

HLA DR2b-binding peptides from human endogenous retrovirus envelope, Epstein-Barr virus and brain proteins in the context of molecular mimicry in multiple sclerosis

Ramasamy, Ranjan; Mohammed, Fiyaz; Meier, Ute-C

DOI:
[10.1016/j.imlet.2019.10.017](https://doi.org/10.1016/j.imlet.2019.10.017)

License:
Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version
Peer reviewed version

Citation for published version (Harvard):
Ramasamy, R, Mohammed, F & Meier, U-C 2019, 'HLA DR2b-binding peptides from human endogenous retrovirus envelope, Epstein-Barr virus and brain proteins in the context of molecular mimicry in multiple sclerosis', *Immunology Letters*, vol. 217, pp. 15-24. <https://doi.org/10.1016/j.imlet.2019.10.017>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

1 **Title:** HLA DR2b-binding peptides from human endogenous retrovirus envelope, Epstein-
2 Barr virus and brain proteins in the context of molecular mimicry in multiple sclerosis.

3 **Authors:** Ranjan Ramasamy^{1*}, Fiyaz Mohammed², Ute-C. Meier³

4 ¹ ID-FISH Technology Inc., 556 Gibraltar Drive, Miltipas, CA 95035, United States of
5 America

6 ² Cancer Immunology and Immunotherapy Centre, Institute of Immunology and
7 Immunotherapy, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United
8 Kingdom

9 ³Department of Neuroscience and Trauma, Blizard Institute, 4 Newark St, Whitechapel,
10 London E1 2AT, United Kingdom

11

12 * Corresponding author (RR)

13 Email: rjr200911@yahoo.com

14

15 **Running Title:** Molecular mimicry in multiple sclerosis

16 **Abbreviations:** ABP - α , β Crystallin; β SYN - β Synuclein; BLAST - Basic Local Alignment
17 Search Tool; CNS – Central Nervous System; EAE- Experimental Autoimmune
18 Encephalomyelitis; EBV – Epstein Barr Virus; EBNA1 - Epstein-Barr nuclear antigen 1; env
19 – envelope; HERV – Human Endogenous Retrovirus; IEDB – Immune Epitope Data Base;
20 MAG - Myelin-associated glycoprotein; MBP – Myelin Basic Protein; MOG - Myelin
21 Oligodendrocyte Glycoprotein; MS – Multiple Sclerosis; MSR - Multiple Sclerosis
22 Associated Retrovirus; NCBI – National Center for Biotechnology Information; OSP –
23 Oligodendrocyte Specific Protein; PLP - Proteolipid Protein; SMM - Stabilised Matrix Method;
24 SYN1 - Syncytin-1; SYN2 - Syncytin-2; TCR – T cell receptor.

25 **Abstract**

26 Multiple sclerosis (MS) is a complex autoimmune disease in which T cells and
27 antibodies damage the myelin sheath in the central nervous system. The aetiology of the
28 disease is poorly understood. HLA Class II DR2b (DRB1*1501 β , DRA1*0101 α) is the
29 strongest genetic risk factor for MS. Genetic remnants of ancient retroviruses, termed human
30 endogenous retroviruses (HERV) that have been incorporated into the human genome and
31 Epstein-Barr virus (EBV) infection have also been associated with MS. *In silico* analyses of
32 human endogenous retroviral envelope (HERV env) proteins and three myelin proteins
33 (myelin basic protein, myelin oligodendrocyte glycoprotein and proteolipid protein) that are
34 principal targets of the autoimmune response showed homologies between potential T_H
35 epitopes within pairs of viral and myelin peptides predicted to bind HLA DR2b. This led to the
36 proposal that such molecular mimicry may potentially trigger MS. To further test this
37 hypothesis, the HLA-DR2b binding characteristics of the three myelin proteins and HERV
38 env peptides as well as *in silico* predicted peptides from other encephalitogenic brain
39 proteins and EBV proteins were investigated. Peptides containing potential T_H epitopes from
40 the myelin oligodendrocyte glycoprotein and HERV env previously predicted to bind HLA
41 DR2b as well as other pertinent potential HLA DR2b-restricted epitopes were shown to be
42 able to do so in a cell-free binding assay. Molecular modelling of HLA-DR2b in complex with
43 high affinity peptides derived from MOG and HERV env proteins highlighted that prominent
44 surface exposed amino acids, which potentially interface with the T cell receptor, are
45 conserved. A **structurally similar pair** of potential T_H epitopes from the EBV protein EBNA1
46 and β synuclein, a brain protein implicated in MS, were shown to be similarly capable of
47 binding HLA DR2b molecules. Our findings justify future investigation of T_H cell responses to
48 the candidate peptides.

49

50 **Key Words:** autoimmunity; Epstein-Barr virus; HLA DR2b-peptide complex; human
51 endogenous retroviruses; molecular mimicry; multiple sclerosis.

52 1. Introduction

53 Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central
54 nervous system (CNS) that involves progressive damage to the myelin sheath and axons
55 leading to neurodegeneration [1 - 3]. Studies on MS patients and experimental allergic
56 (autoimmune) encephalomyelitis (EAE) in rodents have implicated several CNS proteins
57 present in oligodendrocytes and myelin, including the myelin basic protein (MBP), myelin
58 oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP), as targets of the
59 autoimmune response in MS [1 - 3]. However, the aetiology of MS is not well understood.
60 Cells of the innate immune system, CD4⁺ helper T cells, CD8⁺ cytotoxic T cells and
61 autoantibodies are involved in the immunopathology of MS, while CD4⁺ helper 1 cells (T_H1)
62 among all types of antigen-specific cells, are considered to play the critical role in initiating
63 the autoimmune process [1 - 3]. Environmental factors, e.g. vitamin D deficiency [4], and
64 infections especially with Epstein Barr virus (EBV) [1-2, 5-8], have been implicated in
65 predisposition to MS. Genome-wide association studies demonstrate that the HLA Class II
66 allele DRB1*1501 β chain variant, which pairs with the relatively invariant DRA1*0101 α
67 chain to form the HLA DR2b heterodimer in antigen-presenting cells (APCs), is the strongest
68 genetic risk factor for MS [9]. The production of virions and expression of envelope protein
69 (env) of a member of the genome-encoded human endogenous retrovirus W-family (HERV-
70 W), termed the MS- associated retrovirus or MSRV [10], has also been implicated in MS [11
71 - 15]. However, the molecular mechanisms linking T_H cells to the genetic elements in the
72 etiology of MS are not established. It has been recently hypothesized that epitopes in MSRV
73 and other HERV family env proteins that cross-react with epitopes in myelin proteins, and
74 presented by HLA DR2b on APCs to T_H1 cells in an inflammatory milieu, provide the
75 requisite link [16].

76 Sequence homologies have been demonstrated by BLAST analysis between
77 MBP, MOG and PLP on one hand and MSRV env on the other [16, 17]. In addition,
78 **structurally related sequences** were found between the myelin proteins and syncytin-1

79 (SYN1) [16], another HERV-W family-derived env protein that has evolved to perform an
80 essential role in forming the syncytiotrophoblast of the placenta [18]. SYN1 is 87% identical
81 in amino acid sequence to MSRV env [16] and also more distantly related to syncytin-2
82 (SYN2), another essential fusogenic placental protein derived from a different HERV family
83 termed HERV-FRD [19]. SYN2 also possesses regions of amino acid sequence similarity
84 with the three myelin proteins [16]. SYN1 has an additional fusogenic role in the
85 development of myotubes from myoblasts [20] and possibly osteoclasts [21].

