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CHAPTER 26

MOUSE HEPATITIS VIRUS: MOLECULAR BIOLOGY AND IMPLICATIONS FOR PATHOGENESIS

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I. HOST FACTORS IN CORONAVIRUS PATHOGENICITY

The outcome of coronavirus infections frequently depends upon the age, immune status and genetic background of the host and on the strain of virus. Coronavirus diseases of man and domestic animals have been summarized in a recent review (1). In general, coronaviruses have very stringent species and tissue tropisms which affect both the epidemiology and pathogenesis of coronavirus-induced disease. Bovine coronavirus (BCV) and human respiratory coronavirus infections are strictly limited to epithelial cells of the respiratory or gastrointestinal tracts. Other coronaviruses can cause systemic diseases and may exhibit tropisms for the liver, lymphoid tissue or brain. These include mouse hepatitis virus (MHV), feline infectious peritonitis virus (FIP) and hemagglutinating encephalomyelitis virus of swine (HEV).

It has long been recognized that host factors play an important role in coronavirus-host interactions (2,3). Age is an important determinant of coronavirus virulence. Infections with most enterotropic coronaviruses cause much more severe disease in neonatal animals than in older animals (1). Bang and his colleagues (2,4) showed nearly 20 years ago that certain host genes could determine the outcome of infection with MHV. The murine genes for susceptibility or resistance to MHV which they identified determine both susceptibility of the mouse to death from MHV infection and permissiveness for MHV replication of cultured peritoneal macrophages from susceptible or resistant animals. In later studies, genetically determined host resistance to MHV was also found in cultured oligodendroglial cells or hepatocytes (5,6). The molecular mechanisms for host genetic resistance to coronavirus replication have not yet been elucidated.

The integrity of the host's immunological system is another important determinant of the outcome of coronavirus infection. Immunosuppression enhances susceptibility to some coronaviruses, such as MHV (2). Mice given transplanted tumors or immunosuppressive drugs, or infected with other viruses, bacteria or parasites may develop fulminating hepatitis and die from what would normally have been a mild or inapparent infection with MHV (7). Nude mice, which lack functional T lymphocytes, show greater morbidity and mortality from MHV infection than do immunologically intact animals.

In an MHV epidemic in a mouse colony, the spectrum of disease ranges from inapparent infection to overwhelming infection and death. Host factors undoubtedly play important roles in this variation in virulence of virus infection. Animals with inapparent MHV infections are carriers which can transmit infection to susceptibles. In such carriers, the sites and extent of virus replication and the duration of virus shedding have not yet been clearly defined. There is widespread belief that coronaviruses can cause asymptomatic persistent infection with virus shedding over a period of months. MHV can cause persistent infection *in vitro* (8) and *in vivo* (9), but many infections *in vivo* appear to resolve rather quickly without virus persistence (10,11). However, a temperature-sensitive mutant of MHV-JHM was observed to persist for at least one year in BALB/c mice (12). The host and virus factors which permit persistence of MHV have not been defined.

MHV epidemics in laboratory animal colonies have been implicated as a possible cause of variability in diverse experimental protocols. The time has come to progress from

anecdotes about the effects of MHV on murine research to documentation of specific effects of MHV infection on well defined experimental protocols and to characterization of the pathogenic mechanisms responsible. In this paper we will summarize current concepts of coronavirus structure and replication (13-15), and then evaluate the critical role of the host in determining the outcome of MHV infection.

II. MOUSE HEPATITIS VIRUS STRUCTURE AND ANTIGENICITY

MHV, like all coronaviruses, is an enveloped RNA virus with a helical nucleocapsid. The genome is single stranded, non-segmented RNA of positive or message sense (14). It interacts with a 50K phosphoprotein, N, to form the long flexible, helical nucleocapsid. Figure 1 shows the characteristic long, petal-shaped peplomers or spikes on the viral envelope which give the coronaviruses their name. The viral envelope is composed of two viral glycoproteins in a lipid bilayer derived from intracellular membranes. The peplomers are composed of a glycoprotein, E2, in both the intact form (180K) and its proteolytically cleaved products (90A and 90B) (16). The E2 glycoprotein is responsible for attachment to receptors on susceptible cells and for virus-induced cell fusion (3,16-18). The other glycoprotein of the coronavirus envelope, E1, is an unusual transmembrane

