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FRAGMENTATION AND REARRANGEMENT OF THE GOLGI APPARATUS DURING MHV INFECTION OF L-2 CELLS

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INTRODUCTION

The Golgi apparatus-complex (GA) plays a seminal role in the transport, processing and targeting of polypeptides synthesized in the rough endoplasmic reticulum (RER). Important insight into the function of the GA has been gained with the use of viruses or their coat glycoproteins which are processed through the GA. Coronavirus mouse hepatitis virus (MHV), possesses a membrane protein (M) which is targeted to the trans-Golgi network (TGN) [1].

EXPERIMENTAL DESIGN AND METHODS

In this study, we examined the morphologic aspects of the GA in syncytia formation during infection of L-2 cells rat fibroblasts with MHV-A59. At 4 hour intervals cells were fixed in 2% paraformaldehyde, permeabilized and processed for immunohistochemistry with ABC Vector elite kit according to manufacturer's recommendations. Other cells were grown on therminox, fixed with Karnowski's fixative, post-fixed in osmium tetroxide and processed for electron microscopy as previously described [2].

RESULTS

Immunostaining with anti MG-160 antibodies, a GA specific marker[3], revealed fragmentation and translocation of the GA in the center of the syncytia 16-24 hours post infection. Electron microscopy confirmed the presence of a fragmented GA in the center of

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the syncytia. Antibodies against a RER protein [4], and against alpha and beta tubulin, revealed no significant changes in the distribution of the RER and cytoskeleton in MHV infected cells until the end stage of cell death (48 hours). Two fusion-defective, infectivity-competent, mutant MHVs, which contain an identical amino acid alteration in the cleavage signal sequence of the spike (S) glycoprotein [5] caused fragmentation of the GA but without complete aggregation of the GA in the center of the syncytia. Revertant viruses had fusion properties and GA staining as MHV-A59.

DISCUSSION

MHV-induced fragmentation of the GA is independent of the formation of syncytia. However, the translocation of the fragmented GA toward the center depends on syncytia formation. This translocation is associated with, and maps like fusion to the cleavage site of the S glycoprotein of the virus. Fragmentation and/or rearrangement of the GA has been previously shown to occur under several conditions: during mitosis[6], in certain cell lines infected with herpes simplex virus [7], and in motor neurons in the human disease amyotrophic lateral sclerosis [8]. The molecular mechanism(s) of the fragmentation of the GA induced by these conditions remains to be elucidated.

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