies enhance monocyte infection by forming complexes with the infecting virus, and that the infected monocytes are responsible for release of factors that increase vascular permeability. Enhancement of dengue infection in these cells has been demonstrated *in vitro* with a number of flavivirus antibodies as well as with heterologous dengue antibodies. It should be noted, however, that as yet there is no solid evidence that this phenomenon influences dengue virus infection or replication in the human host. In Thailand, as well as in other parts of Southeast Asia, the Pacific, and the Americas, documented severe and fatal hemorrhagic disease may be associated with primary infections of all dengue serotypes (27, 29, 34, 35 and San Juan Laboratories, unpublished data), thus suggesting that virulence characteristics of the virus may also be an important risk factor for DHF (29, 30, 36). Finally, evidence is accumulating that suggests that host genetic factors also play a role in the severity of disease associated with dengue infection. Thus, there are many questions to be answered regarding risk factors associated with DHF that are very important in terms of epidemiology and future plans for prevention and control.

In 1979, the World Health Organization (WHO) sponsored a study group meeting to design prospective epidemiologic studies in high-risk DHF areas (Thailand and Indonesia) and low-risk DHF areas (Sri Lanka) with the objective of identifying risk factors associated with epidemic dengue and DHF. Two of these studies in Thailand and Sri Lanka are on-going and have produced some interesting results. In Thailand, the first year of the

study supported conclusions from earlier work suggesting that secondary infection with dengue 2 was the most important risk factor for DHF (31). By contrast, a dengue epidemic in Colombo, Sri Lanka in 1982 was clinically mild and of the classical type, with no documented DHF cases despite the fact that the majority of confirmed cases in that outbreak were dengue 2 (37). This is reminiscent of the Tonga epidemic of dengue 2 in 1974, in which disease was clinically very mild (29). In Indonesia, from 1975 through 1978, most of the severe and fatal hemorrhagic disease was associated with dengue 3, not dengue 2 (38–40).

The studies in Asia are continuing and have now been expanded to include Puerto Rico, an area in the Caribbean that has experienced repeated epidemics of dengue without DHF (San Juan Laboratories, unpublished data). In the latter study, emphasis is also being directed toward the possibility that there are endemic and epidemic strains of dengue viruses. Limited field data already exist in support of this hypothesis (29, 30), and are consistent with the concept that forest and peridomestic species of *Aedes* may be primarily responsible for maintenance of endemic virus strains, while *Ae. aegypti* selects out and transmits epidemic virus strains.

Molecular Epidemiology

Dengue virus strain variation has been investigated by comparing the oligonucleotide "fingerprints" produced following RNase T1 digestion of genomic RNA and separation of the labeled oligonucleotides by two-dimension gel electrophoresis (41, 43). Oligonucleotide fingerprints of each of the four dengue virus serotypes are unique (41). Analysis of multiple isolates of dengue 1 and 2 viruses during epidemic conditions, during interepidemic years, and from different geographic areas has indicated that strains of virus from each major geographic region are similar to each other and distinct from viruses isolated from other areas (42–43). Comparison of fingerprints has facilitated separation of dengue 1 virus isolates into three distinct genotypic varieties representing Africa, Pacific/Southeast Asia, and the Caribbean. On the basis of fingerprint similarity, dengue 2 viruses have been divided into six genotypic varieties representing the Caribbean Basin/South Pacific/Central America/Mexico, Jamaica/Trinidad/Haiti, the Philippines, Thailand/Burma/Vietnam, West Africa/Upper Volta, and Kenya/Egypt (42, and D.W. Trent, personal communication). In both studies, the virus strains analyzed do not represent all areas of the world where dengue 1 and 2 virus infections occur and, therefore, it is likely that additional genotypic varieties exist. The data suggest that the evolution of dengue virus within a specific geographic region is much slower than that observed for other non-insect-transmitted RNA viruses (44–50), and, therefore, it may be postulated that the mosquito vector plays an important role in selecting viruses within populations conferring genetic stability on

the population of virus transmitted. A second observation relating severity of disease with virus genotype suggests there is no direct relationship between fingerprint type and disease severity; mild to severe disease with hemorrhagic symptoms and shock have been associated with viruses having almost identical fingerprint patterns (D.W. Trent, personal communication).