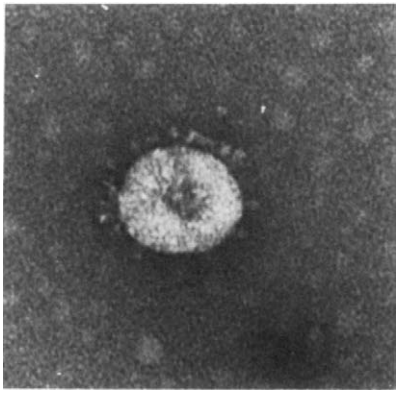

Figure 1. Structure of mouse hepatitis virus (MHV-A59). Negative staining with phosphotungstic acid shows the characteristic long petal shaped peplomers or spikes on the viral envelope. Magnification: X 200,000.

glycoprotein. Its unique structure was deduced from the nucleotide sequence of the clone E1 gene (19) and from enzymatic digestion of domains of E1 on the inner and outer surfaces of the lipid bilayer (20,21). In its small external domain, short oligosaccharides are linked to serine andthreonine residues by O-glycosidic bonds (22,23). E1 contains two hydrophobic intramembranous domains separated by a short loop which extends into the cytoplasm. The carboxy terminal domain of the E1 molecule lies on the cytoplasmic side of the lipid bilayer (20). E1 appears to be responsible both for formation of the viral envelope on intracellular membranes and for interaction of the viral envelope with nucleocapsids (17).

Neutralizing and type-specific antibodies are directed against E2, although antibody to E1 can neutralize virus in the presence of complement (18). Monoclonal antibodies to various MHV strains can distinguish at least five epitopes on E2, and at least two major epitopes on E1 (18,24,25). Some of these monoclonal antibodies will permit identification of specific strains of MHV associated with epidemics in mouse colonies.

III. REPLICATION OF MOUSE HEPATITIS VIRUS

The replication of coronaviruses has been studied in many laboratories using MHV as a model system (15). A diagram of the events in a productive infection with MHV is shown in Figure 2. When the positive-sense viral genomic RNA enters the host cell, it is apparently translated to form one or more 200K polypeptides which may include the viral RNA-dependent RNA polymerase (26). This enzyme transcribes the genomic RNA to form a full-length minus strand template (27,28). Transcription of this template results in formation of both full length, plus-strand genomic RNAs and six overlapping sub-genomic mRNAs which form a nested set and have a common 3' polyadenylated end (29,30). The 5' end of each of these mRNAs and the genomic RNA have the same RNA sequence. This leader sequence is composed of a cap with approximately 70 nucleotides and is apparently encoded by the 3' end of the negative strand template (29,30). The mechanism by which the leader is added to each of the mRNA species is not yet certain, but it is believed that the leader acts as a primer for plus strand RNA synthesis (31,32).

