Autoimmune Reactions Against Myelin Basic Protein Induced by Corona and Measles Viruses[a]

VOLKER TER MEULEN

Institut für Virologie und Immunbiologie
Universität Würzburg
D - 8700 Würzburg, West Germany

Many major human diseases derive from autoimmune reactions directed against particular organ or tissue antigens, as can be demonstrated by immunohistologic studies. One disease in which a virus-triggered autoimmune phenomenon has been postulated is multiple sclerosis (MS).[1] The strongest evidence in support of a viral etiology of MS comes from epidemiologic studies that suggest that MS derives from environmental factors operating years before the onset of the clinical manifestations of the disease. This evidence consists of age-specific tables, geographic distribution, and patterns of migration and family clustering of the cases of MS. The hypothesis derived from these studies holds that a patient with MS is exposed at puberty, or shortly thereafter, to an infectious agent that triggers the disease. Besides the epidemiologic evidence, many investigators have found an increase in virus-specific antibodies against certain enveloped viruses in patients with MS, suggesting that these viruses could be candidate causative agents. However, thus far, all attempts at isolation or identification of a particular virus as the cause of MS have failed. This failure is the more perplexing, because a number of viruses have been isolated from the brains of patients with MS, none of which could be unequivocally related to the disease.

Despite these failures, the hypothesis of a viral etiology of MS remains viable, because of the available circumstantial evidence. It is reasonable to assume that a virus (or several viruses) can trigger an autoimmune reaction against brain antigens, which can then continue and thus can perpetuate the disease, even when the virus is no longer around.

In an attempt to test the validity of this theory, we developed an animal model, the Lewis rat infected with either the murine coronavirus JHM or a neurotropic measles virus. Both viruses cause central nervous system (CNS) disease and trigger an autoimmune reaction mediated by T cells.

MURINE CORONAVIRUS JHM INFECTION IN WEANLING LEWIS RATS

When rats are infected with JHM virus at the age of 21 to 35 days, they develop either acute encephalomyelitis (AE) or subacute demyelinating encephalomyelitis

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(SDE).[2,3] The acute disease has an average incubation period of 6-12 days and follows a rapidly progressing clinical course characterized by paralysis and early death. Examination of the neurologic tissues of these animals reveals changes limited to gray matter of both the brain and spinal cord, consisting of necrosis of the neurons and consequent infiltration by granulocytes, lymphocytes, and macrophages. Viral antigen is evident in neurons and glia, and the culprit virus is readily isolated from the brain and spinal cord tissues.

In contrast, SDE has an incubation period of 2 weeks to 8 months, followed by a slow onset of disease characterized by paralysis of the hind legs and ataxic gait, eventually culminating in tetraplegia. The most prominent neuropathologic changes include primary demyelination in the white matter of the optic nerves, midbrain, pons, cerebellum, and spinal cord. Within the demyelinated plaques are well-preserved axons and neurons. There is also perivascular cuffing by lymphocytes and other mononuclear cells, as well as infiltration by macrophages. Here, too, the viral antigen can be recognized in the tissues, but it is primarily found in the glia and in the proximity of the plaques. The virus can also be isolated by conventional techniques, regardless of the length of the incubation period. Many of these animals eventually recover and show evidence of remyelination.

RELAPSING SUBACUTE DEMYELINATING ENCEPHALOMYELITIS OF LEWIS RATS

Some animals that survive SDE and recover later experience a second attack of the neurologic disease.[4] Between 60 and 120 days following the first episode of disease, the animals get sick again, with identical symptoms that are now more severe. Histopathologic abnormalities consist of new demyelinative lesions infiltrated by mononuclear cells located primarily in the brain stem and the spinal cord. Moreover, there are old lesions where they were previously noted, revealing extensive remyelination indicative of a repair mechanism that had developed after the initial disease. In contrast to the first attack of SDE, following the second attack, isolation of virus is a rarity, even as viral antigen is readily detected in glia and the region surrounding the plaque.

INFLUENCE OF GENETICS ON CNS INFECTION

Because JHM infection in the Lewis rat resembled some characteristics of experimental allergic encephalomyelitis (EAE), we decided to compare the infection with this virus to that of another inbred rat, BN, which is resistant to EAE. Weanling BN rats can be infected with the JHM virus, but the majority of these animals remain clinically well despite histologic changes of subacute demyelinating encephalomyelitis. Virus can be isolated from their brains, but only up to 10 days after infection, even as fresh CNS lesions continue to develop and viral antigen persists beyond that period. In contrast to SDE in Lewis rats, in BN rats the main neuropathologic lesions consist of small nodular demyelinated plaques predominantly located in the periventricular white matter, an area in which Lewis rats only rarely develop changes. These nodular lesions, which differ from the demyelinated plaque seen in infected Lewis rats, contain

microglia, phagocytes, and astrocytes with swollen cytoplasm. In addition, the inflammatory response is expressed mainly by plasma cells.[5] Obviously, therefore, an important pathogenic mechanism in response to the JHM infection depends on the genetic characteristics of the animal.

