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報 文

Effect of caffeine on antigen-specific immune responses of mouse splenocytes

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Abstract

Caffeine, a natural component of coffee and tea, is one of the most widely consumed drugs in the world. Caffeine can interact with several cell-surface receptors (e.g., adenosine receptors) and can modulate various cellular functions by binding to these receptors. However, there are few reports on its immunological functions. Therefore, we investigated the effects of caffeine on *in vitro* antigen-specific immune responses of unsensitized (naïve) immune cells from transgenic mice expressing the ovalbumin-specific T-cell receptor (TCR). Caffeine treatment significantly suppressed the proliferation of splenocytes and the secretion of interleukin (IL)-2, IL-4, and IL-10 elicited by ovalbumin stimulation. Moreover, immunoglobulin (Ig) G1 secretion was downregulated. In contrast, antigen-specific secretion of IL-12p40, IL-5, IgG2a, and IgA were not affected. These results suggest that caffeine treatment alters antigen-specific immune responses of primary naïve immune cells.

Key words: caffeine; Th1; Th2; naïve immune cells; immune responses

Introduction

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid that occurs naturally in many plant materials (i.e., coffee beans and tea leaves). Because it acts as a central nervous system stimulant, caffeine is commonly consumed as a psychoactive drug. It is now known that caffeine has various biological functions, such as anti-inflammatory, anti-obesity, and diuretic functions. Caffeine is an antagonist that binds to adenosine receptors (Yang, A. *et al.* 2010). Moreover, this compound has affinity for the inositol-3-phosphate receptors (IP3Rs) (Szatkowski, C. *et al.* 2010) and ryanodine receptors (RyRs) (Herrmann-Frank, A. *et al.* 1999). These receptors exist on the surface of immune cells (Hasko, G. *et al.* 2009, Premack, B.A. and Gardner,

P., 1992, Kong, H. *et al.* 2008). Therefore, it is possible that caffeine modulates the immune system through the binding of these cell-surface receptors. However, its effects on the immune system are not well understood.

Elevation of intracellular calcium concentration is an essential triggering signal for T cells, and this leads to changes in antigen-specific immune responses. Recent studies have suggested that caffeine alters intracellular calcium signaling in lymphocytes (Ritter, M. *et al.* 2001). Indeed, caffeine suppresses the activation of immune cells (Rosenthal, L.A. *et al.* 1992, Ritter, M. *et al.* 2005).

In these previous studies, caffeine was used at relatively high doses (1.5–10 mM) and concanavalin A or lipopolysaccharide was utilized as a non-specific immune stimulator. Therefore, the cellular responses observed in these experiments may not reflect the intrinsic antigen-

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specific immune responses *in vivo*. In addition, in some reports, cloned T cell lines were employed to assess the effects of caffeine on antigen-specific T cell responses. The establishment of antigen-specific T cell lines requires repeated stimulation with specific antigens. Hence, the immunological characteristics of established immune cell lines may be different from those of naïve cells.

In this study, we used primary immune cells from DO11.10 mice that express a transgenic T cell receptor (TCR) that recognizes chicken ovalbumin (OVA), which is presented by the major histocompatibility complex class II molecule. Given that the T cells in these mice cells properly respond to OVA without sensitization to this antigen, our experimental system seems to be more appropriate for investigating immune modulatory activities of drugs in naïve immune cells. Here, we describe the effects of low-dose caffeine treatment on antigen-specific responses of splenocytes.

Materials and Methods

Mice DO11.10 mice that express TCR specific for the OVA 323-339 peptide bound with I-Ad were purchased from Jackson Laboratory (Boston, MA, USA) and maintained in our specific pathogen-free animal facilities. Homozygotic male mice were mated with BALB/c female mice and the heterozygotes were used in these experiments. The animal studies were reviewed and approved by the Animal Care and Use Committee of the National Food Research Institute, National Agriculture and Food Research Organization (NARO), Japan. All surgical processes were conducted after cervical dislocation.

