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Volker ter Meulen

Institut für Virologie und Immunbiologie
Universität Würzburg, Versbacher Straße 7
D - 8700 Würzburg

Since 1980 two international symposia on coronaviruses have been held bringing together scientists interested in molecular biology and pathogenesis of this large group of viruses. Progress made during this period has revealed many features of these viruses which are unique. Studies on the structure and mechanisms of viral RNA synthesis as well as on the virus-cell interactions in infected tissue were main research topics during the past three years and the considerable advances made were discussed at this third symposium. In the area of viral pathogenesis the majority of studies were concerned with murine coronavirus infections in mice and rats. In these hosts coronaviruses induce a variety of diseases which allow the analysis of virus-cell and virus-host interactions including those viral and host factors contributing to the different disease processes. Interest was focussed on the pathogenetic role of the E2 peplomer protein, virus-target cell interactions, the immune modulatory role of coronaviruses as well as immunopathological reactions of the host.

Pathogenetic role of E2 peplomer protein of the murine coronavirus JHM

The viral structural protein E2 representing the main viral envelope protein, has important structural and biological functions. Its biological activity includes: binding to receptors on cell membranes, induction of neutralizing antibody as well as cell fusion and eliciting T cell mediated cytotoxicity (1). It is therefore not surprising that several groups characterized this protein, analyzed the antigenic epitopes and correlated virulence of neurotropic and non-neurotropic JHM viruses with

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respect to antigenic domains. Different antigenic epitopes have been identified on the E2 protein which are associated with different biological activities (Buchmeier et al., Fleming et al., Goto et al., Knobler et al., Leibowitz et al., Wege et al., this volume). Both gp150 and gp90 of the E2 protein of JHM virus mediate attachment to cell receptors, cell fusion and induction of neutralizing antibodies and with monoclonal antibodies (MAB) these functions can selectively be inhibited. However, the majority of antigenic sites found on the E2 protein are probably of no biological significance since MAB to these domains do not interfer with the biological activity of this protein.

In in vivo experiments, the role of the E2 protein was studied by two approaches. Either epitope mutants resistant to various MABs were isolated and subsequently used for infecting susceptible animals or infections in mice with JHM wild type virus were treated with monoclonal antibodies to define determinants on the E2 protein important to neurovirulence (Buchmeier et al., Fleming et al., Goto et al., Knobler et al., Leibowitz et al., Wege et al., this volume). In these experiments it was found that epitope changes on the E2 protein in the area of attachment, cell fusion and induction of neutralizing antibodies are associated with virulence and neurotropism. However, these differences in pathogenicity are best observed when two or more topographically distinct epitopes are changed. Obviously, molecular biological characterization of these mutants will define the changes which influence neurotropism and virulence of JHM virus.

Virus-host cell interaction: target cells in CNS infections with MHV

In the past, studies have been carried out to identify in infected mice as well as in rats the target cells in acute and subacute demyelinating encephalomyelitis since a CNS disease pattern resulting from viral infection would appear to depend upon the selective tropism of the infectious agent for a particular brain cell. In infected brain tissue this analysis has proven to be technically difficult since double immunofluorescent staining of viral and brain cell specific antigens has to be applied. Nevertheless, studies of infected mice have suggested that the oligodendrocyte (2,3) may be one important target in chronic infections whereas in acute infections neurons are involved shown by immunofluorescent techniques or by electron microscopy (4,5). Similar observations

have been made in mouse brain tissue cultures where again a selective vulnerability of neural cells are found for certain JHM virus mutants (6). However, in rats the main target cell for JHM virus may either be the oligodendrocytes as proposed by Beushausen and Dales (7) or the type I astrocytes or brain macrophages (microglia) as described by Massa et al. (8). Studies on primary Lewis rat brain cultures with MHV JHM showed selective infectivity of microglia and type I astrocytes. Oligodendrocytes and neurons appeared resistant to direct infections and only rarely became infected by JHM virus as a result of fusion with infected astrocytes or microglia. Obviously, the genetic background of the host and the biological properties of the JHM variant used determine susceptibility of the target cell in the rat system.

