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Histamine inhibits advanced glycation end products-induced adhesion molecule expression on human monocytes

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References 37

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d) **Abbreviations: AGEs, advanced glycation end products; BSA, bovine serum albumin ; cAMP, cyclic adenosine monophosphate ; dbcAMP, dibutyryl cAMP; ELISA, enzyme-linked immunosorbent assay ; FITC, fluorescein isothiocyanate ; 4-MH, 4-methylhistamine dihydrochloride ; HDC, Histidine decarboxylase ; ICAM,**

intercellular adhesion molecule ; IFN, interferon; m, monoclonal ; IL, interleukin, LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; PKA, protein kinase A ; RAGE, receptor for advanced glycation end products ;

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ABSTRACT

Advanced glycation end products (AGEs) are modifications of proteins/ lipids that become nonenzymatically glycated after contact with aldose sugars. Among various subtypes of AGEs, glyceraldehyde-derived AGE (AGE-2) and glycolaldehyde-derived AGE (AGE-3) are suggested to play roles in inflammation in diabetic patients. Since the engagement of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40 on monocytes with their ligands on T-cells plays roles in cytokine production, we examined the effects of AGE-2 and AGE-3 on the expression of adhesion molecules and cytokine production in human peripheral blood mononuclear cells (PBMC) and their modulation by histamine in the present study. AGE-2 and AGE-3 induced the expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes and the production of interferon (IFN)-γ in PBMC. Histamine concentration-dependently inhibited the action of AGE-2 and AGE-3. The effects of histamine were antagonized by an H_2-receptor antagonist, famotidine, and mimicked by H_2/H_4-receptor agonists, dimaprit and 4-methylhistamine. Histamine induced cyclic adenosine monophosphate (cAMP) production in the presence and absence of AGE-2 and AGE-3. The effects of histamine were reversed by a protein

kinase A (PKA) inhibitor, H89, and mimicked by a dibutyryl cAMP (dbcAMP) and an adenylate cyclase activator, forskolin. These results as a whole indicated that histamine inhibited the AGE-2- and AGE-3-induced adhesion molecule expression and cytokine production via H_2-receptors and the cAMP/PKA pathway.

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INTRODUCTION

AGEs are products of the non-enzymatic glycation/oxidation of proteins/lipids that accumulate during natural aging and are also greatly augmented in disorders such as diabetes, renal failure and Alzheimer's disease (Brownlee, 1995; Takedo et al., 1996; Schmidt et al., 1994). AGEs are also implicated in the pathogenesis of atherosclerotic vascular disease of diabetic etiology (Takeuchi and Yamagishi, 2004). Direct immunochemical evidence for the existence of five distinct AGEs structures including AGE-2, AGE-3, AGE-4 and AGE-5 was identified, within the AGE-modified proteins and peptides (Takeuchi and Yamagishi, 2004). Among various subtypes of AGE, toxic AGE structures, AGE-2 and AGE-3, are the main structures of AGEs that are detectable in the serum of diabetic patients (Takeuchi and Yamagishi, 2004). AGE-2 is reported to induce diabetic microangiopathy (Takeuchi et al., 2000). AGE-2 and AGE-3 also have diverse biological activities on vascular wall cells, mesangial cells, Schwann cells, malignant melanoma cells and cortical neurons (Yamagishi and Imaizumi, 2005; Okamoto et al., 2002). AGEs and the receptor for AGEs (RAGE) are detected in atherosclerotic plaque of diabetic patients (Cuccurullo et al., 2006). The stimulation of

RAGE induces plaque rupture in diabetic patients. *In vitro* work has shown the involvement of RAGE, leading to oxidative stress and vascular damage, particularly in atherosclerosis (Vlassara and Palace 2002) and in diabetes (Ruderman et al., 1992).

Microinflammation is a common major mechanism in the pathogenesis of diabetic vascular complications. It is reported that diabetes has greater infiltration of macrophages and T-cells in atherosclerotic plaques (Burke et al., 2004). Activation of monocytes/macrophages and T-cells induces the progression of inflammatory atherosclerotic plaques (Stoll and Bendszus, 2006). It has been found that the enhancement of ICAM-1, B7.1, B7.2 and CD40 expression on monocytes results in the activation of T-cells (Camacho et al., 2001; Ranger et al. 1996; Durie et al., 1994). Therefore, the blockade of engagement of adhesion molecules by antibodies against ICAM-1, B7.1, B7.2 and CD40 reduced the production of IFN-γ by PBMC (Takahashi et al., 2003; Morichika et al., 2003). In a previous study, we found that AGE-2 and AGE-3 induced the expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes and production of IFN-γ in human PBMC, but AGE-4 and AGE-5 had no effect (Takahashi et al., in press). The effect of AGE-2 and AGE-3 on the production of IFN-γ was dependent on

cell-to-cell interaction via engagement between ICAM-1, B7.1, B7.2 and CD40 on monocytes and their ligands on T-cells, and the stimulation of RAGE on monocytes was involved in the actions of AGE-2 and AGE-3 (Takahashi et al., in press).

Histamine has immunoregulatory properties as it modulates cytotoxic T-cell activity (Khan et al., 1989), NK-cell activity (Hellstrand et al., 1994) and cytokine production in PBMC (van der Pouw Kraan et al., 1998; Elenkov et al., 1998; Dohlsten et al., 1987). Histamine exerts its effects through the stimulation of H_1-, H_2-, H_3- and H_4-receptors (van der Pouw Kraan et al., 1998; Elenkov et al., 1998). In general, immunoregulatory effects of histamine depend on the stimulation of H_2-receptors (van der Pouw Kraan et al., 1998; Elenkov et al., 1998; Hough, 2001). H_2-receptor stimulation is coupled with the activation of adenylate cyclase and the cAMP/PKA pathway in monocytes (Shayo et al., 1997). However, little is known about the effect of histamine on the AGEs-induced activation of monocytes. In the present study, we examined the effect of histamine on the expressions of ICAM-1, B7.1, B7.2 and CD40 and the production of IFN-γ induced by AGE-2 and AGE-3.

METHODS

Reagents and drugs

Recombinant human IL-18 was purchased from Medical & Biological Laboratories (Nagoya, Japan). Histamine dihydrochloride was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dimaprit dihydrochloride and 4-methylhistamine dihydrochloride (4-MH) were gifts from Drs. WAM Duncan and DJ Durant (The Research Institute, Smith Kline and French Laboratories, Welwyn Garden City, Herts, UK). *d*-Chlorpheniramine maleate, ranitidine and famotidine were provided by Yoshitomi Pharmaceutical Co. Ltd. (Tokyo, Japan), Glaxo Japan (Tokyo, Japan) and Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan), respectively. Thioperamide hydrochloride was provided by Eisai Co. Ltd. (Tokyo, Japan). AGE-modified bovine serum albumin (BSA) (Sigma Aldrich, St Louis, MO) was prepared as previously described (Takeuchi et al., 2000; Takahashi et al., in press). Briefly, each protein was incubated under sterile conditions with glyceraldehyde 3-phosphate (AGE-2) (Sigma Aldrich) or glycolaldehyde (AGE-3) (Sigma Aldrich) in 0.2 M phosphate buffer (pH 7.4) at 37 ^{o}C for 7 days. AGE-BSA was dialyzed for 2 days at 4°C. The endotoxin concentration of AGEs at 100

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μg/ml described above was measured at SRL (Okayama, Japan) and was found to be 1.2 pg/ml. AGE-specific fluorescence was measured at 450 nm after excitation at 390 nm with a fluorescence spectrophotometer (Hitachi, Tokyo, Japan). DbcAMP and forskolin were purchased from Wako (Tokyo,Japan). H-89 was purchased from Sigma Chemical (St. Louis, MO). For flow cytometric analysis, fluorescein isothiocyanate (FITC)-conjugated mouse IgG1 monoclonal (m) Ab against ICAM-1/CD54 and phycoerythrin (PE)-conjugated anti-CD14 mAb were purchased from DAKO (Glostrup, Denmark). FITC-conjugated mouse IgG1 mAb against B7.2 and CD40 were purchased from Pharmingen (San Diego, CA), and FITC-conjugated IgG1, as an isotype-matched control was obtained from Sigma Chemical.

