Investigation of Humoral Immune Responses towards Epstein-Barr Virus in Multiple Sclerosis using Peptide Microarrays

A. Castro[1], B. Wunderlich[2], N. Pawlowski[1], J. Jansong[1], U. Reimer[1], M. Löbel[3], K. Ruprecht[2#], C. Scheibenbogen[3#]

[1]JPT Peptide Technologies, Volmerstr. 5, 12489 Berlin, Germany
[2]Clinical and Experimental Multiple Sclerosis Research Center, Charité - Universitätsmedizin Berlin,
Charité Campus Mitte Charitéplatz 1, 10117 Berlin, Germany
[3]Charité Institute for Med. Immunology, Charité Campus Virchow Klinikum, Föhrerstr. 15/Südstr. 2, 13353 Berlin, Germany
[#]authors contributed equally

Introduction

Most humans carry a considerable number of persisting herpesviral infections, frequently in a latent state. Chronic herpesviral infections may intermittently reactivate especially under immune suppression and may play an important role as trigger or cofactor for autoimmune diseases or cancer. Correlation of such infections with diseases is difficult. Analysis of antiviral antibody repertoires may provide information about the immunological control of herpesviral infections. Following primary infection, Epstein-Barr virus (EBV, human herpes virus 4) persists in the infected host as a mostly asymptomatic latent infection. About 95% of the adult population are EBV-seropositive. EBV has been implicated in various diseases such as lymphomas, multiple sclerosis (MS), and chronic fatigue syndrome. The detailed mapping of the humoral immune response in human serum samples allows the high resolution analysis of the antibody repertoire against EBV antigens. We developed a peptide microarray platform with peptide libraries of 6900 members.

Here, we present data of an EBV antibody screen using a library of peptide scans through eight major EBV antigens with serum samples of healthy human donors and patients with MS.

Experimental Design

- Generation of overlapping peptide scans through major EBV antigens (BLRF2, BZLF1, EBNA1, EBNA3, EBNA4, EBNA6, LMP1, VP26) resulting in 1465 peptides
- Peptide synthesis and printing on microarray slides (Fig. 1)
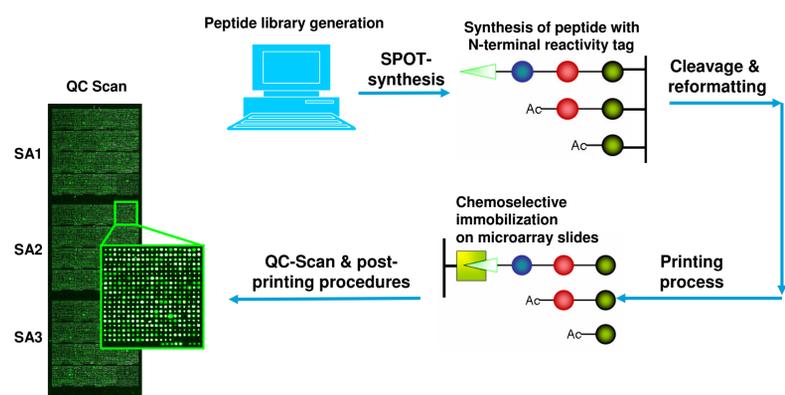- Screening of sera from healthy volunteers (HC) and MS patients

Fig. 1 Schematic representation of the microarray production process. Three subarrays (SA) are used for improved data quality.

References

[1] Ruprecht et al. (2014) Multiple sclerosis: the altered antibody response to Epstein-Barr virus primarily targets, but is not confined to, the glycine-alanine repeat of Epstein-Barr nuclear antigen-1. *J Neuroimmunol*. in press.
[2] Haynes et al. (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med*. **366**:1275-86.
[3] Gaseitsiwe et al., (2010) Peptide microarray-based identification of Mycobacterium tuberculosis epitope binding to HLA-DRB1*0101, DRB1*1501, and DRB1*0401. *Clin Vaccine Immunol*. **17**:168-75.

