Chapter C7

CORONAVIRUSES AND NEUROANTIGENS:
myelin proteins, myelin genes

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Abstract: Multiple sclerosis (MS) is an autoimmune disease in which autoreactive T cells specific to central nervous system (CNS) myelin antigens are activated. Although disease etiology remains unknown, coronaviruses are suspected to be involved in MS pathology. Molecular mimicry, the recognition of two antigens by a single immune cell, could be the mechanism explaining the link between a viral infection and MS through activation of myelin-reactive T cells by a virus infection in a genetically predisposed individual. Evidence supporting this hypothesis in humans has been accumulated in our laboratory. Human coronavirus (HCoV) – myelin cross-reactive T-cell lines (TCL) were predominantly found in MS patients compared to patients with other neurological or inflammatory diseases, or healthy controls. Moreover, virus-myelin T cell cross-reactivity was confirmed at the clonal level. Molecular mimicry between infectious pathogens such as the ubiquitous human respiratory coronaviruses could, in genetically susceptible individuals, play a role leading to the development of MS. Together with other possible mechanisms such as bystander effects, epitope spreading or even superantigenic activities, this pathogen-associated immune induction could play a role in maintaining and broadening the autoimmune response associated with MS pathology.

Key words: autoimmunity, T cells, central nervous system, virus, molecular mimicry

1.   INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by inflammation and myelin destruction. Even

though MS was described more than 130 years ago, its exact etiology remains unknown. A genetic predisposition is suggested and HLA genes are suspected to be involved (1, 2). Environmental factors are also associated with the triggering of this pathology. Indeed, epidemiological studies with identical twins demonstrate a low MS concordance between siblings and specific patterns of worldwide disease distribution point out the role for environmental factors, such as microbial agents (3, 4).

Amongst environmental factors potentially related to MS is a long list of more than 20 viruses. These viruses have been associated with MS by different experimental approaches: isolation of virus particles from tissues of MS patients (5), antibodies specific to virus found in central nervous system (CNS) of MS patients (6, 7) or demonstration of virus gene expression in the CNS of MS patients (8-14).

2.   NEUROANTIGENS

Tolerance is described as a mechanism established to create and maintain a state of non-response to autoantigens. In the CNS, some proteins were found to activate the immune system: myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), myelin oligodendrocyte basic protein (MOBP), CNPase (2',3'- cyclic nucleotidyl 3'-phosphodiesterase), transaldolase, S100β and α-B-crystallin (15, 16). All of these proteins were found at one time or another to activate autoreactive T lymphocytes in MS patients and therefore are suspected in maintaining or amplifying autoimmune reactions observed in the disease (17-20). Those neuroantigens, previously cryptic, might have been liberated in the CNS milieu following damages to the myelin sheath, and made available for antigen presentation.

How tolerance is broken in MS is still not understood. The suggested potential mechanisms are related to the initiation or propagation of the autoimmunity observed in MS. Superantigens could lead to a potent non-specific activation of a given T-cell receptor (TCR) Vβ family. Non-specific bystander effects of inflammation could also hold a key role. Moreover, determinant (epitope) spreading would broaden the immune response towards the recognition of new autoantigens. Finally, molecular mimicry between self-proteins and microbes represents another possible mechanism that could be involved in breaking tolerance and could explain the association of several infectious pathogens with MS etiology and exacerbations.

3.   MS AND CORONAVIRUSES

Coronaviruses are enveloped virus with a diameter of approximately 120 nm and that exhibit characteristic crown-shaped projections on their surface (21). Their genome consists of a very long positive-stranded RNA. Coronaviruses are responsible for 10 to 35% of common cold in humans (22) and were associated with more serious diseases such as respiratory distress in newborns (23), severe acute respiratory syndrome (SARS), and severe diarrhea (24).

The association between coronavirus and MS is strengthened by observations in mice. Indeed, murine coronavirus infection of susceptible mice leads to an inflammatory demyelination. This virus-induced demyelination is similar to MS in several aspects, providing a widely studied model for this neurological autoimmune disease (23, 24).