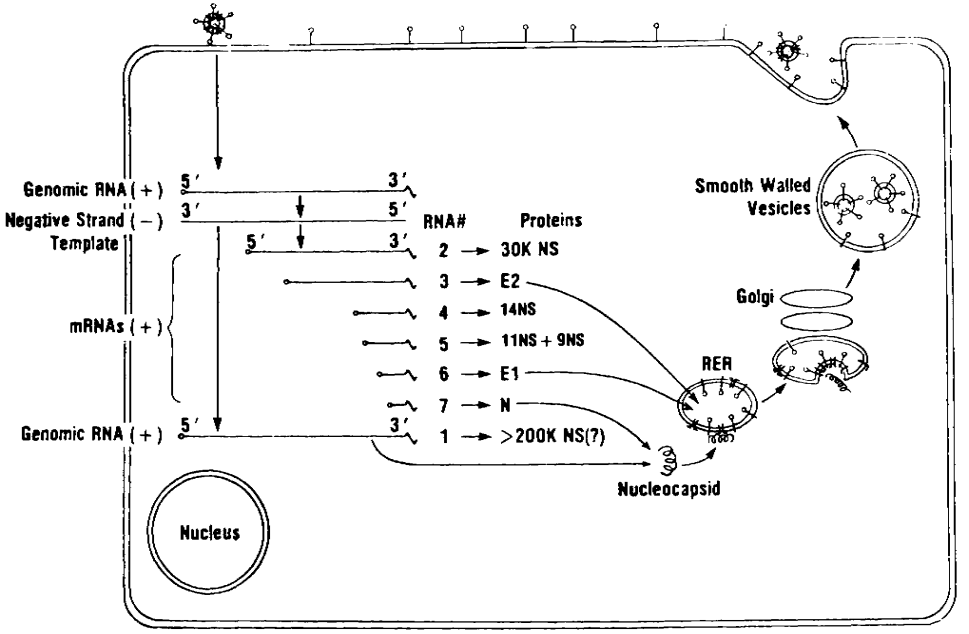

Figure 2. Model of coronavirus replication. Following adsorption and penetration, viral genomic RNA serum as mRNA, and RNA polymerase (200K) is made. This directs synthesis of a full length, negative strand RNA template, six subgenomic mRNAs and new genomic RNA. Each mRNA is translated to yield one protein, except that mRNA #5 yields two non-structural proteins. The virus assembles at intracellular membranes, and virions are released from smooth-walled vesicles by cellular secretion. Adapted from Sturman and Holmes, 1983 with permission of Academic Press.

Translation of each of the viral mRNAs yields a single protein encoded by the gene at the 5' end of the mRNA. An exception is mRNA 5, which encodes two nonstructural proteins whose amino acid sequences have been deduced (33). The functions of these and the other non-structural proteins are not yet known.

The nucleocapsid (N) protein is synthesized on free ribosomes and is then phosphorylated (34-36). Phosphorylated N interacts with viral genomic RNA to form helical nucleocapsids which are either assembled into virions or accumulated in cytoplasmic inclusions. The majority of the N protein synthesized in infected cells is not released in virions, but remains cell-associated (17). N is processed to form several faster migrating species of unknown functional significance (37).

The two envelope glycoproteins of MHV are synthesized, transported and processed differently in infected cells (17,38). E2, the peplomeric glycoprotein, is synthesized on the rough endoplasmic reticulum (RER). Mannose-rich core sugars are added co-translationally, and E2 is transported to the Golgi apparatus where N-linked oligosaccharides are trimmed and terminal sugars such as fucose and sialic acid are added by cellular enyzmes (22). Also in the Golgi vesicles, palmitic acid is covalently bonded to E2 (39).

Glycosylation of E2 is inhibited by tunicamycin (38). E2 is incorporated into virions which bud at the RER and Golgi membranes, and excess E2 is carried by a host glycoprotein transport mechanism to the plasma membrane (17). There it may participate in virus-induced cell fusion and make the infected cell a target for an antiviral immune response.

In vitro translation studies have demonstrated that the E1 glycoprotein can be synthesized on free ribosomes, but in the presence of microsomes, it can insert through the lipid bilayer by means of an internal signal sequence (20,21,36). Glycosylation of serine and threonine residues in the external domain of E1 occurs only after the molecule has reached Golgi membranes and is not inhibited by tunicamycin (23,38). In infected cells, E1 accumulates in vesicles of the Golgi apparatus but is not transported to the plasma membrane (Figure 3). We have postulated that the restruction of E1 to intracellular membranes determines the site of coronavirus budding in infected cells (17).

