



Human Autoantibody Screening with the PEPperCHIP® Autoimmune Epitope Microarray 2.0

Introduction

The PEPperCHIP® Autoimmune Epitope Microarray is based on 3,359 linear autoimmune epitopes including 192 citrullinated peptides of the Immune Epitope Database (www.iedb.org). The epitopes correlate with a variety of autoimmune diseases including Crohn's disease, diabetes mellitus, rheumatoid arthritis, Sjögren's syndrome, systemic lupus erythematosus, vitiligo and many more.

Numerous research institutes, biotech and pharma companies meanwhile successfully applied the PEPperCHIP® Autoimmune Epitope Microarray to e.g.

- analyze autoantibody profiles in human and murine serum samples
- differentiate IgG and IgM autoantibody profiles
- detect various viral infections correlating with autoimmune diseases
- discover new autoantibody serum markers in a uniquely multiplexed manner
- screen for differential autoantibody profiles in patient vs. control groups

"Through the help of researchers at PEPperPRINT, we have identified several important lupus and arthritis associated autoantigen epitopes in a complex mouse model of autoimmunity. The comprehensive database behind these epitopes has enabled us to identify that these epitopes are mostly associated with nucleosomal or ribonucleosomal proteins. The PEPperCHIP® epitope analysis has now helped us to move on to determine the immunopathogenic mechanisms in our autoimmune mice" says Prof. Hui-Chen Hsu of the University of Alabama at Birmingham.

In this application note, we describe the analysis of IgG and IgM autoantibody profiles of the serum of a 56 year old female diabetes mellitus type 1 patient with the PEPperCHIP® Autoimmune Epitope Microarray 2.0. We could identify a number of antibody responses against various viral and human antigens including hits that are linked to the development of type 1 diabetes and Sjögren's syndrome.

Results & Discussion

The PEPperCHIP® Autoimmune Epitope Microarray 2.0 was incubated with 4 µl of human serum 181641 (56 year old female patient with diabetes mellitus type1, in.vent Diagnostica, Hennigsdorf, Germany) at a dilution of 1:500 in PBST buffer. Subsequently, the microarray was stained with goat anti-human IgG (Fc) conj. DyLight680 and goat anti-human IgM (µ-chain) conj. DyLight800 secondary antibodies together with a monoclonal anti-HA (12CA5)-DyLight800 control antibody. The microarray was scanned with a LI-COR Odyssey Imaging System. Quantification of spot intensities and peptide annotation were done with PepSlide® Analyzer.



The IgG (red) and IgM (green) antibodies of patient serum 181641 showed a strong reaction with a number of autoimmune epitopes at high spot intensities and signal to noise ratios (Fig. 1).

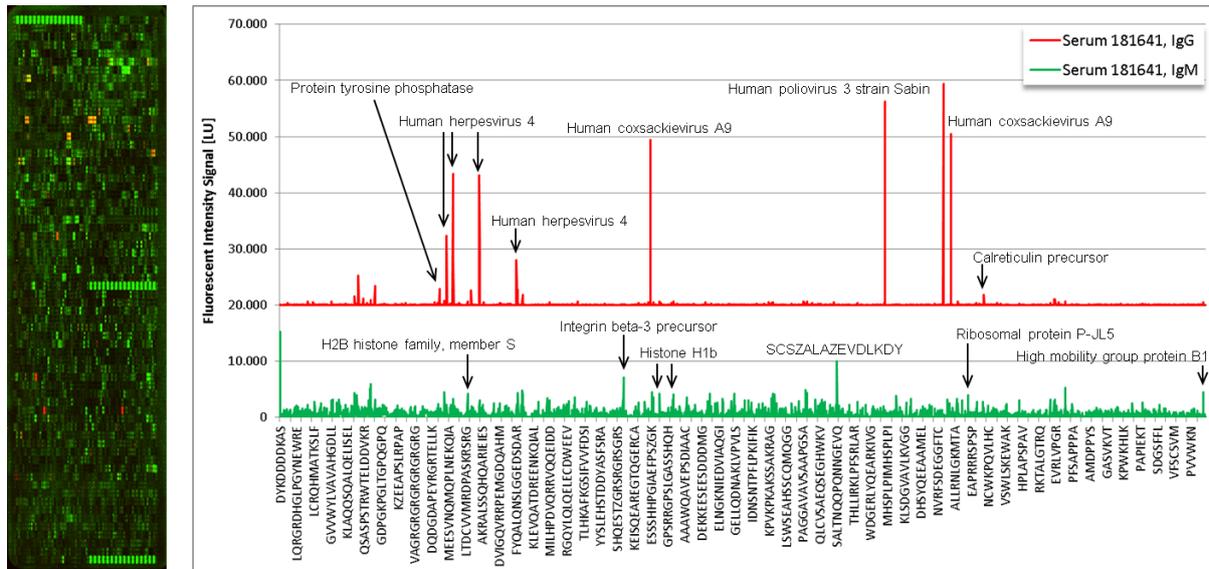


Fig. 1: The PEPperCHIP® Autoimmune Epitope Microarray 2.0 was assayed with human serum 181641 (1:500) followed by staining with the secondary anti-IgG (red) and anti-IgM (green) antibodies and data read-out. Simultaneous staining of the HA control peptides (green signal stretches) gave rise to the expected and well-defined spot pattern and validated the overall peptide microarray integrity and assay quality.