86 *In silico* analyses of myelin and HERV env sequences utilizing the Immune
87 Epitope Data Base (IEDB) [22] to predict HLA DR2b-binding 15mer peptides showed
88 homologies between potential nonamer T_H epitopes within the 15mers from the HERV env
89 proteins and all three myelin proteins that are predicted to bind to DR2b with high
90 (IC₅₀<50nM) or intermediate affinity (50nM<IC₅₀<500nM) [16]. Homologies between a
91 potential nonamer epitope in MOG and those in MSRV env, SYN1 and SYN2 were
92 particularly significant [16]. Interestingly, some predicted higher affinity DR2b-binding
93 peptides lie within longer regions of sequence homology between myelin proteins and HERV
94 env proteins whilst others do not [16]. Since SYN1 and SYN2 have evolved to perform
95 essential physiological functions in humans, it is possible that T_H cells that react with them
96 may be deleted in the thymus and/or regulatory T cells (T_{regs}) that dampen an immune
97 response are selected against them. This may not apply to MSRV env which is not expected
98 to be normally expressed during development. However MSRV env is expressed within
99 innate immune cells in an inflammatory situation e.g. during EBV infection [23] and is a
100 potent stimulant of Toll-like receptor 4 present on macrophages and microglia, leading to
101 impaired functional maturation of myelin-producing oligodendrocytes [24]. While existing
102 data are consistent with an initiating role for molecular mimicry between the MSRV env and
103 myelin proteins in MS, it is unclear whether this extends to the related SYN1 and SYN2
104 molecules. Once MS has been initiated in the proposed manner [16], further damage could

105 arise from T_H cells recognising other myelin epitopes presented by different HLA Class II
106 molecules as a result of epitope spreading [16, 25].

107 This study experimentally investigated HLA DR2b binding of 15mer peptides
108 derived from MBP, MOG, PLP and HERV env proteins earlier identified *in silico* as
109 potentially able to bind to HLA DR2b [16]. It also examined the binding to HLA DR2b of
110 selected peptides from additional CNS proteins reported to be encephalitogenic [1] and
111 corresponding **structurally related** peptides present in the three HERV env proteins as well
112 as EBV proteins that have been described to elicit prominent human CD4⁺ T cell responses
113 [2]. The binding of selected peptides with high HLA DR2b binding affinity were additionally
114 examined by molecular modelling of peptide–HLA DR2b complexes.

115

116 2. Materials and Methods

117 2.1 Selection of CNS proteins for investigation

118 The three myelin proteins previously used for *in silico* analysis of peptides
119 capable of binding to HLA DR2b [16] and six other potentially encephalitogenic myelin and
120 oligodendrocyte-associated CNS proteins [1, 26] selected for the present study are listed in
121 Table 1.

122 **Table 1. CNS proteins selected for investigation**

Protein	Abbreviation	NCBI sequence ID
Myelin basic protein	MBP	P02686.3
Myelin oligodendrocyte glycoprotein	MOG	Q16653.2
Phospholipid protein	PLP	P60201.2
α, β Crystallin	ABP	ACP18852
Myelin-associated oligodendrocyte basic protein	MOPB	NP_001265251.1
Oligodendrocyte-specific protein	OSP	AAC25187

2'3' Cyclic nucleotide 3' phosphodiesterase	CNPase	P09543
Myelin-associated glycoprotein	MAG	AAH53347.1
β -Synuclein	β SYN	Q16143

123

124 2.2 Selection of HERV and EBV proteins for investigation

125 The three HERV env proteins used previously for predicting HLA DR2b-restricted
 126 peptides through the IEDB *in silico* procedure [16] and EBV proteins reported to elicit strong
 127 human CD4⁺ T cell responses [2] were selected for the present study (Table 2).

128 **Table 2. Virus-derived proteins selected for investigation**

Virus protein	Abbreviation	NCBI sequence ID
HERV-W Syncytin-1	SYN1	Q9UQF0
HERV-FRD Syncytin-2	SYN2	NP_997465
HERV-W Multiple sclerosis-associated retrovirus envelope protein	MSRV env	AAK18189.1
Epstein-Barr nuclear antigen 1	EBNA1	YP_401677.1
Epstein-Barr nuclear antigen 2	EBNA2	ALV83014.1
Epstein-Barr nuclear antigen 3C	EBNA3C	CEQ33769.1
Epstein-Barr virus transactivator BZLF1	BZLF1	CAD53423
Epstein-Barr virus glycoprotein BZLF2	BZLF2	CEQ33770.1
Epstein-Barr virus envelope glycoprotein H	BXLF2	ATE89094.1

129

130 2.3 Sequence homologies between CNS and virus-derived proteins

131 The predicted additional CNS and virus-derived protein coding sequences
 132 obtained from the US National Center for Biotechnology Information (NCBI) data base were
 133 compared by pairwise Basic Local Alignment Search Tool (BLASTp) analysis online using
 134 default parameters (<https://www.ncbi.nlm.nih.gov/blast>), as previously described for MBP,

135 MOG, PLP and the three HERV env proteins SYN1, SYN2 and MSRV env [16]. Additionally,
136 every one of the selected CNS proteins shown in Table 1 were individually tested in BLASTp
137 searches for homology against all non-redundant protein sequences of EBV (human herpes
138 virus 4 strain B95-8) with NCBI taxonomy ID 10377.

139

140

141 **2.4 Prediction of peptides potentially binding to HLA DR2b molecules**

142 Prediction of potential peptides binding to HLA DR2b molecules was performed
143 as previously described [13] using the IEDB analysis resource (www.iedb.org) [22, 27, 28].
144 The default peptide length of 15 amino acids was used in the analysis but the results also
145 show the core nonamer peptides that are expected to bind to the HLA DR2b molecule and
146 constitute the major portion of the T cell epitope [22, 27, 28]. The Stabilised Matrix Method
147 (SMM) was used to rank the peptides according to their predicted binding affinities or IC_{50}
148 which indicates the nM concentration of peptide expected to bind and achieve 50%
149 saturation of the HLA DR2b molecules [22, 27, 28]. Structural similarities between core
150 nonamer sequences in 15mer peptides from different proteins that were predicted to bind
151 HLA DR2b with high or intermediate affinity ($IC_{50} < 50nM$) were determined manually.

152

153 **2.5 Determination of the binding affinity and stability of HLA DR2b-peptide complexes**

154 Peptides (15mers) were synthesized by Fmoc solid-phase chemistry and quality
155 checked with matrix assisted laser desorption ionization-time of flight mass spectrometry
156 (MALDI-TOF MS) by ProImmune (Oxford, UK). Binding characteristics of the peptides to
157 HLA DR2b were determined by ProImmune using the cell-free REVEAL® MHC class II
158 binding assay [29]. The REVEAL® assay measured the ability of a peptide to stabilize the
159 MHC-peptide complex based on the detection of the native conformation of the MHC-peptide

160 complex by a specific monoclonal antibody [29]. After an initial incubation with peptide for
161 determining the proportion of MHC molecules binding the peptide (affinity), an additional
162 measurement was taken after a further 24h incubation at 37°C to determine the stability of
163 binding (stability index). The stability index provides information on whether peptide can be
164 presented long enough to serve as a T cell epitope. The affinity and stability index were
165 measured as a percentage of the signal generated by the test peptide in comparison to a
166 proprietary ProlImmune positive control peptide. A well characterised 15mer MBP peptide
167 with the sequence ENPVVHFFKNIVTPR (hereafter referred to as MBP_3) that is presented
168 by HLA DR2b and activates CD4⁺ T cells [30] was chosen as the internal comparative
169 standard in the assays.

