REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

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3.	Frontline area of research in which training/research was carried out	:

Introduction

Primary infection with herpes simplex virus-1 (HSV-1) results in productive replication of the virus at the site of infection. During this initial phase, virus enters trigeminal ganglia and virus replication occurs there but within a few days no virus can be detected. The genome, however, persists in neurons in a latent state from which it reactivates periodically to resume replication and produce infectious virus. In mice, latency can be established efficiently after inoculation with HSV-1 in the cornea. The steps of the latency-reactivation cycle have been operationally divided into three major steps: establishment, maintenance, and reactivation. Establishment of latency includes entry of the viral genome into a sensory neuron and acute infection. Viral gene expression is then extinguished, with the exception of the latency-associated transcript (LAT). Maintenance of latency is a phase that lasts for the life of the host. In general, abundant expression of viral genes that are required for productive infection does not occur. LAT is abundantly expressed during this stage of latency. The promoter that directs expression of the latency-associated transcript (LAT) is activated in sensory neurons.

Therefore, I started my work with purification of available HSV-1 virus by picking single plaque through Agarose overlay method and through multiple passages in Vero cells (Fig.1), titer of virus was increased to $10^{8.5}$. Since latency associated transcripts (LAT) play role in establishment, maintenance and reactivation of virus from latency and is also involved in antisense suppression mechanism because it overlaps the 3′ end of infected cell protein 0 (ICP0) mRNA, an immediate early gene of virus lytic cycle responsible for productive infection therefore, proposed work was planned on understanding the mechanism of latency. Virus genomic DNA was isolated by standard kit. Primers were designed for amplification of LAT gene. For development of latency, mouse neuroblastoma cell line was used and infected with 0.1 MOI (Multiplicity of Infection) and cells were subcultured three times and titer of virus was calculated in pfu and there was no cytopathic effect seen in this cell line unlike Vero cells since virus donot replicate in neuronal cells. US3, One of the important gene of HSV-1, a serine/threonine protein kinase gene, is responsible for neuroinvasiveness and reactivation from latency. It phosphorylates UL47, a major virion protein responsible for HSV-1 nuclear egress. UL47 enhance the efficiency of alpha TIF (VP16)-mediated alpha gene expression through an unknown mechanism of action and exhibits altered viral thymidine kinase gene expression and thus affects the virulence and pathogenesis.