Another important aspect of the virus cell interaction in CNS infection is the establishment and maintenance of virus persistence in mice or rat brains. Only little information is available on the subject. In both infected mice and rats, infectious virus can be isolated from brain tissue within the first to second week post infection and during the acute or subacute stage of encephalitis. Later on infectious virus disappears but may be recovered from brain tissue during stages of exacerbation (9,10) However, in subclinical persistent infections either in mice or rats only viral antigen may be detectable in the absence of infectious virus. Moreover, by in situ hybridization with cDNAs to JHM viral RNA, specific JHM RNA could be detected in brain cells which was not always associated with antigen expression (11). These observations resemble to some extent those seen with other RNA viruses in particular morbillivirus infections in brain tissue cultures or brain cells in situ (12). These viruses induce in their host acute, subacute or chronic CNS diseases with demyelination on the basis of a persistent infection. So far, no common mechanism for the establishment and/or maintenance of morbillivirus persistence has been found and factors such as defective interferring particles, interferon, temperature sensitive mutants or viral antibodies causing antigenic modulations on cell membranes have been suggested to play a regulative role in these infections. In addition, host cell factors may influence morbillivirus replication as shown in measles virus infection in neural cells (13,14). It could be shown that in such virus cell systems increase of endogenous cAMP by papaverine treatment caused a marked suppression of measles virus growth in neural cells. This reduction in replication was due to inhibition of viral genomic RNA and

mRNAs. Similar observations were made with JHM virus infection in brain tissue cells in which experimental elevation of intracellular cAMP JHM replication in oligodendrocytes was suppressed without affecting that of MHV3 in astrocytes (Beushausen, this volume). These observations suggest that replication of these viruses in brain tissue may easily be modified by changes in cellular conditions such as cell differentiation, regeneration hypertrophy or hyperplasia and that such modifications may contribute to the establishment of coronavirus persistence.

Immunemodulatory role of coronaviruses

One of the most interesting features of coronaviruses is their potential to induce class I (Lavi et al., this volume) and class II (Ia) (Massa et al., this volume) antigens of the major histocompatibility complex (MHC) on the surface of brain cells. The phenomenon of Ia-antigen induction on astrocytes is independent of viral replication since ultraviolet inactivated virus is effective. It appears that the Ia antigen inducing capacity of the virus particles depends on direct interaction of the virus with the cell membrane of astrocytes. This interpretation rests on the observation that monoclonal antibodies directed against the E2 glycoprotein, with neutralising capacity prevent the induction whereas the non-neutralizing antibody to this viral structural protein is uneffective (15). These experiments suggest that either binding of the virus to the surface of astrocytes through specific cell surface receptors or phagocytosis of the virus particles initiates expression of Ia. This mechanism which is interferon-gamma independent, may be similar to the one described for bacterial endotoxin (16). In the case of class I antigens different observations were made. Whereas class II antigens are induced only on astrocytes, class I antigens are induced not only on astrocytes but also on oligodendrocytes. Moreover, this phenomenon can also be transferred by a soluble factor (17). Studies on the inducibility of class I surface antigen revealed that the transfer of virus free supernatant from JHM infected astrocytes led to the expression of class I antigen on enriched cultures of individual CNS cells.

These two observations represent an outstanding phenomenon and explain for the first time how an immune response in the CNS is initiated. The CNS is considered an immunologically priviledged site, because 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 interferon-gamma (18). In case of a CNS virus infection, the induction of MHC class II antigens has to be interferon-gamma independent since interferon-gamma is not synthesized by cells of the brain. The inducibility of class I and class II antigens by coronavirus is an important immune regulatory event. The induction of class II molecules allows the initial presentation of viral antigen to T helper and class II restricted cytolytic lymphocytes and class I molecules provide the appropriate receptor for class I restricted cytolytic T 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 presented at this meeting may hopefully allow studies of this aspect in the future (Holmes et al., this volume).

Autoimmune reactions in the course of coronavirus infections

It is well established that humoral and cell mediated immune (CMI) reactions play a major role in the control of a virus infection. Viral antibodies are important to neutralize extracellular virus, to provide immunity against reinfection and under certain conditions to eliminate an infected cell by antibody dependent cytotoxicity. CMI reactions are the main mechanism in destroying infected cells and it has been observed that a genetic defect in CMI in association with viral infection leads to major complications. On the other hand, loss of immunotolerance may cause immunopathological complications which perpetuate a disease process in the absence of the infectious agent. Recently, it has been observed that in the course of a persistent coronavirus infection in Lewis rats a CMI reaction to basic myelin protein (MBP) occurs (19). Lymphocytes collected from spleen, thymus and peripheral blood and cultured in the presence of MBP yielded proliferation responses which were similar to those obtained with lymphocytes from experimental allergic encephalomyelitis (EAE). Moreover, when lymphocytes from SDE Lewis rats were intravenously inoculated into normal rats, the recipients came down with the clinical symptomatology of EAE consisting of abnormal sensitivity in touch and slight ataxic gait within five days after transfer. Histologically, the transferred animals revealed perivascular mononuclear cell infiltrations in the dorsal area of the white matter of the spinal cord, in the pons, cerebellar white matter and thalamus. In contrast, infected BN rats did

not develop such a cell mediated autoimmune reaction as determined by in vitro analysis or by adoptive transfer experiments indicating that the immunogenetic background controls such a reaction (20). Studies on the intrathecal humoral immune response in these two infected rat strains supported the interpretation of an autoimmune reaction. In Lewis rats, only occasionally an intrathecal virus specific immune response could be found despite the presence of oligoclonal IgG of restricted heterogeneity directed against non-viral antigens. In contrast to these observations BN rats with a persistent JHM infection in brain tissue revealed a strong intrathecal humoral immune response against this infectious agent which probably prevents spread of virus infection in CNS tissue (Dörries et al., this volume). Similar observations were made with JHM virus infection in mice emphasizing the importance of these immunopathological reactions in maintaining the disease process (Koolen et al., this volume).

