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Guidelines for pre-clinical assessment of the acetylcholine receptor-specific passive transfer myasthenia gravis model—Recommendations for methods and experimental designs

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ABSTRACT

Antibodies against the muscle acetylcholine receptor (AChR) are the most common cause of myasthenia gravis (MG). Passive transfer of AChR antibodies from MG patients into animals reproduces key features of human disease, including antigenic modulation of the AChR, complement-mediated damage of the neuromuscular junction, and muscle weakness. Similarly, AChR antibodies generated by active immunization in experimental autoimmune MG models can subsequently be passively transferred to other animals and induce weakness. The passive transfer model is useful to test therapeutic strategies aimed at the effector mechanism of the autoantibodies. Here we summarize published and unpublished experience using the AChR passive transfer MG model in mice, rats and rhesus monkeys, and give recommendations for the design of preclinical studies in order to facilitate translation of positive and negative results to improve MG therapies.

Introduction

The postulates of Witebsky–Rose–Koch require an antibody-mediated autoimmune response be recognized by specific characteristics; presence of autoantibody, the identification of the corresponding antigen, the ability to induce the production of the antibody in an experimental animal and demonstrate disease manifestations similar to the human disease (Witebsky et al., 1957).

These criteria still form a solid basis for defining an antibody-mediated autoimmune disease and provide for two experimental models, i) the injection of antigen to elicit an 'active' immune response and ii) the injection of antibodies as a 'passive' transfer of autoimmunity. Experimental autoimmune myasthenia gravis (EAMG) produces autoantibodies by the injection of AChR usually with an immunostimulator. Active immunization against other proteins found at the neuromuscular junction (NMJ) can also cause weakness. The passive transfer myasthenia gravis (PTMG) model is the injection of those autoantibodies into another animal, which will also demonstrate weakness. MG was one of the first diseases that fulfilled the Witebsky–Rose–Koch criteria for autoimmunity (Toyka et al., 1975, 1977). Subsequently, transfer of monoclonal AChR antibodies produced by hybridomas cloned from EAMG model induced similar disease characteristics (Lindstrom et al., 1976; Engel et al., 1979; Lennon and Lambert, 1980; Richman et al., 1980). The robustness and clear-cut phenotype of PTMG have made it a useful model for characterizing the immunopathogenesis of AChR-MG (~80% of the MG cases) and for testing medication that reduces the pathogenic effect of autoantibodies. Although PTMG with antibodies to muscle specific kinase and low-density lipoprotein receptor-related protein 4 have also been performed, the majority of PTMG studies have involved antibodies to the AChR. Over the years, the purpose of the model has shifted from the investigation of the pathology induced by AChR antibodies towards preclinical studies aimed at testing therapeutic interventions. Here, we provide recommendations for the design of preclinical studies using AChR-PTMG model (referred to as PTMG in the text below) in order to facilitate translation of positive and negative results in order to improve MG therapies in clinical practice.

Purpose of the passive transfer model of myasthenia gravis

MG is a T cell dependent-B cell mediated disease (Conti-Fine et al., 2006). Activation of $CD4^+$T cells is required for the autoimmune process

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by impairment of T regulatory cells, cytokine secretion and B cell activation. B cells, and in particular plasma cells, on the other hand are the source of the autoantibodies. The EAMG model utilizes the autoimmune cellular processes, the afferent arm of the immune response, to produce autoantibodies directed at the AChR, the efferent arm of the response. The PTMG model removes the highly variable response of the afferent arm thereby allowing the efferent effects of the antibodies to be studied in a reproducible way. The use of PTMG model for pre-clinical evaluation of a therapeutic is justified when the effect is limited to inhibiting the autoantibody binding or preserving the function and structure of the neuromuscular junction (NMJ) during antibody attack.

Pathophysiology of AChR antibodies

By the transfer of purified immunoglobulins from MG patients to mice and the subsequent muscle weakness developed in the mouse, Toyka and colleagues demonstrated that MG is an antibody mediated autoimmune disease (Toyka et al., 1975). Complement-activating antibodies against the extracellular domain of the AChR induced rapid, dose dependent myasthenia as early as 8 h and death by 48 h. The source of antibodies transferred to animals can be serum IgG of MG patients, polyclonal IgG from chronic EAMG animals, or monoclonal antibodies produced by B cell hybridomas or by heterologous expression (Lennon and Lambert, 1980; Richman et al.; van der Neut Kolfschoten et al., 2007). The main immunogenic region (MIR) on the alpha subunit of the AChR binds a high proportion of antibodies from MG patients (Tzartos and Lindstrom, 1980; Whiting et al., 1986), and it is the target recognized by monoclonal antibodies that produce PTMG. Furthermore, the α subunit antibodies are more pathogenic than the antibodies against the β subunit (Kordas et al., 2014) probably because the alpha subunit is represented twice among the five AChR subunits.

