Contribution of EBV infection to Multiple Sclerosis Pathogenesis: Human leukocyte antigen restricted, EBV epitope-specific T cell responses in Multiple Sclerosis

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Background:

Multiple sclerosis (MS) is the most common chronic inflammatory disorder of the central nervous system in young adults and a prototypic autoimmune disease. Similar to other autoimmune diseases the precise aetiology of MS is unknown but genetic, viral and environmental factors have been implicated. Research, including our own, has shown that presence of human leukocyte antigens (HLA-) DRB1*15, *16 and *08 predispose for MS, while the alleles HLA-DRB1* 04, *07, *09 have a protective effect. Efficient control of EBV infection typically requires action of virus specific CD8+ and CD4+ T cells recognizing viral peptides through MHCI and II respectively and epitopes derived from EBNA-1 protein seem to be specifically targeted in EBV immune response. Next to T cell responses there is increasing evidence for the role of B cells in MS. Antibodies against Epstein-Barr virus (EBV) and in particularly directed against EBV nuclear antigen-1 (EBNA-1) protein have been shown to be significantly elevated in MS cases. A small study found enriched antibodies against a short B-cell-epitope (amino acids 401-411) within EBNA-1 in MS discordant identical twins and we could confirm antibodies against this EBV epitope to be independently contributing to MS risk.

Objectives:

To investigate the contribution of HLA-restricted, epitope-specific, T cell responses in MS cases associated with active and inactive disease and their role in disease pathogenesis.

Materials and Methods:

A total of 426 MS patients of the West Australian Demyelinating Disease Database were included in the study. The control cohort (n=186) was established from the population of Busselton, Western Australia and additional controls were obtained from the Australian Red Cross. To review individual and combined effects on disease risk comparing MS patients and healthy controls from WA, host genetic profiles were determined using Sanger sequence based Human Leukocyte Antigen (HLA) high resolution typing using heterozygous ambiguity resolving primers where applicable.

Immune responses to EBV infection (IgG antibodies) directed against viral capsid antigen (VCA), Epstein-Barr nuclear antigen-1 (EBNA-1) and EBNA-$1_{(short)}$ (peptide within EBNA-1 [aa 401-411]) were detected by using standard commercial and in-house ELISAs.

Patient derived EBNA-1 genomes were amplified without requirement for primary culture using in-house nested PCR. Samples were sequenced using conventional Sanger sequencing (n=73) and for a subset using deep sequencing Roche 454-FLX technology (n=23) (see Figure 1).

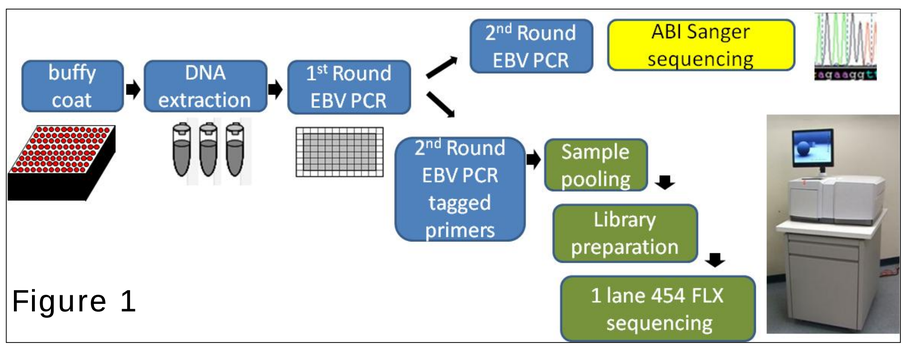


Figure 1

Fig 1. Flow chart of DNA extraction, EBNA-1 amplification, Sanger- and FLX 454 sequencing of patient derived Epstein Barr virus.

HLA binding algorithms NetMHCII and NetMHCIIpan were utilized to predict potential HLA-DRB1*1501 class II HLA epitopes within the EBNA-1 Sanger derived sequences. Additionally, a selection of predicted EBV peptides have been subsequently functionally tested for responses in class II IFNy-ELISpot assays. Samples were tested in duplicate or triplicate using 10ug/ml EBNA-1 peptide and 100,000 - 200,000 CD8+ depleted PBMC/well.

Results:

Comparing class II HLA-DRB allele prevalence in patients and controls, we were able to identify a combined contribution of low risk alleles (HLA-DRB1* 04, *07, *09), a group of neutral HLA risk and a group of high MS risk alleles (HLA DRB1*15,*16 and *08).

Individuals with high risk HLA-DRB alleles (DRB1*08, *15, *16) had significantly higher antibody titres against EBV nuclear antigen 1 (EBNA-1)(long) and against the shorter B cell epitope EBNA-1(short) [aa401-411] in comparison to low-risk carriers ($p<0.0001$; Figure 2A & B), but not significantly different anti-viral capsid antigen (VCA) titres (Figure 2C).

Figure 2

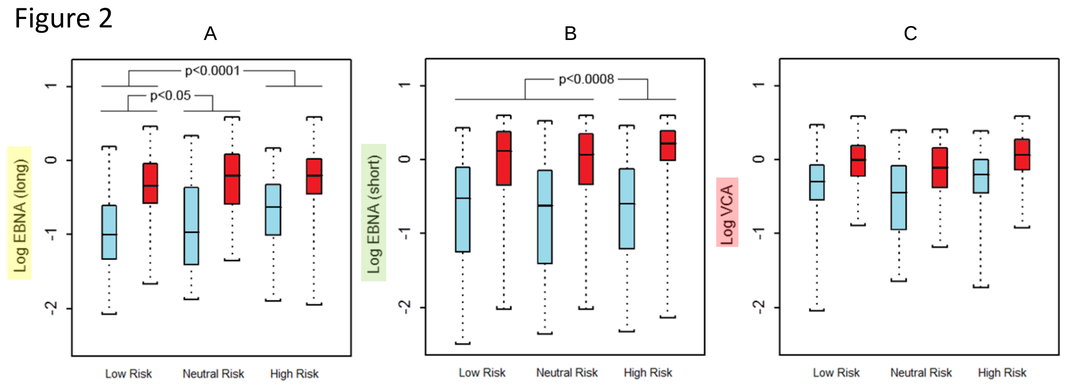


Fig 2. ELISA results for MS patients (red) and healthy controls (blue) stratified according to HLA-DRB1 associated MS risk.

