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MOLECULAR DETERMINANTS IN AUTOIMMUNITY

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INTRODUCTION

Many theories have been proposed to explain the pathogenesis of multiple sclerosis. From epidemiologic studies the occurrence of disease can be associated with genetic and environmental factors (reviewed by 1). Some of the environmental factors include infectious agents (37). Thus far, many agents including a whole host of viruses have been implicated, however, none have been shown definitively to be the causative agent (2-4, 12, 19, 21, 25, 29, 31, 41, 44). In addition, immunologic factors may play an important role in the pathogenesis (30). Along this premise some investigators have been using immunosuppressive regimes to modify the course of the disease (reviewed by 10). These studies have suggested modest improvement in some of the patients. Several models have been put forth to delineate the role of virus and immune responses in reproducing the observed pathologic features of multiple sclerosis.

One of these is experimental allergic encephalomyelitis (EAE) (32). This is an autoimmune disease of the central nervous system (CNS). Several CNS proteins have been determined to be encephalitogenic. It is when these proteins (derived usually from myelin) are injected into a suitable animal host with adjuvant an immune response ensues that cross-reacts with brain components. This disease is characterized by a perivascular mononuclear infiltrate that leads to demyelination. The disease is immunopathologically mediated. Many of the features of EAE can be transferred from animal to animal with the use of sensitized T lymphocytes. As yet the disease cannot be passively transferred with antibodies from EAE animals or animals immunized with myelin components. The relapsing and remitting form of chronic EAE has a lesion distribution and clinical course that can closely approximate multiple sclerosis (43).

Another model for multiple sclerosis is Theiler's murine encephalomyelitis virus (TMEV) infection of mice. Here, there are both genetic and virologic contributions to the demyelinating disease. This model was first championed by Lipton and colleagues in the 1970's as a system to study virus induced demyelination (22-24). They described a biphasic disease in Swiss outbred and later in SJL mice using the Daniels (DA) strain of TMEV. The early disease was an acute phase that resembled poliomyelitis where infection of neurons was prevalent and these cells went on to die. In the early phase mice became paralyzed and depending

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on the dose of the virus and strain of mouse would go on to die or would become persistently infected. In the SJL mouse strain the mice developed more of the chronic disease with less of the acute disease. This chronic late disease was characterized by a perivascular mononuclear cellular infiltrate and demyelination (8). Chronically infected mice have a spastic gait and difficulty righting themselves.

Various genetic components influence the production of clinical disease and demyelination. Both the susceptibility to disease and extent of demyelination have been correlated to H-2 and non H-2 regions in the mouse genome. In mice with the H-2^S alleles susceptibility correlated with the D region but not the K or I-A (5-7, 33). In addition, non H-2^S contributions of a region encoding for the constant portion of the ß-chain of the T cell receptor on mouse chromosome 6 can also contribute to disease production (27). Virus titers in the CNS from resistant and susceptible mouse strains correlating to the extent demyelination have been controversial. There is mounting evidence that delayed type hypersensitivity reactions which are class II restricted are involved in the demyelinating disease (Miller, 1978). Treatment of infected mice with anti-IA and L3T4 sera has been reported to influence the pattern of disease (13, 33, 42).

We have been combining the two aspects of viruses and autoimmune disease; i.e., exploring ways viruses could induce immune reactivities to "self". One hypothesis is that viruses in some way could trigger or initiate autoimmune attack on myelin components leading to demyelination. Along this line there is strong suggestive evidence that infections may precede relapses (37). Our initial studies were to define if viruses could have cross-reacting determinants with host cells. In raising monoclonal antibodies to measles virus it was noted that the monoclonal antibodies from the various fusions could be divided into three groups. First, and the largest group, contained monoclonal antibodies which were viral specific. These monoclonal antibodies reacted only with viral proteins by immunofluorescent staining, immunoprecipitation, or Western blot analysis. The second group of monoclonal antibodies was found to react with just self components and not viral proteins. These antibodies probably arose due to self reactive B cells present in the spleens of these mice. The last group of monoclonal antibodies which contained the smallest number of monoclonal antibodies, reacted with both viral proteins and host cell determinants. These monoclonal antibodies comprised approximately 1-3% of our total antibodies depending on the fusion.