The antibody effector mechanisms are antigenic modulation and complement-mediated focal lysis of the postsynaptic membrane (Lennon et al., 1978; Tzartos et al., 1985; Loutrari et al., 1992). Transfer of IgG from MG patients to mice reduced the number of functional AChR, although AChR synthesis rose to compensate (Wilson et al., 1983a,b; Sterz et al., 1986). Monovalent AChR antibodies without complement binding capacity are not pathogenic unless they interfere with ion channel function (Lagoumintzis et al., 2010). The PTMG model revealed that the influx of mononuclear cells into the NMJ was antibody and complement-dependent, an event also seen during the acute phase (occurring one week after AChR induction in rats) of EAMG. The deposition of IgG and complement components was associated with a large influx of macrophages and a loss of both AChR and postsynaptic folds (Lindstrom et al., 1976; Engel et al., 1979).

PTMG has been used to determine the effects of sex, strain and age on susceptibility to antibody-mediated AChR loss (Hoedemaekers et al., 1998), the importance of the expression levels of AChR-associated proteins like rapsyn in the susceptibility of the AChR to antigen modulation (Losen et al., 2005), and the beneficial effects of complement inhibitors (Morgan et al., 2006; Kusner et al., 2013).

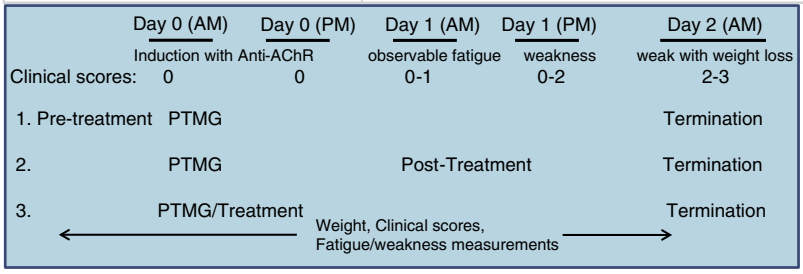
Therapeutic strategies using the PTMG model

Passive transfer of antibodies has also been used for therapeutic development (Lagoumintzis et al., 2010) (Fig. 1). Due to the 48–72 hour experimental timeframe, the PTMG model can function to determine dose–response and offer go-forward information to active immunization/EAMG experiments which require longer experimental periods. Therapeutics that target antibody turnover have shown efficacy in proof-of-concept studies. The increased turnover of antibodies has been facilitated by the use of proteolytic enzymes or antibodies to FcRn (Poulas et al., 2000; Liu et al., 2007). RNA aptamers (Hwang et al., 2003) and antibodies to denatured AChR (Krolick et al., 1996) have been shown effective in inhibiting binding of MIR antibodies. In AChR-specific PTMG mouse models, monovalent Fab fragments have been demonstrated to protect the AChR against the action of intact pathogenic antibodies (Toyka et al., 1980; Barchan et al., 1998; Papanastasiou et al., 2000). Complement depletion by cobra venom

A

Experimental design	Example
Animal species	Rat
Animal number	Power analysis based on clinical score
Randomization of animals	Stratify by weight and randomly assign to group
Number of groups and controls	Treatment response group
Antibody type	mAb35
Dose of antibody	20pmol/100g
Method of injection	Intraperitoneal
Analysis of Data	ANOVA

B



Schematic of PTMG experimental design. A. To properly plan a PTMG study, each aspect of the experiment must be determined prior to initiation. The table above provides some of these aspects with examples. B. The figure demonstrates the potential treatment schematic and course of clinical scores of PTMG induced animals. The pre-treatment would occur prior to initiation of PTMG. The treatment can occur after weakness is observed (24hrs.). Or, the treatment and initiation of PTMG can occur at the same time.

Fig. 1. Schematic of PTMG experimental design. A. To properly plan a PTMG study, each aspect of the experiment must be determined prior to initiation. The table above provides some of these aspects with examples. B. The figure demonstrates the potential treatment schematic and course of clinical scores of PTMG induced animals. The pre-treatment would occur prior to initiation of PTMG. The treatment can occur after weakness is observed (24 h). Or, the treatment and initiation of PTMG can occur at the same time.