In humans, it was demonstrated using different experimental approaches that coronaviruses are neuroinvasive, i.e. that they can reach the CNS. First, virus was isolated from the brains of MS patients (27). Second, titers of antibodies specific to human coronaviruses were described to be higher in cerebrospinal fluids of MS patients than controls (28). Third, coronavirus RNA was detected in the brain of MS patients by RT-PCR and by *in situ* hybridization (11, 12, 14).

Furthermore, coronavirus neurotropism, the capacity to infect CNS cells, has also been demonstrated in humans. An acute infection by human coronaviruses was demonstrated in primary cultures of human microglia and astrocytes (29) as well as oligodendrocytes (Talbot *et al.*, unpublished data). In addition, a persistent infection by coronavirus was demonstrated in cell lines from nervous system (30, 31).

These observations demonstrated that coronavirus could reach the CNS and infect neural cells. Although it cannot be concluded from these observations that coronaviruses do cause MS, it suggests an association between CNS viral infection and this neurologic disease. In addition, epidemiological studies have shown that MS relapses were often preceded with respiratory tract infections (30). Interestingly, coronavirus reinfections are in fact possible (22).

4.   T CELL CROSS-REACTIVITY TO CORONAVIRUS AND MYELIN PROTEINS

Several mechanisms have been proposed to explain how a viral infection could lead to an autoimmune disease. Amongst these mechanisms is molecular mimicry. According to this model, a non-self agent first activates

an immune cell. Then, the cell recognizes a self-element that shares antigenic conformation with the pathogen and directs a response towards it. Individuals who are genetically predisposed to respond to this antigenic determinant of a pathogen having a similar conformation to a determinant on a self-antigen could develop an autoimmune response following infection. Shared sequences or similar determinant conformations between coronavirus and myelin proteins such as myelin basic protein (MBP) and proteolipid protein (PLP) have been identified (33-35). Molecular mimicry provides a unifying mechanism that could explain both the genetic and environmental aspects in the triggering of MS.

The molecular mimicry hypothesis would indeed explain observation of T cell lines (TCL) cross-reactive to both myelin antigen (Ag) and human coronavirus (HCoV) we have reported in MS patients (36). In this study, peripheral blood lymphocytes from MS patients and healthy controls were used to select long-term MBP- and HCoV-reactive T cell lines. A summary of these results is shown in Table 1. MBP T-cell lines were found as frequently in MS patients than in healthy controls. All the participants tested seropositive for coronavirus, as expected (22). Interestingly, TCL from ten of sixteen MS patients and two of fourteen control subjects showed cross-reactivity between myelin and viral antigens, for a proportion of such lines of 29% in MS patients and only 1.3% in healthy donors. Such cross-reactivity is thus highly significantly observed in MS patients. Therefore, in genetically predisposed individuals, T cells could be primed and activated following a systemic infection; the activated lymphocyte would enter the CNS and could establish a response towards myelin antigens. Myelin-reactive T lymphocytes could also have a role in broadening the immune response and increasing tissue damage. Coronavirus-myelin cross-reactive lymphocytes could thus initiate, maintain and/or amplify autoimmunity.

Table 1. Overall number of coronavirus-myelin cross-reactive TCL produced from MS patients and healthy donors. Cross-reactive TCL are obtained in a highly significant proportion (p<0.0001) in MS patients versus controls, with 29% out of TCL generated from the peripheral blood compared to only 1.3% (Talbot, 1996).

Donors	n	Lines (CD4+)	Cross-reactive T-cell lines		
			Lines	n	%
MS	16	134	39	10	29
Healthy Controls	14	155	2	2	1.3

Although these findings regarding T-cell cross-reactivity between human coronavirus and myelin antigen are consistent with the molecular mimicry hypothesis, experiments at the clonal level were needed to prove that a single T cell was indeed activated by both viral and myelin antigens.

