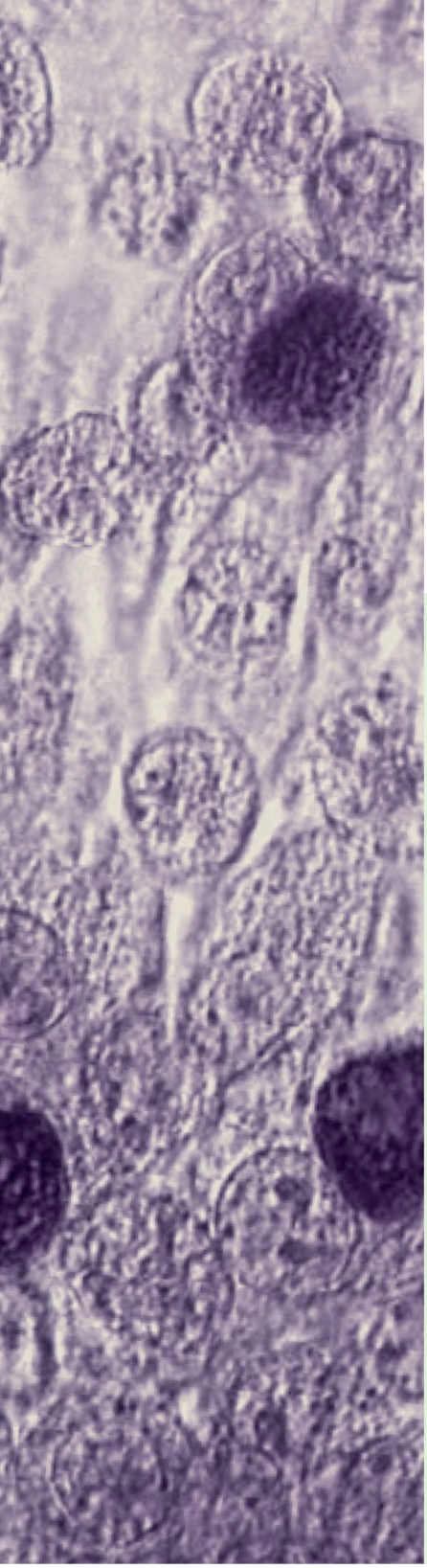

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Application Note

Uncovering immunogenic epitopes of *Chlamydia trachomatis* with the PEPperCHIP® Neglected Tropical Disease Peptide Microarray

ABSTRACT

Chlamydia trachomatis (*C. trachomatis*) is an obligate intracellular bacterial pathogen. It is the most prevalent sexually transmitted bacterium in the United States, and genital tract infections are a leading cause of pelvic inflammatory disease, tubal factor infertility, and ectopic pregnancy. Eye infections with serovars A-C are also linked to trachoma, which belongs to the diverse group of neglected tropical diseases (NTD). Trachoma is the leading cause of infection-induced irreversible blindness. Ongoing attempts aim to develop a safe vaccine that protects from pathologies and prevents spreading of the pathogen in the longer term.

Here we present a case study in which the PEPperCHIP® Neglected Tropical Disease Microarray was applied to identify immunogenic, antibody-inducing epitopes in *C. trachomatis*. The high-density peptide microarray covers 3,390 different linear B-cell epitopes of the Immune Epitope Database that are associated with causative organisms of ten different NTDs including Onchocerciasis (river blindness), lymphatic filariasis (elephantiasis), schistosomiasis (bilharzia), tachoma, helminthiases, leprosy (Hansen's disease), leishmaniasis, dracunculiasis (Guinea worm disease), Chagas disease and human African trypanosomiasis (sleeping sickness). The epitopes are converted into 5,356 different 15 amino acid peptides, from which 631 peptides are assigned to *C. trachomatis*-mediated B-cell responses. Sera of 5 individuals, diagnosed with *C. trachomatis* infection, were tested for IgG and IgA responses and analyzed for frequently recognized epitopes. The data shows that a group of overlapping peptides derived from the translocated actin recruiting phosphoprotein (tarp) was recognized by serum-derived antibodies of all tested patients. As the secreted tarp is mandatory for the entry of the pathogen into human epithelial cells, a facilitated tarp-neutralizing antibody-response might prevent spreading of the pathogen and help clearing the infection.

