Arboviruses have evolved a remarkable capacity to complete their life cycles alternately under conditions as diverse as those in the organs and tissues of arthropods and vertebrates. Perhaps even more remarkable than this capacity to adapt such differing cell materials for virus replicative functions is their success in overcoming or circumventing the diverse specific mechanisms and environmental conditions involved in host resistance. In considering resistance, we tend to focus on the environment generated by the complex machinery of vertebrate inflammation and immunity, but the environment faced by a virus in the arthropod may be no less hostile.1

To complete its life cycle, an arbovirus must invade arthropod tissues and survive anatomic, physiologic, and biochemical barriers in several organs before progeny virus is delivered to salivary secretions for injection into the vertebrate host. The infectious processes that must occur in arthropod tissues and organs for successful perpetuation of virus seem, on the one hand, to reflect highly developed, highly productive adaptive patterns that are well entrenched in nature. The widespread occurrence and importance of arboviruses argues this point. On the other hand, these infectious processes in arthropods seem to involve the most tenuous aspects of life cycle continuity. An impasse in the processes of viral spread or proliferation in the earliest stages of infection in an arthropod host can be most effective because the fewest infectious units are available. During these earliest stages of infection, the relatively few virus particles must survive the most adverse extracellular conditions within the host body before being able to initiate replication.

**Absolute Refractoriness**

Failures in the establishment of arthropod infections most often result from impasses at the earliest stages of viral invasion. Such intrinsic failures have been attributed to the presence of a "gut barrier" or "threshold," although whether these occur as physiologic or anatomic impasses to the movement of virus from an infected blood meal in the gut lumen or as impasses in viral adsorption, penetration, or infection of gut epithelium is not known.2 Absolute refractoriness may be an expression of either active destruction of virus by means unknown or of the gradual dying off of ingested virus in the absence of favorable environmental requirements. In particular, such factors as toxicity of digestive fluids, impermeability of a peritropic membrane, physicochemical deficiencies of gut cell membranes, and activities of arthropod "surface defense
mechanisms" of unknown nature have been considered. In view of more recent knowledge, the fundamental contributions of host factors in the replication of arboviruses in arthropods seem to be more subtle than previously thought. The failure of poliovirus RNA to initiate infection in mosquito cell cultures, which are quite susceptible to togavirus RNA, exemplifies the need for a more comprehensive understanding of the biochemical parameters of viral replication. In this case, viral RNAs with similar replication, transcription, and translation mechanisms behave differently in a system where viral surface functions (adsorption, penetration, and so on) are not factors. The expression of intrinsic defense mechanisms may be genetically determined, and being so, these mechanisms probably have had predominant roles in the evolution of vectorborne infections and in their positions in nature. In other words, it is the narrow range of competence and the widespread occurrence of refractoriness of potential vectors that keep us from literally being overrun by viruses that use invertebrate hosts indiscriminately.

**Modulation of Arthropod Infection**

Mechanisms that cause absolute arthropod refractoriness must be different from those that quantitatively limit or modulate virus yield within an effective arthropod vector species. There is a wide variation in the resistance of vertebrate hosts to particular arboviruses; this variation in the probability of vertebrate infection is often directly related to the amount of infectious virus delivered by the biting arthropod. Therefore, the quantal modulation in the productivity of infection in arthropod tissues, particularly in salivary glands, has an important role in nature. An understanding of the mechanisms of this modulation may provide a key to future human intervention and disease control.

