Discovery and characterization of C1q-associated mimotopes in SLE patients

Sarah Schott[1], Dagmar Hildebrand[1], Elke Hoffner[1], Fiordiligie Casilag[1], and Volker Stadler[1]

[1]PEPperPRINT GmbH, Heidelberg, Germany

KEY FINDINGS

- Via PEPperCHIP® Peptide Microarrays we identified significant C1q-derived B-cell epitopes in SLE patients
- The epitope mimicking mimotopes blocked binding of serum-derived autoantibodies efficiently
- The mimotopes did neither induce cytotoxicity in PBMCs nor a harmful T-effector cell activation

BACKGROUND AND AIMS

A safe and effective SLE treatment should suppress the immune system as less as possible and target-oriented, to prevent side effects. Recently, antigen-specific treatment options came into focus. Peptides that mimic self-epitopes (mimotopes), block auto-antibodies and prevent mediated tissue damage are a promising tool to restore antigen-specific tolerance and to reduce clinical manifestations. Here we aimed to identify blocking mimotopes for anti-dsDNA autoantibodies and subsequently validate a possible harmful T-cell modulating effect of the mimotopes.

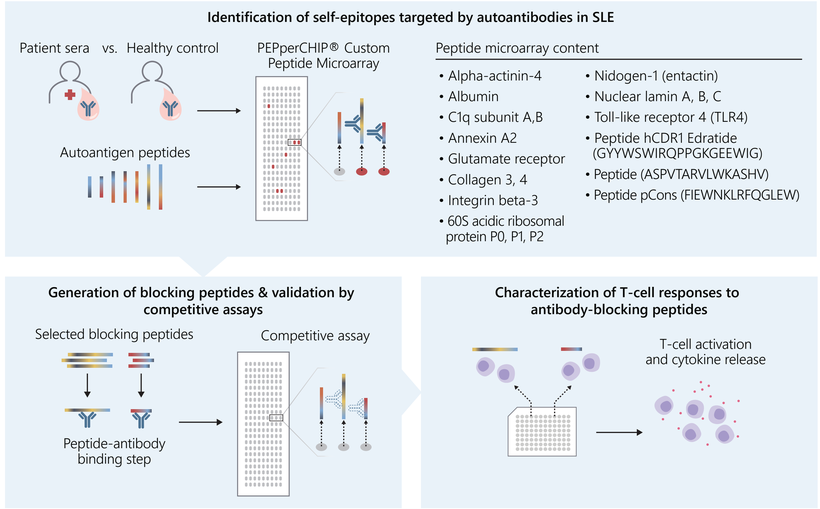

Figure 1. Graphical workflow. 1. For the identification of self-epitopes, sera are screened with PEPperCHIP® Peptide Microarrays. 2. Autoantibody-targeted epitopes are mimicked with synthetic peptides. Blocking values of peptides are evaluated in a competitive approach. 3. Functional blocking peptides are tested for mediated T-cell-activation.

METHODS

To identify self-epitopes, we designed a peptide microarray with non-nucleic acid targets of anti-dsDNA autoantibodies: 4309 linear peptides, 13 aa, overlap 10 aa, including 169 peptides of C1q. We analyzed the anti-IgG antibody response of SLE patients in comparison to healthy donors. Promising peptides were tested for specific blocking of antibodies and validated for T-cell activation via ELISpot assays (Fig.1).

RESULTS

1. Identification of B-cell self-epitopes in SLE patients

The analysis of the IgG responses revealed an increased expression of autoantibodies in SLE patients compared to healthy controls, as expected (Fig.2).

