Linear and Conformational Epitope Mapping of the Myasthenia Gravis Antigen Acetylcholine Receptor

Introduction

Myasthenia gravis (MG) is a neuromuscular autoimmune disease associated with antibodies against the acetylcholine receptor (AChR) and/or other proteins of the neuromuscular junction such as muscle-specific kinase (MuSK) or lipoprotein receptor-related protein 4 (LRP4). The clinical features of MG are heterogeneous, depending on the nature of the targeted autoantigens. Each MG form requires different therapies. Autoantibodies against AChR are present in about 85 % of MG cases, less frequently observed are antibodies against MuSK or LRP4. The role of epitope specificity in the pathophysiology of MG is yet not fully understood.[1] However, the characterization of epitopes in MG would help to elucidate the mechanisms underlying the disease and may provide the basis for novel, more targeted therapies.

In this application note, we performed a high-resolution epitope analysis of IgG and IgA responses towards the most common MG antigen, the α_1-subunit of the AChR. Linear and conformational epitope mappings were performed on customized PEPperPRINT microarrays with sets of overlapping linear and cyclic constrained peptides derived from the antigen sequence. We compared two MG patient sera and one healthy control serum and observed well-defined polyclonal IgG and IgA responses both in the linear and the conformational search area. Unique linear and conformational epitopes were identified for each patient, emphasizing the efficiency of the combined approach for a comprehensive epitope characterization.

Results & Discussion

For high-resolution epitope mapping, customized linear and cyclic constrained peptide microarrays were generated. The amino acid sequence of the α_1-subunit of AChR was translated into sets of 7, 10 and 13 amino acid peptides at maximum peptide-peptide overlap, and peptides were synthesized on the array either as linear or cyclic constrained peptides. Peptide cyclization was performed via a thioether linkage between a C-terminal cysteine thiol and an appropriately modified N-terminus. The resulting microarrays comprised 1,400 different peptides printed in duplicate and were framed by HA (YPYDVPDYAG) positive control peptides.

Peptide microarrays were incubated with patient and healthy control sera (MG sera 01637 and 00456, in.vent Diagnostika, Henningsdorf, Germany) at a dilution of 1:1000 in PBST buffer. Subsequently, the

[1] M.G. Huijbers et al., J. Intern. Med. 2014; 275:12-26

microarrays were stained with secondary antibodies goat anti-human IgG DyLight680 or goat anti-human IgA DyLight800 together with monoclonal anti-HA-DyLight680 or monoclonal anti-HA-DyLight800 control antibodies. Microarrays were scanned with a LI-COR Odyssey Imaging System. Quantification of spot intensities and peptide annotation was done with PepSlide® Analyzer.

Whereas no IgG and IgA responses were detectable in the healthy control serum, the sera of MG patients showed clear defined signal pattern ranging from moderate to strong fluorescence intensity, as exemplified in Figure 1.

