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RESEARCH PAPER

Histamine inhibits adhesion molecule expression in human monocytes, induced by advanced glycation end products, during the mixed lymphocyte reaction

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Background and purpose: Post-transplant diabetes mellitus is a frequent complication among transplant recipients. Ligation of advanced glycation end products (AGEs) with their receptor on monocytes/macrophages plays important roles in the genesis of diabetic complications. The enhancement of adhesion molecule expression on monocytes/macrophages activates T-cells, reducing allograft survival. Out of four distinct AGE subtypes (AGE-2, AGE-3, AGE-4 and AGE-5), only AGE-2 and AGE-3 induced expression of intercellular adhesion molecules (ICAMs), output of cytokines and proliferation of lymphocytes, during the mixed lymphocyte reaction (MLR). Here we have assessed the role of histamine in the actions of AGEs during the MLR.
Experimental approach: Human peripheral blood cells were used in these experiments. Flow cytometry was used to examine the expression of the ICAM-1, B7.1, B7.2 and CD40. Production of the cytokine interferon-γ, and levels of cAMP were determined by ELISA. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake.
Key results: Histamine concentration dependently inhibited the action of AGE-2 and AGE-3. The actions of histamine were antagonized by an H_2-receptor antagonist, famotidine, and mimicked by H_2/H_4-receptor agonists, dimaprit and 4-methylhistamine. The effects of histamine were reversed by a protein kinase A (PKA) inhibitor, H89, and mimicked by dibutyryl cAMP and an adenylate cyclase activator, forskolin.
Conclusions and implications: Histamine down-regulated AGE-2- and AGE-3-induced expression of adhesion molecules, cytokine production and lymphocyte proliferation via histamine H_2 receptors and the cAMP/PKA pathway.
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Keywords: histamine; advanced glycation end products; adhesion molecule; human mixed lymphocyte reaction; transplantation; diabetes; monocytes; cyclic adenosine monophosphate; H_2 receptor

Abbreviations: AGEs, advanced glycation end products; ICAM, intercellular adhesion molecule; MLR, mixed lymphocyte reaction; PTDM, post-transplant diabetes mellitus

Introduction

Diabetes mellitus is characterized by hyperglycemia, which facilitates the formation of advanced glycation end products (AGEs), both in blood and intracellularly (Vlassara and Palace, 2002; Schiekofer *et al.*, 2003). AGEs are formed by a non-enzymatic reaction between a carbonyl group of reducing sugars and free amino groups from macromolecules such as proteins, lipoproteins and nucleic acids. AGEs accumulate in the plasma and tissues of patients with diabetes, leading to the pathogenesis of many of the complications of diabetes (Brownlee *et al.*, 1988; Cooper, 2004). Tissue deposition of AGEs induces macrophage-mediated injury in diabetic complications that correlate with the severity and duration of hyperglycemia (Tanji *et al.*, 2000; Swamy-Mruthinti *et al.*, 2002). Direct immunochemical evidence for the existence of

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four distinct AGE structures, AGE-2, AGE-3, AGE-4 and AGE-5, is provided from the analysis of AGEs within modified proteins and peptides (Takeuchi and Yamagishi, 2004). Recently, two AGE structures, glyceraldehyde-derived AGE (AGE-2) and glycolaldehyde-derived AGE (AGE-3), have been shown to exert diverse biological activities on vascular endothelial cells, vascular smooth muscle cells, mesangial cells, Schwann cells, malignant melanoma cells and cortical neurons (Okamoto *et al*., 2002; Yamagishi *et al*., 2002). AGE-2 and AGE-3 are the main forms of AGEs detectable in the serum of diabetic patients (Takeuchi and Yamagishi, 2004). Loss of glycemic control induces vascular complications of diabetes, leading to allograft loss (Sumrani *et al*., 1991; Miles *et al*., 1998). Many risk factors for atherosclerosis, including hypertension, hyperlipidemia and hyperglycemia, play important roles in the development of chronic allograft nephropathy and graft loss (Arnalich *et al*., 2000; Thomas *et al*., 2001). However, the mechanism of impaired graft survival in patients with post-transplant diabetes mellitus (PTDM) is uncertain.

Monocyte-derived co-stimulatory signals are important in eliciting maximal T-cell growth, differentiation, T-cell proliferation and cytokine production, lowering the concentration of antigen required for stimulation and promoting more sustained signalling from T-cell receptors. The interaction of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40 on monocytes with their ligands on T-cells produces important co-stimulatory signals (Dustin and Springer, 1989; Greenfield *et al*., 1998). Blockade of these co-stimulatory signals has great therapeutic potential for controlling inflammatory and immune responses, and prolongs allograft survival in a variety of animal models and human patients (Shimizu *et al*., 2000; Zhu *et al*., 2000). These results suggested that the down-regulation of adhesion molecule expression on monocytes might decrease lymphocyte proliferation and cytokine production during a mixed lymphocyte reaction (MLR) (Rizzo *et al*., 2000; Tamura *et al*., 2004; Takahashi *et al*., 2005). In an earlier study, we found that AGE-2 and AGE-3, but not AGE-4 and AGE-5, induced the expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, the production of interferon (IFN)-γ and lymphocyte proliferation during human MLR (Ohashi *et al*., 2010).