In summary, this case study on *C. trachomatis* shows exemplary that the PEPperCHIP® Neglected Tropical Disease Microarray enables the finding of immunogenic B-cell epitopes in NTD-causing pathogens for the development of new vaccines.

INTRODUCTION

Neglected tropical diseases (NTDs) are a group of diseases that occur under tropical and sub-tropical climate conditions and are intimately linked to poverty (1). The World Health Organization (WHO) lists 25 NTDs that comprise a diverse group of diseases caused by bacteria, helminths, protozoa or viruses (2). These diseases affect more than 1 billion people worldwide, and global efforts are undertaken to prevent, control, eliminate and eradicate NTDs (2). One member of the NTD family is trachoma, a disease of the eye caused by infection with the bacterium *Chlamydia trachomatis* (*C. trachomatis*). As a public health problem in 42 countries, trachoma is responsible for the irreversible blindness or visual impairment of about 1.9 million people, mostly children and women (3). Infection spreads through personal contact (via hands, clothes, bedding or hard surfaces) and by flies that have been in contact with nasal or eye discharge from infected individuals. With repeated episodes of infection, the eyelashes may be drawn in and permanently mediate irritations. This causes pain and long-term damage of the cornea. Current control measures are based on surgery, antibiotics, facial cleanliness and environmental improvement. However, for elimination of blinding trachoma, safe and effective vaccinations are mandatory (3). A protective vaccine should induce a T-cell response that targets infected host cells as well as a B-cell response that provides neutralizing antibodies.

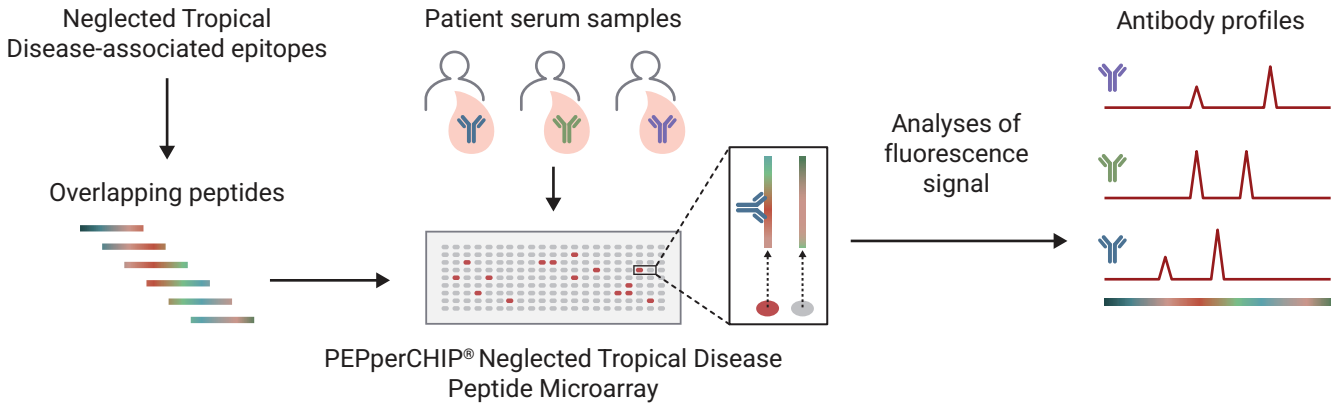

Figure 1. Workflow. A total of 3,390 epitopes from 24 different pathogens associated with 10 neglected tropical diseases were printed in duplicates on the microarray. Epitopes were represented by peptides with up to 15 amino acids in length, with a 14 amino acid overlap for sequences longer than 15 residues. Poliovirus and hemagglutinin control peptides framed the epitope content. Sera from 5 confirmed *Chlamydia trachomatis*-infected patients were incubated on the PEPperCHIP® Neglected Tropical Disease Peptide Microarrays. Serum antibody binding was visualized using fluorescence labeled secondary antibodies (anti-human IgG and anti-human IgA). After washing, microarray image acquisition and data processing was performed to identify immunogenic peptide epitopes.

