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 MHC class I 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 particular against EBNA 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 sequencing 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(early) (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).

**Results:**
Comparing class II HLA-DRB allele prevalence in patients and controls, we could identify five peptides reactive in MS patients only compared to two peptides reactive in healthy control samples at the same time.

**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 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.

**Acknowledgments:**
We would like to thank patients and clinical staff for assistance and contribution to this project. Many thanks also to the Australian Red Cross for providing valuable control samples. Special thanks to the McCusker Foundation and MSRA Australia for supporting this project.

---

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

**Figure 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 affinities of peptides.

**Figure 3A**
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).

**Figure 4A**
A) Example of HLA-DRB1*1501 peptide response in a Multiple Sclerosis (MS) sample: 117 spot forming cells/1mcg CD8 depleted PBMC, neg/NMDD: no peptide controls; CD3: positive control using CD3 peptide pool; BJ 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.

**Figure 4B**
A) T cell clone specific for EBV peptide 1501.

**Figure 4C**
A) T cell clone specific for EBV peptide 1501.

---

**References**

1. Institute for Immunology & Infectious Diseases, Murdoch University. School of Veterinary and Life Science, Murdoch University. Clinical Immunology, Royal Perth Hospital. Department of Neurology, Sir Charles Gairdner Hospital, Perth, Western Australia.