Beside the CNS murine coronaviruses infect other organs as well and depending on the animal strain and on the biological properties of the virus strain used it has been observed that thymic epithelial cells and thymic lymphocytes can be infected which may have severe consequences for the host (Knobler et al., this volume). Depending on the extent of infection an immunosuppression or activation of lymphocyte subpopulations may occur which could be followed by adverse immune reactions. Such mechanisms could be responsible for MHV3 infection in Balb/cJ mice in which a fulminant hepatitis develops (Abecassis et al., this volume). This hepatitis can be prevented by treating the animals with dimethyl-prostaglandin E2 or with immunosuppressive drugs suggesting that an immunopathological reaction contributes to this disease process.

In the context of autoimmune reactions the most important question concerns the mechanism by which coronaviruses induce an immune reaction against host antigens. This problem is still unsolved, but a number of formal possibilities exists:

1. During replication the virus incorporates host antigens into its envelope and inserts, modifies or exposes cellular antigen on the cell surface. The cellular antigens are considered foreign to the immune system since they are ordinarily not encountered (21).

2. The virus interacts with the immune regulatory system by destroying subpopulation of lymphocytes or stimulating generation of lymphocyte

clones which are autoreactive. In general, many viruses are lymphotropic and one of the prime example is Epstein Barr virus which infects and transforms human B lymphocytes. Such immortalized cells may secrete under certain conditions autoantibodies which could react with cellular constituants (22). That murine coronaviruses do indeed infect lymphocytes has been shown at this meeting and therefore this mechanism has to be considered.

3. Another mechanism could be molecular mimicry by which an immune response is raised against certain viral antigens which may cross react with normal host cell antigens. By computer analysis of a variety of viral sequences it was recently found that a number of viruses contains the sequence of basic myelin protein in the viral genome (23). Moreover, the immunization of a rabbit with a synthesized peptide from such a sequence of the hepatitis B virus polymerase led to the induction of EAE lesions in rabbits (24). However, so far, an MBP sequence has not been found in the viral genome of JHM, in particular, in the E2 sequence which represents the peplomer protein of this virus (Siddell, personal communication).

4. An autoimmune response could be triggered by the development of antiidiotypic antibodies. In the reovirus model such antibodies directed against antibodies to the reovirus type 3 hemagglutinin have been found to react with receptors for reovirus on the surface of lymphocytes and nerve cells, and it is conceivable that this could lead to an immunopathological reaction (25).

5. A last possibility for the development of an immunopathological reaction in the course of a CNS viral infection is the induction of class II antigens on astrocytes by the virus and a consequent pathological delayed type hypersensitivity reaction (DTH) in genetically susceptible animals. Class II antigen has to be present on the astrocytes to allow helper T lymphocytes to recognize viral antigens. However, in cases of an extremely high constituitive level of class II antigen expression on astrocytes an inappropriate or accessive presentation of self-antigens and viral antigens may develop, as it is thought to occur in autoimmune processes directed against the thyroid gland (26). This mechanism could probably play a role in the JHM virus induced chronic demyelinating disease of Lewis rats since

such hyper-expression of class II molecules on astrocytes after treatment with interferon gamma or JHM viral particles is genetically regulated (Massa et al., this volume).

CONCLUSIONS

Coronaviruses induce in their respective hosts subacute or chronic disease processes which are of complex nature. Many viral and host factors play a pathogenetic role and at this meeting some of the unique features of these diseases have been demonstrated and discussed. For some of the diseases the availability of susceptible and resistant inbred animal strains which react differently to viral and host antigens as well as to viral mutants with different biological properties will permit the identification of parameters which influence these various disease types. The progress made in the area of molecular biological characterization of coronaviruses as well as in immunology of some of these animals used in the experiments will make it possible to define pathogenetic events leading to these disorders. Progress made in the future will not only improve our knowledge for these experimental model diseases, but will certainly influence studies of related human disorders.

This third international symposium has proven again the usefulness in bringing together scientists from different disciplines involved in the study of coronavirus infections. The meeting place selected, the excellent programme put together, the relaxed atmosphere provided and the endless scientific and personal discussions among the participants have greatly supported scientific exchange and indirectly coronavirus research. I think that all participants are very grateful to Dr. Michael Lai and Dr. Steven Stohlman as well as to their staff for organizing this conference.

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