Highlights

- Fcα/μR enhances, rather than suppresses, autoantibody production.
- Fcα/μR on the MRL/MpJ-*Fas*$^{lpr/lpr}$ background mice decreased survival rate.
- IgM prevents autoantibody production independently with Fcα/μR expression.

Short communication

Involvement of Fcα/μR (CD351) in autoantibody production.

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ABSTRACT

Antibody exerts various immune responses via binding to Fc receptors expressed on immune cells. Although several reports have demonstrated that IgM prevents autoantibody production, the role of IgM Fc receptors is largely unknown. To analyze the involvement of Fcα/μR (CD351), an Fc receptor for IgM and IgA expressed on B cells and follicular dendritic cells (FDCs), in IgM-mediated suppression of autoantibody production, we generated mice deficient in Fcα/μR on the background of MRL/MpJ-*Fas*$^{lpr/lpr}$ (*Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$) mice. *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice showed significantly lower titers of IgG autoantibodies against double strand (ds) DNA, histone and cardiolipin in the sera than did *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice. Moreover, *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice showed higher survival rate at the ages of 28, 32 and 40 weeks old, compared with *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice. These results suggest that Fcα/μR enhances, rather than suppresses, autoantibody production.

Keywords;

Fc receptor, IgM, autoantibody

1. Introduction

After binding to an antigen, immunoglobulin (Ig) initiates various immune responses via binding to a certain receptor for the Fc portion, called Fc receptor (FcR) (Daeron, 1997; Ravetch, 1997). Extensive studies demonstrated that FcRs for IgG (FcγRI, FcγRIIb, FcγRIII and FcγRIV) and IgE (FcεRI and FcεRII) play pivotal roles in immune responses, such as inflammations, cytotoxicity and allergic reactions (Ravetch and Clynes, 1998; Takai, 2002). In contrast to the FcγR and FcεR, molecular and functional characteristics of Fc receptor for IgM remain incompletely understood. Fcα/μR (CD351) is an Fc receptor for IgM as well as IgA (Sakamoto et al., 2001; Shibuya et al., 2000). Fcα/μR forms an atypical dimer, for which the cytoplasmic region of Fcα/μR is required (Cho et al., 2010; Takagaki et al., 2013). Fcα/μR is moderately expressed on B cells and macrophages (Sakamoto et al., 2001; Shibuya et al., 2000), and most strongly on follicular dendritic cells (FDCs) (Cho et al., 2006; Honda et al., 2009; Usui et al., 2012), which is involved in germinal center (GC) formation (Chaplin and Zindl, 2006; Park and Choi, 2005). Fcα/μR-deficient mice showed increased GC formation and antibody production after immunization with T-independent (TI), but not T-dependent (TD), antigens (Honda et al., 2009). Thus, Fcα/μR negatively regulates humoral immune responses against TI antigens.

IgM exists in the sera of naïve host as a natural antibody. IgM is also the first antibody to be produced by naive B cells upon antigen recognition. Thus, IgM may play an important role in the early phase of host defenses against variable bacterial and viral infections (Boes et al., 1998; Ochsenbein et al., 1999). In addition, IgM prevents autoantibody production (Boes, 2000; Carroll, 2004; Ehrenstein et al., 2000). Mice deficient in serum IgM showed increased anti-DNA IgG production, leading to

glomerulonephritis due to deposition of an immune complex with age (Ehrenstein et al., 2000). Moreover, serum IgM deficiency in MRL/MpJ-*Fas*$^{lpr/lpr}$ mice, which spontaneously develop an autoimmune disease that has many features resembling human systemic lupus erythematosus due to lymphoproliferation (lpr) mutation on the *Fas* gene, showed significantly increased serum IgG autoantibodies against dsDNA and histone, and shortened life span caused by severe glomerulonephritis, compared with MRL/MpJ-*Fas*$^{lpr/lpr}$ mice with normal serum IgM (Boes et al., 2000). Thus, serum IgM prevents autoantibody production and ameliorates immunological disorders (Boes, 2000; Carroll, 2004; Ehrenstein et al., 2000). However, how IgM is involved in autoantibody production is largely unknown.