Histamine is known to modulate cytotoxic T-cell activity (Khan *et al*., 1989), NK-cell activity (Hellstrand *et al*., 1994) and cytokine production in human peripheral mononuclear cells (PBMCs) (Elenkov *et al*., 1998; van der Pouw Kraan *et al*., 1998), through the stimulation of H_1, H_2, H_3 and H_4 receptors (Elenkov *et al*., 1998; van der Pouw Kraan *et al*., 1998; receptor nomenclature follows Alexander *et al*., 2009). Immunoregulatory effects of histamine are reported to depend on the stimulation of H_2 receptors (Elenkov *et al*., 1998; van der Pouw Kraan *et al*., 1998; Hough, 2001), and such stimulation is coupled with the activation of adenylate cyclase and the cAMP/protein kinase A (PKA) pathway in monocytes (Shayo *et al*., 1997). We have found histamine to inhibit AGE-2- and AGE-3-induced expressions of ICAM-1, B7.1, B7.2 and CD40 and the production of IFN-γ in PBMCs via H_2 receptors and the cAMP/PKA pathway (Wake *et al*., 2009). However, little is known about the effect of histamine on the AGE-induced activation of monocytes during the MLR, which is frequently used to assess immune reactions in transplantation. In the present study, we examined the effect of histamine on AGE-2- and AGE-3-induced expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and lymphocyte proliferation during MLR, using human cells.

Methods

Culture conditions during the MLR

Normal human PBMCs were obtained from 10 healthy volunteers with consent and institutional review board (IRB) approval (Okayama Univ. IRB No.106). Samples of 20–50 mL peripheral blood were withdrawn from the forearm vein, after which PBMCs were prepared (Ohashi *et al*., 2010; Takahashi *et al*., 2009). PBMCs at 1×10^6 cells·mL^{-1} from an individual volunteer were mixed with cells from an unrelated person (mixed cells), and the final concentration was adjusted to 2.0×10^6 cells·mL^{-1}. PBMCs (not mixed cells) and mixed cells were subsequently suspended in RPMI 1640 medium (Nissui, Co. Ltd, Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 20 μg·mL^{-1} kanamycin and 100 μg·mL^{-1} streptomycin and penicillin (Sigma Aldrich, St Louis, MO, USA). Cells were incubated under various conditions for 48 h at 37°C in a humidified atmosphere of 5% CO_2 in air. All reagents were added to the media at the start of the MLR.

Flow cytometric analysis for adhesion molecule expression

For flow cytometric analysis, fluoresceinisothiocyanate isomer-1 (FITC)-conjugated mouse IgG1 monoclonal antibody (mAb) against ICAM-1/CD54 and phycoerythrin (PE)-conjugated anti-CD3, CD14 and CD19 mAb were purchased from DAKO (Glostrup, Denmark). FITC-conjugated mouse IgG1 mAb against B7.1 was purchased from IMMUNOTECH (Marseille, France), FITC-conjugated mouse IgG1 mAb against B7.2 and CD40 were from Pharmingen (San Diego, CA, USA), and FITC-conjugated IgG1 isotype-matched control was obtained from Sigma Chemical. Changes in the expression of human leucocyte antigens, ICAM-1, B7.1, B7.2 and CD40, were determined by anti-ICAM-1, anti-B7.1, anti-B7.2 or anti-CD40 Ab with anti-CD14 Ab. In addition, changes in the expression of the receptor for AGEs (RAGE) on human monocytes were examined by multicoloured flow cytometry using a combination of PE-conjugated anti-CD14 (monocyte) mouse IgG (DAKO) and anti-human RAGE rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by FITC-conjugated anti-rabbit IgG goat IgG (DAKO) respectively. Mixed cells at 2×10^6 cells·mL^{-1} were incubated for 48 h. Cultured cells at 5×10^5 cells·mL^{-1} were prepared for flow cytometric analysis as previously described (Ohashi *et al*., 2010; Takahashi *et al*., 2009) and analyzed with FACS Calibur (BD Biosciences, San Jose, CA, USA). Data were processed using the CELL QUEST program.

ELISA assays

Mixed cells at 2×10^6 cells·mL^{-1} were used for analyzing IFN-γ production. After culturing for 48 h at 37°C in a 5% CO_2/air mixture, the cell-free supernatant was assayed for IFN-γ protein by ELISA employing the multiple Abs sandwich

principle (R&D Systems, Minneapolis, MN, USA). The detection limit of ELISA for IFN-γ was 10 pg·mL^{-1}.

Cell proliferation assay

Mixed cells were treated under various conditions. Cultures were incubated for 48 h, during which they were pulsed with [^{3}H]-thymidine (3·3 Ci per well) for the final 16 h. Cells were then divided into 96-well microplates, 200 μL per well, resulting in 1 μCi [^{3}H]-thymidine per well, and harvested by a 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.).

