Case Study: IgG and IgA Antibody Profiling with the PEPperCHIP® Infectious Disease Epitope Microarray

Introduction

The diagnosis of infectious diseases is often hampered by the display of identical symptoms caused by different pathogens. To make a definitive diagnosis, individual assays are needed for each suspected pathogen leading to greater costs and increased time consumption. A better approach would be a highly multiplexed assay that enables the analysis of antibody responses against variety of pathogens at once. Therefore, we developed the PEPperCHIP® Infectious Disease Epitope Microarray based on 3,857 B-cell epitopes associated with 191 different pathogens to enable the multiplexed screening of IgG, IgM and IgA antibodies that can be assigned to more than 100 infectious diseases in a single assay.

The pathogen epitopes were selected from the Immune Epitope Database (www.iedb.org) and correlate with a variety of frequent but also less common infections such as various hemorrhagic fevers, Lyme disease, measles, Chagas, RSV, tuberculosis, candidiasis, malaria, hepatitis, influenza, and many more.

In this application note, we describe the analysis of IgG and IgA serum antibodies of a 56 year old female diabetes mellitus type 1 patient with the PEPperCHIP® Infectious Disease Epitope Microarray. We identified a number of antibody responses against various viral and bacterial antigens. They included previously unknown Helicobacter pylori and Toxoplasma gondii infections, which were validated by standard diagnostic tests or in-depth epitope mapping.

Results

The PEPperCHIP® Infectious Disease Epitope Microarray was incubated with human serum 181641 (56 year old female patient with diabetes mellitus type1, in.vent Diagnostica, Hennigsdorf, Germany) at a dilution of 1:250. Subsequently, the microarray was assayed with goat anti-human IgG (Fc) conj. DyLight680 and goat anti-human IgA (alpha) 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.

A detailed material and methods section as well as supplemental material are included in the full report "Case Study: IgG and IgA Antibody Profiling with the PEPperCHIP® Infectious Disease Epitope Microarray" that can be downloaded from http://www.pepperprint.com/science/application-notes/.

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

IgG Response: The strongest interactions were directed against epitopes of human coxsackievirus (VPALTAVETGHT, SASVPALTAVET, EAIPALTAVETGHTSQV) and human poliovirus (HSKEIPALTAVETGA, KEVPALTAVETGAT). Due to the pronounced sequence similarity of the coxsackievirus and poliovirus epitopes, we assume that the main response was directed against
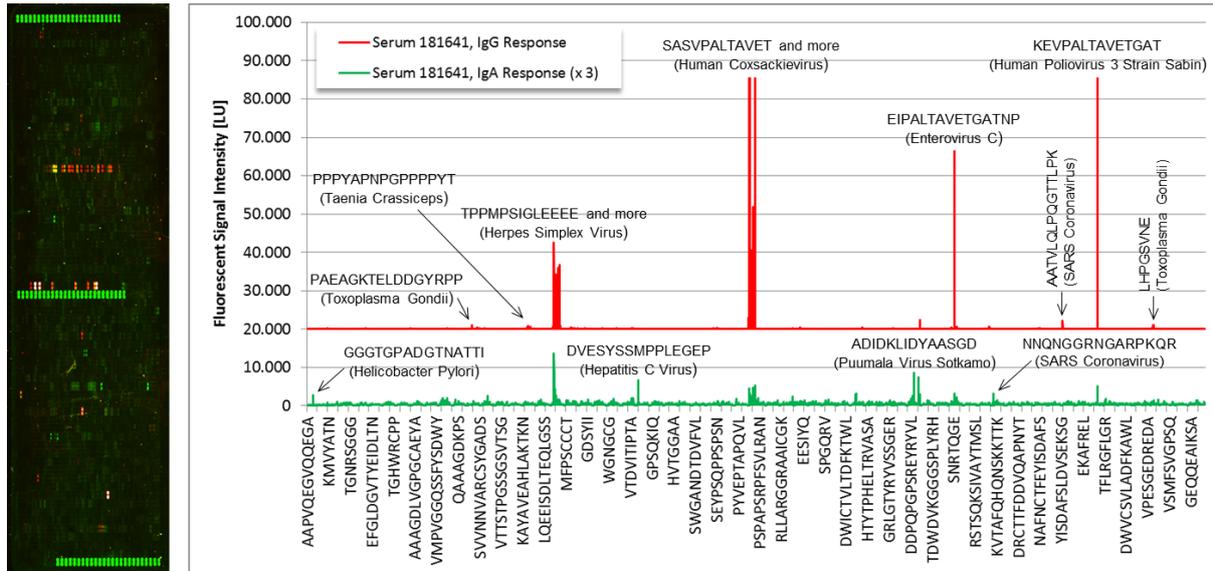
Fig. 1: The PEPperCHIP® Infectious Disease Epitope Microarray was assayed with human serum 181641 (1:250) followed by staining with secondary and control antibodies. The microarray scan showed a well-defined IgG (red) and IgA (green) response pattern with the control peptides (green stretch) on top, in the middle and on bottom of the microarray (left). The IgG and IgA response patterns were visualized by intensity plots (right); for a better data overview, the plot of the IgG response (red) was leveled, and the spot intensities of the IgA response (green) tripled. The main IgG and IgA responses were annotated next to the corresponding signals in the intensity plots.

epitope KEVPALTAVETGAT of human poliovirus. Most likely, the response raised by a polio immunization, whereas the coxsackievirus responses resulted from cross-reactions of the corresponding antibody. Other strong IgG responses were directed against various epitopes of herpes simplex virus, a common pathogen with a very high rate of infestation.

