Tropical Medicine, 26 (4), 173−179, December, 1984

A Hypothesis on the Geographical Distribution of Arboviruses

Akira IGARASHI

Department of Virology, Institute for Tropical Medicine, Nagasaki University

Abstract: Observations on cultured mosquito cells or whole mosquitoes infected with arboviruses in laboratories and in nature led to a hypothesis which might well explain the geographical distribution of various but related arboviruses in the world. The hypothesis of "biological filter" takes into consideration of the high mutation rate of RNA genome combined with 2 different kinds of selective pressures which could be conferred on the virus population by alternative growth in 2 phylogenetically remote hosts, that is vertebrates and arthropods. Such a mode of virus growth could result in a limited range of variation of the virus in spite of the high rate of mutations during growth cycles in both hosts. Since different species of arthropods or vertebrates have been existing in different parts of the world, the selective pressures on arboviruses in various geographical areas could have been different. Thus, arboviruses, which might have originated from a single or limited numbers of ancestor(s) could have diverted, through enormous generations of growth cycles, into many related but different species existing in different geographic areas of the world at present. Although the viruses coud have undergone vast numbers of mutations through their growth in vertebrates and arthropods, only a certain range of selected mutants could have survived the evolutional process, when they possessed selective advantages to grow both in vertebrate hosts and arthropods vectors living in that area. The hypothesis appears to explain the reason why there are so many different but related arboviruses in the world now, possessing their own geographical distributions.

Key word: arbovirus, mutation, selection, geographical distribution

CONSIDERATIONS

There have been more than 350 species of arboviruses listed in the "International Catalogue of Arboviruses" (Berge, 1975), each possessing its unique geographical distribution. Arboviruses are grouped according to their characteristic mode of transmission in nature. They grow in vertebrate hosts, showing viremia and serves as infecting source in the blood meal to the hematophagous arthropods, called vectors. The virus then multiply in the tissues of arthropod and after a certain extrinsic incubation period appears in the

Received for Publication, November 21, 1984.
Contribution No. 1513 from the Institute for Tropical Medicine, Nagasaki University.

salivary gland and then capable of infecting another vertebrate hosts when the infective vector bites on them (WHO, 1967).

It has been well-established that cultured mosquito cells persistently infected with certain arboviruses could generate temperature-sensitive (*ts*) mutants, which showed different biological characteristics from the "standard" virus that had originally been used to initiate the infection (Stollar and Shenk, 1973; Shenk *et al.*, 1974; Stollar *et al.*, 1974; Maeda *et al.*, 1979; Igarashi, 1979; Mah and Westaway, 1980; Kuno, 1982). Such *ts*-viruses generally exhibited reduced virulence to mice, the most widely used vertebrate host in laboratories (Rehacek, 1968; Banerjee and Singh, 1968; Buckley, 1973; Sinarachatanant and Olson, 1973; Stollar *et al.*, 1974; Davey and Dalgarno, 1974; Peleg, 1975). Since these *ts* mutants possessed lower affinities to vertebrate rather than mosquito cells, their interfering activity on the growth of standard virus was more evident in mosquito cells than in vertebrate cells (Table 1). Therefore, such *ts* viruses will continue to be dominant population as long as persistently infected mosquito cells are maintained. However, once such virus population was transferred to vertebrate cells, small fraction of non-*ts* virus in the population possessing higher affinities to and higher growth capacity in vertebrate cells than the *ts*-virus could become dominant proportion of the virus population. *Ts*-mutant of Sindbis virus (an alphavirus of mosquito-borne togavirus) which was generated from persistently infected mosquito cells, was shown to "revert" easily to the "wild" type after a few passages in vertebrate cells (Shenk *et al.*, 1974).