Chemicals and Reagents RPMI 1640 medium and OVA (Fraction V) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeine was purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest purity available from commercial sources.

Isolation of immune cells Single-cell suspensions of splenocytes were prepared from individual mice by mechanical dispersion. Splenocytes were counted and the viability was assessed with trypan blue exclusion. The viability of the splenocytes from all treatment groups was 95%.

Cell culture The medium used for the cell cultures was RPMI 1640 containing 2-mercaptoethanol (5×10^{-5}

mol/L), penicillin (100 U/mL), streptomycin (100 µg/mL), and 10% fetal calf serum (Biowest, Nuaille, France).

Caffeine treatment Caffeine was dissolved in PBS at a concentration of 100 mM and stored at -30 °C as a stock solution. The caffeine stock solution was diluted with the culture medium immediately before use. Pooled splenocytes from 4 mice were treated with 6.25, 25, and 100 µM of caffeine and stimulated with intact OVA (0, 1, 2, 5, or 10 µM) for up to 1 week in a culture medium. The data shown are the means (SE) from cultures measured in triplicate (Similar results were seen in 2 additional experiments with 3–4 mice).

Analysis of antigen-specific immune responses in vitro Splenocytes from the mice were suspended at a concentration of 2×10^6 cells/mL in culture medium and stimulated with OVA in 96-well culture plates (NUNC, Boston, MA, USA). An antigen-specific cell proliferation assay was conducted in a total volume of 100 µL with 1×10^5 cells per well. After 72 h, cell proliferation was measured by a cell proliferation enzyme-linked immunosorbent assay (ELISA) and BrdU (chemiluminescent) kit (Roche Molecular Biochemicals, Basel, Switzerland). An antigen-specific cytokine and antibody secretion assay was performed in a total volume of 300 µL with 3×10^5 cells per well. The culture supernatants were collected at 48 h (for the measurement of IL-2, IL-4, and IL-5) and at 72 h (for the measurement of IL-10, IL-12p40, and IFN-γ) and at 1 week (for the measurement of IgG1, IgG2a, IgM, and IgA) after the OVA stimulation and stored at -30 °C. The cytokine concentrations were measured using Mouse Cytokine ELISA Ready Set Go! kits (eBioscience, San Diego, CA, USA) and the antibodies were measured using Mouse Ig ELISA Quantitation Kits (Bethyl, Montgomery, TX, USA).

Statistical analysis We compared the data for OVA-stimulated cells treated with the same concentrations of OVA. Statistical analyses were performed by ANOVA with Tukey's multiple comparison of means test.

Results

Effect of caffeine on antigen-specific proliferation of naïve splenocytes First, we confirmed that the concentrations of caffeine used in this study did not have result in acute toxicity to the splenocytes prepared from the DO