Infectious processes in arthropods have primarily been studied by sequential assay of viral transmissibility and sequential titration of arthropod organs and tissues. These approaches have been complemented by immunofluorescence and, more recently, by electron microscopy of arthropod tissues. In general, such studies have shown early infection of midgut epithelium and later invasion of salivary glands. In each of these organs, a rapid rise in virus infectivity, virus antigen accumulation, and virus particle concentration has been followed by either a "plateau" phase or a gradual decline through the remainder of the life-span of the arthropod host. Electron microscopic studies performed in our laboratory on *Aedes triseriatus* mosquitoes infected with eastern equine encephalitis (EEE; an alphavirus) virus and of *Culex pipiens* mosquitoes infected with St. Louis encephalitis (SLE; a flavivirus) virus clearly showed that the plateau and decline in the proportion of gut cells infected occurred before theoretic limits were reached. The yield of virus particles was also low relative to the potential of the available cell population through the time course studied. For example, SLE virus never infected more than one of every five gut cells, and EEE virus plateaued after infecting one of every three cells. In the salivary glands of *A. triseriatus*, EEE virus cumulatively reached high concentrations late in infection, and in *C. pipiens*, SLE virus became so concentrated that particles formed paracrystalline arrays (see Figure 1); however, there was no instance where more than one in every 20

**Figure 1.** Electron micrograph of St. Louis encephalitis virus in salivary gland of a *C. pipiens* mosquito 25 days after infection. Virus concentration in a luminal diverticulum is so great that a paracrystalline array is formed. × 41,000.
salivary gland epithelial cells was infected. No morphologic evidence to explain this homeostatic condition was found, and no cytopathic effect of infection was ever identified.

**MECHANISMS OF INFECTION MODULATION**

As with infectivity titration and immunofluorescence studies, the electron microscopic studies performed in our laboratory have not indicated any one hypothetic modulating mechanism, nor have any of these studies localized the mechanism to the arthropod, the virus, or the arthropod-virus interaction. There has been no evidence of hemocytic migration, proliferation, or accumulation in arthropod tissues that carry the highest infectivity titers. We found no evidence of physical degradation of progeny virus in arthropod tissues. This result must be contrasted with one study of *Aedes albopictus* cells grown in culture and infected with Ross River virus (an alphavirus). After a virus proliferation phase, these mosquito cells "cured" themselves of residual infection via a unique endophagocytic process; this phenomenon needs to be studied further.

Because we cannot explain arbovirus modulation as a function of arthropods themselves (other than to associate it with a limitation in the proportion of potential host cells actually productively infected), the several general virus-limiting mechanisms known in vertebrates need to be examined for appropriate analogies. These vertebrate cell mechanisms also act by limiting virus spread and may be considered "primitive," in that they are active in undifferentiated cells and cells in culture. First, a defense elicited by the vertebrate host itself but triggered by virus infection, namely, interferon synthesis, may effectively prevent continuing virus replication cycles. There is preliminary evidence of interferon-like activity in mosquitoes and mosquito cells in culture, but the suspected suppressor of viral replication must be further characterized physicochemically before we can conclude that arthropods make and use interferon.

Another general mechanism that can be effective in restricting viral spread is homologous autointerference. Homologous autointerference may be mediated by the progeny of infecting virus in several ways: progeny viral constituents indistinguishable from those of parental type (wild type) may mediate interference; mutants of the parental virus that can still replicate under some conditions may interfere with wild-type replication; defective-interfering viral progeny or constituents, which can only replicate in the presence of intact virus, may competitively interfere with wild-type replication; and, finally, the distinct phenomenon called "intrinsic interference" may be operative in arthropods.

**Wild-Type Virus Autointerference**

Regulation failure that results in a shortage of virion constituents would favor a buildup of progeny RNA and double-stranded replicative intermediate species; the latter are known in several circumstances to be inhibitory to further RNA replication. This sort of feedback inhibition acting at the level of viral RNA replication might be particularly effective in arthropod cells in the absence of cytopathology; it might also be active over the long course of infection in
arthropods. A further inhibitory effect could be associated with normal wild virus replication; the continuing translation from viral RNA messenger species might yield concentrations of viral products that would affect further transcription and even further replication of viral RNA. The "toxic" properties of accumulated viral proteins are now being widely considered as a mechanism of terminating infection. Experimental proof of viral protein buildup in arthropod tissues is minimal, but viral protein buildup seems to occur in mosquito cell cultures.