One of these monoclonal antibodies was further investigated to characterize its specificity (16). It was first analyzed by immunofluorescent staining. HeLa cells infected with measles virus and mock infected cells were subjected to examination. The monoclonal antibody derived from mice immunized with measles virus was incubated with measles virus infected HeLa cells and immunofluorescent antibody procedure conducted. The pattern of staining was one of a cytoplasmic reaction. The monoclonal antibody bound to prominent cytoplasmic viral inclusions. This type of staining pattern was one typical of the distribution of measles virus phosphoprotein or nucleocapsid protein. When the monoclonal antibody was incubated with mock infected cells the staining pattern was one of a network-like appearance. In cells that were undergoing mitosis the pattern was markedly different, the uninfected cells had a speckled appearance. This type of staining was very characteristic of intermediate filament proteins, particularly vimentin or cytokeratin. Similar patterns of reactivity were found when this monoclonal antibody was tested in BHK and mouse L929 cells. Knowing

this, a preparation enriched for intermediate filament proteins was prepared from uninfected HeLa cells and this preparation was used to adsorb with the monoclonal antibody preparation. The adsorption procedure removed the reactivity to infected cells. Thus, an intermediate filament preparation contained a common determinant with a viral protein.

To further define the viral and host proteins the monoclonal antibody reacted with biochemical methods were employed. Western blotting experiments were initially performed. First, an intermediate filament rich protein preparation from uninfected HeLa cells was prepared and the proteins separated by SDS-PAGE. The proteins were then transferred to nitrocellulose strips and strips incubated with the monoclonal antibody. The monoclonal antibody was found to react with a 52-54,000 molecular weight protein. With the use of other monoclonal antibodies to cytokeratin and vimentin, the cellular protein was identified as one of the cytokeratin proteins. Similarly, cytosols from infected HeLa cells were prepared in which the intermediate filaments proteins were depleted and these preparations were electrophoresed on SDS-polyacrylamide gels and proteins transferred to nitrocellulose paper. The monoclonal antibody which reacted with cytokeratin from uninfected cells was also found to bind to a 70,000 molecular weight viral protein, that co-migrated with measles virus phosphoprotein. Next, purified measles virus was electrophoreses and Western blot analysis performed. Again the monoclonal antibody reacted with the measles virus phosphoprotein that was incorporated into virions and not just present in infected cells. Therefore, a monoclonal antibody had the ability to define an epitope on a viral protein as well as a host cell component.

In like manner, additional monoclonal antibodies were studied: one against a herpes virus protein and another to vaccinia virus hemagglutinin (9, 16). The monoclonal antibody that reacted with a herpes virus protein was found to also bind with an intermediate filament protein. The vaccinia virus hemagglutinin monoclonal antibody bound to vimentin. Thus, additional viruses were shown to have common determinants with self proteins.

Further, Sairenji et al described a murine monoclonal antibody that recognized a filamentous structure in Epstein-Barr virus-producing lymphoblastoid cell lines (35). By immunofluorescent staining, the monoclonal antibody appeared to react with vimentin or a closely associated intermediate filament protein. The expression of this antigen was induced by superinfection with Epstein-Barr virus or treatment with tumor promoting agents, and its appearance may be similar to the induction of stress proteins (36). Along this line Sheshberadaran and Norrby described monoclonal antibodies against measles virus fusion protein that also reacted with cellular stress proteins. This was demonstrated by immunoprecipitation and immunofluorescent staining of infected and uninfected cells. This host stress protein was induced by infection of cells with paramyxoviruses, heat shock of uninfected HeLa cells, and treatment of various cell lines with 2-deoxyglucose, tunicamycin, L-canavanine. Other monoclonal antibodies have been identified to have similar reactivities in many viral systems. Recently, Srinivasappa et al reported that roughly 3-4% of all antiviral monoclonal antibodies reacted with host cells components (39).

As described, many of the monoclonal antibodies cross-react with intracellular determinants or filaments. This probably reflects the fact that viruses are intracellular parasites and assemble at very discrete sites within the infected cell (11). By having common sites or

determinants with cellular proteins these viral proteins could be transported to similar areas as the intermediate filament proteins. Many viruses assemble and mature in association with intermediate filament proteins.

In producing monoclonal antibodies to another paramyxovirus, Goswami et al found that an antibody against the Simian virus 5HN glycoprotein bound to an antigen found in Purkinje cells of the adult rat brain (18). The monoclonal antibody had the ability to prevent infectivity. In addition, this monoclonal antibody could bind to white matter.

Tardieu et al have found a common determinant between reovirus types 1 and 3 and lymphocytes. The monoclonal antibody reacted with the Lyt 2,3 subset of murine lymphocytes (40). In addition, this monoclonal antibody had the ability to initiate complement dependent lysis of Lyt 2,3 positive lymphocytes (40).