Less strong IgG responses were observed against multiple epitopes of Toxoplasma gondii, a parasitic protozoan that causes toxoplasmosis, and Taenia crassiceps, a tapeworm that can be found in immunocompromised patients. Surprisingly, we also identified a number of responses against SARS coronavirus epitopes.

IgA Response: Compared to the IgG response, the IgA response was significantly weaker and less clear. The main interactions were, again, found for various epitopes of human poliovirus/coxsackievirus and herpes simplex virus, what is in accordance with the IgG response.

In addition, we also identified IgA responses against epitopes of hepatitis C virus, Helicobacter pylori and, surprisingly again, against SARS coronavirus. A response against SARS coronavirus was also found in the IgG profile and needs to be validated, since a corresponding infection was regarded as unlikely. The same applied to the IgA responses against epitopes of hepatitis C virus without any accompanying IgG response. In contrast to this, an IgA response against Helicobacter pylori appeared to be reasonable due to its high prevalence particularly in the gastrointestinal tract.

Diagnostic Test: To validate the HCV and Helicobacter pylori infections, 800 µl of human serum 181641 were provided to a diagnostic lab; the standard tests revealed that the serum was actually HCV negative, what was expected from the missing IgG response. However, the IgA response against Helicobacter pylori could be validated with 58 U/ml total antibody.

Additional Epitope Mapping: To further validate the IgG responses against Toxoplasma gondii and Taenia crassiceps as well as the observed IgG and IgA interactions with SARS coronavirus epitopes, we performed an additional multiplexed epitope mapping against the corresponding antigens of these
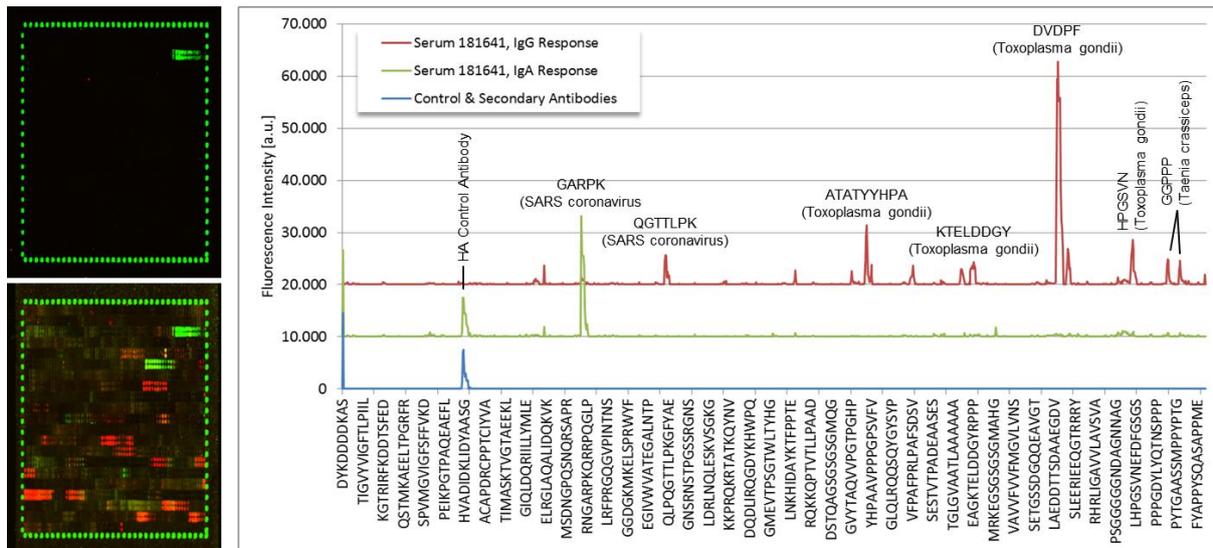
Fig. 2: The PEPperCHIP® Custom Peptide Microarray included selected Toxoplasma gondii, Taenia crassiceps and SARS coronavirus antigens translated into overlapping peptides. The array was pre-stained with secondary and control antibodies, and revealed a cross-reaction of the anti-HA control antibody (green) with peptides with the consensus motif DKLIDYA (left on top). Incubation of a second peptide microarray with human serum 181641 (1:250) followed by staining with secondary and control antibodies revealed strongly polyclonal IgG and IgA responses against virtually all antigens covered by the microarray (left on bottom). The intensity plots (right) highlighted the corresponding IgG and IgA response patterns in comparison with the pre-staining; the main epitopes were annotated next to the corresponding signal.