The epidemiology and spread of dengue viruses are now better understood because of genetic analysis of virus strains by oligonucleotide mapping. Introduction of different strains of virus into a new geographic area can be rapidly detected and the spread of disease caused by the newly introduced virus documented. This capability is of considerable value where genetically distinct, but antigenically similar strains can be distinguished, and their transmission separately followed. Because viral antigens tend to be more conserved than the overall genetic sequence, fingerprinting is more sensitive than detecting antigenic divergence. However, the availability of monoclonal antibodies promises to improve greatly the precision and discrimination of viral serodiagnosis (51, 52). Fingerprinting of the genome is direct in that it does not require new reagents to detect the presence of a new viral genotype. Furthermore, fingerprinting analysis presents an average representation of the entire genome and is not restricted to those genomic sequences specifying viral surface polypeptides.

In the last few years, our knowledge of the molecular biology of viruses has been greatly expanded by the development of techniques for cloning and sequencing viral nucleic acids. This new knowledge has elucidated the organization of the viral genome, replication and translation strategies, the nature of the viral proteins, and the biochemical processing of viral proteins that are involved in virion morphogenesis. Despite the wealth of knowledge about the alphaviruses (53–55), the molecular structure of the flavivirus genome has yet to be determined, although it is now known that the coding sequences for the structural proteins are located at the 5′ end of the viral genome in the order 5′-capsid-membrane-envelope glycoprotein-3′ (J.H. Strauss, personal communication). It is anticipated that molecular cloning and nucleotide sequence data will soon be available for several of the dengue viruses. Genome sequence data must be collected that will permit construction of hybridization probes for the analysis of genetic variation and rapid identification of virus genotypic variants or types. The larger oligonucleotides of some of the dengue 2 geographic genotypic variants have already been sequenced and synthetic oligonucleotide hybridization probes complementary to the unique oligonucleotides have been constructed (J. Kerschner, personal communication). These probes, specific for each of the topotypes, will facilitate rapid and specific genetic characterization of new dengue 2 isolates and provide the information needed to facilitate primer extension nucleotide sequencing in regions of the genome where genetic variation related to antigenic structure and virulence occurs.

Vector Competence

Another important aspect of dengue ecology involves genetic heterogeneity in the mosquito vector populations. It has been documented that geographic strains of both *Ae. albopictus* and *Ae. aegypti* vary considerably in their susceptibility to oral infection with dengue viruses, and thus in their ability to transmit the infection (23, 24). These studies also showed that susceptibility to oral infection was dose-related and, therefore, a mosquito strain could be infected by increasing the amount of virus ingested, regardless of how resistant they were to dengue virus infection. It was also observed that in both mosquito species, the factors controlling susceptibility to oral infection were the same for all four dengue serotypes, that susceptibility was controlled by a midgut or mesenteronal barrier, and that there was no evidence of dissemination or salivary gland barriers. Finally, susceptibility to oral infection with dengue viruses was genetically controlled in both species (23, 24).

The data suggest that selective pressures in the environment may cause changes in susceptibility of the mosquito population and that vector competence may be an important risk factor for epidemic dengue transmission. (56).

Investigators in several laboratories are studying natural genetic variation among strains of *Ae. aegypti* by isozyme analysis, using gel electrophoresis (57–61). Since susceptibility to dengue viruses in *Ae. aegypti* is genetically controlled, attempts have been made to characterize strains of this mosquito with high and low dengue susceptibility by isozyme analysis using gel electrophoresis in an effort to identify markers for susceptibility and refractoriness. Preliminary results, however, have failed to show any conclusive relationship between dengue susceptibility and either enzyme banding patterns among geographic strains or morphotype of *Ae. aegypti* (San Juan Laboratories, unpublished data). These studies have shown that this mosquito species can be separated into geographic groups based on genetic relationships, an observation that has obvious importance in determining the origin of mosquito strains that have reinfested an area after eradication. Whether this type of genetic analysis of mosquito populations will increase our understanding of mosquito vector competence and the role it plays in the distribution and spread of epidemic dengue must be the subject of further study.

