BIOLOGY OF CORONAVIRUSES 1983

Volker ter Meulen

Institute of Virology and Immunobiology
University of Würzburg
D - 8700 Würzburg, F.R.G.

INTRODUCTION

At the first international symposium of coronaviruses held in Würzburg in 1980, studies on the biology of coronaviruses were mainly concerned with two subjects: coronavirus persistent infections in tissue cultures and the description of different disease models, in a variety of hosts, with the particular emphasis on subacute or chronic conditions. Much of the discussion centered around the possible mechanisms of persistence and the pathogenic factors playing a role in the development of the different disease types. However, due to the lack of basic information on the molecular biology of coronaviruses the majority of studies reported three years ago were of a more descriptive nature, and in the animal studies immunological and genetic aspects were mainly analysed. In the intervening period, although surprisingly little virological information has been obtained on coronavirus persistent infections in vitro and in vivo, the animal work has been concentrated on two virus groups, namely the murine and feline coronaviruses. Obviously, the involvement of multiple organs in different diseases associated with infections of the two virus groups has turned out to be the most interesting basis for the study of virus-cell and virus-host interactions and the analyses of viral and host factors involved in the development of different disease processes.

MURINE CORONAVIRUS INFECTIONS IN MICE AND RATS

The mouse hepatitis viruses (MHV) cause a broad spectrum of diseases in their natural hosts including hepatitis, pneumonitis,

nephritis, enteritis and encephalitis. These different disease types are influenced by the age and genetic backgrounds of the animals, the route of infection and the biological properties of the MHV serotypes used in the experiments. Of particular interest have been subacute and chronic CNS diseases associated with the development of demyelination. Demyelination is a neuropathological hallmark of many CNS disorders in animal and man which can occur in association with virus infection. However, the exact mechanisms by which these lesions develop are unknown. In general, it has been suggested that a direct virus effect on oligodendroglia cells, in the course of a lytic or persistent infection, can be followed by the destruction of myelin (1). Oligodendroglia cells support myelination and their dysfunction has severe consequences. On the other hand, it has been shown that an immune response against myelin also causes demyelination as demonstrated in experimental allergic encephalomyelitis (EAE) (2). In addition, in the event of a virus infection of oligodendroglia cells it is conceivable that an immune response either directed to the infectious agent, or to modified host antigens located on infected oligodendroglia cells, may be another way by which demyelination occurs. Yet, these hypotheses are only based on circumstantial evidence (3). In the case of MHV infections of mice and rats, the different types of demyelinating encephalomyelitides provide for the first time an experimental basis to test these different hypotheses.

The CNS disease models based on coronavirus infection (MHV or SD) presented at this meeting revealed certain similarities. In all instances, a subacute or chronic CNS infection with demyelination was dependent, up to a certain extent, on the neurotropic properties of the virus strain used for infection, on the route of infection as well as on the age and genetic background of the animals. Moreover, in A59 infection of C57 Bl/6 mice the virus dose applied was an additional determining factor (Lavi et al., this volume) in contrast, in JHM infection of rats the type of CNS disease was dose independent (Wege et al., this volume). Of interest is the observation that after MHV inoculation in the majority of animals a biphasic disease process develops (Lavi et al., Wege et al., Jankovski et al., Sørensen et al., this volume). It seems that first of all an acute CNS infection occurs which may or may not be clinically silent. This acute infection is survived by a different proportion of animals, but later some of the survivors come down with a second disease course after varying incubation times. This disease is of a more subacute or chronic nature and differs in the degree of inflammatory demyelinating lesions. This second disease develops as a result of a persistent infection. Viral antigen can be found in glia cells and reisolation of infectious virus is possible at least in JHM infection in rats (Wege et al., this volume). However, persistence also occurs in brain tissue without the detection of antigen. In A59 infection in mice (Lavi et al., this volume) and in JHM infection in Wistar Furth

rats (Sörensen et al., this volume) coronavirus RNA can be found by in situ hybridization with specific cDNA in the absence of viral antigens in diseased brain areas. Moreover, under certain conditions an immunosuppressive treatment leads to reactivation of the virus infection (Sörensen et al., this volume) indicating that different forms of persistent infection with and without the production of infectious virus or antigens develop after coronavirus infection in brain tissue in association with subacute or chronic demyelinating encephalomyelitides.