Several reports have demonstrated that autoantibodies are produced in a T cell-independent manner (Groom et al., 2007; Herlands et al., 2008). Because Fcα/μR negatively regulates humoral immune responses against TI antigens (Honda et al., 2009), we hypothesized that IgM suppresses autoantibody production via interaction with Fcα/μR. To test this idea, we generated mice deficient in Fcα/μR on the background of MRL/MpJ-*Fas*$^{lpr/lpr}$ (*Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$) mice, and investigated the role of Fcα/μR in IgM-mediated suppression of autoantibody production.

2. Materials and methods

2.1 Mice

Mice deficient in Fcα/μR on BALB/c background were previously described (Honda et al., 2009). The Fcα/μR-deficient mice were bred with MRL/MpJ-*Fas*$^{lpr/lpr}$ (MRL/MpJ-*Fas*^{lpr}/JJmsSlc) mice purchased from Japan SLC, Inc. (Tokyo, Japan), and their offsprings were intercrossed to yield *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice. In all the experiment, we analyzed littermate mice to exclude the influence of the genetic backgrounds. Mice were maintained under specific pathogen free conditions. All experiments were performed according to the guidelines of the Animal Ethics Committee of the University of Tsukuba Animal Research Center.

2.2 ELISA

2.2.1 Antibody

Horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG antibody (GE Healthcare, Little Chalfont, UK), goat anti-mouse IgG1, IgG2a, and IgG2b antibodies (Bethyl, Mongomery, TX, USA) and goat anti-mouse IgG3 antibody (Southern Biotech, Birmingham, AL, USA) were used as secondary antibodies for detection.

2.2.2 Measurement of autoantibodies

96-well ELISA plate (Maxi Soap, Nunc, Roskilde, Denmark) was coated either with purified double-strand DNA (dsDNA, Sigma, St. Louis, MO, USA) in PBS at 5 μg/ml or histone (Roche, Mannheim, Germany) or cardiolipin (Sigma, St. Louis, MO, USA) in carbonate buffer (pH9.6) at 10 μg/ml. In the case of dsDNA, plate was

pre-coated with protamine sulfate from salmon (Wako, Osaka, Japan) diluted in PBS at 10 μg/ml. After blocking with 3 % FCS-containing PBS, serially diluted serum samples were added. HRP-conjugated antibodies specific for each IgG isotype were then applied, followed by ABTS substrate (KPL, Gaithersburg, MD, USA). Antibody titers were determined relative to those of pooled sera obtained from mice with *lpr* mutation at 8~32 weeks old.

2.3 Biochemical examination of renal function

Sera were obtained from $Fcamr^{+/+}Fas^{lpr/lpr}$ and $Fcamr^{-/-}Fas^{lpr/lpr}$ mice at the ages of 30~33 weeks old and from BALB/c mice at the ages of 8~12 weeks old, and measured for creatinine and blood urea nitrogen concentrations by using clinical chemical analyzer (Fuji Dri-Chem 3500, Fujifilm, Japan).

2.4 Statistical analyses

For statistical analysis of autoantibody production, student's unpaired t-test was applied. For statistical analysis of mice, Gehan-Breslow-Wilcoxon test and log-rank Mantel Cox test were applied for analyses of indicated ages and total survival rate, respectively.

3. Results and discussion

To analyze the involvement of Fcα/μR in autoantibody production, we generated *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice. We collected sera from the both mice at the ages of 12, 16 and 20 weeks old, and measured the titers of IgG autoantibodies against double strand DNA (dsDNA), histone and cardiolipin. *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice showed elevated the titer of total anti-dsDNA IgG, which was further increased with age (Fig. 1A). However, anti-dsDNA IgG titers of *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice were significantly lower than those of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice at the ages of 16 and 20 weeks old (Fig. 1A). Among IgG isotypes, anti-dsDNA IgG1 and IgG2b of 20 weeks old *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice were significantly decreased, compared with those of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice (Fig 1A). Similarly, anti-histone and anti-cardiolipin IgG1 and IgG2a titers of *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice were also significantly lower than those of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice at the ages of 16 and/or 20 weeks old (Fig 1B, C). Collectively, these results suggest that Fcα/μR enhanced, rather than suppressed, IgG autoantibodies production in mice on the MRL/MpJ-*Fas*$^{lpr/lpr}$ background. Moreover, *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice showed significantly higher survival rate at the ages of 28, 32 and 40 weeks old, compared with *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ mice, although the survival at the age of 52 weeks were comparable between *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice (Fig. 2). We also measured serum creatinine and blood urea nitrogen (BUN) concentrations of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice at the ages of 30~33 weeks old. We did not observe elevated creatinine and BUN in the sera compared with BALB/c mice, indicating that renal dysfunction was not severe in either *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ or *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ mice at those ages, although the development of autoantibody deposition and/or glomerulonephritis could not be excluded (Fig. 3).