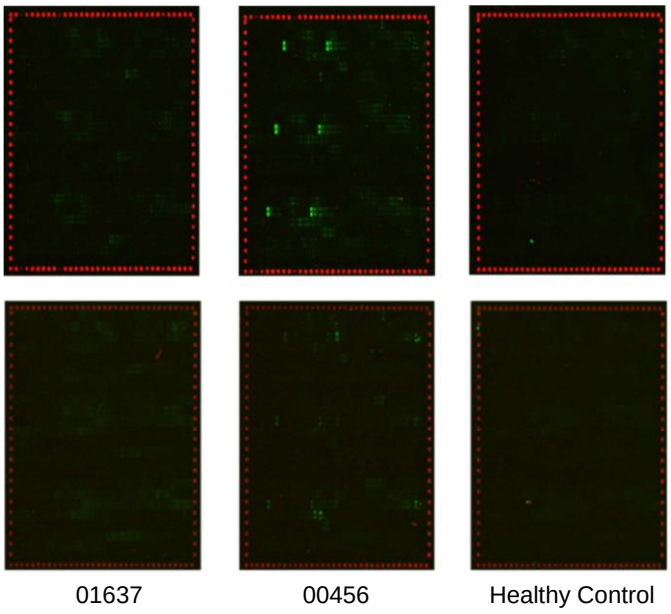

Fig. 1: IgA Response on linear and conformational peptide microarrays: PEPperCHIP® Peptide Microarrays displaying the α_1-subunit of the AChR translated into overlapping peptides of 7, 10 and 13 aa. The arrays were assayed for IgA responses with healthy control serum and MG sera 00456 and 01637, followed by staining with labeled antihuman IgA (green spots) and anti-HA antibodies (red spots). While the healthy control did not show any signal, both MG patient sera exhibited clear epitope stretches. HA positive control peptides framing the peptide array showed the expected well-defined spot pattern. Top row: conformational peptide arrays. Bottom row: linear peptide arrays.

IgA Profile: While the IgA reactivity of MG serum 01637 was rather weak towards linear peptides and conformational peptides, MG serum 00456 exhibited a pronounced IgA response. Major conformational epitopes were the cyclic peptides QYTGHITWTP and IIVTHFP, both motives binding at strong signal intensity. Peptide IIVTHFP was also recognized on the linear peptide array, albeit at a far lower signal intensity. This epitope cannot be considered as strictly conformational and points to a more flexible binding site on the antibody. The healthy control did not show any IgA immunoreactivity towards the α_1-subunit of AChR (Figure 2).

IgG Profile: Serum 01637 exhibited a distinct IgG response. Major conformational epitopes were the cyclic peptides VTHPFD and NNADGDFFAIVT, interacting at medium to strong signal intensity. Two linear epitopes with the core motifs MESGEWV and SEKIWRPDLVLYN were identified, both peptides showing medium spot intensities. The IgG profile of MG serum 00456 was clearly different. Whereas the IgG response towards linear epitopes was very weak, several conformational epitopes were observed at weak to medium signal intensity. The main conformational epitope was the cyclic peptide NEQWVDY. The healthy control did not show any IgG immunoreactivity towards the α_1-subunit of AChR (Figure 3).

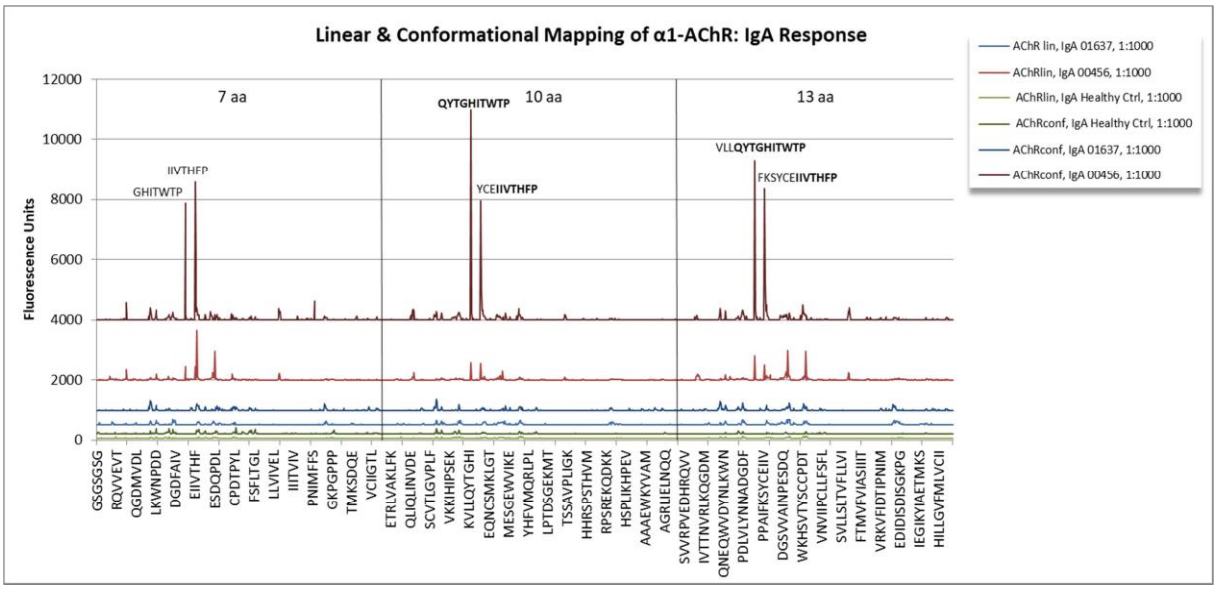

Fig. 2: IgA profiles of healthy control and MG sera on linear and conformational peptide arrays. The intensity plots of the AChR peptides clearly highlight the differential IgA responses of the analyzed MG sera both towards linear and conformational epitopes. X-axis from left to right: sequences of α_1 subunit of AChR derived peptides, translated into 7 aa, 10 aa and 13 aa overlapping peptides.