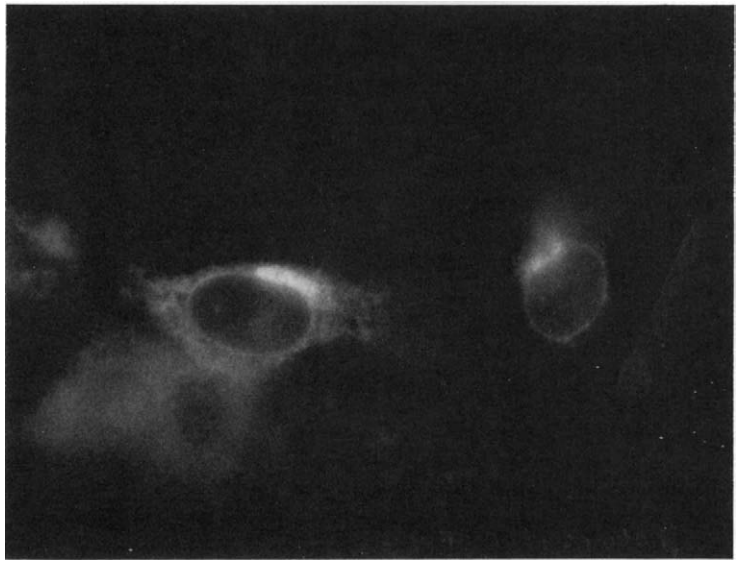

Figure 3. Accumulation of E1 membrane glycoprotein in Golgi apparatus. A 17 C1 1 cell was fixed with acetone 7 hours after infection with MHV-A59, and stained with monoclonal antibody to the E1 glycoprotein. E1 is found in small amounts in the RER, accumulates in large amounts in a perinuclear region which is the Golgi apparatus, and does not migrate to the plasma membrane. In contrast, E2 is found in the RER and is readily transported to the plasma membrane (data not shown).

Assembly of virions occurs in the RER or Golgi apparatus where viral nucleocapsids interact with regions of membrane containing viral glycoproteins E1 and E2 (Figure 4). Virions released into smooth-walled vesicles apparently escape from intact cells by cellular secretion. Released virions are frequently observed adsorbed to the plasma membrane of infected cells (38).

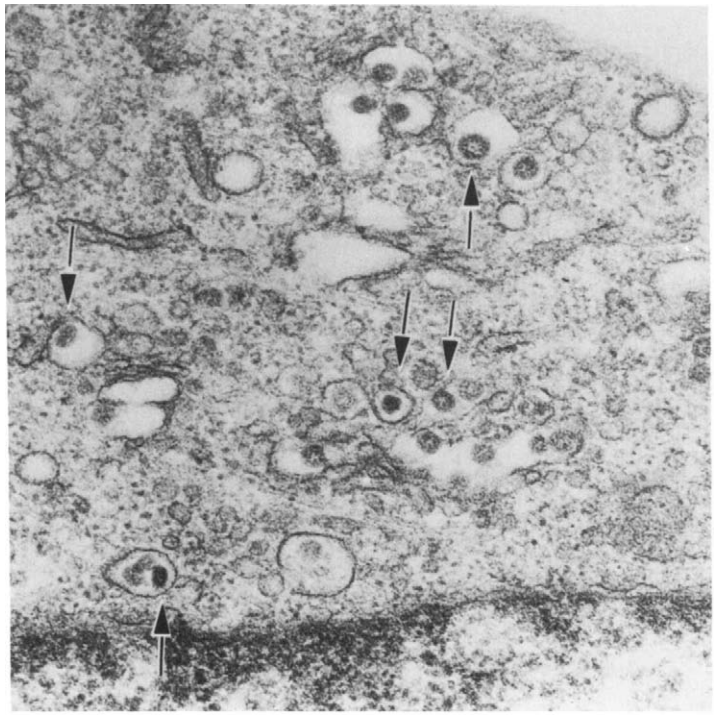

Figure 4. Intracellular budding of MHV. Coronavirus virions are seen in the RER and in intracytoplasmic vesicles in 17 Cl 1 cells infected with MHV-A59. Virions bud from these intracellular membranes, probably because the E1 glycoprotein required for budding is concentrated in this site. Released virions may adsorb to the plasma membrane, but coronaviruses do not bud from the plasma membrane. Magnification: X 50,000.

IV. HOST DEPENDENCE OF MHV REPLICATION

The preceding discussion shows that coronavirus replication depends upon a large number of host cell processes (Table 1). It is therefore not surprising that the ability of different cell types to support coronavirus replication varies markedly. Many coronaviruses can only be isolated

TABLE I. Host Cell Functions Used by Coronaviruses

Protein Processing	
O Linked Glycosylation	E1
N Linked Glycosylation	E2
Acylation	E2
Phosphorylation	N
Proteolytic Cleavage	E2, N
Protein Transport	
To Golgi	E1
To Plasma Membrane	E2
Secretion	Virions

initially by growth in fetal organ cultures of the normal host species (40), presumably because of stringent requirements for functions of differentiated host cells.

V. TISSUE TROPISM

The molecular basis for the tissue tropism of different coronavirus strains *in vivo* has not been elucidated. In several other virus model systems, tissue tropism depends at least in part upon availability of cell membrane receptors for virus attachment proteins. The nature of the coronavirus receptor has not been elucidated. However, the specific cell tropisms of several MHV strains *in vivo* can also be demonstrated *in vitro*. Dubois-Dalcq et al. (41) observed that MHV-JHM replicated in glial and neuronal cells both *in vivo* and in primary cultures of mouse spinal cord, whereas MHV-A59 replicated in glial cells, but not in neuronal cells both *in vitro* and *in vivo*. Analysis of model systems such as these by *in situ* hybridization may permit detection of entering viral genomes in cells in which viral RNA replication is inhibited.