When Sindbis virus was serially transferred undilutedly in vertebrate cells, defective-interfering (DI) particles were shown to accumulate in the virus population (Schlesinger *et al.*, 1972; Shenk and Stollar, 1972) This is rather a general phenomenon with animal viruses which was described by von Magnus (1954) for influenza virus and later found for other viruses as well (see reviews by Huang and Baltimore, 1977; Stollar, 1980). Such DI particles interfered the growth of standard virus in vertebrate cells, but the interfering activity was not efficient in mosquito cells (Eaton, 1975; Igarashi and Stollar, 1976). Therefore, as long as Sinbdis virus was undilutedly passaged in vertebrate cells,

Table 1. Growth inhibition of standard Sindbis virus (SV_{STD}) by Sindbis virus from persistently infected *Ae. albopictus* cells (SV_{PI}) in *Ae. albopictus* and BHK-21 cells

Virus inoculated	Yield of SV_{STD} (PFU/ml) in; (percent)		
	Ae. albopictus	BHK-21	
	28°C, 2 days	28°C, 2 days	37°C, 2 days
SV_{STD}	6.1×10^8 (100)	2.0×10^8 (100)	1.0×10^9 (100)
$SV_{STD}+SV_{PI}$	3.9×10^6 (0.64)	1.1×10^8 (55.0)	5.4×10^8 (54.0)

Each of the SV_{STD} and SV_{PI} was simultaneously inoculated at input multiplicity of 0.1 PFU/ml.

the virus population as a whole showed reduced infectivity compared with standard virus. However, when such virus with reduced infectivity was once passaged in mosquito cells, the progeny virus appeared to show exalted infectivity because of the low interfering activity of DI in mosquito cells. Serial undiluted passage of Sindbis virus in cultured mosquito cells by the same time schedule as in vertebrate cells did not apparently produce significant levels of DI particles (Igarashi and Stollar, 1976). However, later study using prolonged incubation after virus infection could produce significant levels of DI particles (King *et al.*, 1979). Such DI particles generated in mosquito cells did not efficiently interfere the growth of standard virus in vertebrate cells. Therefore, alternative passage of Sindbis virus, which was used extensively as a model of arboviruses, in vertebrate and mosquito cells appears to act as a kind of "biological filter" clearing *ts* mutants generated in mosquto cells and DI particles generated in vertebrate cells, resulting in the elevated levels of virus infectivity as a whole (Fig. 1).

The reasoning mentioned above is coming from a limited observation on mosquito cell cultures *in vitro*, however, could be potentiated by several field observations. Human beings could demonstrate the presence of certain viruses in nature through virus isolation procedures. In the case of arboviruses, inoculation of test materials to brains of suckling mice (SMB) was the most commonly used method. Our study group tried to use cultured mosquito cells to isolate Japanese encephalitis (JE) virus from field-collected mosquitoes. By using virus-sensitive clone C6/36 (Igarashi, 1978), isolation rate of JE virus was equal to or even higher than SMB-inoculation (Igarashi *et al.*, 1981a, b). Moreover, certain