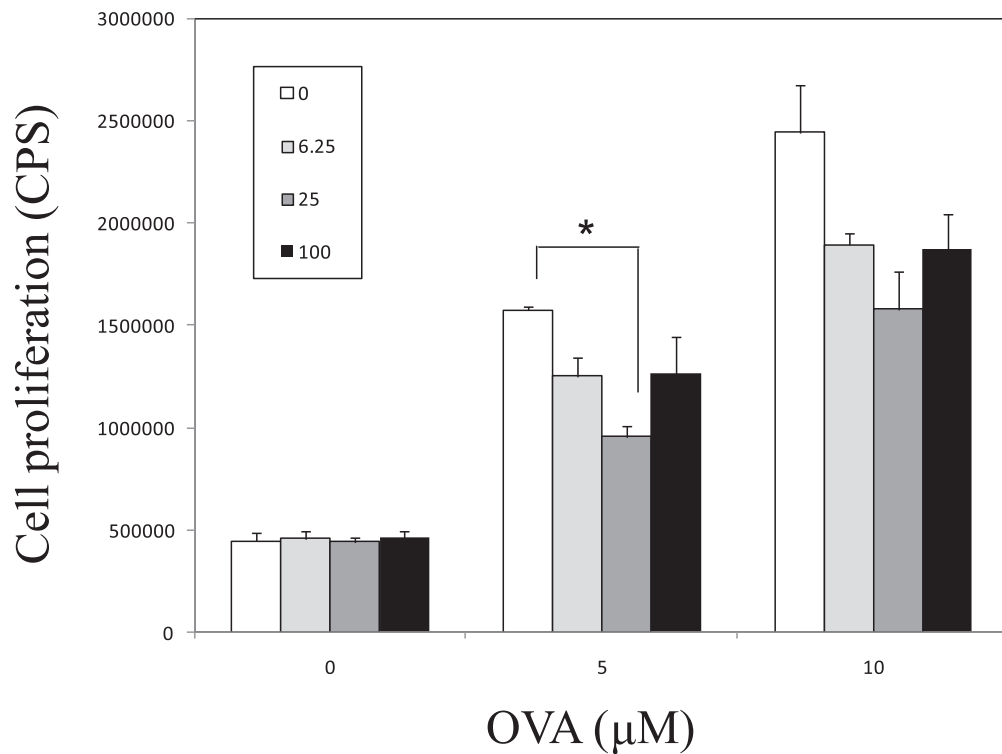


Fig. 1 Proliferation of splenocytes from naïve transgenic mice with specific-antigen in the presence of caffeine

Pooled splenocytes from four DO11.10 mice were cultured for up to 72 h with treatment of 0 (control), 6.25, 25, and 100 µM of caffeine in the presence of 5 and 10 µM of OVA. Cell proliferation was measured by intake of BrdU. Comparison was done among the data of OVA stimulation by equal concentration.

*: p 0.05

11.10 mice (data not shown). Then, the effects of caffeine on antigen-specific cell proliferation of splenocytes prepared from DO11.10 mice were determined. Caffeine exposure (25 µM) significantly suppressed the proliferation of splenocytes stimulated with 5 µM OVA (Fig. 1), indicating that caffeine suppressed the antigen-specific proliferation of these cells (Fig. 1).

Effects of caffeine on antigen-specific Th1-type cytokine secretion from splenocytes The Th1-type cytokines (IL-2, IL-12p40, and IFN-γ) are critical for the development of Th1-type T cells, and IL-2 is critical for the proliferation of T cells. Moreover, IL-2 and IFN-γ are secreted from T cells and IL-12p40 is secreted from antigen-presenting cells. Thus, we analyzed their concentrations in the culture supernatant of OVA-stimulated splenocytes (Fig. 2). Antigen-specific IL-2 secretion decreased significantly following treatment with 100 µM of caffeine. Suppression of IFN-γ secretion was also observed, though it was not significant. In contrast, the suppression of IL-12p

40 secretion was not observed.

Effects of caffeine on antigen-specific Th2-type cytokine secretion from splenocytes. Because the Th2-type cytokines (IL-4, IL-5, and IL-10) that are secreted from Th2 cells are also important for proper immune responses, we examined the concentrations of these cytokines. As shown in Fig. 3, IL-4 and IL-10 secretion, but not IL-5 secretion, was significantly suppressed by caffeine treatment (at 25 and 100 µM).

Effects of caffeine on antigen-specific antibody secretion from splenocytes Finally, we investigated the effect of caffeine on antibody secretion from splenocytes. The concentrations of antibodies (IgM, IgG1, IgG2a, and IgA) in the culture supernatant of specific-antigen-stimulated splenocytes are shown in Fig. 4. Caffeine, at the concentration of 100 µM, significantly suppressed the secretion of IgG1 stimulated with 1 µM OVA. The suppression of IgM secretion was observed following treatment with 100 µM of caffeine and 5 µM of OVA, but the suppression