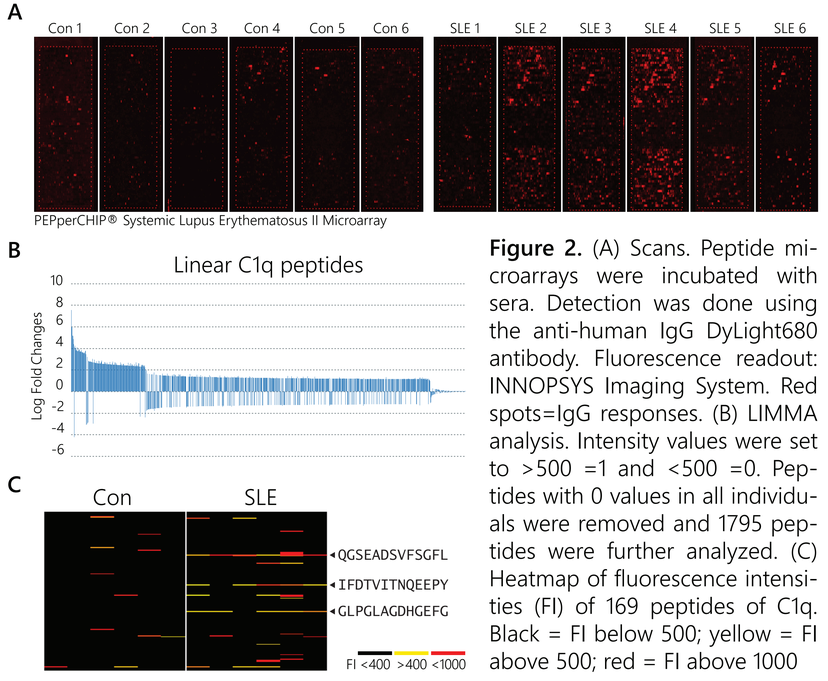

Figure 2. (A) Scans. Peptide microarrays were incubated with sera. Detection was done using the anti-human IgG DyLight680 antibody. Fluorescence readout: INNOPSYS Imaging System. Red spots=IgG responses. (B) LIMMA analysis. Intensity values were set to >500 =1 and <500 =0. Peptides with 0 values in all individuals were removed and 1795 peptides were further analyzed. (C) Heatmap of fluorescence intensities (FI) of 169 peptides of C1q. Black = FI below 500; yellow = FI above 500; red = FI above 1000

Three of the C1q-derived peptides (IFDTVITNQEEPY and QGSEADSVFSGFL from the alpha subunit and GLPGLAGDHGEFG from the beta subunit) showed significantly more response in SLE patients and were selected for further validation (Fig.2).

2. Validation of blocking potential of C1q-associated peptides

The mimotopes were synthesized and used in competitive assays. The sera of SLE patients were preincubated with the three peptides before the screen on the peptide microarray. All three mimotopes could effectively block binding of serum-derived autoantibodies to C1q-derived peptides.

3. Analysis of peptide-mediated T-cell activation

The mimotopes were first tested for mediated cytotoxicity on PMCS. At this, no alarming change in cell viability was observed. Subsequently perfomed IFNg Fluorospots excluded a harmful activation of T effector cells (Fig.3).

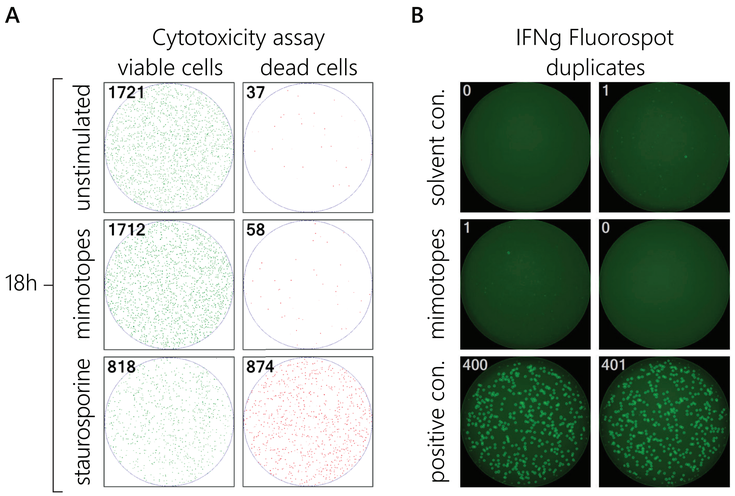

Figure 3. (A) PBMCs were treated with mimotopres (50µg/ml) or staurossporine and stained with CTL-LDC™ Live/Dead Cell Counting Kit. Viable cells: green; dead cells: red. (B) Results of an IFNg Fluorsopot assay. PBMCs were treated with solvent or pooled mimotopes or an CEF control Peptide Pool (10µg/ml).

SUMMARY AND CONCLUSION

We identified three C1q-derived self-epitopes in SLE patients. The respective soluble mimotopes blocked antibody-binding epitope-specific and showed no alarming activation of effector T-cells.

Peptide microarrays are a powerful tool to investigate the misdirected humoral immune response in autoimmune diseases and can support the development of peptide-based therapeutic strategies.