The strongest IgG responses were directed against multiple epitopes of the EBNA-1 protein of human herpesvirus 4 (Epstein-Barr virus), which is explainable by the high level of endemic infection, and against epitopes of human coxsackievirus A9 (e.g. VPALTAVETGHT) and human poliovirus 3 (KEVPALTAVETGAT). Due to the pronounced sequence similarity of the coxsackievirus and poliovirus epitopes, we assume that the main response was directed against epitope KEVPALTAVETGAT and raised by a polio immunization, whereas the coxsackievirus responses resulted from cross-reactions of the corresponding antibody. Strong IgG responses against human antigens were observed for epitopes KIKDPDASKP of Calreticulin precursor and LGPEGAHGDTTFEYQDL of protein tyrosine phosphatase, also known as IA-2. Since IA-2 is reported as autoantigen of type 1 diabetes,¹ the IgG responses against epitope LGPEGAHGDTTFEYQDL correlates very well with the medical condition of the patient. The IgG response against epitope KIKDPDASKP of Calreticulin may be attributed to systemic lupus or Sjögren's syndrome.²

The IgM antibody profile of human serum 181641 corresponded to less mature immune responses. Although the general intensity level was slightly lower, the response pattern was more complex possibly due to the cross-reactivity of IgM antibodies. As with the IgG profile, we also observed some clear IgM responses against epitopes of the EBNA-1 protein of human herpesvirus 4. Interestingly, there was only little overlap with the IgG responses against EBNA-1, what hinted at a new immune response against human herpesvirus 4.

¹ E. Bonifacio *et al.*, J Immunol. 1998 Sep 1;161(5):2648-54.

² <http://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=811>



Strong IgM responses against human antigens were found for epitopes of Integrin beta-3 precursor (APESIEFPVSEARVLE), Trinucleotide repeat containing 6A (PGNRPTGWEEEDVE), High mobility group protein B1 (DAAKKGVVKA EKSKKKK) and Histone H1b (KLNKKAASGEAKPKAK, AAKPKTAKPKAAKPKK). According to the references in the Immune Epitope Database, the corresponding epitopes were also attributed to systemic lupus and/or Sjögren's syndrome. Taking the IgG response against KIKDPDASKP of Calreticulin precursor into account, the IgM response may hint at an onset of one of both autoimmune diseases. However, the age and the sex of the diabetes patient correlates better with Sjögren's syndrome than with systemic lupus.³

It should be pointed out that particularly single epitope hits can also result from cross-reactions of other antibody species against a similar peptide motif; in contrast to this, frequent responses against a single antigen or pathogen indicate a polyclonal response and can be regarded as internal validation. The same applies to peptides with the same consensus motif, e.g. APESIEFPVSEARVLE and CAPESIEFPVSEARVLE of Integrin beta-3 precursor.

Outlook

Further validation of particularly single and unexpected epitope hits can be done by [PEPperMAP® Epitope Mappings](#) of the same sample against the full length antigen to identify e.g. polyclonal responses or to analyze the specific epitopes in detail (see also application note "[High Resolution PEPperMAP® Epitope Mapping of Autoimmune Sera](#)").

Another option for epitope validation, in-depth epitope analysis and particularly the identification of conserved and variable amino acid positions is given by a [PEPperMAP® Full Substitution Scan](#) of all amino acid positions with the 20 main amino acids (see also application note "[PEPperMAP® Full Substitution Scan of HA and M13 Epitopes](#)").

With higher sample numbers and, particularly, a comparison of patient and control groups, the PEPperCHIP® Autoimmune Epitope Microarray 2.0 can be used to identify differential serum biomarkers for autoimmune patient groups, or to discover autoantibody profiles that correlate with a prognostic value.

It should also be pointed out that PEPperCHIP® Peptide Microarrays are fully compatible with standard ELISA tests and immunoassays. Moreover, the peptide and epitope content of PEPperCHIP® Peptide Microarrays can be easily adjusted with respect to custom antigens, epitopes or organisms in a uniquely flexible manner.

³ Y.T. Konttinen et al., J Autoimmun. 2012 Aug;39(1-2):49-56.