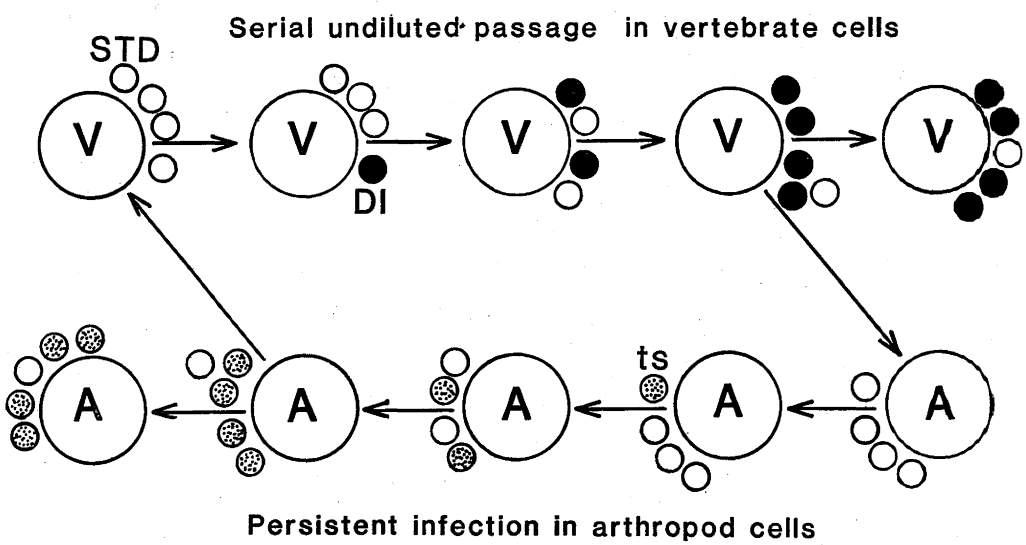

Fig. 1. Biological filter hypothesis on the growth of arbovirus in arthropod and vertebrate cells
A: arthropod cells; V: vertebrate cells; STD: standard virus; DI: defective-interfering particles; ts: temperature-sensitive virus

"mutants" of JE virus and Getah virus (an alphavirus) were detectable as plaque progenies from several virus strains isolated by C6/36 cells (Igarashi *et al.*, 1981c). Such mutants, however, could not be detectable from virus strains isolated by SMB, and possessed host-dependent *ts* characteristics, because their growth was restricted in BHK21 cells at 37°C but not in C6/36 cells at the same temperature. Their genome RNA was found to be different from that of the parental strain from which the mutants were isolated (Morita and Igarashi, 1984). Single SMB passage of the virus strain, which contained "mutant" viruses, appeared to have cleared "mutant" from the virus population. This is a kind of "biological filter" and conventional virus isolation by SMB was using such "filter" losing naturally occurring "mutant viruses".

The "biological filter" hypothesis could further be generalized as follows. Since arboviruses possess RNA as genetic materials, their mutation rate is expected as high enough (Holland *et al.*, 1982) to generate numerous mutations through their growth processes either in vertebrates or arthropods, resulting in the accumulation of various mutants. Growth cycle in vertebrates could confer a kind of selective pressure on the virus population, enabling those mutants which possessed selective advantages in the vertebrates to become major population with many other as minorities or eliminated. Subsequent growth cycle in arthropods could confer another kind of selective pressure, which is different from the previous one, resulting in the virus population consisting of different proportions of various mutants. Also, it is conceivable that the direction of "mutation" in vertebrates will be different from that in arthropods, because these 2 kinds of hosts are phylogenetically quite remote from each other, providing quite different intracellular environments and host factors which will be necessary to support virus growth. Therefore, a number of alternative growth in 2 different kinds of hosts could result in an enormous numbers of mutations with genetically quite heterogenous populations of the virus. However, 2 selective pressures towards quite different directions by vertebrate and arthropod could have eliminated most of the "mutants" letting only limited kinds of the mutants to survive as major population of the virus. Since the selective pressures and also, intracellular events could be different from one species of vertebrate or arthropod to another, the resulting population of the virus would be dependent on the vertebrate hosts and arthropod vectors in which the virus could grow. Since different parts of the world have enabled different species of vertebrates and arthropds to survive, the selective pressures as well as the directions of the arbovirus mutations in these hosts and vectors could have been different from one geographic area to another, resulting in the divergence to many species of arboviruses. These considerations appears to give an easy way to explain the fact that on the earth, there exist many different but related arbovirus species as listed in the "Catalogue", each occupying different geographic "territories" of distribution. Even when there could be a chance of other related and different viruses to invade into the territory of another virus, the invading ones could certaliny possess lower selective advantages compared with preexisting one as long as the environmental factors including the species of vertebrate and arthropod remain

the same. Such "invasion by alien viruses" could be successful only when the "aliens" possess higher selective advantages over preexisting one.

The consideration mentioned above, thus favours the idea that the stability of biological entities, including arboviruses, in a given geographic area is dependent on the environment in which they are now existing. Drastic alteration of the environment, either natural or artificial, could thus alter the existence of life styles of many creatures, including human beings and their pathogens.

REFERENCES

1) Banerjee, K., & Singh, K. R. P. (1968). Establishment of carrier cultures of *Aedes albopictus* cell line infected with arboviruses. Ind. J. Med. Res., 56, 812−814.

2) Berge, T. O. (ed.). (1975). International Catalogue of Arboviruses including certain other viruses of vertebrates. 2nd. ed. U. S. Department of Health, Education, and Welfare, Public Health Service, Washington.

