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CORRESPONDENCE

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Role of type 2 ryanodine receptor stabilisation in autoimmune cell modulation

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Ryanodine receptors (RyRs) are intracellular Ca^{2+}-release channels located in the sarcoplasmic/endoplasmic reticulum membrane, which assume a crucial role in facilitating the deliverance of intracellular Ca^{2+} [1]. A recent investigation by Wang et al. involving type 2 RyR (RyR2)-knockdown experiments reported that RyR2-depleted conventional T cells exhibit regulatory T cell-like suppressive functions in multiple inflammatory or autoimmune contexts [2]. Nonetheless, RyR2 knockdown in patients can be challenging in clinical practice. Therefore, this study aimed to propose RyR2 stabilisation as an alternative strategy for the acquisition of a comparable effect.

Prior research has reported that Ca^{2+} leakage via RyR2 is primarily driven by a reduction in RyR2-calmodulin binding. Based on these findings, we created V3599K-RyR2 knock-in ($RyR2^{V3599K}/RyR2^{V3599K}$) mice by replacing the endogenous gene with a gene in which the Valine at 3599th amino acid in the calmodulin binding domain on RyR2 was replaced with Lysine. These mice have indeed a higher affinity for calmodulin, an important modifying protein for RyR2 [3, 4], stabilizing RyR2 and preventing Ca^{2+} leakage from RyR2. $RyR2^{V3599K}/RyR2^{V3599K}$ mice show inhibition of Ca^{2+} leakage via enhanced RyR2-calmodulin binding, which is associated with improvements in RyR2-related disorders, such as catecholaminergic polymorphic ventricular tachycardia and Alzheimer's disease [4, 5]. These findings provide evidence to indicate the genetic stabilisation of RyR2.

Regarding autoimmune disorders and immune-mediated inflammatory arthropathies, we have reported that treatment with dantrolene, an RyR stabiliser, contributes to a substantial reduction in serum anti-type II collagen immunoglobulin G (IgG) levels in mice with collagen-induced arthritis (CIA), thereby preventing CIA [6]. Furthermore, we and others have reported that dantrolene is a candidate immunomodulator for treating autoimmune disorders and immune-mediated inflammatory arthropathies, including rheumatoid arthritis and multiple sclerosis [6–8].

In addition to the aforementioned findings, we recently established that RyR2 stabilisation assumes a crucial role in suppressing autoantibody induction via antigen sensitisation. Summarily, 8–10-week-old female wild-type *C57BL/6* and $RyR2^{V3599K}/RyR2^{V3599K}$ mice were initially immunised with a subcutaneous injection of 100 μg ovalbumin (OVA) emulsified with complete Freund's adjuvant; an emulsion comprising Freund's incomplete adjuvant and 100 μg of OVA was administered on day 21 to achieve second immunisation. Serum anti-OVA IgG levels were measured 63 days after the initial immunisation using an enzyme-linked immunosorbent assay kit (Chondrex, Woodinville, WA, USA; cat. no. 3011).

OVA-immunised $RyR2^{V3599K}/RyR2^{V3599K}$ mice exhibited a marked reduction in serum anti-OVA IgG levels (Fig. 1), suggesting that RyR2 stabilisation may help to ameliorate autoimmune disorders and immune-mediated inflammatory arthropathies.

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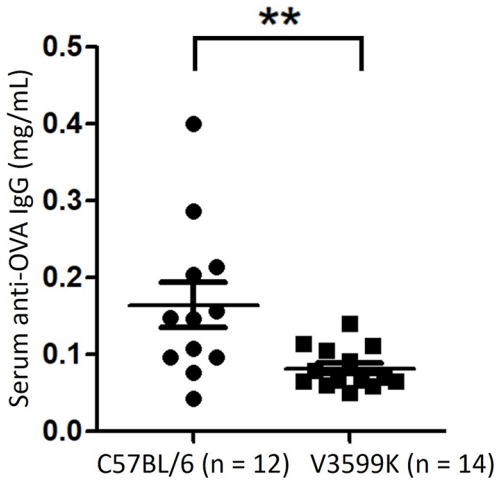

Fig. 1 Serum anti-ovalbumin immunoglobulin G levels on day 63 following an initial immunisation. Data were collected from two independent experiments, with values being presented as the means ± standard error of the mean (SEM). A Mann–Whitney U test was performed to compare differences between two independent groups. $^{**}p < 0.01$; n, number of animals in a group; IgG, immunoglobulin G; OVA, ovalbumin; V3599K, $RyR2^{V3599K}/RyR2^{V3599K}$ mice

Conclusively, our findings and the report by Wang et al. [2] suggest that RyR2 stabilisation may augment the suppressive effect on abnormal immune cells, including pathological T cells. Although the association between RyR2 and immune cells warrants further elucidation, we posit that RyR2 stabilisation may be effective in autoimmune cell modulation.

Abbreviations

CIA	Collagen-induced arthritis
IgG	Immunoglobulin G
OVA	Ovalbumin
RyR2	Type 2 ryanodine receptor
RyR	Ryanodine receptor
$RyR2^{V3599K}/RyR2^{V3599K}$	V3599K- type 2 ryanodine receptor knock-in

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Author contributions
Takashi Nawata: writing the original draft, conceptualization, methodology, data curation; Takeshi Honda: methodology, data curation, supervision; Akihiko Sakamoto: supervision; Hitoshi Uchinoumi: supervision; Takeshi Suetomi: supervision; Yoshihide Nakamura: supervision; Shunya Tsuji: data curation; Hiroki Sakai: methodology, data curation; Shigeki Kobayashi: supervision; Takeshi Yamamoto: supervision; Masataka Asagiri: methodology, supervision; Motoaki Sano: supervision, Masafumi Yano: methodology, supervision. All authors reviewed the manuscript.

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Data availability
All data generated and/or analysed during this study are presented in this article.

Declarations

Ethics approval and consent to participate
The protocols and animal care procedures followed the guidelines of the Animal Ethics Committee of Yamaguchi University (control number: 23-007).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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