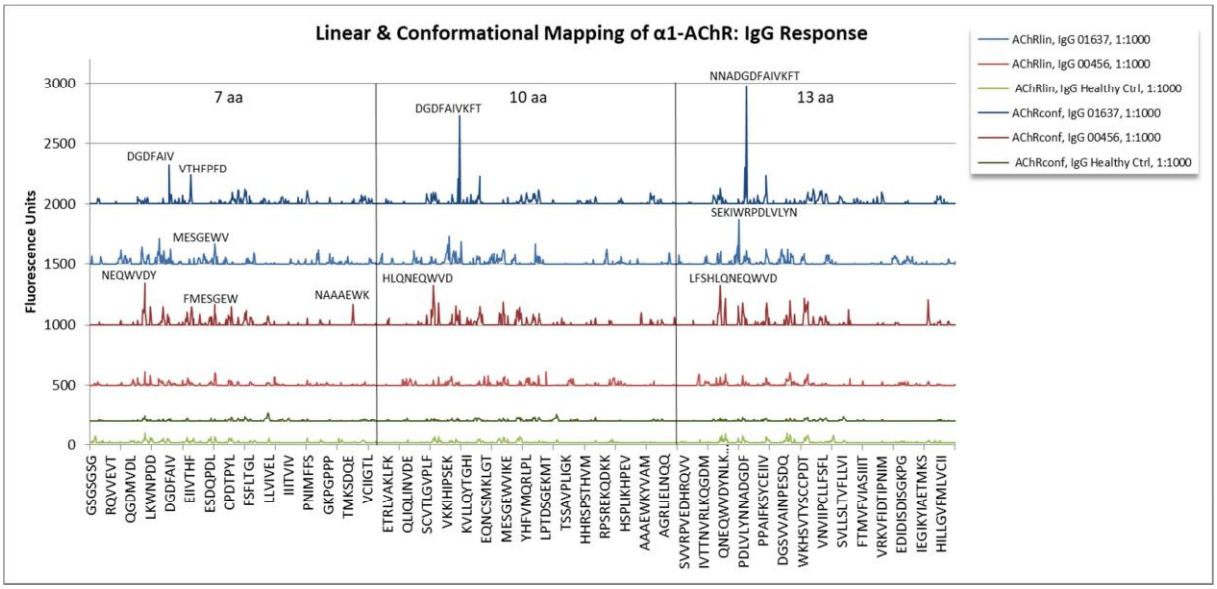

Fig. 3: IgG profiles of healthy control and MG sera on linear and conformational peptide arrays. The intensity plots of the AChR peptides clearly highlight the differential IgG responses of the analyzed MG sera both towards linear and conformational epitopes. X-axis from left to right: sequences of α_1 subunit of AChR derived peptides, translated into 7 aa, 10 aa and 13 aa overlapping peptides.

Conclusion

Here we performed a linear and conformational epitope mapping of the main MG associated antigen, the α_1-subunit of AChR. Peptide microarrays with linear and cyclic constrained peptides were screened for IgG and IgA immunoreactivities using two patient sera and one healthy control sera. We identified individual combinations of linear and conformational epitopes for both patients, with stronger reactivity towards conformational epitopes. Both IgG and IgA specific epitopes were identified for serum 00456. In contrast, serum 01637 responded predominantly to IgG specific epitopes. The pathophysiological significance of IgA autoantibodies in MG, if any, needs to be elucidated. However, our analysis suggests that IgA just as IgG autoantibody profiles could provide a source for novel biomarkers in autoimmune diseases. Our study further underlines the necessity of combining linear and conformational epitope mapping for in-depth epitope analyses and for a comprehensive biomarker discovery.