Statistical analysis

Statistical significance was evaluated using ANOVA followed by Dunnett's test. A probability value of less than 0.05 was considered significant. The results are expressed as the means ± SEM of triplicate findings from five donors

Materials

Histamine dihydrochloride was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Dimaprit dihydrochloride and 4-methylhistamine dihydrochloride 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) was prepared as previously described (Ohashi *et al*., 2010; Takahashi *et al*., 2009). 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°C for 7 days. AGE-BSA was dialyzed for 2 days at 4°C. The endotoxin contamination in the AGEs prepared as

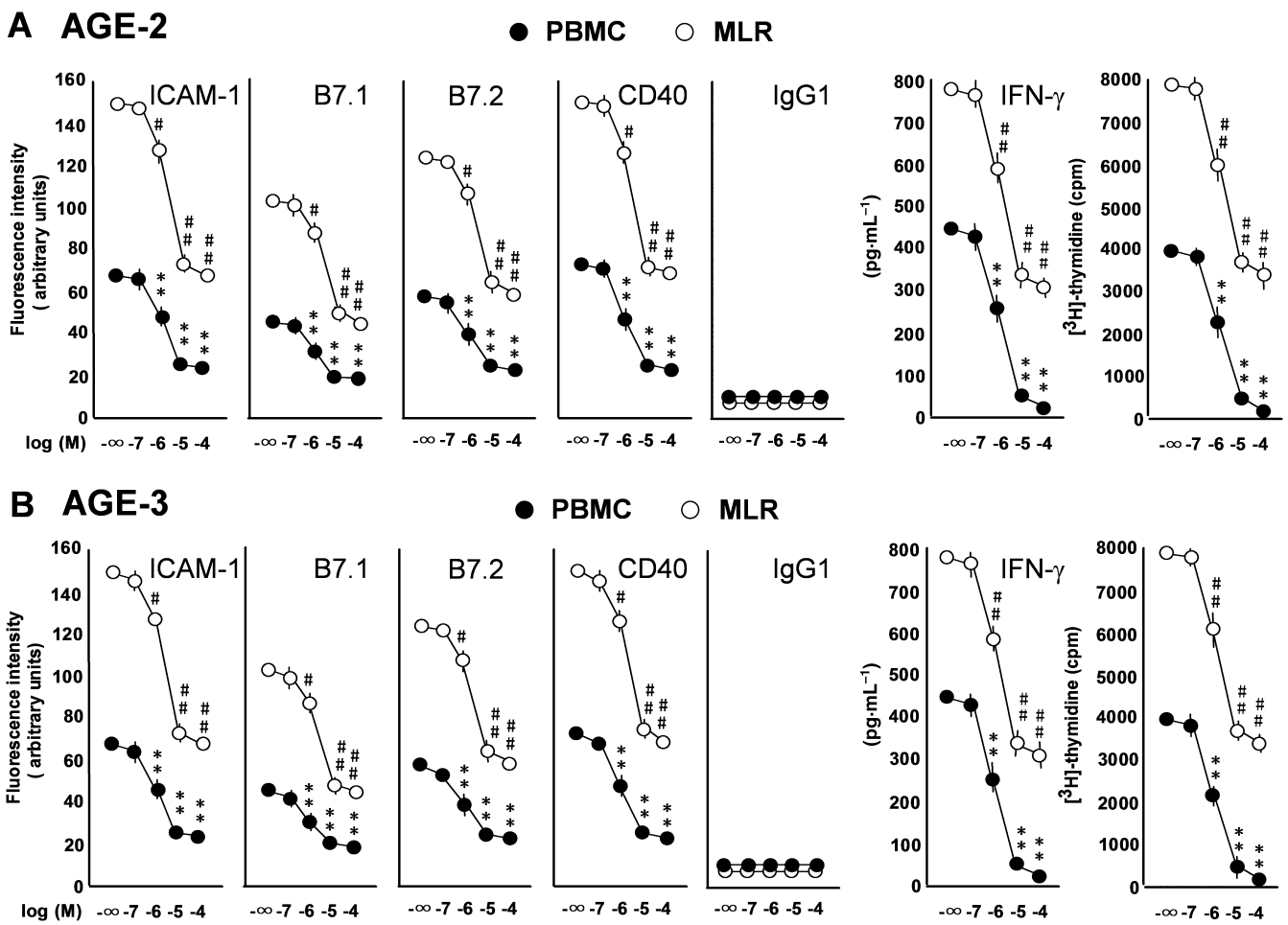

Figure 1 The effects of histamine on advanced glycation end product (AGE)-2- and AGE-3-induced expression of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40 on monocytes, the production of interferon (IFN)-γ and lymphocyte proliferation during the mixed lymphocyte reaction (MLR). Peripheral mononuclear cells (PBMCs) (not mixed cells) and mixed cells at 2×10^6 cells·mL^{-1} were incubated with AGE-2 (A) and AGE-3 (B) (100 μg·mL^{-1}) and histamine (0.1–100 μM) for 48 h. The expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes were determined by flow cytometry. FITC-conjugated IgG_1 was used as an isotype-matched control Ab. IFN-γ concentration in conditioned media was determined by ELISA. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake. The results are expressed as the means ± SEM of five donors with triplicate determinations. $^{**}P < 0.01$ compared with the value for AGE-2 and AGE-3 in PBMCs. $^{\#}P < 0.5$; $^{\#\#}P < 0.01$ compared with the value for AGE-2 and AGE-3 during MLR. When an error bar is within a symbol, the bar is omitted.

described above was 1.2 pg per 100 μg AGE in 1 mL (determined by SRL, Inc., Okayama, Japan). AGE-specific fluorescence was measured at 450 nm after excitation at 390 nm with a fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Dibutyryl cAMP (dbcAMP) and forskolin were purchased from Wako Co., Ltd. (Tokyo, Japan). H89 was purchased from Sigma Chemical.