High-density peptide microarrays are a powerful tool to investigate B-cell-mediated antibody responses. Such microarrays allow the simultaneous screening of serum antibodies against thousands of peptides, enabling the identification of immunogenic B-cell epitopes in a high-throughput context (4). In this case study, we applied the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray based on 3,390 different database epitopes of the Immune Epitope Database (5) that are associated with 10 NTDs, including Onchocerciasis (river blindness), Lymphatic filariasis (elephantiasis), Leishmaniasis, Chagas disease, human African trypanosomiasis (sleeping sickness) and trachoma. 93 of these in part very long database epitopes are assigned to trachoma-causing *C. trachomatis*, and were converted into 631 different 15 amino acid peptides with a peptide overlap of 14 amino acids for high resolution epitope data. To identify immunogenic *C. trachomatis* epitopes, sera of 5 infected individuals were tested for peptide-specific IgG and IgA responses. The analyses highlighted a peptide motif from translocated actin recruiting phosphoprotein (tarp) which was recognized by all tested patients. Tarp is responsible for the entry of the pathogen into the host cell. As *C. trachomatis* is completely dependent on the intracellular localization for replication and a new infection cycle, neutralizing antibodies against tarp could prevent spreading of the bacterium and a severe course of infection.

RESULTS AND DISCUSSION

In the case study, we mapped the humoral immune responses of individuals that were diagnosed with *C. trachomatis* infection with high epitope resolution. To determine the IgG and IgA antibody responses against *C. trachomatis* raised by infection, we screened sera of 5 patients (3 female, 2 male) with PEPperCHIP® Neglected Tropical Diseases Peptide Microarrays (Figure 1). The PEPperCHIP® Neglected Tropical Diseases Peptide Microarray covers 5,356 different peptides printed in duplicate that are associated with pathogens of 10 NTDs. The array is framed by poliovirus VP1 protein and influenza hemagglutinin (HA) derived control peptides. The sera were diluted 1:500 and incubated overnight at 4°C. The next day, binding of serum IgG and IgA antibodies was detected in parallel with fluorescently labeled secondary anti-IgG and anti-IgA antibodies. The frame of the HA peptide microarray was stained with a fluorescently labeled mouse monoclonal anti-HA antibody (Figure 2A). Fluorescence readout was performed using an Innopsys InnoScan 710-IR Microarray Scanner. The red spots in the microarray images represent IgG responses; the green spots