170 Details of the two sets of 40 peptides from CNS and viral proteins that were
171 tested in the HLA DR2b binding assays are provided in Supplementary Table S1. The first
172 set of 40 contained peptides derived from MBP, MOG and PLP and **structurally similar**
173 peptides from SYN1 and MSRV env previously identified *in silico* as being potentially
174 important for molecular mimicry by the IEDB algorithm [16]. Staggered arrays of 15mers
175 were used to identify the best binding peptide. The first set also contained a 15mer derived
176 from EBV DNA polymerase shown to cross-react at the CD4⁺ T cell level with the control
177 peptide MBP_3 on presentation by HLA DR2b [30]. A HLA DR2b-restricted MOG epitope
178 shown previously to stimulate CD4⁺ T cells to produce IFN γ [31] was also included in the first
179 set of peptides. Other first set peptides were comprised of closely related signal sequence
180 peptides of SYN1 and MSRV that encompassed **structurally similar** nonamers to those in
181 internal peptides of MBP (including the control peptide MBP_3) and PLP [16], PLP peptides
182 that contained the nonamer FFFLYGALL that were predicted to strongly bind DR2b [16], and
183 four MSRV env peptides with the nonamer sequence TSVLVGPLV that exhibited weaker
184 homology to MOG nonamer IVLPVLGPLV [16].

185 The second set of 40 peptides (Supplementary Table S1) were chosen to
186 replicate and further examine the binding characteristics of the more promising HLA DR2b-

187 binding peptides identified from first set. They were independently synthesised and tested in
188 REVEAL® binding assays. The second set additionally tested structurally similar pairs of
189 HLA DR2b-binding peptides identified through IEDB *in silico* analysis in EBNA1 and HERV
190 env proteins on one hand and different CNS proteins on the other. They included peptide
191 pairs from EBNA1 and β SYN, as well as EBNA1 and OSP, that had also been independently
192 predicted to bind HLA DR2b using a different *in silico* algorithm [32]. The second set also
193 included a different MBP peptide reported to be recognised by T_H cells in the context of HLA
194 DR2b [33].

195

196 **2.6 Modelling of 15mer peptides binding to HLA DR2b**

197 Molecular modelling of the HLA DR2b-peptide complexes were performed using
198 the *in silico* docking program HADDOCK (high ambiguity driven protein-protein docking) [34].
199 Coordinates for the HLA DR2b complex were retrieved from the Protein Data Bank entry
200 1YMM [35]. Initial coordinates for the DR2b-restricted peptide moieties were extracted from
201 the crystal structure of the T cell receptor(TCR)/HLA DR2b/MBP_3-peptide complex (entry
202 1YMM), and then used to build models of peptides with the molecular builder tool in COOT
203 [36]. Each HLA DR2b-restricted peptide was subsequently subjected to a short
204 regularisation protocol to ensure that the geometry of the peptide residues conformed to
205 known bond lengths and angles.

206 The docking procedure was driven using only ambiguous intermolecular
207 restraints, which were defined based on previously determined HLA DR2b-peptide
208 complexes [35, 37, 38]. These structures revealed that the MBP_3 peptide is bound in the
209 DR2b peptide-binding groove with peptide side chains P1, P4, P6 and P9 occupying pockets
210 within the groove. Hence residues that line the P1, P4, P6 and P9 pockets of DR2b were
211 selected as active residues (comprised of E11 α , F24 α , F32 α , W43 α , F54 α , N62 α , D66 α ,
212 R76 α , R13 β , F26 β , D28 β , Q70 β , A71 β , Y78 β , D57 β and W61 β). For the peptide only the

213 anchor residue side chains at P1, P4, P6 and P9 were defined as active residues. Passively
214 involved residues were selected automatically. The 200 structures obtained after water
215 refinement were analysed and ranked according to their HADDOCK score, a weighted sum
216 of electrostatic, van der Waals, and restraint energy terms [34]. The lowest energy structure
217 solutions were visualised and analysed using Pymol (The PyMOL Molecular Graphics
218 System, Version 1.8 Schrödinger, LLC).

219

220 **3. Results**

221 **3.1 Sequence homologies between additional selected CNS and EBV or HERV env** 222 **proteins**

223 Sequence homologies between the three HERV env proteins and the three
224 myelin proteins MBP, MOG and PLP observed in BLASTp analysis have been previously
225 described [16]. BLASTp **analysis of the non-redundant protein sequences coded** in whole
226 EBV genome against each of the selected CNS proteins only revealed a weak homology
227 between α , β crystallin (ABP) and a 53 residue segment of the EBV protein EBNA4 with an
228 E value of 0.95 (Supplementary Table S2). Pairwise BLASTp analysis of each of the other
229 selected CNS proteins **against the three HERV env proteins demonstrated** homologies with
230 $E \leq 0.5$ only between the pairs ABP and SYN2, ABP and MSR env, and myelin-associated
231 glycoprotein (MAG) and MSR env (Supplementary Table S3).

232

233 **3.2 Structurally related peptides in brain and EBV or HERV env proteins predicted to** 234 **bind to HLA DR2b molecules**

235 IEDB analysis of 15mer peptides containing structurally similar nonamers
236 predicted to bind to HLA DR2b in MBP, MOG, PLP on one hand and the three HERV env
237 proteins on the other, have been previously described [16]. Similar IEDB analysis performed

238 on the determined **structurally similar** regions of ABP/SYN2, ABP/MSRV env and
239 MAG/MSRV env (Supplementary Tables S2 & S3) did not identify 15mer peptides of
240 potentially high or intermediate affinity of binding to HLA DR2b that also contained
241 **structurally similar** nonamer sequences in the three pairs of proteins (**Supplementary Table**
242 **S4 and reference 16**).

243 Because of the homology observed between ABP and a 53 residue sequence of
244 EBNA4 and **perceived sequence similarities independently predicted between HLA DR2b-**
245 **binding peptides of EBNA1 and several CNS proteins [32]**, HLA DR2b binding potential of
246 15mer peptides from EBNA1 and EBNA4 were also analysed by the IEDB procedure
247 (Supplementary Table S5). **These results when examined together with those in**
248 **Supplementary Table S4 and data in reference 16 for HERV env**, showed potential pairs of
249 DR2b-binding peptides of high or intermediate affinity in EBNA1 and OSP, EBNA1 and
250 β SYN, as well as OSP and MSRV env. **These peptides whose sequences are given in**
251 **Supplementary Table S6 were subsequently investigated in DR2b binding assays.**

252

253 **3.3 Experimental binding to HLA DR2b of CNS and viral peptides predicted *in silico* to** 254 **bind HLA DR2b**

255 The results of REVEAL binding assays **on the selected peptides** (Supplementary
256 Table S6) showed that the pairs of peptides from MOG and the corresponding **three** HERV
257 env proteins containing **sequence-related** nonamers previously predicted to engage HLA
258 DR2b [16], and implicated in molecular mimicry, are able to bind HLA DR2b with comparable
259 binding characteristics to the MBP_3 peptide.

260 The results also show that some OSP peptides **with similar sequences** to EBNA1
261 and MSRV env peptides and with predicted *in silico* intermediate binding affinity are able to
262 bind well to HLA DR2b. However, the corresponding **structurally related** viral peptides did not
263 reveal strong binding to HLA DR2b despite homology within the predicted nonamer

264 sequences. For example, the OSP 15mer STTLRALAPRLMRRV which bound strongly had
265 five identities in its predicted nonamer DR2b-binding sequence (LRALAPRLM) to the
266 corresponding nonamer (LRALLARSH) in two 15mer EBNA1 peptides that however only
267 showed weak binding to DR2b (Supplementary Table S6).

268 Peptides from the closely related signal sequences of SYN1 and MSRV that
269 contained **sequence-related** nonamers to those in internal peptides of MBP (including the
270 control peptide MBP_3) and PLP identified in the previous study [16] did not bind strongly to
271 DR2b in the assays. Only one PLP peptide TASFFFLYGALLLAE that contained the
272 nonamer sequence FFFLYGALL that was predicted to bind strongly to DR2b [16] was
273 confirmed to bind strongly to DR2b. Four MSRV env peptides tested containing the nonamer
274 sequence TSVLVGPLV with weaker homology to the MOG nonamer IVLPVLGPLV did not
275 bind strongly to DR2b.