MEASLES VIRUS INFECTION OF LEWIS AND BN RATS

Infection of weanling Lewis rats with neurotropic measles virus CAM/RBH results in either acute measles encephalitis or subacute measles encephalitis (SAME).[6] The acute encephalitis is characterized by a short incubation period and focal infiltration by mononuclear cells of gray matter of both cerebral hemispheres and basal ganglia. There are usually few or no degenerative changes. SAME develops in about 20% of the infected animals, after an incubation period of 3 weeks to 3 months. The earliest sign of the disease is an arrest of weight gain or even weight loss. This is followed by generalized hyperexcitability, unsteadiness, abnormal posture, paresis of the limbs, and occasional seizures. This phase of the disease lasts for several days up to 3 weeks and has a case fatality rate of 50%. Histopathologic changes consist of prominent lymphomonocytic perivascular cuffing. There are no demyelinated plaques. The survivors recover completely and remain well for at least 8 months.

Infection of weanling BN rats results in acute encephalitis in a small percentage only; the majority develop clinically silent encephalomyelitis (CSE). The histopathologic lesions in animals with CSE consist of inflammatory lesions, widespread proliferation of the glial cells, and multicystic parenchymal degeneration.

Infectious virus is easily isolated from the cerebral hemisphere of both types of rats with acute encephalitis. However, in contrast, neither in SAME in the Lewis rats nor in CSE in the BN rats could infectious virus be isolated by conventional techniques or by co-cultivation, despite the detection of viral antigen. This failure of isolation could be attributed to restriction of gene expression of the measles virus occurring at the level of virus envelope proteins. As a result, infectious virus particles cannot be assembled, because the matrix protein required for the budding process is not synthesized. At no time during the course of the disease has virus been recovered from their lungs, liver, kidneys, spleen, or thymus.

IMMUNE REACTION IN INFECTED RATS

Host response, both humoral and cell-mediated immunity (CMI), plays a major role in controlling virus infections. Specific antibodies neutralize extracellular virus and protect against reinfection; in some circumstances they also eliminate infected host cells by antibody-dependent cytotoxicity. CMI reactions, however, are the predominant mechanism that destroys infected cells. Individuals with an inherited defect in the CMI develop severe complications of those infectious diseases that tend to be benign in normal individuals.

In CNS infections, intrathecal synthesis of virus-specific antibodies is an important defense mechanism and is well documented in human patients.[7-12] It is the B lymphocytes that are responsible for the intrathecal synthesis of the antibodies, which are

of an oligoclonal nature as determined by agarose electrophoresis or isoelectric focusing. It is possible to determine viral specificity of the IgG clones in the CSF specimens by immunoprint fixation[13] or immunoblot technique.[14] This determination is important in diagnosis.

In rats infected with either JHM or measles virus that develop SDE or SAME, the same diagnostic techniques have been applied by us[15–17] with the following findings. In the Lewis rats there was only an occasional intrathecal virus-specific response despite the presence of oligoclonal IgG. It is therefore probable that these immunoglobulins of restricted heterogeneity are directed against nonviral antigens, which are likely to be antigens of the central nervous tissues. Conversely, in the BN rats the oligoclonal intrathecal antibodies were specific against the virus used in the original infection. It is likely, therefore, that in the BN rat these antibodies tend to play a protective role, whereas in the Lewis rat no such protection is afforded.

An analysis of the CMI response in SDE and SAME revealed a reaction against the JHM, or measles, virus and in the diseased Lewis rats a reaction also against myelin basic protein (MBP).[18,19] Lymphocytes, whether collected from the spleen, thymus, or peripheral blood, all had a proliferative response in the presence of MBP, akin to that in EAE. Moreover, when lymphocytes from rats with SDE or SAME were infused intravenously into normal rats, the recipients developed symptoms of EAE within 5 days, consisting of hypersensitivity to touch and a slightly ataxic gait. Histologic examination of the CNS tissues of these animals revealed perivascular cuffing with mononuclear cells in the white matter of the spinal cord, where the lesions were in the dorsal columns—the pons, the cerebellum, and the thalamus. Infected BN rats did not develop such an autoimmune reaction when tested either by *in vitro* analysis or by the adoptive transfer of lymphocytes.[5,19] We can therefore conclude that the cellular autoimmune reaction is of pathogenic significance in the infected Lewis rat.