Isolation of PBMC and monocytes

Normal human PBMC were obtained from ten healthy volunteers after acquiring IRB approval (Okayama Univ. IRB No.106). Twenty to fifty ml of peripheral blood were withdrawn from a forearm vein, and PBMC were prepared from buffy coat as previously described (Takahashi et al., 2003). When monocytes were isolated from PBMC,

counterflow centrifugal elutriation was used for separation as previously described (Takahashi et al., 2003). The PBMC and monocytes were then suspended at a final concentration of $1x10^6$ cells/ ml in the medium as previously described (Takahashi et al., 2003).

Flow cytometric analysis

Changes in the expression of human leukocyte antigens, ICAM-1, B7.1, B7.2 and CD40, on monocytes were examined by multi-color flow cytometry using a combination of anti-CD14 Ab with anti-ICAM-1, anti-B7.1, anti-B7.2 or anti-CD40 Ab. PBMC at $1x10^6$ cells/ml were incubated for 24 h. Cultured cells at $5x10^5$ cells/ml were prepared for flow cytometric analysis as previously described (Takahashi et al., 2003) and analyzed with a FACS Calibur (BD Biosciences, San Jose, CA). The data were processed using the CELL QUEST program.

Cytokine assay

PBMC at $1x10^6$ cells/ml were used to analyze IFN-γ production. After culturing for 24 h

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at 37 ^{o}C in a 5%CO2/air mixture, cell-free supernatant was assayed for IFN-γ protein by enzyme-linked immunosorbent assay (ELISA) employing the multiple Abs sandwich principle (R&D Systems, Minneapolis, MN). The detection limit of ELISA for IFN-γ was 10 pg/ml.

Proliferation assay

PBMC were treated with various conditions. Cultures were incubated for 24 h, during which they were pulsed with [^{3}H]-thymidine (3·3 Ci/well) for the final 16 h. Cells were then divided into 96-well microplates, 200 μl/well, resulting in 1μCi [^{3}H]-thymidine per well, and harvested by the Micro-Mate 196 Cell Harvester (Perkin Elmer Life Science Inc., Boston, MA, USA). Thymidine incorporation was measured by a beta-counter (Matrix 9600, Perkin Elmer Life Science Inc.).

Measurement of cAMP production in monocytes.

Monocytes at 1x10^{6} cells/ ml were incubated at 37 ^{o}C in a 5%CO2/air mixture under different conditions. When the effects of histamine receptor antagonists were examined,

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the antagonists were added to the media 30 min before histamine addition. AGEs and histamine were simultaneously added to the media. After 24 h, cells at $2x10^5$ cells/200 µl/well were supplemented with trichloroacetic acid to a final concentration of 5% and 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase at 100 µM and frozen at –80 ^oC. Frozen samples were subsequently sonicated and assayed for cAMP using a cAMP enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions, for which no acetylation procedures were performed. The results are expressed as the means $\pm$ S.E.M. for five donors.

Statistical examination

Statistical significance was evaluated using ANOVA followed by Dunnett's test. A probability value of less than 0.05 was considered to indicate statistical significance. The results are expressed as the means $\pm$ S.E.M. of triplicate findings from five donors.

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RESULTS

The effects of histamine on AGE-2 and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes, the production of IFN-γ and the lymphocyte proliferation in PBMC

In a previous study, to evaluate the binding of AGE subtypes to RAGE, we established an *in vitro* assay by using immobilized AGE subspecies and His-tagged sRAGE protein (Takahashi et al., in press). AGE-2 and AGE-3 showed relatively high affinity binding for sRAGE, whereas AGE-4 and AGE-5 showed moderate affinity for sRAGE. The proper incubation time and concentration of AGEs were determined according to the study reported (Takahashi et al., in press). AGE-2 and AGE-3 at 100 μg/ml significantly induced the expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ at 16 h and thereafter up to 24 and 48 h. As shown in Fig. 1, we observed the effects of histamine at concentrations ranging from 0.1 to 100 μM on the expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ in the presence (100 μg/ml) or absence of AGE-2 and AGE-3 at 24 h. Moreover, AGE-2 and AGE-3 significantly induced the lymphocyte proliferation at 16 h and thereafter up to 24, 48 and 72 h (Fig. 2). The effect

of histamine on lymphocyte proliferation was determined at 24 h.

Histamine concentration-dependently inhibited the AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and the lymphocyte proliferation. IC_{50} values for the inhibitory effect of histamine on the expressions of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and the lymphocyte proliferation in the presence of AGE-2 were 1, 1, 1.5, 2, 0.8 and 0.8 μM, and those in the presence of AGE-3 were 1, 1, 1.7, 1.5, 0.8 and 0.8 μM, respectively. In the absence of AGE-2 and AGE-3, histamine induced the production of IFN-γ, but had no effect on the adhesion molecule expression and the lymphocyte proliferation. ED_{50} values for the effect of histamine alone on the production of IFN-γ was 4 μM, respectively.

The involvement of H_2-receptor in the actions of histamine

To determine the histamine receptor subtypes involved in the effects of histamine on the expressions of ICAM-1, B7.1, B7.2 and CD40 and the production of IFN-γ in the presence of AGE-2 and AGE-3, the effects of an H_1-receptor antagonist, *d*-chlorpheniramine, an H_2-receptor antagonist, famotidine and an $H_{3/4}$-receptor

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antagonist, thioperamide at concentrations ranging from 0.1 to 100 μM on the adhesion molecule expression and cytokine production were examined in the presence of histamine at 100 μM (Fig. 3). Famotidine concentration-dependently inhibited the action of histamine, but *d*-chlorpheniramine and thioperamide had no effect. Another H_2-receptor antagonist, ranitidine exerted a substantially similar effect to famotidine (data not shown).