The preliminary data presented on the coronavirus persistence in mice or rat brains resembles to some extent those seen in morbillivirus infections in animal and man (4). These viruses induce in their host acute, subacute and chronic CNS diseases with demyelination and establish with some ease persistent infections in tissue cultures. So far, no common mechanism for the establishment and/or maintenance of morbillivirus persistence has been found. Persistency varies greatly from infections where progeny virus is continually shed to those in which no virus is released and only a small percentage of cells are found to express viral antigens. Factors such as defective interfering particles, interferon or temperature sensitive mutants have been suggested to play a regulative role in these infections. In addition, translational defects of specific viral mRNAs have been detected which prevent assembly of virus particles (5). However, viral antibodies also modify morbillivirus infection changing a lytic infection to a persistent one or causing antigenic modulations on cell membranes as studies in tissue cultures have revealed. It is conceivable that similar mechanisms are operative during the infection of an organism. In general, any factor which promotes cell survival of an initial lytic infection, or diminishes the virulence of a carried virus, will contribute towards morbillivirus persistence and disease. Similar events may also be of importance in coronavirus infection of CNS tissue.

It has been shown at this meeting that host factors also contribute to a disease process in coronavirus infections and to virus persistence. Viral antibodies are capable of modifying a lytic, acute infection to a subacute-chronic, persistent infection or even prevent formation of a chronic disease (Levy et al., this volume) (6). The effectiveness of humoral immunity seems to depend on the titre, the avidity and the neutralising capacity of the antibody. Such antibodies were effective as maternal antibodies or in passive transfer experiments. Of particular interest is the observation of specific cell-mediated immune reactions developing in the course of coronavirus infection in rats. It could be shown that spleen cells of diseased animals not only proliferate in the presence of basic myelin protein (BMP) in vitro but that adoptive transfer of such cells is followed by the occurrence of EAE-like lesions in CNS tissue (Wege et al., this volume) (7). Moreover,

animals which recovered from JHM virus infection revealed a reduced susceptibility to the induction of EAE when challenged with BMP in combination with Freund's complete adjuvants suggesting the presence of specific T suppressor cells. These findings are similar to that seen in EAE, where the induction of an acute or chronic form of an inflammatory demyelinating CNS process is pathogenically linked to the presence of T lymphocytes sensitized against basic myelin protein (8). However, it has to be stressed that this auto-immune disease of the CNS can only be induced when immunization against brain antigen is carried out in the presence of Freund's complete adjuvants. In nature, such an immunopathological phenomenon does not develop by itself, since basic myelin protein alone is immunologically inactive, yet, JHM virus infection of brain cells overcomes this immunological barrier. How this phenomenon is achieved is at present unknown but it is conceivable that JHM replication in CNS tissue may lead to alterations of myelin, to cell membrane changes by insertion of viral proteins which trigger an immune response against CNS tissue or antigenic crossreactivity exists between JHM structural proteins and BMP. Certainly, JHM virus infection of CNS tissue not only directly destroys the infected host cell, but also initiates an autoimmune reaction which could perpetuate CNS damage. Whether sensitization of T-lymphocytes against JHM viral antigens also plays a pathogenic role is unknown, but such cells have been found after coronavirus infection in rats and mice (Wege et al., Stohlman, this volume).

It is generally agreed, that the neurotropic MHV strains which cause a subacute or chronic CNS disease process show a distinct cell tropism. In each model discussed at this meeting, the viral mutant used infected oligodendroglia cells predominantly. This selective vulnerability of oligodendroglia cells to coronaviruses explains the marked lesions of demyelination, since viral replication interferes with cell function. In in vitro studies it could be shown for example that the RN2 Schwannoma cell line has the unique ability to discriminate between non-neurotropic MHV3 and neurotropic JHM strains (Flintoff, this volume). These results suggest that the host cells have a profound influence in regulating replication of these agents which certainly not only takes place in vitro but also in brain infections. These experiments underline the importance of viral and host factors which determine the fate and the outcome of a viral infection.

FELINE CORONAVIRUS INFECTIONS OF CATS

Coronavirus infection of cats is another interesting disease model of great complexity. Several organs of these animals are infected resulting in different disease forms. At the present time little pathogenic information is available. It has been proposed that the immune response to the virus infection especially the

