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IgG1 deficiency exacerbates experimental autoimmune myasthenia gravis in BALB/c mice

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ABSTRACT

Myasthenia gravis is an autoimmune disease characterized by muscle weakness due to neuromuscular junction (NMJ) damage by anti-acetylcholine receptor (AChR) auto-antibodies and complement. In experimental autoimmune myasthenia gravis (EAMG), which is induced by immunization with Torpedo AChR in CFA, anti-AChR IgG2b and IgG1 are the predominant isotypes in the circulation. Complement activation by isotypes such as IgG2b plays a crucial role in EAMG pathogenesis; this suggested the possibility that IgG1, which does not activate complement through the classical pathway, may suppress EAMG. In this study, we show that AChR-immunized BALB/c mice genetically deficient for IgG1 produce higher levels of complement-activating isotypes of anti-AChR, especially IgG3 and IgG2a, and develop increased IgG3/IgG2a deposits at the NMJ, as compared to wild type (WT) BALB/c mice. Consistent with this, AChR-immunized IgG1$^{-/-}$ BALB/c mice lose muscle strength and muscle AChR to a greater extent than AChR-immunized WT mice. These observations demonstrate that IgG1 deficiency leads to increased severity of EAMG associated with an increase in complement activating IgG isotypes. Further studies are needed to dissect the specific role or mechanism of IgG1 in limiting EAMG and that of EAMG exacerbating role of complement activating IgG3 and IgG2a in IgG1 deficiency.

1. Introduction

Muscle weakness and fatigue in autoimmune myasthenia gravis (MG) is caused by decreased activation of postsynaptic muscle AChR by ACh, due to antibody- and complement-mediated AChR destruction (Ha and Richman, 2015; Huda et al., 2014). A mouse model of experimental autoimmune MG (EAMG), which mimics MG, is induced by immunizing mice 2–3 times with Torpedo AChR in CFA (Christadoss et al., 2000). The predominant anti-AChR Ig isotype that develops after two immunizations of these mice is IgG2b, followed by IgG1 (Allman et al., 2011), which have features in common with human IgG1 and IgG4, respectively (Hussain and Kifayet, 1995; Nimmerjahn and Ravetch, 2010). By immuno-histochemical methods and radioimmunoassay, we and others have previously shown that IgG2b binding to AChR at the neuro-muscular junction (NMJ), triggers complement activation that leads to formation of the membrane attack complex (MAC), which depletes functional muscle AChRs at the NMJ (Nakano and Engel, 1993; Kusner and Kaminski, 2012; Tüzün and Christadoss, 2013). Complement activation by anti-AChR antibody is required for AChR depletion and EAMG development (Michaelsen et al, 2006). However, while mouse IgG2b (as well as mouse IgG2a and IgG3 and human IgG1 and IgG3) effectively activates the classical complement cascade, mouse IgG1 and human IgG2 and IgG4 have little or no ability to do this (Redpath et al, 1998). Consequently, while IgG2b anti-AChR is the critical isotype for EAMG pathogenesis (Tüzün et al, 2012), IgG1 anti-AChR antibody might suppress the development of this disorder by competing with complement-activating isotypes for AChR epitopes and/or inhibiting the production of complement-activating isotypes through negative feedback mechanisms. To test our hypothesis, we compared the responses of WT and IgG1-deficient BALB/c mice to Torpedo AChR/CFA immunization. BALB/c mice are relatively less susceptible to EAMG (Graus et al, 1993; Fuchs et al, 1976; Berman and Patrick, 1980). We find that IgG1 deficiency increases production of complement-activating isotypes of anti-AChR Ab, including IgG2a, IgG2b and IgG3, in Torpedo AChR/CFA-immunized BALB/c mice and increases their EAMG susceptibility.

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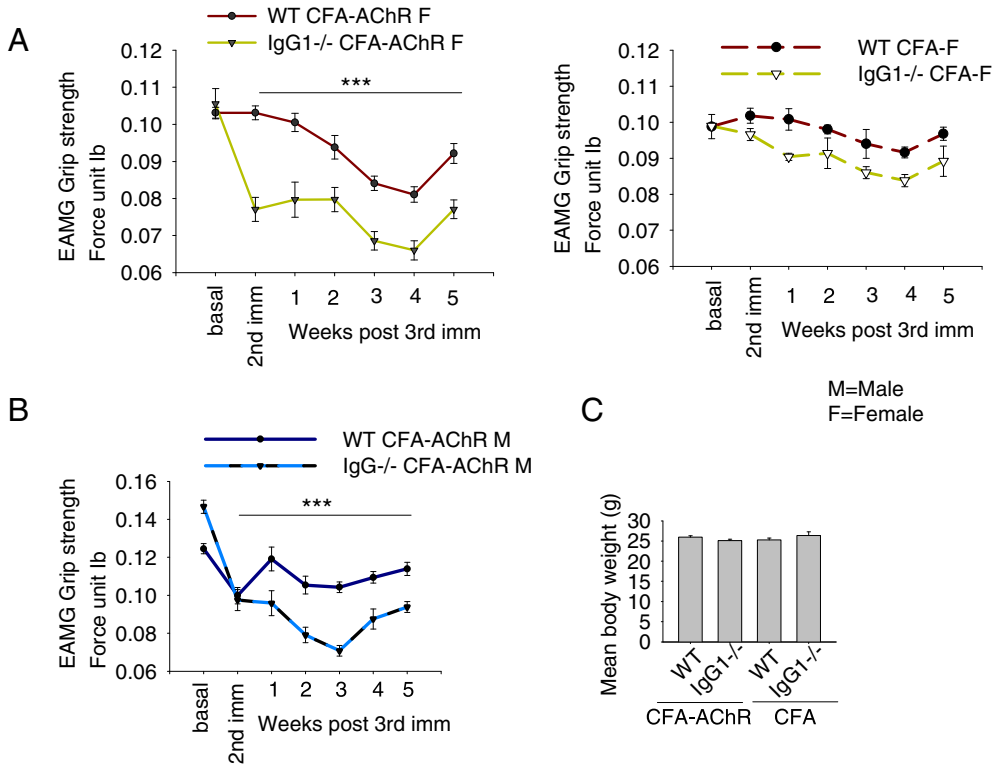

