Identification of immunogenic epitopes of *Chlamydia trachomatis* via PEPperCHIP® Neglected Tropical Disease Peptide Microarray

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INTRODUCTION

Neglected tropical diseases (NTDs) are a group of diseases, intimately linked to poverty, that occur under tropical and sub-tropical climate conditions. NTDs are caused by bacteria, helminths, protozoa, or viruses. They affect more than 1 billion people worldwide. One member of the NTD family is Trachoma, a disease of the eye caused by infection with the bacterium *Chlamydia trachomatis (C. trachomatis)*. Trachoma is responsible for the irreversible blindness or visual impairment of about 1.9 million people, most of them children and women. To eliminate Trachoma induced blinding, safe and effective vaccinations are mandatory.

A protective vaccine should induce a T-cell response that targets infected host cells, as well as a B-cell response that guarantees neutralizing antibodies. For the investigation of B-cell-mediated antibody responses and the identification of immunogenic epitopes, high-density peptide microarrays are a powerful tool. This format allows simultaneously screening tens of thousands of peptides against serum antibodies, enabling the identification of immunogenic B-cell epitopes in a high-throughput context.

KEY FINDINGS

We identified a peptide sequence within the translocated actin recruiting phosphoprotein (Tarp) of *Chlamydia trachomatis* as highly immunogenic. As Tarp is responsible for the entry of the pathogen into the host cell, vaccination-induced neutralizing antibodies against Tarp could prevent the spreading of the bacterium and a severe course of infection.

WORKFLOW

In this case study, we applied the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray that covers 5,356 different epitopes associated with 10 NTDs, including Onchocerciasis (river blindness), Lymphatic filariasis (elephantiasis), Leishmaniasis, Chagas disease, human African trypanosomiasis (sleeping sickness) and Trachoma. It includes 93 different epitopes converted into 631 peptides assigned to Trachoma-causing *C. trachomatis*. To investigate immunogenic epitopes in *C. trachomatis*-derived peptides, sera of 5 infected individuals were tested for peptide-specific IgG and IgA occurrence.