Focusing on the C-terminal region of EBNA-1 demonstrated sequence variation within four major clusters. Interestingly, patient derived viral sequences showed only in a minority of cases strong similarity to the EBV reference strain B95-8 (9/53, 16.9%) and as expected no sequence of the WA cohort clustered with the type 2 AG876 strain.

After predicting potential EBNA-1 HLA-DRB1*1501 binders using patient derived EBV sequences, we selected EBNA-1 epitopes of interest based on HLA binding affinity. We then identified candidate central nervous system protein epitopes that would be predicted to bind HLA-DRB1*15 with sufficient affinity to allow antigen presentation and which exhibited homology with the selected EBNA-1 epitopes.

The more sensitive nature of FLX was emphasized by detection of low level sequence polymorphisms leading to additional predicted DRB1*15 restricted T-cell epitopes within EBNA-1 (Figure 3), but only two samples showed minor sequence variants at a level of ≥10%.

Figure 3

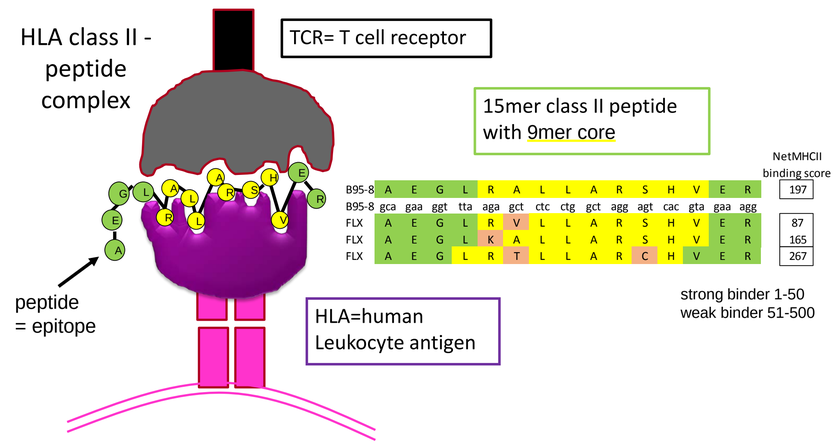
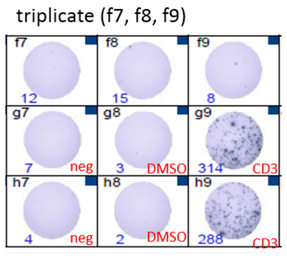


*Fig 3. Example of predicted EBNA-1 HLA-DRB1*1501 epitopes. Single letters: amino acids; yellow: peptide core; red: amino acid variation detected with FLX technology compared to reference strain B95-8 (top); numbers: NetMHCII predicted binding affinities of peptides*

A selection of predicted EBV peptides have been subsequently functionally tested for responses in class II IFNy-ELISpot assays. Although IFNy responses were low and showed high basic activation of patient cells, we could identify distinct positive responses for several epitopes (Figure 4).

So far we have tested five patient and five healthy control samples and could identify five peptides reactive in MS patients only compared to two peptides reactive in healthy control samples only. Of note, none of the peptides were positive for MS and healthy control samples at the same time.

Figure 4 4A



4B

cohort	peptide	
MS	EBNA-1 483T	EGLRTLLARCHVERT
MS	EBNA-1 476P	PKFENIAEGLKLLLA
MS	EBNA-1 560B	IKDLVMIKPAPTCNI
MS	EBNA-1 520I	RRGIGLAIPQCLLTP
MS	EBNA-1 476P	PKFENIAEGLKLLLA
HC	EBNA-1 514B	KTSLYNLRRGTALAI
HC	EBNA-1 513V	SKTSLYNLRRGVALA

*Fig 4. **A)** Example of HLA-DRB1*1501 peptide response in a Multiple Sclerosis (MS) sample: 117 spot forming cells/1mio CD8 depleted PBMC, neg/DMSO: no peptide controls; CD3: positive control using CD3 peptide pool ; **B)** positive peptide responses in MS and healthy control (HC) samples; peptides named after amino acid starting position with EBNA-1, B: B95-8 sequence; T/P/I/V amino acid differences to B95-8 reference sequence*

Conclusions:

In this study we have proven the feasibility of obtaining EBNA-1 sequences directly from buffy coat samples and demonstrated that the majority of autologous sequences do not align closely with the widely used B95-8 reference strain. We were able to identify low-level EBNA-1 sequence variation using FLX technology (8.3% of nucleotides at a 1% threshold) leading to additionally predicted HLA-DRB1*15 binders, but our results do not support a strong influence of intra-individual EBV sequence variation on MS disease risk. A selection of predicted peptides were successfully tested and functionally confirmed in class II IFNy ELISpot assays.

Future work will aim to generate antigen-specific T cell lines and T cell clones in order to investigate the potential for EBNA-1-specific T cells to cross-react with auto-antigens. Additionally, putative cross reactive DRB1*1501 epitopes for central nervous system proteins following the same approach will be further investigated.

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