From these data investigators have found cross-reacting determinants between viral determinants and host CNS and immune tissues. Using antibodies to define common determinants is very useful however it is very difficult to determine the actual epitope; i.e., the sequence involved in the cross-reaction. Thus, experiments defining common amino acids were instigated. These are described below.

A protein capable of inducing an autoimmune disease of the CNS was chosen since the encephalitogenic disease inducing determinants for a wide variety of species is known (20, 26, 38). This is myelin basic protein. It has been sequenced and has been widely studied. Using computer assisted analysis known viral protein sequences were compared to the encephalitogenic sites described for myelin basic protein (14). The original analysis revealed several sequence similarities between various animal viruses proteins and myelin basic protein. One of the best common sequence in tandem was between the myelin basic protein encephalogenic site for the rabbit and hepatitis virus B polymerase. This was:

myelin basic protein	T T H Y G S L P Q K
hepatitis virus	I G C Y G S L P Q E

These peptides were synthesized. Studies looking for the production of autoantibody, cellular reactivity and disease production were undertaken.

Seven rabbits were immunized with the hepatitis virus peptide (HVP) and antibody monitored for the presence of antibody to HVP and myelin basic protein. Five of the seven animals made detectable antibody as measured by ELISA to whole myelin basic protein. Competitive inhibition experiments with increasing amounts of HVP blocked the binding of HBP antibodies to myelin basic protein in a dose dependent manner. As expected all seven rabbits made antibodies that reacted with HVP.

To test for cellular reactivity in rabbits, eight animals were immunized once with HVP and peripheral blood mononuclear cells were obtained. These mononuclear cells were then cultured in the presence of HVP or myelin basic protein. The peripheral blood mononuclear cells from all eight rabbits proliferated when cultured with HVP peptide. Peripheral blood mononuclear cells from half of the rabbits proliferated in the presence of myelin basic protein. Thus, 4/8 animals immunized with HVP reacted with myelin basic protein.

Histologic evaluation was performed in 11 rabbits immunized with HVP. Brain and cervical spinal cords of four animals had scattered lesions that consisted of perivascular mononuclear and meningeal infiltrates characteristic of experimental allergic encephalomyelitis. None of the

rabbits immunized with the HVP developed clinical signs of experimental allergic encephalomyelitis. Similarly, one out of four animals injected with the encephalogenic peptide from myelin basic protein developed clinical signs and three of the four had histologic lesions of perivascular infiltrates in brain and spinal cord, thus, viral infection has the potential to trigger the production of autoantibodies and mononuclear cells that cross-react with self by a mechanism termed "molecular mimicry". The tissue injury from the viral initiated autoallergic event could take place in the absence of infectious virus.

Recently a monoclonal antibody against TMEV has been described to react with galactocerebroside (17). This virus has the ability to cause a chronic demyelinating disease in mice. The cross-reacting monoclonal antibody neutralizes the virus and binds to oligodendrocytes in newborn mouse CNS cultures. The presence of such an antibody could contribute to the observed demyelinating pattern of disease.

Similarly, a peptide (L G R P N E D S S S S S S S C) from the immediate-early region of human cytomegalovirus was analyzed by computer (17). It was found that the first five amino acids of this peptide had sequence similarity to the ß-chain of the human MHC HLA-DR protein. The common amino acids were located in a region that was conserved between the human and mouse histocompatibility antigens on the ß chain. The shared regions from the immediate-early region of human cytomegalovirus and HLA-DR had similar hydrophobicity and predicted ß-turn potential. This data suggested that the determinant would be on the surface of the protein. The IE-2 viral peptide induced antibodies that recognized the human DR ß-chain by western blot analysis. This suggests a mechanism to explain how human cytomegalovirus infection contributes to graft rejection and immunosuppression.

It is easy to speculate that in those instances where autoimmunity occurs, viral determinants reflect host cell determinants which have the capacity to induce disease, such as the encephalitogenic epitopes of myelin basic protein. Actual disease induction would not occur if the common site did not involve a disease inducing region. Immune responses involving non-disease inducing determinants may invoke autoantibody but actual autoimmune disease would not result. Another scenario pertains to those autoimmune afflictions that are of a chronic or relapsing and remitting nature. Here, viruses with the ability to persist may continually or cyclically express viral antigens. Even though the expression of the viral genome and therefore replication may be restricted, translation of the protein having the determinant in common with that of the host could continue. The resulting antigen, properly presented, may then evoke a smoldering immune response leading to chronic and progressive autoimmune disease.

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