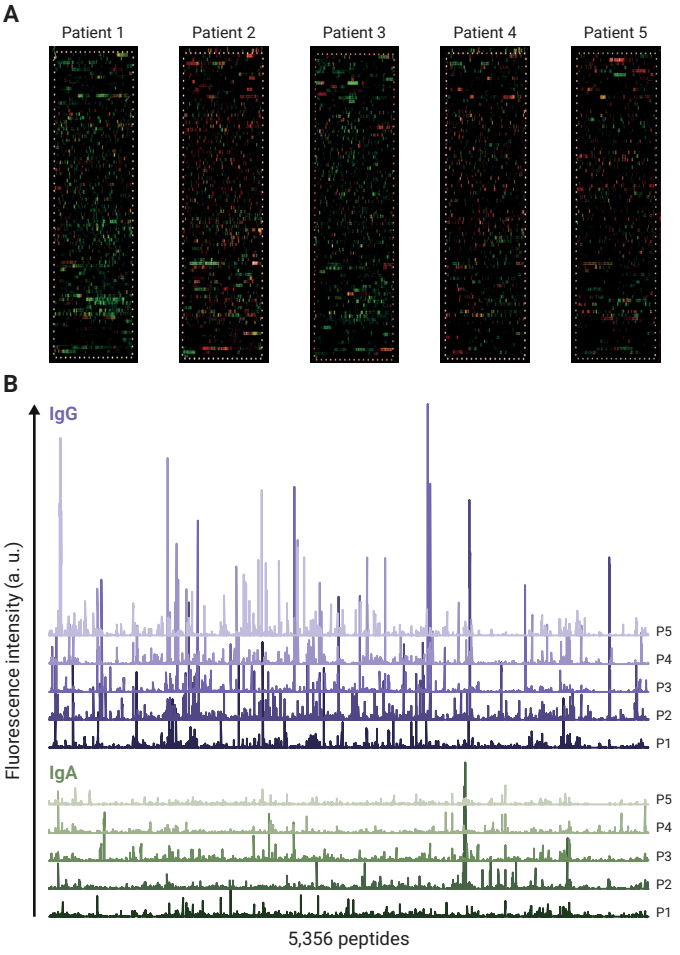

Figure 2. Discovery of B-cell receptor epitopes with high-density peptide microarrays. (A) Microarray scan images of the PEPperCHIP® Neglected Tropical Diseases Peptide Microarrays with IgG responses in red and IgA responses in green; (B) IgG and IgA fluorescence intensity plots plotted in a row wise manner from top left to bottom right of the microarray. For clarity in the intensity plots, the baselines were shifted up. The microarrays were incubated with sera of 5 patients diagnosed with *Chlamydia trachomatis* infection at a dilution of 1:500 followed by detection with secondary goat anti-human IgG (Fc) DyLight680 and goat anti-human IgA (alpha chain) DyLight800 antibodies. To stain the frame of HA control peptides, a mouse monoclonal anti-HA DyLight800 antibody was used. Fluorescence readout was performed using an INNOPSYS Imaging System. Fluorescence intensities were quantified with Mapix Analyzer.

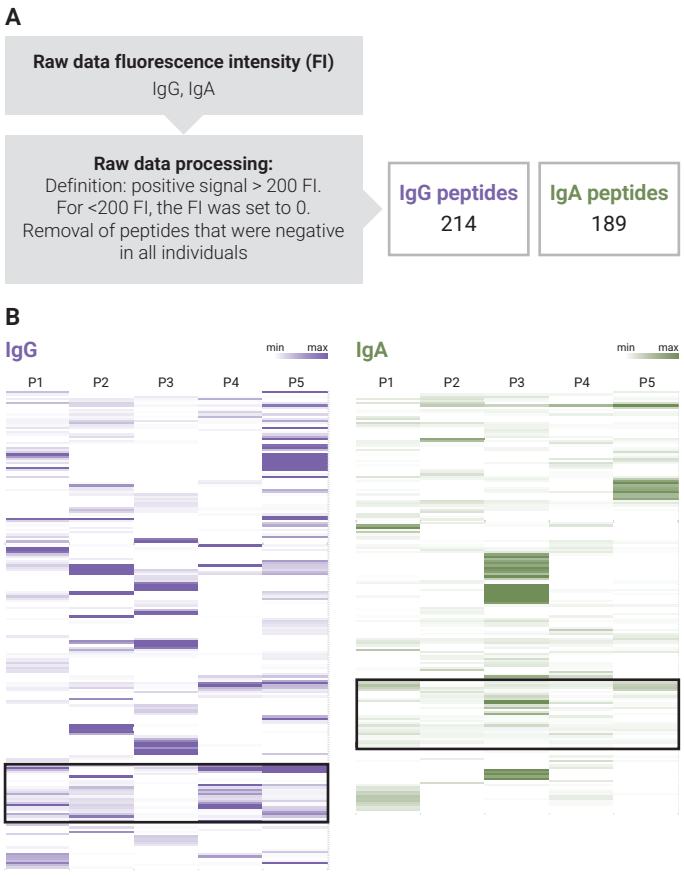

Figure 3. Data processing and results with the *C. trachomatis* peptides. (A) Intensities with >200 fluorescence units were regarded as real signals, all intensity values below this threshold were zeroed and excluded from the data set. This resulted in 214 IgG and 189 IgA responses for further analysis (B) Heat map of the IgG and IgA responses of the remaining peptides with violet (IgG) or green (IgA) for high and white for low intensities. The columns correspond to the different patients, the rows to the individual peptides. The black frames highlight translocated actin recruiting phosphoprotein (tarp) peptides.