VI. GENETIC RESISTANCE

In cells from mice genetically resistant to MHV, virus replication appears to be arrested at a very early step, since no virus specific antigens can be detected (6). It is not yet clear whether this inhibition is at the level of virus adsorption and penetration or early RNA synthesis. Complementation studies indicate that at least 7 genes are required for coronavirus RNA synthesis (26,42). If some host cell functions are also necessary for transcription of viral RNA, this could provide another mechanism for host control of coronavirus replication. The fate of the genomic RNA of the input virus in an abortive infection is not known. If this RNA could survive within an abortively infected cell, it might later be reactivated, providing a mechanism for persistence of coronaviruses *in vitro* and *in vivo*. Possible persistence of unexpressed viral RNA could be explored using cell cultures persistently infected with MHV. In such cultures although viral antigens are not detectable in the majority of cells, cloning of the cells leads to recovery of infectious virus (8).

VII. RELATIVE ABUNDANCE OF VIRUS mRNAs

When different strains of MHV are used to infect the same cell line, the relative abundance of different mRNA species varies markedly (43). The host or viral factors which determine the relative abundance of these mRNA species are not understood, but the resulting differences in abundance of viral specific polypeptides in the infected cells may affect the outcome of infection.

VIII. MHV CYTOPATHIC EFFECTS

The cytopathic effects of coronaviruses are host cell dependent, and may include either cell lysis, cell fusion, or no morphological change. Certain bovine cell lines infected with BCV normally show no CPE, but fuse extensively if trypsin is added to the medium (44,45). This suggests that proteolytic cleavage of a viral glycoprotein might be required to activate coronavirus cell fusing, as has been

demonstrated with paramyxoviruses and orthomyxoviruses (46,47). Trypsin treatment of MHV virions released from the 17 Cl 1 line of BALB/c 3T3 cells results in quantitative conversion of the 180K form of E2 into 90K species and also renders concentrated virions able to cause fusion from without (FFWO), that is immediate fusion of uninfected cells (16).

The extent and time course of MHV-A59 induced fusion caused by virus replication (fusion from within, FFWI) vary considerably among different permissive cell types. Infection of L2, DBT and Sac- cells results in extensive fusion and death by 10 to 12 hours post inoculation. In contrast, fusion of the 17 Cl 1 cell line involves only a small proportion of the cells until 17 to 24 hours after infection. Comparison of the proteins of virions released from these four cell lines revealed significant differences in the proportion of the 180K E2 which had been cleaved to 90K (Figure 5). The more slowly fusing 17 Cl 1 cells released virions containing E2 that was only approximately 50% cleaved, whereas virions released from Sac- cells, which fused rapidly, contained E2 completely cleaved to 90K. This suggests that cleavage of E2 by a host cell protease during intracellular transport of the glycoprotein might be a virulence factor influencing the extent of virus-induced CPE. However, other cell lines which fuse extensively, such as L2 and DBT cells, show incomplete cleavage of E2. In repeated experiments the E2 cleavage products from virions from the Sac- cells differed slightly in molecular weight from those on virions produced by the other three cell types. This suggests that the protease cleavage site on the E2 molecule may differ from one host cell to another and that the specificity of the host protease which cleaves E2 may also be an important determinant of virulence. Alternatively, host dependent differences in glycosylation could explain the observed differences in molecular weights of the E2 cleavage products. Incorporation of E2 into the plasma membrane depends upon cellular transport mechanisms. Thus, depending upon the relative amount of cleaved E2 carried to the plasma membrane, different MHV-infected cell lines show more or less fusion. The amount of E2 on the plasma membrane may also determine susceptibility of infected cells to attack by anti-viral antibody or immune lymphocytes.