cell-mediated immune reactions (CMI) play a crucial role for the disease form developing (Pedersen et al., this volume). In animals without a detectable cell-mediated immune reaction to the virus infection the "effusive" form of disease develops. These animals reveal a swelling of the abdomen as a result of peritonitis. Fibrin is deposited on abdominal organs and granulomatous inflammatory reactions, vasculitis, and plaques of focal necrosis are scattered through the panenchyma of the liver, kidneys, lung, spleen and lymph organs. In contrast, cats developing a partial cell-mediated immunity to feline coronaviruses come down with the "non-effusive" form of the disease. Here localized granulomatous lesions are found without ascites which also involve the eye and the central nervous system. However, in animals which are capable of mounting a humoral and complete cell-mediated immune response probably no disease is observed. In such animals the virus is either eliminated or a latent infection is established which can be reactivated when such animals are infected by feline leukaemia virus. It is believed that the feline leukemia viruses have an immune suppressive effect leading to reactivation of feline coronaviruses. Such a reactivation may then be followed by either the effusive or non-effusive form of the disease depending on the state of CMI reaction. Of interest is also the observation that high levels of coronavirus antibodies do not prevent disease (9). Experimentally infected seronegative kittens survive significantly longer and develop a less fulimant disease than seropositive kittens. Moreover, treatment of seronegative kittens with purified anti FIPV-IgG results in an aggravation of the disease. In such animals viral immune complexes can be found in renal glomeruli tissue.

It is unknown whether the impaired cell-mediated immune response observed in the course of feline coronavirus infection results from the coronavirus infection itself, or existed already in a subclinical form before infection. Certainly, this model reveals pathogenically certain similarities to other acquired immuno-deficiency-syndromes in association with an infectious agent. Further analyses of the interaction between feline coronaviruses and the immune system will provide important answers to these disease processes.

OUTLOOK

The studies on the pathogenesis of coronavirus infections presented and discussed at this meeting present only parts of the disease spectrum which this large virus group can induce. The majority of studies has concentrated on the murine coronavirus group because of the interesting diseases associated with these agents and the detailed virological and molecular biological information available concerning these viruses which is a prerequisite for further analysis. In my judgement, future research in

this field has to concentrate on the following three topics.

Firstly, it is of great importance that the molecular biologists define the virus-cell interactions in the different diseases observed, in particular in those which are based on a persistent infection. Since the development of a clinical disease is a result of an organ dysfunction it is necessary to learn in what way persistence is established in brain tissue, whether full viral replication occurs, or whether transcriptional or translational defects exist as shown for CNS persistent infections with other virus groups. With the methodology available to search for viral nucleic acids it is possible to identify the infected host cells and to determine the state of infection. The central nervous system reveals anatomical and physiological peculiarities which may permit persistence and inhibit virus elimination. Brain tissue consists of a highly differentiated cell population with complex, functionally integrated cell to cell connections and highly specialised cytoplasmic membranes which probably result in great variability in virus receptor sites and cellular capacity to support virus replication (10). Little is known about the spread of viruses within the CNS and in what way host factors may influence viral replication.

Secondly, the impact of a persistent infection on CNS cells has to be evaluated. In vitro studies with different viruses on neuronal cells in culture revealed the development of cellular dysfunctions in the course of infection. It could be demonstrated that persistent lymphocytic choriomeningitis, measles or rabies virus infection influence luxury functions such as production of acetylcholine, acetyltransferase or acetylcholinesterase without effect on cell morphology, growth rate or protein synthesis (11-13). However, in the case of measles persistence in a particular cell line the presence of measles antibodies in the supernatant can overcome a specific virus-induced cellular dysfunction (14). In C6 rat glioma cells, measles virus persistence interferes substantially with the synthesis of catecholamine-dependent β -adrenergic receptor-stimulated cAMP which can be restored when virus-specific proteins inserted into the membrane of infected cells are removed by viral antibodies in a capping process. It is conceivable, that such interactions occurring in infected CNS tissue stabilise the balance between virus and host cells and may explain prolonged incubation periods until a disease with a defined clinical symptomatology eventually develops.

Thirdly, the immune responses are one of the most important host factors in the complex virus host interactions. As it became obvious at this meeting we are just at the beginning of realising how important immune responses are for both murine or feline coronavirus infections. The immune system does not have easy access to the brain which may account for a failure to control