Clinical Studies

Evidence, mainly from Thailand, suggests that the primary pathophysiologic change that occurs in DHF/DSS is increased vascular permeability leading to a loss of plasma from the vascular compartment, hypovolemic shock, and death if not corrected (32, 33, 62). Although this leaky capillary syndrome has also been described in the majority of confirmed DHF cases

in other areas of Asia, patients with a more severe bleeding tendency have been observed in Indonesia (40). Thus, only 53% of virologically confirmed fatal cases had a confirmed leaky capillary syndrome with hemoconcentration, while an additional 10% had some evidence of hemoconcentration, but with massive gastrointestinal (GI) hemorrhage. Thirty percent of these cases in Indonesia, however, died of shock due to blood loss from GI hemorrhage. This type of hemorrhagic disease is very rarely observed in Thailand, but appears to be more common in recent epidemics in the Americas (63) (San Juan Laboratories, unpublished data). Thus, considerable controversy still exists over what actually constitutes a case of DHF. One group states that only those patients with documented hemoconcentration and thrombocytopenia should be called DHF and all others, regardless of the extent of hemorrhagic manifestations and outcome of the illness, be called dengue with hemorrhagic manifestations (2, 32, 33). Others have called for a redefinition of the disease (36, 40).

More detailed studies are needed to clarify the spectrum of illness associated with dengue infection. The disease should be characterized in different parts of the world, in association with different human populations and strains and serotypes of dengue viruses. This is especially important in the American region, where hemorrhagic disease has not been common. Unanswered questions requiring answers include whether there are two or more pathogenetic mechanisms involved in causing severe and fatal dengue disease, or whether dengue disease simply represents a spectrum ranging from inapparent or mild febrile illness on one end to severe hemorrhagic disease with shock and death on the other. We also need to know whether the severity of illness varies with infection by different dengue virus strains and serotypes, and whether some persons (or populations) are genetically more susceptible to the severe and fatal forms of hemorrhagic disease. Last, we need to know the pathogenetic mechanism(s) that results in severe neurologic disorders associated with dengue infections, and whether encephalitic signs observed in patients with dengue infection are associated with certain virus strains or serotypes, or with persons or populations who are genetically predisposed to neurologic disorders.

Laboratory Diagnosis

Virus Isolation and Identification

Dengue viruses have been among the most difficult of the arboviruses to isolate and propagate in the laboratory. None of the standard laboratory animals nor mammalian cell cultures are sufficiently susceptible to dengue virus infection to use for routine dengue virus isolation. It was not until the development of the mosquito inoculation technique that a highly sensitive virus isolation system became available and for the first time allowed

routine isolation of dengue viruses from clinical specimens (64). The technique is easy, relatively rapid, and economical. It has the disadvantage, however, of being labor-intensive and requires rearing and maintenance of mosquitoes, the expertise for which is not available in most virology laboratories.

In recent years, several mosquito tissue culture cell lines have been developed that are much more susceptible to dengue virus infection than mammalian cell cultures (65–67). Three cell lines, the C6/36 clone of *Aedes albopictus* cells, the AP-61 cell line from *Ae. pseudoscutellaris,* and the TRA-284 cell line from *Toxorhynchites amboinensis,* have been the most widely used (65–70). Comparative studies carried out with these three cell lines by two different groups, however, have shown that the TRA-284 cells have a higher susceptibility to dengue viruses than either the AP-61 or C6/36 cells (71, 72). Furthermore, the TRA-284 cells have been adapted to serum-free medium (TRA-284-SF) without a reduction in their susceptibility to dengue viruses (67). Because good-quality bovine serum is very expensive and frequently unavailable in dengue endemic areas, and because the TRA-284-SF are more sensitive, they are the culture system of choice for isolation of dengue viruses (71).

Dengue virus infection in cell cultures can be detected by immunofluorescence or by cytopathic effects (CPE). Reports of CPE in mosquito cells have been variable, but most frequently described in AP-61 and C6/36 cell cultures. In our experience, however, development of CPE is very unreliable, especially in C6/36 cells. CPE can be induced in infected C6/36 cells, however, by increasing incubation temperature to 36°C and using medium with pH 6.8 (73). Even in AP-61 cells, which are most consistent in developing CPE, it is likely that slow-growing viruses that infect only a few cells do not produce CPE. In these cultures, even after 10 days' incubation, it is not uncommon to have less than 5% of the cells infected with virus (69, 71). This type of infection, however, is easily deteced by either direct or indirect immunofluorescence. (69).