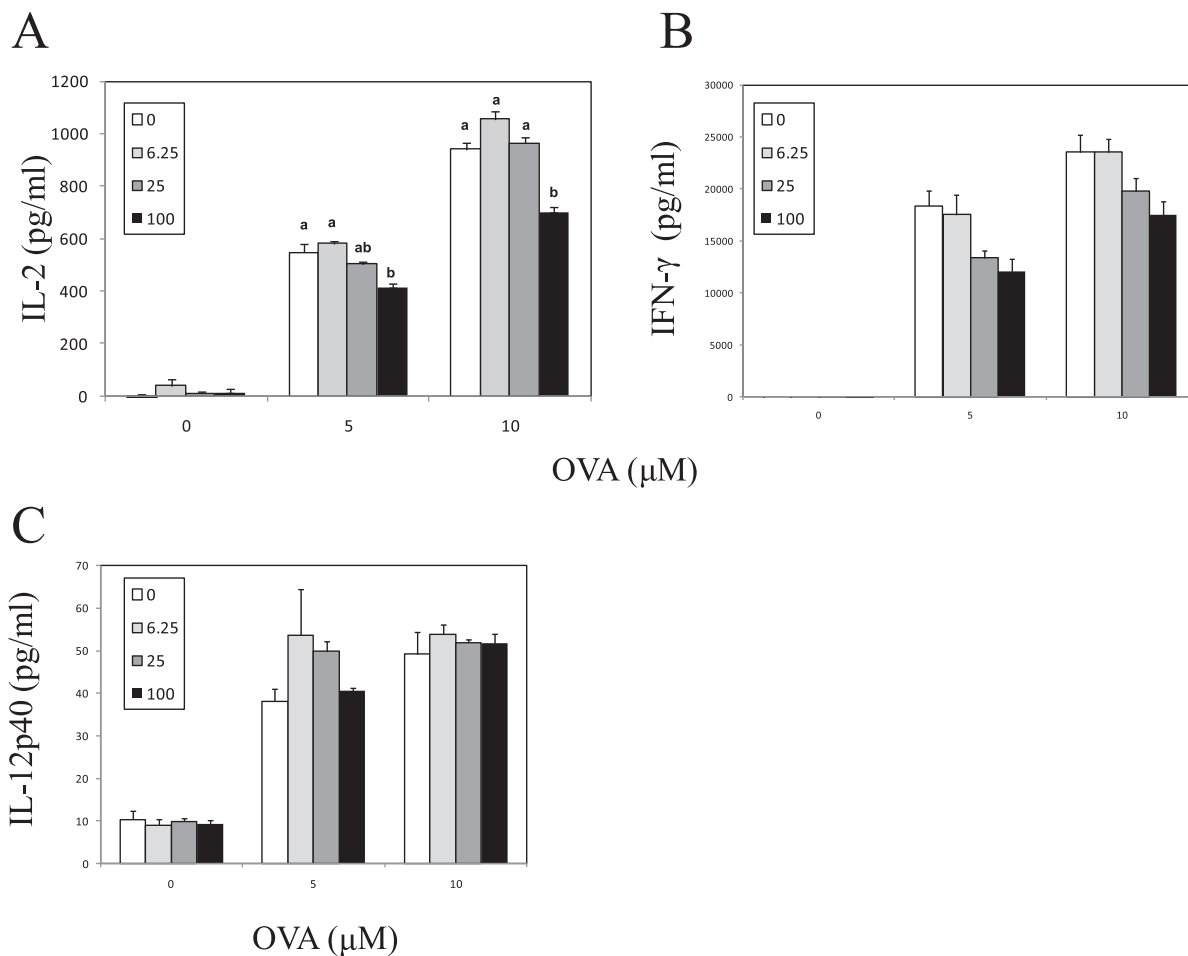


Fig. 2 Effects of coffee on antigen-specific Th1 type cytokines (A: IL-2, B: IFN- γ , C: IL-12p40) secretion of splenocytes from naïve transgenic mice

Pooled splenocytes from four DO11.10 mice were cultured with 0 (control), 6.25, 25, and 100 μ M of caffeine in the presence of 5 and 10 μ M of OVA for 48h (A) and 72 h (B and C). Concentrations of cytokines were measured by ELISA. Comparison was done among the data of OVA stimulation by equal concentration. Different letters above each bar indicate statistical difference ($p < 0.05$).

was not significant. The secretion of IgG2a and IgA did not change.