Results

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

In a previous study, to evaluate the binding of AGE subtypes to RAGE, we established an *in vitro* assay using immobilized

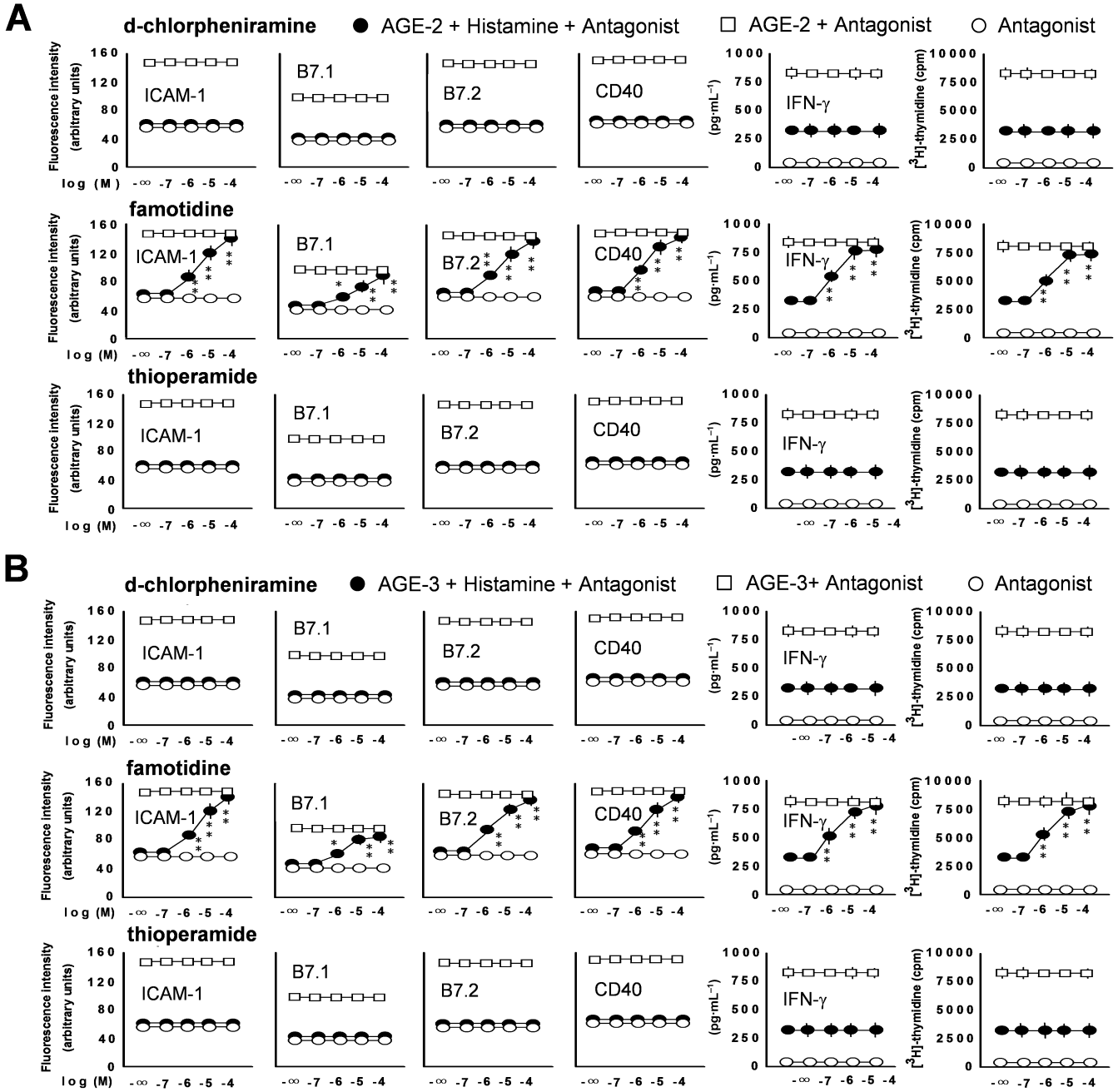

Figure 2 The effects of histamine receptor antagonists on the actions of histamine. Mixed cells at 2×10^6 cells·mL^{-1} were incubated with different classes of histamine receptor antagonists, including *d*-chlorpheniramine (H_1 antagonist), famotidine (H_2 antagonist) and thioperamide (H_3/H_4 antagonist), at increasing concentrations from 0.1 to 100 μM. The antagonists were used alone (antagonist), with advanced glycation end product (AGE)-2 or AGE-3 (100 μg·mL^{-1}; AGE + antagonist) or with AGEs and histamine (100 μM; AGE + Hist + Ant). The expressions of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40 on monocytes were determined by flow cytometry. Interferon (IFN)-γ concentration in conditioned media was determined by ELISA. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake The results are expressed as the means ± SEM of five donors with triplicate determinations. $^*P < 0.05$; $^{**}P < 0.01$ compared with the value for histamine. When an error bar is within a symbol, the bar is omitted.