276 Peptides from EBV DNA polymerase and a different MOG region that had been
277 shown to be presented on DR2b and stimulate CD4⁺ T cells [30, 31] revealed significant
278 binding affinity to DR2b in the assay. A MBP peptide (GTLSKIFKLGGRDSR) containing a
279 putative DR2b-restricted T cell epitope but with a weak predicted IC₅₀ of 940nM based on
280 IEDB analysis, only demonstrated marginal binding to DR2b.

281 An exact correlation between the *in silico* predicted affinity (IC₅₀) and the
282 experimentally determined affinity by the REVEAL® binding assay for DR2b was not
283 observed. For example, some PLP peptides with high predicted affinity (IC₅₀<1nM) showed
284 poor experimental binding while four SYN2 peptides with predicted intermediate affinities
285 (IC₅₀ of 130 to 149nM) reveal experimental binding comparable to MBP_3 (Supplementary
286 Table S6).

287 The details of binding assay results with staggered arrays of significant pairs of
288 CNS and viral peptides are also shown graphically in Supplementary Table S6. Data on the

289 best binding 15mer peptides with nonamers relevant for molecular mimicry grouped together
 290 and compared with the binding of the control MBP peptide are listed in Table 3.

291

292 **Table 3. Binding characteristics and Haddock scores of the best pairs of DR2b-**
 293 **binding 15mer peptides containing sequence-related nonamers relevant to molecular**
 294 **mimicry**

Homology Group	Peptide	Peptide Sequence	Relative affinity	Relative stability	HADDOCK model score
1. MOG & HERV env	MOG_4	ITLFV <u>IVPVLGPLVA</u>	151	110	-123.7±2.5
	MSRV env_5	MPW <u>LPFLGPLAII</u>	69	33	-158.1±2.1
	SYN1_2	MPW <u>LPFLGPLAII</u>	144	124	-152.5±4.4
	SYN2_5	KWFSW <u>VLPLTGPLVS</u>	348	181	-137.8±5.3
2. βSYN & EBNA1	β synuclein	EKTKE <u>GVLVVGSKTR</u>	91	95	-128.7±3.0
	EBNA1_2	VAG <u>VFVYGGSKTSLY</u>	118	43	-131.5±5.1
3. Control	MBP_3	ENPV <u>VHFFKNIVTPR</u>	100	100	-163.7±1.6

295 **Legend to Table 3.** Results show the experimentally determined relative affinity and stability
 296 of binding of peptides expressed as a percentage of that observed with the control MBP_3
 297 peptide assigned values of 100. The nonamers sequence predicted to bind in the peptide-
 298 binding groove in HLA DR2b are shown in bold letters and underlined. The docking scores
 299 for the HADDOCK-derived lowest energy HLA DR2b-peptide complex models are shown.

300

301 **3.4 Molecular models of structurally similar peptides binding to HLA DR2b**

302 Previously identified HLA DR2b-restricted peptides of similar sequences
 303 containing potential T_H epitopes from MOG and HERV env were shown to bind HLA DR2b in
 304 the cell-free binding assay. We employed *in silico* molecular docking strategies to
 305 understand the molecular mechanisms governing binding of the structurally related pairs of
 306 peptides by HLA DR2b and their potential recognition by TCR. To evaluate the feasibility of
 307 using such approaches we first modelled the binding of the control MBP_3 peptide

308 ENPVVHFFKNIVTPR to HLA DR2b using HADDOCK and then compared with the available
309 crystallographic structure (PDB entry 1YMM) [35, 37, 38]. The HLA DR2b-MBP_3 complex
310 model corresponding to the lowest intermolecular energy (with a HADDOCK score of -163.7)
311 shows substantial similarity with the published structure in terms of epitope conformation and
312 docking mode (Figure 1A). Superposition of the MBP_3 peptides derived from the published
313 and model complex structures show that the main chain conformation is highly conserved
314 (Figure 1A). In addition, similar to the published structure, the modelled MBP-3 peptide side
315 chains at P1, P4, P6 and P9 serve as anchors slotting into the DR2b antigen binding cleft
316 (Figure 1B&C). Finally, in both the published and modelled complexes, the peptide was held
317 in the DR2 antigen-binding cleft by a conserved network of hydrogen bonding and non-polar
318 interactions (Figure 1B&C). These observations justified the use of the HADDOCK docking
319 approach to generate models of HLA DR2b bound to peptides that are relevant to MS.

320 To address the molecular mimicry hypothesis we generated models of HLA
321 DR2b in complex with peptides of the highest affinity derived from MOG and the HERV env
322 proteins MRSV env, SYN1 and SYN2 that are shown in Table 3. Superposition of the MOG,
323 MSRV env, SYN1 and SYN2 peptides show that they all adopt a very similar back-bone
324 conformation (Figure 2A). Similarly to the control MBP_3 peptide, the P1, P4, P6 and P9
325 peptide side chain positions serve as anchors inserting into the DR2b antigen binding cleft
326 (Figure 2B-D). The HLA DR2b-peptide interactions were remarkably conserved between the
327 different complexes including the control HLA DR2b-MBP_3 complex. In addition, positions
328 P-1, P2, P5, and P8 are predicted to be surface exposed in the HADDOCK derived HLA
329 DR2b-peptide complex models, and therefore potentially involved in binding to the TCR. The
330 chemical characteristics of these prominent solvent exposed residues were either identical
331 or structurally related in the relevant pairs of peptides. Taken together, these findings
332 support the molecular mimicry hypothesis between MOG and HERV env proteins in
333 triggering MS.

334 To further test the molecular mimicry hypothesis, *in silico* predicted peptides from
335 other encephalitogenic brain proteins and EBV proteins were also investigated using
336 modelling approaches. To address this HADDOCK derived models of HLA DR2b in complex
337 with the β SYN and EBNA1 peptides shown in Table 3 were generated (Figure 3). These
338 peptides adopted similar main chain conformations (Figure 3A) and mediated a conserved
339 network of polar and non-polar interactions with side chains of DR2b (Figure 3 B&C). As with
340 comparisons between MOG and HERV env proteins, the most prominent surface exposed
341 residues (at P-1, P2, P5, and P8) and hence potential TCR contacts were mainly conserved
342 or semi-conservatively substituted between the β SYN and EBNA1 peptide pair. The non-
343 anchoring residues were however different between the two sets of unrelated peptide pairs
344 β SYN/EBNA1 and HERV env/MOG, and between each of these and MBP_3 (Table 3 and
345 Figures 1-3).

346 The HADDOCK docking scores of the best binding 15mer peptides possessing
347 the relevant **structurally related nonamer** pairs are listed in Table 3. The HADDOCK scores
348 do not correlate with experimentally measured REVEAL[®] binding affinities or stability indices
349 for the peptides but the high negative values point towards energetically favourable binding
350 to DR2b molecules.

351

352 4. Discussion

353 The molecular mimicry hypothesis proposed previously [16] attempted a unified
354 explanation for the involvement of CD4⁺ T cells, HLA-DR2b, HERV env proteins and EBV
355 infection in the origin of MS. It was supported by the *in silico* identification of **structurally**
356 **related** pairs of 15mer peptides predicted to bind DR2b in myelin-associated MBP, MOG and
357 PLP proteins on one hand and HERV env proteins on the other. Homologies between
358 predicted MOG and HERV env peptides were particularly prominent [16]. The present study
359 extended the *in silico* predictions by examining the experimental binding of candidate 15mer

360 peptides to DR2b as well as generating molecular models of such complexes. It also
361 investigated the presence of potential DR2b-binding, **structurally related**, peptide pairs
362 between HERV env and other CNS proteins as well as between CNS and EBV proteins.