Discussion

In this study, we investigated the immunological effects of caffeine on the antigen-specific responses of naïve splenocytes. Our data indicate that caffeine suppresses antigen-specific cell proliferation of splenocytes (Fig. 1) and the secretion of IL-2 (Fig. 2A), which is the most important cytokine for T cell proliferation, without any adverse effects on cell viability. Splenocytes include various types of cells that may have various sensitivities to caffeine, and therefore, suppression of the whole batch of

splenocytes caused by caffeine might be inconsistent. Additionally, we demonstrated that caffeine treatment reduces the secretion of IL-4 (Fig. 3A), IFN- γ (Fig. 2B), and IL-10 (Fig. 3C). These results are consistent with previous observations of non-specific stimulated T cells (Rosenthal, L.A. *et al.* 1992, Ritter, M. *et al.* 2005).

Caffeine alters calcium signaling and thereby induces a transient increase in intracellular calcium (Ritter, M. *et al.* 2001). The cell-surface receptors IP3R and RyR are thought to be involved in calcium signaling in T cells. Caffeine has been shown to block IP3Rs, which are involved in calcium signaling in T cells (Premack, B. A. and Gardner, P., 1992); perturbation of IP3R-dependent, but not RyR-dependent, calcium signaling is considered to

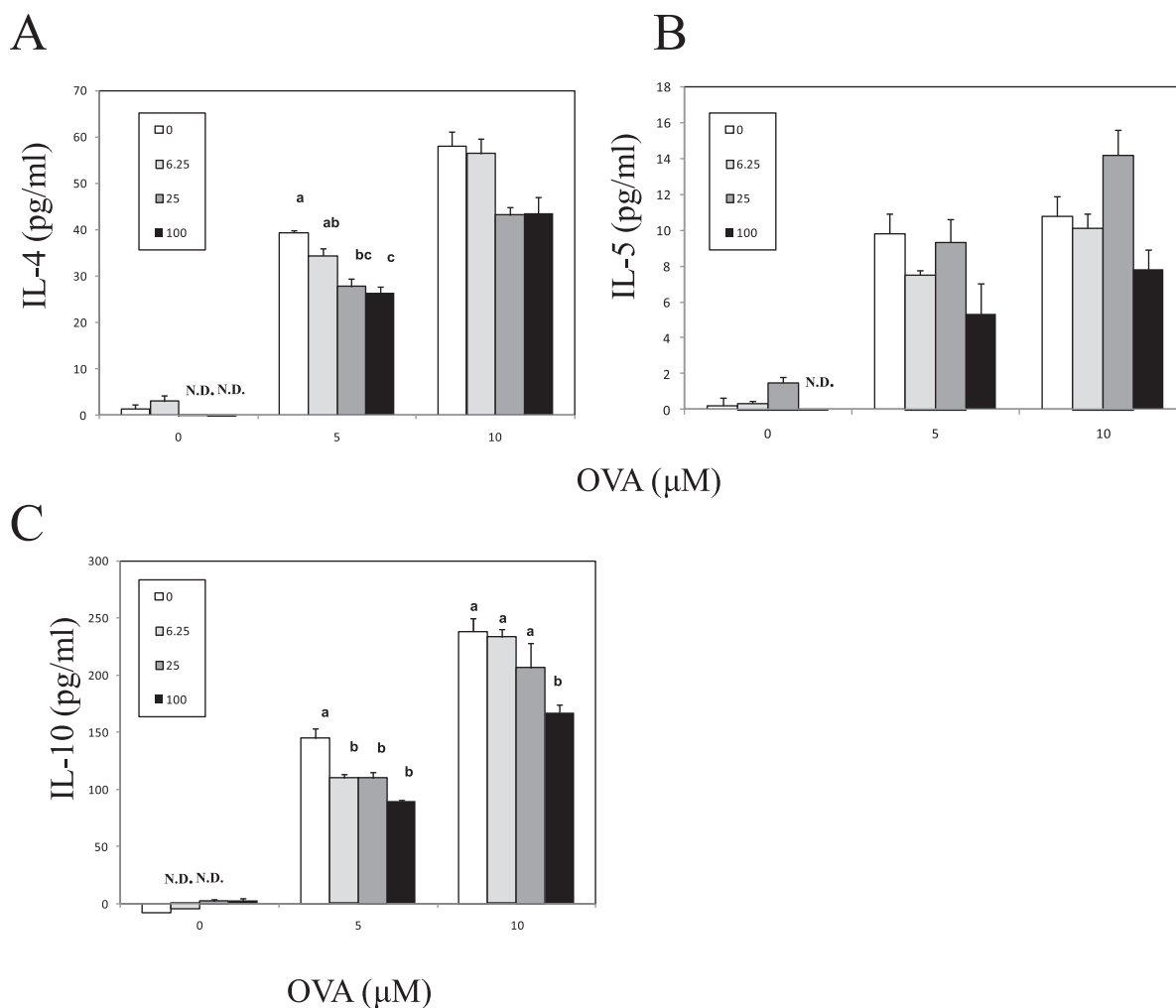


Fig. 3 Effects of caffeine on antigen-specific Th2 type cytokines (A: IL-4, B: IL-5, C: IL-10) secretion of splenocytes from naïve transgenic mice