AGE subspecies and His-tagged soluble RAGE (sRAGE) protein (Takahashi *et al*., 2009). AGE-2 and AGE-3 showed relatively high affinity binding for sRAGE, whereas AGE-4 and AGE-5 showed moderate affinity for sRAGE. To determine the appropriate incubation time, we examined the kinetics at 0, 4, 16, 24, 48 and 72 h. AGE-2 and AGE-3 (100 μg·mL^{-1}) induced the expression of ICAM-1, B7.1, B7.2 and CD40 on monocytes, the production of IFN-γ and the proliferation during MLR at 16 h and thereafter up to 24 and 48 h, whereas AGE-4, AGE-5 and BSA at the same concentration had no effect (Ohashi *et al*., 2010). Moreover, to determine the appropriate concentration of AGEs, the effects of AGE-2 and AGE-3 at concentrations ranging from 100 ng·mL^{-1} to 100 μg·mL^{-1} for 48 h were examined. AGE-2 and AGE-3 at 1, 10 and 100 μg·mL^{-1} significantly induced the expressions of ICAM-1, B7.1, B7.2 and CD40 on monocytes, the production of IFN-γ and proliferation during MLR (Ohashi *et al*., 2010).

As shown in Figure 1, we observed the effects of histamine (0.1–100 μM) on AGE-enhanced expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and lymphocyte proliferation, using 100 μg·mL^{-1} AGE-2 or AGE-3 for 48 h in PBMC and MLR. The expression levels of ICAM-1, B7.1, B7.2 and CD40, production of IFN-γ and lymphocyte proliferation were higher during the MLR than in PBMCs cultured alone. Histamine concentration dependently inhibited the effect of the AGEs on adhesion molecule expression, cytokine production and lymphocyte proliferation in both cell systems. IC_{50} values for the inhibitory effect of histamine in the presence of AGE-2 during MLR were 2, 2, 2, 1.5, 1.5 and 1.5 μM, and those in the presence of AGE-3 were 2, 2, 2, 1.5, 1.5 and 1.5 μM respectively. Incubation with histamine alone had no effect on the variables we measured in PBMC or in the MLR (data not shown).

The involvement of H_2 receptors in the actions of histamine

To determine the histamine receptor subtypes involved in the effects of histamine on these cellular responses to AGE-2 and AGE-3, the effects of an H_1-receptor antagonist, we used a range of histamine receptor antagonists, *d*-chlorpheniramine, an H_2-receptor antagonist, famotidine, and an H_3/H_4-receptor antagonist, thioperamide, at concentrations ranging from 0.1–100 μM. In these experiments, we used a fixed concentration of histamine (100 μM). As shown in Figure 2, among these histamine receptor antagonists, only famotidine inhibited the effects of histamine. Another H_2-receptor antagonist, ranitidine, exerted a substantially similar effect to famotidine (data not shown). As shown in Figure 3, the effects of the H_2/H_4-receptor agonists, dimaprit and 4-methylhistamine (Parsons *et al*., 1977), at concentrations ranging from 0.1 to