pathogens. The antigens were selected based on the observed epitope responses and included nucleocapsid protein (Puumala virus), nucleocapsid protein (SARS coronavirus), dense granule protein GRA4 (Toxoplasma gondii p89), granule antigen protein (Toxoplasma gondii) and protective recombinant antigen (Taenia crassiceps). The proteins were translated into 15 aa peptides with a maximum peptide-peptide overlap of 14 amino acids resulting in a PEPperCHIP® Custom Peptide Microarray with 1,357 different peptides printed in duplicate including HA control peptides surrounding the array. For the following epitope mapping, less than 10 µl serum was used, which is a fraction of the volume (400 - 500 µl) typically used for standard diagnostic tests for each pathogen.

IgG and IgA Responses: The IgG profile highlighted a strong and clear epitope-like spot pattern against SARS coronavirus, a very strong and polyclonal response against Toxoplasma gondii as well as two epitope-like spot patterns against Taenia crassiceps. The two Taenia crassiceps spot patterns exhibited the same short consensus motif GGPPP. However, a short epitope can hint at cross-reactions with other antibody species. Taking the missing polyclonality against protective recombinant antigen of Taenia crassiceps into account, it is reasonable to assume that the observed IgG response of the PEPperCHIP® Infectious Disease Epitope Microarray was in fact caused by such cross-reaction, and not by an actual Taenia crassiceps infection.

In addition to the IgG response against SARS coronavirus epitope QGTTLPK, we further identified a clear IgA response against peptides with the consensus motif GARPK, validating the interactions of the PEPperCHIP® Infectious Disease Epitope Microarray. Since an actual SARS coronavirus infection has to be regarded as very unlikely, we run an NCBI Protein Blast search of both epitopes QGTTLPK and GARPK (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify other pathogens with similar epitopes. Interestingly, we found two very similar sequences QGTTLP and GARPK in Escherichia coli. Similar to Helicobacter pylori, Escherichia coli has a high prevalence and is frequently found in the gastrointestinal tract, what would be in accordance with the clear and strong IgA response. Therefore,

we concluded that the presumable SARS coronavirus responses of the PEPperCHIP® Infectious Disease Epitope Microarray were likely raised by Escherichia coli.

Discussion & Outlook

We used the new PEPperCHIP® Infectious Disease Epitope Microarray based on 3,857 B-cell epitopes of the Immune Epitope Database associated with 191 different pathogens to screen for IgG and IgA antibody profiles in human serum 181641 of a 56 year old female patient, who suffers from Diabetes mellitus Type 1. We identified very strong IgG responses against human poliovirus/coxsackievirus epitopes as well as against herpes simplex virus. Weaker IgG interactions were found for epitopes of Toxoplasma gondii, Taenia crassiceps and surprisingly SARS coronavirus. The two latter, however, were possibly based on cross-reactions with other antibody species. Particularly, the apparent SARS coronavirus was likely caused by a more reasonable Escherichia coli infection, as validated by a subsequent high resolution epitope mapping and a protein blast search of the conserved core epitope.

The main IgA responses of the PEPperCHIP® Infectious Disease Epitope Microarray were also directed against human poliovirus/coxsackievirus and herpes simplex virus epitopes. Moreover, we found a weak IgA response against Helicobacter pylori that was previously unknown and validated by a standard diagnostic test, albeit with a high sample consumption of ~400 µl. As suspected, the IgA responses against HCV epitopes without any accompanying IgG response were false-positive hits due to e.g. cross-reactions with other antibodies or non-specific binding events. The surprising IgA response against SARS coronavirus could be confirmed with the epitope mapping peptide microarray, but was most likely caused by an Escherichia coli infection with an identical conserved core motif.

Due to the high serum need for standard diagnostic tests, some of the less expected IgG and IgA hits were investigated in a subsequent multiplexed epitope mapping of 5 different pathogen antigens with a total sample consumption of only about 3 µl. It turned out that all responses of the PEPperCHIP® Infectious Disease Epitope Microarray were actually based on specific interactions, albeit in part induced by other pathogens with similar epitopes, as shown for the apparent SARS coronavirus infection. However, the rather weak response against Toxoplasma gondii could be validated by a strong and polyclonal IgG response on the epitope mapping peptide microarray.

Another option for epitope validation including an in-depth epitope analysis and 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 SMN Epitope").

With higher sample numbers and the comparison of patient and control groups, the new PEPperCHIP® Infectious Disease Epitope Microarray can be used to identify differential serum biomarkers with a prognostic value, for a correlation of autoimmune diseases with B-cell responses raised by infections, or to differentiate between infected and vaccinated patients in vaccine research and development.

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.