Pooled splenocytes from four DO11.10 mice were cultured with 0 (control), 6.25, 25, and 100 µM of caffeine in the presence of 5 and 10 µM of OVA for 48 h (A and B) and 72h (C). Concentrations of cytokines were measured by ELISA. Comparison was done among the data of OVA stimulation by equal concentration. Different letters above each bar indicate statistical difference ($p < 0.05$).

be responsible for the suppression of cytokine production (Ritter, M. *et al.* 2005). Therefore, it is likely that caffeine caused the suppression of antigen-specific IL-2 secretion and cell proliferation of naïve cells through a similar mechanism.

These findings imply that the signal-transduction pathways that govern the expression of these cytokines are different. Indeed, it has been shown that the regulation of IL-5 expression is different from that of IL-4 and IL-10 (Zhu, J. *et al.* 2004).

IgG1 secretion from splenocytes was significantly suppressed by caffeine treatment (Fig. 4A). The secretion of IgG1 is influenced by the production of Th2-type cy-

tokines, particularly IL-4 (Purkerson, J. and Isakson, P., 1992). Therefore, caffeine might decrease IgG1 through IL-4 suppression. Additionally, it was reported that IP3R is also involved in signal transduction that regulates Ig production in B cells (Sugawara, H. *et al.* 1997, Dellis, O. *et al.* 2006). Hence, caffeine might generally affect Ig secretion through a synergistic effect from its interaction with IP3R on B cells and with T cells. However, this assumption is less likely because the secretion of IgA, IgG2 a, and IgM was not significantly affected by caffeine treatment.

Future clinical and epidemiological studies on caffeine consumption are needed to elucidate the exact physi-

