PERSPECTIVE

Glad Tidings from Yellow Fever Research

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From the 17th to the early 20th century, epidemics of yellow fever have caused economic paralysis, suffering, and death in the Americas, Europe, and Africa. The panic and chaos that pervaded the early epidemics were due in large measure to confusion and dispute regarding etiology, mode of spread, and prevention. The epidemic that occurred in Philadelphia in 1793 resulted in complete dissolution of society. One of America's most prominent physicians at that time, Benjamin Rush, proclaimed that the epidemic arose from a pile of decaying coffee deposited on a wharf by a ship from the West Indies (*1*).

Suggestions by Nott (1848), Beauperthuy (1854), and Finlay (1881) that the disease was transmitted by mosquitoes were generally ignored, and theories of contagion or airborne spread of toxins abounded well into the 19th century. Sanarelli's announcement in 1897 (*2*) that the etiologic agent of the disease was a bacillus stimulated intensive research. In June of 1900, the U.S. Army Yellow Fever Commission, under the leadership of Major Walter Reed, initiated studies in Havana. In October Reed, Carroll, Agramonte, and Lazear verified Finlay's conception that the disease was mosquito-borne and dispelled Sanarelli's claim of bacillary etiology (*3*). Subsequent studies confirmed mosquito transmission and showed that the causative agent was present in blood and was filtrable (*4*).

Because there was no recognized susceptible laboratory host, 27 years passed before the responsible virus was isolated. This interval was marked by yet another false lead, when Noguchi announced that the causative agent was a leptospire (*5*). In May 1927, workers of the Rockefeller Foundation's West Africa Yellow Fever Commission initiated attempts to isolate the agent by inoculation of blood from patients into Indian monkeys. Two months later they succeeded in establishing by monkey-monkey passage a virus from the blood of a young Ghanian named Asibi (*6*); this virus later served as the parent of 17D vaccine.

The early years of laboratory research on yellow fever virus were pervaded by tragedy as well as the exhilaration of success. Three years after isolation of the virus, Bauer wrote, "no infective agent has been discovered throughout medical history which, when brought into the laboratory, has caused so high a rate of accidental infection among research workers. . ." (*7*). Thirty-eight laboratory infections and eight deaths have been attributed to the virus (*8*). The determination and intrepidity of the yellow fever pioneers is remarkable to us in this age when research on the virus is conducted by vaccinated workers, often on the attenuated vaccine virus itself, and under relatively safe conditions of biocontainment.

Development of the live, attenuated 17D vaccine in the Rockefeller Foundation's New York laboratories in the 1930's (*9–11*) was an achievement of global significance. The 17D strain was obtained by serial propagation in cultures of embryonic mouse tissue, minced whole chick embryos, and finally minced chick embryos without nervous tissue. Present-day vaccines that are produced (in 12 countries) by inoculation of whole chick embryos are derived from two distinct substrains (designated 17D-204 and 17DD) and represent independently maintained passage series from original 17D. Since 1945, when passage level was stabilized by a seed-lot system (*12*), these vaccines have proved to be safe and highly efficacious. Both the neurotropic and viscerotropic properties of parent Asibi virus are markedly reduced. Fewer than 20 cases of encephalitis have been associated with immunization. Nearly all of these have occurred in infants under 4 months of age (for whom the vaccine is now contraindicated), and the vaccine virus does not cause hepatocellular injury or dysfunction. Nevertheless, certain problems remain, as mentioned below.

As a result of the successful campaigns in the Americas against the urban vector of yellow fever, *Aedes aegypti*—the first campaign in Havana in 1901 by William C. Gorgas was followed by others throughout the hemisphere—the disease retreated to its enzootic cycle in the forests of South America. From 50 to 300 cases are reported annually, acquired by individuals exposed to tree-hole breeding *Haemagogus* mosquitoes. Although the practice of vaccination is widespread in enzootic areas, the situation is considered volatile because of the renewed potential for urbanization of the disease. Relaxed surveillance, reinvasion of territories once freed of *Aedes aegypti*, and senescence of vector control programs account for the danger. Africa has experienced repeated devastating epidemics (a total of more than 150,000 cases since 1965), due, in large part, to interruption of immunization programs. Surveillance of the disease in most areas is nonexistent, and official reports underestimate the true incidence by 20- to 200-fold (*13*).

