Eastern Equine Encephalitis Virus
Quantitative Study of the Effects of Interferon on Virus Replication

By

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Summary

Eastern equine encephalitis (EEE) virus replication in rabbit kidney (RK) cells was inhibited by interferon (IF). Interferon protected against the virus-induced shutoff of host protein synthesis and partially suppressed the synthesis of EEE structural proteins in infected cells. High doses (300–3000 units) inhibited the labeling of viral RNA in interferon-treated RK cells by as much as 98 per cent, as measured by TCA precipitation following 10, 30 or 60 minute labeling period. Sucrose gradient analysis confirmed that incorporation of $^3$H-uridine into 23S and 40S viral RNA was inhibited by interferon, the latter being slightly more sensitive. The logarithm of the surviving fraction of each virus-specific event persisting in the interferon-treated infected cell was a linear function of the logarithm of the IF dose over a 1000-fold concentration range. The relative interferon sensitivities of the viral events measured were from highest to lowest: EEE yield > EEE RNA synthesis > EEE-induced CPE > EEE-induced shutoff of RK cell protein synthesis = EEE structural protein synthesis.

1. Introduction

The interferon-induced antiviral state in cell culture has been reported to lead to suppression of a number of activities concerned with virus replication. However, these virus-specific events appeared to differ in their sensitivities to interferon (IF). As this report is primarily concerned with group A arboviruses, the effect of IF on the replication of these agents should be reviewed. Wagner (29) found that IF...
inhibited both progeny virus yield and cytopathology in eastern equine encephalitis (EEE) virus-infected chick embryo fibroblasts (CEF), the former being more sensitive to IF. In a similar system, Ho (13) found that the production of infectious viral RNA was considerably more resistant to IF than virus yield. Employing Semliki Forest virus (SFV) in CEF, Mees et al. (20) repeated the result of Ho (13), and also demonstrated that a dose-response relationship existed between IF dose and amount of viral RNA found. They also found that the viral RNAs did not respond identically to IF, that the 45S virion RNA was more sensitive, and the 20S double-stranded form was less so. Armstrong et al. (2), using EEE virus in a continuous line of rabbit embryo (RE-ICH) cells, found that the 40S virion RNA was quite sensitive to IF while the 22S double-stranded form was not inhibited by IF during short pulse labeling procedures.

Other aspects of arbovirus replication have been studied. Friedman (8) found that IF virtually abolished virus-directed protein synthesis in SFV-infected CEF; however IF did not prevent virus-induced shutoff of host protein synthesis (6). In contrast, Muggay et al. (21) reported only partial suppression of Sindbis virus-induced shutoff of CEF protein synthesis by IF. The dose response of these two virus-specified events to IF was not determined. This review of the literature is not intended to be exhaustive, but rather to point out that some discrepancies exist between investigators concerning the effects of interferon on various arbovirus replicate events. Undoubtedly, many of these can be assumed to result from employment of different viruses, cells, and interferon preparations. One goal of our research was to study many parameters of viral inhibition under constant conditions in one virus-cell-interferon system, which has not been previously reported. Valuable insight may be gained on the mode of inhibitor action by a careful comparison of the dose-response curves of sensitive events. In addition, the dose response to interferon of virus-specific protein synthesis and virus-induced shutoff of host protein synthesis have not been previously described for this virus group. Therefore, the relationship of their dose responses to those of other viral processes, particularly virion production and viral RNA synthesis, was of interest. Another aim of this work was to examine the biochemistry of EEE virus replication, especially viral protein synthesis.

2. Materials and Methods

2.1. Cells

Preparation of weanling rabbit kidney (RK) cell cultures (15) and chick embryo fibroblast (CEF) cultures (14) have been described previously. At the time of use, confluent RK monolayers generally contained 2.5–4.0 × 10⁶ cells/5.0 cm culture plate.

2.2. Virological Procedures

Eastern equine encephalitis (EEE New Jersey E-27) virus stocks were prepared in CEF cultures in 32 oz. prescription bottles, as described previously (14). Monolayers of cultures in 5 cm plates were infected with an input multiplicity of 50–80 PFU/cell; infected or mock-infected cultures were maintained in 2.0 ml of minimal medium (17). Virus plaque assays were performed on monolayers of CEF in plastic Petri dishes, basically as previously reported (14).