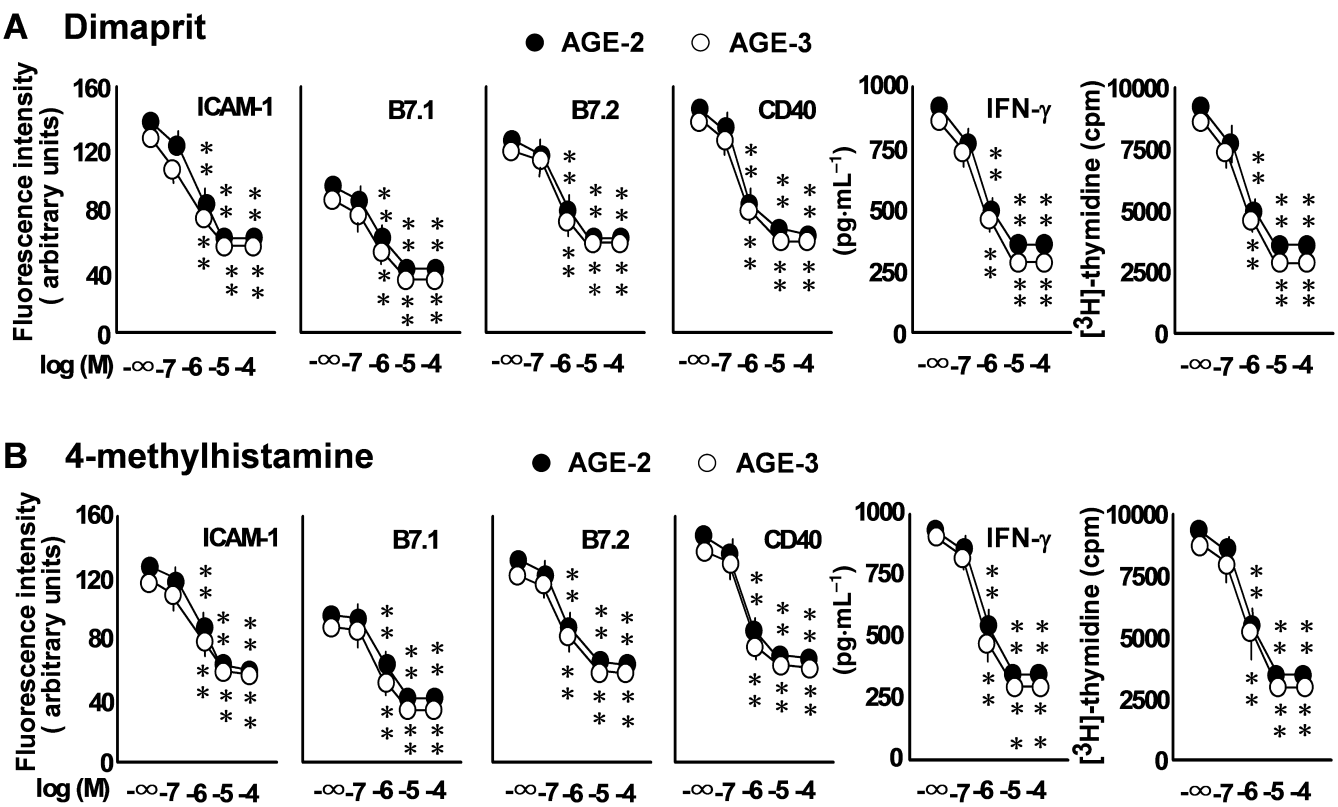

Figure 3 The effects of histamine receptor agonists on the advanced glycation end product (AGE)-2- and AGE-3-induced expressions of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40, the production of interferon (IFN)-γ and lymphocyte proliferation. Mixed cells at 2×10^6 cells·mL^{-1} were incubated with histamine H_2/H_4-receptor agonists, dimaprit (A) and 4-methylhistamine (B) at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 (100 μg·mL^{-1}; 48 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. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake The results are expressed as the means ± SEM of five donors with triplicate determinations. $^{**}P < 0.01$ compared with the value for AGE-2 or AGE-3 alone. When an error bar is within a symbol, the bar is omitted.

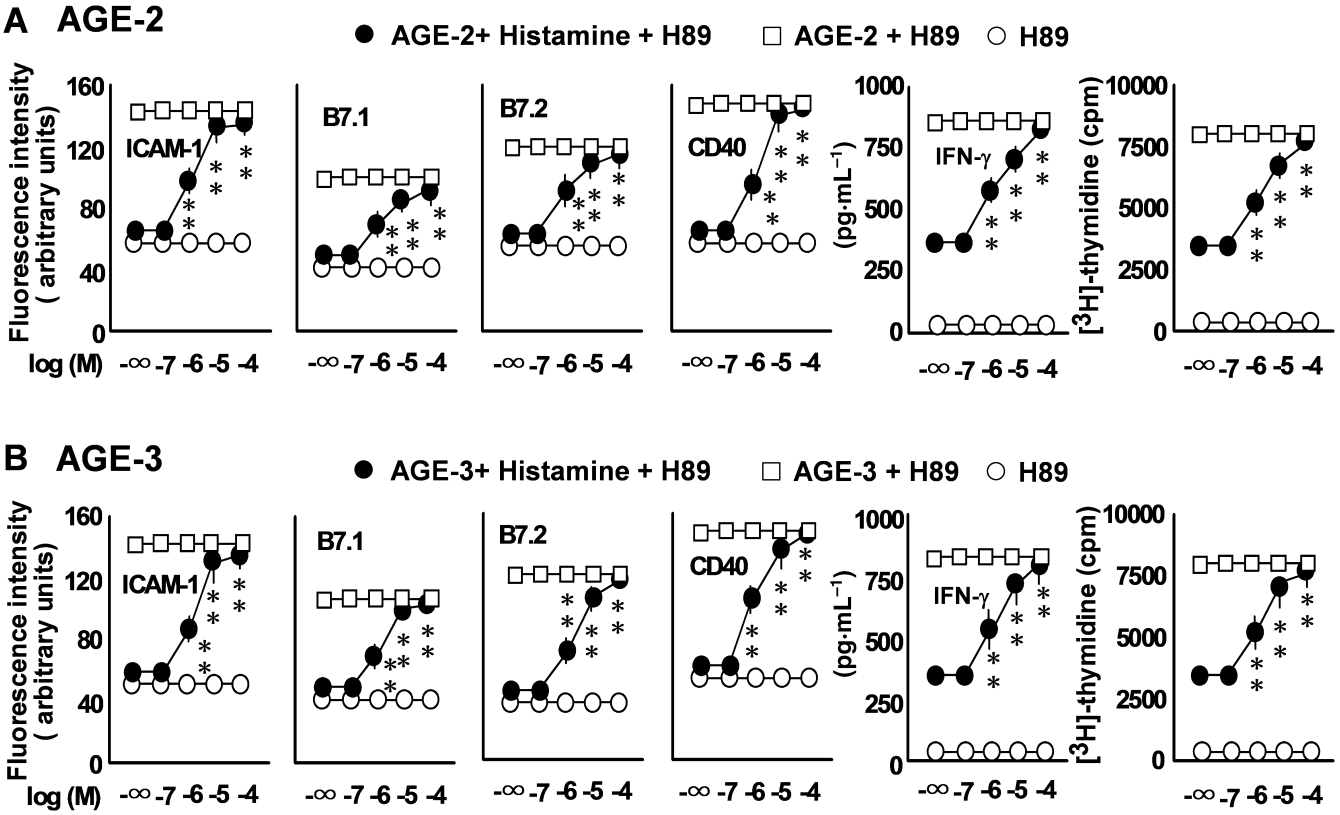

Figure 4 The effects of the protein kinase A (PKA) inhibitor, H89, on the histamine-induced down-regulation of expression of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40, the production of interferon (IFN)-γ and lymphocyte proliferation. Mixed cells at 2×10^6 cells·mL^{-1} were incubated with a PKA inhibitor, H89, (0.1–100 μM) in the presence of advanced glycation end product (AGE)-2 (A) and AGE-3 (B) (100 μg·mL^{-1}) and histamine (100 μM) for 48 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. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake. The results are expressed as the means ± SEM of triplicate findings from five donors. $^{**}P < 0.01$ compared with the value in the presence of histamine and AGEs. When an error bar is within a symbol, the bar is omitted.