The article in this issue by Rice, Lenches, Eddy, Shin, Sheets, and Strauss (p. 726) reporting the nucleotide sequence of 17D virus represents a new milestone in the historical sequence of yellow fever research. The article has wide-ranging implications for future research on the molecular biology of flaviviruses, including other medically important agents such as dengue, and St. Louis and Japanese encephalitis. The recent elevation to family taxonomic status (Flaviviridae) of these serologically related (formerly group B) arboviruses is justified further by the newly found differences in genome organization and replication strategy from the Togaviridae with which they were previously classified.

The yellow fever 17D RNA genome is 10,862 nucleotides in length and contains a single, extremely long open reading frame (10,233 nucleotides) which encodes all three structural proteins and up to 12 nonstructural proteins. The genes for the structural polypeptides (C, M, and E) lie in order at the 5′ terminus. The E (envelope) glycoprotein contains all antigenic structures with biological functions, including yellow fever type-specific determinants involved in neutralization (*14*). Downstream lie nonoverlapping gene segments for the nonstructural proteins. The first of these (NS1 in the

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proposed new terminology of Rice *et al.*) lies adjacent to the E segment and is also glycosylated. This polypeptide is of considerable interest as a potential immunogen. Monoclonal antibodies against NS1 mediate complement-dependent lysis of yellow fever–infected cells, and immunized mice and monkeys resist challenge with viscerotropic virus (*15*). Induction of immunity without stimulating antibodies to virion components is especially interesting for the development of vaccines against the four dengue serotypes, since enhancement of replication in macrophage target cells by heterologous nonneutralizing antibodies to structural (E protein) antigens is implicated in the pathogenesis of dengue hemorrhagic fever (*16*).

Controversy has surrounded the process whereby flaviviral RNA is translated. On the basis of a number of studies, Westaway and colleagues (*17*) concluded that translation occurs at multiple independent internal initiation sites on the flaviviral genome. Although not definitive, the results presented by Rice *et al.* support the alternative view that translation proceeds from the 5′ end sequentially to produce one precursor polyprotein which is rapidly cleaved during translation. Rice *et al.* point out several other features of flaviviral genome organization which help to explain earlier observations, such as the absence of a polyadenylated [poly(A)] tail. Formation of a stable secondary structure at the 3′ terminus appears to explain its resistance to enzymatic modification, including polyadenylation.

Implications for flaviviral evolution are intriguing. Short sequence homologies are present between 17D and polymerase genes of a diverse group of plant and animal viruses, indicating either parallel evolution or common ancestry and preservation of critical functional domains subserving replication. Compared to nucleotide sequences of insect cells, insect viruses, and alphaviruses, yellow fever has an unexpectedly low frequency of CG doublets, possibly revealing a high degree of adaptation to vertebrate host cells. Indeed, other biological attributes appear to support this concept; unlike alphaviruses, flaviviruses may cause persistent infections and many are transmitted between vertebrate host-reservoirs without intermediate arthropod vectors.

A point of concern for future analyses is the origin and manipulation of the 17D virus used by Rice *et al.* The virus obtained from the American Type Culture Collection in the form of chick embryo homogenate at passage 234 corresponds exactly to the 17D-204 substrain vaccine produced by several suppliers (*18*). However, the virus was plaque-purified in Vero cells and amplified in both BHK and SW-13 cells prior to RNA extraction, procedures that potentially introduce genetic variation. The 17D vaccine contains a mixed population of variants having distinct plaque morphology, neurovirulence for mice (*19*), growth in human macrophages (*20*), and T_1-oligonucleotide maps (*21*). Even single passages in chick embryos have been shown to cause detectable changes in RNA nucleotide patterns (*18*). Since minute alterations of gene sequence may radically affect biological functions, caution will be necessary in comparative studies to reveal intratypic variation of 17D, the basis for residual neurotropism, and the origin of attenuation of parent Asibi virus.