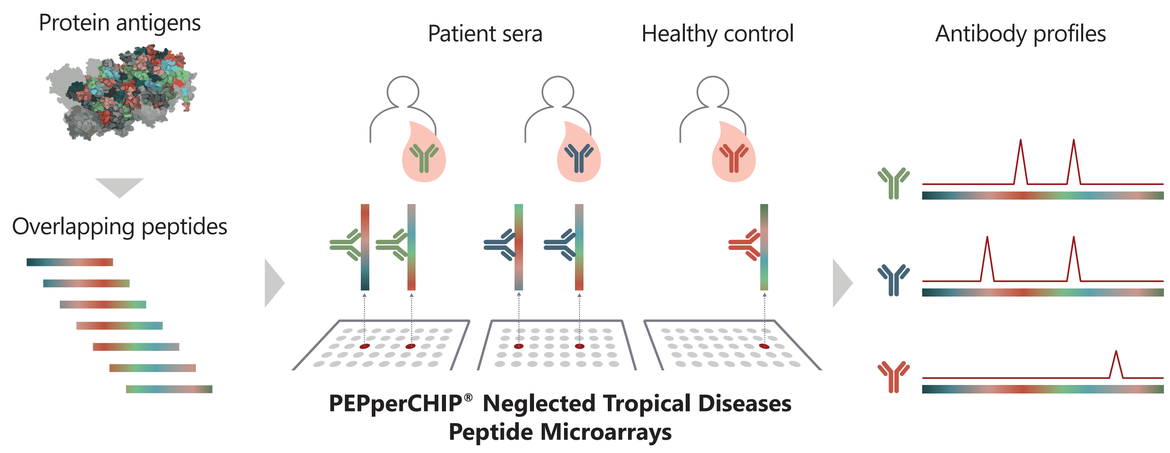


Figure 1. Workflow. Antigens from 10 neglected tropical diseases were translated into 15-mer overlapping peptides with a peptide-to-peptide overlap of 14 amino acids. The resulting 5356 individual peptides were printed in duplicates on the microarray and framed by poliovirus and hemagglutinin control spots. Sera from 5 confirmed *Chlamydia trachomatis*-infected patients were incubated on PEPperCHIP® Neglected Tropical Disease Peptide Microarrays. Serum antibody binding was visualized using respective fluorescence-labeled secondary antibodies (anti-human IgG and anti-human IgA). Image acquisition and data processing were performed to identify immunogenic peptide epitopes.

RESULTS

The in-depth analysis of the IgG and IgA responses to NTD-associated peptides revealed a diverse antibody profile varying between the 5 patients, as expected (Figure 2).

In the following, analyses of data were concentrated on 631 *Chlamydia trachomatis*-derived peptides. The raw data of fluorescence intensity (mean of double determination) of IgG and IgA binding was processed as shown in Figure 3A. The data processing resulted in 214 remaining IgG-binding peptides and 189 remaining IgA-binding peptides. The FIs assigned to the remaining peptide-antibody complexes are shown as heat map in Figure 3B.

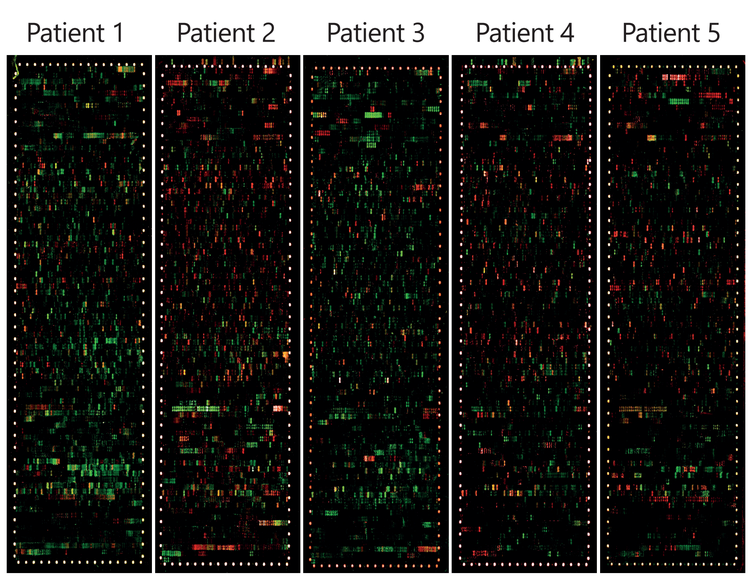
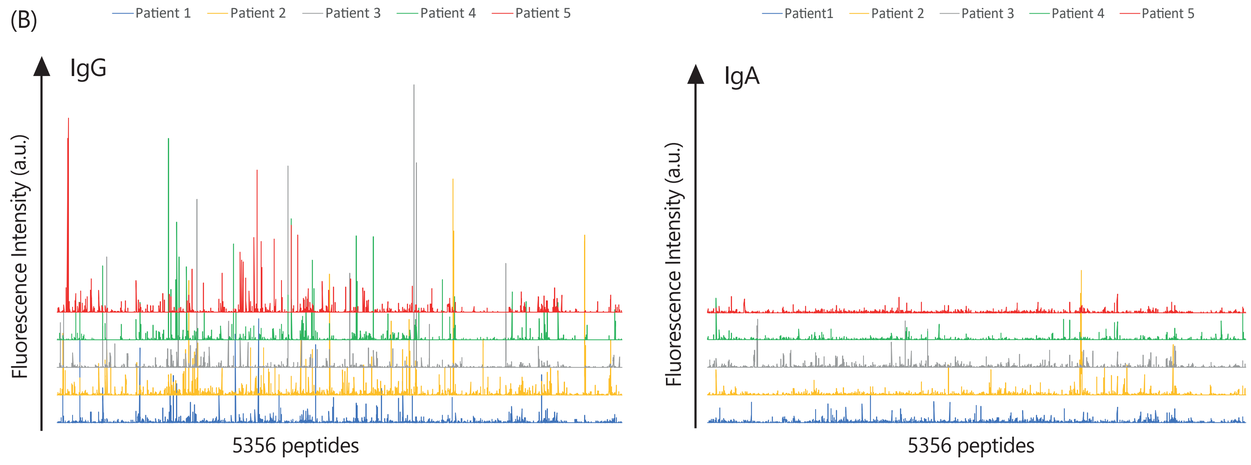