correspond to binding of IgA antibodies. IgG and IgA fluorescence intensities were quantified with Mapix Analyzer and plotted in a row wise manner from top left to bottom right of the microarray (Figure 2B). As expected for human serum samples, the in-depth analysis of the IgG and IgA responses to NTD-associated peptides highlighted diverse antibody profiles varying between the 5 patients, reflecting repertoire of circulating antibodies.

In the following, data analysis was focused on the 631 *C. trachomatis*-derived peptides that are included in the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray. We determined mean IgG and IgA fluorescence intensities of peptide double spots. In a pre-processing of the microarray data, all intensity values below 200 fluorescence units were zeroed, followed by removal of peptides with an intensity value = 0 in all samples. This pre-processing resulted in 214 remaining IgG-binding peptides and 189 remaining IgA-binding peptides (Figure 3A). The IgG and IgA fluorescence intensities of the remaining peptides are visualized as heat maps in Figure 3B. The color scheme uses red (IgG) or green (IgA) for high and white for low intensities.

The heat map shows that a group of overlapping peptides (epitope stretch) was recognized by IgG and IgA antibodies in the majority of patients. These peptides are derived from *C. trachomatis* translocated actin recruiting phosphoprotein (tarp). Tarp is a chlamydial type III secreted effector protein implicated in the bacteria-induced cytoskeletal changes that permit the entry of the infectious elementary bodies (EBs) into the host cell (6). Once internalized, the EB differentiates into a reticulate body (RB) within a membrane-bound vacuole called inclusion, and undergoes several rounds of replication before differentiating back to an EB, which is released from the infected cell and can initiate a new infection cycle (7,8).

Moreover, the immunogenic tarp epitope was evaluated in more detail with the original microarray data. Table 1 depicts the mean fluorescence intensities of all tarp peptides of the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray as data bars (red = IgG, green = IgA). Peptides 1-16 and 18-33 are overlapping sequences (15 aa, peptide overlap of 14 aa) of published longer immunogenic sequences (9). According to our results, the most consistent antibody recognition was observed for the overlapping peptides SSNYDDAAADYEPIR, SNYDDAAADYEPIRT, NYDDAAADYEPIRTT, YDDAAADYEPIRTTE sharing the common motif YDDAAADYEPIR as proposed epitope. The strongest overall antibody response was directed against tarp peptides SSNYDDAAADYEPIR and SNYDDAAADYEPIRT.

Tarp includes three distinct domains; the tyrosine rich repeat region (amino acids 125–424), a proline rich domain required for tarp multimerization (amino acids 625–650), and an actin binding domain (amino acids 748–758) (10). The proposed tarp epitope YDDAAADYEPIR is located in the tyrosine rich repeat region of tarp with eight repeat units (UniProt E0A8V6). Interestingly, it was shown previously that individuals infected with *C. trachomatis* in the urogenital tract or the eye can develop a robust antibody response to tarp, and that immunization with tarp induces protection against challenge infection in mice (11). Therefore, our results are in line with previous published data and provide further information on the actual immunogenic epitope of the antigen. In order to verify the results, a large cohort study should be performed. Such studies could include further antigens of *C. trachomatis* that have not been described as immunogenic so far. The peptide microarray format, such as with the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray, represents the optimal platform to investigate a large amount of potential vaccine candidates in a high-throughput manner.