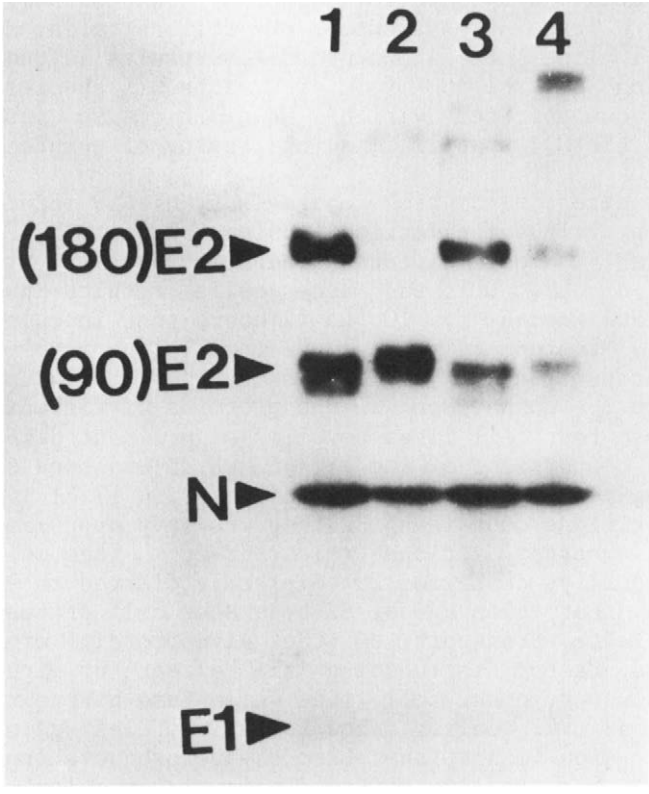

Figure 5. Structural proteins of virions released from four cell lines. Released virions were purified from the supernatant medium over 17 Cl 1 cells (lane 1), Sac- cells (lane 2), L2 cells (lane 3) or DBT cells (lane 4), and analyzed by the Western blot technique with antibody specific for E2 and N.

IX. VIRUS INFECTIVITY

Proteolytic cleavage of a paramyxovirus envelope glycoprotein is required not only for fusion, but also for virus infectivity (46,47). Trypsin treatment of BCV virions can significantly enhance virus infectivity (44,45), but protease treatment of other coronaviruses results in only minor changes in infectivity (15). Possibly the E2 on these viruses is already sufficiently cleaved by enzymes of the

cells in which the virus was made to permit fusion of the viral envelope with the membrane of another cell. Such fusion may be required for infectivity. No cell line which yields MHV with completely uncleaved E2 has yet been identified. The availability of a host cellular protease which can cleave the coronavirus E2 glycoprotein and activate virus infectivity may be another host determinant of coronavirus virulence. For example, human enteric coronaviruses (HECV) may be difficult to propagate for more than one cycle even in human fetal intestinal organ cultures due to inability of the cells to activate viral infectivity (48).

X. PROCESSING OF N PROTEIN

The synthesis and processing of N are dependent upon both the host cell and the virus strain. In virions of different strains of MHV, the apparent molecular weight of the band of N protein varies from about 45 to 55K. However, in 17 Cl 1 cells infected with three different strains of MHV, 3 polypeptides antigenically related to N were observed (Figure 6). The slight strain-specific differences in the mobility of the bands labeled N reflect those seen in N proteins from virion. Two faster migrating intracellular species of N were observed in infected 17 Cl 1 cells, and their mobilities and relative abundance were also strain dependent. Infection of the macrophage-derived cell line J774A.1 with the same three strains of MHV resulted in detection of up to five intracellular species of N which showed strain-specific differences in mobility (Figure 6). The strain-specific differences in the mobilities of virion-associated N and the intracellular N species must be due to differences in the amino acid sequence of N proteins of different strains. The N proteins of MHV-A59 and MHV-JHM share 93% amino acid homology (19,49), but specific amino acid changes which account for the different electrophoretic mobility of N proteins of different strains have not yet been identified. The host cell dependent differences in the intracellular forms of N may be due to differences in mRNA transcription, translation, phosphorylation or proteolytic cleavage. The biological significance of these multiple forms is unknown.