363 More recent findings are pertinent to the original HERV-related molecular
364 mimicry hypothesis. EBV, which primarily infects B cells, has been further implicated as a
365 necessary but not sufficient cause of MS, partly because of its increased and dysregulated
366 expression in peripheral blood and brain [39 -43]. In addition, antibody titres to EBNA1 have
367 lately been confirmed to be higher in MS patients compared to controls [44]. EBNA1 has
368 recently been reported to promote alternative splicing of cellular genes [45]. Since EBNA1 is
369 widely expressed in EBV infected cells [46], it is intriguing to speculate that its splicing
370 activity has a role in the *trans* splicing that has been postulated to produce functional MSR
371 env molecules [17]. This adds to the many different mechanisms proposed to explain why
372 EBV infections are a predisposition for MS [1-2, 5-8].

373 HERVs and their putative role in autoimmunity have been lately reviewed [47-49]
374 and cross-reactive B cell epitopes in MOG and HERV-W env have been documented [50].
375 The presence of antibodies to HERV-W env proteins have recently been reported to
376 differentiate MS from related neurological diseases [51, 52].

377 Evidence that human GDP-L-fucose synthase peptides are recognised by CD4⁺
378 T cells in the context of HLA DRB3 *0202 in MS patients, and that gut bacterial GDP-L-
379 fucose synthase may be cross-reactive has led to a different proposal for molecular mimicry
380 in MS [53]. RAS guanyl releasing protein 2 in peripheral memory B cells driving the
381 proliferation of brain-infiltrating CD4⁺ T_H1 in a HLA DR2b-restricted manner that then
382 recognise epitopes from the same protein expressed in brain cells has been proposed as
383 another autoimmune mechanism explaining the association between MS and HLA DR2b
384 [54].

385 Recent data also suggest that MSRV env is present in microglia associated with
386 myelinated axons in MS lesions, MSRV env induces inflammatory myelin and neuron
387 damaging activity in vitro in microglia and that antibodies to MSRV can be neuroprotective in
388 MS patients [12]. These observations are pertinent to further examining molecular mimicry
389 between MSRV env and MOG.

390 The present study suggests that peptides containing nonamers with potential T
391 cell epitopes in MSRV env, SYN1, SYN2 and MOG have the capacity to bind to HLA DR2b
392 molecules with comparable affinities and similar binding topology to the well characterised
393 MBP_3 peptide containing a T cell epitope. The molecular modelling suggests that potential
394 surface exposed residues that contact TCR are relatively conserved between the MOG and
395 HERV env peptides which is consistent with the proposed molecular mimicry hypothesis.
396 The MOG peptides identified that possessed DR2b-binding nonamers that were related in
397 sequence to those in the three HERV env proteins are located in the predicted C terminal
398 transmembrane domain of MOG. The corresponding DR2b-binding nonamers of related
399 amino acid sequence from MSRV env, SYN1 and SYN2 are also sited in predicted
400 transmembrane domains. A longer peptide from the transmembrane region of MOG, that
401 contained the MOG peptide identified in the present work, has independently been shown to
402 stimulate CD4⁺ T cells from MS patients to proliferate and secrete IFN γ in a DRB-restricted
403 manner [55]. It is possible that SYN1 and SYN2 may normally elicit tolerance as they may be
404 recognised as self-proteins, while MSRV env may function as a foreign protein that can
405 generate autoimmunity through molecular mimicry under certain circumstances as
406 previously outlined [16]. Studies on CD4⁺ T cell response to the peptides identified in this
407 study will help clarify the potential roles of MOG and the HERV env proteins in the
408 immunopathogenesis of MS. It is relevant in this context that TCR recognition of MBP_3
409 bound to DR2b has been shown to involve skewed binding, not typical of TCR binding
410 foreign peptide-Class II MHC complexes, which can result in potentially weaker interactions
411 that may permit autoimmune T cells to escape deletion in the thymus [37].

412 This study did not find evidence for **structurally related** DR2b-restricted T cell
413 epitopes between HERV env proteins and other encephalitogenic CNS proteins. **This was**
414 **also the case in our limited analysis of EBNA1 and EBNA4 against the selected CNS**
415 **proteins except for a pair of sequence-related** nonamers derived from β SYN and EBNA1
416 that showed binding affinity and stability comparable to MBP_3 in the REVEAL[®] assay.
417 Modelling of the β SYN and EBNA1 peptides with HLA-DR2b revealed binding to the peptide
418 binding cleft similar to MBP_3 and relative conservation of the surface exposed, potential
419 TCR contact residues in the two peptides. This suggests the molecular mimicry is possible
420 between β SYN and EBNA1. It is relevant in this context that β SYN-reactive T_H cells have
421 recently been suggested to be responsible for autoimmune damage to CNS grey matter in
422 the progressive stage of MS [26]. **The possibility that EBNA1 generated, β SYN-reactive T_H**
423 **cells induce additional autoimmune pathology, after the potential initiation of MS by**
424 **molecular mimicry between MOG and HERV env proteins, therefore justifies investigation.**
425 **Investigations on other pairs of potential HLA DR2b-binding peptides in EBNA1 and different**
426 **CNS proteins predicted independently [32] may also be useful in this context as the present**
427 **study was limited to CNS proteins with high encephalitogenic potential and restricted by the**
428 **numbers of peptide pairs that could be studied in the HLA DR2b binding assay.**

429 The HLA DR2a molecule is formed by pairing of the DRB5*0101 β chain variant,
430 whose gene is closely linked to the DRB1*1501 gene in many individuals, with the relatively
431 non-polymorphic DRA1*0101 α chain. The previous *in silico* based predictions failed to
432 identify strong DR2a binding pairs of potential **sequence-related** T_H cell epitopes in HERV
433 env and myelin proteins MBP, MOG and PLP [16]. However because of the close genetic
434 linkage of the two β chain loci, the investigation of potential DR2a binding **structurally similar**
435 epitopes in the extended set of CNS proteins and EBV or HERV proteins is warranted
436 because DR2a and DR2b molecules bind complementary sets of peptides through different
437 binding motifs [56].

438

439 **5. Conclusions**

440 The results of the cell free HLA DR2b binding assays and molecular modelling
441 show that **sequence-related** MOG and HERV env as well as β SYN and EBNA1 peptide
442 pairs, with each set of pairs containing related potential T_H epitopes, are able to bind to HLA
443 DR2b with similar affinity and conformation to a peptide MBP_3 containing an experimentally
444 confirmed T_H epitope. These findings support the previous *in silico* analysis-based prediction
445 that pairs of **sequence-related** peptides in HERV env proteins and MOG are potential
446 candidates for a molecular mimicry origin of MS. Kinetic studies of HLA DR2b binding with
447 highly purified peptides and determination of the crystal structure of HLA DR2b-peptide
448 complexes can provide more comprehensive binding information in the future. However,
449 definitive support for molecular mimicry will require detailed studies on CD4⁺ T_H cell
450 responses to the candidate peptides characterised in this study. Such investigations may
451 also contribute to the variety of immunomodulatory approaches presently being explored for
452 treating MS [12, 24, 57 - 63].

453

454 **Conflict of interest statement**

455 The authors declare no conflict of interest.

456

457 **Acknowledgements**

458 This research was supported by the US National Multiple Sclerosis Society (PP-
459 1711-29350). FM is funded by the Wellcome Trust grant 099266/Z/12/Z. The authors are
460 grateful to Professor Nick Willcox of the MRC Weatherall Institute of Molecular Medicine at
461 the University of Oxford for his continuing encouragement.

462

463 **Author contributions**

464 RR and UM initiated the project, FM performed the modelling studies, and RR
465 did the IEDB analysis, collation of data and drafting of the manuscript. All authors read and
466 approved the final manuscript.

