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Use of cell-based assays in myasthenia gravis and other antibody-mediated diseases

P.M. Rodriguez Cruz [1], S. Huda [1], P. López-Ruiz, A. Vincent *

Nuffield Department of Clinical Neurosciences and Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK

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ABSTRACT

The increasing demand on diagnostic assays that are sensitive and specific for pathogenic antibodies, and the interest in identifying new antigens, prompted the development of cell-based assays for the detection of autoantibodies in myasthenia gravis and other autoimmune disorders. Cell-based assays were initially used to show that clustering the AChR improved the positivity in myasthenia gravis, and similar assays have now been applied to detection of antibodies to neuromuscular junction candidate proteins such as LRP4 and agrin. In addition cell-based assays have been used in the routine detection of antibodies to proteins expressed on the surface of neurons (NMDAR, LGI1, CASPR2, AMPAR, GABA-A/B, GlyR, and DPPX) and glia (AQP4, MOG). Here, we summarize the findings in myasthenia and discuss the advantages, disadvantages and controversial issues of using cell-based assays in the detection of these antibodies, and their relevance to the testing of preclinical models of disease.

Introduction

Classically, the diagnostic antibody tests in myasthenia gravis (MG) and other antibody-mediated diseases of the neuromuscular junction depend on radioimmunoprecipitation assay (RIA) and enzyme-linked immunoassay (ELISA). These techniques limit the sensitivity of antibody detection, as some antibodies may bind poorly to recombinant or soluble antigens, but could bind more effectively to the antigen when expressed on the cell surface as they are, of course, at the neuromuscular junction. These limitations, added to the increasing demand on diagnostic assays, which are sensitive, and specific for pathogenic extracellular epitopes, have prompted the development of diagnostic cell-based assays (CBA). These assays involve expressing antigens, which are usually membrane proteins, on the surface of human embryonic kidney (HEK) cells, which have been transfected with the appropriate cDNAs encoding the antigen required. The binding of human (or experimental animal) antibodies can then be scored visually using indirect immunofluorescence (Vincent et al., 2012). CBAs, unlike other diagnostic antibody tests, permit high-density expression levels of antigens in a natural membrane environment, where they adopt native conformational states and appropriate glycosylation levels. The expression of proteins on intact live cells avoids the detection of antibodies to intracellular epitopes, which are unlikely to be pathogenic. An additional advantage is that the antigen may be able to be clustered as will be discussed below.

Cell based assays in myasthenia gravis

The neuromuscular junction (NMJ) is a highly specialized synapse, requiring multiple proteins for the appropriate development and maintenance of its structural and functional integrity (Fig. 1A). In recent years, a number of CBAs have been developed to detect antibodies against these proteins in particular those with extracellular domains that would be accessible to antibodies present in sera. This candidate antigen approach has led to the identification of autoantibodies in patients lacking AChR and MuSK antibodies on RIA (SNMG).

Acetylcholine receptor

The detection of muscle acetylcholine receptor (AChR) antibodies has traditionally been performed using RIA (Vincent and Newsom-Davis, 1985) or less commonly ELISA (Kawanami et al., 1984). At the NMJ, AChRs are tightly clustered, thus to optimize the CBA for AChR antibodies, the AChRs are expressed on the surface of the live HEK cells, and clustered by co-expression with the intracellular anchoring rapsyn as they are in vivo (Leite et al., 2008) (Fig. 1B). This allowed identification of "clustered AChR-antibodies" in a proportion of patients who previously had been consistently negative by RIA or ELISA assays. Although not formally described, it is likely that the IgG antibodies

* Corresponding author at: Nuffield Department of Clinical Neurosciences, University of Oxford, Level 6, West Wing, John Radcliffe Hospital, Headley Way, Headington OX3 9DU, UK. Fax: +44 1865 222402.
E-mail address: Angela.vincent@ndcn.ox.ac.uk (A. Vincent).
[1] Equal contributions.