As shown in Fig. 4, the effects of H_2/H_4-receptor agonists, dimaprit and 4-MH (Parsons et al., 1977), at concentrations ranging from 0.1 to 100 μM were determined in the presence of AGE-2 and AGE-3 at 100 μg/ml. Both dimaprit and 4-MH inhibited the expressions of ICAM-1, B7.1, B7.2 and CD40 and the production of IFN-γ in a concentration-dependent manner. The potency and efficacy of two agonists were quite similar to those of histamine in each response. Moreover, we found that an H_1-agonist, 2-(2-pyridyl)ethylamine dihydrochloride (Durant et al., 1975) and an H_3-agonist, (*R*)-α-methylhistamine dihydrochloride (Arrang et al., 1987) had no effect on the adhesion molecule expression and cytokine production induced by AGE-2 and AGE-3 (data not shown).

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The effects of histamine on the production of cAMP in monocytes in the presence or absence of AGE-2 and AGE-3

The effects of histamine at 100 μM on the production of intracellular cAMP in monocytes isolated from PBMC in the presence (100 μg/ml) or absence of AGE-2 and AGE-3 were determined (Fig. 5). Histamine induced the production of cAMP in monocytes with a peak 30 min after stimulation. The presence of AGE-2 and AGE-3 did not influence the production of cAMP induced by histamine. The H_2-receptor antagonist, famotidine at 100 μM inhibited the effect of histamine on the production of cAMP (Fig. 5). Also, the H_2/H_4-receptor agonist, dimaprit at 100 μM induced the production of cAMP (Fig. 5).

The involvement of cAMP in the action of histamine

To investigate the involvement of the cAMP/PKA pathway in the action of histamine, the effects of a PKA inhibitor, H89, at concentrations ranging from 0.1 to 100 μM on the action of histamine at 100 μM were determined (Fig. 6). In the absence of histamine, the PKA inhibitor had no effect on the adhesion molecule expression and cytokine expression. H89 reversed the inhibitory effect of histamine on the expressions of ICAM-1, B7.1, B7.2

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and CD40 and the production of IFN-γ in the presence of AGE-2 or AGE-3. As shown in Fig. 7, the effects of a membrane-permeable cAMP analog, dbcAMP, and an adenylate cyclase activator, forskolin, at concentrations ranging from 0.1 to 100 μM on the expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes and the production of IFN-γ in PBMC were examined. Both dbcAMP and forskolin inhibited the AGE-2 and AGE-3-induced adhesion molecule expression and cytokine production in a concentration-dependent manner.

The effect of addition of IL-18 on modulatory effects of histamine on AGE-2- and AGE-3-induced ICAM-1 expressions on monocytes.

The addition of increasing concentrations of IL-18 to the culture medium at the start of incubation antagonized either the inhibitory effect of histamine at 100 μM on AGE-2- and AGE-3-induced ICAM-1 expression (Fig. 8).

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DISCUSSION

It is reported that the AGE-BSA enhances dendritic cell-induced stimulation of lymphocyte proliferation and cytokine production (Ge et al., 2005). On the other hand, histamine inhibits lymphocyte proliferation and cytokine production via H2-receptors (Nakane et al., 2004). However, little is known about the effect of histamine on the actions of AGE-2 and AGE-3 in human PBMC. In the present study, we clearly demonstrated that histamine inhibited the AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 on human monocytes, the production of IFN-γ and the lymphocyte proliferation in PBMC (Figs. 1 and 2). The action of histamine was inhibited by the H_2-antagonist, famotidine but not the H_1-antagonist, *d*-chlorpheniramine and the $H_{3/4}$-antagonist, thioperamide (Fig. 3). The H_2/H_4-receptor agonists, dimaprit and 4-MH mimicked the action of histamine (Fig. 4). Since the IC_{50} values of histamine and H_2/H_4-receptor agonists to prevent the up-regulation of adhesion molecule expression and cytokine production were consistent with the affinity of those agonists to typical H_2-receptors (Johnson 1982; Elenkov et al., 1998; Kohka et al., 2000; Morichika et al., 2003: Takahashi et al., 2002), it was concluded that the inhibitory effect of histamine was

mediated by the stimulation of H_2-receptors but not H_1-, H_3- and H_4-receptors.

As shown in Fig. 5, histamine induced the production of cAMP in monocytes via H_2-receptor irrespective of the presence of AGE-2 or AGE-3. The findings that the PKA inhibitor, H89, inhibited the action of histamine (Fig. 6) and that the cAMP analog, dbcAMP, and the adenylate cyclase activator, forskolin, mimicked the effect of histamine (Fig. 7) strongly suggested the involvement of the cAMP/PKA pathway in the action of histamine.

We observed a similar pattern of inhibitory effects of histamine on lipopolysaccharide (LPS)- and IL-18-induced activation of monocytes in human PBMC via H_2-receptors (Morichika et al., 2003: Takahashi et al., 2002). IL-18 is reported to induce the expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes (Takahashi et al., 2002; Takahashi et al., 2003). While AGE-2 and AGE-3 does not induce production of IL-18 in PBMC (Takahashi et al., in press), histamine induces production of IL-18 via H_2-receptor and the cAMP/PKA pathway in monocytes (Takahashi et al., 2006). Thus, there may be a common pathway triggered by LPS, IL-18 and AGEs that was regulated by the H_2-receptors-cAMP/PKA system. Further work is necessary on this issue.

While AGE-2 and AGE-3 does not induce production of IL-18 in PBMC (Takahashi et al., in press), histamine induces production of IL-18 via H_2-receptor and the cAMP/PKA pathway in monocytes (Takahashi et al., 2006). The amount of IL-18 production induced by histamine at 100 μM was 2.5 ng/ml. IL-18 is reported to induce the expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes (Takahashi et al., 2002; Takahashi et al., 2003). As shown in Fig. 8, the requirement of relatively higher concentration of exogenous IL-18 for reversing the inhibitory effect of histamine on AGE-2- and AGE-3-induced action may reflect the IL-18 concentration needed for the functional antagonism of histamine action on ICAM-1 expression. The regulatory mechanisms for ICAM-1 expression in the presence of plural stimuli should be clarified.

In the previous study, we found that AGE-2 and AGE-3 have higher affinity for RAGE than AGE-4 and AGE-5 using an *in vitro* binding assay (Takahashi et al., in press). AGE-2 and AGE-3, but not AGE-4 and AGE-5, induced the up-regulation of their receptor RAGE expression on the cell surface of monocytes. An NF-κB activation inhibitor, SN50, inhibits AGE-2- and AGE-3-induced adhesion molecule expressions and the cytokine production (Takahashi et al., in press), and the interaction of AGE and

RAGE enhances the expression of RAGE through activation of NF-kB in monocytes (Li and Schmidt, 1997). However, histamine had no effect on the expression of RAGE in the presence and absence of AGE-2 and AGE-3 (data not shown). Thus, it might be possible that the downstream pathways of NF-kB activation leading to up-regulation of adhesion molecules and RAGE are probably differentially regulated by the cAMP-PKA system.