Recently, a new IgM Fc receptor, designated FcμR, was identified (Kubagawa et al., 2009; Shima et al., 2010). Ouchida et al. have reported that aged FcμR-deficient mice showed increased serum levels of rheumatoid factor, anti-dsDNA and anti-nuclear antibodies (Ouchida et al., 2012). Thus, FcμR, rather than Fcα/μR, might be involved in IgM-mediated suppression of autoantibody productions.

As for the molecular mechanisms of Fcα/μR-mediated enhancement of autoantibody production, two possibilities can be considered. First, since Fcα/μR expressed on B cells mediates internalization of IgM-coated antigen (Shibuya et al., 2000), Fcα/μR may also internalize IgM immune-complex (IC) with dsDNA containing hypomethylated CpG DNA, resulting in TLR9 stimulation for anti-dsDNA IgG autoantibody production. Supporting this idea, evidences demonstrated that, despite relatively low abundance, hypomethylated CpG motif was included in mammalian DNA (Viglianti et al., 2003). Moreover, TLR9 activates autoreactive B cells, resulting in autoantibody production (Christensen et al., 2005; Leadbetter et al., 2002; Peng, 2005; Viglianti et al., 2003). Second, recent reports demonstrated that FDCs internalize and retain IC, and then recycle them back to the cell surface periodically (Heesters et al., 2013). Prolonged retention of self-antigens on FDCs leads to autoantibody production (Kranich et al., 2010). Since Fcα/μR is strongly expressed on FDCs (Honda et al., 2009), Fcα/μR on FDCs may mediate internalization of IC of self-antigens with IgM autoantibody and prolong self-antigens retention on FDCs, leading to expansion of self-reactive B cells. Further studies should be required to clarify whether and how Fcα/μR and FcμR is involved in autoantibody production.

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Figure legends

Fig. 1. Autoantibody titers in the sera of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ (WT) and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ (KO) mice. Titers of anti-dsDNA (A), anti-histon (B) and anti-cardiolipin (C) antibodies of indicated IgG isotypes in sera of 12 (WT, n=37 ; KO, n=37), 16 (WT, n=37 ; KO, n=37) and 20 (WT, n=35 ; KO, n=36) weeks old mice were measured by ELISA. Each dot represents individual mice. Error bars indicate SEM. *, $P < 0.05$; **, $P < 0.01$.

Fig. 2. Survival of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ (WT, n=38) and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ (KO, n=37) mice. *, $P < 0.05$; **, $P < 0.01$.

Fig. 3. Creatinine (CRE) (A) and blood urea nitrogen (BUN) (B) concentrations were measured in the sera of *Fcamr*$^{+/+}$*Fas*$^{lpr/lpr}$ (WT) (n=5) and *Fcamr*$^{-/-}$*Fas*$^{lpr/lpr}$ (KO) (n=3) mice at the ages of 30~33 weeks old, and BALB/c mice at the ages of 8~12 weeks old (n=3).