Figure 2. Discovery of B-cell receptor epitopes via peptide microarray. (A) Scans of the PEPperCHIP® Neglected Tropical Diseases Peptide Microarray and (B) fluorescence intensity plots. The microarrays were incubated with sera of 5 patients diagnosed with *Chlamydia trachomatis* infection at a dilution of 1:500 overnight at 4°C. Detection was done using the secondary goat anti-human IgG (Fc) DyLight680 antibody and goat anti-human IgA (light chain) DL800. For the control frame, a rabbit anti-HA DL800 antibody was used. Fluorescence readout was performed using an INNOPSYS Imaging System. (A) Red spots=IgG responses. Green spots=IgA responses. Fluorescence intensity was quantified via the PepSlide Analyzer software and shown as line graphs (one color per donor) in (B).



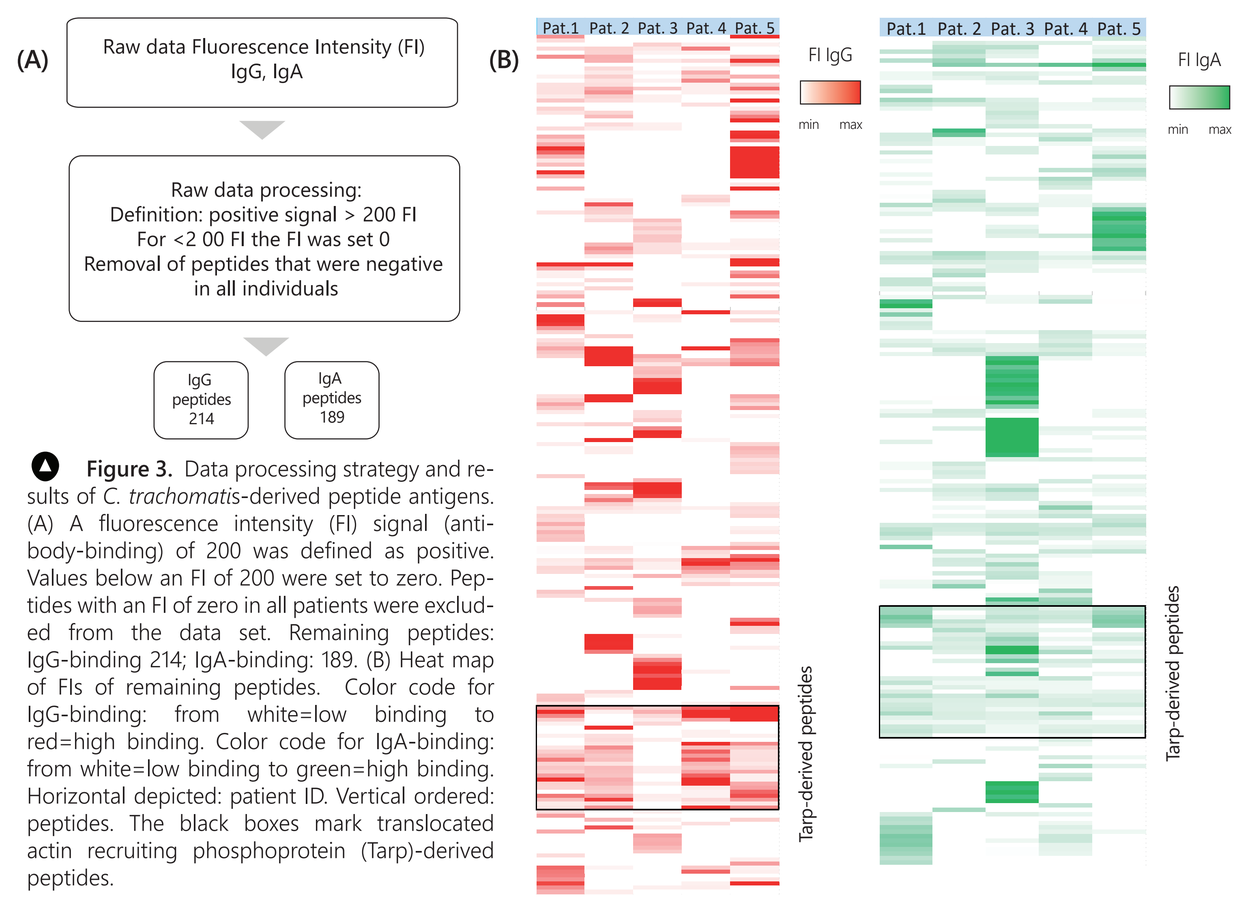


Figure 3. Data processing strategy and results of *C. trachomatis*-derived peptide antigens. (A) A fluorescence intensity (FI) signal (antibody-binding) of 200 was defined as positive. Values below an FI of 200 were set to zero. Peptides with an FI of zero in all patients were excluded from the data set. Remaining peptides: IgG-binding 214; IgA-binding: 189. (B) Heat map of FIs of remaining peptides. Color code for IgG-binding: from white=low binding to red=high binding. Color code for IgA-binding: from white=low binding to green=high binding. Horizontal depicted: patient ID. Vertical ordered: peptides. The black boxes mark translocated actin recruiting phosphoprotein (Tarp)-derived peptides.