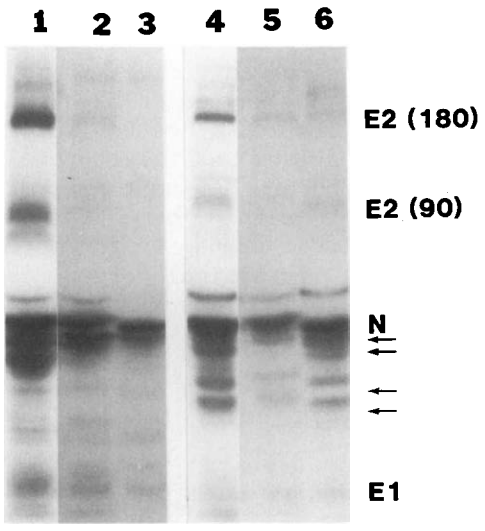

Figure 6. Different intracellular processing of the nucleocapsid protein of three strains of MHV in two cell lines. 17 Cl 1 cells (lanes 1-3) or J774A.1 cells (lanes 4-6) were infected with different strains of MHV, and viral proteins in cytoplasmic extracts were analyzed by Western blotting with antibody to the three structural proteins of MHV. MHV-A59 is shown in lanes 1 and 4; MHV-S, in lanes 2 and 5; and MHV-Yale, in lanes 3 and 6. MHV specific proteins are labeled. Arrows indicate faster migrating intracellular species antigenically related to N.

XI. RELEASE OF VIRIONS

Another factor which may affect the yield of infectious virus from different cell types is the rate of secretion from smooth-walled, post-Golgi vesicles. Thus, cells which secrete cellular products rather slowly may accumulate virions in intracellular vesicles and release relatively little infectious virus. The preceding discussion indicates that coronavirus replication may be influenced by many cellular processes that differ from one cell type to another (TABLE I).

XII. INTERACTION OF MHV WITH LYMPHOCTYES

The outcome of coronavirus infection in vivo depends not only upon virus interactions with individual cells as described above, but also upon interactions between virus antigens and the immune system. The virulence of MHV infection may be enhanced by immunosuppression, and MHV infection may change the immunologic responsiveness of the host. In this section we will discuss recent studies on the interactions of MHV with cells of the immune system and consider their implications for pathogenesis of MHV.

In mice infected with some strains of MHV, viral antigens are observed in the spleen, and occasionally splenolysis results (7). Thus, MHV can infect lymphoid cells. We have demonstrated the availability of cell surface receptors for MHV-A59 on splenic lymphocytes. Interaction of viral E2 glycoprotein on the surface of an infected cell with receptors on splenocytes resulted in an unusual form of natural cell-mediated cytotoxicity (50). The effector cell for this rapid, H2 independent natural cytotoxicity for MHV-infected targets was a B lymphocyte (51). Antibody to E2 prevented cytotoxicity. Thus, the unique ability of the E2 glycoprotein of MHV to bind to a receptor on B lymphocytes has revealed a previously unsuspected cytotoxic activity of these cells.

Levy and his co-workers (52) demonstrated that intravenous inoculation of MHV-3 virions rapidly induces monocyte procoagulent activity (MPC) which results in alteration of the hemodynamics in the livers of infected mice. Microthrombus formation and swelling of hepatocytes were detected within a few hours after virus inoculation. The susceptibility of different mouse strains to death from MHV-3 was correlated with the ability of MHV-3 to induce MPC activity in the strains. Thus MPC may be an important determinant of genetic susceptibility of different mouse strains to MHV. It will be of considerable interest to identify the viral component which stimulates MPC activity.

The extensive use of mice for production of hybridoma antibodies in ascites fluid may present a new opportunity for dissemination of MHV in animal colonies. We have found that MHV-A59 can readily infect hybridoma cells generated by fusion of murine splenic lymphocytes with myeloma cells. Viral antigens were detected in the cytoplasm by immunofluoresence, numerous coronavirions were observed in the RER and Golgi and adsorbed to the plasma membrane (Figure 7), and infectious virus was released from infected hybridoma cultures. It is therefore possible that hybridomas may become

