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Meeting Report

Coronaviruses: a group with unique features

Third International Coronavirus Symposium
Organized by M. Lai and S. Stohlman
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Recent progress in the study of coronaviruses has revealed many unique features of these viruses, which were presented at this International Symposium. Progress was evident in both the area of molecular biology and in viral pathogenesis. Of particular interest were studies revealing the novel structure and the unique mechanism of synthesis of coronavirus RNA, as well as the characterization of envelope glycoproteins, target cell specificity and the immune modulatory role of these viruses.

During the first half of the meeting molecular biological aspects were discussed. Coronaviruses form a large group of infectious agents whose key members are: mouse hepatitis virus (MHV); avian infectious bronchitis virus (IBV); porcine transmissible gastroenteritis virus (TGEV); feline infectious peritonitis virus; and human coronavirus. These viruses contain an infectious single-stranded RNA genome, until recently thought to be 18–20 kilobases (kb) long. In infected cells, six to seven species of mRNA are made, which form a 3′ coterminal nested set structure. Each mRNA is physically polycistronic but functionally monocistronic. Only the unique 5′ terminal sequences which do not overlap with the next smallest RNA are used for translation. Thus, coronavirus genomes contain only six to seven genes, despite the large size of the virion RNA.

The fact that coronaviruses do contain a very large genome was demonstrated by the complete sequencing of the IBV genome (M. Boursnell, Houghton Poultry Research Station, U.K.). Surprisingly, this RNA is 27.6 kb long, considerably greater than previously estimated; it is the largest viral, and stable, RNA species known. Eighteen kb of this RNA is a single gene at the 5′ end that probably encodes the RNA-dependent RNA polymerase. Analysis of the predicted translation products reveals two overlapping open reading frames, with the capacity to code for two proteins of 440 kDa and 300 kDa. It is conceivable that ribosomal frameshifting could result in a single protein product. These proteins have yet to be identified in IBV-infected cells. Why does coronavirus need such a huge polymerase? It might be because the virus utilizes a complex process for synthesizing its mRNAs. The exceptionally large size of the potential proteins suggests multiple functions and possible post-translational processing.

One of the most interesting features of coronavirus mRNAs is the presence of a 5′ leader sequence of 50–70 nucleotides. Evidence was presented that this leader

RNA is not derived by the conventional pre-mRNA splicing mechanism, but rather involves a process of "leader RNA-primed transcription", in which a leader RNA is synthesized from the 3′ end of the negative-stranded RNA template, dissociates, and then reassociates at the sequence where transcription of the various mRNA species initiated (Baric, R.S. et al., 1983 J. Virol. 48, 633). This concept is further supported by the observation that leader sequences can be exchanged with very high frequency between co-infecting viruses and by the fact that the 3′ end of the leader sequence is complementary to sequences in the intergenic regions (Makino, Soe, University of Southern California, Los Angeles). From these data a transcription model was proposed which involves an endonucleolytic activity in the RNA polymerase. This aspect of coronavirus transcription reveals similarities not only to the CAP-snatching transcription of influenza viruses (Plotch, S.J. et al., 1981 Cell 23, 847), but also to transcription in African trypanosomes (Campbell, D.A. et al., 1984 Nature (London) 311, 350).

Two other novel phenomena associated with coronavirus RNA synthesis concerning recombination and defective interfering (DI) particles were presented. Despite the fact that coronavirus contains a single molecule of nonsegmented RNA, genetic exchange between coronaviruses, specifically MHV, can occur at high frequency, approaching that of RNA reassortment of segmented RNA viruses (Keck, University of Southern California, Los Angeles). RNA recombination can be shown to occur in every part of genomic RNA. What distinguishes coronaviruses from other RNA viruses that do not recombine, or recombine at very low frequency? It was speculated that coronavirus RNA replication proceeds by a discontinuous and nonprocessive "stop-and-go" mechanism, thus yielding free RNA intermediates which become precursors for RNA recombination.

The other interesting observation was made while analyzing DI particles. Unlike the DI particles of other viruses. DI particles of coronaviruses synthesize distinct subgenomic RNAs which contain sequences derived from several discontinuous parts of the DI genome (Makino, University of Southern California, Los Angeles). These defective intracellular RNAs are not incorporated into virions, thus must be transcribed de novo from DI particle genomes in infected cells, probably by a mechanism of jumping transcription. This complex process of coronavirus RNA transcription suggests that coronavirus RNA polymerases possess unique properties.