100 μM were determined in the presence of AGE-2 or AGE-3 (100 μg·mL^{-1}). Both agonists inhibited the expression of adhesion molecules, the production of IFN-γ and lymphocyte proliferation in a concentration-dependent manner. The potency and efficacy of the 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 responses to AGE-2 and AGE-3 in our system (data not shown).

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 (0.1–100 μM), on the action of histamine (100 μM) were determined (Figure 4). In the absence of histamine, the PKA inhibitor had no effect on adhesion molecule expression, cytokine production and lymphocyte proliferation, but it did reverse the inhibitory effect of histamine on these responses to AGE-2 or AGE-3. We also assessed the effects of a membrane-permeable cAMP analogue, dbcAMP, and an adenylate cyclase activator, forskolin (0.1–100 μM), on these responses. As shown in Figure 5, both dbcAMP and forskolin inhibited AGE-2- and AGE-3-induced adhesion molecule expression, cytokine production and lymphocyte proliferation in a concentration-dependent manner.

Discussion

The levels of AGE-2 are about 17 μg·mL^{-1} in the serum of diabetic patients (Enomoto *et al*., 2006; Nakamura *et al*., 2007). AGEs at concentrations ranging from 50 to 200 μg·mL^{-1} markedly induced human monocyte adhesion to bovine retinal endothelial cells (Mamputu and Renier, 2004). AGEs at 200 μg·mL^{-1} induced the expressions of CD40, CD80 and CD86 and the production of IFN-γ in dendritic cells (Ge *et al*., 2005). In the previous study, we found that AGE-2 and AGE-3 over a range of concentrations (1–100 μg·mL^{-1}) significantly up-regulated the expression of ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and lymphocyte proliferation during MLR (Ohashi *et al*., 2010). Therefore, the concentration (100 μg·mL^{-1}) used in the present study may not be far above the pathological concentration of AGEs in the serum of diabetic patients previously reported (Enomoto *et al*., 2006; Nakamura *et al*., 2007).

In the present study, we clearly demonstrated for the first time that histamine inhibited AGE-2- and AGE-3-induced