Identification of dengue viruses has been greatly facilitated by the recent development of hybridomas that produce serotype-specific monoclonal antibodies (74, 75). The monoclonal antibodies have been most frequently used in an indirect fluorescent antibody test (IFAT) (52, 69, 75), but can also be used in an enzyme-linked immunosorbent assay (ELISA) (76). They have been used successfully to identify dengue viruses in a variety of mammalian and mosquito cell cultures as well as in mosquito brain tissue (52).

In general, the monoclonal antibodies have been very specific. However, caution should be exercised, because not all dengue viruses, in particular dengue 1 and 2, react well with the monoclonal antibodies (52, 76). Preliminary data suggest that strain variation among viruses may influence reactivity, thus leading to inaccurate identification. On the other hand, it should be possible to produce monoclonal antibodies that can be used to distinguish different virus strains (76).

The serotype-specific monoclonal antibodies used in conjunction with sensitive mosquito cell cultures have provided, for the first time, a rapid, simple, economical, and accurate method for routine isolation and identification of dengue viruses (69). The use of these methods by laboratories in dengue endemic areas should facilitate development of more effective virologic surveillance systems that will be necessary to prevent epidemic dengue and DHF.

Serology

Three basic tests are routinely used for serologic diagnosis of dengue infections in most laboratories. These are the hemagglutination-inhibition (HI), complement fixation (CF), and neutralization (NT) tests. Unfortunately, no new diagnostic tests have been developed that have the sensitivity and specificity to replace any of these.

Research on dengue diagnosis in recent years has emphasized rapid diagnostic tests that would help clinicians establish etiology in severely ill patients. Most emphasis has been given to immunofluorescent assays, radioimmunoassays, and enzyme immunoassays, but to date, no test has been developed that is rapid, specific, and sensitive enough to be useful on a routine basis. Antibody detection assays show the least promise for rapid diagnosis because in primary infections (those persons experiencing their first flavivirus infection) antibody is relatively slow in developing.

The antibody-capture ELISA probably shows the most promise, especially when used for IgM-class antibody (MAC-ELISA). This test was developed for Japanese encephalitis virus (77) and has been adapted for dengue (78, 79, and San Juan Laboratories, unpublished data). However, the results obtained in Puerto Rico have been rather disappointing, in that the test is not as specific for the dengue viruses as was expected. Furthermore, the test has only limited use for rapid diagnosis of dengue, because detectable levels of anti-dengue IgM are not present in all patients before day 5 of illness. When used in conjunction with an IgG-capture ELISA, however, primary and secondary dengue infections can generally be differentiated with confidence.

Like the HI, the MAC-ELISA is not specific enough to identify the infecting virus serotype, even though monotypic reactions may be occasionally observed in both primary and secondary infections. Because anti-dengue IgM antibody persists for only 60 to 90 days in most patients, however, the MAC-ELISA has become a very useful test for surveillance for dengue and DHF. Moreover, it is nearly as sensitive as the HI test, and can be used in seroepidemiologic studies as well (San Juan Laboratories, unpublished data).

Several studies have suggested that peripheral blood leucocytes (PBLs) may be a primary site of virus replication (80–83). If this is the case, detection of infected PBLs by immunofluorescent assay might provide a

method for rapid diagnosis of dengue infection. A study carried out on 19 patients in Puerto Rico, however, suggested that PBLs were not a primary site of dengue virus replication in patients with dengue fever (84). Thus, virus was more frequently isolated from plasma than PBLs, and dengue antigen was detected by DFA in the PBLs of only one patient.

Prevention and Control

There have been no new breakthroughs in the control of *Aedes aegypti* for over 15 years. Moreover, the economic problems of many tropical countries of the world have led to a near breakdown in routine mosquito control for dengue as well as for other diseases. While epidemic control is still considered high priority in most endemic areas, little effort has been made to develop programs designed to actually prevent epidemic transmission. The normal course of events has been to wait until an epidemic occurs, and then implement control measures. This approach generally has little effect on the course of an epidemic because ineffective surveillance programs usually do not detect increased dengue activity until peak transmission has already been reached.

Mosquito Control

A major problem with using mosquito control for prevention of epidemic dengue is that to be effective, it must be continuous, even during periods of low or no dengue transmission. During interepidemic periods, most people, including control agencies, lose interest in mosquito control and as a result, large mosquito population densities are allowed to build up. Equally unfortunate is that surveillance programs are generally too insensitive to detect increased dengue activity until the epidemic has reached near peak transmission. By that time, it is too late to effectively intervene and control the epidemic.