The second half of the meeting was devoted to pathogenesis. The majority of studies centered on the murine coronaviruses with particular emphasis on central nervous system (CNS) infection by JHM virus. This virus induces in different rat or mouse strains a persistent CNS infection which is the basis for a chronic inflammatory demyelinating disease that morphologically has some resemblance to experimental allergic encephalomyelitis (EAE) and multiple sclerosis (MS). This is further supported by the observation that in infected Lewis rats an intrathecal oligoclonal immune response occurs which is not only directed against JHM virus, but also against unknown antigens (Dörries, University of Würzburg). It has been shown recently that in the course of such infection in Lewis rats a cell-mediated immune response to myelin basic protein develops which is of pathogenetic significance (Watanabe et al., 1983 Nature (London) 305, 150). This observation has now been

confirmed in CNS infection of mice (Koolen, Scripps Clinic, La Jolla), emphasizing the importance of these immunopathological reactions in maintaining the disease process.

These observations pose the question of how coronavirus induces autoimmune reactions to myelin. The most likely mechanisms would include: (1) virus-induced target cell changes with presentation of autoantigens; (2) interaction of virus with lymphocytes leading to activation of autoclones; or (3) molecular mimicry. At the meeting some of these possibilities were discussed and interesting data presented. The primary target cells for JHM virus in the CNS seem to be microglia and astrocytes (Massa, University of Würzburg) or oligodendrocytes (Dales, University of Western Ontario, London), depending on the animal strain and virus variant used. A persistent infection is established in these cells and, in the case of a developing dysfunction, primary demyelination can occur. The neurotropism of JHM virus depends on molecular determinants of the E2 peplomer glycoprotein, as studies with JHM variants isolated with and characterized by monoclonal antibodies reveal (Buchmeier, Scripps Clinic, La Jolla; Fleming, University of Southern California, Los Angeles; Knobler, Jefferson Medical College, Philadelphia; Leibowitz, University of Texas, Houston; Wege, University of Würzburg). However, besides infecting microglia and astrocytes, JHM virus has the potential to induce class I (Lavi, University of Pennsylvania, Philadelphia) and class II (Massa, University of Würzburg) antigens of the major histocompatibility complex (MHC) on the surface of these cells. Whereas class II antigens are induced only on astrocytes, class I antigens can also be induced on oligodendrocytes. This novel phenomenon explains for the first time how an immune response in the CNS is initiated. The CNS is considered an immunologically privileged site and those MHC class II antigen-presenting cells (macrophages, dendritic cells, etc.) found outside this compartment are not detectable in normal brain tissue. However, recent evidence indicates that astrocytes have the potential to present antigens if stimulated with gammainterferon (γ-IFN) (Fontana et al., Nature (London) 1984 307, 273). In case of a CNS virus infection the induction of MHC-antigen has to be γ-IFN independent since γ-IFN is not synthesized by cells of the brain. The inducibility of class I and class II antigens by coronaviruses is an important immunoregulatory event. The induction of class II molecules allows the initial presentation of viral antigen to T helper lymphocytes, and class I molecules provide the appropriate receptors for T killer cells in destroying infected brain cells. No information is available about the receptor of JHM virus on brain cells, but a solid phase receptor binding assay was presented which it is hoped will allow studies of this aspect in the future (Holmes, Uniformed Services University, Bethesda).

Murine coronaviruses infect other organs beside the CNS, depending on the animal strain used and on the biological properties of the virus strain. It was reported that JHM virus can in certain mouse strains infect thymic epithelial cells and thymic lymphocytes, which has severe consequences for the host (Knobler, Jefferson Medical College, Philadelphia). Depending on the extent of infection, an immunosuppression may develop which prevents recovery from infection. This lymphoid tropism of coronaviruses may play an important pathogenetic role since it

has been shown for other virus groups that viruses not only interfere with a normal immune response by destroying lymphocytes, but also suppress or activate lymphocyte subpopulations, which may be followed by adverse immune reactions. This aspect was also discussed in MHV3 infections in BALB/cJ mice in which a fulminant hepatitis develops. This hepatitis can be prevented by treating the animals with dimethylprostaglandin E2 or with immunosuppressive drugs; this suggests an immunopathological reaction contributes to this disease process (Abecassis, Mount Sinai Hospital, Toronto). With the progress made in molecular biology of coronaviruses, it is likely that the pathogenesis of these interesting diseases, which provide models for human diseases such as multiple sclerosis or hepatitis, will be revealed before too long.

Volker ter Meulen

Institute of Virology and Immunobiology
University of Würzburg
Versbacher Strasse 7
8700 Würzburg, F.R.G.