特 別 講 演

I Dengue hemorrhagic fever: a critical appraisal of current hypotheses
Leon Rosen（ハワイ大）

（英文参照）

II 科学技術と国際交流
岡本 道雄（科学技術会議議員）

西洋の科学技術は目覚ましく進歩し発展し、今や世界を制覇してしまった。日本も明治維新以来、西洋から科学技術を導入しこれを工夫し発展させて来た。先ず明治の改革では、科学技術によって武器をつくり、軍国主義をもって世界に乗り出すようになった。次に第二次世界大戦後の改革では、科学技術を企業化することによって、経済的に繁栄し今では世界第2の経済大国になった。しかし歴史的めで、武力のみでまた経済のみで国際社会に住して長く栄えた国はないのである。国際社会で信頼される事柄大切なである。その為に日本はどうすればよいか。私は次の方目立重要なと考える。

第1に、外国人を受け入れることである。人が外国へ行った時間、所懐りな花である。国民はその国で見てももらうことで、本当に理解が得られる。日本人が本当に美しいというのは、日本の繊細な美ではあり、その土に生えた花がはやらしい。切り花ははやらく切り花だけである。その意味でこれからの国際交流というのは、日本に受け入れるという方向に最大の努力をすべきである。そこで政府は今、二十一世紀までに10万人計画というのを立てている。

第2に、日本は今まで世界から科学技術を受け入れてここにまで繁栄したのであるから、基礎科学で人類の為になることを積み重ねなくてはいけない。これは単に日本の国益のためだけでなく、基礎科学で世界の為にやるべきことがある。砂漠化を防ぐとか、酸性雨をどうするとか言った地球的な問題になると恐らく金がかかり、アメリカか日本でないと出来ない基礎科学がある。そういうものにしっかりと金を出して、世界に報いる事が大事である。

第3は、昔からよく「和魂洋才」という事が言われたが、日本人の魂とは何かであるかを、今よく考えてみなければならないという事である。今や西洋の科学技術が人間に直撃して、資源枯渇、自然破壊、公益等の多くの深刻な問題を起こしている。また目には見えない人間の心に、どういう影響をもたらしているかというと、これは計り知れない恐ろしい問題であると考えられる。これを救うのはどうしても東洋の精神だ、西洋では考えられている。なぜなら西洋の科学技術その本質は、自然と人間が対立するところから始まっている。人間の理系だけ働かせて、理性を自然を観察して自然の中の法則を見つけて自然を利用し活用し、さらに征服しようというのが近代科学技術の精神である。ところが東洋の考え方では、自然と人間は対立していないのである。自然の中に人間がいて、人間も動物も同じく自然であるという考え方である。調和の中にあるのである。この様な精神をもつ日本や西洋を世界は熱い目で見ている。

この点日本という国は、東西の文化的な接点であるし、また科学技術の価値を日本人は知っている国民ではないのである。日本は科学技術で生きて来たのであり、その功を最もよく知と共に、また原子爆弾を体験し科学技術が誤って使われた時の罪、これほど知っている国民はいないわけではない。
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日本の世界における立場は大きく変化し、今や
日本の内政はまさに日本の外交であり、日本の内
部でやっていることは、すぐ日本の外交になって
いる。その日本の外交は世界の内政、世界政策と
なっているのである。それくらいの立場に変わっ
ているということの自覚が、日本人一人一人の中
にあってこそ、国際交流というもののが政府の命令
でなく、また政府の政策でなしに国民一人一人の
自らのものとして、この時代の科学技術、国際交
流の方向が出て来るのだと思う。
Special lecture

DENGE HEMORRHAGIC FEVER: A CRITICAL APPRAISAL
OF CURRENT HYPOTHESES

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The clinical manifestations encompassed by the term “dengue hemorrhagic fever” are responsible for most of the hospitalizations and deaths among persons infected with dengue viruses. Since there is wide variation in the incidence of these forms of the disease from one dengue outbreak or endemic area to another, it is possible that a better understanding of their pathogenesis could lead to strategies that might prevent severe disease and death from dengue, even if the infection itself could not be prevented.