Results

- Comparison of 22 controls and 29 MS patients, all of which are EBNA-1 positive in ELISA measurements
- 39 out of 1465 peptides show significantly higher reactivities in MS patients ($p < 10^{-3}$, Mann Whitney U-Test)
- 44% of these peptides are derived from EBNA-1, but also significant differences for EBNA-3, EBNA-4, EBNA-6, VP26, and LMP1 (but not BLRF2 and BZLF1) are observed
- The majority of significantly different EBNA-1 peptides is located in GA-rich region (Fig. 2)
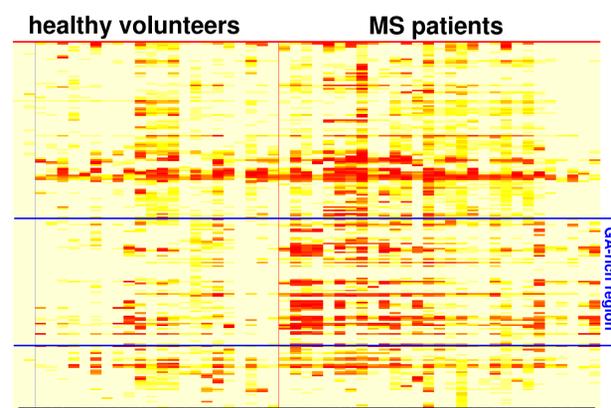

Fig. 2. Heatmap representation of screening results for EBNA-1 peptides. The upper band represents the maximum assay signal (red), light yellow represents the noise level. Patients are represented on the x-axis, the peptides from an EBNA-1 spanning scan (15-meres, 11 amino acids overlap) are shown on the y-axis. The two columns on the left show two healthy volunteers with a negative result in an EBNA-1 protein ELISA.

Correction for multiple testing (Bonferroni) leaves three peptides with significant differences (Fig 3).
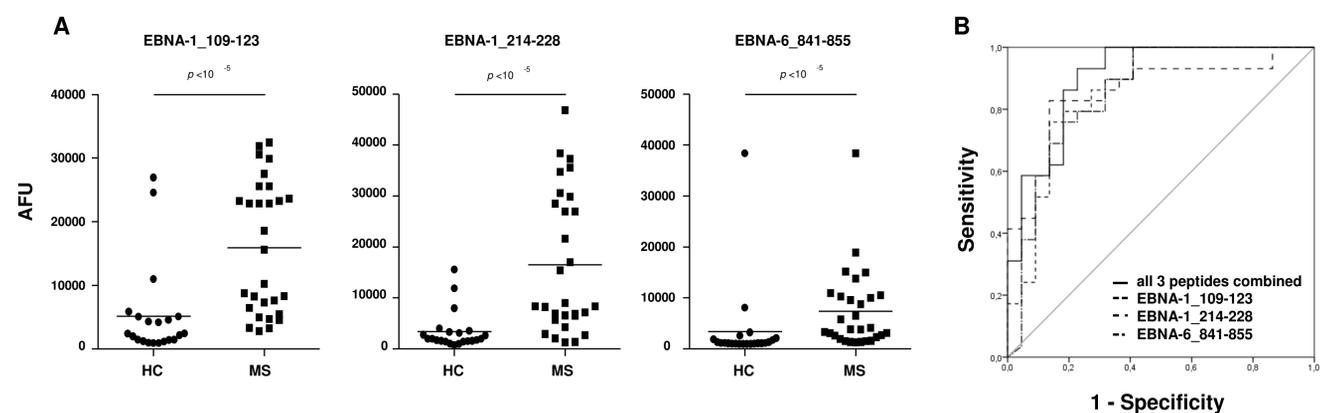

Fig. 3
A The three most significantly ($p < 10^{-5}$) different anti-EBV peptide antibody reactivities between healthy controls (HC, n=22) and patients with multiple sclerosis (MS, n=29). Peptides are designated by the name of the EBV protein followed by the position of the peptide in the respective EBV protein amino acid sequence.
B Receiver operating characteristic curves for the individual EBNA-1_109-123, EBNA-1_214-228 and EBNA-6_841-855 peptides or for all 3 peptides combined. The areas under the curve is 0.9 for antibody reactivities against all 3 peptides combined.

Conclusion

- Screening of low volume serum samples results in specific signal patterns[1,2,3]
- Results confirm predominant role of EBNA-1 in altered antibody response in MS but also show increased reactivities in other latent proteins[1]
- Main differences in reactivities for GA-rich region of EBNA-1
- Study provides possible information on pathogenetic mechanism and for diagnostic biomarkers for MS and the prediction of MS-risk.

* Correspondence should be addressed to Ulf Reimer: reimer@jpt.com

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