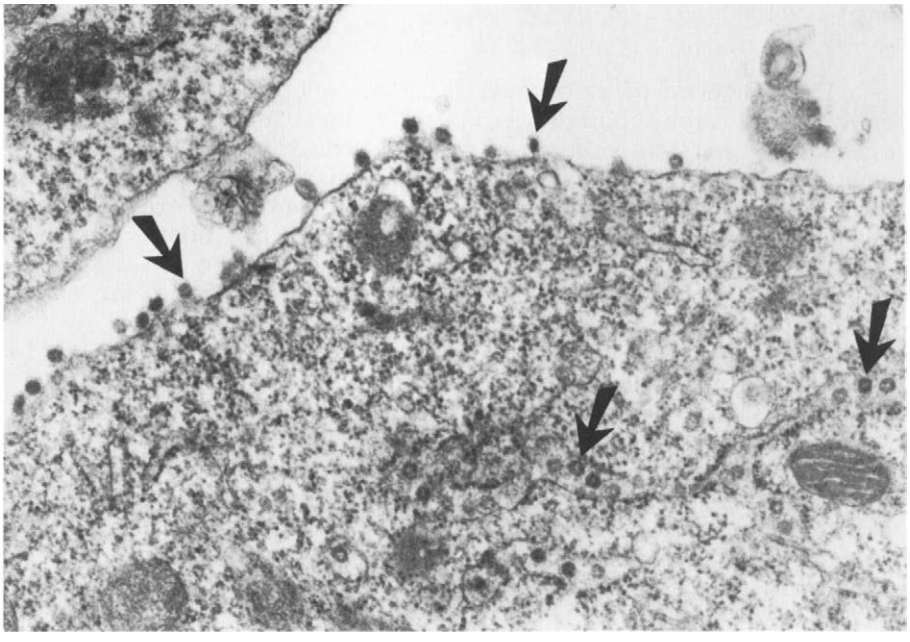

Figure 7. Replication of MHV-A59 in murine hybridoma cells. A thin section of a hybridoma cell producing monoclonal antibody to an unrelated antigen shows coronavirus virions (arrows) in the RER and adsorbed to the plasma membrane of an infected cell. The MHV virions are clearly distinguishable from intracisternal type A virus-like particles (not shown) which are found in both hybridoma cells and the parental myeloma cells. Magnification: X 25,000.

infected when, during preparation of hyperimmune ascites fluids, they are passaged in the peritoneal cavity of mice with inapparent MHV infections. The contaminated hybridoma cells might then transmit MHV to other mice, thus becoming a source for spread of infection within a mouse colony.

Inapparent MHV infection in a mouse colony can also cause another problem for hybridoma research. We have prepared many hybridomas which produce antibody against one or another of the three structural proteins of MHV-A59. Although antibody in the supernatant medium from each hybridoma clone reacted with only one viral protein, ascites fluids induced by some of the hybridoma clones contained antibody to several MHV antigens. We found that the mice in

which these ascites fluids had been prepared had been infected asymptomatically with an enterotropic strain of MHV. The ascites fluids thus contained both monoclonal anti-MHV A59 produced by the hybridoma cells and polyclonal antibodies to the enterotropic strain of MHV produced by resident B cells. We have also found anti-MHV antibody in ascites fluids elicited by hybridomas producing antibodies to non-viral antigens, when the mice had been previously exposed to MHV endemic in the animal colony. Unless immunoglobulins prepared from ascites fluids from MHV-immune mice are purified by immunoaffinity chromatography with the specific epitope, contamination of the monoclonal antibody with anti-MHV antibodies could complicate interpretation of experimental results.

XIII. CONCLUSION

We have discussed some of the ways in which coronavirus replication may depend upon host cell processes. This marked dependence of virus replication on the host cell may explain in part why coronaviruses show such pronounced species and tissue tropisms. Differences in the yields of virus and the extent of viral cytopathic effects may be correlated with the ability of the cells to secrete virus and to transport appropriately processed and cleaved viral E2 glycoprotein to the host cell membrane. Further studies on the functions of the coronavirus non-structural proteins and analysis of temperature-sensitive virus mutants (53,54) should elucidate additional virus-host interactions which may affect coronavirus virulence.

Perhaps due to special properties of coronavirus proteins, MHV infection can have unusual effects on the immune system, resulting in monocyte procoagulant activity or B cell-mediated cytotoxicity. Inapparent MHV infection of a mouse colony can result in appearance of MHV antibodies in ascites fluids prepared by i.p. inoculation of hybridomas producing antibody to unrelated antigens. Accidental infection of hybridoma cells with MHV could result in further spread of the virus through mouse colonies. These studies indicate new insights into viral immunology which can result from studies on MHV and illustrate the importance of eliminating MHV from mouse colonies in order to prevent compromise of research data.

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