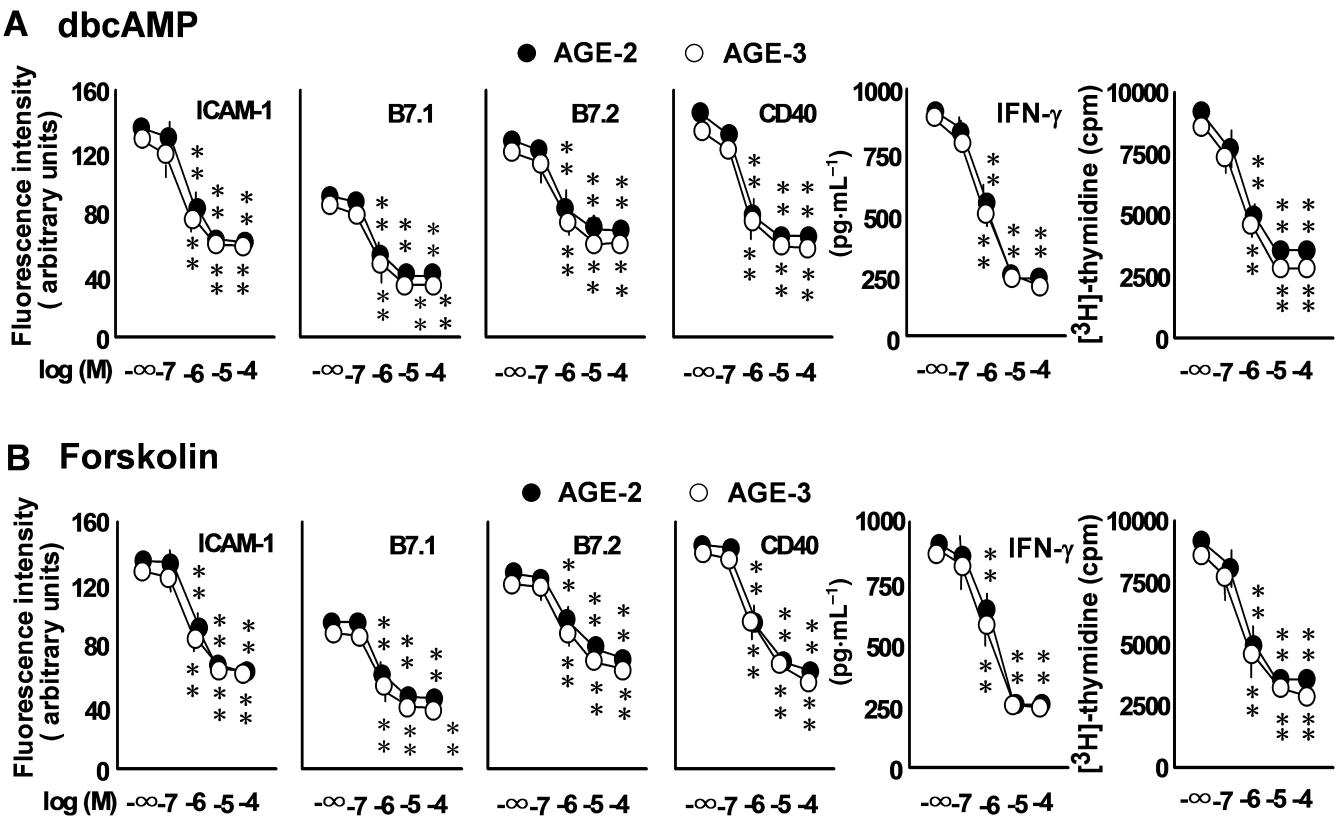

Figure 5 The effects of forskolin and dibutyryl cAMP (dbcAMP) on advanced glycation end product (AGE)-induced expression of intercellular adhesion molecule (ICAM)-1, B7.1, B7.2 and CD40, the production of interferon (IFN)-γ and lymphocyte proliferation. Mixed cells at 2×10^6 cells·mL^{-1} were incubated with a cAMP analogue, dbcAMP (A), or an adenylate cyclase activator, forskolin (B), at increasing concentrations from 0.1 to 100 μM in the presence of AGE-2 and AGE-3 (100 μg·mL^{-1}; 48 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. Lymphocyte proliferation was determined by [^{3}H]-thymidine uptake The results are expressed as the means ± SEM of five donors with triplicate determinations. $^{**}P < 0.01$ compared with the value for AGE-2 or AGE-3 alone. When an error bar is within a symbol, the bar is omitted.

expressions of ICAM-1, B7.1, B7.2 and CD40 on human monocytes, the production of IFN-γ and lymphocyte proliferation during MLR (Figure 1). The action of histamine was inhibited by an H_2-receptor antagonist, famotidine, but not an H_1-receptor antagonist, *d*-chlorpheniramine, or an H_3/H_4 antagonist, thioperamide (Figure 2). The histamine H_2/H_4-receptor agonists dimaprit and 4-methylhistamine mimicked the action of histamine (Figure 3). As the IC_{50} values of histamine and the 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 histamine H_2 receptors (Elenkov *et al*., 1998; Itoh *et al*., 2002; Wake *et al*., 2009), it was concluded that the inhibitory effect of histamine was mediated by the stimulation of H_2 receptors rather than the H_1, H_3 and H_4 receptors for histamine.

In the previous study, we found that histamine induced the production of cAMP in monocytes via H_2 receptors (Wake *et al*., 2009). Here we found that the PKA inhibitor, H89, inhibited the action of histamine (Figure 4) and that the cAMP analogue, dbcAMP, and the adenylate cyclase activator, forskolin, mimicked the effect of histamine (Figure 5), suggesting the involvement of the cAMP/PKA pathway in these actions of histamine. We observed a similar pattern of inhibitory effects of histamine on IL-18-induced activation of monocytes during MLR via H_2 receptors (Itoh *et al*., 2002). Thus, a common pathway triggered by IL-18 and AGEs may be regulated by the H_2-receptor cAMP/PKA system. Further work is necessary on this issue.

Although histamine alone had no effect on basal levels of the adhesion molecules, the production of IFN-γ and lymphocyte proliferation during MLR, that is, in the absence of AGE-2 and AGE-3, dbcAMP and forskolin inhibited these basal responses (data not shown). Moreover, none of the histamine receptor antagonists affected this lack of response to histamine alone, and no histamine receptor agonists altered the basal responses (data not shown). These results indicated that histamine used alone inhibited cAMP-initiated regulation during the MLR, in a histamine receptor-independent manner.

It is known that PTDM is a common side effect of cyclosporin A and tacrolimus (Marchetti, 2005). Many patients develop diabetes mellitus early after transplantation when they are exposed to tacrolimus and steroids (Filler *et al*., 2000; van Hooff *et al*., 2004). The cause of impaired graft survival in PTDM patients depends on the use of lower dosages of immunosuppressive agents in diabetic patients. The accumulation of AGEs was elevated in recipients with chronic renal dysfunction and cardiovascular disease after renal transplantation (Hartog *et al*., 2006). Immunological

rejection and ischaemia was reported as mechanisms of the induction of mast cell infiltration in renal allograft (Shoskes *et al*., 1990). The relation between mast cells and rejection has been described in the heart (Ly *et al*., 1992), lung (Yousem, 1997), intestine (Walgenbach *et al*., 1996), graft-versus-host disease (Nagler *et al*., 1995) and acute cellular rejection of the human kidney (Lajoie *et al*., 1996). However, little is known about the effect of histamine and H_2-receptor stimulation on AGE-induced monocyte activation during MLR.

In conclusion, histamine inhibited the AGE-2- and AGE-3-induced expression of the adhesion molecules ICAM-1, B7.1, B7.2 and CD40, the production of IFN-γ and lymphocyte proliferation, via histamine H_2 receptors and the cAMP/PKA pathway. Through the inhibition of toxic AGE-induced responses in monocytes, the stimulation of histamine H_2 receptors may partially contribute to down-regulating the immune response in patients with PTDM.

Conflict of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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