3) Buckley, S. M. (1973). Modification of chikungunya virus in Singh's *Aedes albopictus* cells. p307. *In* J. Rehacek, D. Blaskovic, and W. F. Hind (eds.). Proc. 3rd Intern. Colloquium on Invertebrate Tissue Culture. Slovak Academy of Sciences, Bratislava.

4) Davey, M. W., & Dalgarno, L. (1974). Semliki Forest virus replication in cultured *Aedes albopictus* cells: studies on the establishment of persistence. J. Gen. Virol., 24, 453−463.

5) Eaton, B. T. (1975). Defective interfering particles of Semliki Forest virus generated in BHK cells do not interfere with viral RNA synthesis in *Aedes albopictus* cells. Virology 68, 534−538.

6) Holland, J., Spindler, K., Horodyski, F., Graban, E., Nichol, S., & Van de Pol, S. (1982). Rapid evolution of RNA genomes. Science 215, 1577−1585.

7) Huang, A. S., and Baltimore, D. (1977). Defective interfering viruses. Comprehensive Virol., 14, 243−261.

8) Igarashi, A. (1978). Isolation of a Singh's *Aedes albopictus* cell clone sensitive to dengue and chikungunya viruses. J. Gen. Virol., 40, 531−544.

9) Igarashi, A. (1979). Characteristics of *Aedes albopictus* cells persistently infected with dengue viruses. Nature, 280, 690−691.

10) Igarashi, A., & Stollar, V. (1976). Failure of defective interfering particles of Sindbis virus produced in BHK or chicken cells to affect viral replication in *Aedes albopictus* cells. J. Virol., 19, 398−408.

11) Igarashi, A., Buei, K., Ueba, N., Yoshida, M., Ito, S., Nakamura, H., Sasao, F., & Fukai, K. (1981a). Isolation of viruses from female *Culex tritaeniorhynchus* in *Aedes albopictus* cell cultures. Amer. J. Trop. Med. Hyg., 30, 449−460.

12) Igarashi, A., Makino, Y., Matsuo, S., Bundo, K., Matsuo, R., Higashi, F., Tamato, H., & Kuwatsuka, M. (1981b). Isolation of Japanese encephalitis and Getah viruses by *Aedes albopictus* clone C6/36 cells and by suckling mouse brain inoculation, in Nagasaki 1980. Trop. Med., 23, 69−78.

13) Igarashi, M., Sasao, F., Fukai, K., Buei, K., Ueba, N., and Yoshida, M. (1981c). Mutants of Getah and Japanese encephalitis viruses isolated from field-caught *Culex tritaeniorhynchus*