INTERACTIONS OF JHM AND MEASLES VIRUSES WITH ASTROCYTES

Our knowledge of the specific immune responses in the CNS to viral infections is quite incomplete. The CNS is an immunologic island, considered a privileged site. In order for the lymphocytes to find an antigen in the CNS, they must invade its domain and, once there, they must secure a mechanism for identification of the antigens. Recent evidence has been brought forth that astrocytes can act as cells presenting antigens.[20] On the basis of the information, we endeavored to investigate the effects of the two viruses on astrocytes. Both viruses can induce class II molecules on cultured astrocytes,[21,22] a property independent of viral replication in the astrocytes because viruses inactivated by ultraviolet irradiation are also effective. Apparently this capacity to induce the Ia antigen depends on direct interaction of the viruses with the cell membranes of the astrocytes. This interpretation has been derived from the observation that monoclonal antibodies directed against the E2 glycoprotein of the JHM virus or against the hemagglutinin of the measles virus prevent the induction of class II antigens. It appears, therefore, that either the viruses bind to specific receptors on the cell surface or the cells phagocytize the viruses and the expression of the Ia antigen follows. This mechanism is independent of gamma-interferon. It may be similar to the one described for bacterial endotoxin.[23]

The phenomenon of Ia induction by these two neurotropic viruses has important implications on the mechanisms of pathogenesis. Until recently it was assumed that gamma-interferon released by activated T lymphocytes was indispensable to the induction of Ia on certain antigen-presenting cells,[24,25] including astrocytes. Because the brain lacks lymphatic drainage and the so-called blood-brain barrier restricts traffic of lymphocytes and macromolecules, gamma-interferon would not be readily available for the induction of Ia, especially in the early phase of the infection. What apparently happens is that the viruses themselves induce Ia expression on the astrocytes, enabling these cells to present the viral antigens to the lymphocytes and allowing the host to mount an effective immune response to control the infection.

POSSIBLE MECHANISMS OF VIRUS-INDUCED AUTOIMMUNE REACTIONS

Although the means by which viruses induce T-lymphocyte responses against host antigens are still unclear, there are a number of speculations to account for this phenomenon.

1. The fact that viruses require living cells for replication has major consequences for the host. During replication, the viruses can incorporate into their envelopes host antigens and they insert, modify, or expose internal cellular antigens on the cell surface. These heretofore "hidden" antigens, now exposed, could appear foreign to the host's immune system.

2. The viruses can interact with the immune regulatory systems by destroying subpopulations of lymphocytes or by stimulating generation of lymphocyte clones that are autoreactive. Many viruses are lymphotropic, as exemplified by measles and Epstein-Barr virus. Measles virus replicates in T or B lymphocytes, which leads to lymphocyte destruction and subsequently to an immunosuppressive effect clinically documented by abolition of skin reactions in delayed hypersensitivity. Epstein-Barr virus infects and transforms human B lymphocytes. The transformed, or immortalized, cells could under certain circumstances secrete antibodies reacting with cellular constituents.

3. Molecular mimicry could also be operating. An immune response might be raised against certain viral antigens that cross-react with some native host antigens. A variety of viral sequences, including those of measles virus, were recently compared by computer analysis with myelin basic protein (MBP).[26] The comparison showed amino acid homology between viral proteins and MBP. This observation led to an interesting experiment in which a rabbit was immunized with a synthetic peptide from such a sequence of the hepatitis B virus polymerase.[27] Peripheral blood lymphocytes from the immunized rabbits proliferated in the presence of either MBP or hepatitis B polymerase, and their CNS tissue revealed similar neuropathologic changes similar to those in EAE. In this context it is of interest to note that in postinfectious encephalomyelitis in man associated with rubella, varicella, or measles, an MBP-specific lymphoproliferative response has been observed.[28]

4. An autoimmune response could be triggered by the development of an anti-idiotypic antibody. In a reovirus model these antibodies directed to type III hemagglutinin react with receptors for reovirus on the surfaces of the lymphocytes and nerve cells, and it is possible that this could trigger an autoimmune reaction.[29]

5. The last possible mechanism for the development of an immunopathologic

reaction in the course of a CNS viral infection is the induction by the virus of class II antigens on astrocytes and a consequent delayed type hypersensitivity reaction (DTH) in genetically susceptible animals. In order for the helper T lymphocytes to recognize viral antigens, class II antigens probably have to be present on the astrocytes. However, in extremely high constitutive levels of class II expression on astrocytes, an inappropriate or excessive presentation of self-antigens and viral antigens may develop, similar to that in autoimmune processes directed against the thyroid gland.[30] This mechanism could well play a role in the JHM- and measles-virus-induced CNS disease in Lewis rats, because as recently shown by us,[31] these hyperexpressions of Ia molecules on astrocytes after contact with gamma-interferon or viral particles are genetically regulated.

CONCLUSIONS

JHM and measles virus infections in rats are models of a persistent viral infection of the CNS with and without demyelination, associated with a cell-mediated immune reaction to MBP. Availability of inbred susceptible and resistant rat strains, which react differentially to viral and host antigens, makes possible a variety of experimental permutations aimed at defining the causal mechanisms of CNS diseases. These mechanisms can be studied from the points of view of molecular biology and immunology, because both viruses used are well characterized and there are appropriate immunologic markers for the lymphocytes and the brain cells of these rats. This approach can be expected to bring us towards an understanding of the pathogenesis of persistent CNS infections that are associated with demyelination and mediated by immunologic reactions. Our hope is that information gained from these animal models will have a bearing on studies of related human diseases, particularly parainfectious encephalitis and multiple sclerosis.

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