CONCLUSION

NTDs such as *C. trachomatis*-mediated trachoma cause devastating health, social and economic consequences to more than one billion people (1, 2). Great effort is undertaken to improve this situation. However, without urgently needed safe and effective vaccines, the control of NTDs will remain a challenge. By the exemplary identification of B-cell epitopes in *C. trachomatis* antigens, this application note highlights peptide microarrays as powerful tool to investigate the immune response towards the pathogen, and to identify immunogenic peptide sequences for the development of new vaccines.

Table 1. Antibody responses to tarp (translocated actin recruiting phosphoprotein). Mean fluorescence intensities of the peptides are shown as numbers with data bars (violet = IgG, green = IgA). Peptides 1-16 and 17-30 are overlapping sequences (15 aa, peptide overlap of 14 aa) of published longer immunogenic sequences (9).

			IgG					IgA				
	Protein	Peptide	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
1	TarP	SSNYDDAAADYEPIR	720	458	283	1,394	5,409	666	266	355	194	490
2	TarP	SNYDDAAADYEPIRT	2,076	100	555	2,567	5,069	694	13	308	298	668
3	TarP	NYDDAAADYEPIRTT	936	0	241	3,722	5,521	1,060	99	440	359	869
4	TarP	YDDAAADYEPIRTTE	310	0	115	686	5,786	814	394	329	252	961
5	TarP	DDAAADYEPIRTTEN	89	21	252	0	0	574	244	130	0	797
6	TarP	DAAADYEPIRTTENI	0	5,867	0	0	0	0	497	172	124	0
7	TarP	AAADYEPIRTTENIY	0	0	0	91	0	0	201	405	13	206
8	TarP	AADYEPIRTTENIYE	94	203	0	92	93	0	288	703	112	336
9	TarP	ADYEPIRTTENIYES	142	137	0	0	0	179	218	537	28	128
10	TarP	DYEPIRTTENIYESI	0	232	102	0	0	461	246	2,519	206	0
11	TarP	YEPIRTTENIYESIG	0	0	99	146	0	0	189	2,080	538	125
12	TarP	EPIRTTENIYESIGG	0	0	0	0	0	0	0	512	0	110
13	TarP	PIRTTENIYESIGGS	0	0	0	5	86	0	182	1,097	254	324
14	TarP	IRTTENIYESIGGSR	0	0	0	0	0	0	0	0	160	0
15	TarP	RTTENIYESIGGSRT	0	0	0	6	0	0	0	492	0	0
16	TarP	TTENIYESIGGSRTS	0	0	150	0	98	0	0	1,445	0	113
17	TarP	SSDHIPSDYDDVGSN	456	370	0	756	198	75	114	151	92	109
18	TarP	SDHIPSDYDDVGSNS	1,055	428	0	1,327	205	136	172	81	88	99
19	TarP	DHIPSDYDDVGSNSG	277	176	0	517	149	235	0	244	95	157
20	TarP	HIPSDYDDVGSNSGD	377	469	113	416	220	487	294	652	193	203
21	TarP	IPSDYDDVGSNSGDI	290	432	116	686	599	223	222	199	248	203
22	TarP	PSDYDDVGSNSGDIS	708	404	0	1,323	96	311	209	193	251	303
23	TarP	SDYDDVGSNSGDISN	13,538	587	0	5,886	99	349	204	96	104	97
24	TarP	DYDDVGSNSGDISNN	296	524	106	6,840	596	572	243	196	580	110
25	TarP	YDDVGSNSGDISNNY	281	360	0	135	690	169	229	193	234	0
26	TarP	DDVGSNSGDISNNYD	0	303	5	82	1,256	106	97	146	91	102
27	TarP	DVGSNSGDISNNYDD	1,078	677	116	86	1,506	332	239	100	187	201
28	TarP	VGSNSGDISNNYDDV	416	1,746	98	244	742	383	251	97	237	201
29	TarP	GSNSGDISNNYDDVG	131	199	0	133	151	246	0	0	96	0
30	TarP	SNSGDISNNYDDVGS	165	840	7	2,776	916	0	0	0	221	0

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