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Potential molecular mimicry between the human endogenous retrovirus W family envelope proteins and myelin proteins in multiple sclerosis

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ABSTRACT

Multiple sclerosis is an autoimmune disease caused by the destruction of the myelin sheath in the central nervous system. The major target molecules for the immune response are the myelin basic protein, myelin oligodendrocyte glycoprotein and proteolipid protein but the aetiology of the disease is as yet poorly understood. The HLA Class II allele DRB1*1501 in particular as well as DRB5*0101 and the expression of human endogenous retroviral envelope proteins have been linked to multiple sclerosis but the molecular mechanisms relating these remain to be elucidated. We hypothesised that cross-reactive peptide epitopes in retroviral envelope proteins and myelin proteins that can be presented by the two Class II DR molecules may play a role in initiating multiple sclerosis. Sequence homologies between retroviral envelope and myelin proteins and *in silico* predictions of peptides derived from them that are able to bind to the two Class II alleles were examined to test the hypothesis. The results support the hypothesis that molecular mimicry in peptide epitopes from envelope proteins of the HERV-W family of endogenous retroviruses and myelin proteins is possible and could potentially trigger multiple sclerosis. Mimicry between syncytin-1, a HERV-W envelope protein that is expressed during placentation, and myelin proteins may also explain the higher prevalence of multiple sclerosis in women. Experiments to test the ability of the identified peptide epitopes to activate T_H cells are required to confirm the present findings.

1. Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) that involves the progressive destruction of the myelin sheath and axons resulting in neurodegeneration [1]. Antibodies, T cells and pro-inflammatory innate immune cells contribute to the complex immunopathology of MS [2,3]. Experimental autoimmune encephalomyelitis (EAE) in rodents, despite showing some differences to MS, has been a useful animal model to investigate particular aspects of MS [2]. Studies on EAE helped identify the main target molecules in myelin for the autoimmune responses in MS. EAE can be induced in naïve mice by active immunization with the myelin basic protein (MBP), proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG), and peptides derived from them, or by passive transfer of immune T cells from animals with EAE [4].

Large, multi-population genome-wide association studies show that amongst all MHC alleles, the MHC Class II allele DRB1*1501 has the strongest association with MS [5]. DRB1*1501 is also associated with early onset of MS [6]. DRB1*1501 is one of the many alleles coding for the DR β chain. The DRB1*1501 β chain pairs with the relatively non-polymorphic HLA DR α chain from DRA*0101 to form the HLA DR2b heterodimer. Furthermore, DRB5*0101, which is in linkage disequilibrium with DRB1*1501, is also associated with MS [7,8] and binds to DRA*0101 to form the HLA DR2a heterodimer. Both HLA DR2a and DR2b, often referred to as representing the HLA DR2(15) haplotype, have been shown to present the encephalitogenic MBP peptide 83–99 to T cell clones from MS patients [7,8]. HLA DR2a and DR2b may also present other peptide epitopes associated with MS to CD4+ T cells. Evidence suggests that CD4+ T cells with a T_H1 phenotype have an important role in the initia-

Abbreviations: BLAST, basic local alignment search tool; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; EBV, Epstein Barr virus; HERV, human endogenous retrovirus; IEDB, immune epitope data base; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MSRV, multiple sclerosis associated retrovirus; NCBI, National Centre for Biological Information; PLP, proteolipid protein; SMM, stabilised matrix method.

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tion of MS and its progression, the generation of autoantibodies and self-reactive CD8+ T cells, and MS-associated inflammation [2,9,10]. HLA DR2a and DR2b restricted T_H1 cells are able to directly lyse target cells through the perforin or Fas/FasL pathway [11]. Proinflammatory CD4+ T_H17 are also reportedly important in the pathogenesis of MS [2,12,13]. Regulatory CD4+ T cells (T_{reg}), which also recognise peptides presented by HLA Class II molecules, have a role in regulating the autoimmune response in MS [2].

Besides genetic factors, viral infections have been associated with MS and three mechanisms that are not mutually exclusive have been proposed to explain this [3]. Firstly, viral infection of the CNS can potentially cause inflammation and damage oligodendrocytes that produce myelin, thereby releasing myelin fragments that activate autoreactive T cells in an inflammatory milieu. Subsequent epitope spreading can produce more demyelination and axon death. Secondly, persistent viral infection of the CNS can produce inflammatory demyelinating disease caused by the immune response attempting to eliminate infected cells within the CNS. Thirdly, viral infection outside the CNS can activate cross-reactive T cells that then enter the CNS and cause inflammatory demyelinating lesions leading to MS. Accumulating evidence suggests that human endogenous retroviruses (HERVs), generally inactive remnants of exogenous retroviruses that became integrated into primate genomes, are important in the aetiology and pathogenesis of MS [14]. An MS associated retrovirus (MSRV), a member of the HERV-W family, has been particularly implicated in MS because virus particles and reverse transcriptase activity are detected in MS patients [14–16]. A role in MS pathogenesis has been ascribed to the MSRV *env* gene product which is nearly identical to syncytin-1 of the HERV-W family [15]. HERV-W encoded syncytin-1, itself a viral envelope protein remnant and a membrane glycoprotein, has evolved to perform an essential fusion function in forming the placental syncytiotrophoblast layer in humans [17,18]. Syncytin-1 is highly conserved between members of the HERV-W family including MSRV [19]. Syncytin-1 is homologous to syncytin-2, another fusogenic envelope glycoprotein encoded in a different HERV family *viz*. HERV FRD, which has also evolved to play an important role in forming the syncytiotrophoblast [18]. However there is no evidence supporting a role for syncytin-2 in MS to our knowledge. The expression of the MSRV *env* gene product is significantly higher in brain lesions in MS plaques and correlates with the extent of active demyelination and inflammation [15,16]. Furthermore, the observed temporal relationship between Epstein Barr Virus (EBV) infection and MS has been ascribed to activation of the expression of MSRV envelope protein/HERV-W syncytin-1 by EBV infection [16].