The importance of Rice *et al.*'s contributions to basic and applied virology deserves final comment. Our understanding of flavivirus organization and replication at the molecular level has lagged behind that of many other virus groups because of inherent and technical difficulties; thus this article provides new expectations of more rapid progress. It is fitting that the breakthrough should come in studies on the prototype flavivirus. Practical applications should be possible in diagnostic virology (nucleic acid hybridization probes) and in the development of new vaccines. An intriguing possibility, for example, is the eventual use of 17D virus as a vector of other flaviviral genes.

Complacency with regard to the worldwide situation of yellow fever is widespread in the biomedical community, largely because of the availability of 17D vaccine; yet international and national health authorities recognize many limitations. Among these are the antiquated and cumbersome methods of present-day vaccine manufacture (growth of the virus in eggs), the limited capability for increased production, the thermal lability of the live vaccine, and the neurotropic potential limiting use in very young children. The total annual production on a worldwide basis is only about 15 million doses, an insufficient number should unforeseen disaster strike (such as the introduction and spread of the virus in Asia, where it has not occurred). As efforts to modernize production of the current vaccine proceed, parallel efforts based on molecular biology seem warranted.

References and Notes

1. H. Bloch, *N.Y. State J. Med.* **73**, 2606 (1973).
2. J. Sanarelli, *Ann. Inst. Past.* **11**, 433 (1897).
3. W. Reed, J. Carroll, A. Agramonte, J. W. Lazear, *Phila. Med. J.* **6**, 790 (1900).
4. W. Reed, *Med. Rec.* **60**, 201 (1901).
5. H. Noguchi, *J. Trop. Med.* **28**, 185 (1925).
6. A. Stokes, J. H. Bauer, N. P. Hudson, *Am. J. Trop. Med.* **8**, 103 (1928).
7. J. Bauer, *ibid.* **11**, 337 (1931).
8. W. F. Scherer *et al.*, *Am. J. Trop. Med. Hyg.* **29**, 1359 (1980).
9. M. Theiler, in *Yellow Fever*, G. K. Strode, Ed. (McGraw-Hill, New York, 1951), pp. 46–136.
10. H. H. Smith, *ibid.*, pp. 546–628.
11. M. Theiler and H. H. Smith, *J. Exp. Med.* **65**, 767 (1937).
12. K. C. Smithburn *et al.*, *WHO Monogr. Ser. No. 30, Geneva* (1956).
13. T. P. Monath, in *Tropical and Geographic Medicine*, K. S. Warren and A. A. F. Mahmoud, Eds. (McGraw-Hill, New York, 1984), pp. 636–651.
14. J. J. Schlesinger, M. W. Brandriss, T. P. Monath, *Virology* **125**, 8 (1983).
15. J. J. Schlesinger *et al.*, unpublished data.
16. S. B. Halstead, in *The Togaviruses*, R. W. Schlesinger, Ed. (Academic Press, New York, 1980), pp. 107–173.
17. E. G. Westaway, *ibid.*, pp. 531–581.
18. T. P. Monath, R. M. Kinney, J. J. Schlesinger, M. W. Brandriss, P. Bres, *J. Gen. Virol.* **64**, 627 (1983).
19. F. Liprandi, *ibid.* **56**, 363 (1981).
20. _____ and R. Walder, *Arch. Virol.* **76**, 51 (1983).
21. C. M. Brand and J. H. Bennett, *Intl. Congr. Virol. 6th*, Sendai (1984), p. 263.

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