Histidine decarboxylase (HDC), which produces histamine from L-histidine, is detected in monocytes/macrophages located in the arterial intima in human atherosclerotic lesions (Higuchi et al., 2001). In a rat model of streptozotocin-induced diabetes mellitus, histamine levels are elevated in various tissues, including the aorta (Gill et al., 1990). Moreover, vascular smooth muscle cells (SMCs) express HDC after injury to endothelial cells (Sasaguri et al., 2005). The resultant production of histamine may regulate vascular contraction directly or indirectly via nitric oxide production (Tanimoto et al., 2007). In addition to the control of vascular contraction, the modulatory effects of histamine on micro-inflammation in atherosclerotic intima through the regulation of monocytes/macrophages are shown in the present study. In fact, AGEs have been demonstrated in atherosclerotic plaque, where macrophages with up-regulated RAGE are

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present (Cuccurullo et al., 2006). Therefore, the regulatory action of histamine through the stimulation of H_2-receptors may inhibit the macrophage-mediated events stimulated by AGE-2 and AGE-3, including the production of IFN-γ and adhesion-molecule-dependent activation of T-cells (Takahashi et al., in press). Thus, locally produced histamine may exert inhibitory influence on the secretory response of macrophages and T-cells activation, leading to the reduction of atherosclerotic plaque formation. Such a possibility should be evaluated by an *in vivo* atherosclerotic model. It is reported that atherosclerotic leisions are reduced in HDC-knockout mice, as compared with wild-type mice (Tanimoto et al., 2006). However, in HDC-knockout mice, the deficiency of granule formation and proteinases expression in mast cells is also observed (Ohtsu et al., 2002). Therefore, attension should be paid to obtaining a straightforward explanation for the phenotype of knockout mice.

It is reported that an H_1-receptor antagonist, diphenhydramine, reduces the formation of intimal hyperplasia in a mouse model with endothelial injury, however, an H_2-receptor antagonist, cimetidine, is ineffective (Miyazawa et al., 1998). Cimetidine inhibits neither the proliferation nor migration of mouse vascular smooth muscle cells stimulated by

platelet-derived growth factor, while diphenhydramine significantly inhibited proliferation, but did not inhibit migration (Miyazawa et al., 1998). Therefore, stimulation of H_1-receptor may induce the proliferation of vascular smooth muscle cells, while H_2-receptor stimulation may inhibit the activation of monocytes, leading to the prevention of atherosclerosis. Further study of the role of H_1- and H_2-receptor stimulation should be continued.

In conclusion, histamine inhibited the AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ via H_2-receptors and the cAMP/PKA pathway. Through the inhibition of toxic AGE-dependent responses in monocytes, the stimulation of H_2-receptors may partially contribute to regulating the development of atherosclerotic plaques in diabetes.

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Footnotes

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LEGENDS FOR FIGURES

Figure 1 The effects of histamine on the AGE-2 and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes and production of IFN-γ in PBMC

PBMC at $1x10^6$ cells/ml were incubated with AGE-2 (A) and AGE-3 (B) at 100 mg/ml and histamine at increasing concentrations from 0.1 to 100 μM for 24 h. The expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes were determined by flow cytometry. FITC-conjugated IgG1 was used as an isotype-matched control Ab. IFN-γ concentration in conditioned media was determined by ELISA. Filled circles (●) represent the effect of histamine on the adhesion molecule expression and cytokine production in the presence of AGE-2 and AGE-3. Open circles (○) represent the effect of histamine in the absence of AGE-2 and AGE-3. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ***P< 0.01* compared with the value for AGE-2 and AGE-3. *##P< 0.01* compared with the value for medium alone. When an error bar was within a symbol, the bar was omitted.

Figure 2 The effects of histamine on the AGE-2 and AGE-3-induced lymphocyte

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proliferation

PBMC at $2x10^6$ cells/ml were incubated with AGE-2 (A), AGE-3 (B) or BSA (C) at 100 μg/ml for the indicated periods, and the lymphocyte proliferation was determined by [^{3}H]-thymidine uptake as described in Methods. The concentration-response relationships for the effects of AGE-2 (D), AGE-3 (E) or BSA (F) on the lymphocyte proliferation were determined at 24 h. The effects of increasing concentrations of histamine on 100 μg/ml AGE-2 (G)-, AGE-3 (H)- or BSA (I)-induced lymphocyte proliferation were determined at 48 h. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ***P< 0.01* compared with the value for 0 h (A,B,C) or 0 μg/ml (D,E,F). *##P< 0.01* compared with the value in the presence of AGE-2 or AGE-3 alone.

Figure 3 The effects of histamine receptor antagonists on the histamine-induced inhibition of expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ

PBMC at $1x10^6$ cells/ml were incubated with different classes of histamine receptor antagonists, including *d*-chlorpheniramine (H_1-antagonist), famotidine (H_2-antagonist)

and thioperamide (H3/4-antagonist), at increasing concentrations from 0.1 to 100 μM in the presence or absence of AGE-2 (A) and AGE-3 (B) at 100 μg/ml. The expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes were determined by flow cytometry. In addition, IFN-γ concentration was determined by ELISA. Filled circles (●) represent the effect of antagonists on histamine-inhibited adhesion molecule expression and cytokine production in the presence of AGE-2 and AGE-3. Open squares (□) represent the effect of antagonists in the presence of AGE-2 and AGE-3 without histamine stimulation. Open circles (○) represent the effect of antagonists on adhesion molecule expression cytokine production in the absence of histamine, AGE-2 and AGE-3. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. **P< 0.05, **P< 0.01* compared with the value for histamine. When an error bar was within a symbol, the bar was omitted.

Figure 4 The effects of histamine receptor agonists on the AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ

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PBMC at $1x10^6$ cells/ml were incubated with histamine H_2/H_4-receptor agonists, dimaprit (A) and 4-MH (B) at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 at 100 μg/ml for 24 h. The expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes were determined by flow cytometry, and IFN-γ concentration in the conditioned media was determined by ELISA. Filled circles (●) represent the effect of agonists on AGE-2-induced responses, and open circles (○) represent AGE-3-induced responses. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ***P< 0.01* compared with the value for AGE-2 or AGE-3 alone. When an error bar was within a symbol, the bar was omitted.

Figure 5 The effects of histamine on the production of cAMP in monocytes in the presence or absence of AGE-2 and AGE-3

Monocytes at $1x10^6$ cells/ml were incubated with histamine at 100 μM in the presence (filled circles; ●) and absence (open circles; ○) of AGE-2 (A) and AGE-3 (B) at 100 μg/ml, and time course changes in the levels of cAMP in monocytes were determined at the indicated time points. (C) The effects of histamine and dimaprit at 100 μM in

combination with famotidine on the production of cAMP were determined in the presence or absence of AGE-2 at 100 μg/ml. (D) The effects of histamine and dimaprit at 100 μM in combination with famotidine on the production of cAMP were determined in the presence or absence of AGE-3 at 100 μg/ml. ***P< 0.01* compared with the corresponding value in the absence of histamine. *##P< 0.01* compared with the corresponding value in the presence of histamine. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. When an error bar was within a symbol, the bar was omitted. ND, not detected.

Figure 6 The effects of PKA inhibitor on the histamine-induced expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ

The effects of a PKA inhibitor, H89, at increasing concentrations from 0.1 to 100 μM on the 100 μM histamine-induced inhibition of expressions of ICAM-1, B7.1, B7.2 and CD40 and production of IFN-γ in the presence of AGE-2 (A) and AGE-3 (B) at 100 μg/ml were determined. Filled circles (●) represent the effect of H89 on the histamine-induced inhibition of responses in the presence of AGE-2 and AGE-3. Open

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squares (□) represent those in the presence of AGE-2 and AGE-3 without histamine stimulation. Open circles (○) represent the effect of H89 on the responses in the absence of both histamine and AGEs. The results are expressed as the means ± S.E.M. of triplicate findings from five donors. ***P< 0.01* compared with the value in the presence of histamine and AGEs. When an error bar was within a symbol, the bar was omitted.