I should like to begin my presentation by commenting on the question. “Exactly what is dengue hemorrhagic fever and how does it differ from ordinary dengue fever?” In general, the more severe forms of dengue infection are characterized by hemorrhage, by hypovolemic shock, or both. However, there is a difference of opinion as to whether it is appropriate or useful to characterize all dengue infections with hemorrhagic manifestations as “dengue hemorrhagic fever.” For example, the latest guidelines on dengue available from the World Health Organization (WHO) distinguish between “dengue hemorrhagic fever” on the one hand and “dengue fever with hemorrhagic manifestations” on the other. According to the WHO, the former is characterized by thrombocytopenia and hemoconcentration and can be divided into 4 grades. The hemorrhagic aspect of the first of these grades is limited to a positive tourniquet test. Thus, according to the WHO, a patient with a positive tourniquet test, thrombocytopenia, and hemoconcentration would be considered to have “dengue hemorrhagic fever”, whereas a patient with severe, or even fatal, gastrointestinal hemorrhage and thrombocytopenia, but not hemoconcentration, would have only “dengue fever with hemorrhagic manifestations”. Since thrombocytopenia is a common manifestation of all types of dengue infection and since children with fever, vomiting, or diarrhea from any cause can also have hemoconcentration, one can imagine the difficulty that at least some observers have in distinguishing the supposed difference between “dengue hemorrhagic fever” and “dengue fever with hemorrhage”. As far as I am aware, there are no data, published or unpublished, which justify the distinction made by the WHO on either pathogenetic or prognostic grounds.

Fortunately for the purposes of this discussion, everyone agrees that there is an entity called the “dengue shock syndrome”, which is one of the common forms, of life-threatening dengue, and which is usually, but not always, accompanied by hemorrhagic manifestations. In the interest of time, I will address the bulk of my remarks concerning pathogenesis to the dengue shock syndrome, but, in doing so, I do not mean to imply that the pathogenesis of the shock syndrome is necessarily the same as that of the hemorrhagic manifestations of dengue. The latter are so common in both mild and severe dengue infections and, as noted above, their
definition so controversial, that it is difficult to analyze any of the data with respect to their pathogenesis.

When the term "dengue hemorrhagic fever" came into use following the epidemics in the Philippine Islands in the early 1950's, it was at first believed, at least by some, that the severe forms of dengue represented a new clinical entity which had not been observed previously. I believe that it is fair to state that, at present, few persons, if any, hold this view. It is clear that the severe forms of dengue are now more prevalent than they were in the past. Unfortunately, there are no data to indicate whether this is the result, on one hand, simply of a quantitative increase in the incidence of all dengue infections, or, on the other hand, if the increase in severe clinical manifestations is the result of some qualitative change.

The outcome of any infection depends on the interaction between the infectious agent on one hand and host defenses on the other. In the case of dengue, there is a considerable difference of opinion as to whether it is the virus or the host that plays the predominant role in the pathogenesis of severe dengue disease. More specifically, there is a difference of opinion as to whether or not an acquired characteristic of the host, namely, a prior dengue infection with a heterologous dengue serotype constitutes a risk factor for the development of severe dengue disease.

In this review, I should like to start with a discussion of the virus and then proceed to a discussion of the host. To begin with, one of the strongest arguments for an important role of viral virulence in the pathogenesis of severe dengue is that variation in the virulence of strains has been described for most, if not all, viruses for which appropriate assay systems are available. It would be surprising indeed if dengue strains and serotypes were an exception.

Epidemiologic evidence suggestive of differences in overall virulence between dengue serotypes is the observation that dengue types 2 and 3 have been more commonly associated with the dengue shock syndrome in Southeast Asia over the last 20 years than have dengue types 1 and 4. Since laboratory methods in use during that time period were least sensitive to dengue type 3, the association of that serotype with severe disease is especially convincing. Evidence for variation in virulence among dengue strains of the same serotype is more difficult to come by. For example, there have been many instances of relatively avirulent epidemics caused by dengue types 2 and 3, but alternative explanations, which I will discuss later, cannot be excluded. Unfortunately, dengue strains potentially different in virulence have not yet been analyzed by appropriate molecular methods. While oligonucleotide fingerprinting has been used to separate dengue strains of the same serotype into topotypes, the method analyzes only about 15% of the virus genome. Consequently, it cannot be expected to detect small differences of the type which have been found between virulent and avirulent strains of other viruses (such as poliovirus). Another major handicap in studies of dengue viral virulence is the lack of a laboratory animal or an in vitro marker for virulence. At present, the only way to assay the virulence of dengue viruses is in man.