Because of the problems of relying on routine mosquito control for epidemic prevention, an attempt is being made in Puerto Rico to develop an early warning surveillance program that will provide a predictive capability for epidemic dengue and DHF (San Juan Laboratories, unpublished data). The system is absed on virologic surveillance using viral syndrome cases from selected cities on the island, and is designed to detect, without too much delay, the introduction of new dengue serotypes. Closely tied into the surveillance system is the development of a rapid-response emergency vector control unit that can respond to new virus introductions and control incipient epidemics before they spread. The vector control program is integrated and will use a variety of approaches, including community education, environmental sanitation and chemicals for larval control, and space sprays for adult control.

Dengue Vaccines

Considerable effort has been put into dengue vaccine development in the past several years. Unfortunately, little progress has s been made. Most attention has been directed toward dengue 2 virus because that serotype is considered by some to be the most important in causing severe and fatal disease (31). A live dengue 2 candidate vaccine (PR-159/S-1) was developed from a Puerto Rico virus by attenuation in fetal rhesus monkey lung cells (DBS-FRhL-2) which is temperature-sensitive, produces uniform small plaques in cell culture, and has decreased mouse neurovirulence (85–87). Human trials have shown that only about 61% of recipients without previous flavivirus infection develop anti-dengue antibodies, compared to 90% in persons with previous yellow fever vaccination (88). Clinically, recipients have presented with a variety of symptoms, including low-grade fever, chills, abdominal pain, headache, night sweats, nausea, and anorexia (88–89). The PR-159/S-1 vaccine virus infection has been shown to produce viremia in human recipients, a potentially beneficial factor considering the fact that the viruses isolated from vaccine recipients have shown no evidence of reversion to wild-type growth characteristics. (90, 91).

More recently, a dengue 4 candidate vaccine, attenuated by passage in primary dog kidney cells, was prepared in fetal rhesus lung cells and tested in five human volunteers. Only two of the recipients developed neutralizing antibodies, and both of these persons had detectable viremia and symptoms compatible with mild dengue, including a rash. The virus recovered from the two recipients showed characteristics of the parental virus, suggesting reversion to the wild type (92–96).

Attenuation of dengue 1 virus has also been attempted, but results with monkeys indicated lack of immunogenicity. Current work on development of dengue vaccines is ongoing in at least three laboratories, in the United States, Thailand, and China. Unfortunately, progress is slow and it is unlikely that a safe, immunogenic dengue vaccine will be available for general use in the near future.

Conclusions

The increasing public health importance of dengue viruses in many parts of the tropics has led to expanded research support in recent years. Important breakthroughs have come in laboratory diagnosis of dengue viruses, primarily in isolation, assay, and identification. A major problem remaining is development of a rapid diagnostic method that is reasonably economical and simple, but which is also sensitive and specific enough to be useful in a clinical laboratory setting. New-generation diagnostic methods will most likely come from a better understanding of molecular virology and the use of cDNA probes.

Progress in dengue vaccine development has been very disappointing.

Major problems have been with safety and immunogenicity of the attenuated vaccine viruses. It is likely that production and use of a safe, effective, and economical vaccine and/or a polyvalent vaccine will have to await development of the technology to produce a genetically engineered vaccine.

In the area of epidemiology, one of the most important questions deals with risk factors for severe and fatal disease. Long-term prospective field studies are required to determine the relative importance of primary versus secondary dengue as a cause of DHF/DSS, and the role that strain variation among dengue viruses plays in the production of severe and fatal disease. Answers to these questions will be necessary before vaccines can be used in endemic areas for prevention and control of epidemic DHF. Until that time, emphasis should be placed on development of more effective surveillance systems that will provide a predictive capability for epidemic dengue and on more effective mosquito control measures.

Field studies will also be required to determine whether rural or forest maintenance cycles for dengue viruses actually occur in most endemic areas, and the significance of such cycles in the distribution and spread of epidemic dengue and DHF. Also, the role of endemic versus epidemic strains of dengue viruses and the role of mosquito vector competence as a selective mechanism for epidemic virus strains must be studied. Answers to these question have obvious important implications for long-term prevention and control of epidemic dengue and DHF.

Acknowledgments. The author acknowledges with gratitude Dr. Dennis W. Trent's contribution of the section on molecular epidemiology.

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