The heat map shows that one overlapping group of peptides was recognized by IgG and IgA antibodies in the majority of patients. These peptides are derived from *C. trachomatis* translocated actin recruiting phosphoprotein (Tarp).

Figure 4 depicts the FI values of the Tarp-derived peptide sequences (SSNYDDAAADYEPIR, SNYDDAAADYEPIRT, NYDDAAADYEPIRTT, YDDAAADYEPIRTTE) with most consistent antibody recognition. At this, the highest overall antibody response was seen against SSNYDDAAADYEPIR and SNYDDAAADYEPIRT.

Fluorescence Intensity (FI) IgG

Protein	Peptide sequence	Pat. 1	Pat. 2	Pat. 3	Pat. 4	Pat. 5
TarP	SSNYDDAAADYEPIR	720	458	283	1394	5409
TarP	SNYDDAAADYEPIRT	2076	100	555	2567	5069
TarP	NYDDAAADYEPIRTT	936	0	241	3722	5521
TarP	YDDAAADYEPIRTTE	310	0	115	686	5786

Fluorescence Intensity (FI) IgA

Protein	Peptide sequence	Pat. 1	Pat. 2	Pat. 3	Pat. 4	Pat. 5
TarP	SSNYDDAAADYEPIR	666	266	355	194	490
TarP	SNYDDAAADYEPIRT	694	13	308	298	668
TarP	NYDDAAADYEPIRTT	1060	99	440	359	869
TarP	YDDAAADYEPIRTTE	814	394	329	252	961

Figure 4. Antibody responses to translocated actin recruiting phosphoprotein (Tarp)-derived peptides. Shown are the mean FI values of duplicate measurements as stacking data bars. In red IgG-binding. In green IgA-binding.

CONCLUSIONS

This case study shows the great usefulness of peptide microarrays to investigate the immune response toward a pathogen and to identify immunogenic peptide sequences for the development of new vaccines.

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