infection in this compartment. There is little information on the flux of antibodies, macrophages, antigens or antigen-antibody complexes in and out of the central nervous system and the pathogenic role of the local immune response is still unknown. Moreover, the effect of the viral infection on the immune system itself could also be a crucial disease determining factor as studies with feline coronaviruses in cats suggest. In the past studies have shown that viruses can alter immune functions (15). Quite often viruses suppress cellular and humoral immune reactivity, whereas enhancement of the immune reactions is uncommon. In this connection, suppression of delayed type hypersensitivity, prolonged allograft survival or reduced in vitro lymphocyte reactivity have been noticed. Also suppression of antibody formation with a variety of viruses has been observed. Destruction of lymphocytes, abnormalities in macrophage functions, inhibition of lymphocyte activation and proliferation or alteration of lymphocyte traffic have been discussed as possible mechanisms of viral induced immune dysfunctions. Certainly, the diversity of effects which viruses have on immune functions in the course of infections suggest more than one mechanism by which the alteration of the immune system is achieved. In coronavirus infection an additional immune effect has to be understood. The observation of an autoimmune reaction to CNS tissue as a consequence of viral replication in oligodendroglia cells suggests that a CNS disease process could also be maintained by these mechanisms even after the virus infection has waned. It is conceivable that the analysis of this phenomenon will provide information which is relevant to other chronic demyelinating diseases of animal and man including multiple sclerosis.

This meeting has demonstrated the complexity involved in coronavirus infections. Like the meeting in Würzburg, it was of great value to bring together scientists, from different disciplines, involved in the study of coronavirus infections. Certainly, a multi-disciplinary approach is needed in the attempts to elucidate the nature of the different diseases associated with this virus group.

I think, all participants are grateful to Drs. Peter Rottier, Ben van der Zeijst, Willy Spaan and Professor Marian Horzinek for organising this meeting and providing the atmosphere in which scientific and personal discussions as well as friendship could grow for the benefit of coronavirus research.

ACKNOWLEDGMENT

I thank Helga Kriesinger for typing this manuscript.

REFERENCES

1. Weiner, L.P., R.T. Johnson, R.M. Herndon. Viral infections and demyelinating diseases. *N. Engl. J. Med.* 288: 1103-1110 (1973).
2. Paterson, P.Y. Transfer of allergic encephalomyelitis in rats by means of lymphnode cells. *J. Exp. Med.* 111: 119-136 (1960).
3. Dal Canto, M.C., Rabinowitz, S.G. and T.C. Johnson. Virus-induced demyelination. *J. Neurol. Sci.* 42: 155-168 (1979).
4. ter Meulen, V. and M.J. Carter. Morbillivirus persistent infections in animals and man. In: "Virus persistence" (B.W.J. Mahy, A.C. Minson and G.K. Darby, eds). Cambridge University Press 97-132 (1982).
5. Carter, M.J., Willcocks, M.M. and V. ter Meulen. Defective translation of measles virus matrix protein in a SSPE line. *Nature*, in press.
6. Wege, H., Koga, M., Wege H. and V. ter Meulen. JHM infection in rats as a model for acute and subacute demyelinating disease. In: "Biochemistry and Biology of Coronaviruses" (V. ter Meulen, S. Siddell, H. Wege, eds). *Adv. Exp. Med. Biol.* 142: 327-340 (1981).
7. Watanabe, R., Wege, H. and V. ter Meulen. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature*, in press.
8. Gonatas, N.K. and J.C. Howard. Inhibition of experimental allergic encephalomyelitis in rats severely depleted of T-cells. *Science* 212: 672-675 (1974).
9. Horzinek, M.C. and A.D. Osterhaus. The virology and pathogenesis of feline infectious peritonitis. *Arch. Virol.* 59: 1-15 (1979).
10. ter Meulen, V. and S.G. Siddell. Virus infections of the nervous system: molecular, biological and pathogenetic considerations. In: "The Molecular Basis of Neuropathology" (A.N. Davison and R.H.S. Thompson, eds), Edward Arnold Publ. 150-187 (1981).
11. Oldstone, M.B.A., Holmstoen, J. and R.M. Weese. Alterations of acetylcholine enzymes in neuroblastoma cells persistently infected with lymphocytic choriomeningitis virus. *J. Cell Physiol.* 91: 459-472 (1977).

12. Halbach, M. and K. Koschel. Impairment of hormone dependent signal transfer by chronic SSPE virus infection. *J. gen. Virol.* 42: 615-619 (1979).
13. Koschel, K. and M. Halbach. Rabies virus infection selectively impairs membrane receptor functions in neuronal model cells. *J. gen. Virol.* 42: 627-632 (1979).
14. Barrett, P.N. and K. Koschel. Effect of antibody-induced modulation of measles (SSPE) virus membrane proteins on β -adrenergic receptor-mediated adenylate cyclase activity. *Virology*. 127: in press.
15. Woodruff, J.F. and J.J. Woodruff. The effect of viral infections on the function of the immune system. In: "Viral Immunology and Immunopathology" (A.L. Notkins, ed), Academic Press, New York, San Francisco, London 393-418 (1975).