5.   CLONAL T-CELL CROSS-REACTIVITY

Studies conducted at the molecular level reinforce and confirm the molecular mimicry hypothesis in MS (37). It was essential to examine coronavirus-myelin cross-reactivities at the T cell clonal level since TCL are made of a heterogeneous cell population: more than one TCR could have been involved in the measured cross-reactive response. Similar to the previous study involving TCL, long-term primary T cell clones (TCC) were also derived from the peripheral blood of MS patients. Selecting antigens used in this study were both known human coronavirus serotypes (229E and OC43) as well as two CNS proteins, MBP and PLP. Briefly, TCL were first selected with either one of the selecting antigen and positive cells were then cloned by limiting dilution; tritiated thymidine incorporation assays determined antigenic-specitifities (38). Table 2 shows that a total of 145 monospecific TCC were obtained from twenty-two patients, out of thirty-two patients studied. Many TCC were positive to coronavirus antigens, with about 80% of the TCC cultures selected with either HCoV-229E or HCoV-OC43.

Interestingly, ten cross-reactive long-term primary TCC were also cultured from six out of thirty-two MS patients (Table 2). While some patients led to very poor TCC production, other patients could lead to more then one cross-reactive TCC. These TCC were selected with myelin antigens (PLP and MBP, both encephalitogenic in genetically predisposed rodents) and HCoV. Half of these patients were HLA-DRB1*1501, a genetic susceptibility associated with MS (1). TCC produced were CD4+. HLA-typing and antigenic specificities of all ten cross-reactive TCC are described in Table 3. The selecting antigen is identified for each clone. Interestingly, two TCC selected with viral antigens recognized both myelin antigens. The TCR Vβ chains were also identified for six of those TCC and hypervariable regions were also determined through sequencing analysis (37).

Even if the method used to obtain the TCC is reliable, clonality was double-checked by diversity PCR. TCR were sequenced and only one TCR β chain was identified by PCR on several bacterial colonies issued from the same transformation. Assuming that the TCC obtained bear only one TCR, this study shows that a single TCR can recognize two different antigens, one from a human coronavirus, and the other from a myelin antigen that is targeted by T lymphocytes in MS. Such a cross-reactive phenomenon could be an elegant framework to explain the origin of an autoimmune disease as MS.

Thus, the T cell cross-reactivity between HCoV and myelin proteins in MS patients was confirmed at the single cell level. Combined with our previous results of highly significant coronavirus-myelin T-cell cross-

reactivities in MS patients compared to healthy controls (36), it appears that molecular mimicry could represent a signicant pathogenic mechanism associated with MS pathogenesis.

Table 2. Overall number of both monospecific and coronavirus-myelin cross-reactive TCC produced from the peripheral blood of thirty-two MS patients. Selecting antigens used for that experiment were both known serotypes of human coronavirus, HCoV-229E and HCoV-OC43, as well as two CNS proteins, MBP and PLP. Long-term primary human TCC were generated by limiting dilution of antigen-specific TCL. (37, 38)

TCC	Donors (n)	Number of TTC Produced with Viral Antigens		Number of TCC Produced with Myelin Antigens		Total
		HCoV-229E	HCoV-OC43	MBP	PLP	
Monospecific	22/32	80	34	19	12	145
Cross-reactive	6/32	4	2	2	2	10

Table 3. Antigenic specificity patterns of ten coronavirus-myelin cross-reactive TCC obtained from six MS patients. The HLA-typing for each patient from which those TCC were produced is shown. The selecting antigen is identified in bold.

TCC	HLA-DR	Antigenic Specificity	
P7-a	11,17	**HCoV-OC43**	MBP
P8-a	15,17	**HCoV-229E**	MBP
P12-a	13	**PLP**	HCoV-229E
P12-b	13	**PLP**	HCoV-229E
P12-c	13	**MBP**	HCoV-229E
P13-a	15	**MBP**	HCoV-229E
P13-b	15	**HCoV-OC43**	MBP and PLP
P22-a	13,15	**HCoV-229E**	MBP
P22-b	13,15	**HCoV-229E**	MBP and PLP
P24-a	7,17	**HCoV-229E**	MBP