467

468 **References**

- 469 1. M. Sospedra, R. Martin. Immunology of multiple sclerosis. *Annu. Rev. Immunol.* 23
470 (2005) 683–747, doi: 10.1146/annurev.immunol.23.021704.115707.
- 471 2. G.S. Taylor, H.M. Long, J.M. Brooks, A.B. Rickinson, A.D. Hislop. The immunology of
472 Epstein-Barr virus–induced disease. *Annu. Rev. Immunol.* 33 (2015) 787-821, doi:
473 10.1146/annurev-immunol-032414-112326.
- 474 3. C.A. Dendrou, L. Fugger, M.A. Friese. Immunopathology of multiple sclerosis. *Nat.*
475 *Rev. Immunol.* 15 (2015) 545–558.
- 476 4. E. Kocovská, F. Gaughran, A. Krivoy, U-C Meier. Vitamin-D deficiency as a potential
477 environmental risk factor in multiple sclerosis, schizophrenia, and autism. *Front.*
478 *Psychiatry* 8 (2017) 47, doi: 10.3389/fpsyt.2017.00047.
- 479 5. M.F. Cusick, J.E. Libbey, R.S. Fujinami, Multiple sclerosis: autoimmunity and viruses,
480 *Curr. Opin. Rheumatol.* 25 (2013) 496–501,
481 <http://dx.doi.org/10.1097/BOR.0b013e328362004d>.
- 482 6. J.S. Tzartos, G. Khan, A. Vossenkamper, M. Cruz-Sadaba, S. Lonardi, E. Sefia, et al.,
483 Association of innate immune activation with latent Epstein-Barr virus in active MS
484 lesions. *Neurology* 78 (2012) 15-23, doi: 10.1212/WNL.0b013e31823ed057.
- 485 7. S. Sisay, L. Lopez-Lozano, M. Mickunas, A. Quiroga-Fernández, J. Palace, G.
486 Warnes, et al., Untreated relapsing remitting multiple sclerosis patients show antibody
487 production against latent Epstein Barr Virus (EBV) antigens mainly in the periphery
488 and innate immune IL-8 responses preferentially in the CNS. *J. Neuroimmunol.* 306
489 (2017) 40-45, doi: 10.1016/j.jneuroim.2017.02.017.
- 490 8. S.V. Ramagopalan, R. Dobson, U.C. Meier, G. Giovannoni. Multiple sclerosis: risk

- 491 factors, prodromes, and potential causal pathways. *Lancet Neurol.* 9 (2010) 727-739.
492 doi: 10.1016/S1474-4422(10)70094-6.
- 493 9. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control
494 Consortium, S. Sawcer, G. Hellenthal, M. Pirinen, C.C. Spencer, et al., Genetic risk
495 and a primary role for cell-mediated immune mechanisms in multiple sclerosis, *Nature*
496 476 (2012) 214–219, <http://dx.doi.org/10.1038/nature10251>.
- 497 10. H. Perron, B. Lalande, B. Gratacap, A. Laurent, O. Genoulaz, C. Geny, et al. Isolation
498 of retrovirus from patients with multiple sclerosis. *Lancet* 337 (1991) 862–863.
- 499 11. T. Christensen. Human endogenous retroviruses in the aetiology of MS. *Acta. Neurol.*
500 *Scand.* 136 Suppl. 201 (2017) 18-21.
- 501 12. D. Kremer, J. Gruchot, V. Weyers, L. Oldemeier, P. Göttle, L. Healy, et al. pHERV-W
502 envelope protein fuels microglial cell dependent damage of myelinated axons in
503 multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 116 (2019) 15216–15225.
504 [doi/10.1073/pnas.1901283116](https://doi.org/10.1073/pnas.1901283116).
- 505 13. K.K. Nissen, M.J. Laska, B. Hansen, T. Terkelsen, P. Villesen, S. Bahrami, et al.
506 Endogenous retroviruses and multiple sclerosis-new pieces to the puzzle. *BMC*
507 *Neurol.* 13 (111) (2013), <http://dx.doi.org/10.1186/1471-2377-13-111>.
- 508 14. A. Dolei, E. Uleri, G. Ibba, M. Caocci, C. Piu, C. Serra. The aliens inside human DNA:
509 HERV-W/MSRV/syncytin-1 endogenous retroviruses and neurodegeneration. *J.*
510 *Infect. Dev. Ctries.* 9 (2015) 577-587, doi: 10.3855/jidc.6916.
- 511 15. E. Morandi, R.E. Tarlinton, B. Gran, Multiple sclerosis between genetics and
512 infections: human endogenous retroviruses in monocytes and macrophages. *Front.*
513 *Immunol.* 6 (647) (2015), <http://dx.doi.org/10.3389/fimmu.2015.00647>.
- 514 16. R. Ramasamy, B. Joseph, T. Whittall. Potential molecular mimicry between the
515 human endogenous retrovirus W family envelope proteins and myelin proteins in
516 multiple sclerosis. *Immunol. Lett.* 183 (2017) 79-85.
517 dx.doi.org/10.1016/j.imlet.2017.02.003.
- 518 17. G.S. do Olival, T.S. Faria, L.H. Nali, A.C. de Oliveira, J. Casseb, J.E. Vidal, et al.

- 519 Genomic analysis of ERVWE2 locus in patients with multiple sclerosis: absence of
520 genetic association but potential role of human endogenous retrovirus type W
521 elements in molecular mimicry with myelin antigen. *Front. Microbiol.* 4 (2013)172, doi:
522 10.3389/fmicb.2013.00172.
- 523 18. S. Mi, X. Lee, X. Li, G.M. Veldman, H. Finnerty, L. Racie, Syncytin-1 is a captive
524 retroviral envelope protein involved in human placental morphogenesis. *Nature* 403
525 (2000) 785–789.
- 526 19. A.G. Lokossou, C. Toudic, B. Barbeau. Implication of human endogenous retrovirus
527 envelope proteins in placental functions. *Viruses* 6 (2014) 4609–4627,
528 <http://dx.doi.org/10.3390/v6114609>.
- 529 20. B. Bjerregard, I. Ziomkiewicz, A. Schulz, L.I. Larsson. Syncytin-1 in differentiating
530 human myoblasts: relationship to caveolin-3 and myogenin. *Cell Tissue Res.* 357
531 (2014) 355-362, doi: 10.1007/s00441-014-1930-9
- 532 21. K. Søe, T.L. Andersen, A.S. Hobolt-Pedersen, B. Bjerregaard, L.I. Larsson, J.M.
533 Delaissé. Involvement of human endogenous retroviral syncytin-1 in human
534 osteoclast fusion. *Bone* 48 (2011) 837-846.
- 535 22. Y. Kim, J. Ponomarenko, Z. Zhu, D. Tamang, P. Wang, J. Greenbaum, et al. Immune
536 epitope database analysis resource. *Nucleic Acids Res.* 40 (2012) W525-W530, doi:
537 10.1093/nar/gks438.
- 538 23. E. Morandi, R.E. Tarlinton, B. Gran. Multiple sclerosis between genetics and
539 infections: human endogenous retroviruses in monocytes and macrophages, *Front.*
540 *Immunol.* 6 (2015) 647, <http://dx.doi.org/10.3389/fimmu.2015.00647>.
- 541 24. A. Madeira, I. Burgelin, H. Perron, F. Curtin, A.B. Lang, R. Faucard. MSRV envelope
542 protein is a potent, endogenous and pathogenic agonist of human toll-like receptor 4:
543 Relevance of GNbAC1 in multiple sclerosis treatment. *J. Neuroimmunol.* 291 (2016)
544 29-38, doi: 10.1016/j.jneuroim.2015.12.006
- 545 25. K. Raddassi, S.C. Kent, J. Yang, K. Bourcier, E.M. Bradshaw, V. Seyfert-Margolis, et
546 al. Increased frequencies of myelin oligodendrocyte glycoprotein/MHC class II-binding