Figure 1

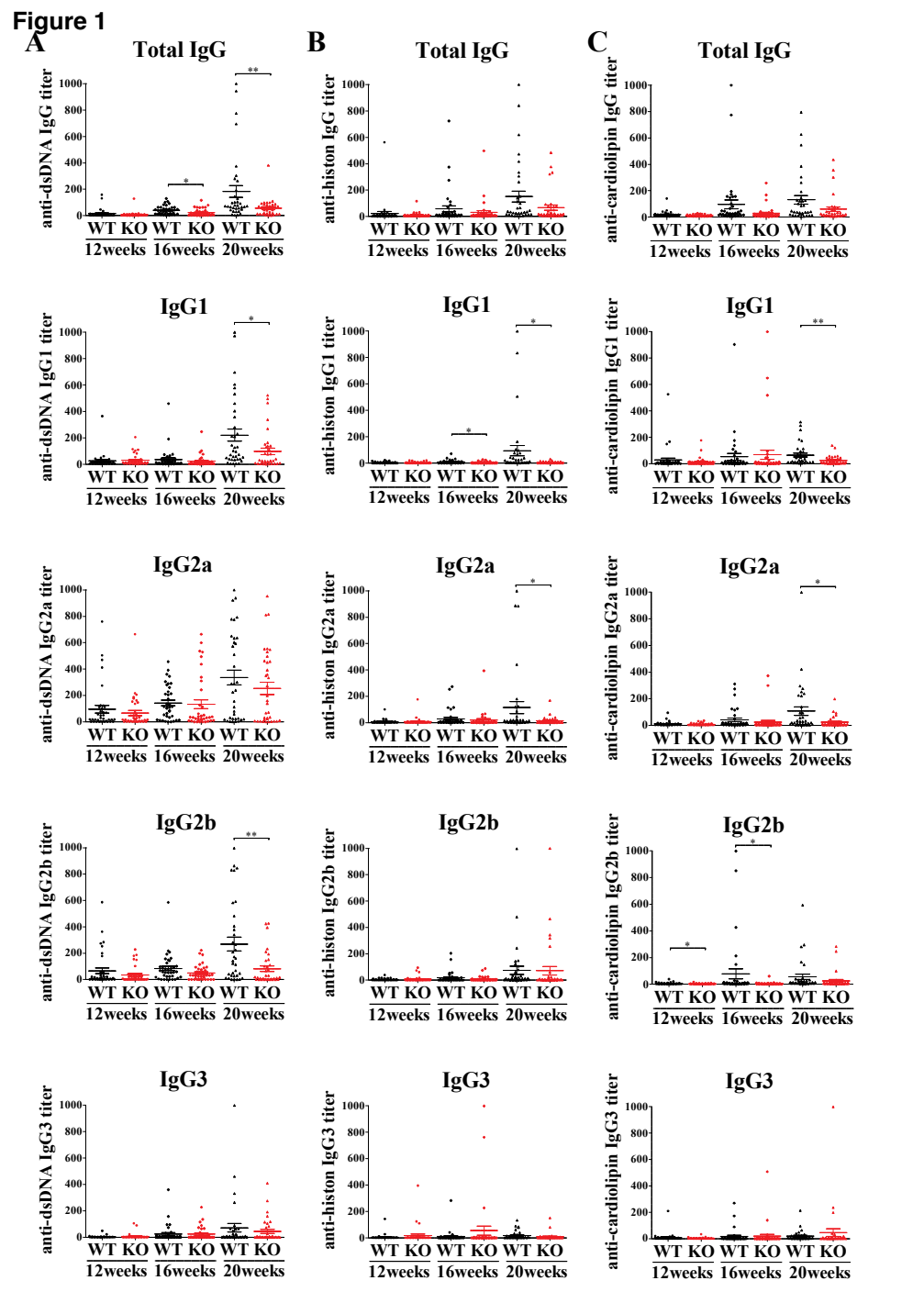
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Figure 2