We hypothesised that the existing data are consistent with the presentation of peptide epitopes derived from the HERV-W family envelope proteins that cross-react with epitopes from myelin proteins by MHC Class II DR2b and DR2a molecules to CD4+ T cells being a molecular mimicry trigger for MS. We therefore determined *in silico* the potential for cross-reactive epitopes between syncytin-1, syncytin-2 as a possible control, and the MSRV envelope protein on one hand and human MBP, MOG and PLP on the other that can be presented to CD4+ T cells by HLA DR2b and DR2a molecules on antigen presenting cells.

2. Materials and methods

2.1. Sequence homologies between HERV-W and HERV FRD envelope proteins and the myelin proteins MBP, MOG and PLP

The predicted protein coding sequences of the 538 amino acid (aa) HERV-W syncytin-1 (Uniprot accession number Q9UQF0), the 538 aa HERV FRD syncytin-2 (NP_997465), the 542 aa MSRV envelope protein (AAK18189.1), the 304 aa human MBP (P02686.3), the 247 aa human MOG (Q16653.2), and the 277 aa human PLP (P60201.2) were obtained from the NCBI data base. Amino acid sequences of the proteins were compared by pairwise Basic Local Alignment Search Tool (BLAST) analysis performed online using default parameters (https://www.ncbi.nlm.nih.gov/blast).

2.2. Prediction of peptides in HERV envelope proteins and myelin proteins potentially binding to HLA DR2a and HLA DR2b molecules

Syncytin-1, syncytin-2, the MSRV envelope protein and the three myelin proteins were analysed for peptides potentially capable of binding to HLA DR2a and HLA DR2b molecules using the Immune Epitope Data Base or IEDB (www.iedb.org) procedures [20–23]. The default peptide length of 15 aa was used in the analysis but the results also show the core nonamer peptides that are expected to bind to the HLA DR molecule and constitute the major portion of the T cell epitope [20]. The analysis method selected was the Stabilised Matrix Method (SMM) where the peptides are ranked according to their predicted binding affinities or IC_{50} which indicates the concentration of peptide in nM expected to achieve 50% saturation of the HLA molecule. Therefore a lower IC_{50} shows a higher affinity. As a guide, peptides with IC_{50} values <50 nM are considered to bind with high affinity, between 50 nM to 500 nM with intermediate affinity and between 500 nM to 5000 nM with low affinity [21–23]. For each peptide, a percentile rank is generated by comparing the peptide's score against the scores of five million random 15mer peptides selected from the SWISSPROT protein database. Therefore smaller percentile rank values, typically <10, also indicate higher affinity and specificity of binding to the HLA molecule [21–23].

The predicted peptides binding with high affinity were then examined to determine whether they were located in regions of homology between the HERV envelope and myelin proteins. Homologies between the predicted HERV envelope protein and myelin peptides were additionally tested by pairwise BLAST analysis of the peptides.

3. Results

3.1. Sequence homologies between HERV envelope proteins and the three myelin proteins by BLAST analysis

A comparison of syncytin-1 and the MSRV envelope protein by BLAST revealed that the two proteins were 87% identical with 90% positives and 4 gaps (Supplementary Fig. S1). A comparison of syncytin-1 with syncytin-2 showed that they were more distantly related with significant homology present only in some regions (Fig. S2). The results obtained with pair-wise BLAST analysis of syncytin-1 with MBP, MOG and PLP are presented in Supplementary Figs. S3–S5 respectively, between MSRV envelope protein with MBP, MOG and PLP in Supplementary Figs. S6–S8 respectively, and between syncytin-2 with MBP, MOG and PLP in Supplementary Figs. S9–S11 respectively.

3.1.1. Sequence homologies between MBP and HERV envelope proteins

There are three regions of homology between syncytin-1 and MBP, with greatest homology of 29% with an E value of 0.06 being found between MBP amino acid residues 223–274 and syncytin-1 8–58 allowing for a total of nine gaps in both proteins (Fig. S3). The region between MBP 223–274 was also homologous to MSRV envelope protein 8–58, with an E value of 1.8 and nine gaps (Fig. S6). MBP 230–273 was more weakly homologous to syncytin-2 residues 170–271 with an E value of 3.8 (Fig. S9). A second region of homology identified between syncytin-1 283–339 and MBP 127–181