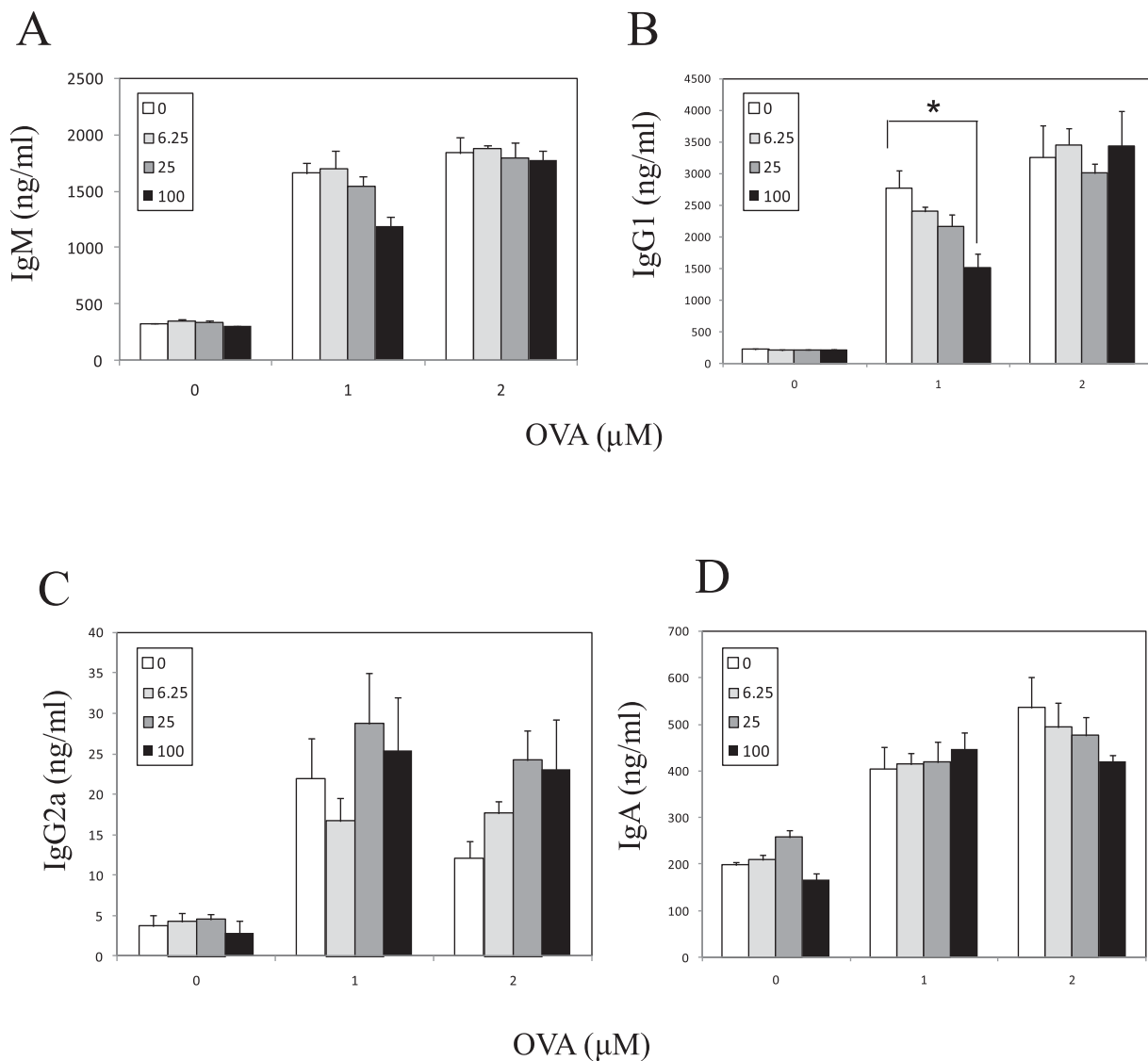


Fig. 4 Effects of caffeine on antigen-specific antibodies (A: IgM, B: IgG1, C: IgG2a, D: IgA) secretion of splenocytes from naïve transgenic mice

Pooled splenocytes from four DO11.10 mice were cultured with 0 (control), 6.25, 25, and 100 μM of caffeine in the presence of 1 and 2 μM of OVA for 1 wk. Concentrations of antibodies were measured by ELISA. Comparison was done among the data of OVA stimulation by equal concentration. Different letters above each bar indicate statistical difference ($p < 0.05$).

ological mechanism of immune response.

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