- 547 CD4 cells in patients with multiple sclerosis, *J. Immunol.* 187 (2011) 1039–1046,
548 <http://dx.doi.org/10.4049/jimmunol.1001543>.
- 549 26. D. Lodygin, M. Hermann, N. Schweingruber, C. Flügel-Koch, T. Watanabe, C.
550 Schlosser C, et al. β -Synuclein-reactive T cells induce autoimmune CNS grey matter
551 degeneration. *Nature.* 566 (2019) 503-508, doi: 10.1038/s41586-019-0964-2.
- 552 27. P. Wang, J. Sidney, C. Dow, B. Mothé, A. Sette, B. Peters. A systematic assessment
553 of MHC class II peptide binding predictions and evaluation of a consensus approach.
554 *PLoS. Comput. Biol.* 4 (2008) e1000048.
- 555 28. P. Wang, J. Sidney, Y. Kim, A. Sette, O. Lund, M. Nielsen, B. Peters. Peptide binding
556 predictions for HLA DR, DP and DQ molecules. *BMC. Bioinformatics* 11 (2010) 568.
- 557 29. https://www.proimmune.com/ecommerce/page.php?page=reveal_class2. Accessed
558 24 May 2019.
- 559 30. K.W. Wucherpfennig, J.L. Strominger. Molecular mimicry in T cell-mediated
560 autoimmunity: viral peptides activate human T cell clones specific for myelin basic
561 protein. *Cell* 80 (1995) 695 – 705.
- 562 31. R. Weissert, J. Kuhle, K.L. de Graaf, W. Wienhold, M.M. Herrmann, C. Müller, et al.
563 High immunogenicity of intracellular myelin oligodendrocyte glycoprotein epitopes. *J.*
564 *Immunol.* 169 (2002) 548-556.
- 565 32. M. Tschochner, S. Leary, D. Cooper, K. Strautins, A. Chopra, H. Clark, et al.
566 Identifying patient-specific Epstein-Barr NuclearAntigen-1 genetic variation and
567 potential autoreactive targets relevant to multiple sclerosis pathogenesis. *PLoS. One*
568 11 (2016) e0147567, doi:10.1371/journal.pone.0147567
- 569 33. M. Pette, K. Fujita, D. Wilkinson, D.M. Altmann, J. Trowsdale, G. Giegerich, et al.
570 Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the
571 context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and
572 healthy donors. *Proc. Natl. Acad. Sci. USA.* 87 (1990) 7968-7672.
- 573 34. G.C.P. van Zundert, J.P.G.L.M. Rodrigues, M. Trellet, C. Schmitz, P.L. Kastiris, E.
574 Karaca, et al. The HADDOCK2.2 web server: user-friendly integrative modeling of

- 575 biomolecular complexes. *J. Mol. Biol.* 428 (2016) 720-725, doi:
576 10.1016/j.jmb.2015.09.014.
- 577 35. M. Hahn, M.J. Nicholson, J. Pyrdol, K.W. Wucherpfennig. Unconventional topology of
578 self peptide-major histocompatibility complex binding by a human autoimmune T cell
579 receptor. *Nat. Immunol.* 6 (2005) 490-496.
- 580 36. P. Emsley, K. Cowtan. Coot: model-building tools for molecular graphics. *Acta.*
581 *Crystallogr. D Biol. Crystallogr.* 60 (2004) 2126-2132.
- 582 37. M.J. Nicholson, M. Hahn, K.W. Wucherpfennig. Unusual features of self-peptide/MHC
583 binding by autoimmune T cell receptors. *Immunity* 23 (2005) 351–360,
584 doi:10.1016/j.immuni.2005.09.009.
- 585 38. K.J. Smith, J. Pyrdol, L. Gauthier, D.C. Wiley, K.W. Wucherpfennig. Crystal structure
586 of HLA-DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human myelin
587 basic protein. *J. Exp. Med.* 188 (1998) 1511–1520.
- 588 39. C. Veroni, B. Serafini, B. Rosicarelli, C. Fagnani, F. Aloisi. Transcriptional profile and
589 Epstein-Barr virus infection status of laser-cut immune infiltrates from the brain of
590 patients with progressive multiple sclerosis. *J. Neuroinflammation.* 15 (2018)18, doi:
591 10.1186/s12974-017-1049-5.
- 592 40. M.P. Pender, P.A. Csurhes, J.M. Burrows, S.R. Burrows. Defective T-cell control of
593 Epstein-Barr virus infection in multiple sclerosis. *Clin. Transl. Immunology.* 6 (2017)
594 e126, doi: 10.1038/cti.2016.87.
- 595 41. A. Afrasiabi, G.P. Parnell, N. Fewings, S.D. Schibeci, M.A. Basuki, R. Chandramohan,
596 et al. Evidence from genome wide association studies implicates reduced control of
597 Epstein-Barr virus infection in multiple sclerosis susceptibility. *Genome. Med.* 11
598 (2019) 26, doi: 10.1186/s13073-019-0640-z.
- 599 42. B. Nourbakhsh, A. Rutatangwa, M. Waltz, M. Rensel, M. Moodley, J. Graves, et al.
600 Heterogeneity in association of remote herpesvirus infections and pediatric MS. *Ann.*
601 *Clin. Transl. Neurol.* 5 (2018)1222-1228, doi: 10.1002/acn3.636.

- 602 43. A. Hassani, J.R. Corboy, S. Al-Salam, G. Khan. Epstein-Barr virus is present in the
603 brain of most cases of multiple sclerosis and may engage more than just B cells.
604 PLoS. One 13 (2018) e0192109, <https://doi.org/10.1371/journal.pone.0192109>
- 605 44. S. Agostini, R. Mancuso, F.R. Guerini, S. D'Alfonso, C. Agliardi, A. Hernis, et al. HLA
606 alleles modulate EBV viral load in multiple sclerosis. J. Transl. Med. 16 (2018) 80, doi:
607 10.1186/s12967-018-1450-6.
- 608 45. S. Boudreault, V.E.S. Armero, M.S. Scott, J.P. Perreault, M. Bisailon. The Epstein-
609 Barr virus EBNA1 protein modulates the alternative splicing of cellular genes. Virol. J.
610 16 (2019) 29, doi: 10.1186/s12985-019-1137-5.
- 611 46. J. McKenzie, A.G. El-Guindy AG. Epstein-Barr virus lytic cycle reactivation. Curr. Top.
612 Microbiol. Immunol. 391 (2015) 237-261, doi: 10.1007/978-3-319-22834-1_8.
- 613 47. N. Grandi, E. Tramontano. HERV envelope proteins: physiological role and
614 pathogenic potential in cancer and autoimmunity. Front. Microbiol. 9 (2018) 462, doi:
615 10.3389/fmicb.2018.00462.
- 616 48. V. Gröger, H. Cynis. Human endogenous retroviruses and their putative role in the
617 development of autoimmune disorders such as multiple sclerosis. Front. Microbiol. 9
618 (2018) 265, doi: 10.3389/fmicb.2018.00265.
- 619 49. G. Morris, M. Maes, M. Murdjeva, B.K. Puri. Do human endogenous retroviruses
620 contribute to multiple sclerosis, and if so, how? Mol. Neurobiol. 56 (2019) 2590-2605,
621 doi: 10.1007/s12035-018-1255-x.
- 622 50. V. de Luca, A. H. Martins, C. M. Romano, G.M. Pimenta, L.A. Peroni. Cross-reactivity
623 between myelin oligodendrocyte glycoprotein and human endogenous retrovirus W
624 protein: nanotechnological evidence for the potential trigger of multiple sclerosis.
625 Micron 120 (2019) 66-73, doi: 10.1016/j.micron.2019.02.005.
- 626 51. G. Arru, G. Mameli, G.A. Deiana, A.L. Rassu, R. Piredda, E. Sechi, et al. Humoral
627 immunity response to human endogenous retroviruses K/W differentiates between
628 amyotrophic lateral sclerosis and other neurological diseases. Eur. J. Neurol. 25
629 (2018)1076-e84, doi: 10.1111/ene.13648.