Figure 7 The effects of forskolin and dbcAMP on AGEs-induced expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes and production of IFN-γ in PBMC

PBMC at $1x10^6$/ml were incubated with a cAMP analog, dbcAMP (A) and an adenylate cyclase activator, forskolin (B) at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 at 100 μg/ml for 24 h. Filled circles (●) represent the effects of dbcAMP or forskolin on AGE-2-induced responses, and open circles (○) represent the effects of dbcAMP or forskolin on the AGE-3-induced responses. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ***P< 0.01* compared with the value for AGE-2 or AGE-3 alone. When an error bar was within a symbol, the bar was omitted.

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Figure 8 The effect of IL-18 on the modulatory effects of histamine on AGE-2- and AGE-3-induced ICAM-1 expression on monocytes..

PBMC at $1x10^6$ cells/ml were incubated with IL-18 at increasing concentrations from 0.01 to 10 ng/ml in the absence or presence of AGE-2 and AGE-3 at 100 μg/ml and histamine at 100 μM for 24 h. At the end of the culture, the expressions of ICAM-1 were determined by flow cytometry. The results are expressed as the means ± S.E.M. of five donors with triplicate determinations. ***P< 0.01* compared with the value for histamine.

Figure 1

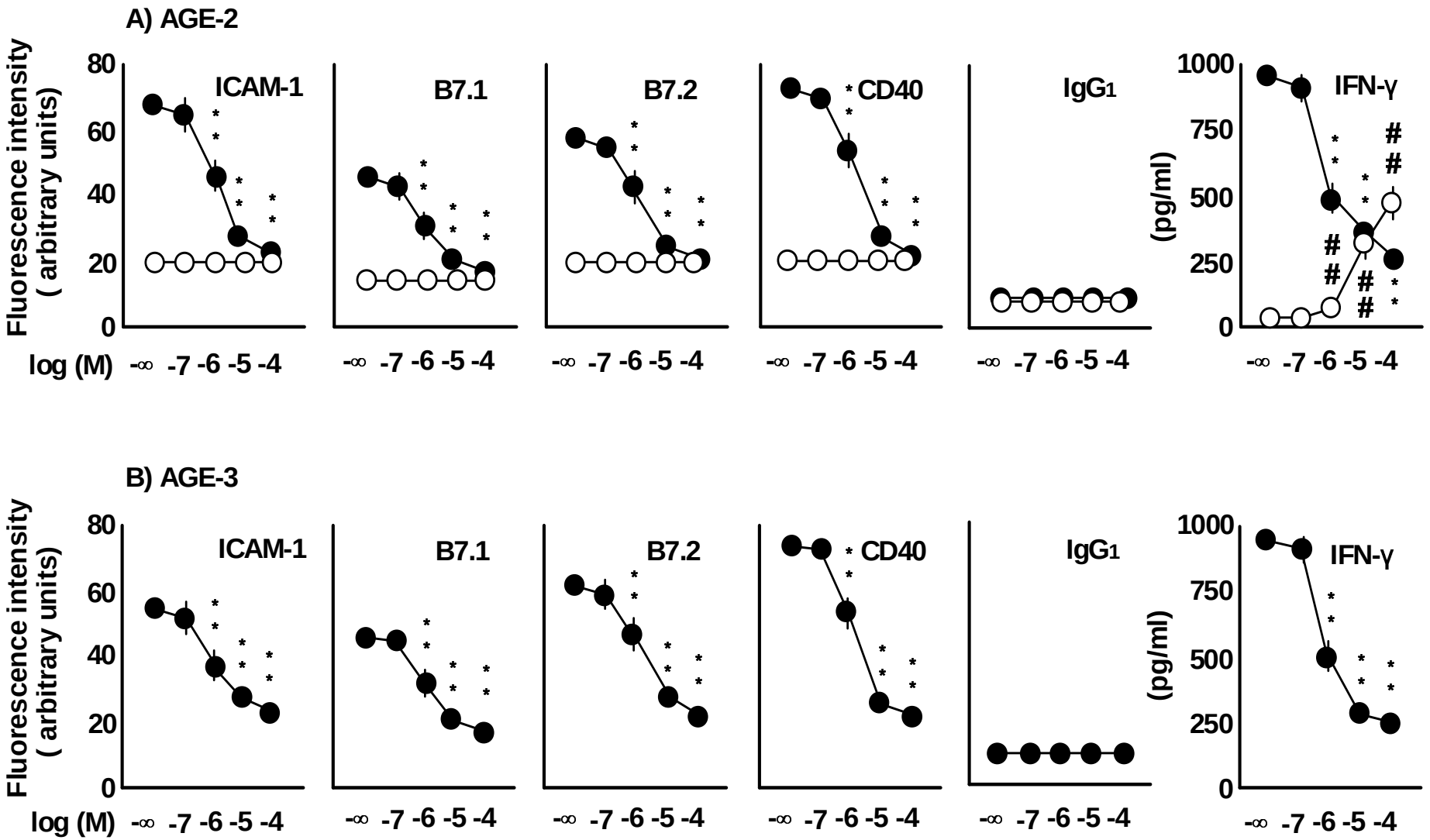

Figure 2

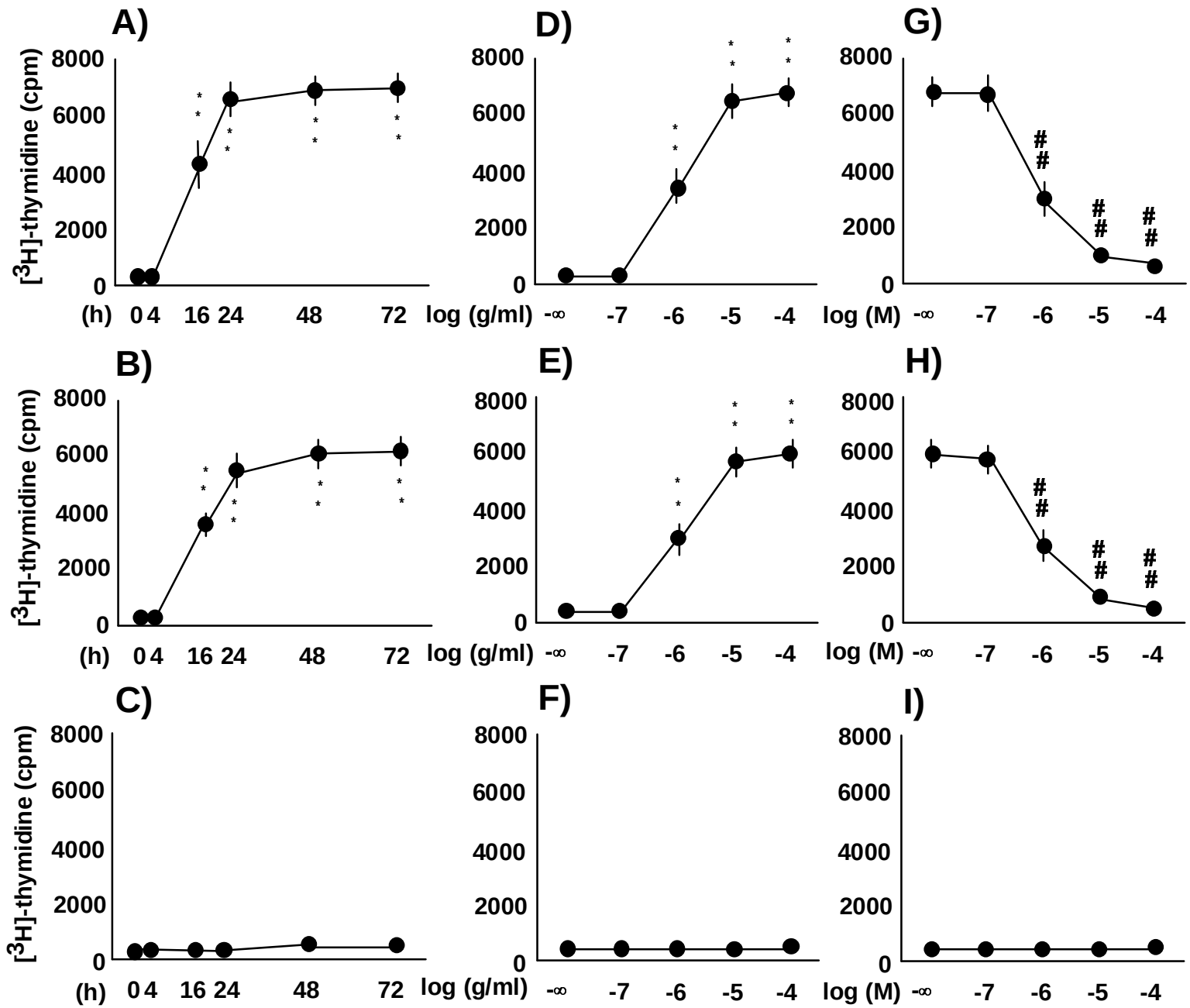

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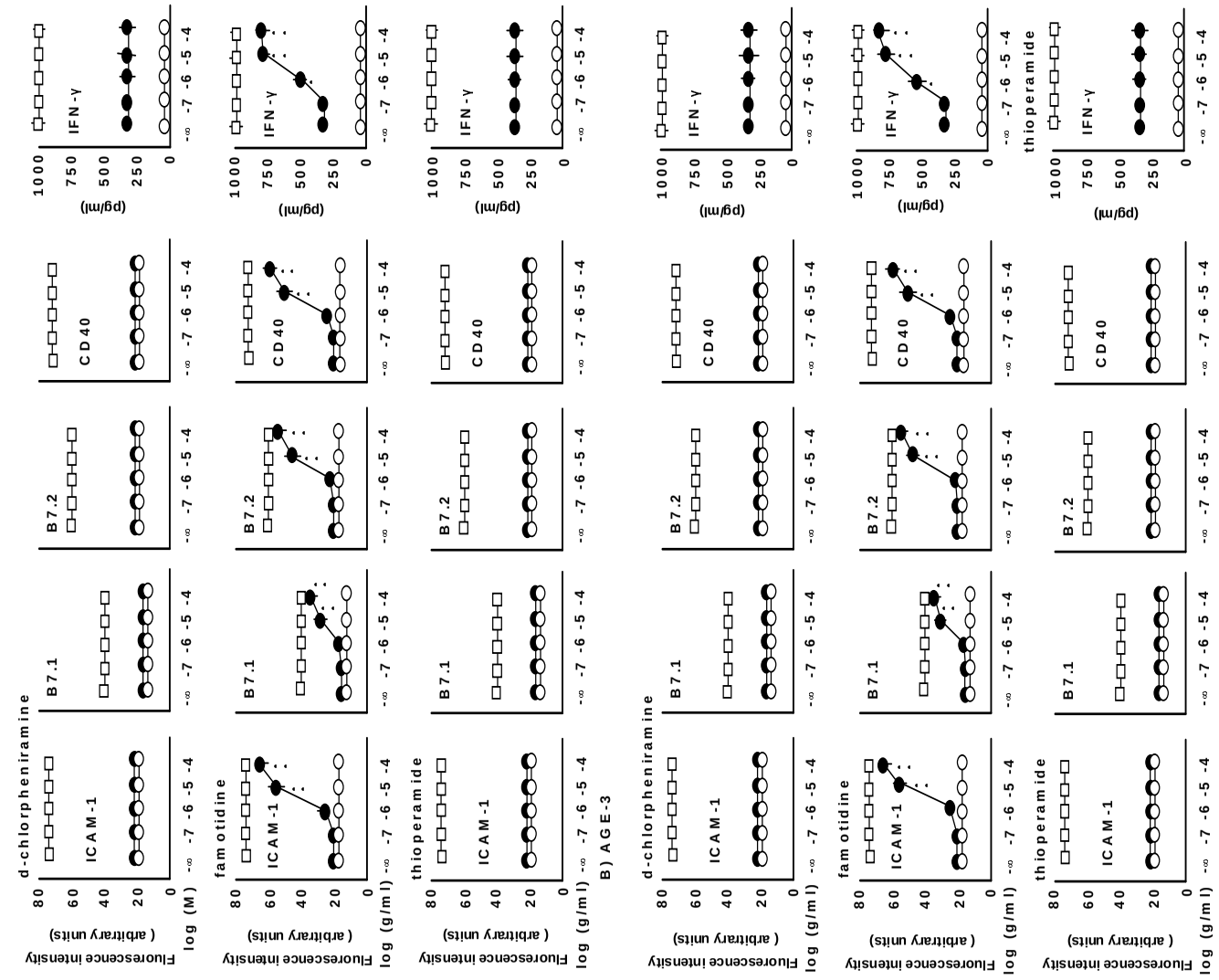
Figure 3