**Mutant Virus Interference**

Temperature-sensitive mutants, small-plaque-sized mutants, virus particle surface antigenicity mutants, and heat-labile mutants have been commonly isolated from mosquito cell cultures infected persistently with several arboviruses. Such mutations are often associated with a decrease in vertebrate host virulence and a decrease in progeny virus yield. These RNA+ mutants can replicate under permissive conditions, and there is evidence that they may interfere with wild-type virus replication. If these mutations are also related to failures in the regulation of viral synthetic processes or in viral constituent formation, the buildup of double-stranded RNA species would inhibit further synthesis, just as was described for wild-type virus interference. Alternatively, if mutations reflect sublethal quantitative or qualitative deficiencies in viral constituents, infection cycles might be affected directly.

**Defective Interference**

This type of modulation is a property of virus particles with defective RNA content that competes successfully for replication of viral RNA but yields non-infectious virus. Defective interference is a special case of mutation in which multiplication of mutant RNA is amplified. It is commonly seen in some infections, such as influenza, parainfluenza, rhabdovirus, and arenavirus. Recently, defective-interfering particles with abnormal RNA have been found in two alphaviruses in cell culture; they are Sindbis and Semliki Forest viruses. These *in vitro* findings should now be tested in intact mosquitoes by sequential assays of infectious:defective particle ratios. In an infected arthropod, the yield of defective virus particles from an initial infection site could seed other cells in the same organ or even elsewhere in the body and competitively inhibit normal viral RNA synthesis.

**Intrinsic Interference**

The initial observation of this phenomenon was that cells in culture infected with rubella virus (a togavirus) were refractory to superinfection with Newcastle disease virus but could be productively infected with several other viruses. The phenomenon does not require new cellular synthesis (as does the interferon response) but depends upon viral proteins blocking early steps in the replication of the superinfecting virus. This mechanism should be further considered in the modulation of arbovirus infections in arthropods; it may also
have a role in the heterologous interference exhibited when two related arboviruses infect the same arthropod. This mechanism and others may also play a role in interference between endogenous arthropod viruses and superinfecting arboviruses.

**Conclusions**

The likelihood that any or all of these modulating mechanisms might be active in arbovirus-infected arthropods may not warrant consideration, because experimental evidence is not available. Nevertheless, modulation is a real phenomenon, and the mechanisms described could, in the absence of real immune mechanisms, greatly affect infectious virus titers and transmissibility. From the standpoint of the natural history of arboviruses, the mechanisms under consideration might keep viral burdens within bounds that are physiologically tolerable to the vector species involved and at the same time provide a survival advantage to vector and virus alike. From another standpoint, we must consider means of intentional genetic manipulations of modulating mechanisms. With further understanding of their modes of action, we may be able to introduce variant characteristics into wild vector populations and, in doing so, make poor virus vectors out of good ones.

**Summary**

When an arbovirus enters an arthropod in an infected blood meal, several mechanisms may interact to affect its life cycle and ultimate transmissibility. Intrinsic absolute failure in the establishment of infection must be contrasted with infection that is successfully established but is variably modulated in its viral yield throughout the vector's life-span. Degrees of vertebrate host resistance make this modulation a central factor in determining whether an arthropod is an important vector in nature; moreover, human intervention that affects modulating mechanisms may become a basis for disease control. In the absence of evidence of real immune resistance to arbovirus infections in arthropods, other more primitive modulating mechanisms must be considered: interferon-like substances may be formed in arthropod cells; arthropod cells may "cure" themselves by a unique endophagocytic digestion of their virus burden; homologous interference with viral replicative processes may be mediated via wild or mutant viral RNA species acting to shut down further RNA synthesis; and homologous interference may be mediated by RNA of defective-interfering virus formed earlier in infection.

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REFERENCES