While the concept of differences in dengue viral virulence is almost certainly true, at least to some extent, there are certain epidemiologic observations from the South Pacific which are difficult to explain, unless virulence is a rather labile characteristic. For example, when dengue type 2 swept through the South Pacific, beginning in 1971, it was almost certain that the same viral strain was carried from one island to another. Yet, the virus caused relatively severe disease on some islands such as Tahiti and Niue and very mild disease on others such as Samoa and Tonga. The same phenomenon was observed with dengue type 1 beginning in 1975.
However, in the type 1 epidemics, severe disease occurred in Tonga and Niue, and relatively mild disease on Tahiti and Samoa. I might add at this point that the differences between the islands could not be explained by differences in prior experience with other dengue serotypes. Anecdotal evidence that dengue strains might change in virulence relatively easily, perhaps on the basis of rapid human to human passage, is the observation that a higher proportion of severe disease is often seen in the second half of an epidemic as compared with the first half. This was noted, for example, in Niue Island epidemic of 1972, Cuban epidemic of 1981 and also was described in the large Greek epidemic of 1927–1928.

I should now like to turn to a consideration of host factors in the pathogenesis of severe dengue. To begin with, I should like to comment on what might be considered two innate characteristics of the host, namely, genetic background and age, and then discuss an acquired characteristic, prior dengue experience.

It has been well documented in both laboratory animal models and observations on man that genetic factors can modulate the immune response of a host and hence the outcome of an infection. It would be surprising if the same were not true for dengue infections. At present, however, for dengue, only anecdotal observations are available on this point. First, one type of anecdotal observation is that it is not uncommon to observe multiple cases of severe dengue in the same family during an outbreak—far more frequently than might be expected to occur by chance. For example, in the Niue Island outbreak of 1972, there were 3 fatal cases among children in a single family out of a total of 12 fatal cases in a population of about 5,000. Second, there have been several examples of differences in attack rates for severe dengue among different ethnic groups during the same epidemic—for example, among Chinese in Malaysia. In most such instances, other explanations, such as differences in exposure to vector mosquitoes cannot be excluded. However, environmental factors have not been cited as an explanation for the relative lack of severe disease among blacks during the recent Cuban outbreak. If it were true that a small proportion of a given population was particularly susceptible to the severe manifestations of dengue because of genetic factors, this might provide an explanation for the dramatic decrease in the incidence of such severe infections after the first few epidemic waves of dengue shock syndrome in certain areas—such as has been observed in Manila in the Philippine Islands. A similar explanation has been suggested for the dramatic decreases in the death rate for measles in island populations during succeeding epidemics.

It is well established that, in virgin soil epidemics of dengue in which persons of all ages are infected, the common clinical manifestations of dengue, such as fever, are more severe among older children and adults as compared with younger children. This is similar to the experience with certain other infectious diseases, such as rubella and poliomyelitis, in which clinical manifestations are more severe in infected adults as compared with infected children. No data are available on possible age differences in susceptibility to the dengue shock syndrome. In those epidemics in which a sufficiently large number of such cases have occurred, data are lacking on the number of individuals in the different age groups susceptible to infection with the responsible dengue serotype or serotypes. It is of interest in this regard to recall the history of poliomyelitis epidemics. Paralytic poliomyelitis was originally called infantile paralysis because in early epidemics only young children were paralyzed. As poliovirus infection was delayed until later in life with improved environmental sanitation, it became apparent that older children and adults were actually more susceptible to the paralytic consequences of a poliovirus infection than were young children. In the first epidemics, paralysis was limited to young children because they
were the only ones not already immune. I will have more to say on this point in discussing the next item on my agenda.

I should now like to discuss the controversial question of whether or not a prior heterologous dengue infection constitutes a risk factor for development of the dengue shock syndrome. Two types of data have been cited in support of this concept. One of the types is experimental. It is said that lower primates experimentally infected with dengue type 2 virus develop higher levels of viremia if they have actively or passively acquired heterologous dengue antibody than they do if they have no such antibody. The other type of data cited in support of what can be called the “sequential infection hypothesis” is epidemiologic. Several studies are said to show that the dengue shock syndrome occurs more frequently during a second dengue infection than it does during a first infection.

Some of you may have noted that I have not mentioned as a supporting argument the voluminous literature on antibody enhancement of the replication of dengue type 2 virus in cell cultures. I do not doubt that such enhancement occurs in cell cultures—just as it does with other flaviviruses and viruses of other taxonomic groups. However, unless antibody enhancement of dengue virus replication occurs in the intact host, the in vitro data are irrelevant. As you will see, it is my opinion that the evidence with respect to intact hosts is far from convincing.