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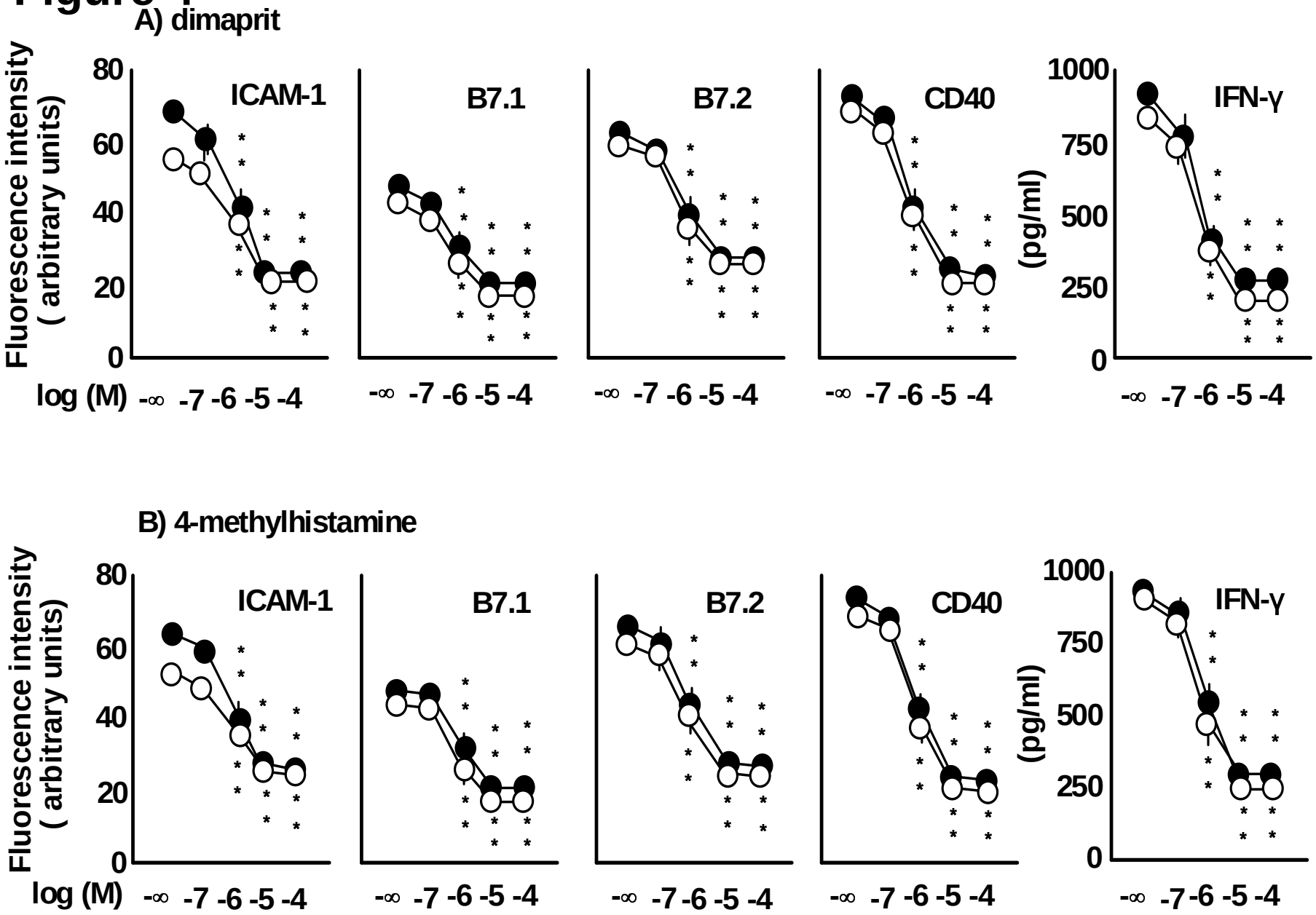

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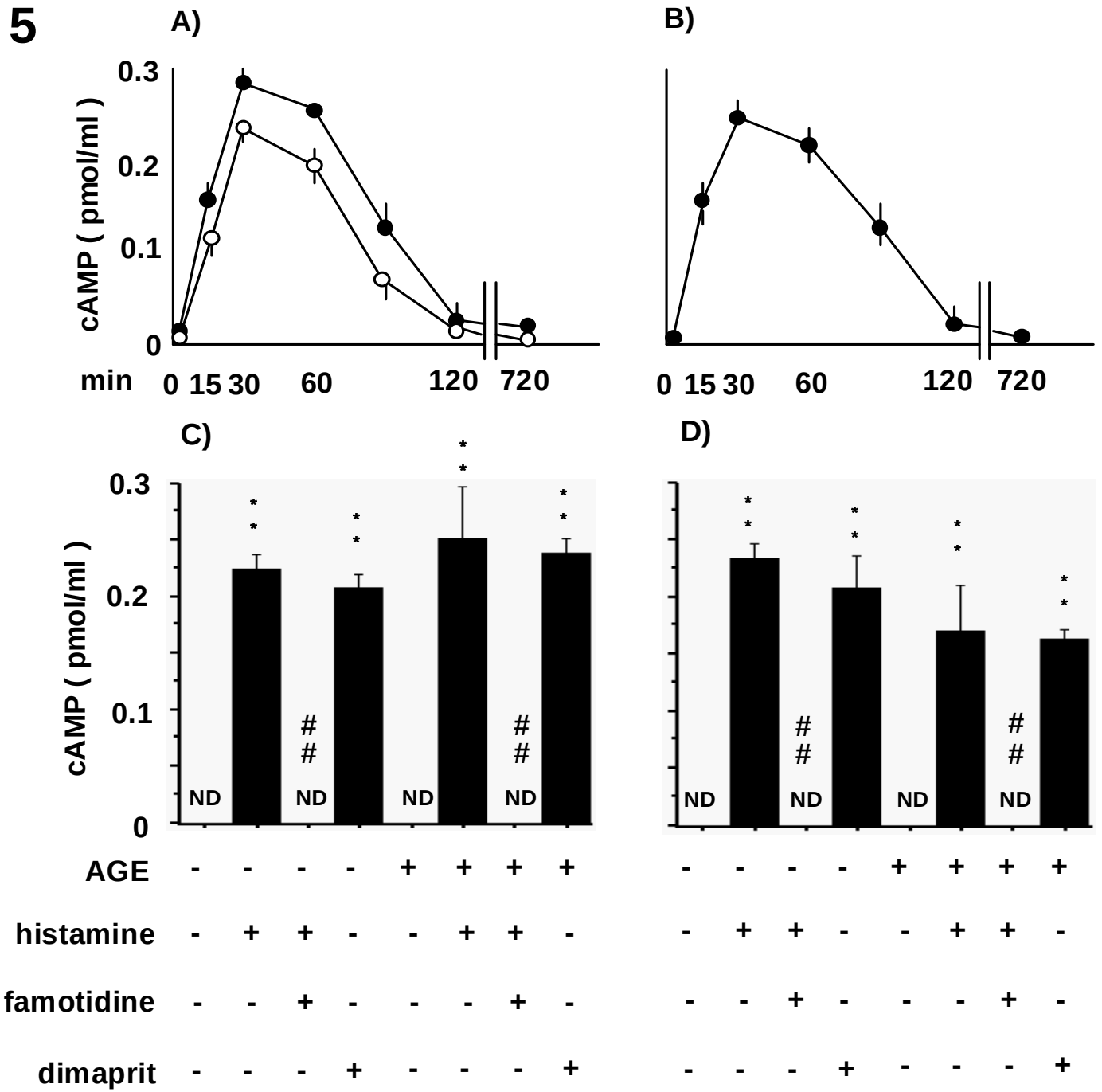