6.   MS SPECIFICITY OF T-CELL CROSS-REACTIVITY

In order to verify whether coronavirus-myelin T-cell cross-reactivity is more significantly associated with MS, TCL were established from the peripheral blood of patients having neurological or inflammatory disease and from healthy controls (Talbot *et al.,* unpublished data). In this study, TCL

cross-reactive for both myelin (MBP, PLP) and human coronavirus antigens were found in four out of eleven MS patients, in a proportion of 3.4% of all TCL derived from the peripheral blood. Only one out of ten patients having neurological or inflammatory disease showed such cross-reactive TCL, for a proportion of 0.5% of the overall TCL produced from the peripheral blood. The latter proportion (0.5%) of cross-reactive TCL was obtained from healthy donors, where a cross-reactive TCL toward myelin and coronaviral Ag was found in only one out of twelve donors. These results are shown in Table 4. Cross-reactive TCL were found more often in MS patients. It is too early to say that such antigenic pattern is clearly related to MS, however, it remains of great interest to explore those antigens regarding molecular mimicry and neurodegeneresence.

Thus, cross-reactive TCL for myelin proteins and HCoV seem to be more abundant in the peripheral blood MS patients than in healthy patients. Moreover, the cross-reactivity observed appears to be found in MS, an autoimmune neurological disease, but not in other inflammatory or neurological diseases.

7.   CONCLUSIONS

The etiology of MS, an autoimmune neurologic disease, is unknown. Coronaviruses are suspected to be involved in MS pathology. Molecular mimicry, the recognition of two antigens by one single immune cell, could be the mechanism explaining the link between a viral infection and MS. Evidence of molecular mimicry between coronavirus and myelin antigens have been described in humans by our laboratory. Cross-reactive TCL were found predominantly in MS patients compared to patients with other neurological or inflammatory diseases, or healthy controls. Moreover, the T cell cross-reactivity was confirmed at the clonal level. However, it is important to keep in mind that the role of molecular mimicry does not exclude the participation of other immune mechanisms such as bystander effects, determinant spreading or even superantigens in the triggering or development of this autoimmune disease. All those mechanisms could play a role in maintaining or broadening the autoimmune response. The association of infection by a ubiquitous virus such as the human coronavirus with the development of multiple sclerosis in genetically susceptible individuals most likely represents the result of an aberrant induction of immune responses towards myelin proteins, possibly coupled to a persistent virus infection of the central nervous system that may activate glial cells (39), induce neuronal loss (40) and be associated with local immunopathology (41).

Table 4. To assess whether coronavirus-myelin cross-reactive TCL were only found in MS or could also be detected in other diseases, 13 MS patients, 10 patients suffering either of a neurological or an inflammatory disease, as well as 12 healthy controls were studied in comparison. For each group, TCL were produced and selected with MBP, HCoV-229E or HCoV-OC43, then were tested with a proliferation assay against both CNS proteins and both human coronavirus serotypes. The coronavirus-myelin cross-reactive TCL are shown in bold; those are also compared to the overall monospecific TCL number produced during the experiment to determine the percentage of cross-reactive TCL generated per studied group of donors. N.D. indicates that the test was not performed and results are not determined (selecting antigen indicated at the top of each column and antigen used for proliferation assay indicated on the left).

	Multiple Sclerosis			Neurological and Inflammatory Diseases			Healthy Controls		
	MBP	HCoV-229E	HCoV-OC43	MBP	HCoV-229E	HCoV-OC43	MBP	HCoV-229E	HCoV-OC43
MBP	N.D.	**3**	**1**	N.D.	**1**	0	N.D.	0	0
PLP	5	0	0	2	0	0	2	N.D.	N.D.
HCoV-229E	**1**	N.D.	0	0	N.D.	5	N.D.	N.D.	1
HCoV-OC43	**2**	2	N.D.	0	1	N.D.	**1**	2	N.D.
Overall Monospecifc TCL produced	123	50	53	55	90	37	71	84	49
Myelin-Virus Cross-reactive TCL/Total	7/226 (3.1%)			1/182 (0.5%)			1/204 (0.5%)		

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