- 630 52. G. Arru, E. Sechi, S. Mariotto, A. Farinazzo, C. Mancinelli, D. Alberti, et al. Antibody
631 response against HERV-W env surface peptides differentiates multiple sclerosis and
632 neuromyelitis optica spectrum disorder. *Mult .Scler. J. Exp. Transl. Clin.* 3 (2017)
633 2055217317742425, doi: 10.1177/2055217317742425.
- 634 53. R. Planas, R. Santos, P. Tomas-Ojer, C. Cruciani, A. Lutterotti, W. Faigle, et al. GDP-
635 L-fucose synthase is a CD4+ T cell-specific autoantigen in DRB3*02:02 patients with
636 multiple sclerosis. *Sci. Transl. Med.* 10 (2018) 462 pii: eaat4301, doi:
637 10.1126/scitranslmed.aat4301.
- 638 54. I. Jelcic, F. Al Nimer, J. Wang, V. Lentsch, R. Planas, I. Jelcic, et al. Memory B cells
639 activate brain-homing, autoreactive CD4+ T cells in multiple sclerosis. *Cell* 175 (2018)
640 85-100 e23, doi: 10.1016/j.cell.2018.08.011.
- 641 55. A. Shetty, S.G. Gupta, M. Varrin-Doyer, M.S. Weber, T. Prod'homme, N. Molnarfi, et
642 al. Immunodominant T-cell epitopes of MOG reside in its transmembrane and
643 cytoplasmic domains in EAE. *Neurol. Neuroimmunol. Neuroinflamm.* 1 (2014) e22,
644 doi: 10.1212/NXI.0000000000000022.
- 645 56. E.M. Scholz, M. Marcilla, X. Daura, D. Arribas-Layton, E.A. James, I. Alvarez. Human
646 leukocyte antigen (HLA)-DRB1*15:01 and HLA-DRB5*01:01 present complementary
647 peptide repertoires. *Front. Immunol.* 8 (2017) 984, doi: 10.3389/fimmu.2017.00984.
- 648 57. C.A. Dendrou, L. Fugger. Immunomodulation in multiple sclerosis: promises and
649 pitfalls. *Curr. Opin. Immunol.* 49 (2017) 37–43.
- 650 58. J. Chataway, K. Martin, K. Barrell, B. Sharrack, P. Stolt, D.C. Wraith, et al. Effects of
651 ATX-MS-1467 immunotherapy over 16 weeks in relapsing multiple sclerosis.
652 *Neurology* 90 (2018) e955-e962, doi: 10.1212/WNL.00000000000005118.
- 653 59. N. Kaushansky, A. Kaminitz, H. Allouche-Arnon, A. Ben-Nun. Modulation of MS-like
654 disease by a multi epitope protein is mediated by induction of CD11c+CD11b+Gr1+
655 myeloid-derived dendritic cells. *J. Neuroimmunol.* 333 (2019) 476953. doi:
656 10.1016/j.jneuroim.2019.04.013.

- 657 60. S. Kasagi, D. Wang, P. Zhang, P. Zanvit, H. Chen, D. Zhang, et al. Combination of
658 apoptotic T cell induction and self-peptide administration for therapy of experimental
659 autoimmune encephalomyelitis. *EBioMedicine*. (2019) S2352-3964(19)30306-8, doi:
660 10.1016/j.ebiom.2019.05.005
- 661 61. C.J. Pickens, M.A. Christopher, M.A. Leon, M.M. Pressnall, S.N. Johnson, S. Thati, et
662 al. Antigen-drug conjugates as a novel therapeutic class for the treatment of antigen-
663 specific autoimmune disorders. *Mol. Pharm.* 16 (2019) 2452-2461, doi:
664 10.1021/acs.molpharmaceut.9b00063.
- 665 62. N. Ji, A. Somanaboeina, A. Dixit, K. Kawamura, N.J. Hayward, C. Self, et al. Small
666 molecule inhibitor of antigen binding and presentation by HLA-DR2b as a therapeutic
667 strategy for the treatment of multiple sclerosis, *J. Immunol.* 191 (2013) 5074–5084,
668 <http://dx.doi.org/10.4049/jimmunol.1300407>.
- 669 63. I. Zubizarreta, G. Flórez-Grau, G. Vila, R. Cabezón, C. España, M. Andorra, et al.
670 Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded
671 tolerogenic dendritic cells in a phase 1b trial. *Proc. Natl. Acad. Sci. USA.* 116 (2019)
672 8463-8470, doi: 10.1073/pnas.1820039116.

673

674 **Figure Legends**

675 Figure 1 Comparison of the HLA DR2b-MBP_3 complex generated by HADDOCK
676 with the reference structure. (A) Superposition of MBP_3 peptides bound to HLA
677 DR2b in the reference (cyan) and modelled structures (black). (B) Ribbon
678 representation of the published crystal structure of HLA DR2b bound to MBP peptide
679 (MBP_3; ENPVVHFFKNIVTPR) (PDB entry 1YMM). (C) Ribbon representation of the
680 lowest energy HLA DRb-MBP_3 complex model structure generated by HADDOCK.
681 The HLA DR2b alpha and beta chains are depicted as pink and blue, respectively. For
682 clarity only the peptide binding groove is highlighted. The peptide side chains (ball
683 and stick format) and positions (red) are shown. Peptide residues P1, P4, P6 and P9
684 serve as anchor residues which slot into the antigen binding groove, whereas side

685 chains at P-1, P2, P5 and P8 are surface exposed. HLA-DR2b residues involved in
686 stabilising peptide binding are also highlighted (ball and stick format). The black
687 rectangle boxes correspond to the core 9-mer sequence for each peptide. Figure was
688 generated with Pymol (The PyMOL Molecular Graphics System, Version 1.8
689 Schrödinger, LLC)

690

691 Figure 2 Comparison of HADDOCK generated models of HLA-DR2b in complex with
692 peptides derived from myelin (MOG) and HERV W-family (MSRVenv, SYN1 and
693 SYN2) associated proteins. (A) Superposition of MOG_4 (red), MSRVenv_5 (blue),
694 SYN1_2 (yellow) and SYN2_5 (green) peptides bound to HLA DR2b. (B) Ribbon
695 representation of the lowest energy HLA DR2b-MOG_4 complex model structure. (C)
696 Ribbon representation of the lowest energy HLA DRb-MSRV_5 complex model
697 structure. (D) Ribbon representation of the lowest energy HLA DR2b-SYN1_2
698 complex model structure. (E) Ribbon representation of the lowest energy HLA DRb-
699 SYN2_5 complex model structure. The HLA DR2b alpha and beta chains are
700 depicted as pink and blue, respectively. For clarity only the peptide binding groove is
701 highlighted. The peptide side chains (ball and stick format) and positions (red) are
702 shown. Peptide residues P1, P4, P6 and P9 serve as anchor residues which insert
703 into the antigen binding groove, whereas side chains at P-1, P2, P5 and P8 are
704 surface exposed. HLA-DR2b residues that contribute to peptide interactions are also
705 highlighted (ball and stick format). The black rectangle boxes correspond to the core
706 9-mer sequence for each peptide.

707

708 Figure 3 Comparison of HADDOCK generated models of HLA-DR2b in complex with
709 peptides derived from a CNS (β -SYN) and an EBV (EBNA1) protein. (A)
710 Superposition of β SYN (grey) and EBNA1_2 (orange) peptides bound to HLA DR2b.
711 (B) Ribbon representation of the lowest energy HLA DR2b- β SYN complex model
712 structure. (C) Ribbon representation of the lowest energy HLA DRb-EBNA1_2

713 complex model structure. The HLA DR2b alpha and beta chains are depicted as pink
714 and blue, respectively. For clarity only the peptide binding groove is highlighted. The
715 peptide side chains (ball and stick format) and positions (red) are shown. Peptide
716 residues P1, P4, P6 and P9 serve as anchor residues which slot into the antigen
717 binding groove, whereas side chains at P-1, P2, P5 and P8 are surface exposed.
718 HLA-DR2b residues involved in peptide binding are also highlighted (ball and stick
719 format). The black rectangle boxes correspond to the core 9-mer sequence for each
720 peptide.
721