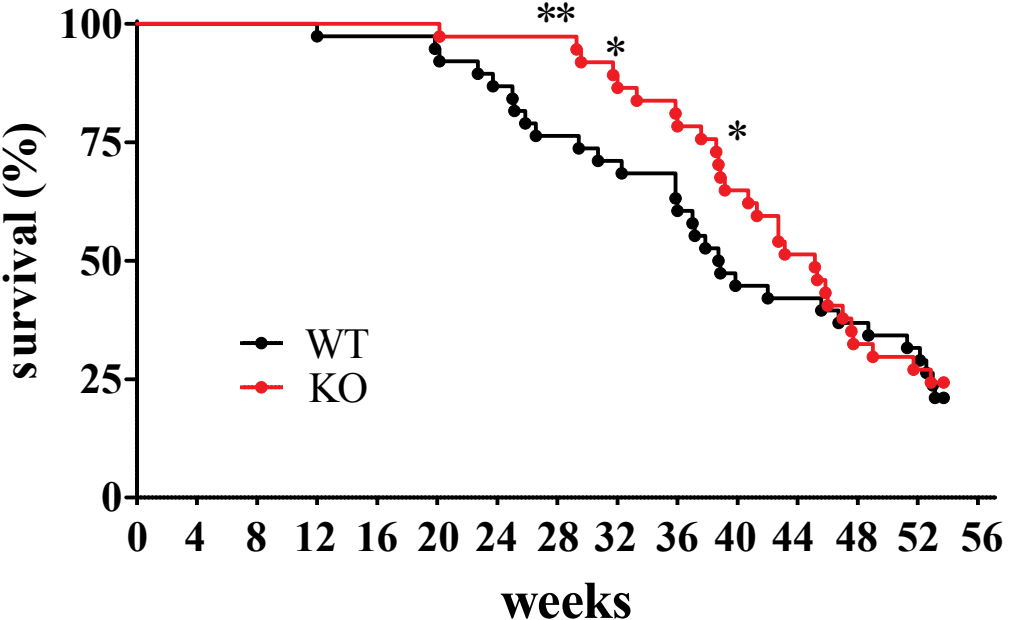
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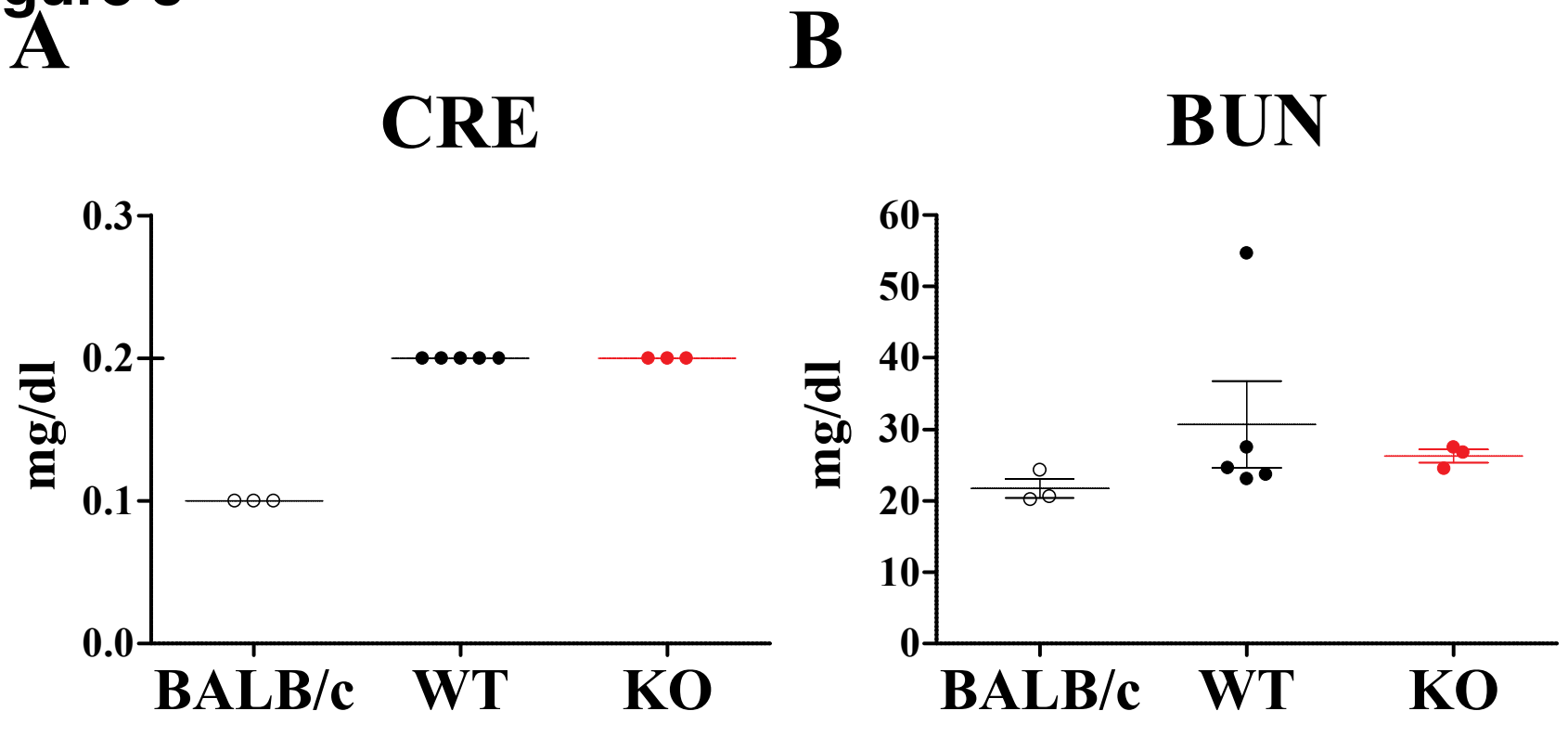
Figure 3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