The data on antibody enhancement of dengue virus replication in lower primates consist of 2 studies from same laboratory. In the first study, it was found that in monkeys infected with dengue type 2 virus after a previous dengue type 1, 3, or 4 infection, mean peak viremia was higher than that of monkeys with primary infection with the same virus. Mean peak viremia for secondary type 1, 3, and 4 infection either was the same or was lower than that for the corresponding primary infection.

I do not find the data on dengue type 2 viremia convincing for the following reasons. First, the magnitude of peak viremia in the monkeys, as measured in this study, was extremely variable. For example, while the mean peak viremia in secondary infections was said to be 13–fold higher than that in primary infections, the variation in peak viremia from one individual animal to another in both primary and secondary infections was a 1,000–fold or more. The second question that I have about this study concerns the way in which viral titers were determined. The viral assay system used, namely plaque assay in LLC-MK2 cells, is known to be relatively insensitive, but that in itself is not a problem in a comparative study of this type. What is a problem is that the sensitivity of the system is known to vary from one test to another. In my opinion, viremia levels can be legitimately compared in this system only if matched pairs of specimens from primary and secondary infections of the same serotype were assayed in parallel. As far as I can determine, this was not done. Moreover, the fact that viremia was found to be higher in secondary infections with dengue type 2, but not with dengue types 1, 3, or 4, calls for an especially rigorous look at the data. Antibody enhancement of virus replication in cell cultures is not limited to dengue type 2, nor is the dengue shock syndrome caused exclusively by this serotype. As a matter of fact, dengue type 3 has been the predominant virus responsible for the syndrome in several recent epidemics in Southeast Asia.

The second experimental study cited in support of antibody enhancement in the intact host consisted of comparing the viremias in 2 groups of 5 monkeys each. Immediately prior to being inoculated with dengue type 2 virus, one group of animals was infused intravenously with a human serum pool known to have an in vitro dengue virus replication enhancement titer of greater than 1 to 2,000,000. The other group of animals received human serum without dengue
antibody. Three- to 50-fold higher peak viremias were observed in the animals which had
received antibody as compared with those which did not.

The problem that I have in accepting this experiment as evidence for antibody enhancement
in the intact host is that I believe that it may represent just another in vitro experiment.
Unfortunately, the sera tested for virus content were not assayed for in vitro antibody enhance-
ment titer. Such tests were carried out on sera drawn from monkeys shortly after they had
been infused. The enhancement titer in those animals which had received antibody was found
to be about 1 to 1,000,000. Given this high titer, I think it quite likely that in vitro enhancing
antibody was still present in the monkeys' sera at the time of their viremia a few days later. If
this were the case, the same data might have been obtained had the antibody not been given to
the monkeys at all, but rather, just added to their sera after the latter were withdrawn for virus
assay! There is no question that dengue plaque counts in LLC–MK2 cells can be increased by
small amounts of antibody. When utilizing such cells for plaque reduction neutralization tests,
we often have observed a larger number of plaques in wells with serum dilutions beyond the
neutralization endpoint than in control wells without antibody.

I am aware of 4 other studies, involving man or lower primates, in which flavivirus viremia
was compared in individuals with and without prior flavivirus infection. In 2 of the studies, the
second infecting virus was a vaccine strain of dengue type 2. In one, vaccine was given to men
who previously either had been vaccinated with yellow fever virus, or had not. In the other,
vaccine was given to monkeys which had previously been infected with dengue types 1, 3, or 4,
or had not been infected with any of the viruses. In the other 2 of the 4 studies, the second
infecting virus was yellow fever. In one, yellow fever vaccine virus was given to individuals
who previously had been infected naturally with Japanese encephalitis virus, or had not. In the
other, virulent yellow fever virus was given to monkeys which previously had been infected with
dengue type 2 virus, or had not. In none of these studies was a higher viremia observed in
previously infected individuals as compared with those previously nonimmune.

In addition to this negative experimental data, in the course of extensive investigations of
dengue epidemics of all 4 serotypes in the South Pacific, our laboratory has never observed that
secondary infections were characterized by viremias that were, on the average, higher than
those in primary infections, nor am I aware that anyone else has data to that effect.

It is, of course, impossible to prove a negative proposition. Thus, it is not possible to
prove that antibody enhancement of dengue infection does not occur in the intact host. It can
always be argued that such enhancement occurs only with certain dengue serotypes or strains,
or only under certain special conditions. However, in view of the alternative interpretations
which I have suggested for the 2 studies which are cited in support of the antibody enhancement
concept. I believe that the burden of proof should still lie with those who believe it to be true.
Confirmation of the experimental data by another laboratory, with modifications to meet the
criticisms expressed, would be especially convincing.