using *Aedes abopictus* clone C6/36 cells. Ann. Virol., 132E, 235−245.
14) King, C.-C., King, M. W., Gary, R. F., Wan, K. M.-M., Ulug, E. T., & Waite, M. R. F. (1979). Effect of incubation time on the generation of defective interfering particles during undiluted serial passage of Sindbis virus in *Aedes albopictus* and chick cells. Virology 96, 229−238.
15) Kuno, G. (1982). Persistent infection of a nonvector mosquito cell line (TRA-171) with dengue viruses. Intervirology, 18, 45−55.
16) Maeda, S., Hashimoto, K., & Simizu, B. (1979). Complementation between temperature-sensitive mutants isolated from *Aedes albopictus* cells persistently infected with western equine encephalitis virus. Virology, 92, 532−541.
17) von Magnus, P. (1954). "Incomplete" forms of influenza virus. pp36-44. *In* F. W. Hartman, F. L. Horsfall, Jr., & J. G. Kidd (eds.). The Dynamics of Virus and Rickettsial Infection. Blakiston, New York.
18) Mah, L. -N., & Westaway, E. G. (1980). Establishment of persistent infections by flaviviruses in *Aedes albopictus* cells. pp389−402. *In* E. Kurstak, K. Maramorosch, & A. Dübendorfer (eds.). Invertebrate Systems *in Vitro*. Elsevier/North-Holland Biomedical Press, Amsterdam.
19) Morita, K., & Igarashi, A. (1984). Oligonucleotide fingerprint analysis of Getah virus isolated in Japan and Malaysia. J. Gen. Virol., 65, 1899−1908.
20) Peleg, J. (1975). *In vivo* behavior of a Sindbis virus mutant isolated from persistently infected *Aedes aegypti* cell cultures. Ann. N. Y. Acad. Sci., 266, 204−213.
21) Rehacek, J. (1968). Persistent infection of mosquito cells grown *in vitro* with Murray Valley encephalitis and Japanese encephalitis viruses. Acta Virol., 12, 340−346.
22) Schlesinger, S., Schlesinger, M., & Burge, B. W. (1972). Defective virus particles from Sindbis virus. Virology, 48, 615−617.
23) Shenk, T. E., & Stollar, V. (1972). Viral RNA species in BHK-21 cells infected with Sindbis virus serially passaged at high multiplicity of infection. Biochem. Biphys. Res. Commun., 49, 60−67.
24) Shenk, T. E., Koshelnyk, A., & Stollar, V. (1974). Temperature-sensitive virus from *Aedes albopictus* cells chronically infected with Sindbis virus. J. Virol., 13, 439−447.
25) Sinarachatanant, P., & Olson, L. C. (1973). Replication of dengue virus type 2 in *Aedes albopictus* cell culture. J. Virol., 12, 275−283.
26) Stollar, V. (1980a). Defective interfering alphaviruses. pp427−457. *In* R. W. Schlesinger (ed.). The Togaviruses. Biology, Structure, Replication. Academtc Press, New York.
27) Stollar, V. (1980b). Togaviruses in cultured arthropod cells. pp 583−621. *In* R W. Schlesinger (ed.). The Togaviruses. Biology, Structure, Replication. Academic Press, New York.
28) Stollar, V., & Shenk, T. E. (1973). Homologous viral interference in *Aedes albopictus* cultures chronically infected with Sindbis virus. J. Virol., 11, 592−595.
29) Stollar, V., Peleg, J., & Shenk, T. E. (1974). Temperature sensitivity of a Sindbis virus mutant isolated from persistently infected *Aedes aegypti* cell cultures. Intervirology, 2, 337−344.
30) WHO Scientific Group (1967). WHO Technical Report Ser. No. 369.

アルボウイルスの地理的分布に関する仮説

五十嵐　章　（長崎大学熱帯医学研究所ウイルス学部門）

実験室および自然界においてアルボウイルスに感染した蚊または蚊の培養細胞に関する観察から，世界において互に関連性をもっている数多くのアルボウイルスの地理的分布を説明し得る仮説が導かれた．「生物学的フイルター」と呼ぶこの仮説は，アルボウイルスの RNA 遺伝子に高頻度で起る突然変異と，アルボウイルスが脊椎動物と節足動物という系統発生的に遠く離れた2つの宿主で交互に増殖する事によってウイルス集団に及ぼされる2種類の相異なる撰択圧力を組合せて考慮するものである．アルボウイルスが自然界でこのような増殖形態をとる結果，その増殖過程で高頻度の突然変異が生ずるにもかかわらずウイルス集団としては或る限られた範囲の変化しか起り得ない事になる．地球上のいろんな地域に生息する節足動物および脊椎動物はそれぞれ異っているから，地理的に異なる地域でアルボウイルスに加えられる撰択圧力も異っていると考えられる．従って，アルボウイルスの起源は一種ないしは限られた数種であったものが，幾多の増殖過程を経る間にいろんな方向に分散的に変異した結果，今日地球上のいろんな地域にはそれぞれ関連性はあるが異なる種類のアルボウイルスが存在するようになったと考てられる．アルボウイルスが節足動物と脊椎動物で増殖する間に数え切れない位の突然変異が起るであろうが，そのうちの限られたものだけがある地域に存在する節足動物と脊椎動物の双方で他のものよりも有利な条件を有していた結果進化の過程において生き残ったのであろう．この仮説によって，現在地球上のいろんな地域にはそれぞれ関連性は有するが別のアルボウイルスが分布している理由を説明することができる．

熱帯医学　第26巻　第4号，173－179頁，1984年12月