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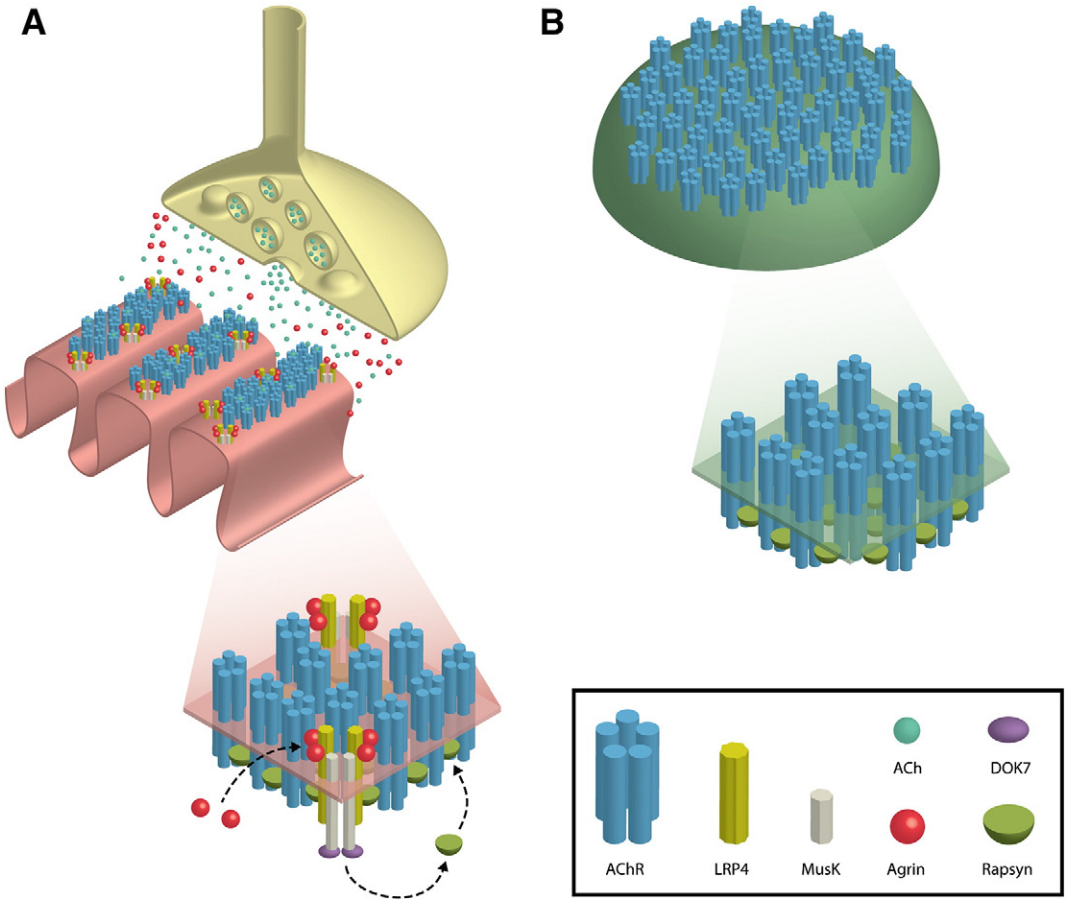

Fig. 1. Schematic representation of the NMJ (A) and the clustered AChR CBA (B). The AChR receptors are clustered at the top of the postsynaptic junctional folds by the anchoring protein rapsyn, which is stimulated through the Agrin–LRP4–MuSK–DOK7 signaling pathway. The clustered-AChR CBA detects antibodies on the surface of live HEK cells by expressing AChRs with physiological conformations clustered by the intracellular anchoring rapsyn as they are in vivo at the NMJ. These conditions allow IgG antibodies to bind more avidly, thus explaining the increased sensitivity of the CBA.

cross-link the AChRs on the surface, which would allow low-affinity IgG antibodies to bind more avidly, thus explaining the increased sensitivity of the CBA.

The proportion of SNMG with autoantibodies to clustered AChRs ranges from 16 to 60% (Devic et al., 2014; Jacob et al., 2012; Leite et al., 2008) but CBAs are not yet widely used for reasons as discussed below (*Advantages and disadvantages of CBAs*). The antibody binding can be scored visually (from 0 to 4) with 1 being the threshold for positivity. The clustered-AChR antibodies detected by these means are mainly IgG1 and can activate complement on the surface of the transfected HEK cells suggesting that they are pathogenic. Indeed, the passive transfer of IgG from patients with only clustered AChR-Abs to mice resulted in reduced AChR expression and defects in neuromuscular transmission very similar to those found concurrently following transfer of typical AChR antibodies, that are known to cross-link AChRs at the NMJ (Jacob et al., 2012). Thus the pathogenic mechanisms appear similar to those of patients with typical AChR-Abs. However, patients with clustered-AChR abs generally have relatively mild disease,

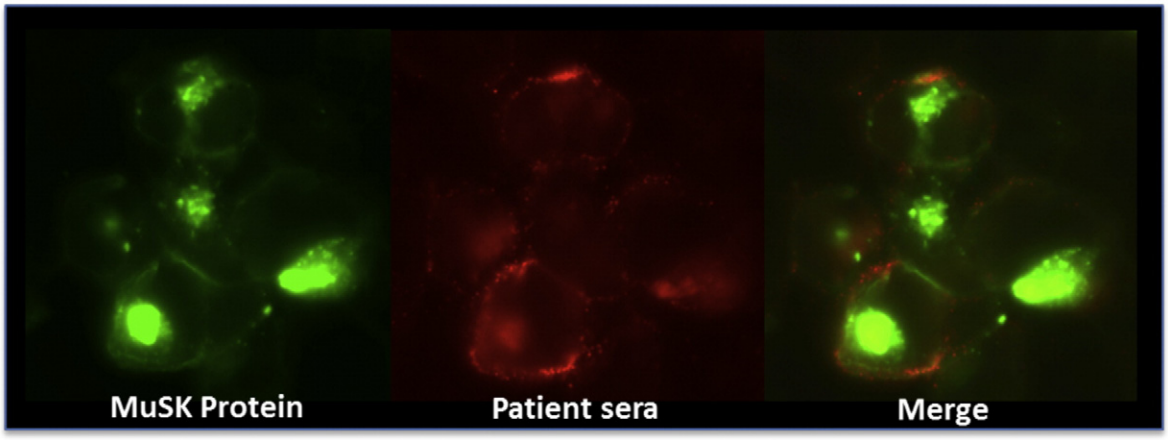

Fig. 2. MuSK cell based assay. Sera from a MuSK-MG patient binds to live HEK cells expressing MuSK on the cell surface.