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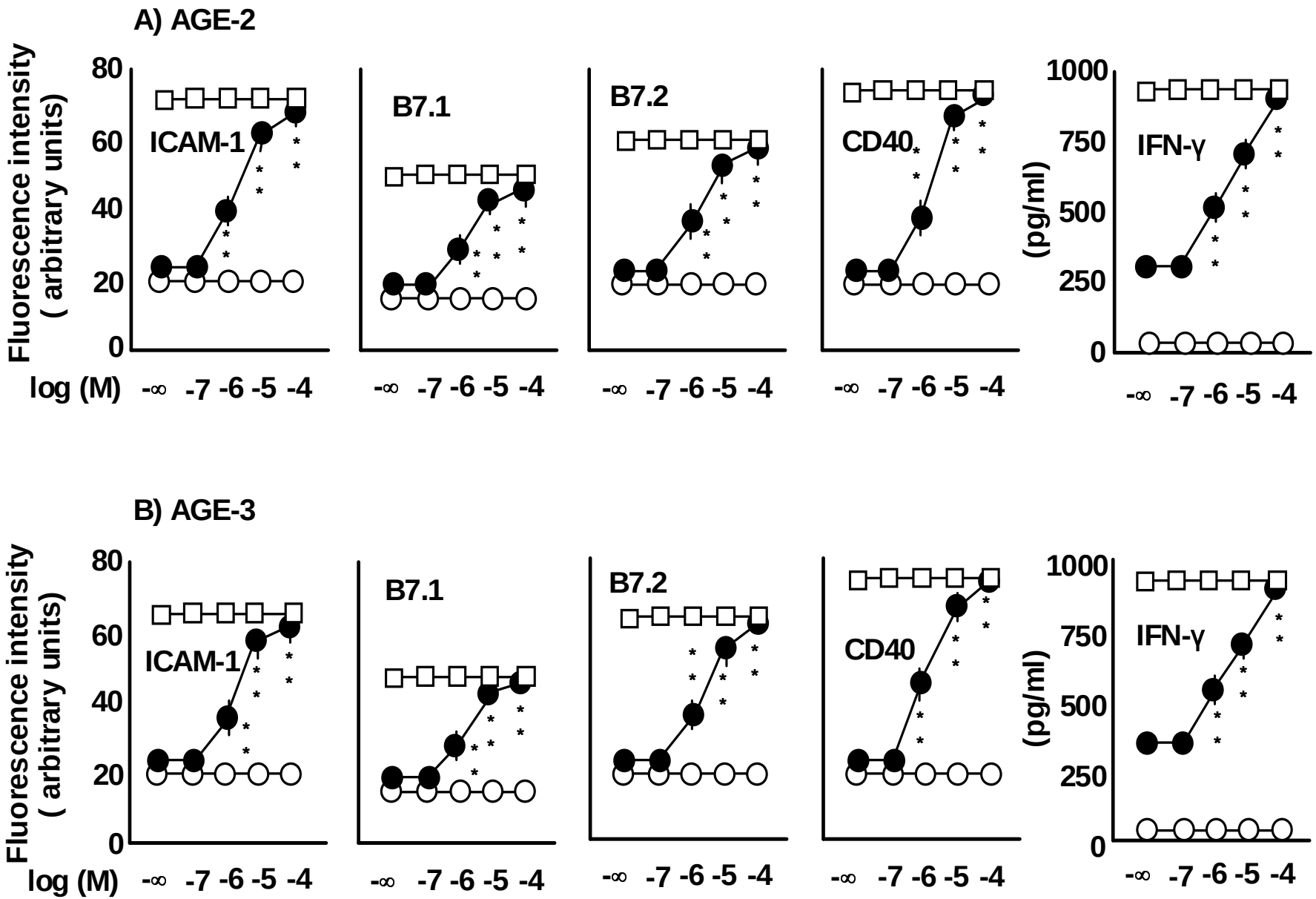

Figure 7

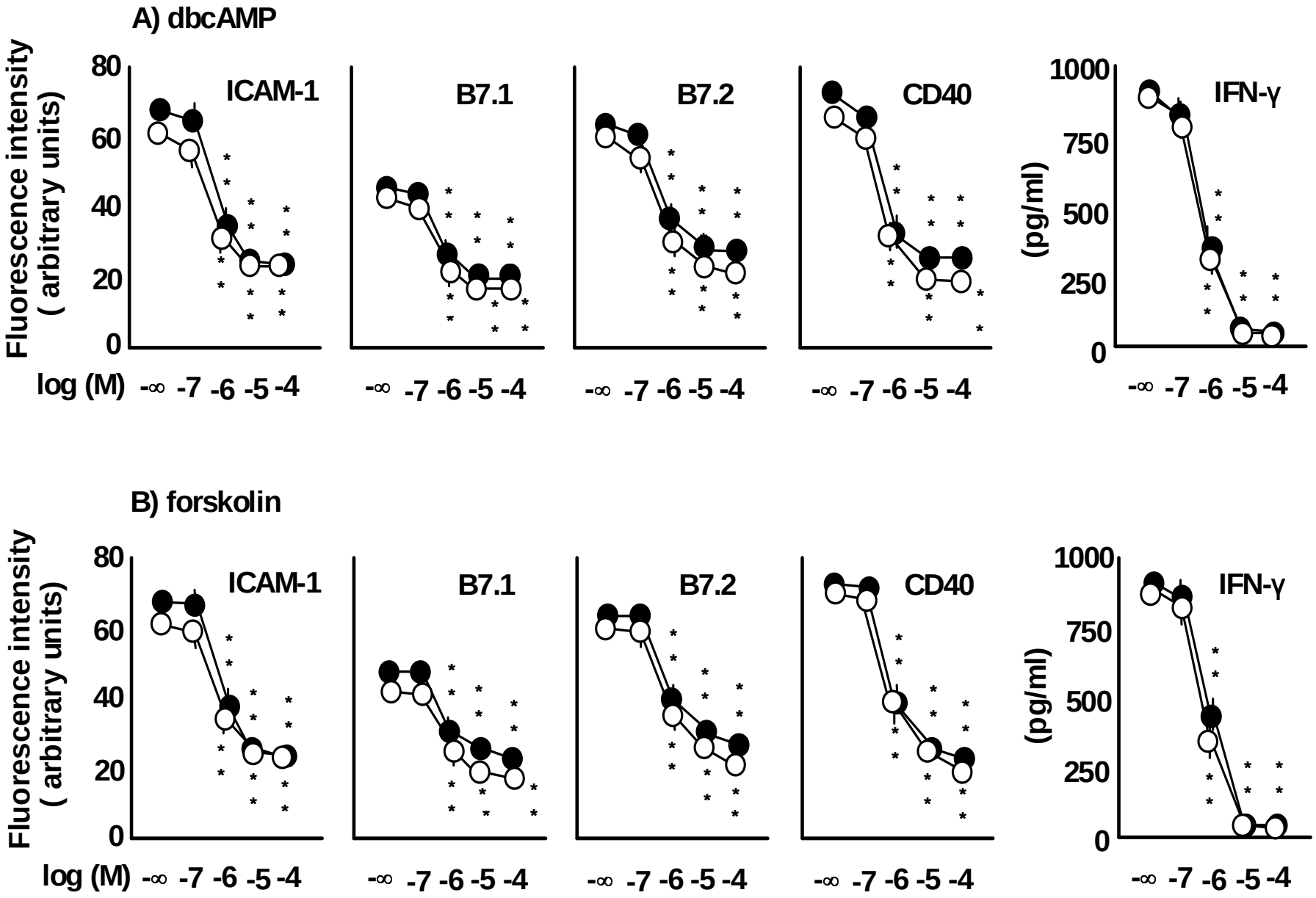

Figure 8