The most impressive type of evidence for the sequential infection hypothesis would be
epidemiologic. Several epidemiologic studies, all from Thailand, are said to be compatible with
the hypothesis or to have demonstrated that certain sequences of dengue infection do increase
the risk of the shock syndrome. I do not find these studies convincing because their conclusions
are based on certain explicit or implied assumptions which either are not supported by data or
are actually contrary to available information. The time available here is not sufficient for a
detailed discussion of all the points of contention, but the following are some of the main issues.
In order to demonstrate that persons who have had a prior heterologous dengue infection are at greater risk of developing the shock syndrome than those who have not had such an infection, it is necessary to be able to define the denominators, that is, the 2 populations at risk. There are several problems in the epidemiologic studies in this regard. For example, at present, there is no serologic procedure which can determine if persons who have had more than one previous dengue (or flavivirus) infection are susceptible to infection with a given dengue serotype. Similarly, in the absence of virus isolation, it is currently impossible to identify the infecting serotype in a secondary dengue infection. Also, dengue viruses are transmitted by a vector with a short flight range and the risk of infection tends to be focal in distribution. Unless substantiating evidence is available, it cannot be assumed that an entire population will be uniformly exposed to infection with each of the dengue serotypes present in a study area. Then, there is the important question of whether or not individuals of different ages are equally susceptible to the risk of developing the shock syndrome. It cannot be assumed that they are. In fact, from what is known of other infectious diseases, such an assumption is unlikely to be correct.

There are also problems in the epidemiologic studies with respect to the numerator in the risk assessment, namely, the identification of cases and their correct classification with respect to prior dengue experience. It is important that there be no bias in the chance of detecting the 2 types of cases (that is, primary or secondary). For example, in the absence studies of virus isolation, which was relatively uncommon in the studies under consideration, any primary infection that was fatal, or one with only a single serum specimen, or one with a second serum specimen collected 7 days or less after onset, would be excluded from the numerator—since there would be no evidence of current dengue infection. Secondary infections can often be identified under those conditions because of the more rapid appearance of high-titered antibody. Finally, there is the very important problem of the serologic criteria by which cases are classified as primary or secondary. It cannot be assumed that the serologic responses of shock syndrome patients with primary infections will be the same as patients with primary infections and milder clinical manifestations. As a matter of fact, there is indisputable evidence that at least some patients with primary dengue infections and severe disease have unusually high antibody responses (usually characteristic of secondary infections).

It is impossible to prove that immune enhancement of dengue does not occur—since it can always be postulated that such enhancement occurs only with certain dengue serotypes, or only with certain strains of those serotypes, or only at very critical intervals of time. A well-designed prospective epidemiologic study would resolve the question—if immune enhancement of dengue was demonstrated. However, the low and unpredictable incidence of the shock syndrome, even in highly endemic areas, poses formidable problems for the design of such a study. There may be a greater chance of obtaining unequivocally positive data from retrospective investigations in particularly favorable environments. In the meantime, each person with an interest in the subject can take a careful look at the data presently available—and then decide for himself if immune enhancement of dengue is myth or reality.
SCIENCE AND TECHNOLOGY AND INTERNATIONAL EXCHANGE

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Since the Meiji Restoration Japan has introduced science and technology from advanced western countries and succeeded economically by exporting the technological products. As it is well known, we have now the friction of technology and trade with advanced countries. Therefore it is the time for us Japanese to make efforts to be trusted by other countries.

First of all, we should invite foreign people to Japan and make them see the Japanese living in Japan instead of the Japanese living or travelling in other countries. And that is the only way to be truly understood by foreigners.

Secondly, we Japanese should create our own science, especially placing emphasis on the basic science research, which shall be not only seeds of our future engineering but also contribution to the whole world.

On the other hand, the problem of the impact of science and technology to human being has become serious, as it is seen in air pollution or destruction of nature. In this respect, too, we feel we have much to do to solve the problem as Japanese who have built our culture by adopting western and eastern culture. And it becomes important to discover the true Japanese spirit, in which especially the westerners are much interested.

In this international community, these are the important tasks for us Japanese; inviting foreign people to Japan, placing emphasis on the basic science research and discovering true Japanese spirit. And each of us should bear in mind that Japan has become a very important country in the world and contribute to the international exchange by ourselves.