Fig. 1. Grip strength in CFA- and CFA/AChR-immunized IgG1$^{-/-}$ vs. WT BALB/c mice. IgG1$^{-/-}$ and WT BALB/c mice were immunized twice with CFA/AChR. Both male and female CFA/AChR immunized IgG1$^{-/-}$ mice had significantly less grip strength than WT mice ($p < 0.001$ after second immunization) (A, B). Grip strengths of CFA immunized IgG1$^{-/-}$ and WT mice did not differ significantly (A, right). Differences in mean body weight of CFA- or CFA/AChR immunized IgG1$^{-/-}$ and WT mice were not significant (C). Fore limb grip strength from each mouse was the average value of 5 repeats, read from the digital dynamometer each time. Grip strength was determined twice a week for 5 weeks (n = 10 for IgG1$^{-/-}$ and WT mice, each gender, M — male; F — female).

2. Materials and methods

2.1. Animals, EAMG model and clinical evaluation of EAMG

BALB/c IgG1-deficient (IgG1$^{-/-}$) mice were generated as described (Jung et al., 1993). Age matched BALB/cJ mice (12 weeks, WT) were purchased from Jackson Laboratories (Bar Harbor, Maine). AChR from the electric organ of *Torpedo californica* was affinity purified with a neurotoxin affinity column (Wu et al., 2013). Mice were immunized and boosted at 4 and 8 weeks with 20 μg of affinity-purified Torpedo AChR emulsified in CFA (heat-killed *Mycobacterium butyricum*) (Wu et al., 2013). All IgG1$^{-/-}$ mice were maintained in a barrier facility according

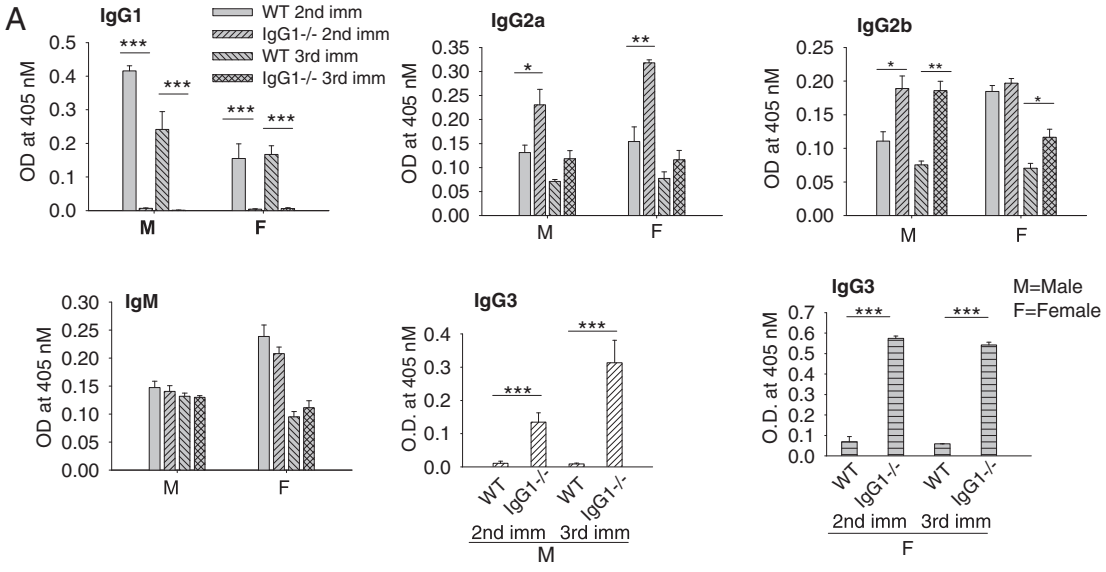

Fig. 2. Serum AChR antibody levels of IgG1$^{-/-}$ and WT mice. Serum level of anti-AChR auto-Ab was determined by ELISA using affinity purified mouse AChR as a coating antigen. A dramatic increase in anti-AChR IgG3 level was seen in all CFA/AChR immunized IgG1$^{-/-}$ mice (male and female), ***$p < 0.001$. The increase in serum IgG2a, IgG2b and IgG3, but not IgM anti-AChR Ab was significant in CFA/AChR immunized IgG1$^{-/-}$ mice compared to CFA/AChR immunized wild type mice, *$p < 0.05$, **$p < 0.01$. Results are representative of 3 independent experiments. Vertical bars represent standard error, n = 10 each for IgG1$^{-/-}$ and WT mice, M — male; F — female.